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Neuronal Control of Swimming in the Medicinal Leech

I. Dynamics of the Swimming Rhythm

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Summary. Leeches swim by undulating their extended and flattened body in the dorsoventral direction, to form a wave that travels backwards along the animal. The troughs and crests of this body wave are produced by a metachronal rhythm of antiphasic contractions of the dorsal and ventral longitudinal musculature of the body wall of successive segments. Cinematographic records of swimming leeches show that over a range of cycle periods from 390 to 1100 msec the animal maintains one full wave along the length of its body. This constant wave form is achieved by compensating for the increase in the cycle period by an increase in the intersegmental travel time of the wave from 19 to 77 msec per segment. Direct muscle tension measurements of the segmental body wall during the swimming movement of a restrained, brainless and partially denervated leech lead to values for the dynamic parameters of the body wave which are in agreement with those abstracted from cinematographic records. In order to study the neuronal control of the swimming movement, a semi-intact leech preparation was developed. This preparation carries out movements that are clearly vestiges of the swimming rhythm while allowing the taking of electrophysiological records from exposed and immobilized parts of its peripheral and central nervous systems. The records reveal a nerve cell activity rhythm whose period matches that of the swimming rhythm. The swimming rhythm and its body wave must be generated by a system of distributed, phase-locked segmental oscillators that cause an antiphasic activity of the motor neurons innervating the segmental dorsal and ventral longitudinal muscles. The cycle of these oscillators can be inferred to consist of a variable time sector whose changes in length are responsible for changes in the cycle period, and of a constant time sector whose length is independent of the cycle period.

Introduction

The central nervous system of the medicinal leech *Hirudo medicinalis* consists of 34 interconnected ganglia. Fused masses of 13 of these ganglia form two brains: a 6-ganglion head brain and a 7-ganglion tail brain. Head and tail brains are linked by a ventral nerve cord consisting of the remaining 21 ganglia and their connectives. Each ganglion of the cord contains the cell bodies of some 175 pairs of bilaterally symmetrical neurons and innervates one of 21 metameric body segments via a system of segmental nerves which issue from the ganglion in bilateral pairs of anterior and posterior roots. The gross anatomy of the iterated segmental leech ganglia is sufficiently stereotyped from segment to segment, and sufficiently invariant from individual to individual, that a large portion of the cell bodies of the approximately $21 \times 2 \times 175 = 7500$ neurons of the segmental central nervous system can be reproducibly identified. Studies carried out in recent years have shown that the leech ventral cord is a particularly favorable material for the functional analysis of neuronal networks. The ganglionic neurons can be penetrated with microelectrodes to obtain recordings of action potentials,

as well as of excitatory and inhibitory synaptic potentials arising from interneuronal contacts in the inner ganglionic neuropil. The favorable electrical properties and disposition of the cells have made it possible to ascertain the modalities, receptive fields and response characteristics of a number of sensory neurons and the fields of peripheral action of a number of motor neurons, and to establish the nature of their intra- as well as interganglionic connections (Nicholls and Baylor, 1968; Nicholls and Purves, 1972; Stuart, 1970). These findings, in turn, have allowed the identification of the specific cells and their synaptic connections that are responsible for generating some simple acts of reflexive behavior (Nicholls and Purves, 1970). The work to be reported in this series of papers is an extension of such analyses to a more complex behavioral act. In particular, an account will be given of the neuronal control of swimming in the leech, in terms of identified cells and their synaptic connections.

The leech swims by undulating its extended and flattened body in the vertical direction (Fig. 1), to form a body wave that travels in the antero-posterior direction. While undulating in the water, the leech controls its attitude so as to have its venter face downward. The moving crests of the body wave are produced by a metachronal rhythm of contraction of the ventral body wall of successive segments and the troughs are produced by a similar but antiphasic metachronal contractile rhythm of the dorsal body wall. The forces exerted against the water by these changes in body form provide the propulsion that drives the animal through its fluid medium. The average period of the body wave ranges from about 350 msec for fast swimming to about 700 msec for slow swimming (Gray *et al.*, 1938; Schlüter, 1933). In 1905, Uexküll described the basic activity pattern of the leech musculature responsible for the changes in body form of the swimming movement. He set forth that the flattening of the body, which optimizes the force that dorsal and ventral body walls exert against the water, is produced by tonic contraction of the *dorsoventral* muscles. These muscles traverse the body between their points of insertion into dorsal and ventral body walls (Mann, 1962). Uexküll proposed, furthermore, that the rhythmic local contractions of dorsal and ventral body wall segments that form the troughs and crests of the wave are produced by a phasic contraction of the *longitudinal* muscles embedded in the body wall (Mann, 1962). By exerting pressure on the body fluids the tonic contraction of the dorsoventral muscles also provides for the general tonus of the leech during swimming and thus can provide for the periodic longitudinal distension of the segmental body wall following relaxation of the contracted segmental dorsal or ventral longitudinal musculature. Both dorsoventral and longitudinal muscles are innervated by motor neurons that reside in the corresponding segmental ganglion, and the activity of these motor neurons causes the local contraction of the segmental musculature (Stuart, 1970). Thus, the problem of the neuronal control of swimming is: how do the relevant segmental motor neurons that control the contraction of dorsoventral, dorsal longitudinal and ventral longitudinal muscles maintain an activity pattern which gives rise to the characteristic body wave?

The presence of the leech brains is not required for the generation of the body wave. Uexküll (1905) had found that decapitated leeches not only still swim but swim more readily and for longer episodes than do intact animals.

Schlüter (1933) later observed that swimming still occurs after surgical disconnection of head and tail brains from the ventral cord. Hence, the body wave of alternating dorsal and ventral contractions is generated by the ganglia of the ventral cord, with the head and tail brains functioning only to modulate the basic swimming rhythm. Schlüter showed, furthermore, that the intersegmental coordination of the contractile rhythm of dorsal and ventral longitudinal muscles requires the integrity of the ventral cord. He found that although after cutting the connective in midbody between two ganglia both anterior and posterior parts of the leech still maintain the swimming rhythm, front and back movements are no longer coordinated. This finding revealed also that maintenance of the swimming rhythm does not depend on a special or unique function of any particular one of the 21 segmental ganglia. Gray, Lissman, and Pumphrey (1938) later demonstrated that the swimming wave is propagated centrally and without the necessary participation of the periphery. They found that a leech preparation from which the body wall of three midbody segments had been removed exhibits complete coordination of swimming movements in the intact front and back parts of the animal connected only by the cord. Thus the rhythm travels through ganglia which can neither command contraction of peripheral muscles nor receive sensory inputs from the segmental body wall. In fact, the rhythm not only travels through such deafferented ganglia but also goes out the segmental nerves. This was shown by use of a preparation in which the brains were severed from the cord and the roots of the first few segmental ganglia were sectioned. The intact rump of this preparation exhibited swimming movements. During such movements extracellular recordings taken from the proximal stump of a sectioned root showed a well-defined rhythm of nerve impulse activity, whose period matched that of the swim cycle (Gray *et al.*, 1938).

The results presented in this first paper extend and refine the earlier studies of the swimming movement, in order to define the parameters of the behavior whose neuronal basis is to be explained. This paper also describes how more detailed electrophysiological records can be obtained from the nervous system of a semi-intact leech preparation similar to one used by Gray *et al.* (1938). The second paper (Ort *et al.*, 1974) presents the results of extra- and intracellular recordings taken from the segmental nerves and ganglia of the semi-intact preparation. These observations demonstrate the existence of a neuronal ensemble that takes part in the generation of the swimming rhythm and of synaptic connections among the members of that ensemble. The third paper (Kristan *et al.*, 1974) presents the results of a dynamic analysis of the activity of these neurons which provide some information concerning the nature of the oscillators which drive the swimming rhythm.

Materials and Methods

Animals

Specimens of *Hirudo medicinalis* were purchased from a commercial supply house. The leeches were stored at 4° C and generally used within six weeks after their arrival. During that storage period the animals gradually deteriorated with regard to their eventual performance as a semi-intact preparation, although there was a great variability in the storage resilience of different batches.

