*THE LIGHT RESPONSE AND THE RHYTHMIC POTENTIALS OF HYDRA**

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Although the hydra was one of the first organisms discovered with the light microscope and it occupies a time-honored place in elementary biology, little is known of its mechanisms of coordination or its sensory physiology. The histology and even the existence of its nervous system are again in dispute,[1] but it is usually credited[2] with possessing a network of morphologically unpolarized nerve-cells overlying the longitudinally oriented epidermal muscles. Similar nerve-cells are associated with the transversely arranged gastrodermal muscles, although here they do not appear to be interdigitated into a nerve-net.[2] No nerve rings or nerve-cell concentrations (ganglia) have been found in hydras, nor have any photoreceptors been described, although the animal's responses to light have been known ever since the classic works of Trembley and Baker. Hydras are most responsive to blue light and are insensitive to light of medium and longer wavelengths.[3]

Compound potentials, often in bursts, have been recorded by Josephson[4] from the athecate hydroids *Cordylophora* and *Tubularia*. In the former, pulses can be obtained after stimulation either from the body of the individual polyps or from the stolons which interconnect individuals of the colony. They are always associated with tentacular movements and hydranth shortening. In *Tubularia*, however, Josephson has found regularly occurring spontaneous potentials. Typically, single pulses reoccur at regular intervals, interrupted by rapid bursts in the same conducting system. He has also found cases where either the bursts or the single pulses alone are found. Coordinated elevation of the proximal ring of tentacles sometimes occurs about the time of these bursts or with single pulses shortly after a burst.

Electrical recordings from coelenterates have otherwise been restricted to the large scyphozoan jellyfish, where nerve action potentials from two nerve-nets can be differentiated.[5] No muscle action potentials have been obtained from any coelenterate, nor have any electrical recordings of photic responses been reported.

Methods.—We have mainly used the large European *Hydra pirardi*,† but have confirmed our results with two American species, probably *H.* (*Pelmatohydra*) *oligactis* and *H.* (*Hydra*) *littoralis*. Recordings are obtained either by penetrating the basal disk of polyps suspended from the surface film or from animals pinned to wax plates with fine spines from the fruits of the cactus *Opuntia*. Rather low impedance glass microelectrodes filled with 3 *M* KCl are used. Their output usually goes directly into high gain capacitance coupled preamplifiers and is recorded with a Grass polygraph.[6] The indifferent electrode, a platinum wire, is put in the water several centimeters from the animal. Hydras are very sensitive to vibration, so that the entire preparation, manipulators, dissecting microscope, etc., must be shielded from all vibration as well as from electrical interference and extraneous light.

Light stimulation is given with a battery-operated American Optical "Universal"

illuminator with a Corning glass heat filter 2 mm thick, usually through a 4 cm water cell and then through at least 2.5 cm of water in the hydra's dish before hitting the animal. Localized light stimulation is given with a polished cone of acrylic plastic, painted black, tapering to a 0.5 mm point. Illuminating the recording electrode does not affect the shape of the pulses. Blue light is obtained either with an Ilford gelatin filter (number 621, maximum transmittance at 450 mμ) or with a GAB narrow band interference filter with a transmittance peak at 427 mμ. Animals are observed under conditions of "hydra darkness" with a red light (Wratten number 25, opaque below 590 mμ).

Results.—Rhythmic potentials: Several kinds of potentials can be obtained from hydras under these recording conditions (Fig. 1). In addition to the rhythmic potentials (RP) described here, there are large slow compound pulses that always occur prior to each coordinated contraction of the epidermal longitudinal muscles (Fig. 1, *C*). These potentials occur on a separate conducting system from that of the RP's. There are also small fast potentials associated with contractions of the individual tentacles (Fig. 1, *T*) and slow potentials of several shapes associated with asymmetrical longitudinal muscle contractions (Fig. 1, *A* and *A'*). These latter potentials are not conducted throughout the body of the polyp but are recorded by electrotonic spread from their active loci, as evidenced by simultaneous recording at two sites.

