J. exp. Biol. (1978), 77, 71–88
With 9 figures
Printed in Great Britain

INITIATION, MAINTENANCE AND MODULATION OF SWIMMING IN THE MEDICINAL LEECH BY THE ACTIVITY OF A SINGLE NEURONE

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(*Received* 6 *February* 1978)

SUMMARY

(1) A neurone (designated cell 204) has been identified in the segmental ganglia of the leech which, when stimulated intracellularly in isolated nerve cords, reliably initiates and maintains the neuronal activity pattern characteristic of swimming. In a minimally dissected leech, cell 204 activity results in normal swimming movements.

(2) Cell 204 is an unpaired, intersegmental interneurone which is present in most, if not all, of the segmental ganglia. Horseradish peroxidase injections indicate that cell 204 has extensive arborizations in its own ganglion and sends an axon both anteriorly and posteriorly via Faivre's Nerve.

(3) Cell 204 is normally quiescent, but during swimming activity becomes depolarized and produces impulse bursts in the ventral contraction phase of its own segment. Such activity is observed in every cell 204 in the nerve cord and is independent of the stimulus used to evoke the swimming episode.

(4) Activity in any cell 204 is *sufficient* for initiation and maintenance of swimming activity, whereas activity in any two of them is not *necessary* for swimming.

(5) During swimming activity, imposed increases in the impulse frequency of any cell 204 cause a decrease in the swim cycle period of the entire nerve cord.

(6) Tactile stimulation of the skin, which is an effective method of eliciting swimming episodes, excites cell 204.

(7) Our findings indicate that cell 204 may activate swimming in the intact leech.

INTRODUCTION

The pattern of neuronal activity which produces a rhythmic behaviour is often generated by the central nervous system in the absence of peripheral afference (Kennedy & Davis, 1977; Kristan *et al.* 1977). An example is the ability of the totally isolated nerve cord of the leech, *Hirudo medicinalis*, to generate the coordinated neuronal activity pattern characteristic of swimming (Kristan & Calabrese, 1976).

The undulations which constitute swimming movements in the intact leech are represented in each segment of the isolated nerve cord by antiphasic bursts of impulses in motor neurones innervating the dorsal and ventral longitudinal muscles

(Kristan, Stent & Ort, 1974*a*; Kristan & Calabrese, 1976). The contractile cycle of each segment has a period of the order of 1 s, and precedes that of the next more posterior segment by 20–80 ms. This results in a front-to-rear progression of the undulation along the animal's body. A number of parametric comparisons indicate that the neuronal activity pattern produced by the isolated nerve cord is essentially identical to the pattern produced during swimming by more intact preparations (Kristan & Calabrese, 1976) and by the intact animal (Kristan *et al.* 1974*a*, *b*). An ensemble of premotor intersegmental interneurones has recently been described which seemingly constitutes the central pattern generator for swimming (Friesen, Poon & Stent, 1978; Poon, Friesen & Stent, 1978).

Tactile and electrical stimulation can initiate swimming episodes in quiescent preparations. Tactile stimulation elicits swimming episodes from intact animals, 'semi-intact' animals, and from isolated cord preparations which still innervate a small flap of body wall. A short train of shocks to a segmental nerve elicits the swimming activity pattern from the isolated nerve cord. This electrical stimulation may be effective because it activates mechanosensory afferents, and therefore mimics tactile stimulation. However, intracellular stimulation of individual primary mechanoreceptors, namely the touch, pressure and nociception neurones (Nicholls & Baylor, 1968) does not reliably initiate swimming activity (R. L. Calabrese, W. B. Kristan and J. C. Weeks, unpublished observations). This observation suggests that many of these neurones must be activated to elicit swimming, or that other sensory neurones are involved. Intracellular stimulation of swimming motor neurones or members of the central pattern generator does not lead to the initiation of swimming (Kristan *et al.* 1974*a*, *b*; Ort, Kristan & Stent, 1974; Friesen *et al.* 1978).

Little has been determined previously about the neuronal pathways by which swimming is evoked in the intact leech. We now report the discovery of a neurone which, when active, elicits swimming activity from the isolated nerve cord or normal swimming movements from the minimally dissected leech preparation.

Materials and Methods

Leeches, *Hirudo medicinalis* or *Macrobdella decora*, were obtained from distributors and maintained as previously described (Kristan and Calabrese, 1976). Except as noted, all the experiments described herein were done on *H. medicinalis*. Three types of preparations were used: (1) an isolated ventral nerve cord preparation consisting of a chain of the 21 segmental ganglia with the head and tail brains left attached, but with the connectives between the brains and the adjacent segmental ganglia crushed (Kristan & Calabrese, 1976); (2) single ganglia or short chains of ganglia; (3) a semi-intact preparation consisting of the complete animal with the head and tail brains disconnected from the nerve cord and the body wall and viscera removed from 4–6 midbody segments so as to expose the nervous system (Kristan *et al.* 1974*a*). All preparations were maintained at 15–20 °C in leech physiological saline (Nicholls & Purves, 1970). Segmental ganglia were numbered sequentially, beginning with the first ganglion posterior to the head brain (Kristan *et al.* 1974*a*).

The electrophysiological methods of recording and stimulating neuronal activity were as described previously (Kristan *et al.* 1974*a*; Ort *et al.* 1974). Briefly, intra-

cellular recordings were made using glass micropipettes filled with 3 M potassium acetate (30–80 MΩ), while extracellular recordings from segmental nerves or connectives were obtained using glass-tipped suction electrodes. Amplified recordings were displayed on a multiple trace oscilloscope and stored on magnetic tape for later playback at $\frac{1}{4}$ or $\frac{1}{8}$ speed on to a pen recorder. Spontaneous contractions of muscles in the nerve cord sometimes made recording difficult. Such contractions were stopped by using saline in which the Mg concentration had been raised to 1–5 mM by replacing an equivalent amount of Na.

Horseradish peroxidase (HRP) injections were made using the general technique of Muller & McMahan (1976). 2% HRP was injected via bevelled glass micropipettes and allowed to diffuse for 1–3 h. The ganglia were then fixed for 15 min in 2% glutaraldehyde (in 0·1 M phosphate buffer, pH 7·4), rinsed 15 min in buffer, incubated with 0·5% diaminobenzidine tetra-HCl (in buffer) for 15 min, and developed under visual observation by adding a few drops of 1% H_2O_2. *Camera lucida* drawings of injected cells were made from ganglia in whole mount.

RESULTS

This report concerns the properties of an identified neurone in the segmental ganglia of the leech, designated cell 204. This neurone's morphology, electrical properties, and role in the generation of swimming are discussed in the following sections.

(1) *Morphology and electrical characteristics of cell 204*

One cell 204 soma is present in each segmental ganglion in the antero-medial cell packet, one of two unpaired cell packets which, together with four paired packets, comprise the ganglion (Fig. 1 A). Cell 204 has been found in every one of the 16 ganglia in which it was sought; therefore, we assume that it is present in all 21 segmental ganglia. This neurone was named according to a previous convention (Ort *et al.* 1974), by which all unidentified neuronal somata in the antero-medial cell packet were numbered consecutively in an arbitrary order, starting with number 201 (Fig. 1 B). The neurone described herein was assigned the number 204 based upon its approximate location the first few times it was found. In fact, its cell body occupies a variable position within the cluster of small neurones, designated cells 201 to 218, that lie posterior to the Retzius cell pair. As is true for other leech neurones designated by such a numerical scheme, positive identification of cell 204 was based on physiological criteria (i.e. action potential shape, effects on swimming) rather than strictly on soma position.

