J. exp. Biol. (1982), **99**, 185–196
With 6 figures
Printed in Great Britain

THE CENTRAL NERVOUS ORIGIN OF THE SWIMMING MOTOR PATTERN IN EMBRYOS OF *XENOPUS LAEVIS*

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(*Received* 11 *November* 1981 – *Accepted* 18 *March* 1982)

SUMMARY

Rhythmic motor nerve activity was recorded in stage 37/38 *Xenopus* embryos paralysed with curare. The activity was similar to the swimming motor pattern in the following ways: cycle period (40–125 ms), alternation of activity on either side of a segment, rostro-caudal phase lag. Episodes of rhythmic motor activity could be evoked by stimuli that evoke swimming and inhibited by stimuli that normally inhibit swimming. On this basis we conclude that the swimming motor pattern is generated by a central nervous mechanism and is not dependent on sensory feedback.

In addition to the swimming pattern, another pattern of motor activity ('synchrony') was sometimes recorded in curarized embryos. In this, the rhythmic bursts on either side of a segment occurred in synchrony, and the rhythm period (20–50 ms) was half that in swimming. This was probably not an artifact of curarization as there were indications of a similar pattern in uncurarized embryos. Its function remains unclear.

INTRODUCTION

There has been much controversy in the past over whether sensory reflexes are necessary for the generation of rhythmic locomotor patterns (e.g. Gray, 1950). However, recently it has been shown in several different vertebrate species that the basic locomotor rhythm can be generated in the absence of rhythmic sensory inputs (fish: Roberts, 1969*a*; Grillner, Perret & Zangger, 1976; Poon, 1980; mammals: Viala & Buser, 1969; Grillner & Zangger, 1974, 1975). Among the invertebrates, also, there are many species in which central pattern generators have been shown to underlie locomotion (insects: Wilson, 1961; Pearson & Iles, 1970; molluscs: Dorsett, Willows & Hoyle, 1973; annelids: Kristan & Calabrese, 1976).

We here report on experiments to test whether, as in the above animals, the swimming motor pattern of *Xenopus* embryos is produced by a central nervous mechanism, or whether it is dependent upon sensory reflexes. When released from their egg membranes, *Xenopus* embryos will swim in response to stimulation (Roberts, 1971, 1978; Roberts & Smyth, 1974). In swimming, waves of bending pass alternately down either side of the body (Kahn, Roberts & Kashin, 1982). Underlying the swimming movements, myotomal muscles are active alternately on opposite sides of a

segment of the body, and myotomes in different segments on the same side of the body are active in a rostro-caudal sequence.

The results indicate that the swimming motor pattern of *Xenopus* embryos is produced by a central nervous mechanism, which is not dependent upon sensory feedback. Some of these results have been reported previously (Roberts, Kahn, Soffe & Clarke, 1981).

MATERIALS AND METHODS

Experiments were carried out on embryos of *Xenopus laevis* at developmental stage 37/38 (Nieuwkoop & Faber, 1956). The methods for obtaining eggs, the saline composition, and the method for recording muscle potentials from restrained, uncurarized embryos have all been described earlier (Kahn *et al.* 1982). Before an experiment, embryos were removed from their egg membranes with fine forceps. Experiments were carried out at temperatures of 18–22 °C.

Extracellular motor nerve recordings in curarized embryos

Embryos were paralysed by bathing in saline containing D-tubocurarine chloride at 10^{-4} M. Curare blocks transmission at the neuro-muscular junction, which in *Xenopus* embryos is known to be a cholinergic synapse (Blackshaw & Warner, 1976; Kullberg, Lentz & Cohen, 1977). To allow curare to enter the body, a gash was made in the belly skin. When paralysed (determined by lack of overt responses to stimulation), embryos were pinned on their sides to a Sylgard layer, with fine pins pushed through the notochord. Skin overlying the myotomes in the area to be recorded from was stripped away using fine pins.

The Sylgard to which the embryo was pinned was attached to a small table (5×10 mm) that could be rotated about its long axis. To make motor nerve recordings from both sides of the body simultaneously, the table was rotated so that the embryo was dorsal side uppermost. To make recordings from two different motor nerves on the same side, the table was rotated so that one side of the embryo was facing upwards.

In *Xenopus* embryos, motoneurone axons innervating myotomal muscle run in the cleft between adjacent myotome segments (Hughes, 1959; Lewis & Hughes, 1960; Kullberg *et al.* 1977). In curarized embryos, motor nerve activity was recorded with a glass suction electrode (tip opening 50–70 μm), placed on the outer surface of the myotomal muscle, over an inter-myotomal cleft. Motor nerve activity could be recorded at all points along the dorso-ventral extent of inter-myotomal clefts. Amplification techniques for motor nerve recordings were conventional.

