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THE NEUROMUSCULAR BASIS OF SWIMMING MOVEMENTS IN EMBRYOS OF THE AMPHIBIAN *XENOPUS LAEVIS*

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SUMMARY

When removed from their egg membranes, *Xenopus* embryos can swim. High-speed cinematography shows that, in swimming, lateral undulations pass rostro-caudally down the body. The swimming rhythm period is 40–100 ms. In swimming, electrical activity in myotomal muscles alternates on opposite sides of a segment and sweeps rostro-caudally in ipsilateral myotomes.

Myotome muscle physiology was examined. Muscle fibres are electrically coupled to each other, and the fibres are able to spike. The possible role of a myotomal conduction pathway in swimming is discussed.

INTRODUCTION

Swimming by means of undulations of the body has been studied most closely in fish. In this type of swimming, bends pass alternately rostro-caudally down either side of the body (Gray, 1933*a*). Electrical recordings from myotomal muscles in swimming animals (Grillner, 1974; Blight, 1976, 1977; Grillner & Kashin, 1976) have shown that there is a basic rhythmic alternating pattern on opposite sides of a segment. Along the body, ipsilateral myotomes are active in a rostro-caudal sequence, and this is thought to be the basis of the caudally propagating waves of bending.

In contrast to these results on fish it has been found (Blight, 1976, 1977) that in embryos of the newt *Triturus* waves of bending are not based on caudally spreading electrical activity in myotomes. Rather, it was found that activity in different ipsilateral segments began simultaneously. Blight suggested that waves of bending in newt embryos arise from the interaction of the passive mechanical properties of the body with the surrounding water.

We here examine swimming movements and underlying myotome activity in embryos of a different amphibian species, the clawed toad, *Xenopus*. This is part of a study of the central nervous mechanisms which underly the generation of swimming in this species. In contrast to the observations on *Triturus* embryos, it was found that

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caudally spreading waves of myotome activity underlie the waves of bending in *Xenopus* embryos at the developmental stage studied. Some of these results have been reported previously (Roberts *et al.* 1981)

MATERIALS AND METHODS

Experiments were carried out on embryos of the clawed toad, *Xenopus laevis*, at developmental stage 37/38 (Nieuwkoop & Faber, 1956). Eggs were obtained by injecting breeding pairs of adults with chorionic gonadotrophin and were left to develop in dechlorinated tapwater. Embryos were removed from their egg membranes before an experiment using fine forceps. All observations and experiments were at temperatures of 18–22 °C, unless otherwise indicated.

High-speed films of swimming

Several embryos were placed in a small dish of water, 80 mm in diameter and 5 mm deep (embryos at stage 37/38 are about 6 mm long). Swimming was evoked by a 2 ms electric shock between electrodes in the dish. This probably had its effect by stimulating a skin impulse (Roberts, 1971). Films were made at 300 frames/s using a Photosonic camera positioned above the dish. Water temperature was 21–22 °C. The swimming filmed was restricted to the initial, shorter cycles of a swimming episode; the cycle period was 44–53 ms.

For measurement of the angle of bending, the film was projected on to tracing paper on a glass table, and in each frame the midline of the body was drawn by eye. Starting from the front of the head, points were marked on this midline at spacing of 0·67 mm (the image was considerably enlarged to enable this). Adjacent points were joined by lines (Fig. 2, inset), thus dividing the body into 8 or 9 'segments' (the number depending on body length). The angle between pairs of segments was measured (Fig. 2, inset, angle θ).

To measure the lateral displacement of different points along the body relative to the base of the dish, points were marked off on the midline as above. The position of each of the points in successive frames was then recorded relative to a grid on the base of the dish. From this the distance between the maximum excursion to the left and to the right on a swimming cycle was measured for each of the different points on the body.

Swimming cycle period measurement in restrained embryos

Embryos were temporarily immobilized by chilling to about 6 °C, and anchored by wedging the gill region between pins pushed into a Sylgard layer in the base of a Petri dish. This left the trunk and tail free to make swimming movements. The embryo was anchored ventral side up to prevent the cement gland coming into contact with the floor of the dish (Stimulation of the gland inhibits swimming: Roberts & Blight, 1975).

