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RHYTHMIC SWIMMING ACTIVITY IN NEURONES OF THE ISOLATED NERVE CORD OF THE LEECH

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SUMMARY

1. Repeating bursts of motor neurone impulses have been recorded from the nerves of completely isolated nerve cords of the medicinal leech. The salient features of this burst rhythm are similar to those obtained in the semi-intact preparation during swimming. Hence the basic swimming rhythm is generated by a central oscillator.

2. Quantitative comparisons between the impulse patterns obtained from the isolated nerve cord and those obtained from a semi-intact preparation show that the variation in both dorsal to ventral motor neurone phasing and burst duration with swim cycle period differ in these two preparations.

3. The increase of intersegmental delay with period, which is a prominent feature of swimming behaviour of the intact animal, is not seen in either the semi-intact or isolated cord preparations.

4. In the semi-intact preparation, stretching the body wall or depolarizing an inhibitory motor neurone changes the burst duration of excitatory motor neurones in the same segment. In the isolated nerve cord, these manipulations also change the period of the swim cycle in the entire cord.

5. These comparisons suggest that sensory input stabilizes the centrally generated swimming rhythm, determines the phasing of the bursts of impulses from dorsal and ventral motor neurones, and matches the intersegmental delay to the cycle period so as to maintain a constant body shape at all rates of swimming.

INTRODUCTION

The swimming movement of the leech, *Hirudo medicinalis*, consists of a wave of rearward moving crests and troughs of the flattened body. These crests and troughs are produced by phasic contractions of sheets of longitudinal muscles which are embedded in the ventral and dorsal segmental body wall, respectively. The period of the segmental contraction cycle (which can vary over a 4-fold range for fast or slow swims) is of the order of 1 s, and the rearward movement of the crests and troughs is the consequence of a rostro-caudal phase delay of the contractile cycles of successive segments. The neuronal control of the swimming movement has been previously

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studied by means of a *semi-intact* leech preparation in which the ventral nerve cord of several mid-body segments was exposed by opening the body and removing the viscera and in which the cerebral and caudal fused ganglia were severed from the cord. The remaining, intact, segments of this preparation still produce the body wave, thus making it possible to take electrophysiological records from the exposed and immobilized parts of the peripheral and central nervous system of the leech while swimming movements are underway (Kristan, Stent & Ort, 1974*a*). A swimming episode of the semi-intact preparation can be evoked by gently stroking the dorsal skin of the rear intact body rump or by delivering electrical stimuli to an exposed segmental nerve. The ensuing episode may consist of from three swim cycles to several hundred swim cycles. The swim cycle periods of semi-intact preparations range from 400 to 2000 ms. Usually the period is shortest immediately after the onset of swimming and then progressively lengthens throughout the episode.

Studies with the semi-intact preparation have shown that the contractile rhythm of the dorsal and ventral longitudinal muscles is generated by an ensemble of rhythmically active motor neurones, of which homologues are situated in each of the segmental ganglia of the ventral cord. This motor neurone ensemble consists of *dorsal* or *ventral excitors* and *dorsal* or *ventral unhibitors*. The excitors cause contraction of the longitudinal muscles in dorsal or ventral territories of the body wall via direct excitatory synaptic input. The inhibitors cause relaxation of the longitudinal muscles in corresponding body wall territories by inhibitory synaptic inputs both to the muscle fibres at the periphery and to the homonymous excitors within the segmental ganglion. Thus during the trough or 'dorsal' phase of the segmental swim cycle, an impulse burst of each of four dorsal excitors contracts the dorsal body wall longitudinally while an impulse burst in at least one ventral inhibitor relaxes the ventral body wall and inhibits the activity of the ventral excitors. During the crest, or 'ventral' phase of the cycle, an impulse burst in each of three ventral excitors contracts the ventral body wall longitudinally, while an impulse burst in each of two dorsal inhibitors relaxes the dorsal body wall and inhibits the activity of the dorsal excitors (Ort, Kristan & Stent, 1974).

The problem of the neuronal control of the swimming movement has thus been reduced to accounting for the source of the phase-locked activity rhythm of the segmental excitors and inhibitors. One possible source of that rhythm was suggested by the findings that the peripheral feedback has a profound effect on the realization of the swimming movement of the intact leech and that there exist segmental sensory-motor reflexes which oppose dorsal or ventral body wall stretch by activating (or disinhibiting) the corresponding dorsal or ventral excitors (Kristan, 1974; Kristan & Stent, 1976). Accordingly, it could be envisaged that the activity rhythm is generated by oscillatory reflex loops between peripheral stretch detectors and the motor neurones (Kristan, 1974). The results to be presented here, however, demonstrate that an impulse burst rhythm of excitors and inhibitors similar to that observed in the swimming semi-intact preparation can occur also in an isolated leech nerve cord deprived of all sensory input from the periphery. Hence, the phasic neuronal activity responsible for the body wave is generated by a *central oscillator* (i.e. by neuronal elements of the central nervous system which do not require sensory input for their rhythm). Thus leech swimming resembles other rhythmic motor acts of invertebrates whose

underlying neuronal activity pattern is similarly of central origin (Kandel & Kupfermann, 1970).

MATERIALS AND METHODS

Leeches, *Hirudo medicinalis*, were obtained from a French distributor. They were kept in glass aquaria partially filled with commercially obtained spring water at 15 °C. They were fed with a bullfrog about once a month. Under these conditions they can be maintained in the laboratory for over 3 months with little or no deterioration in their behaviour.

The nerve cord was isolated from a leech by a dissection procedure similar to that described previously for preparing the midbody ganglia of the semi-intact preparation (Kristan *et al.* 1974*a*), except that the entire nerve cord was exposed and freed from the body wall. The segmental nerves of five to ten ganglia were prepared for extracellular recording and electrical stimulation. The preparation was pinned to the bottom of a chamber that provides for dark field illumination of three adjacent ganglia. The chamber was filled with leech physiological saline (Nicholls & Purves, 1970).

The electrophysiological methods of recording and stimulating neuronal activity, the procedure of assigning spikes recorded extracellularly from segmental nerves to individual indentified motor neurones, and the nomenclature used to identify individual neurones and segmental nerves are as previously described (Kristan *et al.* 1974*a*; Ort *et al.* 1974).

In some preparations, intracellular recording was made difficult by spontaneous contractions of the muscles embedded in the connective tissue surrounding the ganglia and interganglionic connectives. This difficulty can sometimes be overcome by exposure of the preparation for 5 min to leech physiological saline in which 20–40 mM-$MgCl_2$ replaces an osmotically equivalent amount of NaCl. Within 10 min of the return of the preparation to normal saline, the motor neurone activity of the isolated cord usually resumes its normal pattern without resumption of the spontaneous contractions. Moreover, it is often easier to evoke the swimming rhythm in the isolated preparation after this pre-treatment.

To provide a quantitative description of the neuronal activity rhythm, five parameters of the timing of motor neurone impulse bursts were measured. The *burst midpoint* is the time of occurrence of the middle spike of an odd number of spikes in the burst or the time half-way between the occurrence of the two middle spikes of an even number of spikes in the burst. The *period* is the interval between the midpoints of two successive bursts of the same cell. Since the bursts of the dorsal excitor, cell 3, are most easily recorded, the period is usually, though not always, calculated from the records of that cell. The *dorsal to ventral delay* is the interval between the midpoints of successive bursts of a dorsal excitor (usually cell 3) and of a ventral excitor (usually cell 108). The *burst duration* is the interval between the first and the last spike in a burst. The *intersegmental delay* is the interval between the midpoint of a burst of an excitor and the midpoint of the corresponding burst of a homologous excitor in a more posterior ganglion. Although the delay measurements were usually made between homologous excitors four or more segments apart, the results are always expressed as the average delay per segment.

To abstract these parameters from the raw records, the data were digitized, either by

measuring manually the time of occurrence of individual spikes on the chart recorder transcripts to the nearest 10 ms or by play-back of the tape-recorded data through trigger circuits and registering the time of occurrence of trigger pulses evoked by individual spikes to the nearest 1 ms. The digitized data were then processed further in a PDP-11 computer, by means of a program designed to compute the slope (α), parameter axis intercept (π_0) and period axis intercept (P_0) of the linear regression line relating each parameter (π) to period (P) by a least-square fit of the data points to the equation

$$\pi = \alpha P + \pi_0. \tag{1}$$

However, it was often more convenient to express the relationship in the form

$$\pi = \alpha (P - P_0), \tag{2}$$

where

$$P_0 = -\pi_0/\alpha.$$

The program computed also the variance of the points about the regression line and the confidence limits to any given criterion level.

To interpret the observed nature of the dependence of these parameters on the period, according to equation (2), it was essential to ascertain the statistical significance of the finding that a given regression line does or does not pass through the origin, i.e. to establish whether P_0 is or is not significantly different from zero when $\pi = 0$. For this purpose the origin was excluded as a possible point of the relation of equation (2) if it lies outside the 99% confidence limits for the linear regression line; if the origin lies inside the narrower 90% confidence limits, it was not excluded as a possible point of the relation of equation (2).

RESULTS

(1) *Swimming rhythm recorded from motor neurones in the isolated nerve cord*

Delivery of a brief train of electrical stimuli to one or two segmental nerves of an isolated leech nerve cord preparation can elicit a prolonged episode of rhythmic motor neurone activity (Fig. 1). As shown by the upper three traces of Fig. 1A the stimulus train evoked an initial short tonic discharge of the ventral excitors which, after about 1·5 s, gave way to an alternating impulse burst rhythm of dorsal and ventral excitors whose period was slightly more than 1 s. This motor neurone impulse burst rhythm closely resembles that previously observed in recordings taken from the exposed part of the nerve cord of the swimming semi-intact leech preparation (Kristan, Stent & Ort, 1974*b*). In particular, such features of the rhythm as the period, the duration and average interspike interval of the bursts, the antiphasic relation of dorsal and ventral excitor bursts, and the front to back intersegmental delay between bursts of homologous excitors are virtually the same for the isolated cord and the semi-intact preparation. Furthermore, the bottom trace in Fig. 1A shows that the membrane potential oscillations of a dorsal excitor (cell 7) also resemble those seen in intracellular recordings taken from the same cell in a semi-intact preparation (cf. fig. 7 in Ort *et al.* 1974).

