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Circadian Pacemaker in the *Bursatella* Eye: Properties of the Rhythm and Its Effect on Locomotor Behavior

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Summary. The eye of the frilled sea hare, *Bursatella leachi plei*, expresses a circadian rhythm in the frequency of spontaneously occurring optic nerve impulses. The rhythm will free-run for at least 3 cycles in vitro (Fig. 2) and can be entrained by light cycles provided in vivo (Fig. 4A). While both *Bursatella* and *Aplysia* eyes contain circadian pacemakers the two rhythms differ in several respects: (1) the peak impulse frequency for *Bursatella* eyes is only 96/h (± 36 SD) compared with 247/h (±61 SD) for *Aplysia*. (2) The ocular waveform of the *Bursatella* rhythm exhibits a steep rise and fall from peak frequencies and lacks the delayed falling phase which creates a 'shoulder' on the ocular waveform in *Aplysia* (Fig. 2). (3) The in vitro free-running period of the *Bursatella* ocular rhythm is 21.2 h (±0.6 SD) compared with 24.3 h (±0.9 SD) for the *Aplysia* rhythm (Fig. 2). (4) The steady state phase angle for entrainment differs with *Bursatella* eyes showing a median activity peak at +3 Z.T. compared with a median *Aplysia* peak at −1 Z.T. (Fig. 4).

We also investigated the locomotor rhythm. *Bursatella* were found to be predominantly diurnal when exposed to L:*D*, 12:*12* (Fig. 5A) and to exhibit anticipatory locomotor activity when maintained on L:*D*, 9:*15* (Fig. 6). The eyes appear to play a minor role, if any, in timing the locomotor rhythm. Eyeless *Bursatella* remained diurnal on L:*D*, 9:*15* and most animals continued to exhibit anticipatory behavior (Fig. 6). These results suggest that the *Bursatella* eye plays a less prominent role than the *Aplysia* eye in controlling locomotor behavior.

Introduction

The marine mollusc *Aplysia* has become an important model system for studying the physiological basis of circadian rhythms (for reviews see Strumwasser 1974; Lickey et al. 1976; Eskin 1979, Strumwasser et al. 1979). The attractiveness of the *Aplysia* system derives primarily from the presence of a precise circadian pacemaker located within each retinae (Jacklet 1969a), the existence of large, identifiable neurons affording a cellular level analysis of behaviors and the expression of an endogenously timed locomotor rhythm (Strumwasser 1967; Kupfermann 1968). These attributes have provided an opportunity to obtain an understanding of the neurocellular basis of circadian pacemakers as well as an appreciation of the mechanisms by which pacemakers regulate rhythmic behaviors.

We have recently investigated another opisthobranch, *Bursatella leachi plei*, the frilled sea hare. We were motivated to undertake this study for three reasons. First, we wished to determine whether ocular circadian pacemakers were present in other opisthobranchs. Aside from a single report of a circadian rhythm in the eye of *Navanax* (Eskin and Harcombe 1977), only the *Aplysia* eye has been identified as a circadian pacemaker. Secondly, we desired to know whether endogenously timed rhythmic behaviors could be identified in other opisthobranchs and whether these rhythms offered advantages over the *Aplysia* locomotor rhythm for physiological study. Finally, we wished to test in *Bursatella* the model proposed for *Aplysia* that the ocular pacemakers participate in the timing of rhythmic behaviors (Strumwasser 1973; Lickey et al. 1977; Lickey and Wozniak 1979).

In the current paper we discuss experiments which describe the presence of an ocular circadian pacemaker in the eye of *Bursatella* and compare some of its formal and physiological properties to the eye rhythm in *Aplysia*. In addition we identify a circadian rhythm in locomotor activity and attempt to evaluate the role of the ocular pacemakers in timing locomotor behavior.

Abbreviations: *D:D* constant darkness; L:*D*, 12:*12* 24 h light cycles 12 h light, 12 h dark; *EST* Eastern Standard Time; *Z.T.* Zeitgeber Time

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Materials and Methods

Ocular Rhythm Experiments

Two species of opisthobranchs were employed for experimentation. Approximately 60 *Bursatella leachi plei* and 20 *Aplysia californica* were obtained from marine suppliers in Florida (Gulf Specimen Co.) and California (Pacific Biomarine Co., Marine Specimens Unlimited). While in the laboratory all specimens were housed in a 1,200 l recirculating seawater system charged with Instant Ocean artificial seawater (16 °C ± 0.5 °C). After separation from the animal, the eyes were washed with filtered seawater and placed into 100 mm petri dishes containing 60–70 ml of buffered seawater (Instant Ocean, 30 mmol/l HEPES buffer, 100 units/ml penicillin and 100 mcg/ml streptomycin). The optic nerve was inserted into a suction electrode and the dish was placed into a light-tight enclosure maintained at 16 °C ± 0.5 °C. The elapsed time from removal of an animal from the seawater system to placing the isolated eyes into darkness was approximately 15 min.