Intracellular recording of endplate potentials in myotome fibres

The preparation was similar to that used above, with the curarized embryo pinned on its side, and skin overlying the myotomes stripped away. In contrast to the previous method, a lower concentration of curare was used. This had to be sufficiently high to prevent muscle contraction, while still leaving reduced endplate potentials in myotome muscle fibres. The critical curare concentrations ranged, in different preparations, from 10^{-6} to 5×10^{-5} M.

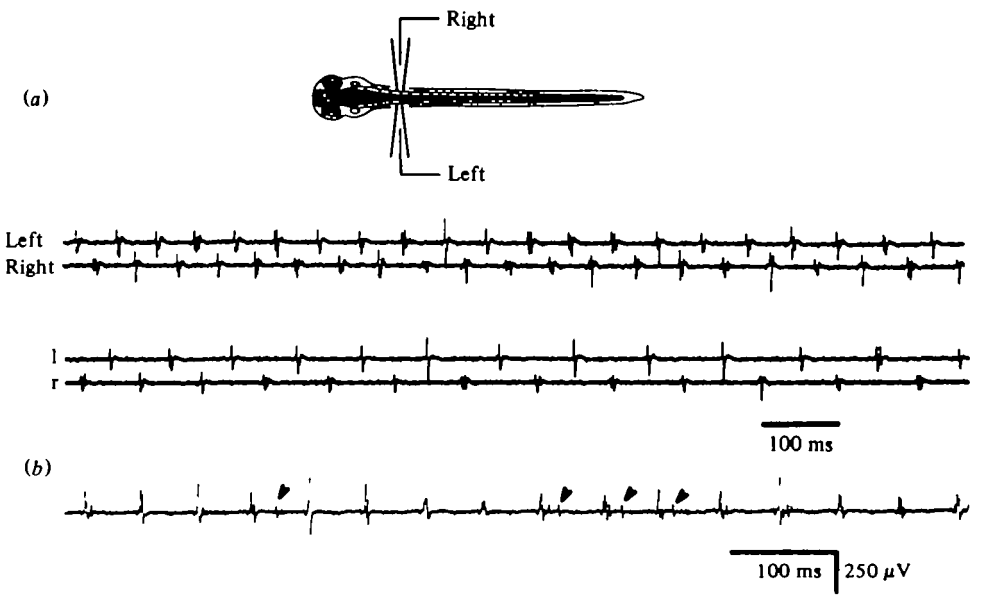

Fig. 1. Rhythmic, alternating motor nerve activity recorded extracellularly from inter-myotomal clefts in curarized *Xenopus* embryos. (*a*) Recordings from left and right sides of a trunk segment. The upper and lower sequences are from the same episode, but are not continuous. (*b*) A recording from a single motor nerve, showing occasional irregular impulses (arrowheads) between the main rhythmic bursts. Vertical scale bar applies only to (*b*).

Endplate potentials were recorded intracellularly with glass microelectrodes, filled with 3 M KCl and having resistances of 20–60 MΩ. Amplification techniques were conventional.

RESULTS

'*Swimming*' *activity in curarized embryos*

In order to determine whether the swimming motor pattern of *Xenopus* embryos is generated by a central nervous mechanism, recordings of motor nerve activity were made in curarized embryos. In these curarized embryos, because the neuromuscular junctions were blocked and there was no movement, there was no possibility of sensory feedback. Even so, episodes of rhythmic, alternating activity were recorded in the motor nerves (Fig. 1 *a*). This rhythmic activity was recorded along the body as far caudally as the cleft between the 24th and 25th myotomes in the tail (more caudal electrode placements were not tried).

The rhythmic bursts recorded at each electrode varied in amplitude and duration from cycle to cycle (Fig. 1 a), indicating that the motor nerve electrodes were recording a compound potential, the summed activity of a number of different individual motoneurone axons. The compound nature of these bursts has meant that single units could not be resolved with certainty in the bursts. However, occasional impulses appeared between the main rhythmic bursts (Fig. 1 *b*, indicated), and these were probably from single units, because they had a simple, often biphasic, waveform, and