The records presented in Fig. 1B show a more prolonged interval between the stimulus and the onset of the impulse burst rhythm than do the records in Fig. 1A;

but once the rhythm had started the impulse burst pattern was the same. The intracellular records taken from the dorsal inhibitor, cell 1, also show the membrane potential oscillations typical of the swimming rhythm. It is to be noted that the electrical stimuli which set off the swimming episode caused an initial *de*polarization of the dorsal excitor (cell 7) (Fig. 1 A), and an initial *hyper*polarization of the dorsal inhibitor (Fig. 1 B). Although both episodes of the swimming rhythm depicted in Fig. 1 start with bursts in ventral excitor, episodes started with bursts in dorsal excitor with equal frequency.

Nerve cord-wide episodes of the swimming activity rhythm can be initiated by brief electrical stimulation of any of the major branches of the segmental nerves or of any site of the interganglionic connective. To elicit a swimming episode, the intensity of the individual stimulus pulses must be sufficient to exert a maximal excitatory effect (a train of stimuli at 10–20 Hz for 0·5–1 s is usually necessary) and stimulation of two nerves in the same suction electrode is more effective than stimulation of a single nerve. Stimulation of the dorsal branch of the posterior nerve produces this rhythm most consistently, particularly if applied to middle or posterior segments. This finding accords with the observation (which we have been able to confirm) that mild electrical stimuli applied to the dorso-caudal body wall, or gentle stroking of the wall, is a highly effective stimulus for initiating swimming or increasing the swimming rate in the intact leech (Gray, Lissman & Pumphrey, 1938).

To ascertain the minimum length of the isolated nerve cord required to produce the swimming activity rhythms, the cord was progressively shortened, by cutting single ganglia from either end while recording from a midbody ganglion and stimulating nerves in an adjacent ganglion. It was found that, after each cut, a given stimulus train was less effective than before in first causing tonic excitor impulse activity and then initiating the swimming activity rhythm (i.e. the total stimulus required to initiate the rhythm increased as fewer ganglia remained connected). Once the rhythm had been initiated, however, its features were relatively independent of the length of the cord. When the number of ganglia fell below some minimum (usually 8–12 depending on the preparation) the swimming activity rhythm could no longer be initiated, no matter now intensely the nerves were stimulated.

Thus, it can be concluded that the ventral nerve cord of the leech contains a *central swimming oscillator* which can generate the basic impulse burst rhythm of the segmental motor neurones responsible for the contractile rhythm of the segmental body wall, without afference from the periphery. This central oscillator does not, however, appear to be capable of function in isolated single ganglia and requires an intact, intersegmental chain of several ganglia for producing its characteristic activity rhythm. However, if the nerve cord is left innervated the minimum number of ganglia necessary to produce the swimming rhythm is reduced to three. This suggests that peripheral receptors may provide some tonic excitatory drive to the central oscillator which is not necessary if the ganglionic chain is long enough.

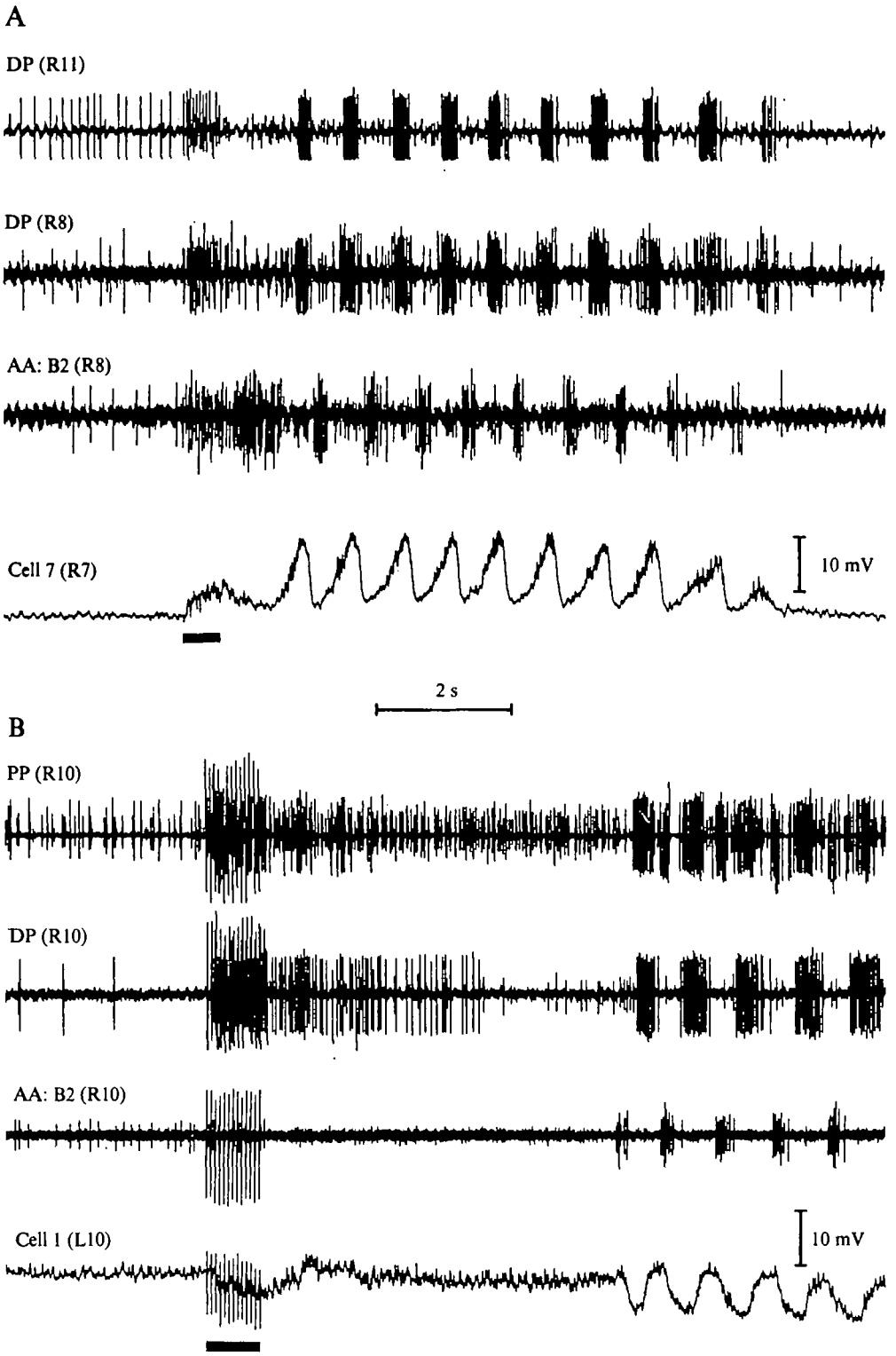

Fig. 1. For legend see opposite

(2) *Comparison of motor neurone activity in the semi-intact and isolated cord preparations*

To provide a quantitative comparison of the swimming rhythm of the isolated cord preparation with that of the semi-intact preparation, comparisons were made of various parameters characterizing the impulse burst patterns in the two types of preparation. Previous analyses of records obtained from semi-intact preparations had indicated that the delay between the occurrence of successive dorsal and ventral excitor bursts (i.e. the dorsal to ventral delay), the excitor burst duration and the average interspike interval during the bursts all increase as the period of the swim cycle increases. The manner in which each of these parameters increases with period led to the conclusion that the duty cycle of the oscillator which drives the motor neurones into their activity rhythm consists of two parts: a variable time sector that rises in proportion to the period, and a constant time sector of about 250 ms which is independent of the period. Just how the phase of the dorsal and ventral excitor bursts in the swim cycle is related to the constant sector of the oscillator duty cycle was found to be an important diagnostic criterion of the semi-intact preparation (Kristan *et al.* 1974*b*; Kristan & Stent, 1976).

The results of an experiment conducted for the purpose of a detailed comparison of the activity rhythms of semi-intact and isolated cord preparations are presented in Fig. 2. The data in Fig. 2A consist of extracellular recordings obtained from suction electrodes attached to the ends of segmental nerves in two exposed segments of a semi-intact leech preparation. In agreement with previous findings, these records show motor neurone impulse bursts whose rhythm matched the swimming movement carried out by the intact body parts of the semi-intact preparation (Kristan *et al.* 1974*a*). As can be seen, the spike bursts of the ventral excitor (cell 108) and of the dorsal excitor (cell 3) alternated, the period of the rhythm being about 1 s. Furthermore, the midpoints of spike bursts recorded from cell 3 two segments posterior occurred approximately 50 ms after the midpoints of the spike burst of the homologous cell 3 in the anterior ganglion, corresponding to an intersegmental delay of 25 ms per segment. This delay is of the same order of magnitude as the intersegmental travel time of the rearward propagation of the body troughs in the intact, freely swimming leech (Kristan *et al.* 1974*a*).

Fig. 1. Rhythmic motor neurone activity characteristic of swimming in an isolated nerve cord.

(A) Extracellular records taken from the dorsal branch of the posterior nerves (DP) in ganglia 11 and 8 and from the anterior branch of the anterior nerve (AA) in ganglion 8. The largest spikes in the DP records represent impulses of the dorsal excitor, cell 3; the largest spikes in the AA record represent impulses of the ventral excitor, cell 108. The bottom trace is an intracellular recording taken from the dorsal excitor, cell 7, in ganglion 7. A train of electrical stimuli was delivered to right and left DP nerves of ganglion 6 during the time marked by the bar below the bottom trace.

(B) Extracellular records taken from the posterior branch of the posterior nerve (PP) and from the DP and AA nerves of ganglion 10. The PP record shows spike bursts representing impulses of at least two dorsal excitors (cells 5 and 7), two ventral excitors (cells 4 and 8) and a dorsal inhibitor (cell 1). The largest spikes in the DP record represent the dorsal excitor, cell 3; the large spikes in the AA record represent the ventral excitor, cell 108. The bottom trace is an intracellular recording from the dorsal inhibitor, cell 1. The bar below the bottom trace marks the time of delivery of an electrical stimulus train to left and right DP nerves in segment 9.