Cell 204 is an intersegmental interneurone, as determined both by visualization of the cell by horseradish peroxidase (HRP) injection (Fig. 1 C) and by extracellular recordings from the connectives and segmental nerves. Fig. 1 C shows the branching pattern typical of cell 204 in its ganglion of origin. The cell body (20–25 μm in diameter) gives off a main neurite which bifurcates into two prominent axons. These axons project both anteriorly and posteriorly via Faivre's Nerve, a small, unpaired bundle of axons which lies ventro-medially to two larger, paired bundles. These three axon bundles are held together by connective tissue and constitute the con-

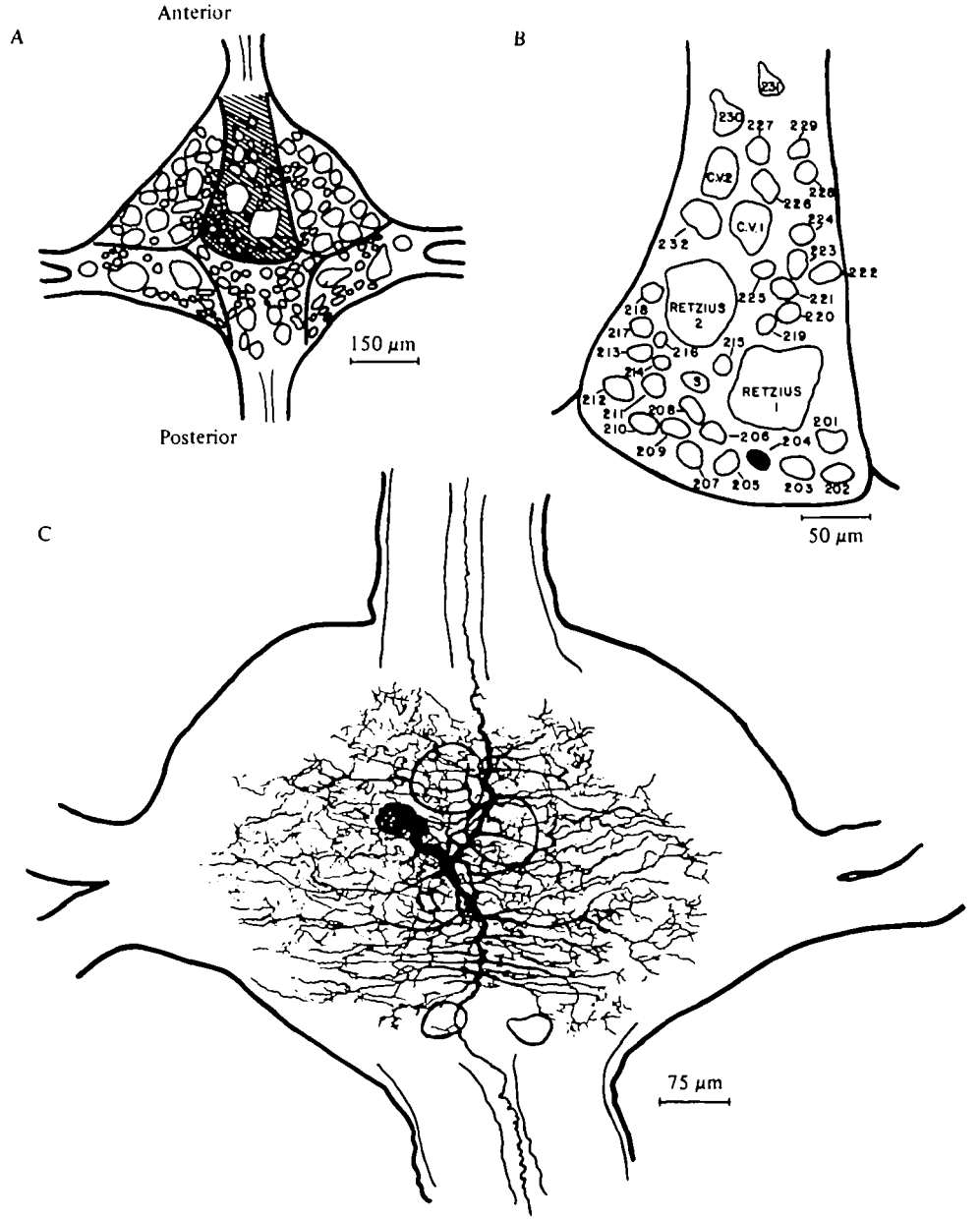


Fig. 1. Location and morphology of cell 204. (A) Cell body map of the ventral aspect of a midbody segmental ganglion of *H. medicinalis*. The anterior and posterior interganglionic connectives are indicated, and the segmental nerves exit at left and right. The six cell packets are outlined, and the antero-medial cell packet is crosshatched. A typical cell 204 location is blackened. (B) Enlargement of the antero-medial cell packet, showing the numbering system used to designate cell body positions. Cells previously identified and named are the S cell (Frank *et al.* 1975), CV 1 and 2 (exciters of the ventral circular musculature, Stuart, 1970), and Retzius cells (Retzius, 1891). (C) Camera lucida tracing of cell 204 injected with horseradish peroxidase. The cell shown is cell 204 in ganglion 14 (204(14)). Ganglion orientation is the same as that shown in (A) and (B). The two cell pairs drawn in for reference are the Retzius cells (antero-medial cell packet) and annulus erector motor neurones (postero-medial cell packet) (Stuart, 1970). The soma position of cell 204 is variable but, as discussed in the text, positive identification is possible in spite of this due to the cell's physiological properties. The small medial axon bundle in the connectives which contains cell 204's axons is Faivre's Nerve.

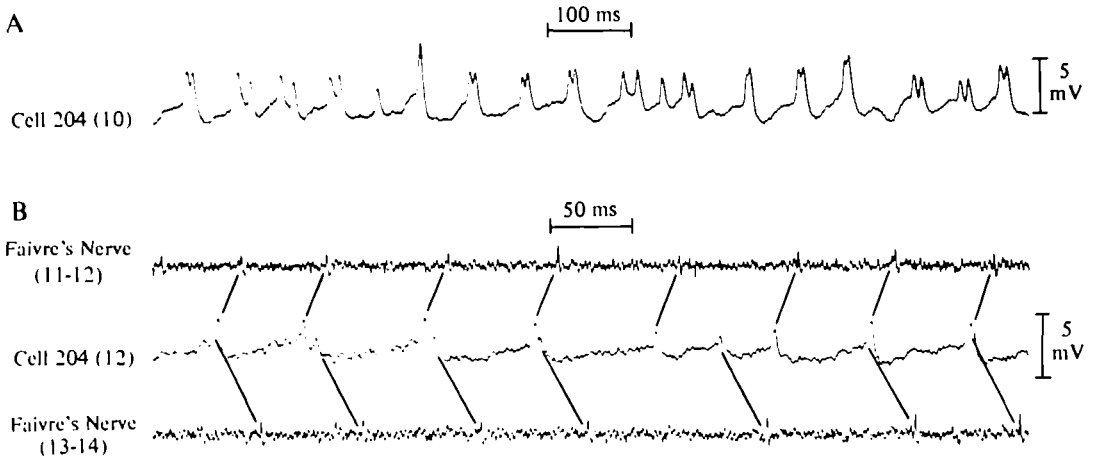


Fig. 2. Cell 204's action potential. (A) Intracellular recording of cell 204, taken from cell 204(10) in an isolated 10th ganglion during injection of depolarizing current. (B) Cell 204's action potentials recorded in Faivre's Nerve. The top and bottom traces are extracellular recordings from suction electrodes placed on the cut end of Faivre's Nerve (FN) anterior to ganglion 12 (FN 11–12) and posterior to ganglion 13 (FN 13–14). The middle trace is an intracellular recording from cell 204(12) during injection of depolarizing current. The lines match intracellular action potentials with their occurrences in Faivre's Nerve, either in the adjacent anterior connective (FN 11–12) or one segment posterior (FN 13–14).