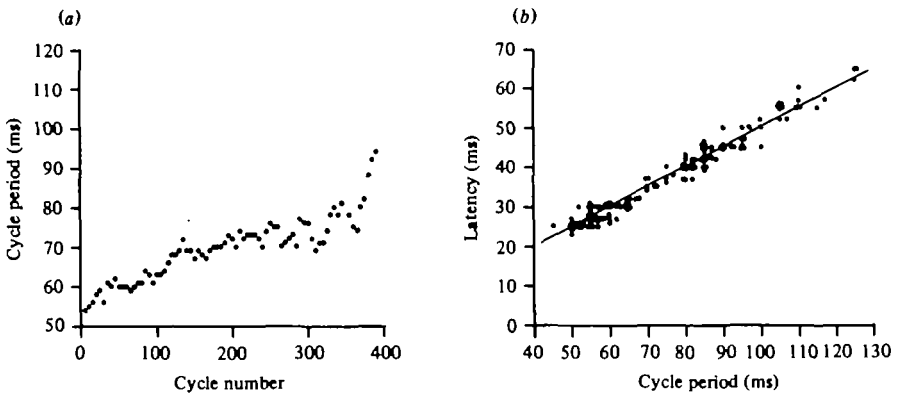

Fig. 2. Characteristics of the rhythmic, alternating pattern of motor nerve activity in curarized preparations. (*a*) Plot of the rhythm period in successive cycles of one episode, which was evoked by dimming the lights and allowed to continue uninterrupted. Period measured between the onset of one motor nerve burst to the onset of the next at the same electrode. Each point represents the mean period for 5 successive cycles. (*b*) Graph showing the latency between the onset of the motor nerve burst on one side of a segment, and the next burst on the other side, plotted against the period of the rhythm. Results are from 150 cycles in one preparation (that shown in Fig. 1*a*). The line indicates the expected values for perfect phase coupling of 0·5 (phase = latency/period) between the left and right motor nerves, and would intersect the origin.

short duration (1·7–3·5 ms). This is similar to extracellular impulse duration in single units of other types of neurones in *Xenopus* embryos (Roberts & Blight, 1975). From these values for impulse durations there are strong indications that on at least some cycles in the alternating rhythm the underlying units spiked only once per cycle. Thus, bursts in the rhythm were sometimes as short as 5 ms in total duration, and in these, units with impulses longer than 2·5 ms could not have spiked more than once. Those with impulses less than 2·5 ms could have spiked twice, but to do so they would need to have spiked at a high frequency; units with impulses of 1·7 ms duration (the shortest measured) would need to have spiked at least at 300 Hz. This frequency is greater than the maximum of 277 Hz found in other types of neurones in *Xenopus* embryos (Roberts & Blight, 1975). It is therefore probable that in bursts of 5 ms total duration, individual motoneurones spiked only once, and different motoneurones spiked in very close synchrony. Results of intracellular recording from probable motoneurones during the alternating rhythm indicate that one spike per cycle is the normal pattern of activity (Roberts & Kahn, 1982). Thus, the variable duration of extracellularly recorded motor nerve bursts was probably due to variations in the degree of synchrony of spiking in different motoneurones in a motor nerve.

Episodes of rhythmic motor nerve activity in curarized preparations appeared after a single, brief sensory stimulus (see below). They often continued for 10–30 s and in some recordings for up to 2 or 3 min. During an episode there was in many cases a gradual increase in the rhythm period (Fig. 2*a*). The period of the rhythmic activity was normally between 40 and 125 ms at room temperature (18–22 °C).

During the rhythmic activity, bursts in motor nerves on the left and right sides of a segment of the body appear alternately (Fig. 1*a*). The motor nerve bursts on the two

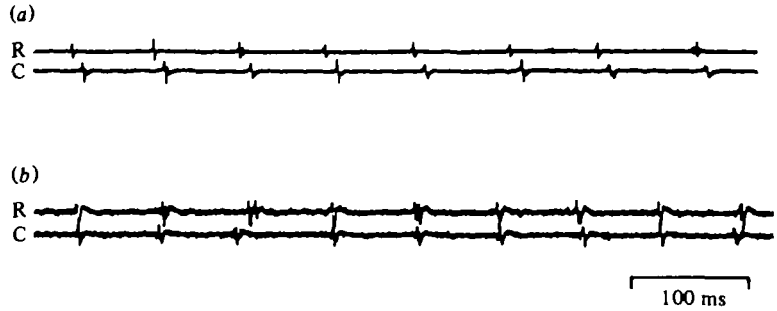

Fig. 3. Coordination between bursts at different motor nerves on the same side of the body during rhythmic, alternating activity in a curarized preparation. (*a*) and (*b*) from different preparations; in each, upper trace is rostral motor nerve, lower trace is caudal motor nerve. Rostral electrode at cleft between post-otic myotomes 3 and 4 in (*a*): 2 and 3 in (*b*). Caudal electrode between myotomes 17 and 18 in (*a*): 14 and 15 in (*b*). In (*a*) electrodes are 1·5 mm apart, in (*b*) 2 mm apart.

sides kept an approximately constant phase relationship of 0·5 (Fig. 2*b*) over the full range of rhythm periods.