Numbering of Segments

In this, as well as our subsequent papers, we use a numbering system for the metameric segments and their ganglia of *H. medicinalis*, which is different from the conventional system of numbering leech body segments (Mann, 1962). We assign No. 1 to the most anterior (non-cephalic) ganglion of the ventral cord and number the remaining ganglia consecutively in an anteroposterior sequence, up to ganglion No. 21 just anterior to the tail brain. To each of the metameric body segments we assign the same number as that given to the ganglion which innervates it, reserving a separate numerical sequence for the six rostral head segments. The conventional segmental numbering system is inconvenient for neurophysiological work because under it the first ganglion of the cord innervates segment No. 7 rather than segment No. 1.

Cinematography

Swimming movements of leeches were recorded on 16 mm film by means of a camera running at 25 to 30 frames per second. The films were reprojected frame by frame by means of a photo-optical data analyzer to give a magnification of about 3 times.

Dissection and Mounting of Preparations

All dissections were carried out while the animal was immersed in cold physiological saline maintained below 10° C. To disconnect the brains from the nerve cord, the ventral blood sinus was exposed by longitudinal incisions through the body wall at the ventral midline of the first and twenty-first body segments. The sinus was then opened anterior to the first and posterior to the twenty-first segmental ganglion and the connective between each ganglion and its adjacent brain was cut. To denervate a segment, the body wall of the segment was similarly opened at the ventral midline and both pairs of ganglionic roots were cut at their point of emergence from the blood sinus. After such operations the body wall incisions were usually sutured closed. To make the semi-intact preparation, the cord is first disconnected from both brains. The leech midbody is then opened for the length of several segments by a longitudinal incision along the dorsal midline and by circumferential cuts at the end of the incision from the dorsal midline to the lateral edge in order to expose the cord. The roots of the most anterior and most posterior exposed ganglia are cut and the segmental nerves of the middle exposed ganglia are dissected and freed from the body wall. The entire body of the leech is then cut away for nearly the length of the exposed cord, resulting in a fore and a hind animal connected only by the cord and its exposed ganglia with their segmental nerves. For some experiments, the segmental nerves of one side of an exposed middle ganglion are left attached to a flap of body wall with its underlying musculature. Depending on the number of segments to be exposed and the extent of segmental nerves to be freed from connective tissue, the dissection takes from one to five hours.

After the dissection, the preparation is placed in a flat glass-bottom dish containing leech physiological saline (Nicholls and Purves, 1970; Stuart, 1970), to a depth of about 1.5 cm. The exposed cord and its ganglia are made to lie on top of a small glass bar which is mounted on the bottom of the dish and surrounded by resin. The body and the cord are secured to the resin by pins inserted into the denervated parts of skin at the cut edges of the fore and the hind animal, and into bits of tissue purposely left attached to the ganglionic roots. This arrangement allows the viewing of the ganglia by convergent transmitted light focused on them from below by a dark-field condenser of short focal length. The clearly visible cell bodies of the immobilized and trans-illuminated ganglia can then be penetrated by capillary microelectrodes. The preparation is usually pinned dorsum down, and hence the ventral aspect of the ganglia presents itself for penetration. But if cell bodies of the dorsal aspect are to be penetrated, the attitude of any ganglion can be reversed by simply twisting the cord by half a turn. The fore and hind parts of the animal are otherwise unrestrained and make swimming movements which result in a periodic up and down motion of the head and tail. That motion is monitored by its recurrent interruption of a light beam focused on a photocell. The preparation is maintained at a temperature of 12–15° C by means of a thermionic cooling device.

For many hours both fore and hind parts of a successful preparation can be induced to carry out episodes of coordinated swimming movements lasting 10–50 cycles, by gently stroking the ventral body wall. This behavior gradually deteriorates in the following manner: (1) swimming episodes become more difficult to induce and consist of fewer cycles; (2) during the swimming episodes, more and more often only the fore part swims; the hind part, however, never swims independently, at least not as long as the cord is intact.

Muscle Tension Measurements

To measure the tension developed by the longitudinal muscles of a single segment of a swimming leech, the body wall of that segment must not be subject to tensile forces exerted by the contraction of muscles of adjacent segments. Such a functional isolation of the segmental body wall was produced by denervating the neighboring two segments on either side. Beads were sewn to the dorsal and ventral skin of the anterior of the two posterior denervated segments and the leech was pinned to small, appropriately spaced corks at the anterior of the two anterior and at the posterior of the two posterior denervated segments. Bionix F100-A tension transducers were placed posteriorly and attached by taut threads to the beads sewn to the skin. This arrangement allows separate measurement of the tension developed by the dorsal or the ventral longitudinal muscles of the innervated segment. To measure separately the tension developed by the musculature of two different functionally isolated segments, three pairs of adjacent segments were denervated. The anterior and middle pair of denervated segments bracketed one innervated segment and the middle and posterior pairs bracketed another. Beads were sewn to the skin of each of the anterior segments of the middle and posterior pairs of denervated segments. The leech was pinned to corks at the anterior of the two anterior, at the posterior of the two middle, and at the posterior of the two posterior denervated segments. Tension transducers were attached as described above.

The tension developed in the body wall flap of a semi-intact preparation can be measured by attaching beads to both anterior and posterior edges of the flap; the anterior beads are pinned to the bottom of the dish and tension transducers attached to the posterior beads by taut threads.

Electrophysiological Recordings

Extracellular records were obtained by means of glass-tipped suction electrodes attached to the dissected segmental nerves. The output of the suction electrodes was amplified 100-fold by a capacity-coupled Grass Model P15 amplifier. Intracellular records were obtained by means of glass capillary microelectrodes filled with 4 M potassium acetate and having resistances ranging from 50 to 120 MΩ. The microelectrodes were connected to a unity-gain, high-input-impedance WPI Model M4A electrometer. Both extracellular and intracellular electrode outputs were further amplified 100 to 1000-fold by means of low-input-impedance D.C. amplifiers possessing a flat frequency response up to 1 kHz. These amplifiers were used also to amplify the signals generated by photocells and tension transducers. The final amplifier outputs were monitored on a multiple trace oscilloscope and recorded on magnetic tape by means of a Vetter-Crown 8-channel FM recorder. Later they were transcribed from tape to paper at a four-fold speed reduction by a Brush-Gould Model 260 penwriter. This arrangement faithfully reproduces signals up to about 300 Hz, so that synaptic and action potentials can be recorded with rather little attenuation of their amplitude or distortion of their time course without use of an oscilloscope camera.

Results

Cinematography of Wave Dynamics

In order to measure some of the parameters of the periodic changes in body form which make up the body wave of the swimming movement, cinematographic records were taken of the lateral aspects of swimming specimens of *H. medicinalis*. Before filming, markers such as thin threads or small beads were attached at several points of the leech body to provide easily recognizable landmarks for later analysis of the records. Fig. 1 is a composite print of 12 successive frames

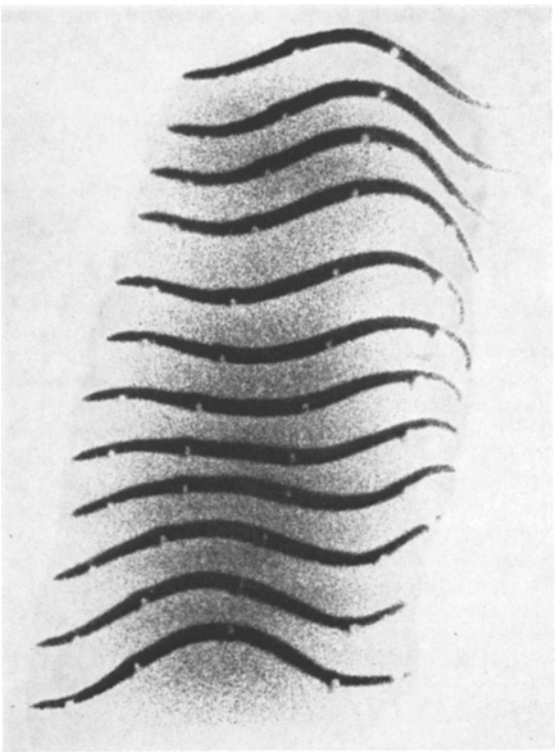

Fig. 1. The body wave of a swimming leech. A composite print of successive frames of a cinematographic record of a free-swimming specimen, with white reference beads attached to the 1st, 5th, 10th and 15th segment. The right-to-left horizontal displacement of the animal depicts its true progress in the water; the vertical displacement is an artifact of the photographic print. The time occupied by this episode, which corresponds to one cycle period, is about 400 msec