nectives between adjacent ganglia. HRP injections showed no cell 204 processes in the segmental nerves, and no cell 204 impulses could be recorded from the nerves of any ganglia. The intersegmental axons were traced histologically only to the nearest ganglia, but recordings indicated that these processes extend at least three segments in each direction. Secondary processes from the main neurite and axons extended into virtually the entire neuropile region and, when viewed in whole mount, were found to lie within a restricted horizontal plane near the dorsal surface of the ganglion. Similar planar arrangements of sensory neurone processes in leech ganglia have been observed (E. R. Macagno, personal communication).

Intracellular recordings from cell 204 revealed a resting potential of approximately −60 mV, upon which was superimposed a variety of synaptic activity. Fig. 2A presents a typical intracellular recording from cell 204 during injection of depolarizing current. The cell body was inexcitable, as is true for most leech neurones, and action potentials recorded in the cell body were attenuated in amplitude to 5–15 mV. Fig. 2B shows that action potentials were associated at constant latency with extracellularly recorded spikes in the anterior and posterior Faivre's Nerve. Small-amplitude action potentials were associated with a spike in one or the other Faivre's Nerve, and when two small action potentials superimposed, the resultant large-amplitude action potential was associated with spikes in both Faivre's Nerves. Among cells in the antero-medial cell packet, this variation in action potential waveform was a unique attribute of cell 204. Thus, unambiguous identification of the neurone is possible independent of its properties related to swimming activity.

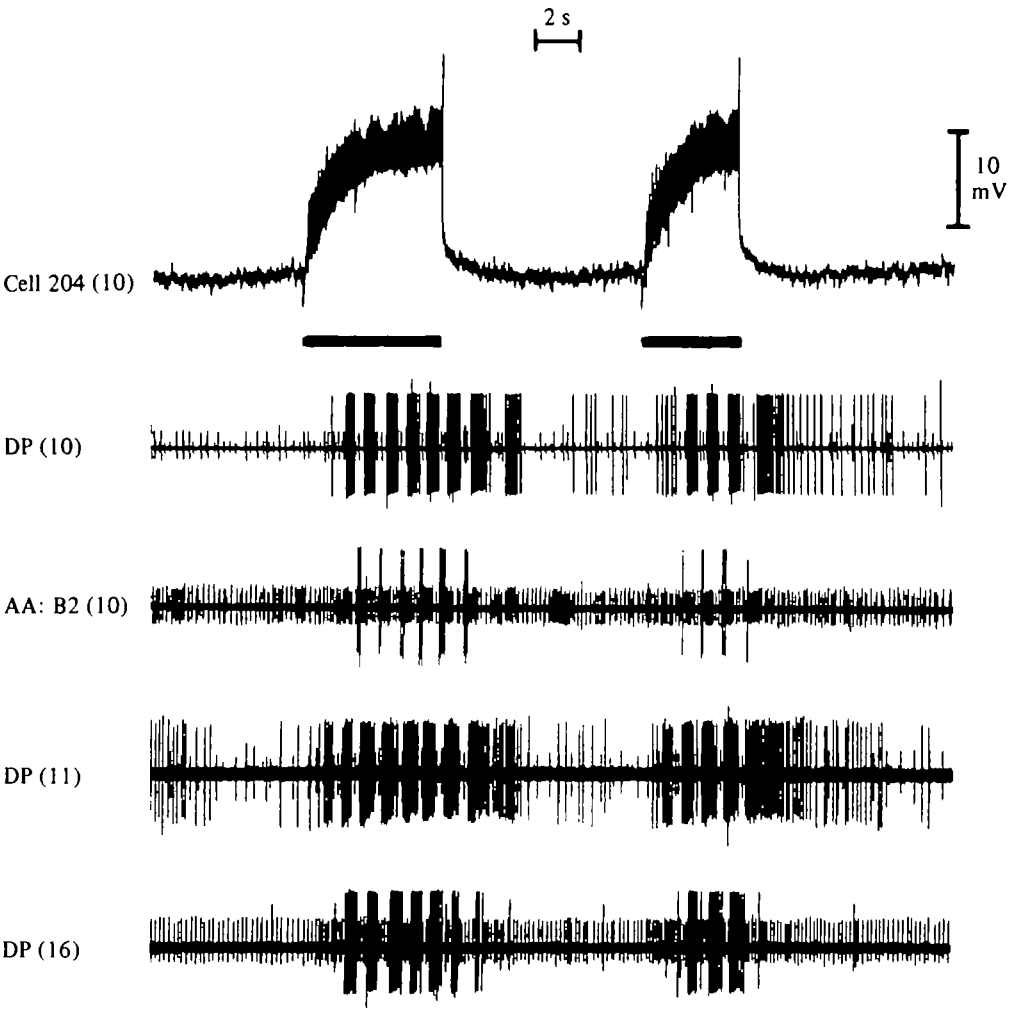


Fig. 3. Swimming episodes produced in the isolated nerve cord by stimulation of cell 204. The upper trace is an intracellular recording from cell 204(10). Bars indicate the times during which a constant level of depolarizing current was injected into the cell. The lower four traces are extracellular recordings from various segmental nerves; the recordings from the dorsal branch of the posterior nerve in segments 10, 11 and 16 (labelled DP(10), DP(11), and DP(16)) show bursts from cell 3, a dorsal longitudinal body wall exciter motor neurone, and the large spikes recorded from nerve AA:B2(10) are from a ventral exciter motor neurone, cell 108.