Optic nerve impulses were amplified and recorded on a Grass polygraph. Isolated eyes were maintained in darkness for 48–96 h during which time a continuous record was obtained. For analysis, optic nerve impulses were counted by hourly intervals and plotted in relation to clock time. The time of peak impulse frequency was chosen as the phase reference point for comparison of different ocular rhythms.

Locomotor Experiments

In order to measure locomotor activity in *Bursatella* activity monitors were fabricated out of 1 liter perforated plastic beakers. An infrared beam was directed down one side of the container and aligned with a phototransistor mounted on the bottom surface. The phototransistors were connected to a discriminator circuit which provided a standard width pulse each time an animal crawled through the infrared beam (Block and Page 1977). Ten activity monitors were suspended inside an aerated 400 l compartment of the seawater system. For data analysis pulses generated each time an animal interrupted the infrared beam were summed into 10 min intervals. A maximum numerical value of '3' was given to 10 min intervals containing 3 or more counts, in an effort to minimize the effects of spuriously high counts caused by *Bursatella* occasionally waving their heads while positioned near the phototransistor. Two techniques employed in data analysis were the production of mean hourly form estimates for animals exposed to light cycles and periodogram analysis for locomotor records generated in D:D (Enright 1965; Binkley 1973).

Results

Gross Morphology and Optic Nerve Activity

The eyes of *Bursatella* are located near the base of the rhinophores. They attach to the cerebral ganglion via a slender optic nerve about 1 cm long. When examined under a dissecting microscope the eyes appear nearly spherical except for a slight convexity formed by a clear 'cornea'. The diameter of the eye is approximately 400 μm and the optic nerve about 100 μm.

Electrical activity in the optic nerve of the isolated *Bursatella* eye consisted of 20–150 μV potentials which could be evoked in response to illumination or occurred spontaneously in darkness. Optic nerve impulses in *Aplysia* are compound action potentials

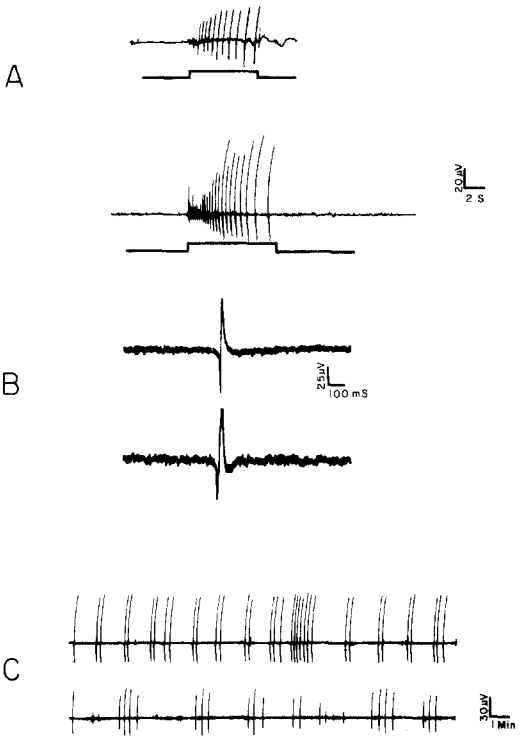

Fig. 1A–C. Optic nerve activity in *Bursatella* and *Aplysia* eyes. **A** Top trace indicates optic nerve response in *Bursatella* to a 2,500 lux light pulse. Lower trace is the response of an *Aplysia* eye to a similar pulse. **B** Oscilloscope trace of spontaneous impulses in D:D from *Bursatella* (*top trace*) and *Aplysia* eyes (*lower trace*). **C** Variability in spontaneous impulse amplitude in two *Bursatella* eyes recorded in D:D

(Jacklet 1969b) and it seems likely that *Bursatella* impulses are also compound as suggested by their graded size in response to illumination of the eye (Fig. 1A), their long duration (Fig. 1B) and the large number of different amplitudes displayed when impulses occur spontaneously in darkness (Fig. 1C).

Free Running Circadian Rhythm

If *Bursatella* eyes were maintained in darkness for more than 24 h a circadian rhythm could be measured in the frequency of spontaneously occurring optic nerve impulses. When two eyes were recorded from the same animal we found the mean phase difference during the first cycle in D:D to be only 8 min (± 21 SD, $n=12$), which is comparable to the 2 min (± 50) separation observed between *Aplysia* eyes on the first cycle (Rothman and Strumwasser 1976).