Rhythmic potentials can be distinguished from other pulses recordable from hydras by several criteria: (1) They always occur in a regular, repetitive manner in animals attached to the substratum or surface film, hence their name. For example, during a 5-min period following a brief light stimulus (Fig. 1, A_2), a *H. pirardi* showed 24 consecutive RP's with a mean interval of 13.0 $\pm$ 0.1 sec. The regularity of the rhythm decreases with decreased RP frequency. (2) Their amplitude is always two orders of magnitude less than that of the potentials preceding coordinated epidermal muscle contractions (Fig. 1, *C*). (The high amplification necessary for RP recording grossly distorts these potentials here.) We have never recorded RP's greater than 0.4 mV. (3) Careful observation with a binocular microscope has failed to show any movements of the animal directly correlated with RP occurrence. (4) RP frequency is inversely proportional to the frequency of polyp contractions. The latter characteristically occur regularly in healthy unfed *H. pirardi* in the dark and consist of a number of coordinated muscle contractions with a characteristic temporal pattern. (5) RP's show a distinctive compound shape. Examination of an individual RP (Fig. 2, *A*) shows that it can be differentiated into two components, an initial short pulse lasting less than 50 msec (and sometimes one-third as long) followed by a slow potential which rises fairly rapidly to its maximum value and then decays slowly with a total duration of about 500 msec. The initial pulse may be either negative, biphasic, or positive to the reference electrode, losing its negativity with increasing distance between the recording electrode and the locus of origin of the RP. The second component is always negative to the reference electrode.

An estimation of the locus of RP initiation can be obtained by simultaneously recording on two separate channels from different places on the animal. This can be done from the basal disk and the hypostome in an animal pinned to the substratum, or in a manner more conducive to a natural situation by surgically creating

