CO-ORDINATING SYSTEMS AND BEHAVIOUR IN HYDRA

II. THE RHYTHMIC POTENTIAL SYSTEM

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In the first paper of this series (Passano & McCullough, 1964) we described the system concerned with the periodic bursts of impulses arising from the subhypostome and conducted throughout the column of several species of the genus Hydra, the familiar freshwater polyp (hydrozoan coelenterate). Each of these bursts of impulses is followed by a co-ordinated contraction of the epidermal, longitudinally arranged muscles, so that the total burst of this contraction-burst system results, in reducing the animal to a stubby ball. Periodic contraction bursts (CB) are the most important overt behaviour of Hydra.

There is a second through-conducting system in these animals which we have called the rhythmic potential system (Passano & McCullough, 1962). It is the aim of the present report to describe this system of pacemakers and conducting elements in detail. As implied by its name, impulses in this system are produced more or less rhythmically rather than irregularly. They also arise from pacemakers and these units show a comparable sensitivity to direct illumination, but the rhythmic potential (RP) impulses are not obviously related to a specific behavioural event. The RP system is a cryptic feature of the polyp’s biology whose existence was not anticipated; it was only by direct recording of the electrical impulses produced by polyps that this system was disclosed.

In spite of their obvious interest as representatives of the most primitive phylum with a well-defined nervous system, there has been very little direct study of behavioural physiology in coelenterates. Most forms are difficult to record from; their neurites are not conveniently arranged in bundles of nerves, but assume two-dimensional felting arrangements termed ‘nerve-nets’. They are nearly all marine and their tissues are aqueous and electrically in continuity with the surrounding medium, causing a great attenuation of any signal. Happily Hydra is somewhat the exception to this generality, but nevertheless work on this animal previous to these studies has been largely qualitative and indirect. A notable exception is the behaviour study of Haug (1933) on their responses to light.

Josephson, in an important series of papers, has recorded electrical activity from several species of hydroids (1961, 1962, 1963). His work with a species of Tubularia is particularly relevant to what we find in species of Hydra, for in both there are several distinct through-conducting systems whose activity may originate from pacemakers, both rhythmic and displaying bursts of activity, and from complex interaction

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between the different systems. It seems reasonable to assume that Tubularia exhibits the more general hydroid behaviour pattern from which that of Hydra has been evolved, so that as our understanding of the former grows it should be possible to homologize their different co-ordinating systems. It is now realized that many, if not all, coelenterates have several overlying conducting systems, some of which utilize nerve-nets and which may or may not be through-conducting (Bullock & Horridge, 1965). Since seemingly endogenous, rhythmic activity patterns are commonplace among animals, there is a reasonable basis for supposing that co-ordinating systems like the RP system of Hydra occur in other coelenterates and other invertebrates as well (Passano, 1963).

Some of the findings reported here have previously appeared in preliminary or abstract form (McCullough, 1962; Passano, 1962; Passano & McCullough, 1962, 1963-

MATERIALS AND METHODS

The majority of our observations have utilized either Hydra littoralis Hyman or the European H. pirardi Brien, but essentially the same results were obtained with H. pseudoligactis Hyman and a small species which is probably H. cornea Agassiz. We believe that our conclusions can be generalized to all species of Hydra. Animals were maintained in mass cultures in versenated tap water in the manner previously described (Passano & McCullough, 1964), and fed with newly hatched Artemia nauplii. Except when noted specifically, only well-nourished mature individuals were used for experiments.

In many experiments it was useful to create polyps with two bases and lower stalks on a single upper body. These animals, shaped roughly like the Greek letter λ and hereafter termed a λ-preparation, are easily formed by cutting a normal animal from the base longitudinally to the middle of the column. In the majority of cases the preparation healed into the desired shape within 24 hr. The opposite extreme, where a polyp with two mouths, rings of tentacles and upper columns was formed by making a longitudinal cut from the hypostome to the mid-column, was also used occasionally (Y-preparation).

A detailed exposition of the techniques used for recording electrical activity from Hydra is contained in the first paper of this series (Passano & McCullough, 1964). Briefly, the animal may be immobilized on the bottom of a wax dish, using fine cactus spines (from a species of Opuntia; they are 2–3 mm. long, about 0.1 mm. in diameter, barbed and with very sharp tips, so that they are only 35 μ in diameter 100 μ from the tips. Alternatively, the animal is kept ‘upside-down’ with its base attached to the surface film of the medium within a circle of platinum wire held immobile with a manipulator. Either way, conventional KCl-filled glass capillary microelectrodes were used, being pushed against or into the tissue on the surface of the animal. A dual-channel ink-writing polygraph was generally used but appropriate low-level a.c. preamplifiers (Tektronix 122) and a double-beam oscilloscope (Tektronix 502) were also employed occasionally.

As RP potentials under our recording conditions are of much lower magnitude than those from the CB system it is necessary to take the greatest care to exclude interference from the recording arrangement. Identification of the RP signals is helped
by recording simultaneously from two places on the animal. We re-emphasize that it is also necessary to shield the animal under observation from extraneous stimuli of all kinds, especially light and vibration. Our results have largely been obtained in an isolated laboratory in quiet surroundings, usually at night.

Stimuli were given as previously described (Passano & McCullough, 1964). Mechanical stimuli often were given simply by touching or inserting an empty glass microelectrode, held in a manipulator; in other cases they were given by stroking the column or tentacles with a fine bristle. The manipulator-held light (previously described) gave a localized spot of light of about 0·5 mm. diameter close to its tip.

Results

The potentials

A. Description of the RP conduction system

Rhythmic potentials can be recognized by the following combination of characteristics: they occur regularly without apparent relation to the movements of the animal, at frequencies varying between approximately 1 and 10 per min.; they usually originate in the lower column adjacent to the basal disk area and are thence conducted throughout the column; their shape is distinct from that of other pulses recorded from these animals. Each impulse is compound, consisting of an initial relatively short monophasic spike, a pause of 80-150 msec., and then a slow compound pulse. Although this pause between components varies slightly from individual to individual, it is found to remain constant in a given recording situation regardless of where the impulse originated relative to the recording site.

Of these criteria probably shape is the most useful once the investigator has learned to recognize it. Text-fig. 1 shows several RP impulses obtained from individual
animals; it is apparent that while the recorded amplitude, polarity and shape of the initial spike vary, the characteristic double nature of the impulse pattern is always evident.

Variation in the recorded amplitude seems clearly to be a function of the position of the recording electrode. In the initial period of setting up a preparation it is often necessary to change the position of the recording electrode many times before satisfactory recording is achieved. From then on, however, pulse amplitudes remain quite constant for considerable periods and usually change drastically only after the animal's movements, usually an attempt at locomotion, displace the recording electrode.

Text-fig. 2. RP impulses recorded from each base of a $\lambda$-preparation of $H. pirardi$. Each impulse originates near one base and is conducted to the other. A. The initiation site changes from near one base to near another, with a correlated shape change in the first component of the potential. B, C. Subsequent pairs of impulses from the same preparation, showing repeated origin from one site. Horizontal bar indicates 2 sec.; vertical bar, 100 $\mu$V.