(2) *Cell 204 activity elicits swimming*

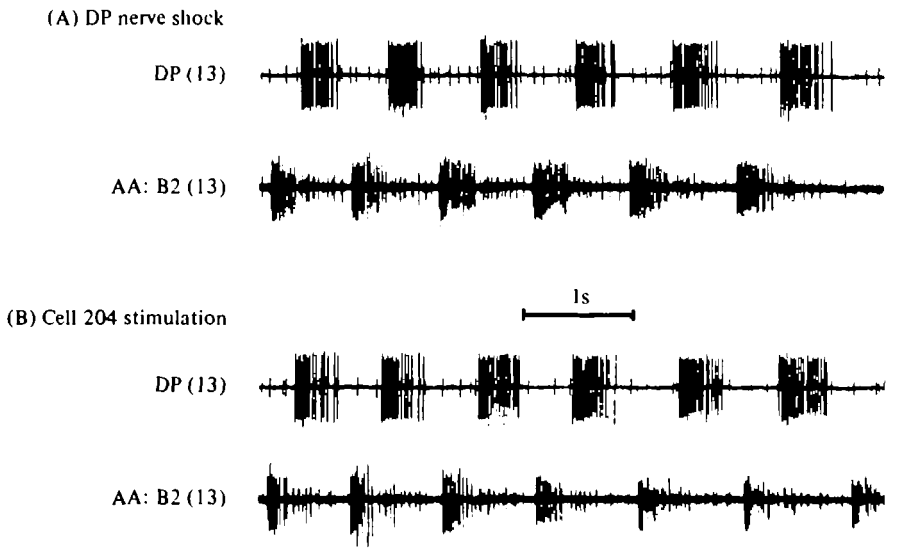
When any cell 204 in the isolated nerve cord was stimulated intracellularly to produce impulses in the range of 10–30/s, motor neurone activity characteristic of swimming was observed in either *H. medicinalis* or *M. decora* preparations. Similar results were obtained whether cell 204 was induced to fire by continuous injection of depolarizing current, by repeated depolarizing pulses, or by post-hyperpolarization rebound. Every cell 204 investigated in 24 isolated cord preparations (85 ganglia) was found capable of driving swimming activity.

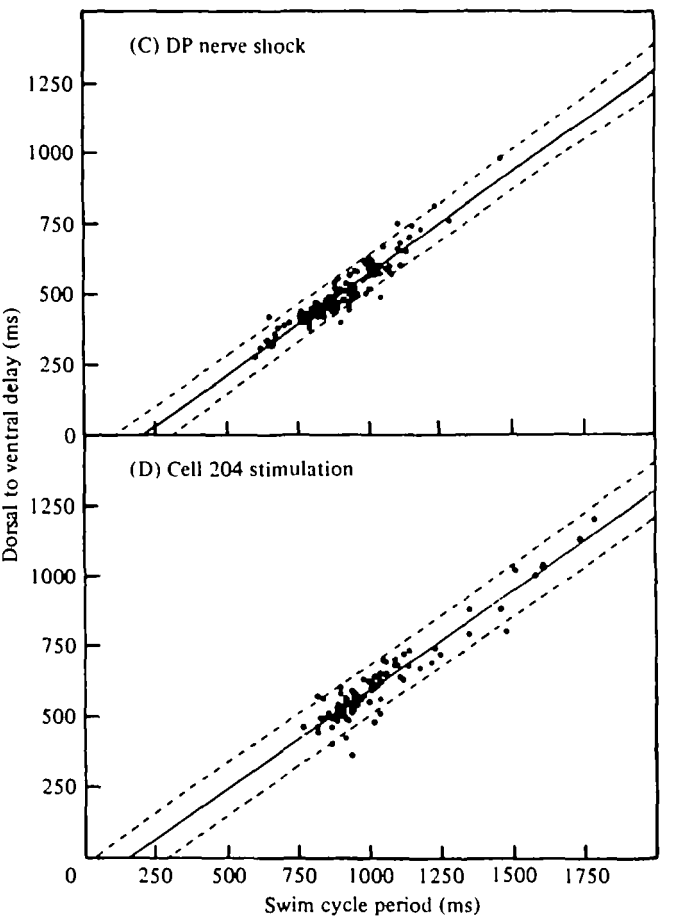
In any ganglion, the neuronal swimming pattern induced by cell 204 activity consisted of a repeating cycle of alternating bursts of impulses in dorsal and ventral longitudinal muscle motor neurones. Fig. 3 illustrates that depolarization of cell 204 in segment 10 (204(10)), to increase its impulse frequency to approximately 20/s, caused the swimming activity pattern in three different ganglia. In fact, such activity in any cell 204 evokes normally co-ordinated swimming activity along the entire nerve cord. The motor neurone pattern always commenced in the most anterior segment of the preparation, and progressed metachronically through successively more posterior segments. This pattern corresponds to the rearward-moving body wave in the swimming animal. Swimming episodes generally continued for at least the duration of cell 204 stimulation.

To assess the degree of similarity between swimming episodes induced by cell 204 stimulation and those previously described in the isolated nerve cord (Kristan & Calabrese, 1976), segmental nerve recordings were obtained from the same isolated cord preparation during episodes initiated by two methods: application of a short train of shocks to the dorsal branch of the posterior (DP) segmental nerve, and sustained depolarization of a cell 204. Kristan & Calabrese found that DP nerve shock was the most effective stimulus for eliciting swimming episodes from the isolated cord or semi-intact preparations. Figs. 4A and 4B illustrate that, for both stimulation methods, an antiphasic relation of dorsal and ventral exciter motor neurone bursts was observed, and that swim cycle periods, burst durations, and average interspike intervals during bursts were similar. Both stimulation methods caused the motor neurone pattern to commence in the most anterior segment. The only consistent difference was a tendency for the ventral exciter impulse bursts to contain fewer impulses during episodes driven by cell 204 stimulation. The duration of swimming episodes following DP nerve shock was generally 10–20 cycles. When cell 204 was kept depolarized, however, episodes lasting hundreds of cycles could be produced.

Figs. 4C and 4D are analyses of the motor neurone impulse patterns shown in Figs. 4A and 4B, respectively. To obtain these figures, the delay from the midpoint of a dorsal exciter burst to the midpoint of the next ventral exciter burst (the dorsal to ventral delay) was plotted against the delay between successive dorsal exciter burst midpoints (swim cycle period). As has previously been shown for semi-intact and isolated cord preparations (Kristan & Calabrese, 1976), dorsal to ventral delay increased with increasing swim cycle period. The similarity between the regression lines for the two data sets indicates that there were no major differences in the relative timing of the dorsal and ventral motor neurone bursts during swimming activity initiated by the two stimulation methods. Since both regression lines had an intercept on the period axis that was significantly greater than zero ($P < 0{\cdot}05$), these activity patterns fall into the 'period dependent' category of Kristan & Calabrese (1976), in which the ratio of the dorsal to ventral delay to the cycle period (termed the dorsal to ventral phase lag) is dependent upon the period.

The comparisons presented in Figs. 4 A–D show that the neuronal activity pattern observed in the isolated nerve cord in response to cell 204 stimulation was essentially the same as the pattern observed in response to DP nerve shock, previously shown to constitute the neuronal activity analogue of swimming. The body movements


(A) DP nerve shock
DP (13)
AA: B2 (13)
1s
(B) Cell 204 stimulation
DP (13)
AA: B2 (13)


(C) DP nerve shock
(D) Cell 204 stimulation
Dorsal to ventral delay (ms)
0
250
500
750
1000
1250
1500
1750
Swim cycle period (ms)

observed during swimming in minimally dissected, semi-intact preparations of both *H. medicinalis* and *M. decora* were the same when produced in response to tactile stimulation, DP nerve shock or cell 204 stimulation, and were the same as those of freely behaving, intact animals.