On each cycle in the rhythm there was usually a rostro-caudal delay in the onset of bursts at motor nerves in different segments on the same side of the body (Fig. 3*a*).

Exceptionally, however, there were some cycles where the bursts began simultaneously at the two motor nerves, or there was even a slight lead at the caudal motor nerve (Fig. 3*b*).

Measurements were made to determine whether there was any correlation between the rostro-caudal delay in burst onset and the rhythm period. In measurements on eight different preparations with electrodes positioned near either end of the trunk, 1·5–2·2 mm apart, results from 6 showed a significant correlation ($P < 0{\cdot}05$) (Figs. 4*a*, *b*1, *b*2). In these preparations then, there was a tendency for the rostro-caudal delay to increase with the rhythm period. By contrast, in two preparations, episodes with no significant positive slope were found; in one there was a slight, but significant negative slope, and in the other no significant slope (Fig. 4*b*3). In each of these two preparations another episode was measured and these did have significant positive slopes. The reason for these differences is not clear but was probably not due to differences in the region of the body from which recordings were made, for electrodes were positioned at approximately similar spacings and at similar segments in each case.

To check that motor nerve recordings could be related to activity in the myotomal muscles, recordings were made intracellularly from muscle fibres in critically curarized animals. These showed the pattern of endplate potentials (EPPs) in the muscle fibres expected from motor nerve recordings. In myotomes on left and right sides of a segment of the body there was a rhythmic, alternating pattern of EPPs (Fig. 5*a*). In muscle fibres in two different segments on the same side there was usually a rostro-caudal delay in onset (Fig. 5*b*).

EPPs had variable rise times (Fig. 5*a*, *b*), and this could be explained if myotomal muscle fibres received synaptic inputs from several different motoneurones. If, as suggested above, motoneurones usually spike once per cycle, then the EPP rise time would vary with the degree of synchrony in impulses in different units. For two