The polarity and shape of the initial spike does vary (Text-fig. 1 A), but in a consistent manner. Text-fig. 2 shows pulses obtained from a single individual with the recording electrodes in the same position throughout. These impulses were obtained from a $\lambda$-preparation of $H. pirardi$ with a recording electrode on each basal disk. It is evident that the shape and polarity of the initial spike is a function of the distance between the initiating pacemaker and the recording site. When the impulse originates close to the recording electrode the initial spike is monophasic and negative relative to the resting potential, but when records are taken at increasing distances from the pacemaker the initial spike becomes biphasic and then monophasic and positive to the resting potential. The shape of the slow second component does not show any change of this sort. Changes in shape of the initial spike even in one-channel recordings thus signify that the lead has changed from one pacemaker site to another. We shall return to this topic below.

Facilitation has been shown to be characteristic of both nerve-nets and effector responses in actinians and scyphomedusans (see Bullock & Horridge, 1965), so that it might be expected to occur in some form in the RP system of Hydra. But at least
as far as conduction through the RP system is concerned, there is no evidence that either component of the RP impulse is facilitated in amplitude or duration by the occurrence of previous activity in the system. In this respect the RP impulse differs from the much larger, longer compound impulse of the CB system whose amplitude does appear to be correlated with the interval elapsing since the previous pulse in the burst (Passano & McCullough, 1964).

Text-fig. 3. RP initiation loci in H. pirard. Three λ-preparations. In B recording was interrupted for 3 min., then resumed for 1.5 min. until locomotion occurred. The differences in arrival time between the two basal recording sites are shown for successive impulses. The horizontal base-line represents simultaneous arrival of the pulses; the lead to the left or right base is plotted respectively above or below this line.

The relative time arrival of the RP impulse at the two recording sites of the λ preparation permits some localization of the active pacemaker, assuming that conduction velocities are relatively uniform (which appears to be so), for if the RP pacemakers were located in the common upper column, or in the hypostome, then the interval between arrival at the two bases would be constant and directly correlated with the difference in length of the two lower columns. In fact, however, the pulse almost always appears at one base before the other; the lead fluctuates between bases in these preparations. The differences in arrival times vary, so that (again assuming conduction velocity constancy) there appears to be no strict localization of pacemakers to one spot in the basal region. This is illustrated by Text-fig. 3 which shows the differences in times of arrival at the two base recording sites for a series of successive RP impulses obtained in several experiments. These data are consistent with the
conclusion that RP's originate near the base and are then conducted upward throughout the column. The same conclusion has been reached as a result of experiments with localized illumination which will be described below. It is also noteworthy, from inspection of Text-fig. 3, that the rhythmic property of the RP system is a property of the system as a whole rather than that of the individual pacemakers. Regular firing is maintained in spite of frequent shifts in the lead from one locus to another. We are unable to determine the exact number of functional RP pacemakers in an individual animal, but believe that the number is greater than four and may be less than ten. This estimate is derived from our attempts, in several recording sessions each lasting several hours, to determine the number of groups into which the RP impulses can be placed with regard to common impulse shape and arrival time at the two recording electrodes. Similarly, our current view is that 'parental' RP impulses are not conducted into the buds of asexually-produced offspring (see §D below). Where they are conducted, impulses always 'get through', so that the system as whole is a through-conducting one and there is no evidence for decremental conduction. This holds even for abnormally long conduction paths created artificially by splitting animals longitudinally nearly to the hypostome, allowing them to heal, and then repeating the split from the opposite end nearly to each new base. The resulting preparation has a total conduction path of nearly four times its original length, but RP conduction velocity remains as constant as it was in the intact animals.

RP impulses originating at opposite ends of a preparation are never seen to pass each other; instead they cancel each other out. But the RP conducting system is completely separate, functionally, from the CB conduction pathways.

Conduction velocity data have been obtained from a number of 'normal' (unoperated) animals of various species by pinning them to wax plates with cactus spines and then recording from two points, near the hypostome at the base of one of the tentacles and in the middle of the column. Under such conditions conduction velocities seem constant. For a series of eighteen determinations, taken without particular regard to constancy of temperature (generally ranged between 16.5 and 19.5°C), the mean conduction velocity (with S.E.) was 4.57 ± 0.09 cm./sec.

RP impulses and body movements

In the recording arrangement that we have normally employed, described in detail in the first paper in this series (Passano & McCullough, 1964), it is possible to keep the animal under observation under conditions of 'hydra-darkness', while recording from it for extensive periods. Particular attention has been paid to the question as to whether or not any movements of the animal, due either to the epidermal or gastrodermal muscle sheets, can be directly correlated with RP system impulses.

The epidermal muscle sheet can be quickly eliminated from further consideration. No movements of any kind can be discerned during or immediately following the passage of an RP potential up the column. Conversely, local asymmetrical contractions or the co-ordinated contractions that follow CB impulses (Passano & McCullough, 1964) have no effect on the appearance or conduction velocity of RP system pulses. Such contractions of course often disrupt recording.

Equally, we have been unable to correlate any contractions of the circularly arranged gastrodermal muscles with activity in the RP system, but here the evidence for lack
of interaction is much less satisfactory than that for the ectodermal muscles. Semal-Van Gansen (1952) has presented evidence that \textit{H. attenuata} elongates in a stepwise jerky fashion, the steps occurring at approximately 10 sec. intervals. We have never observed such rhythmic elongation directly in the species that we have studied, but find a suggestion of such elongation in a single case among scores of body elongations photographed with time-lapse cinephotography. The relationship between RP impulses and such gastrodermal muscle movements remains uncertain, however, for there seems to be no correlation between the occurrence of impulses and the re-elongation of the body after contraction bursts. Commonly re-elongation commences prior to the first RP impulse following a contraction burst although the reverse is also often true. We have been unable to interfere with elongation by illumination of the RP pacemakers (see §B below), although this modifies the RP rhythm.

Chloretone, known to be an effective anaesthetic agent on \textit{Hydra}, was tested in an attempt to separate muscle movements from activity in the RP system. Kepner & Hopkins (1923) described its action as first affecting muscular activity and subsequently the nervous system. When $10^{-6}$ M chloretone is added drop by drop to the recording vessel, the resulting solution at first affects the CB system (Passano & McCullough, 1964). The effective concentration was approximately $5 \times 10^{-6}$ M or 10\% of the mean lethal dose. Five to ten min. after the first appearance of disruption of the CB system (during which time nearly all movements cease and the polyp becomes limply elongated), and without further addition of the anaesthetic, the RP rhythm begins to show the effects of the anaesthetic. The RP rhythm is interrupted, but a few impulses appear erratically before all activity ceases. The effect is almost immediately reversible, for by the time recording is re-established following exchange of the chloretone solution for fresh culture solution, RP impulses are again occurring normally. This immediate and complete recovery is evidence that the chloretone affects the pacemakers rather than the RP conducting system. Further support for this hypothesis comes from the fact that the anaesthetized animal can still show, transiently, rhythmic generation of RP's following illumination. Text-fig. 4 compares
the RP activity before the chloretone has taken effect and after a light stimulus has momentarily re-activated the spontaneous generation of RP's. Such impulses elicited by illumination appear identical with those recorded before anaesthetization, even though the muscle system appeared to be totally inert.