Swimming was evoked by a 2 ms electric shock to the fin in the tail. Movements were monitored photoelectrically (Roberts & Blight, 1975). Recordings were made from five preparations and used to calculate the swimming cycle period.

Myotomal muscle activity in restrained embryos

Embryos were pinned on their sides to a Sylgard block with pins passing through the notochord. The block was mounted on a small perspex table (5 × 10 mm) pivoted to allow rotation of the embryo about its longitudinal axis. To make recordings from either side of the body, the embryo was rotated dorsal side up, and skin overlying the trunk myotomes on either side was dissected away with fine pins. For making recordings from two points on the same side of the body, the table was rotated so the side of the body was facing up, and skin overlying the myotomes in the trunk on this side only was removed. Embryos were bathed in saline, composition: NaCl 115 mM, KCl 2·5 mM, $CaCl_2$, 1·8 mM, $NaHCO_3$ 2·4 mM, saturated with 95% O_2, 5% CO_2 at pH 6·8 to 7·2.

Electrical recordings were made with extracellular suction electrodes (50–70 μm tip opening) positioned over the outer surface of the myotomes. The amplification techniques were conventional, recordings were taped for subsequent analysis. Recordings from the same side of the body were made in two preparations, and from either side of the body in a third preparation.

Electrical coupling between myotome fibres

Embryos were paralysed in saline containing 10^{-4} M D-tubocurarine chloride. The embryo was pinned on its side, and skin overlying rostral myotomes on one side was removed. Myotome fibres were penetrated with glass microelectrodes filled with 3 M-KCl, and of resistance 20–60 MΩ. One electrode was used for passing current into one fibre, a second for recording from a different fibre. Penetrations were made in the rostral trunk, in the third to seventh post-otic myotomes. Amplification techniques were conventional. Experiments were carried out on twelve preparations.

RESULTS

The swimming movements

When stimulated, *Xenopus* embryos swim rapidly through the water, stopping when they bump into the edge of the dish (Roberts, 1975). The embryo swims with lateral undulations of the body (Fig. 1), with forward velocities of between 4·5 and 6·1 cm s^{-1}.

On each swimming cycle, bends in the body developed first rostrally and later caudally, (Fig. 1). To determine more accurately the rate at which bends propagate caudally down the body, measurements were made of the local angle of curvature at different points along the body throughout several swimming cycles (Fig. 2*a*). From the time of bend onset at different points along the body the velocity of bend propagation can be calculated (Fig. 2*b*). In two swimming sequences, the delay in bend onset was 6·3 and 8·0 ms.mm^{-1} of the body. This is equivalent to velocities of 15·9 and 12·5 cm s^{-1} respectively. The mean cycle periods for these two sequences were respectively 44·4 and 53·3 ms, the measurements were made over a period of 4–4½ swimming cycles. In fish the velocity of the travelling wave on the body is faster than the velocity of forwards progression of the animal (Gray, 1933*b*). This was also

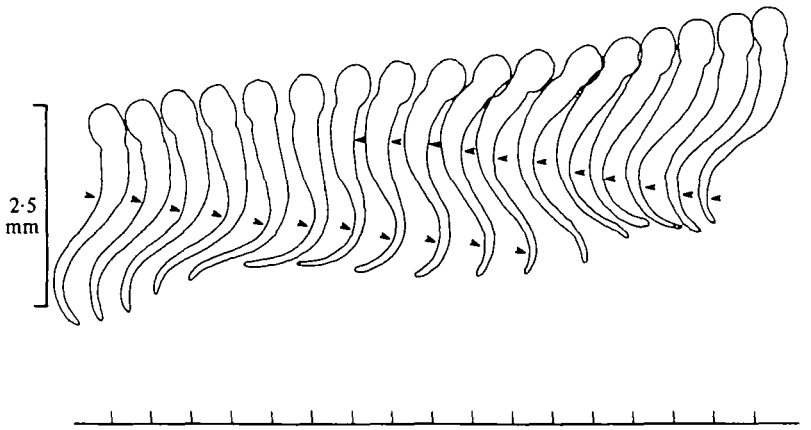

Fig. 1. Tracings of swimming movements in stage 37/38 *Xenopus* embryos, released from egg membranes, from film taken at 300 frames/s. Waves of bending pass alternately down either side of the body (arrowheads). The sequence reads from left to right, each frame is displaced to the right by the distance shown on the scale divisions of the baseline. Axis of progression is inclined slightly to the right.