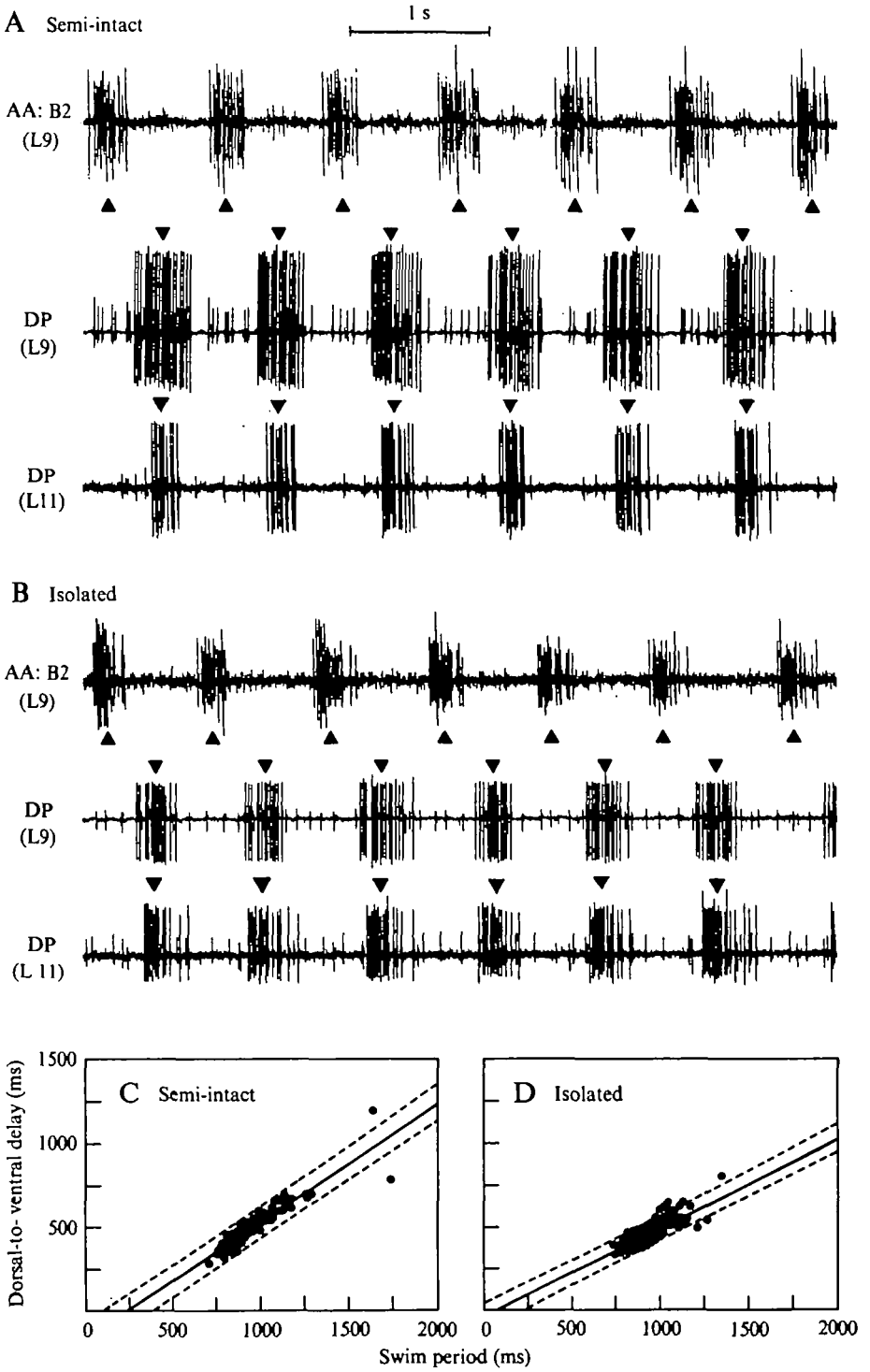

Fig. 2. For legend see opposite.

After securing recordings from many swim episodes of the semi-intact preparation, the remaining intact body parts of the semi-intact preparation were opened and the nerve cord was isolated from the body wall by section of all segmental nerves. Upon reattaching suction electrodes to the same segmental nerves of this isolated cord preparation, recordings were obtained which still showed rhythmic motor neurone impulse bursts similar to those seen in the semi-intact preparation, as is shown in Fig. 2B.

(a) Relative timing of dorsal and ventral excitor bursts

The results of one detailed comparison between the swimming rhythms of the semi-intact and isolated cord preparation are presented in Fig. 2C and D. These figures contain plots of the delay between a dorsal excitor burst and the next ventral excitor burst (the dorsal to ventral delay) against the period, based on data abstracted from records of numerous swim cycles of the preparation whose activity rhythms are presented in Figs. 2A and B. As can be seen, both prior to and after isolation of the preparation, the dorsal to ventral delay d increased with the cycle period P, according to the relation

$$d = \delta(P - P_0), \tag{3}$$

where δ is a dimensionless fraction and P_0 is a constant having the dimension of time. The least-squares fit of the data points to equation (3) results in the linear regression line shown in the plot. It is evident that the two plots of dorsal to ventral delay against period give rise to very different regression lines. In the case of the semi-intact preparation of Fig. 2C the regression line has a slope, $\delta = 0{\cdot}71$, and a period axis intercept, $P_0 = 245$ ms, in good agreement with the corresponding values abstracted from an earlier analysis of the excitor burst rhythm of semi-intact preparations (Kristan *et al.* 1974*b*). That P_0 is greater than zero expresses the fact that the ratio of dorsal to ventral delay to the cycle period, or the dorsal to ventral *phase lag*, increases with the period, from a minimum lag of zero for $P = P_0 = 245$ ms to a maximum of 0·71 when P is large. In the case of the isolated preparation of Fig. 2D, the regression line has a slope $\delta = 0{\cdot}54$ and period axis intercept $P_0 = 74$ ms. That P_0 now has a value near zero expresses the fact that after isolation of the nerve cord, the dorsal to ventral phase lag is nearly independent of the period and is equal to δ (0·54) (i.e. the ventral bursts always occur half-way between two successive dorsal bursts).

Fig. 2. Comparison of the motor neurone impulse burst rhythm in the semi-intact and isolated nerve-cord preparations.

(A, B) Records from the same nerves of ganglia 9 and 11 in the same semi-intact preparation, before and after the nerve cord was isolated from the body wall in all segments. The large spikes in the top trace in each panel are from the ventral excitor, cell 108; the large spikes in the second and third trace are from the dorsal excitor, cell 3. The triangular markers below or above the traces point to the middle of individual impulse bursts.

(C, D) Plots of the delay between the midpoints of dorsal and ventral excitor impulse bursts, as a function of swim cycle period. The data of (C) are based on 244 swim cycles of the semi-intact preparation whose recordings are shown in panel (A). The solid line shown is the regression line for the points shown and the dotted lines are the 99% confidence limits. The slope, δ, of the regression line is 0·71 and the period axis intercept, P_0, is 245 ms. The correlation coefficient, r, is 0·93. The data of (D) are based on 385 swim cycles of the isolated nerve cord preparation whose recordings are shown in (B). In this case the regression line is characterized by the values $\delta = 0{\cdot}54$, $P_0 = 74$ and $r = 0{\cdot}80$; the dotted lines are the 90% confidence limits.

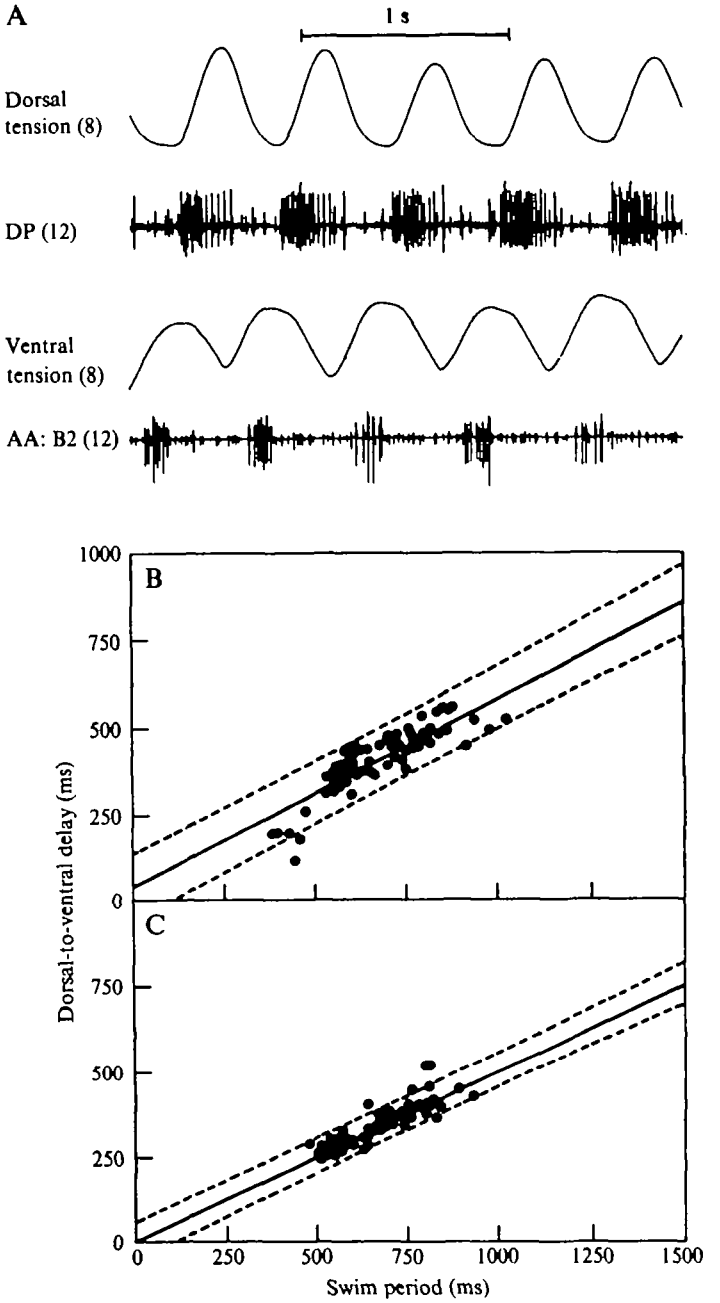

Fig. 3. Comparison of the dorsal to ventral delays determined from dorsal longitudinal muscle contractions and from excitor bursts in a 'nearly-isolated' preparation.

(A) Tension transducer recordings from dorsal and ventral longitudinal muscles in the body wall of the intact segment 8 and extracellular recordings from the DP and AA nerves of the exposed ganglion of segment 12. The large spikes in the DP record are from the dorsal excitor, cell 3, and in the AA record from the ventral excitor, cell 108.