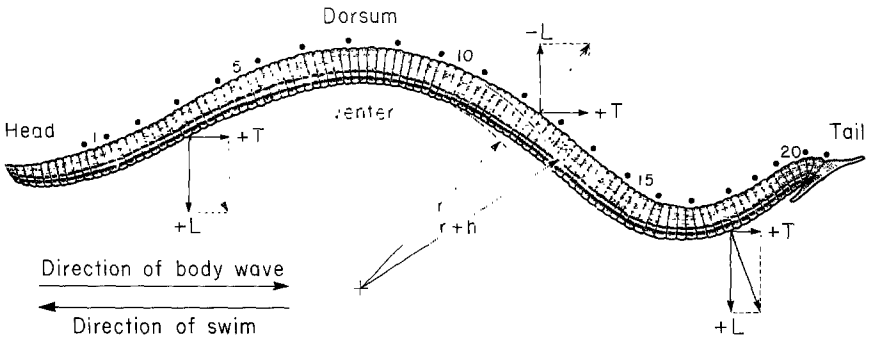

Fig. 2. Dynamics of the body wave. r and $r+h$ are respectively the radii of curvature of ventral and dorsal body walls at a body wave crest, with h being the thickness of the flattened leech body. The three force parallelograms show the contributions to thrust (T) and lift (L) of the force exerted against the water by ventral and dorsal body walls attending the rearward motion of crests and troughs. The dots indicate the borders of the 21 body segments

of such a cinematographic record. In order to evaluate the record, the film was projected frame by frame, and an outline of the lateral body silhouette with its reference markings was traced. Fig. 2 presents a drawing of the lateral aspect of

a leech, the body shape shown in this drawing is based on a silhouette traced from one of the frames of Fig. 1.

It is evident from Fig. 2 that for its swimming movement the leech maintains one full body wave, as do most other aquatic animals, e.g. eels, that use undulations of their body for propulsion (Gray, 1968). The following reasoning can be advanced to explain this general feature. The net movement of the animal is the result of two vectorial components of the propulsive force: the *thrust*, or force in the direction of the longitudinal body axis, and the *lift*, or force in the vertical direction. As can be seen from the parallelogram of forces drawn schematically in Fig. 2, the rearward travel of both wave crests and troughs produces positive, or forward thrust. However, positive lift is produced only by the forces exerted against the water by the ventral body wall (on the leading edge of a trough and the trailing edge of a crest). And negative lift (or sinking) is produced only by the forces exerted by the dorsal body wall (on the leading edge of a crest and the trailing edge of a trough). Detailed analysis of these forces show that the speed of propulsion of the animal in water increases with both the frequency and the amplitude of the body wave (Gray, 1968). To achieve the highest thrust at a given wave frequency, the animal could shape its entire body into a half-wave and thus attain the greatest possible wave amplitude. But the lift vector attending this wave form would cause severe pitching of the head. Such undesirable transverse oscillations of the head about the axis of forward progression can be ameliorated only if at any moment the length of the body comprises at least one full wave (Gray, 1968). Thus, the conflict between the desiderata of large wave amplitude for fast propulsion and short wavelength for rostral dynamic stability is resolved by shaping the body into one full wave.

The radius of curvature of the leech body at the crests and troughs of the wave shown in Fig. 2 allows an estimate of the degree of contraction of the ventral or dorsal segmental longitudinal muscles during the swim cycle. If at a wave crest the ventral body wall has a radius of curvature r and the dorsal body wall a radius of curvature $r+h$, where h is the thickness of the flattened leech body, then the length ratio of contracted ventral to distended dorsal longitudinal muscles of the segment at the trough is $1/(1+h/r)$. Measurement of the body shape shown in Fig. 2 shows that at the wave crests and troughs h/r has a value of about 0.2. Hence, in the contractile phase of the swimming cycle the longitudinal muscles shorten to about $1/(1+0.2)=0.8$ of their length in the distended phase, or by only about 20%. This phasic change in length of the longitudinal muscles in the swimming wave is much smaller than the degree of contraction of about 70% that these same muscles undergo in the "shortening reflex". In that reflex, the leech body changes from the shape of a thin, oblong cylinder to that of a squat elipsoid, upon simultaneous tonic contraction of the entire longitudinal musculature, in response to the activation of cutaneous sensory receptors (Nicholls and Purves, 1970). Thus, it follows that for generating the swimming wave, the longitudinal muscles of individual segments contract and distend rhythmically over only a small fraction of the total change in length of which they are actually capable.

The main purpose for which the cinematographic records were made was to measure the time taken for the swimming wave to travel from one segment to the next, and to ascertain how this time varies with the swim cycle period. To obtain this information, the positions of wave crests and troughs were determined

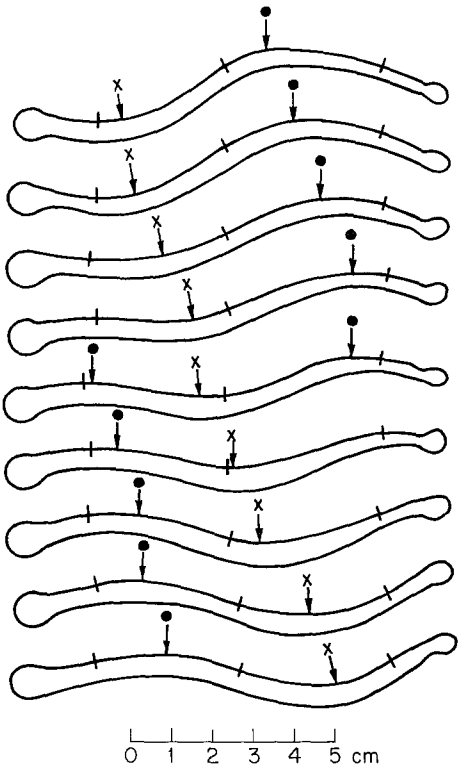

Fig. 3. Sample sequence of outlines drawn from cinematographic records such as those shown in Fig. 1. Head of animal on left; direction of swim from right to left. The three vertical lines appearing on each silhouette represent the position of markers sewn to the body wall of the 5th, 10th and 15th segment of the specimen. The arrows topped by a dot or by a cross indicate the visually identified positions of crests and troughs respectively

by visual inspection on a sequence of lateral body silhouettes, such as those shown in Fig. 3. The linear distances of crests and troughs from the reference markings were then measured by rolling a cartographic distance wheel along the curved edge of the body outline. Sample results of such distance measurements are plotted in Fig. 4. In this plot, the ordinate represents the segmental position of a crest or trough and the abscissa the elapsed time (or frame number). Hence a line connecting successive points represents the rearward progress of individual crests or troughs along the body of the swimming animal. The horizontal distance separating the lines of progression of a trough and its following crest represents the delay between contraction of the dorsal and ventral longitudinal muscles of a particular segment. The horizontal distance separating the lines of progression of two successive crests or two successive troughs represents the cycle period. The slope of an individual progression line represents the speed of rearward travel of a trough or crest, and the reciprocal of that slope is the intersegmental travel time of the wave.