While both *Bursatella* and *Aplysia* eyes expressed circadian periodicities the two rhythms differed in several respects (Fig. 2). First, the peak impulse frequency for *Brusatella* eyes on the first day in vitro was 96/h (± 36 SD, $n=30$) compared with 247/h (± 61 SD, $n=14$) for *Aplysia* eyes evaluated simultaneously. Secondly, there was a consistent difference

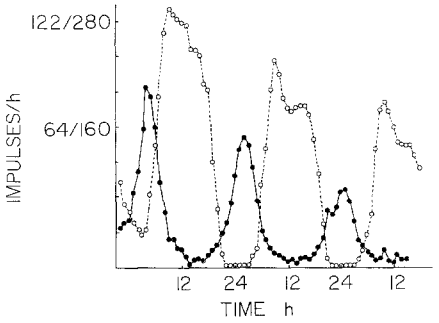

Fig. 2. Free-running ocular rhythms in *Bursatella* and *Aplysia*. *Filled circles: Bursatella* eye. *Open circles: Aplysia* eye. The outer value on the ordinate is *Bursatella* impulse frequency; inner value is for *Aplysia*. Both animals were removed from the same light cycles (L:*D*, 12:*12*)

in the shape of the *Bursatella* and *Aplysia* ocular waveforms. *Aplysia* eyes uniformly exhibited a steep rise to peak impulse frequencies followed by a rapid descent which produced a characteristic hump on the falling phase (Eskin 1971; Rothman and Strumwasser 1976). *Bursatella* eyes, on the other hand, showed a much faster drop off from peak impulse levels.

A third, and striking difference between *Bursatella* and *Aplysia* eyes was the period of the free-running rhythm. In the records shown in Fig. 2 there was a 2 h difference in the free-running period of the two eyes (*Bursatella*=22 h, *Aplysia*=24 h). Overall we measured the free-running period of *Bursatella* eyes to be 21.2 h ($\pm$0.6 SD, n=15) between the first two peaks in vitro compared with 24.3 h ($\pm$0.9 SD, n=10) for *Aplysia* eyes evaluated concurrently. The free-running periods between the second and third peaks in vitro showed similar differences with *Bursatella* eyes exhibiting a period of 21.7 h ($\pm$1.94 SD, n=5) compared with 24.2 h ($\pm$0.45 SD, n=5) for *Aplysia* eyes.

Efferent Modulation of the Eye

The optic nerve of *Bursatella* contains efferent fibers which, when active, modify afferent impulse activity. Efferent activity was detected by leaving one eye attached to the cerebral ganglion and recording electrical activity en passant with a suction electrode. Examples of efferent activity recorded in this fashion from *Bursatella* and *Aplysia* optic nerves are shown in Fig. 3A. In both animals efferent activity consisted of 5–20 μV potentials which disappeared when the cerebral ganglion was detached. In *Aplysia* efferent activity appears to delay the occurrence of afferent impulses and thus disrupts the higly regular burst patterning normally observed in the optic nerve (Eskin 1971; Luborsky-Moore and Jacklet 1976). Efferent activity likewise appeared to alter afferent impulse production in *Bursatella*. The effects were not as pronounced, however, since impulse patterning in the

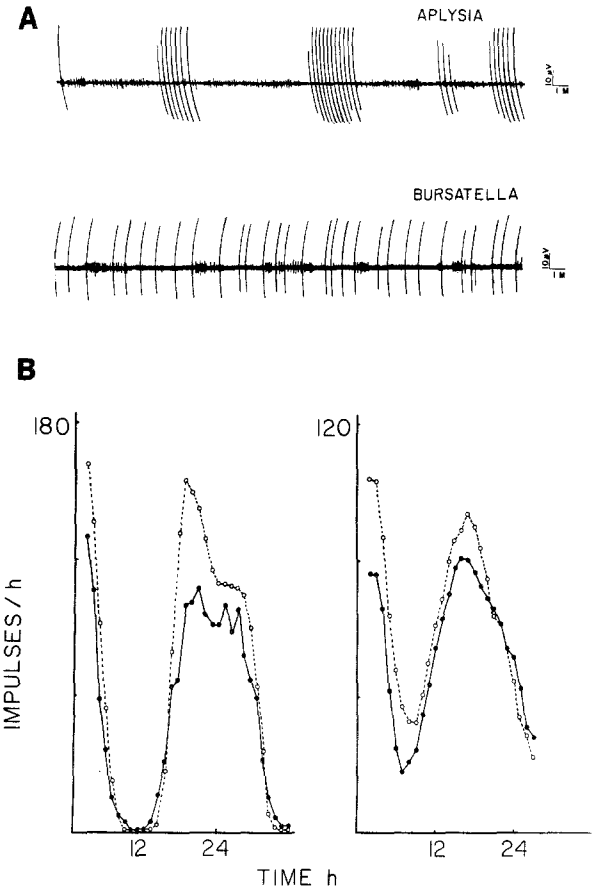

Fig. 3A, B. Efferent optic nerve activity. **A** En passant recordings from the optic nerves of *Aplysia* and *Bursatella*. Large units are afferent impulses. **B** Circadian ocular rhythms in *Aplysia* (*lower left*) and *Bursatella* (*lower right*). *Dotted line:* detached eye. *Solid line*: eye recorded attached to the cerebral ganglion

Bursatella eye tends to be irregular. In order to obtain a quantitative measure of the effects of efferent activity, a *Bursatella* optic nerve recording was selected which contained clearly identifiable efferent spikes. The afferent interspike intervals for the first 2 h of the record were measured and tabulated into one of two categories, depending upon whether any efferent activity was visible during the particular interval. Divided in this fashion the mean interspike period for intervals not containing efferent activity was 38.6 s compared with 61.6 s for intervals in which efferent activity occurred. This difference was statistically significant ($P<0.005$, t-test) and confirmed our impression that efferent activity was associated with longer afferent interspike intervals.

