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CENTRALLY GENERATED RHYTHMIC AND NON-RHYTHMIC BEHAVIOURAL RESPONSES IN *RANA TEMPORARIA* EMBRYOS

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Summary

Embryos of the frog *Rana temporaria* up to and around the time of hatching show a range of rhythmic and non-rhythmic movements. These may occur spontaneously or in response to lightly touching the skin of the trunk or head. The first response to touching one side is usually on the opposite side. Non-rhythmic movements range from weak twitches centred on the mid trunk to strong flexions along much of one side of the body and part of the tail, which result in the animal becoming tightly coiled. Rhythmic movements range from slow, high-amplitude 'lashing' movements to faster, lower-amplitude 'swimming' movements. During rhythmic movements, a wave of bending passes along the animal from head to tail. The longitudinal phase delay in bending is constant for a range of cycle periods (88–193 ms) but is not uniform along the whole body. Bending is maximal along the body and rostral part of the tail, decreases towards the tip of the tail and is lowest at the head. Lateral displacement during rhythmic movements is lowest 0.2 body lengths from the snout, increases rostral and caudal to this level and is highest at the tip of the tail.

In animals immobilised with curare, a range of patterns of motor discharge can be recorded in response to stimulation. Non-rhythmic responses range from single spikes to prolonged bursts, usually on the opposite side to the stimulus. Stronger bursts can alternate briefly between the two sides and are never synchronous on both. Episodes of sustained rhythmic activity can be evoked by touch, electrical stimulation of the skin or, rarely, dimming the lights. Cycle periods within each episode can vary considerably but often shorten as activity proceeds. Discharge on the two sides alternates (phase is approximately 0.5). Motor root burst duration correlates with cycle period, bursts being longer at longer cycle periods. Burst onset is delayed caudally, this delay being longer at longer cycle periods. Stimulating one side of the head evokes a large burst of discharge on the opposite side, often followed by sustained rhythmic discharge. These responses in immobilised animals are judged to constitute centrally generated correlates of the main behavioural responses of *R. temporaria* embryos.

Key words: *Rana temporaria*, swimming, embryo.

Introduction

By the time of hatching, most amphibian embryos have developed some form of rhythmic locomotion to enable them to survive the start of free-swimming larval life. The simple patterns of swimming shown by the late embryos of two amphibians, the urodele *Triturus* (*T. helveticus* and *T. vulgaris)* and the anuran *Xenopus laevis*, have been analysed in some detail (Blight, 1976, 1979; Kahn *et al.* 1982; Soffe *et al.* 1983). The aim has been to seek, in the nervous systems of these relatively simple animals, basic principles of operation for vertebrate locomotor rhythm generation. Patterns of locomotion in *X. laevis* and *T. vulgaris* embryos show strong parallels and both are centrally generated by neural elements situated within the spinal cord (Soffe *et al.* 1983; Roberts *et al.* 1985; for a review, see Roberts *et al.* 1986). Swimming movements in *X. laevis* and *T. vulgaris* occur at relatively high frequency, generally 10–30 Hz, and motor activity in both is characterised by motoneurones firing a single spike per cycle.

As a precursor to more detailed studies of motor pattern generation and selection, the present investigation was carried out to describe the main behavioural responses of the frog *R. temporaria* L. close to hatching and to examine the extent to which these, too, are centrally programmed. Interest in this amphibian was stimulated because its embryos are rather larger and swim with a lower beat frequency than those of *X. laevis* and *T. vulgaris*. Initial studies (Sillar and Soffe, 1989) suggested that swimming in *R. temporaria* embryos is driven, at least in part, by a pattern of rhythmic motor bursts more typical of vertebrate locomotor drive than the rhythmic single spikes seen in *X. laevis* and *T. vulgaris* (see also Stehouwer and Farel, 1980, for results using older *Rana* larvae).

To understand the neural basis of a behaviour pattern, it is important to have a knowledge of the movements involved. This provides a background against which to assess the different contributions of centrally generated neural programmes, sensory modulation and mechanical considerations. In the case of larval anurans, detailed studies have been carried out for both *Rana* and *Xenopus* (Wassersug and von Seckendorf Hoff, 1985; von Seckendorf Hoff and Wassersug, 1986). However, relatively few studies have been carried out on younger amphibians or, indeed, on smaller swimming vertebrates generally. For this, video recording now offers an approach by which relatively large amounts of data can be obtained with ease, in situations where the frame speed gives sufficient resolution (e.g. Batty, 1984). This approach has been adopted here to examine the early movements of *R. temporaria* embryos, including both sustained rhythmic lashing and swimming movements and the simple flexions that precede them in development. The extent of central motor programming for these behaviour patterns is then examined by making electrical recordings of motor responses in paralysed animals. This makes it possible to investigate which properties the motor programmes of *R. temporaria* embryos share with those of other amphibian embryos and older larvae and also with the locomotor patterns of adult vertebrates.