Measurements of the horizontal distances between the lines of progression (at segment No. 10, near the body midpoint) show that, during the swim episode plotted in Fig. 4a, the average cycle period was 405 msec, with individual periods

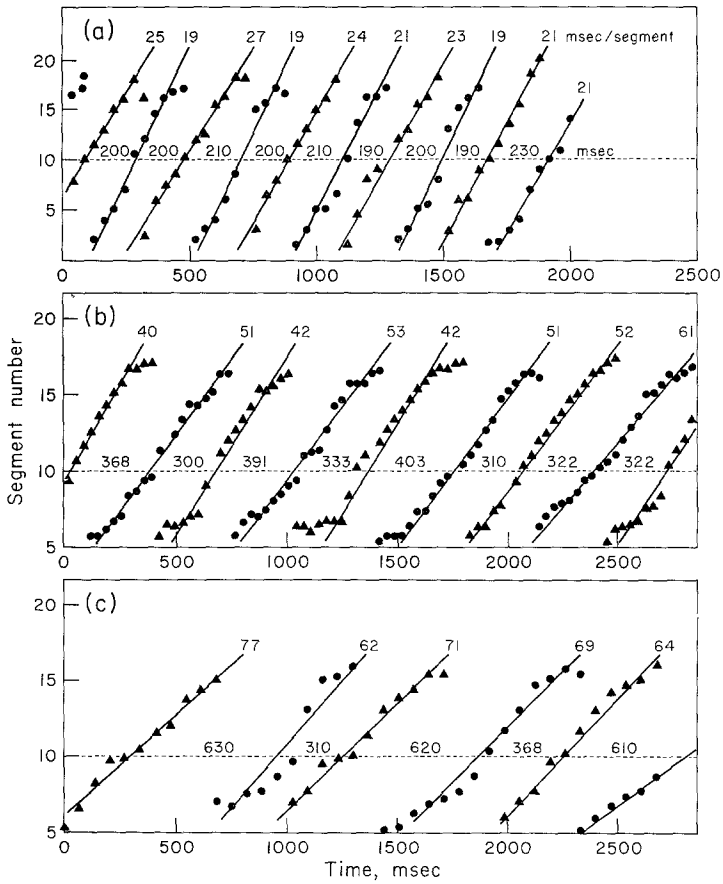

Fig. 4. Rearward progression of the body wave of swimming leeches. Each point represents the segmental position of a wave trough (circle) or crest (triangle) on the outline drawn from an individual frame of the cinematographic record. The abscissa indicates the time during the swim at which each frame was exposed. The numbers written above the dashed horizontal lines indicate the elapsed time in msec from trough to crest, or from crest to trough, at the 10th segment. The numbers written over the top of individual progression lines indicate the reciprocal slope of the line, or the intersegmental travel time in msec/segment of individual troughs or crests. Panel a: Fast swim of an intact, free swimming specimen. Panel b: Intermediate speed swim of a brainless, suspended specimen. Panel c: Slow swim of a brainless, suspended specimen

falling in the range of 390 to 420 msec. Measurement of the slope of the lines of progression yielded an average intersegmental travel time of 22 msec/segment, with individual values lying in the range of 19 to 27 msec/segment.

It is to be noted in the plot of Fig. 4a that the lines of progression of crests and troughs generally end before the 18th segment and do not reach the 21st segment bordering the posterior sucker. This reflects the fact that the body wave is not propagated all the way to the tail of the animal. Instead of undulating, the tail pitches and furnishes a long descending or ascending paddle for the alternating crests and troughs of the posterior half wave.

The numerical relations abstracted from the data of Fig. 4a thus confirm the inference that the swimming leech body maintains one full wave. Under an

average intersegmental travel time of 22 msec/segment, it takes $22 \times 18 = 396$ msec for the wave to travel from the head to the 18th segment, which time is very close to the observed average cycle period of 400 msec. Thus a new wave is initiated rostrally just when the previous wave has reached its caudal destination.

The swimming episode presented in Fig. 4a occurred at a water temperature of 20° C. Cinematographic records of swimming movements were obtained also at a water temperature of 15° C and analyzed in the same manner as the data presented in Fig. 4a. These analyses showed an average cycle period of 640 msec and an average intersegmental travel time of 43 msec/segment. Not unexpectedly the period of the swim cycle is longer and the body wave travels more slowly at 15° C than at 20° C. In addition to having a longer period and traveling more slowly, however, the wave also travels less far along the body at the lower temperature, the lines of progression of troughs and crests generally ending near the 16th segment. The net result of these numerical relations is that the leech maintains also one full body wave during its slower swim at 15° C, since under an intersegmental travel time of 43 msec/segment, it takes $43 \times 16 = 688$ msec for the wave to travel from the head to the 16th segment, a time close to the observed average wave period of 650 msec.

Since most of the work to be reported in the papers of this series was carried out with preparations whose brains had been surgically disconnected from the ventral cord, cinematographic records were taken also of swimming episodes of such animals. These brainless preparations, furthermore, were not filmed during free swims, but while suspended in the water from thin threads attached to the head and the tail. The advantage of observing suspended rather than free-swimming animals is that episodes with very long cycle periods can be recorded. (Free-swimming leeches sink to the bottom if the wave periods become too long because the infrequent undulations provide insufficient positive lift.) The results of analyses of cinematographic records of the swimming movements at 20° C of a suspended brainless preparation are presented in Fig. 4b and 4c. The data shown in Fig. 4b pertain to a swimming episode characterized by an average cycle period of 675 msec and an average intersegmental travel time of 48 msec/segment. As can be seen, the basic features of the swimming rhythm of the suspended, brainless preparation resemble those of the free swim of the intact animal. Here too the wave is propagated at a more or less constant speed until it dies out somewhere between the 16th and 18th segment; furthermore cycle period and intersegmental travel time are matched so that at any moment the body carries one full wave. The data shown in Fig. 4c pertain to an even slower swimming episode. In this episode the average cycle period extended to 950 msec and the average intersegmental travel time to 70 msec/segment.

It is to be noted that slow swimming has an asymmetric feature that is not evident in fast swimming. In the fast, free swim of Fig. 4a the lines of progression of crests and of troughs are evenly spaced. Thus here the contraction of the ventral longitudinal musculature of any segment seems to occur just midway between two successive contractions of the dorsal musculature. But in the slow swim of Fig. 4c, the lines of progression are not evenly spaced, the intervals between a trough and the next crest being much shorter than the intervals between a crest and the next trough. During slow swims, therefore, the ventral musculature

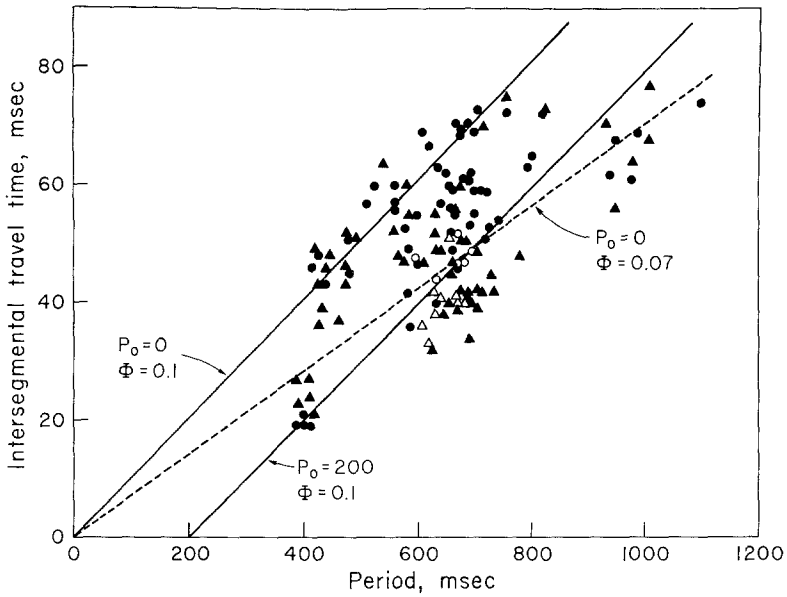

Fig. 5. The relation of intersegmental travel time to cycle period abstracted from cinematographic records. Each point represents an intersegmental travel time of a trough (circles) or a crest (triangle), as determined from the reciprocal slope of individual progression lines on plots such as those shown in Fig. 4. The corresponding cycle period is the sum of the time spans separating the progression line from its immediately preceding and succeeding progression lines. Closed symbols: observations made at 20° C. Open symbols: observations made at 15° C. The straight lines are drawn for different parametric values of the function $t = \Phi (P - P_0)$

contracts after much less than half the cycle has elapsed since the last dorsal contraction. This asymmetric feature of the swimming rhythm will be considered in more detail in the Discussion.