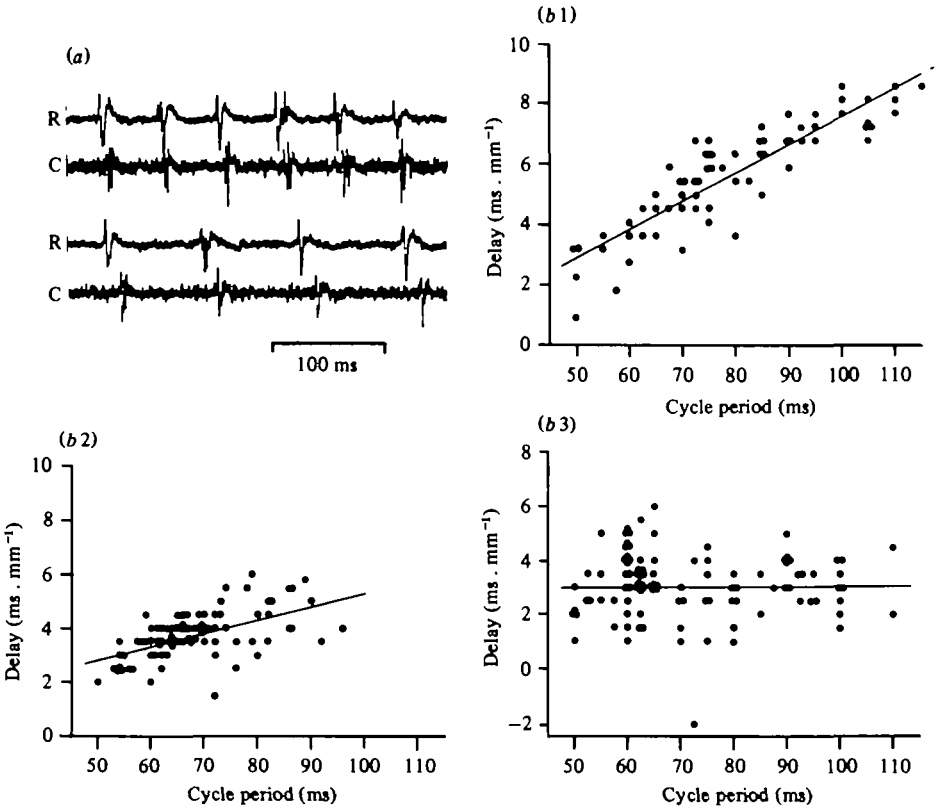

Fig. 4. The delay on one side of the body between the onset of motor nerve bursts at rostral and caudal electrodes during rhythmic alternating activity. (*a*) Two sequences from an episode in a preparation (also shown in *b*1) where there was a marked increase in the delay as rhythm period increased. Electrodes 2·2 mm apart, for positions see below. (*b*) 1–3: plots of delay against rhythm period for three different preparations. Delay was measured from the onset of the burst at the rostral motor nerve to the onset at the caudal motor nerve. Electrodes were 2–2·2 mm apart in different preparations, and delay is plotted as delay per mm. Each graph represents the results from one swimming episode. The lines for linear regression are plotted. (*b*1) Regression line significant, $P < 0{\cdot}001$ ($n = 67$ cycles); (*b*2) regression line significant, $P < 0{\cdot}001$ ($n = 87$ cycles); (*b*3) regression line not significant, $P \gg 0{\cdot}05$ ($n = 103$ cycles). Rostral electrode at cleft between post-otic myotomes 3 and 4 in (*a*) and (*b*1) (same recording); same for (*b*2); between 2 and 3 in (*b*3). Caudal electrode between post-otic myotomes 18 and 19 in (*a*) and (*b*1); 17 and 18 in (*b*2); 14 and 15 in (*b*3). Electrodes 2·2 mm apart in (*a*) and (*b*1), 2 mm apart in (*b*2) and (*b*3).

reasons it is to be expected that muscle fibres received inputs from several different motoneurones. First, fibres are electrically coupled to each other (Kahn *et al.* 1982) and EPPs in one fibre would probably be conducted to neighbouring fibres. Secondly, single myotome fibres probably have synapses at both their ends, for this appears to be the case in older *Xenopus* larvae at stage 45 (Lewis & Hughes, 1960). Each intermyotomal cleft receives axons from different ventral roots (Roberts & Clarke, 1982) and so it is probable that different motoneurones innervate each end of a fibre. Thus the EPPs recorded here were probably summed from endplate regions both on the penetrated fibre and on neighbouring electrically coupled fibres.

If the rhythmical pattern of motor activity in curarized preparations is 'swimming' activity, then it should be evoked and inhibited by the same stimuli that evoke and

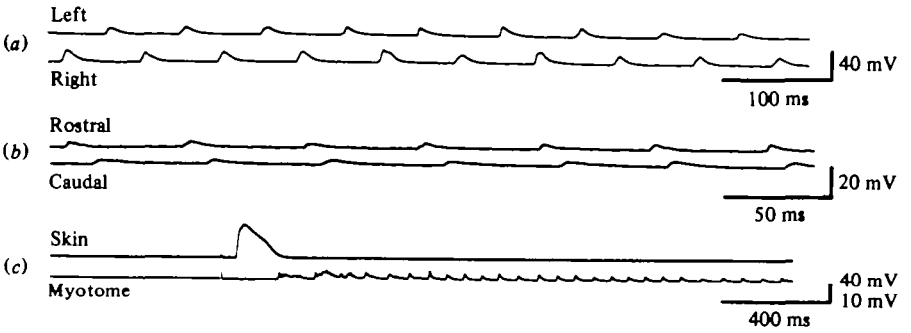

Fig. 5. Intracellular recordings of endplate potentials in myotomal muscle fibres in critically-curarized, paralysed preparations. (*a*) Recording from fibres on left and right sides of a trunk segment (the fifth post-otic myotomes); (*b*) electrodes in myotomes 2 mm apart on the same side of the body; rostral electrode at the 3rd post-otic myotome, caudal electrode at the 15th post-otic myotome; (*c*) a skin impulse, recorded intracellularly from a skin cell (upper trace), evokes rhythmical endplate potential activity in a myotomal muscle fibre (lower trace). The preparation was critically curarized, and the skin impulse was triggered by an electric shock (note stimulus artefact) applied to the skin.

inhibit swimming in the uncurarized embryo. Mechanical stimulation of the skin of the trunk and tail was effective in evoking the rhythmic motor-nerve activity in curarized preparations. This could be either gentle strokes to the skin with a fine hair, an effective stimulus for the sensory endings of Rohon-Beard cells (Roberts & Hayes, 1977), or stronger pokes which indented the skin and probably evoked skin impulses (Roberts, 1971). Electrical stimulation of the skin that evoked a skin impulse was also effective in evoking rhythmic activity (Fig. 5*c*). Dimming the illumination was similarly effective. All these stimuli will evoke swimming in uncurarized preparations (Roberts, 1971, 1978; Roberts & Smyth, 1974). Swimming movements in uncurarized embryos are inhibited by pulling on the cement gland mucus strand (Roberts & Blight, 1975), and the same stimulus inhibited the rhythmical activity in curarized preparations.