(B) Plot of the dorsal to ventral delay as a function of the period of the impulse bursts in the nerve recordings from 107 swim cycles. For the regression line shown, $\delta = 0{\cdot}54$, $P_0 = -76$ ms and $r = 0{\cdot}84$.

(C) Similar plot for the delay between midpoints of the rising phase of dorsal and ventral muscle tension recordings. For the regression line shown, $\delta = 0{\cdot}50$, $P_0 = 6$ ms and $r = 0{\cdot}90$. The dotted lines in both plots are 90% confidence limits.

To test the statistical significance of this difference in phase lag variation with period between the two kinds of preparations inferred from these burst delay data, confidence limits were calculated for the points about the linear regression lines (Fig. 2C, D). They show that for the semi-intact preparation it can be inferred with 99% confidence that the value of P_0 is greater than 100 ms, whereas for the isolated cord preparation the value of P_0 is, within a 90% confidence limit, not significantly different from zero. Hence, it can be concluded that in this experiment total isolation from the periphery of the ventral nerve cord of an initially semi-intact preparation changed the dorsal to ventral phase lag of the swimming rhythm from period dependence to period independence.

To establish that the selected excitor impulse bursts adequately represent the activity pattern of the entire ensemble of motor neurones controlling the swimming movements, dorsal and ventral longitudinal muscle contractions were recorded concurrently with the excitor impulse activity during swimming episodes of a 'nearly-isolated' preparation. In this preparation the entire ventral nerve cord was isolated from the body wall by excision of all segmental nerves, except that a single segment was left completely intact in the midbody region. Tension transducers were attached to the dorsal and ventral body wall in the intact segment to register the development of tension in the longitudinal muscles. Suction electrodes were attached to the exposed nerves of a nearby segment to record dorsal and ventral excitor impulse bursts. Data obtained from such a preparation are shown in Fig. 3.

The traces of Fig. 3A present the dorsal and ventral body wall tension records from the intact segment, as well as the impulse activity in the dorsal posterior and anterior nerves of a dissected ganglion four segments posterior to the intact segment. As can be seen, the dorsal and ventral body walls of the single intact segment manifested the swimming rhythm by an alternation of tension–relaxation cycles. Furthermore, as befits their designation, the dorsal excitors produced their impulse bursts during development of tension in the dorsal body wall and the ventral excitors did so during development of tension in the ventral body wall. (It must be borne in mind, of course, that the corresponding excitor impulse bursts in the intact segment whose tension is being recorded preceded by about 160 ms the impulse bursts recorded from the exposed nerves four segments to the rear.) Fig. 3B and C show plots as a function of the swim cycle period of the delay between dorsal and ventral excitor burst midpoints, and between the development of half maximal tension in dorsal and ventral body walls. The delay between dorsal and ventral body wall tension development increased with the cycle period in the same manner as did the delay between dorsal and ventral excitor impulse bursts. Least-squares fits of the data points of Fig. 3B and C to equation (3) lead to parametric values of $\delta = 0{\cdot}54$ and $P_0 = -76$ ms for the impulse burst delays of Fig. 3B and of $\delta = 0{\cdot}50$ and $P_0 = 6$ ms for the tension delays. Within a 90% confidence limit, neither of these values of P_0 is significantly different from zero. Since the parameters for the two regression lines are very similar, it can be concluded that the impulse burst rhythm recorded during swimming episodes from the axons of the dorsal excitor cell 3 and the ventral excitor cell 108 in a segmental ganglion of the isolated cord preparation does adequately represent the activity of the total population of motor neurones which control the dorsal and ventral longidinutal muscles.

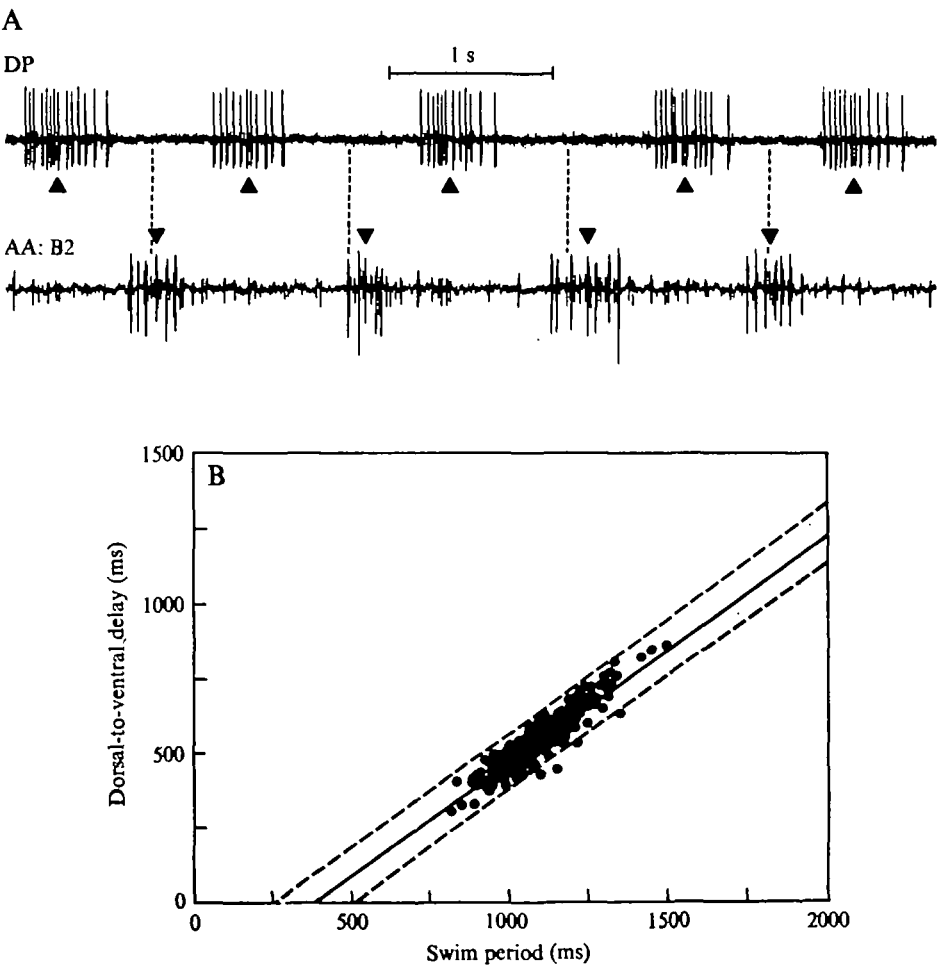

Fig. 4. Isolated nerve cord showing period-dependent dorsal to ventral phase lag.

(A) Extracellular recordings from nerves on left side of ganglion 9 during swim episode. The large impulses in the DP recording are from the dorsal excitor, cell 3, and in the AA: B2 recording from the ventral excitor, cell 108. The midpoint of each burst is marked by a pointer; the midpoint of each cycle period between dorsal excitor bursts is marked by a vertical line.

(B) Plot of the dorsal to ventral delay as a function of cycle period for data from 400 swim cycles of the preparation from which the records of (A) were taken. For the regression line shown, $\delta = 0{\cdot}76$, $P_0 = 377$ ms, and $\delta = 0{\cdot}92$; the dotted lines are the 90% confidence limits.

Not every isolated cord preparation exhibits the period-independent dorsal to ventral phase lag seen in the data of Figs. 2D and 3. For instance, the traces of Fig. 4A represent the dorsal and ventral excitor impulse bursts recorded during a swimming episode from the segmental nerves of another isolated cord preparation. Fig. 4B shows the plot of dorsal to ventral delays against the period for numerous swim cycles recorded from this preparation. The least-squares fit of these data to equation (2) yields a regression line for which $\delta = 0{\cdot}76$ and $P_0 = 377$ ms, with the value of P_0 being greater than 250 ms with 99% confidence. Hence, this particular isolated cord preparation

Table 1. *Relation between dorsal to ventral burst delay and swim-cycle period in a set of isolated cord, nearly isolated cord and semi-intact preparations*

	Isolated cord					Semi-intact and nearly isolated cord					
Preparation no.	No. of cycles	Avg. P (ms)	δ	P_0 (ms)	r	Segments intact	No. of cycles	Avg. P (ms)	δ	P_0 (ms)	r
1	400	1091	0·76	377*	0·92	13	71	857	0·76	251*	0·91
2	207	873	0·39	−146	0·77	13	400	680	0·69	135*	0·87
3	385	901	0·54	74	0·80	13	244	910	0·71	245*	0·93
4	124	1163	0·44	−94	0·97	—	—	—	—	—	—
5	68	1000	0·76	243*	0·95	—	—	—	—	—	—
6	44	728	0·59	64	0·81	—	—	—	—	—	—
7	88	819	0·73	218*	0·92	1	120	649	0·50	−7	0·90
						1	107†	676	0·54	−76	0·84
8	221	990	0·49	3	0·80	1	143	953	0·42	−213	0·55
						1	153†	1001	0·39	−271	0·72
9	66	980	0·44	−56	0·92	1	78†	872	0·67	148	0·90

* P_0 values that are greater than 70 ms with 99% confidence.
† Data derived from muscle-tension recordings.

clearly exhibited a variable rather than constant dorsal to ventral phase lag, and thus retained that characteristic feature of the semi-intact preparation.

To appreciate the significance of the difference in the details of the swim-cycle rhythms found in different preparations, a summary of the dorsal to ventral delay regression line parameters observed in nine isolated cord preparations is presented in Table 1. Three of these preparations were first studied as semi-intact preparations, before their reduction to the isolated cord condition; three were first studied as nearly isolated cord preparations; and three were directly prepared as isolated preparations.

Three points are to be noted. Firstly, although the average swim cycle periods ranged from 650 to 1200 ms, there is no significant difference in average period among the different kinds of preparations. Secondly, the rhythm of all three semi-intact preparations exhibited a variable phase lag between dorsal and ventral excitor bursts (i.e. the value of P_0 for each is significantly greater than zero). To supplement these data, values of P_0 were calculated also for dorsal to ventral delay data obtained in previous studies of semi-intact preparations (Kristan *et al.* 1974*b*). These calculations showed that in the case of five other semi-intact preparations the value of P_0 was greater than zero ms within a 99% confidence limit. Thus it may be concluded that an increase of the dorsal to ventral phase lag with the cycle period is a characteristic of the swimming rhythm of the semi-intact preparation. The third point to be noted is that the swimming rhythm of six of the isolated cord preparations exhibited a constant dorsal to ventral phase lag (i.e. $P_0 \cong 0$ ms), whereas the rhythm of three of the isolated cord preparations exhibited a variable phase lag which increased with the cycle period (i.e. $P_0 > 70$ ms).