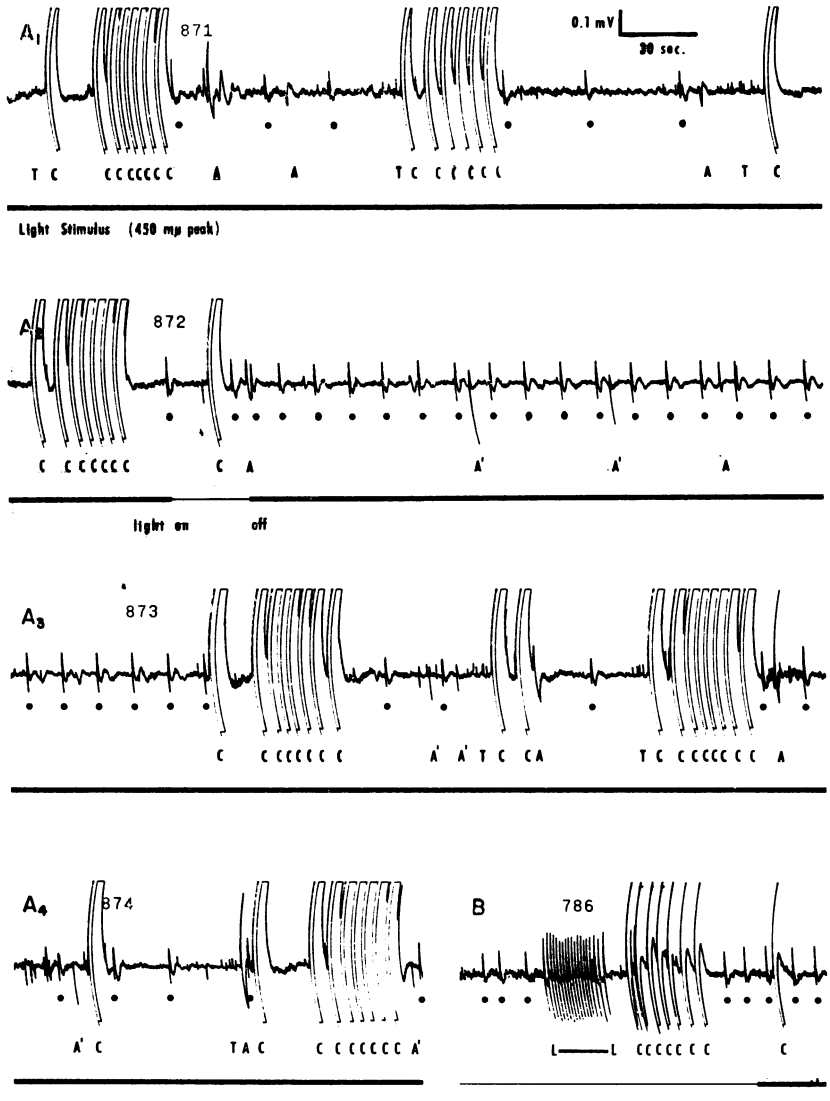

FIG. 1.—Electrical potentials from *Hydra pirardi*. *A*. (continuous record, 1–4. Total length: 18 min). The effect of a 30-sec light stimulus on a dark-adapted animal. *B*. Burst of potentials from a hypostomal pacemaker associated with tentacle attachment to the substratum. (Each rhythmic potential is marked with a ●. C: pulse preceding a coordinated epidermal muscle contraction. T: tentacle contraction. A and A′: asymmetrical epidermal muscle contractions. L–L: burst of pulses associated with tentacle attachment. Light stimulus shown on lower trace.)

a hydra with two basal disks and two peduncles and then recording from each disk simultaneously (Fig. 2). It has been found that RP's usually originate from a single locus for a number of pulses, showing a constant difference in conduction time between the point of origin and the arrival of the pulse at one electrode and the same origin locus and the arrival at the other electrode. The shape of these successive RP's also remains constant. Then, both the conduction time difference and

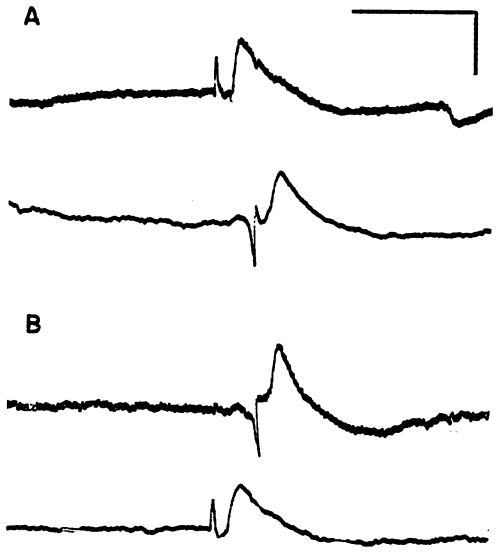

FIG. 2.—Successive rhythmic potentials recorded separately from each base of a two-basal disk preparation, *Hydra pirardi*. *A*. Rhythmic potential preceding stimulation, pacemaker near left (upper) electrode. *B*. Next rhythmic potential, showing pacemaker shift to area near right electrode. Vertical bar equals 200 μV, horizontal bar equals 1 sec.

the shape may change abruptly, showing that the RP has now originated from another locus, i.e., a new pacemaker is now leading. It has not been possible to discern any constant number of pacemakers nor to determine precisely where these are found in the hydra, but RP's may originate from any of several loci in the peduncle. It is often possible to change the functional RP pacemaker either for a single pulse or for a longer period by giving the animal a mechanical, photic, or electrical stimulus (Fig. 2, *B*).

Rhythmic potentials occur during all phases of the animal's behavior in which it has been possible to record. They can occur between the successive epidermal muscle contractions of a contraction burst and also during the slow phases of elongation of the animal. Tentacular twitches and other movements of the tentacles and column do not interfere with RP frequency (Fig. 1). Capturing and swallowing *Artemia* nauplii does not interrupt the RP's. The addition of 10^{-5} *M* reduced glutathione likewise changes the hydra's behavioral pattern but does not greatly change the RP's.

Some individuals show a propensity to move away from the recording situation by somersaulting. This is initiated by a number of tentacles attaching to the substratum or the surface film. The RP's are interrupted, and a burst of from 3 to 20 or more pulses in quick succession originates from the hypostome area (Fig. 1, *B*, *L–L*). The animal then may wrench its basal disk off the surface film (displacing the recording electrode) with a burst of epidermal muscle contractions, or it may fail to somersault, release its tentacles, and resume its previous activity. These pulses seem to be conducted by the same system conducting RP's; however, they are always at a higher frequency and often differ in shape from the typical RP.