While efferent fibers influenced afferent impulse timing, they did not have a major effect on the *Bursatella* ocular waveform. Figure 3B shows ocular rhythms from *Aplysia* and *Bursatella* recorded with one eye attached (solid line) and one detached (dotted

line) from the cerebral ganglion. In *Aplysia*, attachment of the eye to the cerebral ganglion leads to a flattening of the ocular waveform (Eskin 1971) and this effect is visible in the lower left record of the figure. *Bursatella* ocular rhythms, on the other hand, did not change shape when attached to the cerebral ganglion, although there was a slight reduction in the overall impulse frequency (Fig. 3B). The reduction in spontaneous impulse levels occurred in all 7 *Bursatella* eyes evaluated while attached to the cerebral ganglion. This suggests that modulatory effects of efferent activity had not been blocked by optic nerve damage.

Entrainment of the Ocular Pacemakers by Light Cycles

The ocular pacemakers of *Aplysia* can be entrained by light cycles applied in vivo (Jacklet 1969a; Eskin 1971). In order to test for in vivo entrainment of *Bursatella* eyes, 5 animals were placed on light cycles with dawn at 06:00 EST, and an additional 4 animals placed on light cycles (L:*D*, 12:*12*) with the dawn delayed by 6 h. After exposure to 5 complete cycles the eyes of 1 animal from each light cycle were removed at 14:00 (day 6) and the phase of the ocular rhythms assayed. The eyes of the remaining 7 *Bursatella* were then evaluated at successive 48 h intervals until ocular rhythms from all animals had been recorded.

The results indicated that the phase of the *Bursatella* ocular pacemakers can be entrained by light cycles provided in vivo. A summary of ocular peak times is shown in Fig. 4A. In the upper portion of the figure are 2 examples of ocular rhythms recorded from animals exposed to light cycles 6 h apart. The impulse peaks for each rhythm are projected down to the appropriate time on the lower axis. As can be seen in the resultant distribution of ocular peaks there was a clear separation as a function of the phase of the prior light cycles. There was a 6 h difference in the median peak times in response to a 6 h difference in the light cycles.

While *Bursatella* eyes can be entrained in vivo, the inferred steady-state phase angles differed from values reported for *Aplysia*. As seen in Fig. 4A, peak activity in the *Bursatella* eyes occurred about 3 h before projected dawn while peak activity in *Aplysia* eyes occurs close to or just after projected dawn (Eskin 1971; Block et al. 1974). Since this suggested a major difference in the entrained phase angles for eyes in the two species we decided to study the entrainment of *Bursatella* and *Aplysia* eyes maintained and evaluated under identical experimental conditions. Twenty five *Bursatella* and thirteen *Aplysia* were exposed to L:*D* 12:*12* for at least 7 complete