The average values of δ and P_0 calculated for the three isolated cord preparations which showed variable dorsal to ventral phase lag are not significantly different from the corresponding average values calculated for the semi-intact preparations (Table 2A). Furthermore (as shown in Table 2B), the average values of δ and P_0 calculated

Table 2. *Comparison of groups of regression lines whose data are shown in Table 1*

	No. of preparations	Average P (ms)	Average δ	Average P_0 (ms)
(A) Comparison of variable phase-lag regression lines obtained from semi-intact preparations with those obtained from isolated cord preparations				
Semi-intact	3	816 ± 72	$0{\cdot}72 \pm 0{\cdot}02$	201 ± 38
Isolated cord	3	970 ± 80	$0{\cdot}75 \pm 0{\cdot}01$	279 ± 50
		$t = 1{\cdot}44$	$t = 1{\cdot}16$	$t = 1{\cdot}25$
		$p > 0{\cdot}50$	$p > 0{\cdot}50$	$p > 0{\cdot}50$
(B) Comparison of variable phase-lag regression lines with those of constant phase lag irrespective of the type of preparation from which they were obtained				
Variable phase	6	893 ± 51	$0{\cdot}74 \pm 0{\cdot}01$	245 ± 78
Constant phase	8	914 ± 56	$0{\cdot}48 \pm 0{\cdot}02$	-62 ± 40
		$t = 0{\cdot}25$	$t = 8{\cdot}47$	$t = 5{\cdot}65$
		$p > 0{\cdot}50$	$p < 0{\cdot}001$	$p < 0{\cdot}001$

The standard error is shown after all average values.

The symbol t refers to the parameter of the t test of the differences between two means. According to this test differences in the average values listed are considered significant if $P < 0{\cdot}05$.

for all preparations (semi-intact and isolated), which were designated as having a variable phase lag, are clearly different from those calculated for all preparations which were designated as having a constant phase lag. The studied preparations fall, therefore, into two distinct classes with respect to the period-dependence of the dorsal to ventral phase lag.

It may be concluded that the oscillator generating the swimming rhythm of the leech can exist in one of two distinct states in regard to the activity pattern of the relevant motor neurones: in one of these states there is a period-dependent phase lag and in the other a period-independent phase lag between dorsal and ventral excitor bursts. The oscillator of semi-intact preparations consisting of at least 13 intact, fully innervated body segments always appears to exist in the period-dependent phase lag state, whereas the oscillator of isolated cord preparations (or nearly isolated preparations containing only one intact body segment) can exist in either the period-dependent or the period-independent phase lag state.

(*b*) *Burst duration*

A further diagnostic criterion of the character of the swimming rhythm is the duration of the excitor impulse bursts and the dependence of the duration on the swim cycle period. As previously found (Kristan *et al.* 1974*b*), the burst duration b varies with the period P according to the relation

$$b = \beta(P - P_0), \tag{4}$$

where β is the dimensionless slope of the linear regression line and P_0 is, as in equation (2), the period axis intercept. Analysis of the impulse burst data gathered during swimming episodes of semi-intact preparations had shown that the dorsal and ventral excitatory swimming motor neurones fall into at least two distinct classes with respect to their burst duration regression line values β and P_0. For ventral excitatory motor

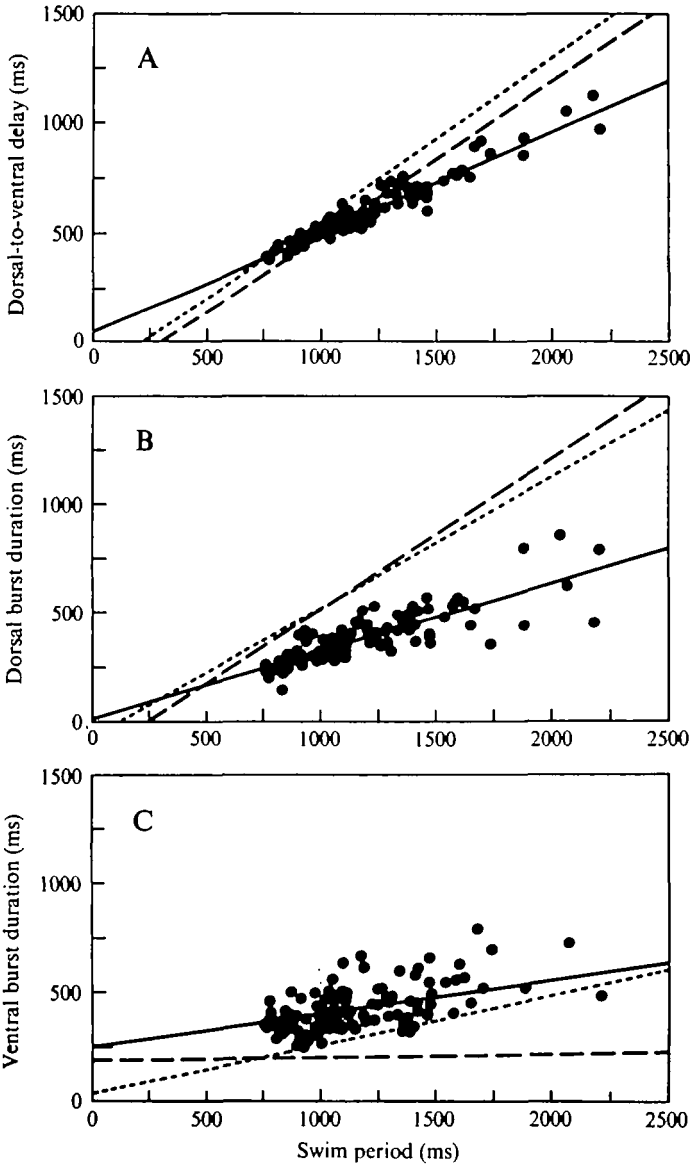

Fig. 5. Dependence of impulse burst duration on swim cycle period in isolated cord and semi-intact preparations. In all plots, solid lines represent regression lines for the data points shown, which were obtained from 124 swim cycles in the isolated cord preparation no. 4 listed in Table 1; dashed lines represent regression lines from data previously published from a semi-intact preparation (figs. 3B and 6B, Kristan *et al.* 1974*b*), and the dotted lines represent regression lines for the data from the isolated cord preparation no. 7 listed in Table 1.

(A) Plot of dorsal to ventral delay as a function of period. For the solid line, $\delta = 0{\cdot}44$, $P_0 = -94$ ms, and $r = 0{\cdot}97$; for the dashed line, $\delta = 0{\cdot}70$, $P_0 = 293$ ms, and $r = 0{\cdot}89$; for the dotted line, $\delta = 0{\cdot}73$, $P_0 = 218$ ms, and $r = 0{\cdot}92$.

(B) Plot of burst duration as a function of period for bursts of the dorsal excitor, cell 3. For the solid line, $\beta = 0{\cdot}30$ and $P_0 = -16$ ms; for the dashed line, $\beta = 0{\cdot}69$ and $P_0 = 242$ ms for the dotted line, $\beta = 0{\cdot}60$ and $P_0 = 115$ ms.

(C) Plot of burst duration as a function of period for ventral bursts. For the solid line, $\beta = 0{\cdot}14$, $P_0 = -1795$ ms, and $r = 0{\cdot}40$; for the dashed line, $\beta = 0{\cdot}0057$, $P_0 = 33300$ ms, and $r = 0{\cdot}33$; for the dotted line, $\beta = 0{\cdot}22$, $P_0 = -170$ ms and $r = 0{\cdot}45$.

neurones the burst duration is nearly independent of the period, and hence their regression lines are characterized by small values of β and large values of P_0. The burst duration of dorsal motor neurones rises sharply with the period, and hence their regression lines are characterized by large values of β, and positive values of P_0 of the order of 200 ms. A comparison was, therefore, made between the dependence on cycle period of the burst duration in the semi-intact and isolated cord preparations. Ideally, this comparison should be made for the preparation before and after isolation of its nerve cord from the periphery, as had been the case in the comparisons of the dorsal to ventral delay in the experiment of Fig. 2. Unfortunately, upon isolation of the cord of preparations initially dissected as a semi-intact condition, the burst duration of the excitors becomes so variable that meaningful comparisons before and after isolation cannot be made. However, if an isolated cord preparation is prepared directly in a single stage dissection, then the variability in burst duration may be no greater than that found in semi-intact preparations. Because of this purely technical complication, comparisons of burst duration were made between data obtained from favourable isolated nerve cord preparations directly dissected in a single stage and the corresponding data previously published for semi-intact preparations (Kristan *et al.* 1974*b*).

Fig. 5 presents plots as a function of swim cycle period of dorsal to ventral delay and of burst duration for dorsal and excitor bursts observed in three different preparations. As illustrated in Fig. 1 A, the oscillator of one of these, an isolated cord preparation, was in the period-independent phase lag state; the oscillator of each of the other two preparations one a semi-intact and the other an isolated cord preparation, was in the period-dependent phase lag state.

Fig. 2 B shows that the burst duration of the dorsal excitor increased linearly with the period in all three preparations. However, the burst duration increased much less with the cycle period in the isolated cord preparation whose oscillator was in the period-independent phase lag state than in the semi-intact isolated cord preparations whose oscillators were in the period-dependent phase lag state. That is to say, the slope of β of the regression line for the period-independent phase lag preparations is only 0·30 compared to the corresponding values of 0·69 and 0·60 for the two period-dependent phase lag preparations. Hence, the degree of variation in dorsal excitor burst duration with the cycle period seems to be related to the phase lag state of the oscillator. In addition P_0 is not significantly different from 0·0 for the isolated preparation which is in the period-independent phase lag state ($p > 0{\cdot}1$) while both period-dependent phase lag preparations show positive P_0's. By contrast, the ventral excitor burst duration shown in Fig. 5 C does not serve to distinguish between the two phase lag states of the preparations. Although the ventral excitor burst duration does vary over a range comparable to that of the dorsal excitor, this variation does not show any consistent dependence on the cycle period. Accordingly, the slopes of the three burst duration regression lines have the low values 0·14, 0·0057 and 0·22. In addition all three preparations show large negative values of P_0.