**Pacemaker properties**

The main characteristic of the RP system, the endogenous rhythmicity of its activity, is emphasized by the use of the term 'pacemaker' for its initiation sites (Tasaki, 1959). The output of the RP system pacemaker can be modified by stimuli (see §§B and C below), but at the same time the pacemakers are capable of initiating RP impulses for extended periods, seemingly without stimuli. We have been able to record RP activity in animals without readjustment of the recording electrode (which disturbs the animal) for periods as long as six hours.

Text-figs. 5 and 6 show examples of polygraph records of RP system activity for an individual *H. pirardi* and a *H. littoralis*, the two species which have been studied most extensively, after an hour or more of dark-adaptation and during illumination.

Examination of typical records such as these show that the RP rhythmicity, while obvious, is variable. At times it can be very regular, so that, for example the standard deviation of the mean interval duration for a series of seventy-seven successive impulses in a light adapted *H. pirardi* the mean interval was $7.7\text{ sec.}$ and the standard deviation was $+0.62\text{ sec.}$, less than $8\%$ of the mean interval. But such regularity is not the rule; in general the faster the rhythm, the more regular, the slower, the more irregular. Text-fig. 6 illustrates the erratic appearance of RP's in a dark-adapted *H. littoralis*. 

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Text-fig. 5. The effect of illumination on RP activity and contraction bursts, *H. pirardi*. A. In the dark and undisturbed for over 1 hr. B. Activity from another animal in the same recording situation but illuminated. • indicates RP impulse; C indicates CB impulse; horizontal bar indicates 1 min.
Co-ordinating systems and behaviour in Hydra. II

Text-fig. 6. The effect of illumination on RP activity and contraction bursts, H. littoralis. A. After 2-5 hr. in the dark without disturbance. Note erratic occurrence of RP impulses. B. A comparable animal under illumination. Record ends in pre-locomotor burst impulses (PLB) and a locomotion contraction burst (LCB). • Indicates RP impulse; C indicates CB impulse; A, asymmetrical column contraction; horizontal bar indicates 1 min.

A  H. pirardi in dark

5 min.

B  H. pirardi in light

20 min. Interruption

C  H. carneae in light

5 min. Interruption

Text-fig. 7. Graphic representation of RP and CB activity for extended periods. A. H. pirardi in the dark. B. H. pirardi under constant illumination. C. H. carneae under constant illumination. Vertical bar indicates single RP; horizontal bar indicates CB.
The nature of RP activity under these extremes of illumination is also easily seen in Text-fig. 7. This shows compressed representations of RP recordings for more extended periods of time in several individual polyps. The interaction between the RP system and the CB system will be the subject for a subsequent paper in this series, but it can be noted here that the RP rhythm appears enhanced after a contraction burst, only to slow down again gradually. Since normal, well-fed animals show more or less regular contraction bursts every 5–10 min., it is obvious that if RP frequency is reset after a contraction burst there will be a correlation between these periodic events and the regularity of the RP rhythm. This is especially relevant in the light, where the CB frequency is usually higher. Clearly the RP rhythms occurring when the animal is showing contraction bursts cannot be considered to be from the ‘unstimulated’ animal, even in the dark. Preparations can be made which do not display these periodic contraction bursts, by extirpation of the hypostome, tentacles and tentacle base-subhypostomal region (Passano & McCullough, 1964); activity of the RP system continues in spite of the elimination of periodic contractions. Text-fig. 8 shows a polygraph record from such a ‘decapitate’ animal soon after the operation and 24 hr. later when CB have become re-established. RP rhythms in such operated preparations tend to be unusually rapid and to continue regularly without abating for long periods of time. Twenty-four hours later RP frequencies remain high. Under these circumstances they proceed during the contraction bursts without alteration.

Text-fig. 8. The effect of hypostome removal on RP activity, *H. pirardi*. A. Hypostome and tentacles removed 1 hr. previously. CB are eliminated temporarily. B. The same animal 24 hr. later. Note RP impulses proceeding during CB. Horizontal bar indicates 10 sec.

Since the recorded RP impulse changes shape whenever pacemakers change, it is possible to detect such shifts even when recording from a single spot on the animal (Text-fig. 2). Examination of records often show that after such shift the intervals between successive impulses tend to be either slightly longer or slightly shorter than
Co-ordinating systems and behaviour in Hydra. II

those between successive impulses from the same locus. The usual pattern of pacemaker lead was shown in Text-fig. 3 above, where it can be seen that individual pacemakers can retain the lead for varying periods up to 10–15 impulses, after which another pacemaker becomes the leader. The situation is reminiscent of the way in which pacemaker leadership is shared by the various marginal ganglia of scyphomedusans. Less commonly (Text-fig. 3A) there occur periods when the activity seems to shift from one locus to another for several minutes before one locus assumes the lead. This usually occurs only during bouts of relatively rapid activity following some form of external stimulation. It is evident just as in scyphomedusans that the impulse coming from the leading pacemaker must suppress activity from all of the other pacemaker sites, for in the \( \lambda \)-preparation where the two bases are too far apart to make a third local interconnecting system between pacemakers an attractive hypothesis, one base at a time ‘calls the tune’ for the entire animal. Impulses in the RP conducting system thus affect the RP pacemakers, even though it is impossible to determine the manner of this control until more direct information about local pacemaker activity is obtained.

We have been unable to extirpate all RP sites. Even with small portions of any area of the column, comprising scarcely one-fifth of the entire length, it is possible to detect RP activity, although it is difficult to record. In this regard the RP system is unlike the CB system, for in the latter one can remove all the pacemakers by hypostomal extirpation (Passano & McCullough, 1964). There must exist many potential RP pacemaker sites throughout the column whose activity is normally repressed by the dominant loci near the base.

B. External stimuli affecting the RP system

‘Natural’ stimuli other than light

There is no evidence that Hydra is directly sensitive to temperature, but this is not unexpected, as neither actinians nor scyphomedusans show responses to these stimuli. While making time-lapse motion pictures of Hydra, we used strong far-red and infra-red illumination, with suitable filters to screen the animals from heat irradiation. If such filters are removed, permitting irradiation which we can easily feel as heat, the animals fail to show any behavioural change. Irradiation with infra-red sources while recording RP system activity has had the same negative results. Ambient temperature would be expected to affect rhythmic processes through its control of the rates of biochemical processes unless the system was compensated, as has been observed for circadian rhythm mechanisms, but systematic experiments to measure this temperature effect or the existence of circadian activity have not been undertaken.