Light effects: Light, either on the entire hydra or localized on the basal disk and its adjacent peduncle, has a compound effect: it immediately interrupts the regular rhythmicity of the RP's and then, after 20 sec or so, RP's reoccur at a more rapid rate than before. A response obtained with white light can be duplicated when a blue-pass filter is interposed between lamp and animal, although the total energy

content of the stimulus is considerably reduced. Evidently, the response is primarily effected by the blue component of white light. Sensitivity falls abruptly above 500 mμ. A light stimulus often shifts the effective RP pacemaker, either for a single pulse or for an extended period, just as do stimuli of other modalities. The shift may not occur immediately. Increases in RP frequency after light stimulation may be quite marked, especially when the animal has been kept in the dark for some time prior to stimulation. In the specific case shown (Fig. 1, *A*), the animal had been in the dark for 12 min prior to a 30-sec stimulation with blue light. RP frequency then increased from 1.8 pulses per min to 4.8 pulses per min, and the enhanced rate lasted at least until epidermal muscle contraction bursts recommenced, over 5 min later. The end of the light stimulus may also delay the first subsequent RP, but this delay is not as marked as that following the onset of light, where even short flashes—not more than 0.2 sec long—can delay the subsequent RP and lead to a pacemaker shift.

Light stimuli can also block coordinated epidermal muscle contractions and their associated pulses; this effect is on a different pacemaker system, for it is restricted to light stimuli striking the hypostome-tentacle base area. Such localized stimulation does not affect the RP frequency directly. The over-all changes in hydra's behavior when it goes from light to darkness include the assumption of a regular reoccurrence of these patterned bursts of coordinated contractions, periodically reducing the polyp to a tight ball. In *H. pirardi*, these contraction bursts occur every 3 to 5 min in the dark. Light thus seems to change this behavioral pattern both by its direct effect on the contraction pacemaker and indirectly by stimulating the RP pacemakers, since frequent RP's depress contraction bursts.

Brief localized light stimulation can have the paradoxical effect of allowing one or two muscle contractions to "escape" during the initial RP inhibition before the subsequently enhanced RP frequency inhibits further contractions. Such paradoxes are often characteristic of the coelenterate nervous system.

Light stimuli apparently act on the pacemakers rather than on the conducting system generally, since localized illumination has never been observed to block through conduction of a pulse. The usual result is to inhibit the lead pacemaker and then to raise the general excitational state of the pacemaker elements so that a new rhythm is established. There is at present no way to decide whether this effect is on the pacemakers directly or indirectly through specialized sensory receptors which modify pacemaker activity without causing any recognizable potentials. We do know that the attachment of several tentacles can excite another pacemaker in the hypostomal region; presumably this pacemaker is excited indirectly.

Discussion.—Electrophysiological recording from hydras shows clearly that the coordinating system of these animals is considerably more complex than has been assumed previously. In the peduncle-base region there are several potential pacemakers within the conducting network. We are unable to state whether or not there is any definite number of pacemakers; indeed, the pacemaker function may be a general property of the elements of the net. Collectively, these pacemakers produce rhythmically reoccurring spontaneous potentials that are conducted throughout the entire animal by a through-conducting system of which they are a part.

It is interesting to compare this system of pacemakers and conducting elements

to the central nervous system of more elaborate animals. Indeed, Josephson[4] has very recently done this with the rhythmically active conducting system of *Tubularia*. He points out how its properties of spontaneity, interaction between different elements, and patterned bursts are all properties normally associated with the central nervous system rather than with nerve-nets.