(*c*) *Intersegmental delay.*

The intersegmental delay of the longitudinal muscle contractions which make up the swimming wave increases along the body of an intact leech with an increase in the period of the swim cycle (Kristan, *et al.* 1974*a*). Thus, during short cycle periods of

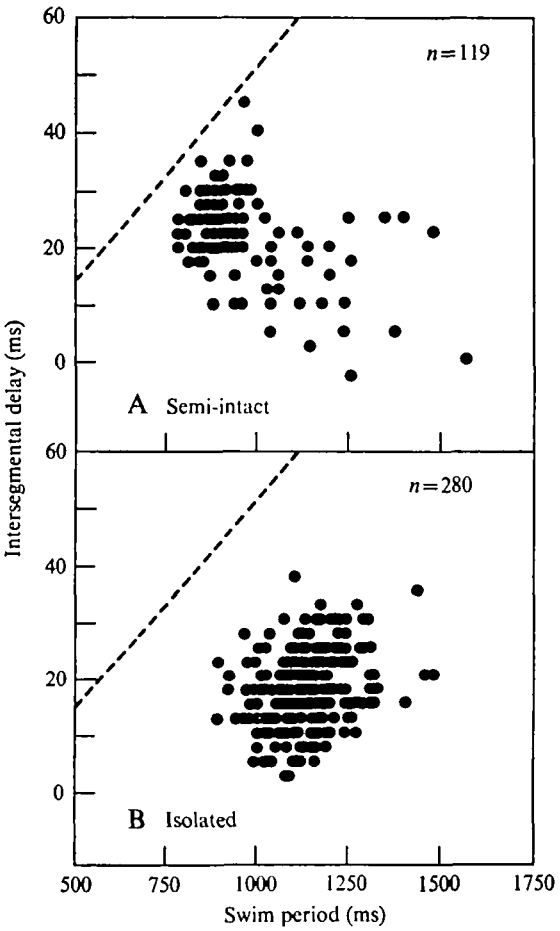

Fig. 6. Intersegmental excitor burst delay as a function of swim period in a semi-intact and isolated cord preparation. The intersegmental delays were measured between burst midpoints of the dorsal excitor, cell 3, in ganglia 9 and 13. The delays were divided by 4 to obtain the average delay per segment and plotted against the period obtained from cell 3 bursts in ganglion 9. The number of swim cycles from which data were used to generate the graphs is indicated by *n*. The dashed line in both graphs is the regression line obtained from tension recordings in a nearly intact preparation (fig. 9, Kristan *et al.* 1974*a*).

about 520 ms, the delay between formation of the wave crest in any two successive segments is about 25 ms; whereas during long cycle periods of about 2000 ms, the corresponding intersegmental delay is about 100 ms. The consequence of this increase in intersegmental delay with the period is that the swimming leech body always takes on the optimal hydrodynamic shape of a single wave-length.

We may now compare this mode of rearward travel of the swimming wave in the intact leech with the front-to-back progression of the activity cycle phases of homologous motor neurones in successive segmental ganglia of the semi-intact and isolated cord preparations. For this purpose, the delay between the onset of the impulse bursts of the dorsal excitor (cell 3) homologues in ganglia 9 and 13 have been plotted in Fig. 6 as a function of the period based on recordings obtained from both semi-

intact and isolated nerve cord preparations. As can be seen, these data are in qualitative accord with the observed rearward travel of the swimming wave, in that there usually is a delay between the onset of the dorsal excitor burst in a posterior and in an anterior ganglion. However, it follows from these data (and from similar delay data obtained with several other preparations) that, from a quantitative viewpoint, the intersegemental impulse burst delays in both semi-intact and isolated cord preparations differ from the intersegmental wave crest delay in the intact swimming animal in two respects. First, the intersegmental excitor burst delay is shorter than is the intersegmental wave crest delay in the intact animal. Secondly, and more importantly, unlike the clear dependence of the intersegmental delay on the swim cycle period of the intact animal (shown by the dashed lines on Fig. 6A and B), the observed variation in the intersegmental impulse burst delay shows no obvious correlation with the period in either the semi-intact or the isolated cord preparation. This finding suggests that the co-ordination of the cycle period and the inter-segmental delay between two adjacent segments depends on peripheral sensory input to the segmental ganglia.

(3) *Resetting of the oscillator*

Previous studies have shown that passage of current into a single motor neurone during a swimming episode of a semi-intact preparation can produce a transient change in the activity pattern of that cell and of the motor neurones to which it is linked via chemical synapses or electrical junctions (Ort *et al.* 1974). However, such passage of current neither alters the length of the period nor does it reset the phase of the rhythm (Kristan & Stent, 1976). Hence, it was concluded either that the motor neurones are not themselves part of the oscillatory circuits that generate the swimming rhythm, or that, if they are, induced disturbances in a single segmental oscillator of a semi-intact preparation are overridden by the normal operation of other homologous oscillators in the many intact body segments to which that oscillator is coupled. Passage of current into the motor neurones of an isolated cord preparation similarly fails to reset the phase of the swimming rhythm, except for passage of depolarizing current into the dorsal inhibitor, cell 1. As the data to be presented now show, passage of current into cell 1 does lengthen the swim cycle period and resets the phase of the rhythm. Thus cell 1 appears to differ from the other excitors or inhibitors in that it has access to the oscillator.

Detecting a change in swim cycle period evoked by passage of current into a motor neurone is complicated by the spontaneous changes in period that occur during a swim episode. As is evident from the dorsal and ventral excitor burst cycle periods recorded during swim episodes of four different semi-intact preparations presented in Fig. 7A, there occur wide variations both in episode duration and in the range of cycle periods. Nevertheless, in all cases the period tends to increase with cycle number within any swim episode.

Fig. 7B shows the variations in period for two swim episodes of a semi-intact preparation while cell 1 of the same ganglion from which the excitor bursts were recorded was impaled with a microelectrode. During the first episode, no current was passed into cell 1, whereas during the middle of the second episode enough depolarizing current was passed into cell 1 to abolish completely any concurrent dorsal excitor

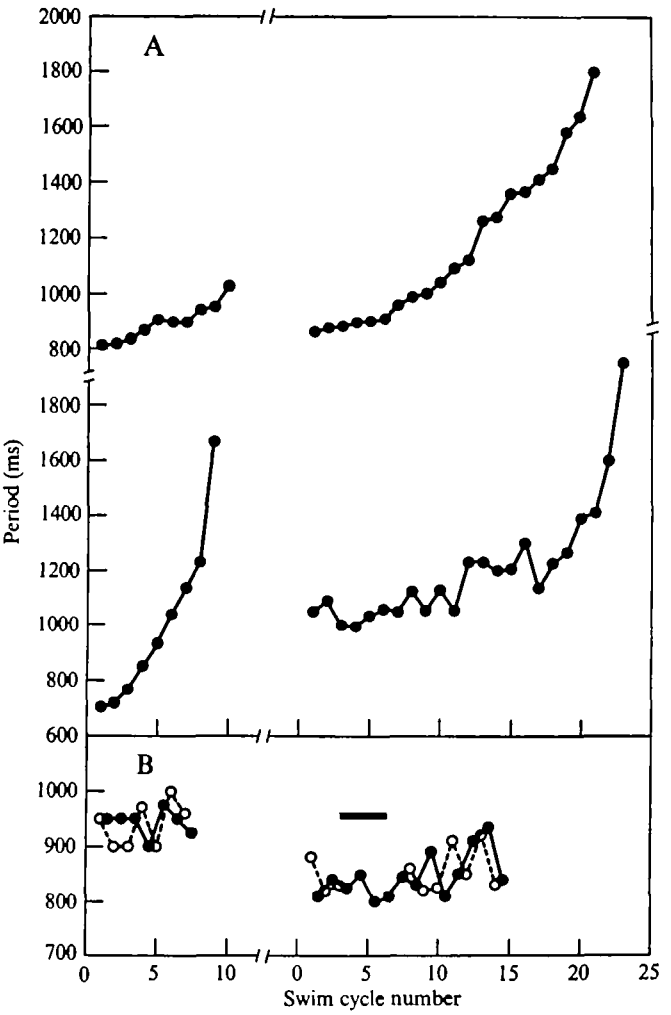

Fig. 7. Variation in cycle period with cycle number during swim episodes in semi-intact preparations.

(A) Cycle periods observed in four different semi-intact preparations, as determined from dorsal excitor, cell 3, impulse bursts recorded from the DP nerve.

(B) Cycle periods during two swim episodes of the same preparation while a microelectrode was in the dorsal inhibitor, cell 1, and extracellular records were obtained from the contralateral DP and AA nerves of the same segment. Periods were determined from impulse bursts in both the dorsal excitor, cell 3 (open circles) and the ventral excitor, cell 108 (closed circles). During the time marked by the black bar in the second swim episode, sufficient depolarizing current was passed into cell 1 to eliminate the dorsal impulse bursts; during this time, swim cycles could be determined only from the ventral excitor impulse bursts.

burst. Nevertheless, despite such current passage, the period, as measured from ventral excitor burst rhythm, continued to maintain its normal gradual increase during the swim episode. This result confirms, therefore, the previous finding that passage of depolarizing current into motor neurones, including the dorsal inhibitor cell 1, does not affect the swim cycle period of a semi-intact preparation.