Materials and methods

Animals

Embryos of the frog *Rana temporaria* were maintained in the laboratory at temperatures between 10 and 20°C until required. Since there appear to be no detailed normal tables for *R. temporaria*, animals were staged using the criteria of Shumway (1942) for *R. pipiens* and the simplified scheme of Gosner (1960). Animals were examined at total lengths between 7 and 10 mm (developmental stages 19 and 20). Embryos of this age are available between approximately February and April each year and the work described here and in the following paper (Soffe and Sillar, 1991) is based on studies performed between 1986 and, 1989.

Movement analysis

Body movements were recorded using a NAC 200 high-speed video recorder at 200 frames s^{-1}. Animals were placed in a Petri dish (diameter 90 mm) containing tapwater maintained at approximately 20°C. The temperature in the recording chamber was monitored continuously by means of a thermistor probe. Animals were kept at the recording temperature for at least 2 h before recordings were made. They were viewed from directly above and were lit from directly below using reflected stroboscopic lighting synchronised to the recorder frame rate.

Movements were evoked by a light touch on one side using a fine etched tungsten needle or, more rarely, by a light pinch of the tail fin. Stimuli were applied as far as possible at a level just sufficient to evoke a response and responses should be considered threshold except where stated otherwise. During swimming, animals were recorded moving across the centre of the dish. On reaching the edge, they sometimes stopped but often continued, following the curve of the edge of the dish. Measurements were made only on animals swimming freely in a straight line across the dish.

The form of body movements was determined from outline tracings of consecutive frozen frames made on acetate sheets placed over the video monitor. For each outline, a series of points one-tenth of the body length ($0.1\,L$) apart was drawn along the midline. Coordinates were assigned to each point using a grid with a definition of approximately $0.005\,L$. Bending angles (θ, in degrees) were then computed for each point except the tip of the snout and tail. A sine curve of the form:

$$y = a\sin\,[b(x+c)] + d\,,$$

where x is time in ms and $y=\theta$, was fitted to the distribution of bending angles at each point during swimming using a least-squares method and the simplex algorithm (Nelder and Mead, 1965) to minimize the difference between the observed and theoretical distributions. Parameter a represents the maximum bending angle; $360b^{-1}$ is the cycle period of swimming, c represents the magnitude of the phase delay in ms and d is the asymmetry of bending between the two sides

of the animal. The starting guesses for the fitting parameters a, b, c and d were calculated automatically from the data. The lateral displacement of each point during swimming as well as the forward velocity were determined from plots of the path of each point using the same coordinates.

Some recordings were also made with a low-band U-matic video recorder at 50 frames s^{-1}. These records were used to obtain some data on cycle periods during whole episodes of swimming and also the form of slower movements. Analysis was carried out as above where appropriate.

Electrophysiology

For electrophysiological recording, animals were anaesthetised initially with MS222 and pinned on their sides onto a Sylgard block. An area of skin overlying the myotomes of the mid-trunk on the right-hand side was then removed using fine etched tungsten pins. Animals were kept immobilised by the addition of 70 μmol l^{-1} *d*-tubocurarine to the saline, the MS222 being washed off immediately after dissection. Extracellular recordings were made by means of glass suction pipettes (60–80 μm in diameter) applied to the intermyotome clefts either at, or near, the same level on the two sides or rostral and caudal on the same side. During experiments, animals were maintained in a bath of approximately 1.5 ml in volume and perfused at a rate of 5–7 ml min^{-1} with saline. This contained the following (in mmol l^{-1}): NaCl, 105; KCl, 2.5; $CaCl_2$, 2 and $NaHCO_3$, 15, equilibrated to pH 7.2 by bubbling continuously with 5 % CO_2, 95 % O_2.

Statistical analysis was carried out using the Minitab software package.

Results

Behavioural responses

Early non-rhythmic movements

Early movements in *R. temporaria* embryos occurred both spontaneously and in response to touch. Movements also occasionally occurred in response to dimming the lights, though with a longer latency. The first movements made by embryos during development were small twitches or larger flexions of the body to one side (Fig. 1). These apparently occurred spontaneously within the egg membranes but could also be evoked by lightly touching the skin of the trunk or head in animals released from their egg membranes. During the onset of each flexion, bending occurred maximally at the level of the trunk. In stronger flexions, this was often associated with an opposite bending of the tail and sometimes head, presumably due to recoil. Only during straightening from the flexion did a wave of bending pass along the tail. The stronger flexion responses involved a near-synchronous and maintained bending along one side (usually the opposite side in the case of a single-sided stimulus). This could involve just a small part of the trunk or, in the tightest flexions, the whole trunk and rostral part of the tail. Even in the strongest flexions, where the total angle of curve of the body could be as much as 325°, the