Food, food extracts and reduced glutathione (Loomis, 1955) cause a marked, abrupt change in the behaviour of Hydra. Text-fig. 9 shows the results of several trials of the addition of reduced glutathione (‘GSH’) to the recording vessel. The final concentration was \( 1.5 \times 10^{-6} \text{M} \). For comparison, there are also shown examples where washed Artemia nauplii were added (‘food’). There were clear ‘feeding’ responses (save perhaps in Text-fig. 9E, where locomotion occurred), yet even the capture and ingestion of prey often failed to cause an abrupt change in the pattern of RP activity. Contraction bursts are usually (but not always) suppressed during feeding, while
there is a great increase in the asymmetrical column-bending and hypostomal 'nodding' which contribute to the 'feeding response'. After this behavioural pattern is well established, there is usually a decline in the RP frequency and it may become less regular (although the recording trace usually becomes disrupted by the vigorous columnar movements so that it becomes more difficult to detect RP impulses). The variability in the effect of feeding or of adding reduced glutathione on the RP pattern, and the fact that the RP frequency is entirely unaffected if it has previously been accelerated (Text-fig. 9F), even though feeding behaviour occurs as usual, strongly suggests that any effect on the RP system is indirect and that the feeding response and the suppression of contraction bursts are not under RP control. A similar freedom from RP dominance occurs once pre-locomotor activity is established (see below).

![Graph](https://example.com/graph.png)

Text-fig. 9. RP activity in several individual *H. pirardi* upon the addition of food (washed *Artemia* nauplii) or reduced glutathione (GSH). Animals previously fed 48 hr. before. In examples A to D inclusive the frequency is diminished, but no effect is seen in E or F (see text). Vertical bar indicates single RP; horizontal bar indicates CB; * indicates end of recording.

Mechanical stimuli can affect the RP system, but it is not very sensitive to the levels of stimuli that must be encountered naturally by the animal. Thus vibrations of the recording vessel which shake the tentacles (and seem to lead to increased tentacle activity), or gentle stroking of the tentacles or column with a plastic filament, may have no noticeable effect on the RP system unless it is prolonged. In the latter case it can lead to an accelerated RP frequency and typical pre-locomotor behaviour, culminating in tentacular attachment and a 'locomotor contraction burst', (Passano & McCullough, 1964). Strong mechanical stimulation can affect the RP system directly, however. In an experiment with a λ-preparation of *H. pirardi* we could shift the leading locus reliably to that base which had a fine glass probe pressed on it, when the RP impulses had previously been coming from the other base. Such shifts are accompanied by an increase in the RP frequency.
Stimulation by light

Diurnal variations in ambient illumination are a prominent feature of the natural environment of *Hydra*. It has been shown that contraction-burst frequency is enhanced twofold with the onset of daylight and remains elevated throughout the day (Passano & McCullough, 1964). Phototropism has been known in *Hydra* ever since the discovery of the animal and has been studied by Wilson (1891) and Haug (1933) among others. Patterns of activity in the RP system are similar in otherwise unstimulated animals whether under constant moderate illumination or in the dark, but certain differences can be seen. The RP frequency is usually lower in the animals kept in the dark, and it may not be as regular. In part, this difference could be due to the direct action of light on the RP pacemakers, but as illumination also affects the CB pacemakers directly it is probable that the depression of contraction-burst frequency is of equal or greater importance to the determination of RP frequency. After being fully dark-adapted the RP rhythm often undergoes slow fluctuations in its degree of rhythmicity, often ceasing entirely for a few minutes and then recommencing again at a slow rate. In the light, however, the rhythm seems to be reset following each contraction burst, so that the RP frequency will show a higher average value but will still be less constant than that in the dark.

Dark-adapted animals show a characteristic response to the onset of illumination (Passano & McCullough, 1963). There is initially an interruption of the RP rhythm with an abnormally long post-stimulatory interval. The impulses then recommence, always from a different pacemaker locus and with a marked increase in frequency. Gradually the frequency declines again, so that after a variable period (from 5 to 15 min. is commonplace) the rhythm is characteristic of that individual’s RP frequency under constant illumination. Several examples of the initial portion of individual responses to illumination are shown in Text-fig. 10. The duration of the interruption of the RP rhythm following the onset of illumination varies widely, even in the same individual for successive trials with repeated dark-adaptation. This variation is due in part to the degree of dark adaptation and to the strength of the light stimulus, but it is also a measure of the level of activity of the leading pacemaker. Animals with abnormally high RP frequencies (as for instance rates of 10–12 RP impulses per minute caused by repeated mechanical stimulation) will fail to show any response to light even though they are fully dark-adapted. When the RP frequency is low or irregular, changes due to a light stimulus can be very dramatic, although it is equally true that systematic appraisal of the effect of light is difficult when pre-stimulus RP intervals have varied widely.

By observing animals under deep red light (see below) or by photographing them with infra-red sensitive film under near infra-red illumination, it has been possible to follow the changes in behaviour occurring upon illumination with white light. Whether the animal is just contracted from a contraction burst (which may have been abruptly interrupted in progress (see Passano & McCullough, 1964)) or moderately extended in the typical ‘inter-burst’ posture, it starts extending within 10–15 sec. of the onset of illumination, so that by 60–100 sec. after illumination it may be over twice as long as it was previously. Such elongation has never been observed in the absence of augmentation of the RP frequency, so that it appears to be causally related to it. This
response is shown in a series of photographs (Pl. 1) of H. pseudoligactis floating in a dish.

During and after this elongation, the animal's behaviour resembles the 'feeding response' that occurs upon the addition of Artemia nauplii to the culture vessel, save that the mouth is not opened. The tentacles writhe, the column shows asymmetrical contractions, the tentacles may show concerted adaxial movements and in some cases the tentacles are held against the substratum or surface film. Attachment of the tentacles by their atrichous isorhiza nematocysts (Ewer, 1947) is followed by an abrupt arching of the column and vigorous peristalsis of the columnar muscles; together with the rapid 'locomotion contraction burst' which then occurs these movements may wrench the base free of its attachment.

When tentacle attachment occurs, there is an abrupt burst of rapid potentials which originate in the subhypostomal area. This is termed a 'pre-locomotor burst' (see §E below) and always precedes the locomotion contraction burst. The amount of disturbance to which the individual animal has been subjected and its nutritional state, as well as variation in response between individuals, determine whether or not illumination will lead to locomotion. If it does not, the post-illumination elongation and swaying will end in a regular contraction burst.

There is sometimes a response of the light-adapted animal to the end of illumination, but such an 'off-effect' is not consistently found. When it does occur (Text-fig. 11), it also consists of a double response; an initial pause followed by a change in pace-
Co-ordinating systems and behaviour in Hydra. II 219

maker locus and a somewhat augmented rhythm. It is evident from the examples shown in Text-fig. 11 that the removal of illumination does not alter the RP frequency as markedly as does sudden illumination.

These responses to moderate levels of light not only occur in response to continuous illumination of the entire animal, but also to short periods of stimulation and to stimuli falling on certain portions of the animal. Such shorter stimuli lasting a few seconds to a minute lead to the same response as does continuous light. Responses also occur to very brief stimuli. An RP response to a 0-6 sec. light flash is illustrated in Text-fig. 10 B. Even flashes lasting no more than 0-2 sec. can affect the RP rhythm, although in general it appears that the degree of response is directly proportional to the duration as well as to the strength of the stimulus. But very short intense flashes (from a photographic flash bulb, lasting less than 50 msec.) had no effect at all. Stimuli just preceding an endogenous RP (see Text-fig. 10 C) even by as much as 2-3 sec. may fail to arrest its occurrence, suggesting that since inhibition is delayed there is a latent period following the minimum adequate stimulus before a normal response, with inhibition and subsequent rhythmic enhancement, is realized. Such latent periods are the rule for dermal photoreceptor in other animals (Steven, 1963).