The analyses presented in Fig. 4 show that there exists a positive correlation between the cycle period and the intersegmental travel time. In order to gain more data on the nature of this correlation, intersegmental travel times have been measured on cinematographic records of a large number of individual swimming cycles over a broad range of periods of a brainless, suspended preparation at 20° C. The results of these measurements are presented in Fig. 5, where the data derived from Fig. 4 have been replotted (as well as the data pertaining to the free swim of an intact animal at 15° C). As can be seen, within the 390 to 1100 msec range of cycle periods observed here, the intersegmental travel time increases from 19 to 77 msec/segment. However, there is so great a scatter of the points on this plot that it is difficult to infer the general function that relates the intersegmental travel time t to the period P. But if that function were linear, then it would have the form

$$t = \Phi (P - P_0) \tag{1}$$

where Φ is a constant having a value between 0.06 and 0.10 per segment, and P_0 is a constant having a value between 0 to 200 msec. The significance of Eq. (1), particularly of the constants Φ and P_0, will be considered in the Discussion.

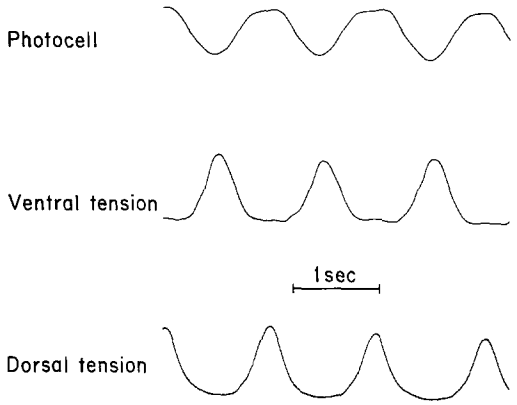

Fig. 6. Contractile rhythm of the ventral and dorsal body wall of the same segment during a swimming episode of a partially restrained, brainless leech. Top trace: output of photocell registering up and down movements of the head. Middle and lower trace: outputs of isometric tension transducers recording the contraction (upward deflection) of the right-hand ventral and dorsal body wall respectively of the 9th segment

Body Wall Tension

Uexküll's inference that the troughs and crests of the body wave derive from antiphasic contractions and relaxations of dorsal and ventral longitudinal muscles of individual segments was based on rather indirect observations of the behavior of mutilated leeches. In order to confirm this inference, and also to observe directly the phasic muscle activity during swimming, longitudinal tension records were obtained from a functionally isolated segment of the body wall of a restrained, brainless denervated leech.

Intrasegmental Dorsal-Ventral Tension Delay. The results of one set of such tension measurements of the dorsal and ventral body wall of the 9th body segment are shown in Fig. 6. The time elapsed between two maxima of the photocell output, which monitors the up and down movements of the head, indicates the period of the swim cycle. In the swimming episode represented by this record, the average period was 960 msec. The outputs of two tension transducers, one connected to the ventral and the other to the dorsal body wall, show that the segmental ventral and dorsal longitudinal muscles undergo contraction cycles whose period matches exactly that of the up and down movements of the head. Furthermore, the ventral and dorsal cycles are locked in antiphase, in that the dorsal body wall contracts while the ventral wall distends, and vice versa.

The phase relation of dorsal and ventral contraction cycles has been investigated in more detail by measuring the delay between the half maximum tension of the dorsal and the ventral body wall of the same midbody segment for fast and slow swimming episodes, ranging in cycle period from 800 to 1500 msec. The result of these measurements is presented in Fig. 7. It can be seen that the delay between dorsal and ventral half maximum tensions increases with the period. In fact, the delay d between dorsal and ventral contractions appears to be linearly related to the period P according to an expression analogous to Eq. (1), namely

$$d = \delta (P - P_0) \tag{2}$$

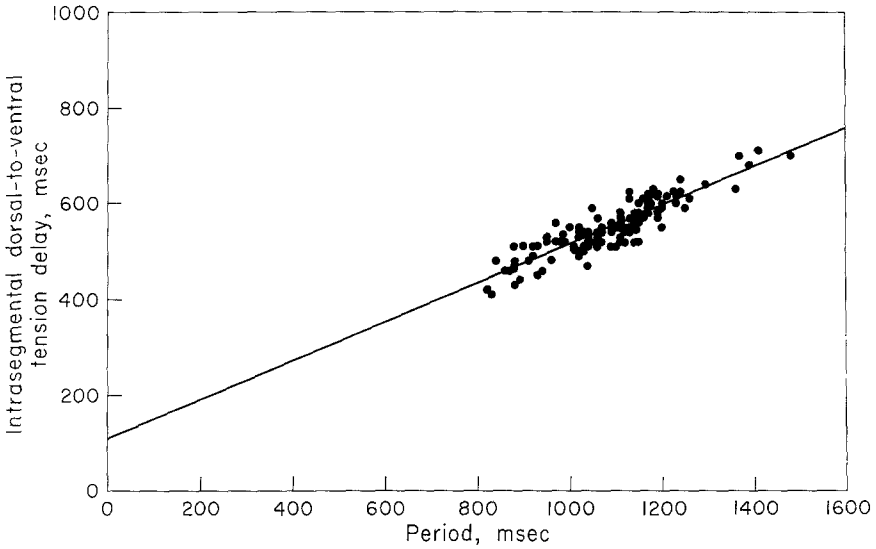

Fig. 7. Delay between the half-maximal tensions of the dorsal and the ventral body wall of the 9th segment in swims of different cycle periods. The data are abstracted from records such as those shown in Fig. 6. The straight line represents a least squares fit to the experimental points and corresponds to the function $d = \delta\,(P - P_0)$, for $P_0 = -230$ msec and $\delta = 0.46$

where δ is a dimensionless fraction and P_0 a constant having the dimension of time. The straight line drawn in Fig. 7 was fitted to the experimental points by the least-squares method and is given by the values $\delta = 0.46$ and $P_0 = -230$ msec.

It is evident that the ratio of the dorsal-ventral tension delay to the period, or d/P, is the intrasegmental *phase lag* between the contraction of the antagonistic dorsal and ventral muscles in the swimming rhythm. It follows from these parametric values of Eq. (2) that the phase lag between the contraction of the antagonistic dorsal and ventral muscles is not constant at all swimming speeds. In a fast swim, say for $P = 300$ msec, d is 250 msec, and the phase lag $250/300 = 0.83$ of the cycle. But in a slow swim, say for $P = 2000$ msec, d is 1030 msec, and the phase lag only $1030/2000 = 0.51$ of the cycle. Thus this relation confirms the inference previously drawn from the cinematographic data of Fig. 4 that the phase lag between dorsal and ventral contractions shortens with an increase in the cycle period.

Intersegmental Ventral-Ventral Tension Delay. The result of a set of tension measurements of the ventral body wall of the 9th and 12th body segments is shown in Fig. 8. The top trace again presents the output of the photocell monitor, which indicates that the average period of the swim cycle was 1100 msec. The output of two tension transducers attached to two antero-posteriorally displaced sites on the ventral body wall show that the ventral longitudinal muscles of the innervated 9th and 12th segments undergo phase-locked contraction cycles, the ventral half-maximum tension of the 12th segment following that of the 9th segment after a delay of about 200 msec. Thus we may reckon an intersegmental travel time of the ventral tension of about $200/3 = 66$ msec/segment. This value is in good agreement with the intersegmental travel time of about 70 msec/segment

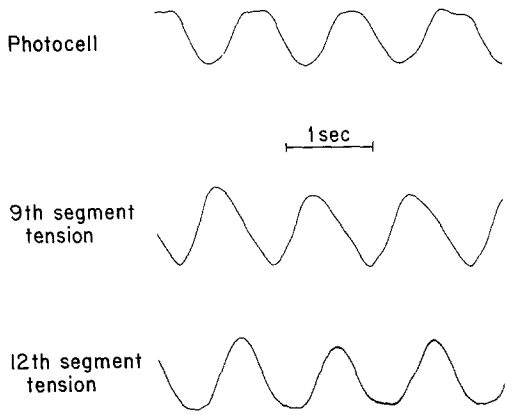

Fig. 8. Contractile rhythm of the ventral body wall of two different segments during a swimming episode of a partially restrained, brainless leech. Top trace: output of photocell registering up and down movements of the head. Middle and lower trace: outputs of isometric tension transducers recording the contraction (upward deflection) of the right-hand ventral body wall of the 9th and 12th segment respectively

for body wave crests estimated from the cinematographic data of Fig. 5 for a slow swim of comparable period.