The Synchronous pattern

In addition to the rhythmic, alternating 'swimming' activity described above, a dramatically different pattern of motor nerve activity has also been recorded in curarized preparations. In this the motor nerves on the left and right sides of a segment were active in synchronous bursts (Fig. 6*a*), in contrast to the alternate bursts at left and right motor nerves during the 'swimming' activity described above. The synchronous bursts occurred rhythmically, with a cycle period of about half the swimming rhythm that immediately preceded or followed them (see Fig. 6*a*). Cycle periods in rhythmic synchronous activity were 20–50 ms. Bursts in this pattern usually continued to occur with a rostro-caudal delay. This pattern will hereafter be referred to as Synchrony, or the Synchronous pattern. The rhythmic bursts in the Synchronous pattern may be similar in amplitude and duration to those in 'swimming' activity (Fig. 6*a*), but often they were smaller in amplitude than in the 'swimming' pattern (Fig. 6*b*).

The Synchronous pattern usually appeared in short sequences of two seconds or less, and it most commonly occurred near the start of the episode of motor nerve

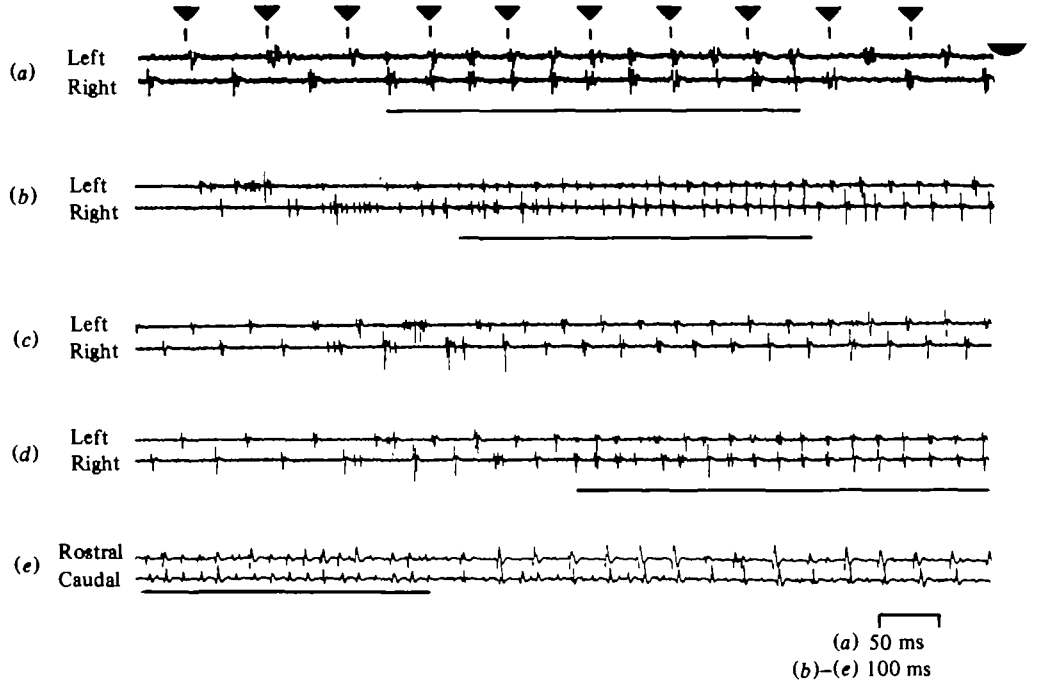

Fig. 6. Synchronous patterns of extracellular motor nerve activity recorded in curarized embryos (periods of Synchrony underlined). (*a*) Motor-nerve recordings from left and right sides of a trunk segment. Marks above the trace are separated by equal time intervals, and they show there to be a halving of the cycle period at the transition from alternation to Synchrony, and (in this case) they also show a reversal of cycle phase between the two periods of alternation. (*b*) Motor-nerve activity was evoked by dimming the lights. After initial irregular activity, rhythmic Synchronous bursts appear, and at the end of the trace, alternation is established. During Synchrony there are occasional cycles where one or other side does not seem to have spiked. (*c*) and (*d*) Near the start of the two traces the tail skin was stroked gently with a fine hair during the alternating rhythm. In (*c*) the alternating rhythm speeds up, whereas in (*d*) the pattern then changes to Synchrony. (*e*) Probable sequence of 'Synchrony' in muscle potential recordings from an uncurarized, restrained preparation. 'Synchrony' (mean cycle period, 29·1 ms) seems to occur above the line, and it is followed by swimming (mean cycle period 59·7 ms on the second line). Recordings from myotomal muscles in different segments on the same side. Rostral electrode, upper trace; caudal electrode, lower trace.