Text-fig. 11. On and off responses to illumination by RP system, H. punctata. A. Response to illumination (CB impulse occurred spontaneously just after stimulus but further CB impulses did not occur). B, C. Responses of the same individual to turning off illumination after several minutes stimulation. Horizontal bar indicates 10 sec.

It has already been noted that the results of recording RP system activity simultaneously from two separated sites on the animal gave strong evidence that the RP pacemakers are located in or near the base of the animal, although it has not proved possible to extirpate all pacemaker sites. The effect of light on the RP pacemakers permits us to confirm this localization by studying the effect of localized illumination of portions of the column of dark-adapted animals. Such experiments show that localized light stimulation of the middle and upper column areas, the tentacles and
their bases, the subhypostome and the hypostome regions never cause any alteration
of the RP rhythm nor any consistent shift in the pacemaker locus. Neither do such
stimulations ever lead to the behavioural sequence of column elongation, and so forth,
just described.

However, the results are very different when we stimulate the base region. In a
specific experiment, five healthy *H. littoralis*, fed 48 hr. previously, were immobilized
on the bottom of a wax dish with fine cactus spines. After being left an hour to recover
from the effects of this stimulation, each animal in turn was illuminated locally on its
extreme base while being observed under deep red light ('hydra darkness'—see below).
Two of the animals which remained in a state of hyper-excitability due to the re-
straining cactus spines were excluded from further light stimulations, but recording
from an area adjacent to the illuminated base was carried out with a microelectrode
(which was repositioned carefully as local stimulation trials were carried out) on each
of the remaining animals in turn. In eight out of nine trials, the localized illumination
caused a pacemaker shift and a clear augmentation of the rhythm. The behavioural
light response (elongation, etc.) commenced within a few seconds of the RP fre-
quency increase. In the single case where there was no effect on the RP system there
was no development of a behavioural pattern either.

In contrast to RP responses to generalized illumination, localized basal stimulation
does not cause the usual post-stimulus pause, perhaps because it does not impinge on
the usual currently active pacemaker site in the lower stalk adjacent to the extreme
base region. Rather, the basal pacemakers, positively light-sensitive, are activated
and come into play about 15 sec. after the 'on' stimulus just as they do when the whole
animal is illuminated. The formerly initiating pacemaker has continued to generate
one or even two post-light RP's independently of basal stimulation, before the basal
units take the lead. It appears that a few rapid RP's from the base almost immediately
lead to a generalized RP augmentation in the other normally active pacemakers, and
from this point on the electrical and behavioural responses to basal stimulation are
indistinguishable from the response to illumination of the whole animal.

Throughout these experiments on the responses of the RP system to light it was
repeatedly observed that illumination with red light (as with a photographic 'ruby'
incandescent bulb, or through a Wratten no. 25 filter) had no noticeable effect on
either the RP system or on the animal's behaviour. This insensitivity to red light
permits continual observation of animals under conditions of 'hydra darkness', as
we have already noted. This confirms the pioneer work of Wilson (1891) on the light-
sensitivity of *Hydra*, who showed that their strongest phototropism is to blue-green
light with no response to red.

The same spectral sensitivity is found when following the response of the RP
system directly. This was determined in the following manner. After reliable recording
from a normal *H. pirardi* was successfully established, the polyp was allowed to adapt
to darkness and to recover from handling for about 30 min. A light source was arranged
to deliver stimuli of various spectral ranges to the entire animal by interposing
different interference filters ('GAB narrow pass') between the lamp and the prepara-
tion. In other respects the arrangement was like that previously described (Passano
& McCullough, 1964), and similar results were obtained using gelatin filters. Pre-
sentation order was random. The animal was allowed to adapt to darkness for at least
15 min. between each stimulus, which was applied 1 sec. after a final pre-stimulus RP and was 15 sec. in duration. The percentage by which the post-stimulus RP interval was delayed relative to the pre-stimulus intervals was chosen as the index of stimulus effectiveness. While the amount of delay and subsequent frequency augmentation are highly correlated, the delay parameter varied through greater ranges and afforded a larger measurable quantity than did degree of frequency increase. No effort was made to equate the total energies delivered through the different filters or to compare the efficiencies of blue wavelengths to that of the unfiltered white light, but the different filters pass approximately equivalent band widths at the same percentage of maximum transmission. The result of these preliminary experiments summarized in Text-fig. 12, indicate that, while responses are variable, sensitivities decline rapidly in the region of 500 mμ and that no RP effects are discernible in the red regions of the spectrum. The effect of light on the RP system is entirely restricted to the pacemakers. Once initiated, an RP impulse is conducted throughout the column in the same manner whether in light or darkness. Illumination of the column does not interfere with conduction.

Text-fig. 12. Spectral sensitivity of RP system. The maximum wavelength of the stimulus is plotted against the percent augmentation of the interval between RP impulses, during which the stimulus intervened.

**Electrical stimuli**

A single brief electrical pulse given to the basal disk (usually 1 msec. duration and about 30% above threshold, given with a low-resistance saline-filled microelectrode and a platinum bare wire reference electrode) usually causes an immediate pulse in the RP system and an accelerated RP rhythm for some minutes before return to the previous level. Like the response to strong mechanical stimuli, but unlike that to light, there is no post-stimulus pause before the accelerated rhythm of firing occurs, although occasionally an electric shock can evoke increased frequency without causing a direct RP. These two types of response, displayed by a single λ-preparation of
**H. pirardi** pinned to a wax plate on the bottom of the recording dish, are illustrated by polygraph records in Text-fig. 13. Electric shocks applied to the leading base accelerate the rhythm at the existing active locus. Stimulating the non-leading side consistently caused a shift to the stimulated region which continued to be active for varying periods following the shock. In a few instances the leading base interjected a single RP immediately after the shock, before the stimulated (and formerly non-leading) base responded, indicating that a latent period can be found to exist for electrical stimuli at near-threshold intensities.

![Text-fig. 13. Effect of electric shock on RP pacemakers, H. pirardi (λ-preparation).](image)

*Text-fig. 13. Effect of electric shock on RP pacemakers, H. pirardi (λ-preparation).* A. Shock to base recorded on lower channel causes direct response, shift of active locus to stimulated base and sustained increase in RP frequency. B. Shock to base recorded on lower channel causes shift of active locus and increase of frequency without direct response to stimulus. Horizontal bar indicates 1 sec.