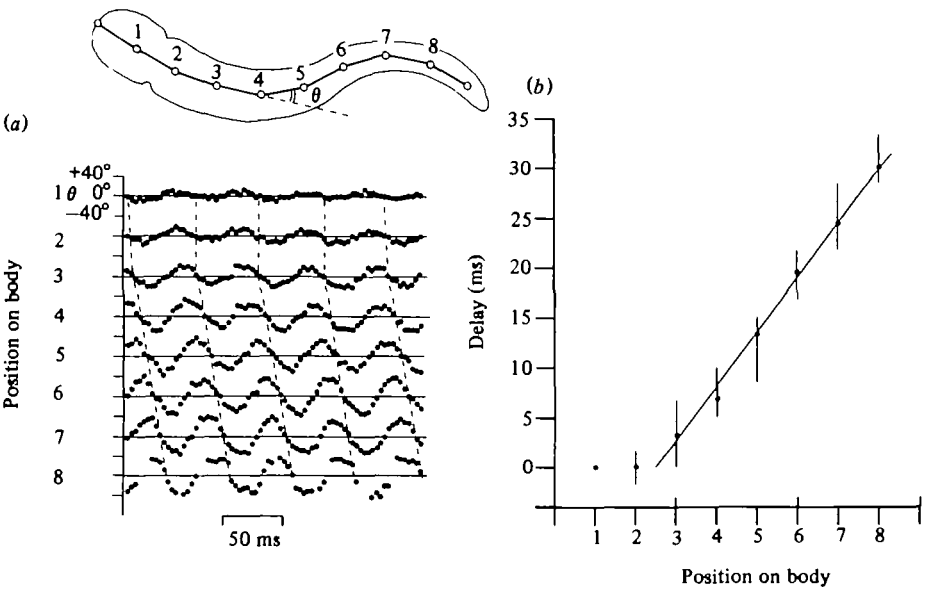

Fig. 2. Measurement of caudal propagation of bends in swimming. (*a*) Angle θ between adjacent 'segments' (Methods) is plotted frame by frame (frame interval 3·3 ms) for 4½ swimming cycles. (*b*) Plot of latency in bend onset (dashed line in (*a*)) relative to onset at position (1) for different positions on the body. Points are means with ranges for each half cycle in sequence in (*a*).

true for the embryos in the present study. In the two sequences measured the velocities of forward movement were fractions of 0·36 and 0·39 of the velocities of the travelling waves.

In swimming, each point on the body moves not only forwards, but also from side to side. In fish, the amplitude of the lateral movement is much less at the head end