activity that followed stimulation (Fig. 6*b*). However, Synchrony could often be induced during an episode of swimming, by an excitatory sensory stimulus (Fig. 6*d*) such as a gentle stroke to the skin of the body with a fine hair. On other occasions (Fig. 6*c*), the same stimulus only speeded up the swimming rhythm.

The Synchronous pattern also seemed to occur in uncurarized embryos. In muscle recordings from restrained, uncurarized embryos a rhythm of muscle potentials with period much shorter than that in swimming was sometimes seen near the start of an episode (prior to the arrow in Fig. 5). The fast rhythm in uncurarized embryos resembled the Synchronous pattern of motor nerve activity in curarized embryos in the following respects: (i) the period was about half that of the swimming rhythm that followed it; (ii) the fast rhythm occurred near the start of an episode; (iii) the muscle potentials in the fast rhythm were often smaller than those in the swimming rhythm

hat follows (Fig. 6), probably because fewer myotomal muscle fibres were active. This evidence therefore indicates that the Synchronous pattern can also occur in uncurarized embryos.

A neural mechanism which can account for the generation of both the Synchronous pattern and the swimming pattern is discussed elsewhere (Kahn & Roberts, 1982).

DISCUSSION

The central nervous origin of the swimming motor pattern

The evidence presented here indicates that the swimming motor pattern of *Xenopus* embryos is produced by a central nervous mechanism and is not dependent upon rhythmic sensory inputs. When embryos were paralysed by blocking the neuromuscular junction with curare, they generated a rhythmic, alternating pattern of motoneurone activity that closely resembled the swimming pattern. In curarized preparations there was no movement, and therefore no possibility of rhythmic sensory inputs.

The rhythmic, alternating pattern of motor nerve activity in curarized embryos had the following features in common with swimming (Kahn *et al.* 1982).

(i) The rhythmic activity in curarized preparations had cycle periods of 40–125 ms, similar to cycle periods (40–100 ms) measured during swimming movements.

(ii) Activity in motor nerves on the left and right sides of a segment alternate on each cycle of the rhythm in curarized preparations. Similarly, in swimming the activity of myotomal muscles on the left and right sides of a segment alternates.

(iii) In curarized preparations, there was usually a rostro-caudal sequence in the activity of motor nerves in different segments on the same side of the body. Similarly, in swimming myotomal muscles in different segments on the same side are active in a rostro-caudal sequence.

(iv) The rhythmic pattern in curarized preparations was evoked by the same stimuli that evoke swimming: skin impulses (Roberts, 1971), dimming the lights (Roberts, 1978), and gentle strokes to the skin of the trunk and tail (Roberts & Smyth, 1974; Roberts & Hayes, 1977).

(v) The rhythm in curarized embryos was inhibited by stimulation of the cement gland, a stimulus that will inhibit swimming (Roberts & Blight, 1975).

The neural mechanism which underlies swimming in *Xenopus* embryos is therefore a central nervous mechanism and is not dependent upon sensory feedback. *Xenopus* embryos are, in this respect, similar to adult fish, for in these also, central pattern generators have been shown to underlie swimming (B. Roberts, 1969*a*; Grillner *et al.* 1976; Poon, 1980). Recently it has been shown that swimming is produced by a central pattern generator in another amphibian, the tadpole of the bullfrog (*Rana catesbeiana*) (Stehouwer & Farel, 1980). However, in the bullfrog tadpole the rostro-caudal phase lag is lost when the dorsal roots are cut. This contrasts with the present results from *Xenopus* embryos, where the rostro-caudal sequence persists in the curarized preparation.

Do proprioceptors play a role in swimming?

It has been shown that sensory feedback is not necessary for the generation of a swimming motor output. Is there any evidence that sensory feedback plays any role in *Xenopus* embryo swimming? Previous anatomical and physiological studies of the sensory systems of *Xenopus* embryos have provided no evidence for a proprioceptive sensory system. In amphibian embryos, Rohon-Beard neurites provide tactile sensory innervation of the skin (Coghill, 1914; Hughes, 1957; Roberts & Hayes, 1977). The earlier reports by Coghill on the axolotl embryo and Hughes on *Xenopus* indicated that the Rohon-Beard neurites might also innervate the myotomal muscle fibres and provide proprioceptive information in swimming. This now seems unlikely, for more recent anatomical studies (Roberts & Hayes, 1977) show that, while the Rohon-Beard neurites do pass through the inter-myotomal clefts to reach the skin, they do not seem to have endings on the muscle fibres.