With the correlations between sensory input, RP system activity, and behavior that we have found in *Hydra*, it is possible to extend this analogy further. Light can inhibit, temporarily, the generation of pulses. Light and mechanical stimulation (as well as electrical shock) usually results in a pacemaker shift and may give rise to a long-lasting post-stimulatory hyperactivity. On the effector side, RP frequency controls a major component of the animal's behavior. In addition to such "reflex" activity as the capture of prey, the glutathione-initiated feeding reflex, and the quick contraction "protection" reflex following strong stimulation, the main behavior of *Hydra* consists of the periodic withdrawal and re-extension of the body, usually in a new direction.[3] As noted above, these contractions are actually made up of a more-or-less regular number of individual coordinated contractions, initiated by a separate pacemaker. This burst pacemaker is inhibited by high-frequency RP's. At the same time, muscle contraction bursts do change RP frequency, slowing down high-frequency patterns and speeding up low-frequency ones, so that there is feed-back control between the main effector and the RP generators.

While the similarities between *Hydra* and *Tubularia* coordinating systems are marked, some differences should be noted. *Tubularia* shows a regular pattern of bursts (at a much higher frequency than we have ever found in *Hydra*) alternating with single RP's. The much slower bursts of very large pulses associated with body contractions in *Hydra* differ from these rapid bursts in that they are not conducted on the same pathway as the RP's, although they achieve conduction without decrement throughout the entire animal. The special bursts of pulses that occur after tentacle attachment, associated with somersaulting, are more like the bursts in *Tubularia*, but this behavior does not occur regularly and seems to be initiated by strong stimulation and consequent high RP frequency. Somersaulting bursts inhibit RP's and are usually followed by a rapid sequence of body contractions.

Because of the complication of two through-conducting systems, RP and muscle contraction, we are unable to refer the RP coordinating system to any specific morphological feature of the animal. The epidermal nerve-net seems to be the most extensive nerve-net, but it is also closely associated with the longitudinal muscles. Possibly this nerve-net is physiologically subdivided into several nerve-nets, but there is no histological support for such a subdivision. Of the remaining possibilities, either one of two, *a priori*, seems most plausible: (1) The pulses that precede coordinated longitudinal muscle contractions are muscle action potentials. This would leave the epidermal nerve-net for the RP system, but it would require the hydra's muscles to have different properties from the subumbrellar muscles of scyphozoan jellyfish, where the coordinated contraction is due to a nerve action potential in the "giant fiber" nerve-net.[5] (2) The RP's are conducted by the gastrodermal nerve-cells, either alone or in combination with the gastrodermal muscle cells. If we are recording the activity of gastrodermal elements, we might expect to find a correspondence between RP occurrence and polyp elongation.

This is not the case. Yet perhaps initial elongation is due to hydrostatic pressure in the coelenteron, so that the polyp could commence elongation before any gastrodermal contractions. If this second alternative is allowed, one could explain the second slow component of the individual RP as being a gastrodermal muscle potential. We prefer this explanation at present.

The discovery of pacemakers in *Hydra* is consistent with their presence in other coelenterates. In addition to *Tubularia* and several other hydroid polyps,[4] they are known to occur in free-living medusae and have recently been postulated for the "swimming" anemone, *Stomphia coccinea*.[7] And in hydromedusae, there are many pacemakers in the nerve ring rather than the discrete number, often eight, found in scyphozoans. But *Hydra* seems to have several systems with pacemaker elements; the system responsible for the RP's, like that of *Tubularia*, is as regular as the swimming beat pacemakers of the medusae but is not directly concerned with a locomotor pattern. Perhaps, the two conducting systems of *Hydra* are analogous to the separate conducting systems of certain hydrozoan jellyfish, for as in *Aequorea*,[8] activity in one net can inhibit activity in the other.

Summary.—Rhythmically reoccurring potentials can be recorded electrophysiologically from intact hydras. Their frequency varies from about 1 per min to 12 per min. Sensory stimuli, notably blue light, can modify their frequency and change their point of origin. Each pulse is compound and of a distinctive shape, originates from any one of several pacemakers within its through-conducting network, is seemingly independent of polyp movement, and is indirectly correlated with the animal's behavior. Other much larger compound potentials, separately conducted, precede coordinated epidermal muscle contractions. The rhythmic potential system fulfills many criteria for a "central nervous system" for these animals. Its light sensitivity provides a mechanism for the hydra phototropic response.

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[6] Closely similar pulses can be recorded with high impedance microelectrodes coupled with a cathode follower preamplifier to a high-gain direct coupled CRO.

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