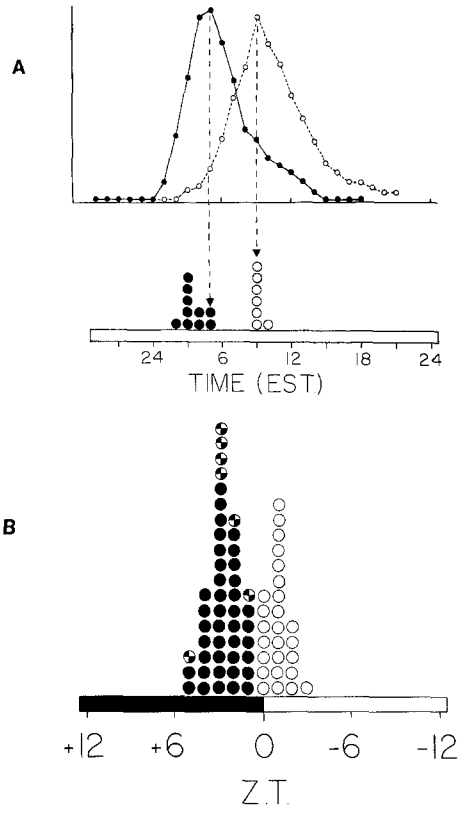

Fig. 4. A In vivo entrainment of the *Bursatella* eye. The two ocular rhythms plotted on the upper axis are from two animals, one exposed to L:*D*, 12:*12* dawn=06:00 (*solid lines*) the other to L:*D*, 12:*12* dawn=12:00 (*dotted lines*). Both eyes were recorded simultaneously in D:D. The impulse peaks are projected down to the appropriate place on an axis indicating peak times for all of the eyes recorded in this experiment. *Filled circles:* eyes from 06:00 dawn. *Open circles:* eyes from 12:00 dawn. One eye (12:00 dawn) failed to show spontaneous activity. **B** Phase angles for entrainment of *Bursatella* and *Aplysia* eyes. Peak times are for the first cycle in D:D and are plotted as hours before (+) or after (−) projected dawn. Peak times from both eyes of each animal are shown. *Filled circles:* peak times of detached *Bursatella* eyes. *Half-filled circles:* peak times of *Bursatella* eyes recorded attached to the cerebral ganglion. *Open circles:* peak times of detached *Aplysia* eyes. Three detached *Bursatella* eyes were not successfully recorded

cycles. The eyes of each animal were then removed and the phase of each rhythm assayed. Dissection times were selected throughout the light-time in an effort to evaluate any differential effects dissection might have on the ocular phase. In 18 of the *Bursatella* both eyes were isolated from the cerebral ganglion during in vitro recording. In the remaining 7 *Bursatella* one eye was left attached during recording to an otherwise isolated cerebral ganglion. This was undertaken in an effort to determine whether severing the *Bursatella* optic nerve spuriously shifted the ocular rhythm.

The results from this experiment confirmed that there is a consistent difference in the entrained phase angles of *Bursatella* and *Aplysia* eyes. Figure 4B

shows peak times for *Bursatella* eyes either detached (filled circles) or attached (half filled circles) to the cerebral ganglion. Also shown are the peak times for detached *Aplysia* eyes (open circles). As can be seen in the figure, *Bursatella* and *Aplysia* eyes formed two non-overlapping distributions with *Bursatella* eyes exhibiting a median peak at +3 Z.T. and *Aplysia* eyes peaking later at −1 Z.T.

No major phase difference was detected between attached and detached *Bursatella* eyes. Attached eyes also showed a median peak at +3 Z.T. Likewise the time of dissection exerted only a small influence on the timing of peak activity in *Bursatella*. There was a mean difference of 1 h in peak times of eyes dissected in the early day compared with the late day.

Locomotor Rhythm

Since *Bursatella* showed evidence of an ocular circadian rhythm we were interested in determining whether a circadian rhythm of behavioral activity was also evident. In order to evaluate locomotor behavior 6 *Bursatella* were placed into activity monitors and exposed to L:*D*, 12:*12* light cycles. Under these conditions all 6 *Bursatella* exhibited a diurnal locomotor rhythm. Two examples of locomotor activity on L:*D*, 12:*12* are shown in Fig. 5A. While overall activity was diurnal, its pattern could be quite variable with occasional bouts of nocturnal activity evident in most records.

In order to determine whether locomotor behavior was timed by an endogenous pacemaker 6 *Bursatella* were exposed to L:*D*, 12:*12* light cycles and then placed into D:D. Unfortunately we were unable to obtain unambiguous evidence for a circadian free-run in the 6 animals tested. Two examples are shown in Fig. 5B. One animal showed scattered bouts of activity and the other showed activity that split into two components.

Since our initial attempts at observing endogenous control of the locomotor rhythm in D:D were unprofitable, we employed an alternate strategy. Ten *Bursatella* were placed on short-day light cycles (L:*D* 6:*18* or L:*D*, 9:*15*). *Aplysia* maintained on similar short days (L*D* 8:*16*) reveal the presence of endogenous timing by initiating locomotor acivity before dawn on most days (Lickey et al. 1976). We found that *Bursatella* likewise exhibited anticipatory activity when exposed to L:*D* 9:*15* although not on the shorter 6 h day (L:*D*, 6:*18*). In general *Bursatella* anticipatory behavior was not as prominent as predawn activity observed for *Aplysia* (Lickey et al. 1976). Nevertheless the initiation of locomotor activity prior to dawn suggested that the *Bursatella* locomotor rhythm was influenced by a circadian pacemaker.