Cell 204 activity was also capable of evoking the swimming pattern from abbreviated lengths of nerve cord containing as few as four ganglia. Previous studies have demonstrated swimming activity only in preparations containing at least 6–8 ganglia (Friesen *et al.* 1978; Kristan & Calabrese, 1976). Thus, cell 204 stimulation reliably evokes what may confidently be identified as swimming in the complete isolated cord, abbreviated isolated cord, and semi-intact preparations.

(3) *Cell 204 activity during swimming episodes*

The activity of cell 204 was investigated during swimming episodes initiated by various means. Figure 5 shows recordings from cell 204 in two ganglia, obtained when swimming was initiated in an isolated nerve cord by shocking a DP segmental nerve. Prior to the stimulation, both cells' membrane potentials were fluctuating due to synaptic input, but impulses were produced only rarely. In response to DP nerve stimulation, both cells became depolarized and began producing impulses. Such excitation of cell 204 in response to DP nerve shock was observed in ganglia up to 6 segments distant from the site of stimulation. During the ensuing swimming episode, cell 204 in both ganglia remained depolarized and produced periodic bursts of impulses in the ventral contraction phase of swimming. In Fig. 5 and successive figures only one DP nerve recording is presented to monitor swimming activity, but in all cases the normal antiphasic dorsal and ventral motor neurone impulse bursts occurred along the entire nerve cord. Simultaneous recordings from various ganglia along the nerve cord during swimming episodes indicated that every cell 204 behaved essentially identically; that is, every one was depolarized and producing bursts of impulses in the ventral contraction phase of its own segment. This pattern of cell 204 activity was seen regardless of the initiating stimulus, whether it was shock to the DP or other segmental nerves, stimulation of a cell 204 (see below), stroking the skin of a semi-intact animal, or no overtly applied stimulus during 'spontaneous' swimming episodes.

Fig. 4. Comparison of swimming activity pattern during episodes initiated by DP nerve shock and cell 204 stimulation. (A, B) Records from the same segmental nerves of an isolated nerve cord preparation during swimming episodes initiated by DP nerve shock (A) and cell 204 stimulation (B). DP(13) segmental nerve recordings show impulse bursts from a dorsal exciter motor neurone, cell 3, and the ventral phase impulse bursts in AA:B2(13) are from cell 108, a ventral exciter. To initiate swimming episodes, the right and left DP nerves in segments 15 and 16 were simultaneously shocked (A) or 204(13) was tonically depolarized (B). (C, D) Plots of the delay between midpoints of dorsal and ventral exciter impulse bursts (dorsal to ventral delay), as a function of the delay between successive dorsal exciter burst midpoints (swim cycle period). The data are based on 135 swim cycles during 21 swimming episodes initiated by DP nerve shock, and 99 swim cycles from 13 episodes during cell 204 stimulation, from the preparation whose recordings are shown in (A) and (B). The solid line in each plot was derived by linear regression. For (C) it is described by $d = 0{\cdot}72P - 154$, and for (D) by $d = 0{\cdot}71P - 115$, where d is the dorsal to ventral delay and P is the swim cycle period. The standard deviation of the slope is 0·023 for (C) and (D) and the standard deviation of the intercept is 21 for (C) and 24 for (D). Dotted lines indicate the 95% confidence limits for the distribution of the points about the lines.

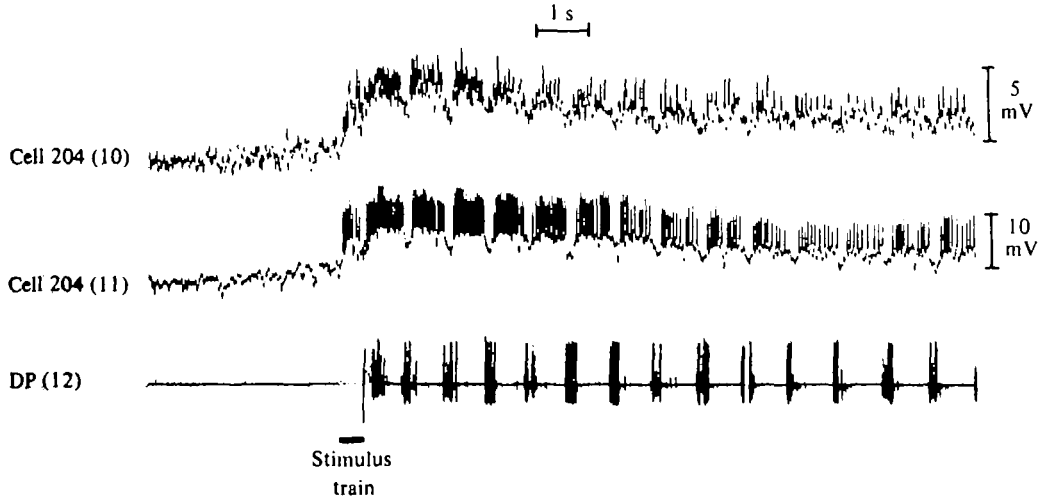


Fig. 5. Activity of cells 204 during a swimming episode initiated by shock to a segmental nerve. The upper two traces are intracellular recordings from cells 204(10) and 204(11) in an isolated nerve cord preparation. The bottom trace is an extracellular recording from segmental nerve DP(12) showing impulse bursts from a dorsal exciter motor neurone, cell 3. The bar indicates the duration of the stimulus train (5 ms pulses at 15 Hz) delivered to DP(12). In this and the following figures, only one segmental nerve recording is presented to indicate when swimming activity is occurring along the entire isolated nerve cord.

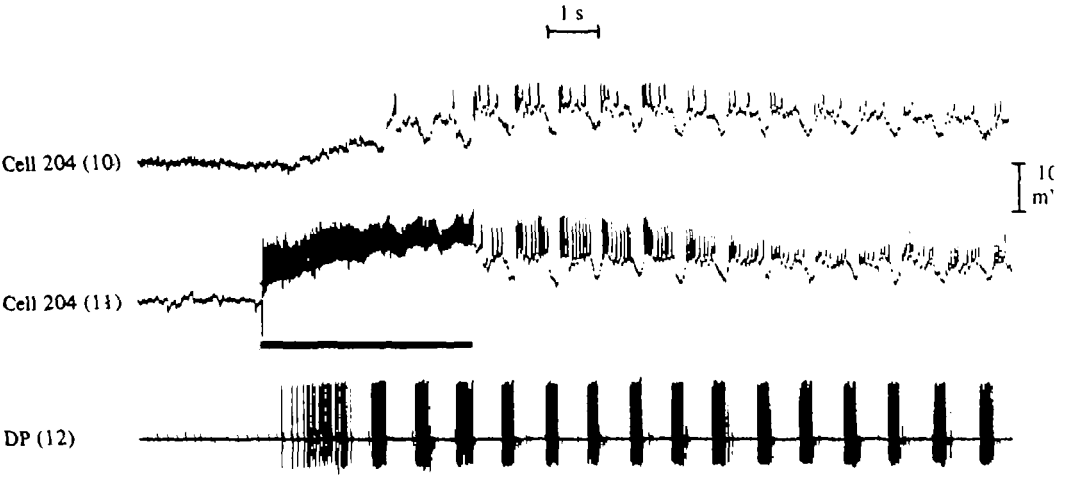


Fig. 6. Activity of two cells 204 during a swimming episode initiated by depolarizing one of them. The upper two traces are intracellular recordings from cells 204(10) and 204(11) in an isolated nerve cord preparation. The bar indicates the time during which a square pulse of depolarizing current was injected into 204(11). The lower trace is an extracellular recording from segmental nerve DP(12) showing impulse bursts from a dorsal exciter, cell 3.