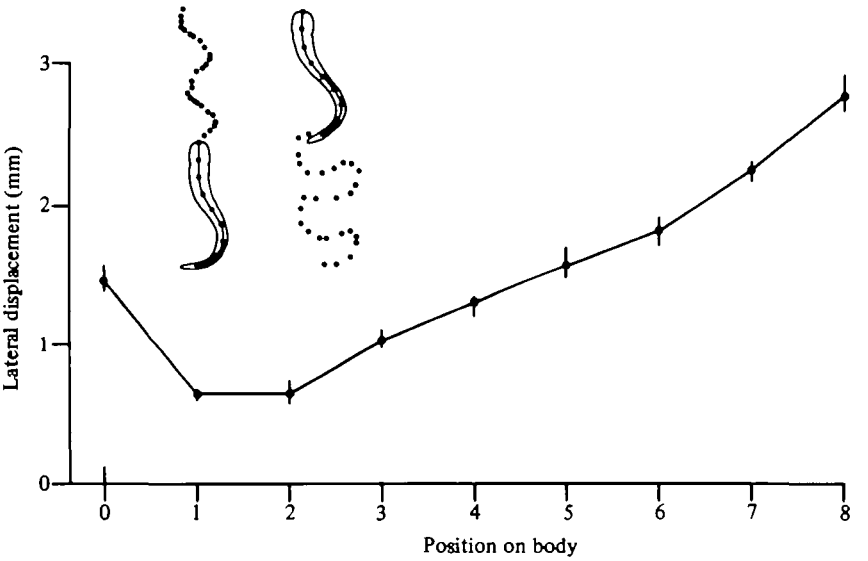

Fig. 3. The amplitude of lateral movements of different parts of the body in swimming, from films at 300 frames/s. Graph plots amplitude (see inset) at nine different points on the body (Methods) for one sequence of four swimming cycles. Points numered 0 at front of head to 8 near tip of tail. Amplitude is lowest just behind head and greatest at end of tail, similar curves were obtained in two other sequences, though absolute values differed. Points on graph are means with ranges.

of the body than in the tail (Bainbridge, 1963). Measurements of lateral movements of points on the body relative to the base of the dish shows that this is also true for the embryo (Fig. 3). However, it is also apparent from Fig. 3 that the lateral movements of the front of the head are quite large; this has also been noted in the newt embryo (Blight, 1976, 1977).

When left to swim freely in a dish, embryos rapidly reach the edge and then stop swimming. Therefore, in order to determine the overall range of swimming cycle periods that embryos are capable of producing in sustained swimming, a restrained preparation was used. These responded to a brief electric shock, which probably evoked a single skin impulse (Roberts, 1971), with an episode of swimming lasting usually between 5 and 65 s. The range of swimming-cycle periods was similar for different episodes in the same preparation and in different preparations. At room temperature, 18–22 °C, cycle periods ranged from about 40 to 100 ms. During a single episode, the cycle period usually increased gradually (Fig. 4*a*).

Cycle periods in the swimming rhythm were shorter at higher temperatures. This is shown for two different embryos in Fig. 4(*b*), where swimming episodes at different temperatures are compared by plotting the shortest cycle period in each episode. The short-cycle periods seen in *Xenopus* embryos are found also in the swimming of other amphibian embryos (*Triturus*; Blight, 1976). In fish embryos and young larvae, even shorter cycle periods, as brief as 20 ms, have been reported (Eaton *et al.* 1977; Hunter, 1972).