The Rohon-Beard neurites that innervate the skin of the trunk and tail do not seem to have properties which would make them likely to respond to swimming movements. They respond best to sharp indentation of the skin (Roberts & Hayes, 1977). During swimming, however, the skin is likely to be alternately stretched and compressed, and Rohon-Beard cells do not respond to stimuli that stretch the skin, such as indentation with blunt probes.

The present results provide no evidence to suggest that there is proprioceptive activity during swimming in *Xenopus* embryos. Thus, in some animals where proprioceptive activity is known to occur during locomotion, removing this proprioceptive input results in a marked increase in the period of the rhythm (Roberts, 1969*a*; Wilson, 1961 but see Grillner *et al.* 1976). In contrast, in *Xenopus* embryos the cycle period of the rhythm in curarized preparations was very similar to that during swimming, and therefore sensory feed-back is not needed for the maintenance of the normal swimming cycle period. The apparent absence of a proprioceptive sensory system is perhaps not surprising in view of the early developmental stage of the embryo. In adult fish, proprioceptors have been found in the dogfish (Roberts, 1969*b*, *c*) and in the ray (Fessard & Sand, 1937; Ridge, 1977) and it may be that at later stages of development in amphibian larvae, proprioceptors will also be found.

Inter-segmental coordination

We have presented here evidence that reflexes are not necessary for establishing a rostro-caudal sequence of motor nerve activity, for this sequence is preserved after curarization. Similar conclusions have come from the studies of the central motor programmes for swimming in the dogfish and the lamprey (Grillner *et al.* 1976; Cohen & Wallen, 1980). However, there are exceptions to this, in particular, studies on the tadpole of the bullfrog have reported that the dorsal roots need to be intact in order for a rostro-caudal sequence to be established (Stehouwer & Farel, 1980).

Whilst the basic rostro-caudal sequence is preserved in *Xenopus* embryos paralysed with curare, it cannot be stated definitely that the delay is not affected in the curarized preparation. There do in fact appear to be some differences in the rostro-caudal pattern in the normal and the curarized preparations. In the uncurarized embryo, the

rostro-caudal delay remained constant at different cycle periods (Kahn *et al.* 1982). In contrast to this, in the curarized preparation there was in general an increase in the delay as the cycle period increased, although two episodes were measured where this did not occur. In addition, the delay in the uncurarized embryo was about 6 ms per mm of the body, longer than most of the delays in the motor nerve recordings in curarized embryos (Fig. 4). These differences are only slight and may be simply sampling errors. A more detailed study would be needed to see if there really is a genuine difference.

One of the simplest suggestions for the neural mechanism which coordinates the activity in different segments is that there is a fixed axonal pathway which descends from the rostral end of the spinal cord, and excites motoneurones in successive caudal segments. This would, however, produce fixed rostro-caudal delays and the evidence presented here demonstrates that the delays, at least in the motor nerve recordings in curarized preparations, are not fixed. Firstly, on certain cycles, albeit a small minority, the bursts in motor nerves did not appear in a rostro-caudal sequence, but began either simultaneously at both motor nerves, or began first at the most caudal motor nerve. Secondly, the rostro-caudal delay in most preparations was related to the cycle period, increasing as the period increased. A similar increase in delay has been observed in the spinal dogfish (Grillner, 1974), spinal lamprey (Poon, 1980), and in several teleosts (Grillner & Kashin, 1976). This has been taken to indicate that there is pattern-generating capacity distributed segmentally along the spinal cord (Grillner & Kashin, 1976). In the spinal dogfish, it has been found that the segment which leads on a swim cycle is not always the most rostral in the preparation, though it usually lies in the rostral part of the spinal cord (Grillner, 1974). This might explain some of the range of delay *v.* cycle period plots in the present study (Fig. 4). In plots such as those in Fig. 4*b*2 and 3, the leading segment might have been located between the two recording points, with the caudal part of the body having a delay similar to that in Fig. 4*b*1, but with the rostral part having a reversed phase coupling. This would result in an underestimate of the change in delay as cycle period increases.

We would like to thank Ken Wood for photographic assistance, Drs B. L. Roberts, S. R. Soffe and R. de G. Weevers for their comments on earlier drafts, and S. Martin for technical help.

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