Fig. 8 presents the corresponding data for an isolated cord preparation. Fig. 8A shows the impulse bursts of dorsal and ventral excitors, as well as the variations in

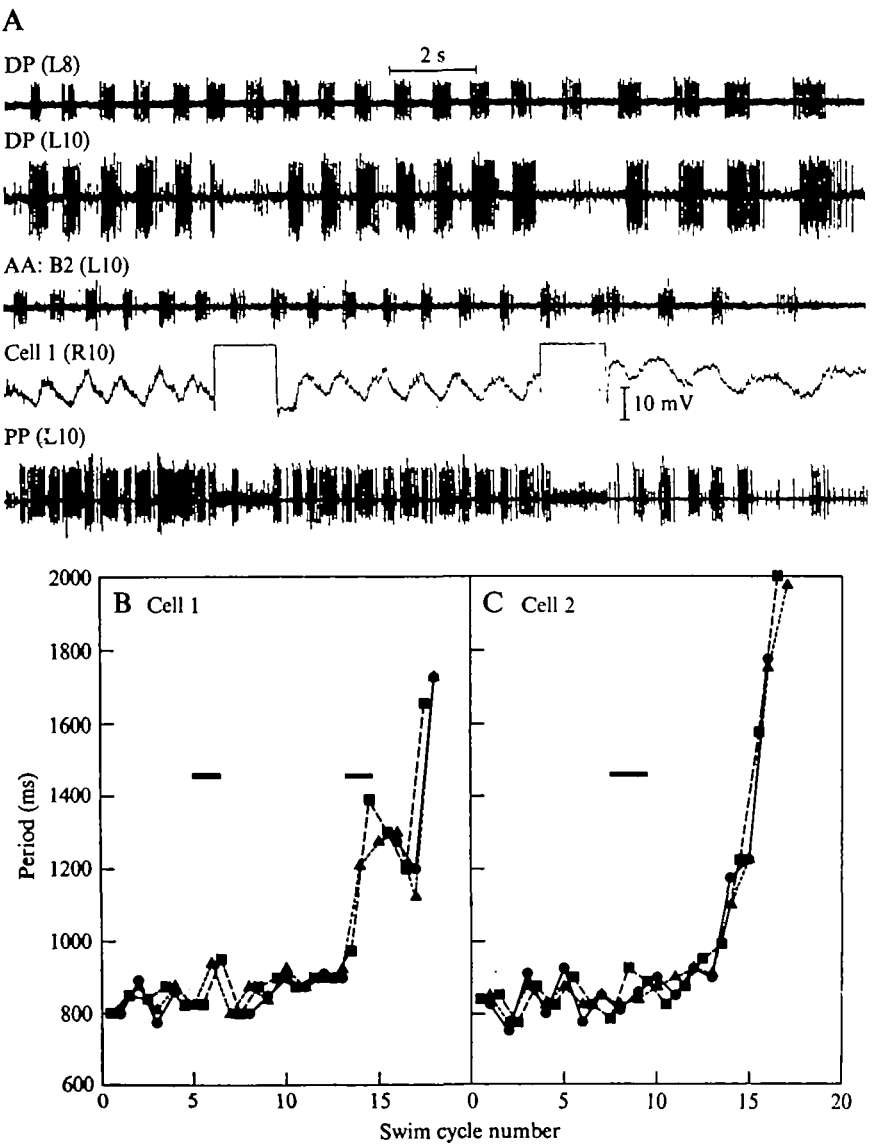

Fig. 8. Effect of depolarization of inhibitors on the swim cycle period in an isolated nerve cord.

(A) Extracellular and intracellular records from isolated cord preparation during complete swim episode. The recordings from the left DP nerves show impulse burst in the dorsal excitor cell 3 homologues in ganglia 8 and 10; the recording from the left AA nerve shows impulse bursts in the ventral excitor, cell 108, in ganglion 10; and the recording from the PP nerve shows impulse bursts from dorsal and ventral excitors, as well as from the dorsal inhibitor, whose small spikes can be seen clearly only during the depolarization of its cell body. The intracellular recording (to which the 10 mV calibration mark applies) was taken from cell 1 on the right side of ganglion 10. The two raised flat portions of the intracellular record, caused by saturation of the amplifier, mark the time of passage of depolarizing current into cell 1. The strength of the depolarizing current was adjusted to be just sufficient to eliminate the cell 3 bursts in the DP (L10) nerve.

(B) Variation of cycle period with cycle number for the episode shown in (A). The bars indicate the lines of passage of depolarizing current into cell 1.

(C) Variation of cycle period with cycle number for another episode while an intracellular electrode was in the ventral inhibitor, cell 2, on the right side of ganglion 10. The bar indicates the time during which depolarizing current was passed into cell 2.

In (B) and (C) the three sets of connected symbols indicate the periods measured from cell 3 bursts in ganglion 8 (▲ - - ▲) and ganglion 10 (● - - ●), and from cell 108 bursts in ganglion 10 (■—■).

membrane potential of cell 1 during a complete swim episode. As can be seen, passage of depolarizing current into cell 1 evoked tonic impulse activity in that cell, manifest as a prolonged, high-frequency train of low-amplitude spikes in the PP nerve record. As in the case of the semi-intact preparation, passage of depolarizing current into cell 1 immediately abolished the dorsal excitor impulse bursts in the same ganglion (but not in the anterior ganglion). However, here, depolarization of cell 1 had also some effect on the ventral excitor bursts, namely it prolonged them in the AA nerve record and shortened them in the PP nerve record.

The effect of passage of depolarizing current into cell 1 on the period is seen clearly in Fig. 8B. During the first current passage, the period increased from about 825 to 940 ms, and then returned to an intermediate level. During the second current passage the period increased from about 900 to more than 1300 ms, and again returned to an intermediate level. The excitor burst records obtained from a ganglion two segments anterior to that into whose cell 1 current was passed showed an entirely analogous transient lengthening of the period. It can be inferred from this finding that depolarization of cell 1 in one ganglion lengthens the swim cycle period in other, and presumably all, ganglia of an isolated cord preparation. It is to be noted that, although the relevant data are not shown here, passage of hyperpolarizing current into cell 1 has no effect on the swim cycle period.

The prolongation of the period evoked by current passage into cell 1 has been observed in three different isolated cord preparations. However, in no preparation did passage of current into any other known excitor or inhibitor produce a change in the period. For instance, as shown in Fig. 8C, passage of depolarizing current into the ventral inhibitor, cell 2, in the same isolated cord preparation as that used in the experiment of Fig. 8B did not alter the normal pattern of cycle period increase during a swim episode.

Another experimental manipulation which can change the swim cycle period of the isolated (or, rather, nearly isolated) cord preparation, but not of the semi-intact preparation, is longitudinal stretch of the body wall. Fig. 9 shows the effect of dorsal body wall stretch during a swim episode on the dorsal and ventral excitor burst rhythm in a semi-intact and in a nearly isolated cord preparation. In both preparations, recordings were taken from the segmental nerves of a ganglion attached to a flap of dorsal body wall by its dorsal branch of the posterior nerve, and from the segmental nerves of a ganglion several segments to the anterior. The posterior edge of the dorsal body-wall flap was pinned down and the anterior edge was attached via a thread to a tension transducer mounted on a rack and pinion. The flap was stretched by turning the pinion of the transducer mount, and the output of the transducer served as a marker for the time at which stretch was applied.

As is evident in Fig. 9A, stretch of the dorsal body wall prolongs the dorsal excitor bursts and shortens, or even abolishes, the ventral excitor bursts in the semi-intact preparation. The plot of cycle period variation with cycle number for this swim episode (Fig. 10A) shows, however, that stretch did not alter the cycle period of the semi-intact preparation.

In the nearly isolated cord preparation, stretch of a flap of dorsal body wall innervated by a single segmental nerve has a more pronounced and more complex effect on the swimming rhythm. Here, a weak body wall stretch prolonged the dorsal excitor

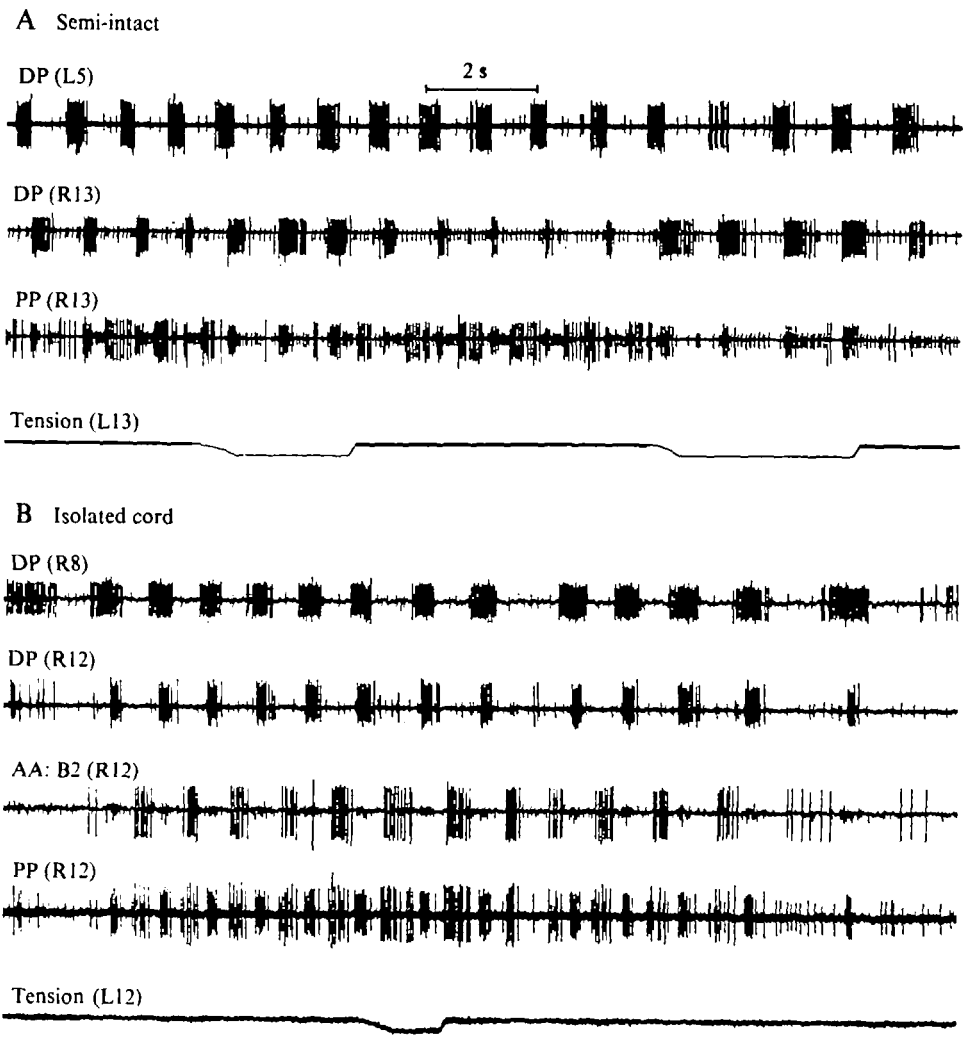

Fig. 9. The effect of longitudinal stretch of the dorsal body wall on the swimming rhythm of semi-intact and isolated nerve cord preparations.

(A) Nerve recordings during complete swim episode of semi-intact preparation with ganglian 4–6 and 12–14 exposed. The DP nerve records show impulse bursts of the dorsal excitor, cell 3, in ganglia 5 and 13. The PP nerve record shows dorsal and ventral excitor impulse bursts as well as dorsal inhibitor bursts in ganglion 13. The downward deflexion of the tension transducer record indicates the time that stretch was applied to a dorsal body wall flap innervated by the DP (L, 13) nerve.