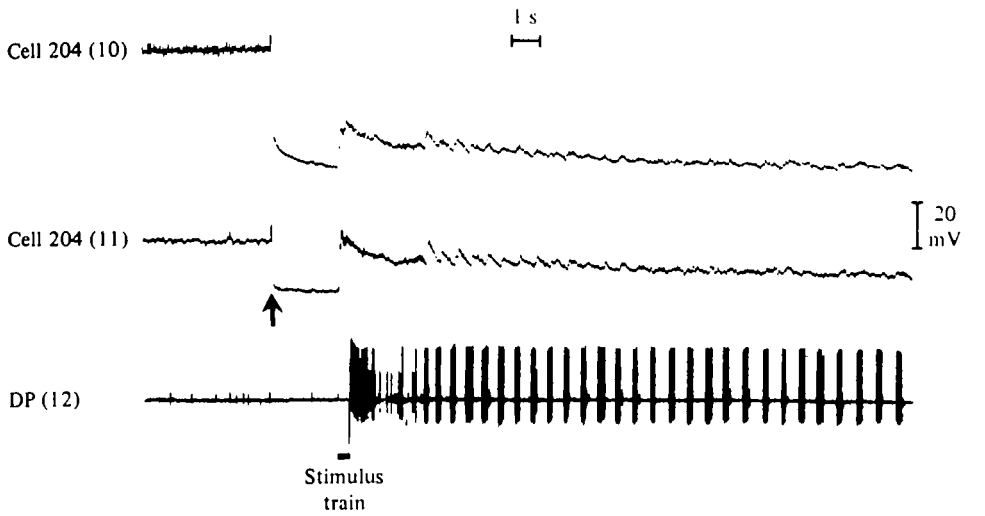


Fig. 7. Swimming episode occurring during hyperpolarization of two cells 204. The upper two traces are intracellular recordings from cell 204(10) and 204(11) in an isolated nerve cord preparation. Beginning at the arrow, hyperpolarizing current sufficient to prevent intracellularly recorded impulse activity was injected into both cells for the duration of the time shown. During hyperpolarization, the bridge circuits for current passage were not accurately balanced. The bottom trace is an extracellular recording from segmental nerve DP(12), showing bursts from a dorsal exciter, cell 3. The bar indicates the duration of the stimulus train (5 ms pulses at 15 Hz) delivered to DP(12).

When swimming was elicited by injection of depolarizing current into a cell 204, activity in the other cells 204 appeared as is shown in Fig. 6. Initially, the stimulated cell discharged tonically and there was no effect on the other cell. Then, after a delay of about 750 ms, both cells became progressively more depolarized and, as motor neurone impulse bursts first appeared in the segmental nerves, membrane potential oscillations began in both cells. Stimulation of one cell 204 had no direct effect upon any other cell 204, as tested by the ability of action potentials or membrane polarizations in one cell 204 to produce a membrane potential change in the cell bodies of other cells 204. The delay between the onset of cell 204 stimulation and the initiation of swimming activity varied inversely with the impulse frequency of the stimulated cell 204. Swimming episodes sometimes persisted after stimulation of cell 204 was discontinued (Fig. 6), and sometimes did not (Fig. 3), even within the same preparation. It is probable that some uncontrolled 'excitability' of the preparation determined which phenomenon would occur at any given time.

Since swimming could be driven by stimulating any cell 204 in the nerve cord, every cell 204 can be considered *sufficient* for the initiation and maintenance of swimming activity. However, this result does not indicate whether the activity of individual cells 204 is *necessary* for the expression of swimming behaviour. This was tested by inactivating cells as effectively as possible by injection of hyperpolarizing current into their cell bodies. Fig. 7 shows that hyperpolarization sufficient to suppress impulse activity in cell 204 in two ganglia did not prevent the isolated cord from producing swimming activity in response to DP nerve shock. The same result was obtained when DP nerve shock was applied in a segment containing a hyperpolarized cell 204. There