**Nutrition**

No direct correlation between recorded RP frequencies and nutritional state has been systematically sought. Indirect evidence from behavioural studies, however, implies that a relationship exists. When extended observations were made of the locomotion behaviour of groups of *H. littoralis* in response to turning on an incandescent white bulb after several hours in the dark, it was found that starved animals tended to conclude the sequence of post-light elongation, swaying, etc., with locomotion rather than simply with a contraction burst as seen more commonly in well-fed individuals. The practical details of these experiments have been given previously (Passano & McCullough, 1964). The results of an experiment on twenty-five well-fed and twenty-five 4-day-starved *H. littoralis* are given in Table 1. Since the RP system and the associated pre-locomotor burst mechanism (PLB) have been linked with locomotion by direct recording during this behaviour, it appears reasonable to assume that the increased tendency to locomotion of moderately (less than a week) starved animals reflects the nutrition levels and metabolic state of the RP pacemakers.
Depression and sexuality

Records were made from several *H. pirardi* which were either in spontaneous 'depression' or had fully developed testes. In both such circumstances the behaviour of *Hydra* is known to change markedly. The animals cease to capture and ingest prey, and their tentacles regress almost completely. They remain unusually elongated and limp, rarely exhibiting spontaneous contraction bursts or responding to shaking or agitation of the medium in their culture vessel. RP rhythms in four such animals were rapid and regular, averaging 8–10 per min. and remaining at high levels for the 20–30 min. of recording. Contraction-burst impulses were not observed in three of these four animals. In these respects such 'depressed' animals resemble the 'decapitate' preparations described above (see Text-fig. 8) in which RP potentials are generated rapidly and regularly without diminishing to low levels or showing much cyclic activity that characterizes normal, moderately well-fed, asexual animals.

<table>
<thead>
<tr>
<th>Light, successive 5 min. periods after illumination</th>
<th>Dark, 5 min.</th>
<th>0-5</th>
<th>5-10</th>
<th>10-15</th>
<th>15-20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fed daily, total number of steps, 25 animals</td>
<td>1</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Starved for 4 days, total number of steps, 25 animals</td>
<td>3</td>
<td>12</td>
<td>18</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

D. Ontogeny of RP co-ordination system

Under the culture conditions used in these investigations reproduction was exclusively asexual. While the coelenteron of the budding juvenile remained continuous with that of the parent we were unable to detect any RP impulses in the bud other than those also occurring in the parent. It has not been possible to determine whether these impulses detected from the surface of the bud were conducted in the bud, or whether we were detecting impulses being conducted in the parental column.

It is generally possible to detect the RP impulses of the bud, as distinct from those of the parent, shortly after the separation of the coelenterons. At first the RP rhythm of the bud only occurs (or is detected) erratically; but as the bud matures in size and the tentacles develop, its RP rhythm becomes more regular. It is never synchronized with the parent rhythm, and is always slower, even when the bud is about to become free of the parent. By this time the bud is exhibiting its own contraction bursts independent of the parent. Text-fig. 14 A shows the simultaneous recording of a parent *H. pirardi* and a young bud that has just begun to display its own RP's.

As shown in Text-fig. 14 B, buds are generally less sensitive to external stimuli than the parent. Their RP pacemakers are not affected by light stimulation at levels which cause the parent to respond.

E. Pre-locomotor burst impulses

In the section above, in which we described the response of a dark-adapted animal to light, we mentioned that a distinctive kind of impulse can be recorded from animals which have attached their tentacles just prior to the locomotion contraction burst
which initiates a somersault. A preliminary account of the pre-locomotor burst (PLB) system is included in this paper otherwise devoted to the RP system largely as a matter of convenience, for in spite of our initial suggestion (Passano & McCullough, 1962) we are unable to say whether or not they are conducted on the same system as are the RP impulses. However, PLB pacemakers are distinct; they are located in the subhypostome close to the points of insertion of the tentacles and, unlike the normally active RP pacemakers, they show rapid bursts of impulses like a contraction burst rather than regular activity. Their frequency (up to one per sec.) is far higher than that from any other pacemaker in *Hydra*.

Text-fig. 14. RP system activity in buds, *H. pirardi*. A. Bud (lower trace) which has recently established its own RP rhythm separate from that of parent (upper). B. Older bud (upper) showing more regular independent rhythm but insensitivity to illumination. C indicates parental CB; (C) indicates bud CB; horizontal bar indicates 10 sec.

Text-fig. 15 shows several examples of PLB impulses recorded from several different individuals (note also Text-fig. 6). It can be seen immediately that under favourable recording conditions PLB impulses always show certain characteristics which distinguish them from RP or CB impulses. They are similar to RP impulses recorded from the same recording site either before or after the pre-locomotor burst, but are always larger. Like the RP impulse, however, the PLB impulse has two components, a brief initial spike whose sign depends upon the distance between pacemaker and recording electrode and a second slower component that is always negative. In the examples shown the recording electrode was in the base, so that the initial spike is strongly positive to the reference electrode.

The exact moment when PLB impulses commence in relation to the animal’s behaviour has not been determined. At first we were under the impression that the PLB occurred as the tentacle were being attached to the substratum or surface film, but further observations now make it appear that the PLB starts after the tentacles are firmly attached. At other times the animal has merely arrayed the tentacles over the attachment site and the actual ‘seizing’ associated with nematocyst discharge appears to take place just prior to the final arching of the column and the wrenching free of the basal disk. The PLB could be triggered by the mechanical stimulation of the PLB pacemakers, by stretch and pull of attached tentacles or by contact of the oral
region pressing against the substratum. Sometimes there are several preliminary impulses from mid-column sites less regular in frequency before the PLB becomes fully established. Just as with the CB the interval between the first and second PLB impulse is often longer than that between succeeding impulses, so that the PLB rhythm appears to require several impulses to become established. Once established it usually proceeds with notable regularity; however, sometimes PLB impulses come in patterned groups of two to four pulses (Text-fig. 15 A) rather than at uniform intervals.

The number of impulses in a pre-locomotor burst varies somewhat, but is usually between 10 and 25 impulses. During this time the circular gastrodermal muscles are fully contracted and a series of peristaltic waves originate below the hypostome and spread down to the base. Asymmetrical contractions of the epidermal, longitudinally arranged, columnar muscles cause the animal to bend its upper column in waves (see illustration in Passano & McCullough, 1963). Towards the end of the PLB there occurs the first impulse of the locomotion contraction burst. We have already shown (Passano & McCullough, 1964) that this vigorous co-ordinated contraction of the body is distinguishable from the usual contraction burst not associated with locomotion. It is often possible (Text-fig. 15 C) to discern PLB impulses in the pause between the first and subsequent impulses of the locomotion contraction burst.

We have been unable to initiate PLB pacemaker activity directly by localized illumination of the subhypostome, or by giving electric shocks to this region. Stretching individual tentacles does not lead to PLB impulses; in fact, it is possible to obtain PLB activity and locomotor behaviour after removal of the tentacles as close to their insertions as possible. Removal of the entire subhypostome temporarily terminates PLB impulses as well as contraction bursts.

Text-fig. 15. Pre-locomotor impulses in Hydra. A. PLB followed by locomotion CB (LCB), H. littoralis. B. The same from H. pirardi. C. PLB recorded at higher recording speed. H. pirardi. Note continuation of PLB impulses into LCB. D. Similar to C, but PLB irregular. Horizontal bars indicate 10 sec.
We have been unable to affect PLB pacemaker activity with sudden illumination. Turning the light on during a pre-locomotor burst does not alter its frequency, nor does it block the subsequent appearance of the locomotor contraction burst which follows it (Passano & McCullough, 1964). Conduction of impulses originating at the PLB pacemakers, just as RP impulses, is not blocked by illumination of the column.