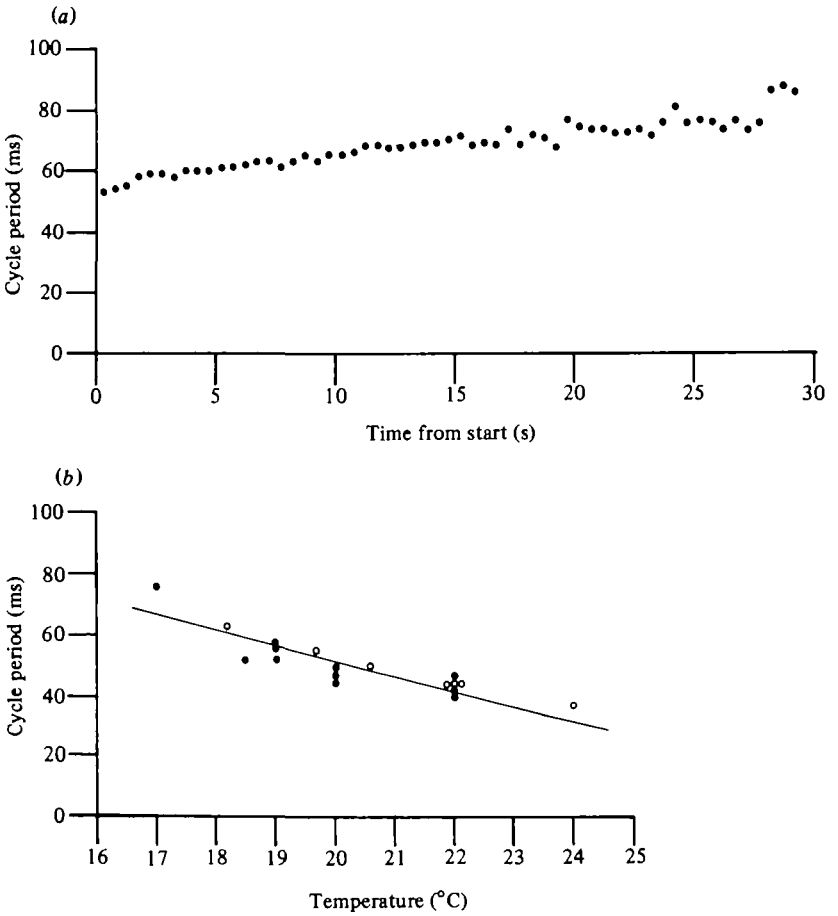

Fig. 4. Measurements of swimming cycle period using restrained embryos. (*a*) Plot of cycle period against time from start of episode for one swimming episode at 18·5 °C. Each point is the average cycle period for each half second interval of the episode. (*b*) Plot of the shortest cycle period in swims at different temperatures. Points represent mean cycle period for 1 s of swimming at the time of episodes when cycle period was shortest. Results from two embryos (○ and ●). Line fitted by eye. Temperature was changed by adding hot or cold water to the bath, measured with a thermistor in the bath. For both embryos the correlation coefficient between temperature and cycle period is significant ($P < 0{\cdot}001$).

The neuromuscular basis of swimming

Myotomal muscle activity in swimming

To determine the pattern of muscle activity that underlies the swimming movements, recordings were made from the myotomal muscles. Because the embryos are very small, it would be difficult to implant electrodes and allow the embryo to swim freely, and so a restrained preparation was used, where sensory inflow could have differed from that in normal animals.

Stimuli that evoke swimming in unrestrained embryos produce, in restrained embryos, a rhythmical oscillation of myotome activity. This activity shows a cycle

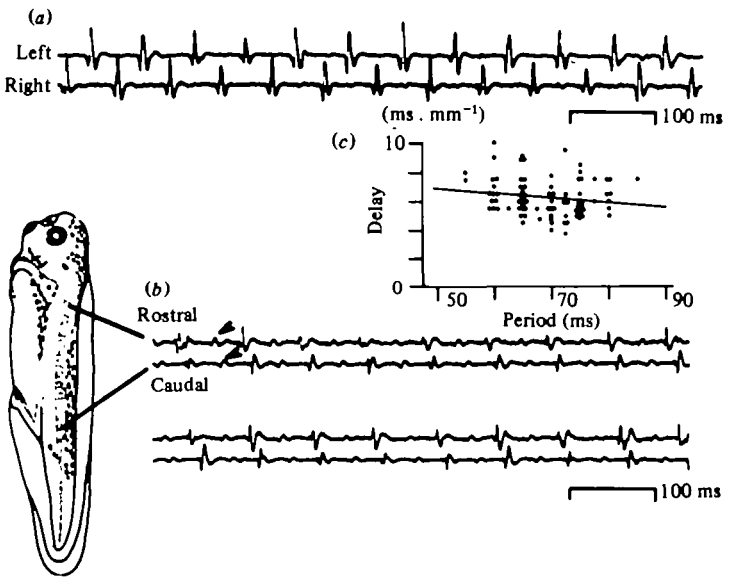

Fig. 5. Myotomal muscle activity during swimming in restrained embryos. (*a*) Recording of muscle potentials from left and right sides of a rostral trunk segment (the seventh post-otic myotome). Muscle potentials alternate. (*b*) Muscle potentials from segments 2 mm apart on the same side (approximate positions indicated), the two lines are continuous.There is a rostro-caudal delay in muscle potentials in each cycle. (Small potentials, arrowed, are picked up from the opposite side in the middle of a cycle). At 18 °C from an embryo 5 mm in length. (*c*) Relationship between rostro-caudal delay in EMG during swimming and the period of the swimming rhythm. Electrodes on the same side, 2 mm apart, delay measured onset to onset. The line of best fit was calculated by linear regression analysis, the slight negative slope is not significant (sample size = 87 cycles, $P > 0{\cdot}05$). Cycle period measured in units of 2·5 ms, and so apparent grouping is artifactual. Temperature was 18 °C.

period within the range seen in swimming, and muscle potentials alternate strictly on the two sides (Fig. 5*a*). Such a pattern can account for the alternate bending of each side of the body in swimming. Variation in the amplitude of muscle potentials on different cycles indicates that electrodes were recording the summed activity of several fibres.