The phase relation of the contraction in successive segments has been investigated in more detail by measuring the delay between the half-maximum tension of the ventral body wall of the 9th and 12th segment for fast and slow swimming episodes, ranging in cycle periods from 750 to 1350 msec. The result of these measurements is presented in Fig. 9. In agreement with the cinematographic data, it can be seen that the intersegmental travel time t increases with the cycle period. In particular, t appears to be related to the cycle period P via Eq. (1), a least-squares fit of the experimental points yielding a straight line with the values $\Phi = 0.08$ and $P_0 = 280$ msec.

Thus the general agreement of the dynamic parameters of the body wave inferred from these tension measurements with those abstracted from the analysis of cinematographic records indicates that a contractile rhythm of the segmental longitudinal musculature is indeed responsible for the swimming movement.

Open Body

The preparation used in the preceding body wall tension measurements was modified in order to examine the extent to which the general tonus maintained during swimming through pressure exerted by the musculature on the body fluids might play an essential role in the contractile rhythm of the segmental body wall. For this purpose, the entire body wall and viscera of the left half of the 8th, 9th and 10th segment of the animal previously used for the experiment of Figs. 6, 7, 8 and 9 were cut away. The right half of the body wall of these same segments and the nerve cord were left intact. Despite the gaping hole in its midbody, the intact front and back parts of this preparation still carry out periodic coordinated swimming movements. Furthermore, measurement of the

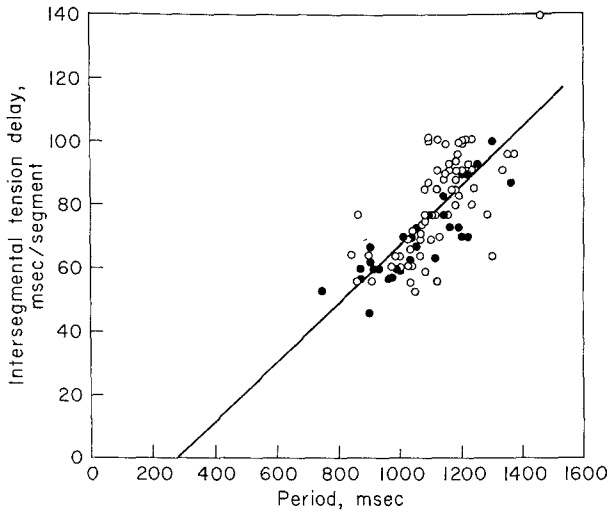

Fig. 9. Delay between (or intersegmental travel time of) the half maximal tension of the ventral body wall of the 9th and 12th segment in swims of different cycle periods. (The ordinate presents the delay in msec/segment, or one third of the actually observed delay between 9th and 12th segments.) The data are abstracted from records such as those shown in Fig. 8. Open and closed points represent data from different swimming episodes of the same preparation. The straight line represents a least squares fit to the experimental points and corresponds to the function $t = \Phi (P - P_0)$ for $P_0 = 280$ msec and $\Phi = 0.08$

tension developed by the dorsal and ventral longitudinal musculature of the innervated remaining right body wall of the 9th segment still shows an antiphasic contraction cycle whose period matches that of the swimming wave (Fig. 10).

However, when the dependence of the phase relation of dorsal and ventral contraction cycles on the cycle period is investigated in more detail in this open-body preparation, the result shown in Fig. 11 is found. It is evident that although the intrasegmental dorsal to ventral tension delay d still increases linearly with the period P, according to Eq. (2), opening the body produces an important change in the values of the parameters δ and P_0 relating d to P in that equation. Whereas the corresponding data presented in Fig. 7 for the same animal prior to opening the body are best described by the values $\delta = 0.46$ and $P_0 = -230$ msec, a least-squares fit of the data of Fig. 11 yields $\delta = 0.72$ and $P_0 = 280$ msec. This change in the values of δ and P_0 means that whereas in the intact animal the intrasegmental phase lag between dorsal and ventral contractions *shortens* with an increase in the period, in the residual hemilateral body wall of an open segment the dorsal to ventral phase lag *lengthens* with an increase in the period. It follows, therefore, that although a general tonus is not needed locally for the maintenance of a contractile rhythm with a period matching that of the swimming wave in the rest of the animal, the phase relation between dorsal and ventral contractions does seem to depend on the integrity of the segmental body. As will be shown in the third paper of this series (Kristan *et al.*, 1974), the lengthening of the dorsal to ventral phase lag with increasing cycle period in an open-body preparation is found not only for the phasic contractions of the body wall but also for the

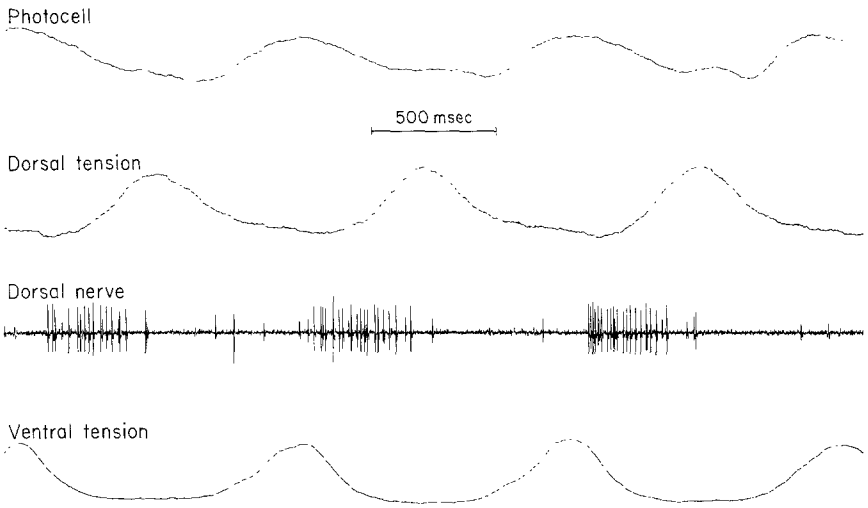

Fig. 10. Contractile rhythm and segmental nerve activity during a swimming episode of an open body preparation. Traces labeled "photocell", "dorsal tension" and "ventral tension" are as in Fig. 6. The trace labeled "dorsal nerve" presents the output of a suction electrode attached to the left dorsal nerve of the 9th segment

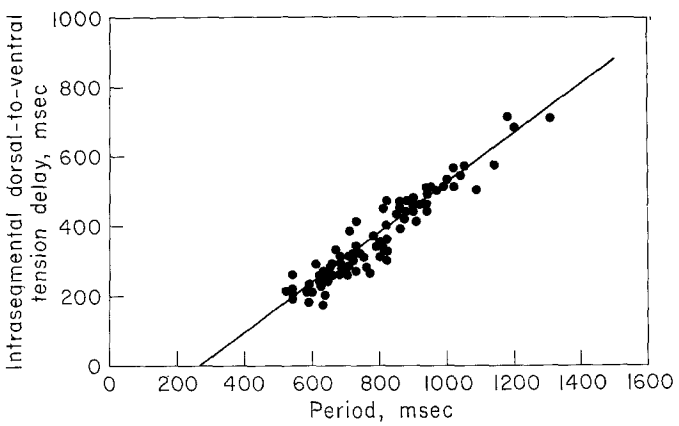

Fig. 11. Delay between the half-maximal tensions of the dorsal and the ventral body wall of the 9th segment in swims of different cycle periods of an open body preparation. The straight line represents a least squares fit to the experimental points and corresponds to the function $d = \delta\,(P - P_0)$ for $P_0 = 280$ msec and $\delta = 0.72$

rhythmic activity of the motor neurons which drive the two sets of longitudinal muscles.