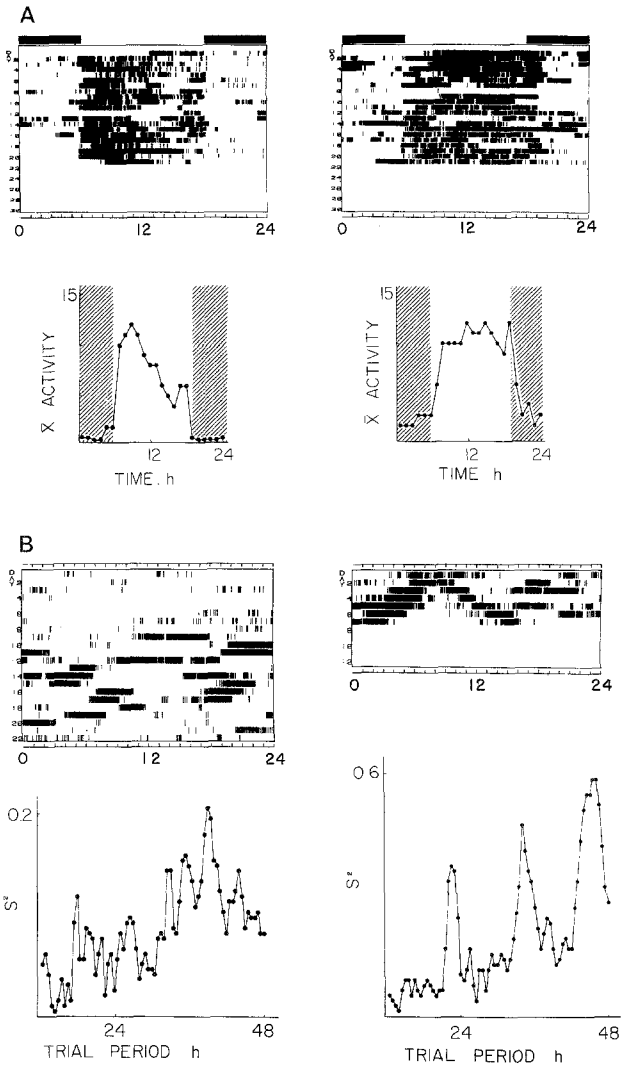

Fig. 5. A *Bursatella* locomotor activity on L:*D*, 12:*12*. Computer generated representation of locomotor activity. Each *vertical deflection* indicates the infrared beam has been broken. *Bar* above each record shows dark-time of the light:dark cycles. Time is Eastern Standard Time. The form estimate below the activity record indicates mean activity (infrared crossings/h). The mean was computed for the entire activity record. *Diagonal hatching* indicates activity which occurred during the dark time of the light cycles. **B** *Bursatella* locomotor activity in D:D. Activity record conventions as in Fig. 5A. Below each record is a periodogram which indicates the relative variance (S^2) at each test period. Relative variance is the variance of each column of the test matrix divided by the variance of the unordered matrix

Role of the Eye in Controlling Locomotor Behavior

The *Bursatella* eye contains both photoreceptors (Fig. 1) and a circadian pacemaker (Fig. 2) and could contribute to locomotor control in one or both capacities. In order to evaluate the contribution of the eye to behavioral control *Bursatella* locomotor activity was compared before and after eye removal. Eight *Bursatella* were placed on short days (L:*D* 9:*15*) and the presence of a clear locomotor rhythm was con-

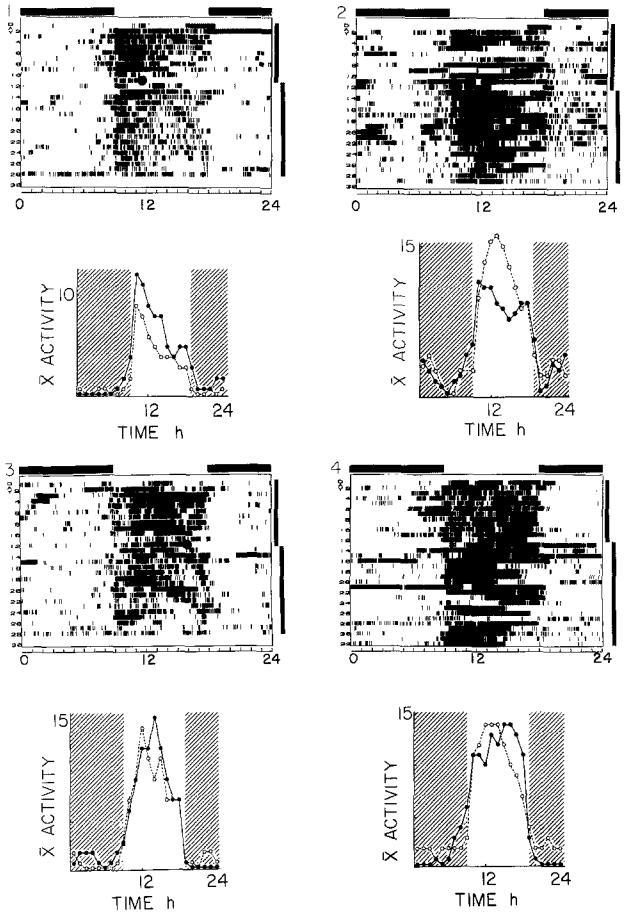

Fig. 6. *Bursatella* locomotor activity on L:*D*, 9:*15* before and after eye removal. Conventions as in Fig. 5A. *Filled circle* on record: time of eye removal. *Vertical bars* to right: days for which form estimates were calculated. *Solid line* on form estimates: mean preoperative locomotor activity. *Dotted line:* mean postoperative activity

firmed. The eyes were then removed from each animal and locomotor activity measured postoperatively.