**DISCUSSION AND CONCLUSIONS**

The main purpose of this paper has been to describe the second co-ordinating system found in *Hydra*, the RP or rhythmic potential system. It might be useful to summarize its properties: (1) RP impulses are initiated and conducted throughout the column in a regular, rhythmic pattern; (2) RP impulses are cryptic, that is to say their occurrence cannot be correlated with any obvious behaviour of the animal; (3) their frequency varies widely, ranging mainly from 0.5 per min. to 12 per min., and is dependent on the state of the animal and the amount of stimulation that it has received; (4) they are conducted on a separate conduction system from that conducting the CB impulses; (5) the shape of the impulse changes with increasing distance from the initiation site; (6) they originate from pacemakers located throughout the column, but those near the base are usually the most active; (7) the RP system is endogenously active but is influenced by both internal and external conditions; (8) single electric shocks or vigorous mechanical stimulation excite adjacent pacemakers and can cause a long-lasting increase in frequency; (9) RP pacemakers are inhibited by sudden light, with their maximum sensitivity in the blue end of the visible spectrum. Inhibition of the leading pacemaker in this manner is followed by a shift to another pacemaker site, usually in the basal disk, and the subsequent RP rhythm remains accelerated for some time; (10) RP conduction, as opposed to impulse initiation, is unaffected by light.

The RP co-ordinating system comprises two components: the pacemakers that initiate impulses and the conducting system that transmits these impulses, without decrement, throughout the column. Since pacemakers are not localized in just one part of the animal as is true for the CB system (Passano & McCullough, 1964) we cannot say with certainty that separate morphological components are involved in conduction and initiation, but the sensitivity of the pacemaker to light, not shared by the conducting components, suggests that here, too, separate elements are involved in the two processes. A similar conclusion emerges from the experiments on the effects of chloretone, where the pacemaker of the system loses its endogenous activity even though still capable of showing a temporary response (with normal conduction) to light stimuli.

The change in shape of the initial component of the RP impulse is also evidence favouring the separateness of pacemaker and conduction functions. Close to the site of initiation the initial spike is 'conventionally' negative, but as it travels from this point it becomes compound and the negative spike becomes reduced and finally disappears, leaving a positive initial RP component. This could be explained by supposing that the initiation of the impulse by the pacemaker involves an impulse that is negative to the surroundings, but that conduction involves the non-decremental transmission of a positive spike. The signs of these two components are not affected by shifting the reference electrode from the surrounding medium to the coelenteron.
Yet although they are separate from the conducting elements, the pacemakers are not unaffected by conducted RP impulses originating elsewhere. To use the terminology of Pantin & Vianna Dias (1952), they are in 'physiological continuity' with the conducting system. Interposing a single artificial stimulus by giving the animal an electric shock causes an immediate and long-lasting augmentation of the active pacemaker's endogenous output. The same conclusion is reached from a consideration of the interaction between the various potential pacemakers when the animal is undisturbed, just as is the case for scyphomedusan's marginal ganglion pacemakers. A single locus leads for a series of impulses; then the lead (active pacemaker) shifts to another spot, and so forth. The overall rhythm remains approximately constant throughout, with only a slight irregularity to mark the shift from one pacemaker to another. Rhythmic activity is a property of the system as a whole as well as of the individual pacemakers, for it persists in spite of continual shifts in initiation loci.

One of the most interesting aspects of the RP system is its sensitivity to blue light. Just as with the CB system discussed earlier (Passano & McCullough, 1964) it is the pacemaker elements and not the conducting (or effector) elements that are light-sensitive. The behavioural responses resulting from these sensitivities are an important component of the animal's responsiveness to its environment (Haug, 1933; Passano & McCullough, 1963).

We have assumed that the pacemaker elements are sensitive, rather than postulating that there are separate specialized receptor cells. We do this simply because such cells have never been identified in Hydra, even though our results indicate that they would have to be so associated, separately, with many pacemaker units or else fashioned together into another co-ordinating system if they occurred. In either case, one would expect to find some electrical adjunct to their activity, yet nothing resembling an ERG has been observed. We are also influenced by the parallel between the light sensitivity of Hydra, and the 'dermal light sense' of the other eyeless animals. Yoshida & Millott (1959) have presented convincing evidence that the dermal sensitivity of the sea-urchin Diadema antillarum (Echinodermata, Echinoidea) is a property of their nerves. It is also a striking fact that the spectral sensitivity of these forms with their maximum sensitivity at 455-460 mμ (Yoshida & Millott, 1960) is similar to that shown by Hydra. The nature of the photosensitive pigment responsible for the dermal light sense is not yet known for any animal (Steven, 1963) and it seems premature to ascribe it to a carotenoid, in Hydra, as Singer, Rushforth & Burnett (1963) have done. However, the recent isolation of a photopigment from the ocelli of the anthomedusan Spirocodon (Yoshida, 1963), whose properties are comparable to rhodopsin systems, suggests that light-sensitive pigments in coelenterates may conform to the general pattern.

The effect of light on the RP pacemakers is twofold when the whole animal is moderately illuminated, for it both inhibits the initiation of RP impulses temporarily, and causes a subsequent enhancement of the RP rhythm when firing recommences. It appears that the spectral sensitivity for both processes is the same, but the techniques that we have employed thus far are inadequate to establish this with certainty. Double effects of this kind are not uncommon; for example the spine shadow reflex of the echinoid Diadema also shows both inhibitory and excitatory components to illumination (Millott & Yoshida, 1960).
It is clear that the PLB pacemakers are distinct from those of the RP system in general. They occur near the hypostome instead of in the lower column and base. They produce rapid bursts of impulses (like the CB pacemakers, but at a higher frequency and with more impulses) under the particular conditions associated with tentacle attachment and are at other times inactive. They directly precede the special locomotion contraction burst, which is recognizably different from the usual contraction burst. The PLB pacemakers have not been shown to be light-sensitive.

Is the PLB conduction system the same as that conducting the RP impulses or are there three separate conducting systems, one each for the CB, RP and PLB impulses? If these latter two were distinct we would hope to be able to detect different conduction velocities, different extents of conduction, differences in the shapes of the two kinds of impulses recorded from a single recording electrode, and to find evidence of simultaneous independent activity in the two conducting systems. Conversely, if the PLB pacemakers were connected to the RP conducting system, we would expect that impulses initiated by either kind of pacemaker would be identical and have the same effect on other pacemakers.

Our findings fail to give an unequivocal answer to this question. In conduction velocity, extent of conduction and general shape, RP and PLB impulses appear the same. Nevertheless, PLB impulses are generally greater in amplitude (Text-fig. 15), no matter where they are recorded. Larger size does not seem to be due to some quick-decaying facilitation, for even the first PLB impulse is recognizably larger than the previous RP impulse without any discernible correlation with the time since the previous RP event.

Sometimes it appears that RP impulses and the PLB burst can continue superimposed on each other. This must mean that a PLB impulse originating at the hypostomal end of the polyp does not affect RP pacemaker loci in the more basal part of the animal in the manner that they are affected by an RP impulse.