The open body preparation makes it possible also to record the impulse activity in the segmental nerves of a swimming leech. To this end, the segmental nerves issuing from the open left side of the 9th ganglion are carefully dissected and freed from connective tissue before cutting away the wall and viscera of the left body half of the 8th, 9th and 10th segments. Suction electrodes are then

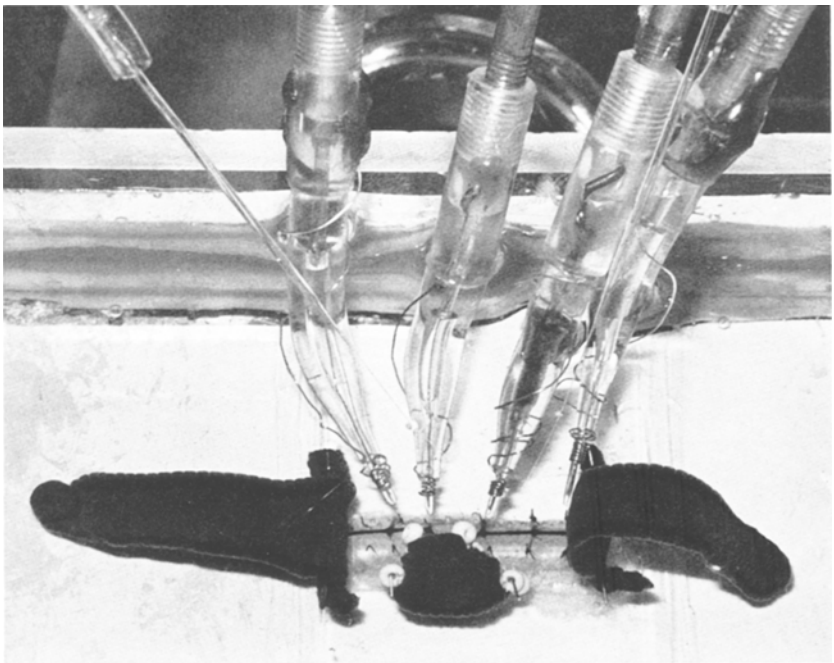

Fig. 12. The semi-intact preparation pinned venter upwards. Fore (right) and hind (left) parts are connected by the exposed ventral nerve cord and its segmental ganglia, of which four are visible. (To improve visibility of the cord for this photograph the ventral blood sinus enclosing the cord has not been removed; the blood sinus must be removed from the ganglia if intracellular recording is to be done). The isolated body wall flap with sewn-on beads for attachment of threads leading to tension transducers is still connected via the left segmental nerves to one of the exposed ganglia. Four glass-tipped suction electrodes for extracellular nerve trunk recordings and two glass capillary microelectrodes for intracellular membrane potential recordings are shown

attached to the dissected segmental nerves. Fig. 10 presents the output of an electrode attached to the left dorsal nerve of the 9th segment. [The branching pattern of the segmental nerves will be described in the second paper of this series (Ort *et al.*, 1974)]. Fig. 10 shows that there occur bursts of spikes of uniform amplitude whose period matches exactly that of the head movements recorded by the photocell and of the antiphasic contractions of the dorsal and ventral longitudinal muscles. It is to be noted that these rhythmic spike bursts occur while the dorsal longitudinal muscles develop their tension and while ventral longitudinal muscles distend. As will be shown in the following paper (Ort *et al.*, 1974), these spike bursts in the dorsal nerve represent impulses carried by the axon of a motor neuron innervating the dorsal longitudinal muscles. It is apparent that the tension developed by the muscle lags behind the impulse activity of its motor neuron by about 100 msec, as estimated from the delay between the spike burst midpoint and the attainment of half-maximal tension. The relative timing revealed by this experiment between the spike bursts of this motor neuron in the

dorsal nerve and the contraction of the longitudinal musculature will be used in the work to be reported in the following papers of this series to identify the phase relation to the swimming cycle of the activity of each member of the ensemble of neurons of the segmental ganglion that is rhythmically active during the swimming movement.

The Semi-intact Preparation

Gray, Lissman and Pumphrey's (1938) leech preparation from which the entire body of several midbody segments has been removed and which consists of intact, swimming front and back parts of the animal connected only by the exposed ventral cord offers a means for probing in more detail the nervous control of the swimming rhythm. With this semi-intact preparation neuronal activity can be recorded, not only extracellularly as single unit spikes in the segmental nerves as in the open body preparation, but also intracellularly as variations in transmembrane potential of identified nerve cell bodies of the exposed segmental ganglia while swimming movements are in progress (Fig. 12).

Fig. 13 presents a sample of both intracellular and extracellular recordings taken from such a semi-intact preparation. The extracellular recording is from the dorsal nerve of the exposed 10th segment, and the intracellular recording is from the cell body of a neuron lying on the dorsal aspect of the 10th ganglion. This neuron is designated as cell 3, according to the nomenclature to be presented in the second paper. While the preparation is at rest (panel A) intracellular and extracellular records show a tonic impulse activity. Moreover, it is evident that the action potentials recorded intracellularly from cell 3 match one-for-one with constant delay the large spikes recorded extracellularly from the dorsal nerve. This correspondence, together with other criteria, will be used in the second paper to establish that the spikes recorded from the dorsal nerve represent impulses carried by the axon of cell 3.

The amplitude of the action potentials recorded from the cell body of cell 3 is quite small, being at most 3 or 4 mV, and the action potential time course is quite long, being about 10 to 12 msec, similar to earlier findings on leech motor neurons (Stuart, 1970). The intracellularly recorded action potential can be seen to precede by a few msec the matching spike recorded in the segmental nerve, the actual lead time observed depending on the point of placement of the extracellular electrode. This indicates that the impulse traffic is efferent. The intracellular records show not only action potentials but also synaptic potentials which reflect both excitatory and inhibitory inputs to cell 3. These synaptic potentials have amplitudes up to 3 mV and durations of 10 to 20 msec, and are therefore distinguishable from action potentials by their somewhat smaller amplitude and slower rise time. That the recorded amplitude of the synaptic potentials is nearly as great as that of the action potentials must mean, however, that the sites of synaptic input to these cells are closer to the cell body than is the axonal point proximal to which active impulse conduction is blocked (Stuart, 1970).

While the preparation is swimming (panel B) a rhythmic impulse activity of cell 3 is observed. The extracellular record shows the periodic spike bursts in the dorsal nerve previously seen in Fig. 10 and the intracellular record shows slow oscillations in potential whose period matches that of the swimming rhythm.

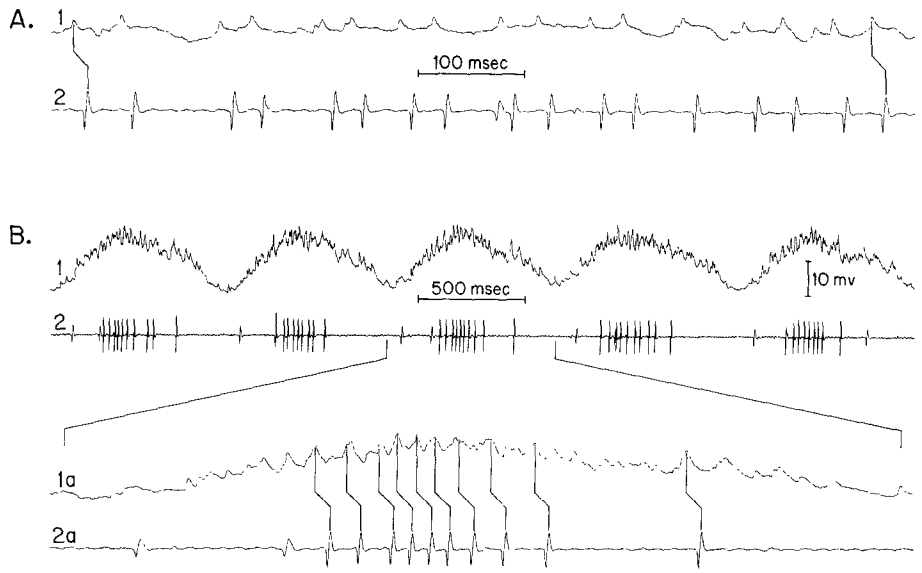

Fig. 13. Extra- and intracellular recordings taken from a semi-intact preparation. Panel A. Preparation at rest. Trace 1: output of microelectrode inserted into left cell 3 of the 10th segmental ganglion. Trace 2: output of suction electrode attached to right dorsal nerve of the 10th segment. Action potentials in intracellular record of trace 1 match one-for-one spikes in extracellular record of trace 2. Panel B. Preparation swimming. Traces 1 and 2 as in panel A. Intracellular record of trace 1 shows rhythmic variations in membrane potential with action potentials occurring during depolarized phase. Extracellular record of trace 2 shows cell 3 spike bursts in dorsal nerve during depolarized phase. Traces 1a and 2a are five-fold time expansions of center segments of traces 1 and 2, showing match of intra- and extracellularly recorded impulses during swimming. Vertical voltage marker applies to all three microelectrode output traces

During the depolarized phase of these potential oscillations there occur action potentials which are, however, difficult to identify as such because of the concurrent abundance of synaptic potentials. But if the records are compared on an expanded time scale, every large extracellularly recorded spike in the dorsal nerve can be seen to be preceded with a fixed lead time by an action potential in the intracellular record.