Comparison of pre- and postoperative locomotor records indicated that the *Bursatella* eye was not critical as either a photoreceptor organ or circadian pacemaker. Activity records from 4 animals which are representative of the group are shown in Fig. 6. All of the *Bursatella* evaluated remained diurnal following eye removal. We failed to observe an increase in nocturnal activity or major changes in locomotor patterning, alterations which often occur following blinding in *Aplysia* (Block and Lickey 1973; Strumwasser 1973; Lickey et al. 1977). We also did not detect an increase in locomotor activity just after light-onset, another common effect of eye removal in *Aplysia* (Lickey and Wozniak 1979). Importantly, we continued to observe anticipatory locomotor activity in eyeless *Bursatella*. As can be seen in records 1–3 of Fig. 6, pre-dawn initiation of activity while variable in its day to day occurrence, persisted following blinding. However, in one of the 8 *Bursatella* studied, anticipatory behavior was attenuated after eye removal (record 4).

Discussion

Ocular Rhythm

Bursatella eyes like the eyes of *Aplysia* and *Navanax* express a circadian rhythm in the frequency of afferent impulses (Fig. 2; Jacklet 1969a; Eskin and Harcombe 1977). The 3 rhythms share several common features. Each eye exhibits the rhythm by impulses which appear to be compound action potentials. These potentials occur spontaneously in darkness, are almost completely absent during the early to middle subjective night and then increase rapidly in frequency during the late subjective night. All 3 rhythms display a remarkable amount of precision in their waveform and free-running period. Two eyes taken from the same animal and recorded in isolation in vitro continue to exhibit nearly identical patterns of activity for several cycles (Eskin and Harcombe 1977; Eskin 1979; Fig. 2). Finally, all 3 ocular rhythms can be entrained by light cycles provided in vivo (Jacklet 1969a; Eskin 1971; Eskin and Harcombe 1977; Fig. 4).

Aside from these general similarities, several features distinguish the *Bursatella* rhythm. The mean impulse frequency is lower in *Bursatella* (96/h) than in *Aplysia* (247/h), although slightly higher than values reported by Eskin and Harcombe (1977) for the *Navanax* rhythm (60/h). The waveforms of the 3 ocular rhythms also differ. *Bursatella* shows a steep rise to peak impulse frequencies followed by a rapid decline in activity. *Aplysia* exhibits a sharp peak but then a sustained falling phase providing a shoulder. *Navanax*, on the other hand, displays a broad peak with near maximum levels of activity persisting for 5–6 h. Aside from the fact that the 3 waveforms are quite reproducible the physiological (or behavioral) significance of the shape of the rhythm is presently unclear. Eskin and Harcombe (1977) point out that the isolated *Navanax* eye displays an ocular waveform similar to an *Aplysia* eye attached to the cerebral ganglion. They suggest that some of the efferent circuitry present in the *Aplysia* cerebral ganglion may be present in the *Navanax* retina. Alternately, differences in the waveform may reflect fundamental differences in the organization of the actual pacemakers.

A third and dramatic difference between *Bursatella* and the other two opisthobranchs was in the free-running period of the ocular oscillator in D:D. In the current experiments the in vitro period of the *Bursatella* rhythm was 21.3 h compared with 24.3 h for *Aplysia* eyes evaluated concurrently. Similar to *Aplysia*, the *Navanax* eye displays a free-running peri-

od of 25.6 h, although a wide range of values were obtained (22–28 h). The *Bursatella* 21.3 h ocular period represents an extremely short value – at least compared to most behavioral rhythms (Aschoff 1979). The significance of this difference is unknown but may also indicate differences in the cellular organization of the two pacemakers. There is some question about how closely this in vitro period reflects the actual in vivo free-running period. For example, in *Aplysia* the period of the ocular pacemaker can range from 22–27 h depending upon whether the eyes are maintained in filtered seawater or culture medium (Jacklet 1971). Yet, the in vivo free-running period measured either indirectly (Block 1975; Hudson and Lickey 1980) or directly by in vivo recording techniques (Block, submitted manuscript) is close to 24 h. While for *Aplysia* the buffered seawater used in the present experiments resulted in an in vitro period close to the in vivo value, we cannot be certain that the same was true for the *Bursatella* ocular rhythm.

The short in vitro free-running period raises a possible complicating factor in evaluating a fourth difference among *Bursatella, Aplysia* and *Navanax* eye rhythms – the steady state phase angle for entrainment. *Aplysia* and *Navanax* show activity peaks just after projected dawn when previously exposed to L:*D*, 12:*12* light cycles (Eskin 1971; Eskin and Harcombe 1977). In distinction, *Bursatella* eyes, exhibit peak activity 3 h before projected dawn (Fig. 4). Since the free-running period of the *Bursatella* eye rhythm is shorter than that of *Aplysia*, entrainment theory would predict a more positive phase angle for the *Bursatella* eye, assuming that the underlying phase response curves were similar (Pittendrigh and Daan 1976). However, the accuracy of inferring in vivo phase angles from in vitro projected relationships on the first cycle in D:D can be affected by at least two sources of error. First, if dissection serves to phase shift the rhythm and second, if there is a discrepancy between ocular free-running periods in vivo and in vitro. Our results suggest that dissection has only a minor effect on the timing of peak activity. Dissection times were varied over a 10 h period with a resultant mean change of only 1 h in peak times. Furthermore the free-running period in vivo and in vitro are probably quite similar. Experiments with animals kept in D:D for two days showed in vitro phase advances of approximately 8 h suggesting that the in vivo free-running period was in fact quite short and close to 21 h.