It is worthwhile considering the possible morphological basis of the RP, PLB and CB systems, even though it is impossible to reach any certain conclusion at present. It may be recalled (Passano & McCullough, 1964) that the CB system includes both a pacemaker or pacemakers in the hypostomal end of the animal and a conducting system which transmits CB impulses throughout the column. Similarly, we have shown here that the RP system consists of both initiating and conducting components with its pacemakers located throughout the column (and the most active loci near the basal disk). The third system, the PLB system, resembles the RP system in many but not all respects; its pacemakers are subhypostomal and while it may prove to share the same conducting system as that used by the RP pacemakers, it seems more likely on the basis of the evidence at hand also to have a separate conducting network in the column.

None of these co-ordinating systems appears to penetrate the tentacles. The tentacles can act as autonomous units, in pairs or groups, or as a co-ordinated unit, showing behavioural sequences that are related to the behaviour of the rest of the animal but still distinct. Further consideration of tentacles must be withheld from this paper. Since the RP system has no direct effect on the longitudinally arranged epidermal muscles, and since the CB conducting system does excite these muscles, it is plausible to consider the former as gastrodermal, whereas the latter is epidermal. From this
line of reasoning the absence of a complete nerve-net (Semal-Van Gansen, 1952) in the gastrodermis must mean that some other component is responsible, at least in part, for conduction. The most likely tissue would be the muscle cells either alone or with the nerve cells. If this is so, one could explain the second, slow, negative pulse of the compound RP potential as being a corollary to the contraction wave. One could postulate further that the pacemaker units are the gastrodermal protoneurons. If this line of reasoning is correct, the PLB system might well be the longitudinal epidermal muscles, and the PLB pacemakers certain protoneurons of the subhypostome epidermal layer. Against this very tentative hypothesis, it must be said that there seems to be no evident correlation between the specific occurrence of RP impulses and elongation of the column following a contraction burst. The beginning of re-elongation before the occurrence of a single RP impulse following the contraction burst has been observed repeatedly. Perhaps, however, this initial recovery of column shape after the contraction burst is due to some other factor or factors, such as a heightened coelenteric pressure, and hence gastrodermal muscle contraction may still be correlated with conducted RP impulses.

A decade ago Semal-Van Gansen (1952) suggested that elongation in Hydra was controlled by a mixed plexus of both muscle and neuronal elements. More recently, Mackie (1960) has proposed that in hydrozoan medusae contractions of the circular muscles are co-ordinated by myoid conduction from pacemaker elements in the marginal nerve rings. He suggests that nerve nets in Hydrozoa are found only where two conducting systems (radial and circular) with separate effectors occur in the same layer and that where there is only one action system (as in the subumbrellar layer of the nectophores of siphonophores) a single myoid conducting system, together with its marginal nerve rings, is sufficient. If the RP system is gastrodermal while the PLB and CB systems are epidermal, Hydra would be a coelenterate with a single action system in one of its epithelia and a double system in the other. Myoid conduction should then suffice for both the RP and the PLB systems if this reasoning is valid; the epidermal nerve-net Hydra could therefore be the third conducting system for the co-ordinated contractions of longitudinal muscle that make up the contraction burst.

It is evident that our present information is insufficient to make possible any definite conclusion about the nature of the conducting system in hydoras. Many alternative schemes could be outlined, so that the following should be considered simply as one possibility: the RP system consists of two components, a series of pacemakers and a conducting mechanism throughout the column. The pacemaker units are the gastrodermal protoneurons which, like many other cell-types of the polyp, are continuously being formed at the region of growth in the subhypostome. They are all potentially active, but are usually led from the mature protoneurons near the base. These cells are in 'physiological continuity' with the conducting component in the sense that action potentials originating elsewhere modify their internal state, but these local non-propagated fluctuations do not affect the conducting system. External stimuli act to elevate this local state of excitation.

The conducting system is functionally a single all-or-none unit occurring throughout the column, capable of transmitting single impulses non-decrementally that are initiated by the protoneuron pacemakers. It is also an action system of circularly arranged gastrodermal muscles so that following each impulse there is a wave of
contraction. The weakness and slowness of the response, and the duration of the intervals between RP impulses, obscures this correlation. This tentative hypothesis also suggests that the PLB system has a parallel morphological basis in the epidermal epithelium. Here the pacemakers are subhypostomal, and unlike those of the RP or CB systems (but like the RP pacemakers of the still-attached asexual bud) they are insensitive to illumination. Supposing of course that the PLB system is a separate entity from the RP system, and that its similarities to the RP system are meaningful, this disposition of the PLB conduction system to the epidermal muscles relegates the CB conduction system to the epidermal nerve-net. This would fit Mackie's (1960) speculation that nervous conduction in addition to myoid conduction is only required in coelenterates when two action systems are super-imposed in the same epithelial layer. The CB conduction system would then be a physiological 'giant-fibre' system for the rapid co-ordinated contraction of the longitudinal muscles.

**SUMMARY**

1. Two further co-ordinating systems, the rhythmic potential (or RP) and the pre-locomotor burst (PLB) systems, are described from several species of *Hydra*.

2. RP impulses may be recorded from anywhere in the column. They are small, distinctive in shape, through-conducted at 4 cm. per sec. and without direct behavioural correlates.

3. RP pacemakers occur throughout the column, but those near the base are the most active. Impulses are initiated endogenously in a more or less regular, rhythmic manner, with a frequency of 1–10 per min.

4. Mechanical or electrical stimulation of the column enhance pacemaker activity and often shift the active loci towards the points stimulated. Starvation depresses activity after several days.

5. Moderate illumination has a dual effect on RP pacemakers: it causes almost immediate inhibition of the currently active locus, and then an extended period of rhythmic enhancement triggered by basal disk pacemakers. Both effects show the same spectral sensitivity with the greatest sensitivity to blue light and total insensitivity to the red. RP conduction is unaffected by light.

6. Chloretone affects first the contraction-burst system and the epidermal muscles, then endogenous RP pacemaker activity, while RP responsiveness and conduction are still unaffected. These effects are reversible.

7. RP impulses can first be detected in still-attached buds, but their rhythms are slower, less regular and not light-sensitive.

8. The PLB system resembles the RP system in the character of its impulses, and the contraction-burst system in its occasional bursts of activity. PLB pacemakers are in the subhypostomal region. These impulses precede (and presumably cause) the distinctive locomotor contraction bursts.

9. It is concluded that the RP system consists of two distinct components, the pacemakers and the conducting network; it is suggested that the pacemakers are protoneurons and that both RP and PLB conduction is myoid.

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Co-ordinating systems and behaviour in Hydra. II

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REFERENCES


EXPLANATION OF PLATE

Elongation of dark-adapted H. pseudoligactis following the onset of illumination. The animals were floating in a shallow dish and photographed from above: (1) 30 sec. after the start of illumination; elongation has already begun. (2) 150 sec. from start; tentacle writhing and elongation evident; (3) 180 sec. from start; tentacle movements are causing the large animal on the left to move away from the edge of the dish. Note also asymmetrical column bend on animal on right. (4) 270 sec. from start; length of animal on right more than double its original length.