The myotomal muscle activity in different segments on the same side of the body occurred in a rostro-caudal sequence on each swimming cycle (Fig. 5*b*). When measured over an inter-electrode spacing of 2 mm, the mean delay in onset per mm was 6·3 ms (range 3·75–10 ms, $n = 87$ cycles). This was measured over a range of cycle periods of 55–85 ms, and there was no strong tendency for the delay to change with the cycle period (Fig. 5*c*) (but see Kahn & Roberts, 1982). There was some contralateral pick-up in these recordings (Fig. 5*b* indicated), suggesting the activity at each electrode might not be from a single segment but also from neighbouring segments, perhaps reducing the accuracy of these measurements.

Electrical coupling of myotome fibres

In *Xenopus*, during embryonic development (Blackshaw & Warner, 1976; Hayes, 1975) and even in early larval stages (as late as stage 39, Hayes, 1975) gap junctions

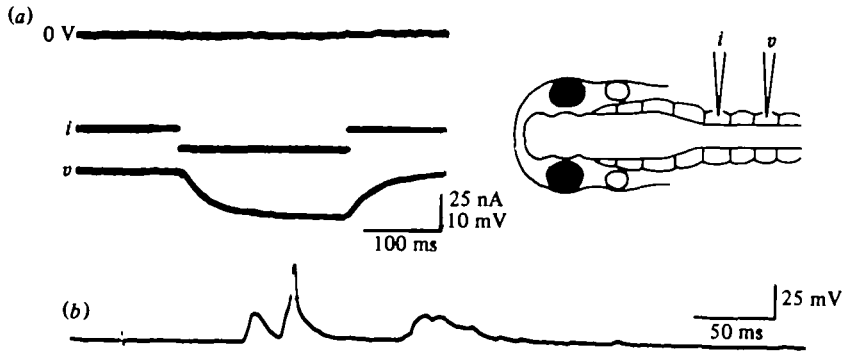

Fig. 6. Intracellular recording from myotomes. (*a*) Test for electrical coupling between myotome muscle fibres in different segments (electrode positions shown in inset). Current passed into one fibre through an intracellular micro-electrode (middle trace (*i*), current monitor), and simultaneous change in membrane potential of a second fibre (*v*) (lower trace) indicates electrical coupling. No potential change during the current pulse after withdrawal of the recording electrode from the second fibre (upper trace). Upper trace also marks zero potential (*b*) Spike potential (arrowhead) in myotome muscle fibres with an intracellular electrode. Endplate potentials, which sometimes exceed spike threshold, appear in the fibre following an electrically evoked skin impulse, at stimulus artifact. The preparation was bathed in saline with curare (6×10^{-4} M) added to reduced movements.

have been found between myotome fibres. This suggests that there might be electrical coupling between the myotome fibres at stage 37/38.

To test for electrical coupling, two different myotome fibres were penetrated with intracellular microelectrodes. When a current pulse was passed into one fibre, a simultaneous change in the membrane potential of the second fibre indicated the presence of electrical coupling between the two fibres (Fig. 6*a*). Both depolarizing and hyperpolarizing current pulses would pass between the fibres. Electrical coupling between fibres, while not always present, was found between pairs in the same myotome segment (30 pairs), in adjacent myotome segments (20 pairs), and in myotomes separated by one or two intervening segments (9 pairs). Tests over greater electrode separations were not carried out.

Muscle spikes

When penetrated with an intracellular microelectrode in partially curarized embryos (Kahn & Roberts, 1982), spike potentials were occasionally recorded in myotome fibres (Fig. 6*b*). In all, five such fibres were found. These spikes were evoked by endplate potentials which appeared after sensory stimulation. From the notch on the rising phase of the spike in Fig. 6(*b*). spike threshold was 25–30 mV below the resting potential (−75 mV) of this fibre. Subthreshold endplate potentials are also present in these recordings.