(B) Nerve recordings during swim episode in nearly isolated nerve cord. The DP nerve records show the impulse bursts of the dorsal excitor, cell 3, in ganglia 8 and 12; the AA nerve record shows impulse bursts of the ventral excitor, cell 108, in ganglion 12; and the PP record shows both dorsal and ventral excitor bursts in ganglion 12. The downward deflexion of the tension transducer record indicates the time that stretch was applied to a dorsal body wall flap innervated by the DP (L, 12) nerve.

bursts and shortened the ventral excitor bursts, just as did a strong body wall stretch n the semi-intact preparation. But a moderate stretch such as that applied while the record of Fig. 9B was taken, is seen to produce not only changes in excitor burst duration but also significant increases in the cycle period. Upon application of a strong

stretch, the swimming rhythm stopped completely but sometimes resumed after release of stretch, if the stretch did not last too long. A very strong stretch not only stopped swimming during the stretch but also prevented its resumption after release of stretch. The data of Fig. 10B document the effect of a moderate stretch on the swimming rhythm of a nearly isolated cord preparation. During this stretch of the dorsal body wall flap the cycle period increased by nearly 20%; after release of the stretch, the period again shortened to a value well below that of the prestretch period and then gradually increased again in the normal manner until the end of the swimming episode. The near congruence of the points obtained from segments 8 and 12 shows that the stretch affected these two segments at the same time and to the same degree.

Since, in the semi-intact preparation, motor neurone burst duration is changed by body wall stretch without changing the swim cycle period, there must be a pathway from peripheral receptors to the motor neurones that by-passes the central oscillator. The fact that body wall stretch in the nearly isolated nerve cord does change the swim cycle period indicates that there must also be a pathway from peripheral receptors to the central oscillator. This pathway might include the dorsal inhibitor, cell 1.

DISCUSSION

(1) *Production of the swimming rhythm in the isolated nerve cord*

Despite earlier unsuccessful attempts (Gray *et al.* 1938; Kristan, 1974) to produce a swimming rhythm in the motor neurones of an isolated leech nerve cord, the rhythm can now be found in at least 75% of such preparations. Several factors can be advanced to account for the previous lack of success. Probably the most important factor concerns an improvement in the physiological state of the animals from which the preparations were made. Previously, the animals were maintained at 5 °C to avoid the necessity of feeding them. Although leeches remain alive as long as a year under these conditions, their vigour and usefulness as even semi-intact preparations deteriorates within 3 or 4 weeks. However, in leeches maintained at 15 °C and fed regularly, the vigour of the dissected preparations increased greatly and the swimming rhythm was first observed in the isolated cord.

With these technical improvements, the isolated cord now provides a much easier and more reliable preparation for neurophysiological studies of the swimming rhythm than does the semi-intact one.

(2) *The effects of sensory input on the swimming rhythm*

Previous experiments using the semi-intact preparation have shown that stretching either the dorsal or ventral longitudinal muscle during a swimming episode increases the intensity (i.e. the duration and impulse frequency) of the bursts in the excitors to the muscles being stretched and decreases the intensity of the excitor bursts to the other, functionally antagonistic muscles (Kristan, 1974; Kristan & Stent, 1976). The present finding that an isolated cord preparation can produce the basic swimming rhythm now shows that these reciprocal stretch reflexes are not necessary for its production. In addition, since the range of observed swim cycle periods, of motor neurone interspike intervals and of burst durations is similar in the semi-intact and isolated cord preparations, it can be inferred that the phasic afference supplied by the

segmental stretch reflexes is not necessary to provide a generalized intersegmental tonic excitation to the central oscillator or to the motor neurones, or to both, to drive the swimming rhythm. Nevertheless, it is likely that these reflexes do intervene in the detailed realization of the body wave. At least three different functions for such intervention may be envisaged.

(*a*) *The stretch reflexes may serve to stabilize the centrally generated rhythm*

In this case the inherent dynamics of the central swimming oscillator would be matched with those of the stretch reflex loops in such a way that the rhythmic sensory afference from the periphery onto the central oscillator is normally in phase with the oscillator. Hence the stretch reflexes would affect the rhythm only if the actual body movements do not match those commanded by the central oscillator. Such an effect has been shown to occur for the phasing of wingbeat in the locust by input from the hinge receptors at the base of each wing (Wendler, 1974). In the leech, sensory-motor mismatch would occur if a physical obstruction were to retard or prevent bending of the body. If this obstruction were to be confined to only one or two segments, the stretch reflexes might simply increase the intensity of the motor neurone bursts in those segments without affecting the period of the overall rhythm. Such a condition is, in fact, mimicked by stretching the body wall of a single segment in the semi-intact preparation. If however, the bending of many segments were to be obstructed, then the period of the rhythm would also be lengthened. This feedback effect would explain why an increased viscosity of the medium lengthens the period of the leech swim cycle (Gray *et al.* 1938), and explain also why an obstruction to trough formation in the middle of the intact leech body affects the regular swimming movements of the unobstructed front and back ends if, and only if, more than eight middle segments are obstructed (Kristan & Stent, 1976).

That the swim period can be lengthened by stretching a single segment in the nearly isolated cord preparation would, of course, be attributable to the lack of stabilization of the swimming rhythm by any stretch reflexes in the wholly deafferented rest of the nerve cord.

Similarly, the fact that depolarization of the dorsal inhibitor, cell 1 in a single ganglion, lengthens the period of an isolated cord but not in a semi-intact preparation would be accounted for by the stabilization of the rhythm by the reflexes in the intact segments of the swimming semi-intact preparation and by the absence of any such stabilizing influence in the isolated cord.

(*b*) *Stretch reflexes may determine the period-dependent phase lag between dorsal and ventral excitor bursts*

According to the data summarized in Tables 1 and 2, the rhythm of all semi-intact preparations and some of the isolated cord preparations thus far examined exhibited a period-dependent dorsal to ventral phase lag, and hence appears to be generated by an oscillator whose duty cycle contains a constant time sector intervening between the ventral and dorsal excitor impulse bursts. By contrast, the rhythm of some of the isolated cord preparations exhibited a period-independent dorsal to ventral phase lag, indicative of the absence of an asymmetrically distributed constant time sector of the

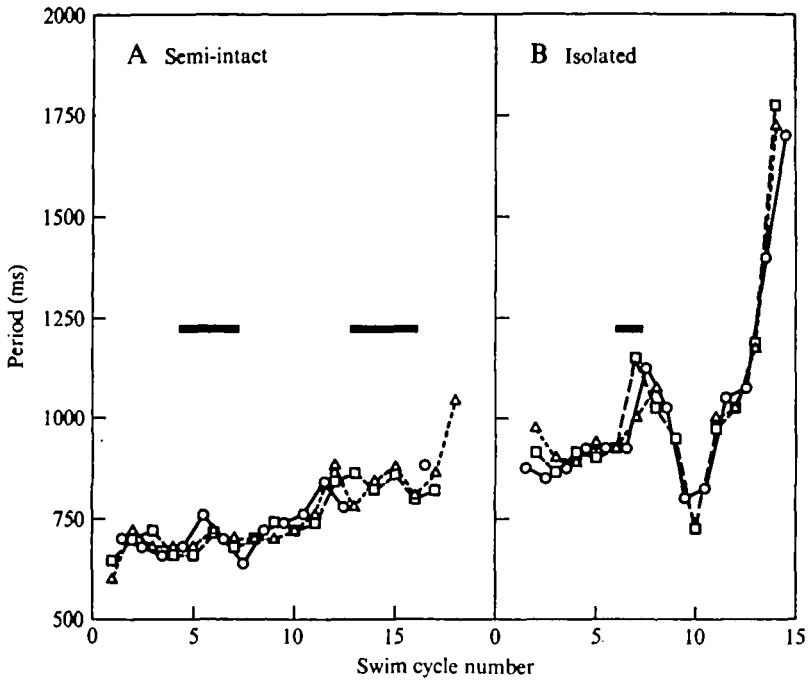

Fig. 10. Variation of cycle period with cycle number for the swim episodes shown in Figs. 9(A) and (B). The bars indicate the time at which the dorsal body wall flaps were stretched.
(A) Periods were measured from cell 3 impulse bursts in ganglion 5 (△ – – △) and ganglion 13 (□ – – □) and ventral excitor bursts in ganglion 13 (○—○).
(B) Periods were measured from cell 3 impulse bursts in ganglion 8 (△ – – △) and ganglion 12 (□ – – □) and cell 108 impulse bursts in ganglion 12 (○—○).

oscillator duty cycle. It was previously suggested that the asymmetrically distributed constant time sector is introduced into the oscillator by the peripheral reflex loops (Kristan, 1974). This interpretation is no longer tenable because a constant time sector of the same size and asymmetric distribution is evident in the data from some isolated nerve cords. Therefore, the constant time sector can be generated by the central oscillator, and its expression is assured by the presence of a large part of the periphery in the semi-intact preparation.

(*c*) *Stretch reflexes may be responsible for the constant intersegmental phase lag of the swimming rhythm*

In the swimming intact leech the front-to-rear intersegmental delay increases with the cycle period so as to produce a constant intersegmental phase lag. The result of this co-ordination is that the body maintains a single wave length at all swimming speeds. As was seen from the data of Fig. 6, neither the isolated cord nor the exposed ganglia of a semi-intact preparation show any such increase of the intersegmental delay with the period. Hence it seems reasonable to conclude that the constant intersegmental phase lag is also attributable to the operation of the peripheral reflexes which the denervated ganglia lack. Figs. 8 and 10 show that the changes in period induced in a segmental ganglion by stretch or inhibitor depolarization in that segment

are exactly duplicated in distant ganglia. This shows that very effective intersegmental reflexes are present; these reflexes may be responsible for matching the intersegmental delay and the cycle period. This function of peripheral reflexes was proposed for the leech on the basis of behavioural experiments (Kristan & Stent, 1976), and has also been proposed for the coordination of the legs of an insect during walking (Pearson & Iles, 1973).

The neuronal nature of the central oscillator must be determined before the cellular mechanism of the sensory effects can be elucidated. However, it is now clear that swimming movements of the leech are produced by a neuronal activity pattern of central origin and that peripheral sensory afference intervenes in the detailed execution of the movements.

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