A final difference among *Bursatella, Aplysia* and *Navanax* eyes was in the effect of efferent activity on afferent impulse patterning and on the waveform of the circadian rhythm. In *Aplysia*, efferent activity consists of small amplitude units (< 20 μV) which disrupt the otherwise regular patterning of afferent impulses. In *Navanax* efferent activity consists of both large and small units which are similar in amplitude to afferent impulses but no effects of these units on afferent impulse production has been reported (Eskin and Harcombe 1977). In *Bursatella*, efferent activity (5–20 μV) serves to reduce ongoing afferent impulse activity although the effects are not as pronounced as efferent actions on the *Aplysia* eye. Furthermore, there is no evidence for major changes in the ocular waveform as a result of *Bursatella* efferent activity.

The exact role of efferent fibers is not fully understood, although there are some hints in the *Aplysia* system. Efferent fibers are known to carry photic information to the eye from receptors in the anterior tentacles (Block 1975), rhinophores (Jacklet 1980) and from the cerebral ganglion (Eskin 1971). In addition, efferent activity has been implicated in entrainment of the eyes by red light cycles (Block et al. 1974) and in the resetting response of the ocular pacemakers to long duration light pulses (Prichard and Lickey 1978). On the other hand efferent action has apparently been ruled out as responsible for differences between in vitro and in vivo entrainment rates (Block and Page 1978). We are not yet certain whether efferents in the *Bursatella* optic nerve perform similar functions.

The Locomotor Rhythm

In addition to the ocular rhythm, *Bursatella* exhibit a robust locomotor rhythm which is predominantly diurnal on L:*D*, 12:*12*. While we were unable to observe the expression of a circadian pacemaker in D:D, we were able to detect an endogenous component on short-day light cycles. Under these conditions *Bursatella*, like *Aplysia*, initiate locomotor activity before dawn (Fig. 6).

Since the eye is an obvious candidate for a pacemaker controlling locomotor activity, attempts have been made in *Aplysia* to define its role in timing locomotor behavior. The current view is that the *Aplysia* eye contributes to locomotor control as a circadian pacemaker. While eyeless *Aplysia* remain diurnal when exposed to light cycles (Block and Lickey 1973), free-running locomotor behavior severely deteriorates following eye removal (Strumwasser 1973; Lickey et al. 1977). In addition, anticipatory locomotor activity which is often present when *Aplysia* are exposed to short-day light cycles (L:*D*, 8:*16*) is attenuated in eyeless animals (Lickey et al. 1976; Lickey and Wozniak 1979).

Since the *Bursatella* eye also exhibits a circadian rhythm we were interested in determining whether eye removal in *Bursatella* leads to similar locomotor deficits. As with *Aplysia* we found that all eyeless *Bursatella* remained diurnal and therefore possessed

sufficient extraocular photoreceptors for locomotor control. However, aside from a single *Bursatella* (record 4, Fig. 6) we were unable to observe any marked changes in locomotor patterning which would suggest the loss of a circadian pacemaker. We are presently unable to explain the loss of anticipatory activity in the single *Bursatella* record. However, since the remaining 7 animals failed to show any marked effects on locomotor patterning, we believe the change in the behavior of this particular *Bursatella* may have been independent of the surgical procedures. These results are in contrast to published reports on the effects of eye removal on *Aplysia* (Lickey et al. 1976) and results we have obtained with *Aplysia* maintained in our laboratory under the same conditions as the *Bursatella* (Roberts, unpublished experiments). These facts lead us to believe that the ocular pacemakers in *Bursatella* play a less prominent role than their counterparts in *Aplysia* in controlling locomotor behavior.

It is intriguing to speculate that closely related opisthobranchs may have evolved alternate strategies for employing endogenous pacemakers for behavioral control. It may be the case that while *Aplysia* ocular pacemakers play a role in controlling locomotor behavior, we may find other, as yet unidentified rhythmic behaviors for which *Bursatella* ocular control is more prominent.

It appears that the ocular pacemaking property is widespread among opisthobranchs. Aside from reports of ocular pacemakers in *Aplysia, Navanax* and now *Bursatella*, we also have evidence for ocular rhythms in *Bulla* and *Dolabrifera* (unpublished experiments). Thus there are significant opportunities for obtaining comparative insights into the employment of circadian pacemakers in controlling rhythmic behaviors.

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