DISCUSSION

The results presented here describe the swimming movements of *Xenopus* embryos and also the muscle activity which occurs during swimming in restrained embryos. The results of recordings of electrical activity in myotomal muscle during swimming

accord well with the observations of the swimming movements. The muscle activity alternates on either side of a segment and this can account for the alternation in bending on swimming cycles. The rostro-caudal delay in muscle activity can account for waves of bending passing caudally. The velocity of the travelling wave of bending (6·3–8·0 ms.mm^{-1}) was similar to the velocity of the wave of electrical activity in the myotomes (3·75–10·0 ms.mm^{-1}, mean 6·3 ms.mm^{-1}). There is therefore no need to propose that mechanical factors play a major role in determining the velocity of the travelling wave of bending. This contrasts with Blight's conclusions (see Introduction) from his study of newt embryos, and indicates that a more detailed study should now be made of the newt embryo.

The electrical coupling that was found between myotomal muscle fibres was to be expected on the basis of previous studies of *Xenopus* embryos (Blackshaw & Warner, 1976*a*; Hayes, 1975). Functionally, it might serve to increase the degree of synchrony in spiking in neighbouring myotomal muscle fibres. A brief, synchronous contraction of the fibres of a segment is likely to be of importance because the swim cycles are very short; they can be as short as 40 ms.

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REFERENCES

BAINBRIDGE, R. (1963). Caudal fin and body movement in the propulsion of some fish. *J. exp. Biol.* **40**, 23–56.

BLACKSHAW, S. E. & WARNER, A. E. (1976). Low resistance junctions between mesoderm cells during development of trunk muscles. *J. Physiol., Lond.* **255**, 209–231.

BLIGHT, A. R. (1976). Undulatory swimming with and without waves of contraction. *Nature, Lond.* **262**, 217–218.

BLIGHT, A. R. (1977). The muscular control of vertebrate swimming movements. *Biol. Rev.* **52**, 181–218.

EATON, R. C., FARLEY, R. D., KIMMEL, C. B. & SHABTACH, E. (1977). Functional development in the Mauthner cell system of embryos and larvae of the zebra fish. *J. Neurobiol.* **8**, 151–172.

GRAY, J. (1933*a*). Studies in animal locomotion. I. The movement of fish with special reference to the eel. *J. exp. Biol.* **10**, 88–104.

GRAY, J. (1933*b*). Studies in animal locomotion. II. The relationship between waves of muscular contraction and the propulsive mechanism of the eel. *J. exp. Biol.* **10**, 386–390.

GRILLNER, S. (1974). On the generation of locomotion in spinal dogfish. *Expl. Brain Res.* **20**, 459–470.

GRILLNER, S. & KASHIN, S. M. (1976). On the generation and performance of swimming in fish. In *Neural Control of Locomotion* (ed. R. M. Herman, S. Grillner and P. S. G. Stein), pp. 181–201. New York, London: Plenum.

HAYES, B. P. (1975). The distribution of intercellular junctions in the developing myotomes of the clawed toad. *Anat. Embryol.* **147**, 345–354.

HUNTER, J. R. (1972). Swimming and feeding behaviour of larval anchovy, *Engraulis mordax*. *Fish. Bull.* **70**, 821–838.

KAHN, J. A. & ROBERTS, A. (1982). The central nervous origin of the swimming motor pattern in embryos of *Xenopus laevis*. *J. exp. Biol.* **99**, 185–196.

NIEUWKOOP, P. D. & FABER, J. (1956). *Normal Tables of* Xenopus laevis (*Daudin*). Amsterdam: North-Holland.

ROBERTS, A. (1971). The role of propagated skin impulses in the sensory system of young tadpoles. *Z. vergl. Physiol.* **75**, 338–401.

ROBERTS, A. (1975). Some aspects of the development of membrane excitability, nervous system and behaviour in embryos. In '*Simple*' *Nervous Systems* (ed. P. N. R. Usherwood and D. R. Newth), pp. 27–65. London: Edward Arnold.

ROBERTS, A. & BLIGHT, A. (1975). Anatomy, physiology and behavioural role of sensory nerve endings in the cement gland of embryonic *Xenopus*. *Proc. R. Soc. Lond.* B, **192**, 111–127.

ROBERTS, A., KAHN, J. A., SOFFE, S. R. & CLARKE, J. D. W. (1981). Neural control of swimming in a vertebrate. *Science, N.Y.* **213**, 1032–1034.