It seems likely that the excitatory and inhibitory synaptic potentials arising in cell 3 are responsible for its rhythmic activity pattern. Unfortunately, during swimming these potentials are so frequent that it is difficult to resolve them as single events.

Discussion

The problem of the neuronal control of the swimming rhythm may be restated in the form of two basic questions. (1) How do the motor neurons innervating dorsal and ventral longitudinal muscles of any one segment oscillate antiphasically between activity and inactivity with a period of the order of 1000 msec? (2) How is the oscillatory activity of the antiphasic motor neurons of a given ganglion

coordinated with that of the other ganglia of the cord, so that its cycle has an appropriate phase lag relative to the cycle of the corresponding motor neurons of the next anterior ganglion ?

With regard to the first question, some general properties of the control system can now be specified. First, it can be inferred that the contractile rhythm is not governed by the output of a unique, central nervous oscillator that serves all ganglia of the cord, since section of the cord in any one of various segments produces two half-animals each capable of producing an independent swimming rhythm. Hence, there must exist a number of oscillators distributed over the cord. In view of the stereotyped character of the segments, it seems most likely that each segment contains its own oscillator. To produce the coordinated swimming wave, these distributed oscillators must be coupled so as to lock their cycles into an appropriate phase relation. Second, it appears that the period of the oscillators can vary over a five-fold range, as indicated by the observed range of cycle periods from 400 to 2000 msec. Third, it can be inferred from the dependence of the intrasegmental dorsal-ventral tension delay on the cycle period expressed by Eq. (2) that the swim cycle of the segmental oscillators appears to consist of two distinct sectors. The time required for completion of one of these sectors is constant and does not vary as the period of the swim cycle is shortened or lengthened. The time required for completion of the other sector is variable, and it is by reducing or increasing the time spent on the variable sector that the period of the swim cycle is shortened or lengthened. Such a division of the cycle period into constant and variable time sectors is a characteristic feature of the class of oscillators called *relaxation oscillators* (Wever, 1965).

In order to state this inference analytically, we may consider an oscillator which requires the variable time T for completing one part of its cycle and the constant time C for the completion of the remainder of the cycle. The period of this oscillator is therefore

$$P = T + C. \tag{3}$$

Let δ and k be the fractions of the variable and constant time sectors of the cycle respectively which the oscillator completes between two periodic events. The observed delay d between these events is

$$d = \delta T + kC. \tag{4}$$

Solving (3) for T and substituting in (4) yields

$$d = \delta(P - C) + kC. \tag{5}$$

Eq. (5) is evidently equivalent to the previously noted empirical relation

$$d = \delta(P - P_0) \tag{2}$$

provided that the identity holds

$$P_0 \equiv C(1 - k/\delta). \tag{6}$$

Thus the slope δ of the straight line of the empirical Eq. (2) represents the fraction of the variable time sector of the cycle which intervenes between the two events. The meaning of the constant P_0 of that equation is not intuitively obvious. However, if the part of the cycle intervening between the two events comprises none of the constant time sector (i.e. if $k = 0$), then P_0 is equal to the constant time sector C.

The data of Fig. 7 show that for the delay between half-maximal dorsal and ventral contractions in the intact animal δ has the value 0.46. In other words, about one half of the variable time sector of the cycle intervenes between these two events. We may now infer also the values of the parameters C and k. Since $T>0$, it follows from Eq. (3) that the maximum possible value of C is the minimum observed value of P, or about 400 msec (cf. Fig. 5). And according to the identity (6) the minimum possible value of C obtains if $k=0$ for positive values of P_0, and if $k=1$ for negative values of P_0. Since the data of Fig. 7 lead to the value of $P_0=-230$ msec, we may solve identity (6) for the minimum value of C

$$C=P_0/(T-k/\delta)=-230/(1-1/0.46)=200 \text{ msec}.$$

We may conclude, therefore, that the constant time sector C of the cycle of the segmental oscillator lasts no less than about 200 msec and no more than about 400 msec. The corresponding maximal and minimal values of k are 1.0 and 0.73. In other words, at least three quarters of the constant time sector of the cycle occurs between the half-maximal dorsal and ventral contractions.

Fourth, it can be inferred from the data of Fig. 11, that the parametric constants δ and k of Eq. (5) change when the body is opened. The value of δ evidently increases from 0.46 to 0.72. And the value of k decreases from the minimum of 0.73 to a maximum of 0.22, as estimated from the new value of $P_0=280$ msec by solving (6) for k and substituting the appropriate parametric values, including the previously inferred maximum value for C of 400 msec, i.e.

$$k=\delta(1-P_0/C)=0.72\ (1-280/400)=0.22.$$

In other words, it appears that opening the body shifts the occurrences of half-maximal dorsal and ventral contractions with respect to the cycle of the oscillator: nearly three fourths, rather than half, of the variable time sector and less than one-fourth, rather than three-fourths of the constant time sector intervene between dorsal and ventral contractions in an open segment of the preparation.

With regard to the second question concerning the manner of coupling of the segmental oscillators we may examine the results presented in Fig. 9. For this purpose, let us consider two homologous oscillators of the type posited for the derivation of Eq. (5), both running with the same variable period P. Let them be coupled in such a manner that the phase of one lags behind that of the other by a fraction Φ of either the variable time sector T or the constant time sector C of the cycle respectively. In these cases, the delay t between the occurrence of an event in the lead oscillator and the corresponding event in the follower oscillator is

$$t=\Phi C \tag{7}$$

if the coupling pertains to the constant time sector, and

$$t=\Phi T \tag{8}$$

if the coupling pertains to the variable time sector of the cycle. Substituting in (8) for T from (3) we obtain

$$t=\Phi(P-C). \tag{9}$$

The results of Fig. 9 indicate clearly that the intersegmental delay of half-maximal ventral contraction does not match the statement of Eq. (7) that the

delay is independent of the period. Instead, the delay can be seen to vary with the period according to Eq. (1), which is formally equivalent to Eq. (9), provided that

$$P_0 \equiv C. \tag{6'}$$

That is to say, the interganglionic coupling of the oscillators appears to be such that they run with a fixed phase lag during the variable time sector of their cycle. The fact that the data of Fig. 9 give rise to the values $\Phi = 0.08$ and $P_0 = 280$ msec indicates, in the light of Eq. (9) and (6'), that during the variable time sector of their cycle the phase of the oscillators of successive ganglia lags by a fraction 0.08 of the cycle and that the constant time sector of their cycle lasts about 280 msec. This latter estimate is thus in good agreement with the previous, and quite independent, estimate of C made on the basis of the observed *intra*ganglionic delays of Fig. 7 and 11 that the invariant time sector of the segmental oscillator is in the range of 200 to 400 msec. The mechanism by which the segmental oscillators are coupled to make them run with the same period and with the same phase lag per segment in the anteroposterior direction is likely to involve inhibition and excitation communicated along one or more "coordinating fibers" (Stein, 1971) of the cord.

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