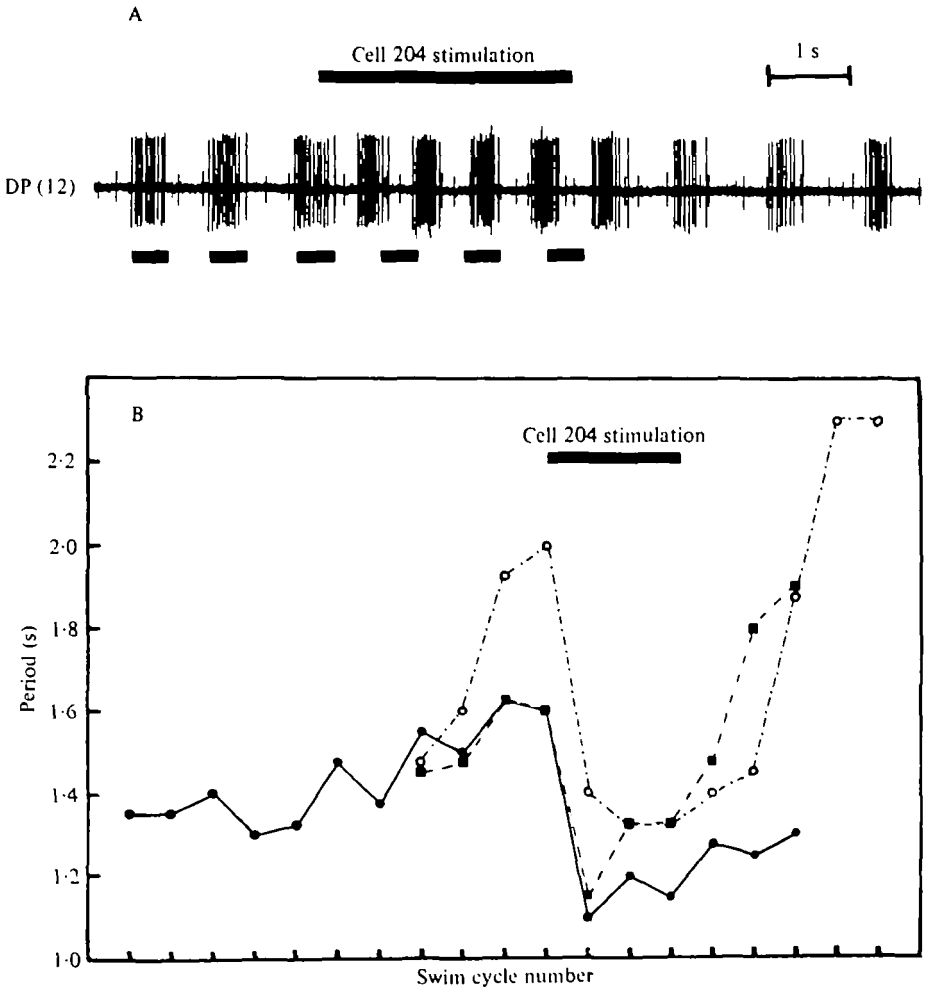


Fig. 8. Decrease of the swim cycle period during an ongoing swimming episode by increasing the impulse frequency of cell 204. (A) Extracellular recording from segmental nerve DP(12) showing impulse bursts from a dorsal exciter motor neurone, cell 3. Simultaneously, intracellular recordings were made from cells 204(10) and 204(11) (not shown). The swimming episode was produced by tonic depolarization of cell 204(11), causing it to produce impulses at ~ 35 Hz. Average swim cycle period was approximately 1·6 s. For the duration of time indicated by the upper bar, the impulse rate of cell 204(10) was increased from ~ 3 to 40 Hz by injection of a train of depolarizing pulses. Short bars beneath the trace indicate the expected occurrences of motor neurone bursts, based on the swim cycle period prior to cell 204(10) stimulation. (B) Graph of swim cycle period vs. swim cycle number for the trial shown in (A) (open circles) and two additional stimulus presentations to the same preparation. The data has been shifted along the swim cycle number axis to line up the onset of the three stimulus trains. Period was measured between midpoints of successive impulse bursts in cell 3(12). In all three cases, swimming activity was produced by tonic depolarization of cell 204(11), and during the time indicated by the bar, depolarizing pulses were passed into cell 204(10) to increase its impulse frequency to 40 Hz. In the episode represented by closed circles, the impulse frequency of cell 204(11) was ~ 20 Hz and unstimulated impulse frequency of cell 204(10) was ~ 10 Hz; and in the episode represented by closed squares, the impulse frequency of cell 204(11) was ~ 20 Hz and unstimulated impulse frequency of cell 204(10) was ~ 5 Hz.

was no disruption of the motor neurone activity pattern in segments containing a hyperpolarized cell 204, nor was there any observable alteration of the co-ordination of the swimming rhythm along the nerve cord. The hyperpolarization delivered was at least as great as that found in other tests to prevent the appearance of cell 204 action potentials in the adjacent connectives. Given the extensive arborizations and intersegmental processes of cells 204, however, it is uncertain whether their interactions with other neurones were entirely eliminated. Regardless, since we have observed that DP nerve shock excites cells 204 in many adjacent ganglia, swimming activity may well have been mediated by unhyperpolarized cells 204 in other ganglia.

We conclude from these experiments that while any cell 204 is *sufficient* to drive swimming, impulse activity in at least two of them is *unnecessary*. The ideal manner in which to test the necessity of all cells 204 would be to hyperpolarize every one of them and attempt to elicit swimming activity by nerve stimulation. However, this approach presents obvious technical difficulties which are currently insurmountable.

(4) *Cell 204 impulses can alter the swim cycle period*

During swimming activity induced by depolarization of one cell 204, intermittent depolarization of another cell 204, to increase the latter's impulse frequency for a few swim cycles, caused a decrease in the swim cycle period. This is exemplified in Fig. 8A by a decrease in period of the impulse bursts of a dorsal exciter motor neurone, cell 3. Since the cycle period in all segments was equal at any given time, the entire cord experienced a resetting of the phase of the swimming rhythm and a decrease in cycle period during the time of stimulation of any cell 204. During swimming episodes initiated by nerve stimulation, imposed increases in impulse frequency in one or more cells 204 also decreased cycle period. Increasing the impulse frequency of two cells 204 during swimming almost invariably accelerated the swimming rhythm more than when just one cell 204 was stimulated, and never caused slowing of the rhythm.

To further illustrate the effect of cell 204 activity on the swimming rhythm, Fig. 8B plots swim cycle period against cycle number for the trial shown in Fig. 8A, along with two other stimulus presentations to the same preparation. For these three trials, the first cycle period following onset of cell 204 stimulation averated 30% less than the cycle period immediately preceding stimulation. This represents a major change in period compared to the normal cycle-by-cycle variation in period observed in the absence of cell 204 stimulation. Typically, this variation is less than 10%, and normally the cycle period tends to *increase* with cycle number during any swimming episode (Kristan & Calabrese, 1976). After termination of cell 204 stimulation, the cycle period increased again towards its unstimulated rate. This increase was not always immediate, and in many cases the decreased swim cycle period outlasted the stimulus duration by several cycles.

The degree of cycle period shortening induced by cell 204 stimulation depended both upon the absolute impulse frequency to which the cell was driven, and upon the percentage increase in impulse frequency that the stimulation produced. For instance, increasing cell 204 activity to 40 Hz led to a greater decrease in the cycle period than did stimulating at 30 Hz. However, the cycle period shortening resulting

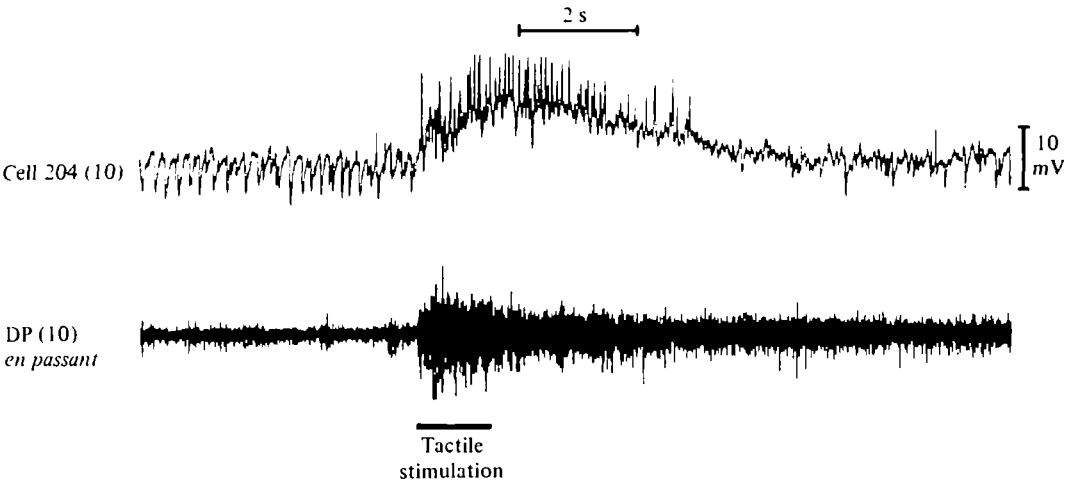


Fig. 9. Excitation of cell 204 by tactile stimulation. The upper trace is an intracellular recording from cell 204(10) in an isolated cord preparation connected by DP(10) to a small flap of body wall. The lower trace is an *en passant* extracellular recording from DP(10). The bar indicates the time during which the skin flap was gently stroked with a blunt forceps.

from a 40 Hz stimulation was greater if the cell's unstimulated impulse rate was 5 rather than 20 Hz. The effect of cell 204 stimulation also depended upon the ongoing swim cycle period, with the same stimulating frequency causing more shortening of the cycle period if the cycle period was long rather than if it were already short. Hyperpolarization of a single cell 204 during swimming so as to decrease or halt its impulse activity tended to increase the swim cycle period of the entire nerve cord or prematurely halt the swim episode, but the effects of hyperpolarization were decidedly less than those of depolarization.

(5) *Tactile sensory input excites cell 204*

Fig. 9 shows the typical response of cell 204 to tactile stimulation of the leech's skin. An isolated nerve cord was left innervating a small flap of body wall via segmental nerve DP(10), and recordings were made from cell 204(10) and *en passant* from DP(10). On this occasion, the head and tail brains were left connected to the nerve cord but similar results were obtained when they were not. During the time indicated by the bar, the skin flap was gently stroked with a blunt forceps. This increased the activity recorded from the DP nerve, and excited cell 204 in that segment. A reduction in the frequency of the large inhibitory potentials observed prior to the tactile stimulation appeared in part responsible for the depolarization by approximately 10 mV of cell 204. Simultaneous intracellular recordings from cell 204 and the touch, pressure or nociception sensory neurones revealed no monosynaptic connexions from these mechanoreceptors on to cell 204, at least in the midbody ganglia thus far examined. Accordingly, activation of cell 204 by tactile sensory imput must be mediated polysynaptically or involve other, as yet unidentified, mechanoreceptor neurones.

DISCUSSION

(1) *Morphology of cell 204*

Most leech neurones occur as paired homologues, with one member of the pair on each side of the ganglion's antero-posterior axis of symmetry. When examined after HRP injection, paired neurones such as sensory neurones (Muller & McMahan, 1976), motor neurones (Muller & McMahan, 1976; Poon *et al.* 1978; Thompson *et al.* 1976*a*), swimming oscillator interneurones (Friesen *et al.* 1976; Friesen *et al.* 1978; Poon, 1976), and heartbeat co-ordinating interneurones (Thompson *et al.* 1976*b*) are seen to restrict their arborizations to one or two localized regions of the neuropile. Each member of such neurone pairs has a branching pattern which roughly mirrors that of its homologue. This is in contrast to the unpaired neurones which have been so examined, all of which lie medially in the two unpaired cell packets and have arborizations that are symmetric *about* the midline. These include cell 204 (Fig. 1), the M cell (Lent & Frazer, 1977; K. J. Muller, personal communication), and the S cell (Frank *et al.* 1975; Muller & Thompson, 1976). Faivre's Nerve contains the axons of both cell 204 and the S cell, whose axons from adjacent ganglia fuse, forming a multisomatic, septate axon called Rohde's fibre (Mistick, 1974) or the fast conducting system (Bagnoli, Bonnelli & Magni, 1972). Thus the possibility exists that unpaired cells are unique to the unpaired cell packets, and that the unpaired Faivre's Nerve may contain axons belonging only to unpaired cells.

(2) *Cell 204 as a 'command neurone'*

Operationally, cell 204 qualifies as a 'command neurone'; that is, when tonically stimulated, a stereotyped behavioural response ensues. Although this term provides a convenient shorthand for describing a cell's properties, we prefer not to use this designation in referring to cell 204, due to current inconsistencies in the literature as to what the term 'command neurone' implies. A recent attempt to define strictly the criteria used to designate neurones or systems of neurones as having a command function includes the requirements that their activity be both necessary and sufficient for the behaviour in question (Kupferman & Weiss, 1978). Since the sufficiency, but not the necessity, of cells 204 has been established, and since the functional relationship of these cells to the swimming central pattern generator is as yet undetermined, the designation of 'command neurone' seems premature.

The effects of cell 204 activity on the swimming motor programme show some similarities with those reported for other neurones involved with the production of rhythmic motor behaviour. For example, in the lobster swimmeret system, an increase in the number of command interneurones stimulated, or in the frequency at which they are stimulated, results in an increase in the motor output frequency (Davis & Kennedy, 1972*a*, *b*). In addition, the latency to onset of motor output is inversely related to the impulse frequency of the stimulated command interneurone (Wiersma & Ikeda, 1964). Recent studies have demonstrated neurones which initiate swimming in *Tritonia* (Getting, 1977), and feeding in both *Helisoma* (Granzow & Kater, 1977) and *Pleurobranchea* (Davis & Gillette, 1978). Because these behaviours are amenable to intracellular analysis, the mechanisms for command functions may soon be elucidated.

(3) *The role of cell 204 in swimming*

Two criteria have previously been used to identify neurones as putative members of the central pattern generator for leech swimming: the cell's membrane potential must oscillate with the swim cycle period, and passage of current into the neurone must reset the phase of the motor neurone activity cycles (Friesen *et al.* 1976). Although cell 204 meets these criteria, it differs from the previously identified oscillator cells in two major ways. First, stimulation of an oscillator cell does not evoke swimming activity. Second, depolarization of cell 204 accelerates the swimming rhythm, whereas depolarization of an oscillator cell slows the rhythm. This effect of oscillator cell depolarization results from the recurrent inhibitory connexions of the pattern generating network, such that depolarization of an individual oscillator cell tends to arrest the swim cycle in a particular phase and thus impedes the swimming rhythm.

The importance of cell 204's phasic activity pattern during swimming is not yet clear, especially since tonic impulse activity is sufficient for initiation, maintenance, and modulation of swimming. Cell 204 may exert its excitatory effects by its average impulse frequency over one or several cycles rather than by the temporal pattern of its activity (Wilson & Wyman, 1965). An analysis of the central oscillator for swimming, using electronic analogues, has shown that swim cycle period is inversely related to the tonic excitatory drive provided to the oscillator cells (Friesen & Stent, 1977). Cells 204 may provide this excitatory drive, since the swim cycle period of the entire nerve cord can be decreased by increasing the firing frequency of any cell 204 (Fig. 8). Since the central oscillator is the ultimate source of rhythmicity during swimming, it must be responsible at some level for cell 204's rhythmicity; for example, cell 204 could receive synaptic input preferentially from oscillator cells active in only one phase of the swimming cycle. This suggests a reciprocal excitatory relationship between cell 204 and the central oscillator which, by positive feedback, could maintain excitatory drive once a swimming episode had begun. Such positive feedback could explain why swimming episodes initiated by cell 204 sometimes outlast the period of cell 204 stimulation. The termination of a swimming episode could result from the waning of the positive feedback cycle which can be reinforced in the whole animal by sensory input, or in the isolated nerve cord by maintained stimulation of cell 204. We are currently seeking evidence for synaptic connexions between cell 204 and the oscillator cells, a technically difficult task because of the small size of the oscillator cells and their location on the opposite surface of the ganglion from cell 204. Evidence for mutual excitation between 'command' elements and pattern generating networks has been presented by Getting (1977) and Davis & Gillette (1978).

Thus far, the necessity of individual cells 204, or the population as a whole, for the generation of swimming has not been determined. Activity in the cell 204 system could represent the unique means of turning on the central pattern generator, in which case these cells would be both sufficient and necessary; alternatively, there could exist several activating systems in parallel, perhaps subserving different sensory modalities. Regardless, by elucidating cell 204's inputs from identified sensory neurones, and outputs to members of the central oscillator, we hope ultimately to characterize completely one pathway by which swimming behaviour is normally activated in the leech.

This study was supported by Public Health Service predoctoral traineeships 5T01 AI 0453-05 and 5T32 GM 07153-03 to J. C. W. and National Science Foundation Grant number BN 575-23567 to W. B. K. We thank Dr Ronald Calabrese for his many helpful discussions during the course of this work, Dr Otto Friesen, Dr Peter Getting, Dr Corey Goodman, Dr Daniel Hartline, Dr Allen Selverston, Dr Gunther Stent and Dr Wesley Thompson for their helpful comments, and Ms Mary Lee for her help in preparing the manuscript.

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