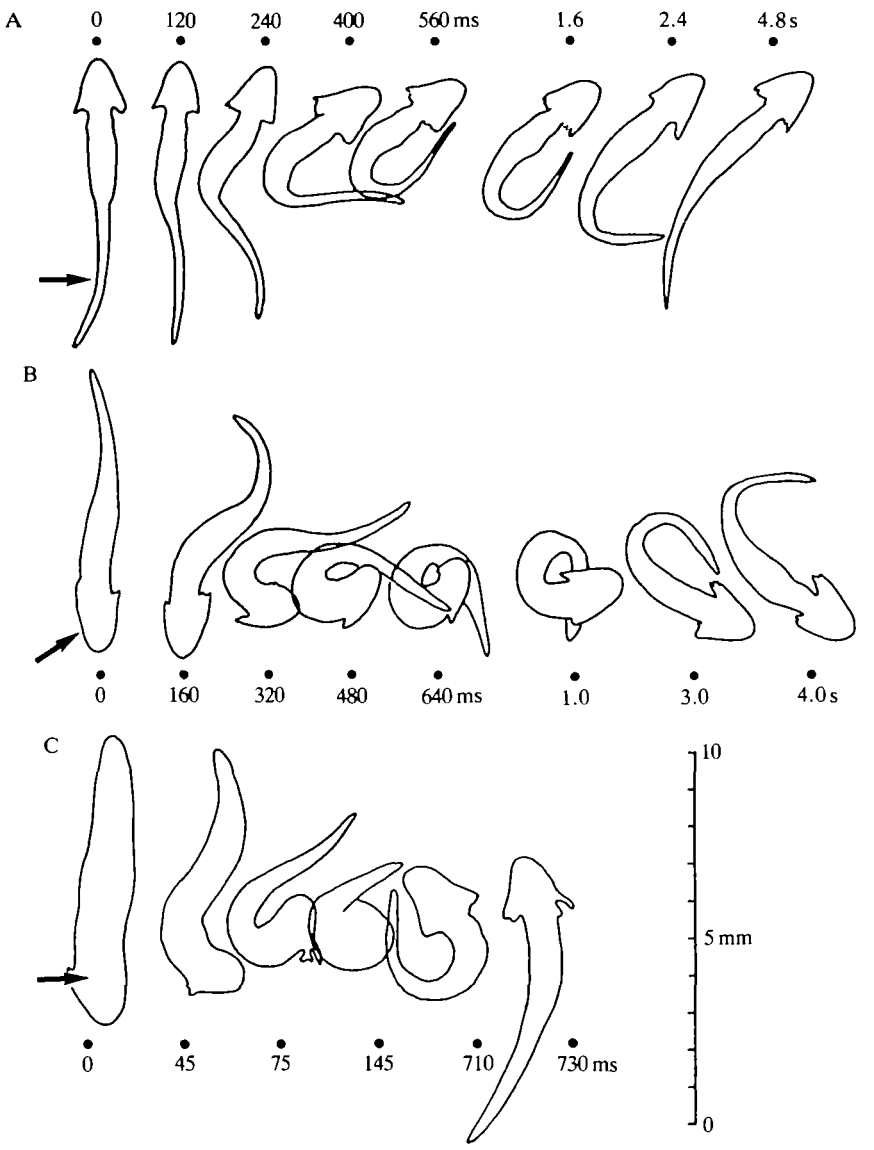


Fig. 1. Non-rhythmic responses to touch in *Rana temporaria* embryos at around the time of hatching. Tracings of single frames from video recordings, each displaced by the distance indicated by dots. Numbers indicate elapsed time. (A) Flexion response on the right side to touching the left side of the tail (arrow). Most bending is restricted to the level of the caudal trunk. There is some initial recoil at the rostral trunk and tail. During relaxation, a bend passes along the tail. (B) Tight flexion of the left side in response to touching the right side of the head (arrow). Bending starts at the level of the rostral trunk and shows recoil at the tail. By the most intense part of the flexion, bending involves the whole head and trunk. Most of the tail shows little bending except during relaxation. (C) Different embryo, as for B but a more intense flexion results in the embryo turning through approximately 180°.

caudal part of the tail showed little bending except as recoil to the initial trunk movement and during the last stage of straightening from the flexion.

When embryos were touched on one side, the first response was usually a bend to the opposite side (chi-square analysis, $N=290$, $P<0.001$) (Fig. 1). If the trunk skin was touched, this bend usually led directly to swimming (in those animals that had developed the ability to swim). In contrast, touching the head usually evoked a strong flexion that could be enough to turn the animal up to 180° away from the stimulus (Fig. 1C) before often leading to swimming.

It was not possible to determine the latency of a response to touch by direct observation or even from video analysis, in most cases, since the precise timing of each hand-held stimulus could not be clearly detected.

Early rhythmic movements

The first sustained rhythmic movements to appear were alternating tight flexions on each side of the body, with the body held maximally flexed for up to 1–2 s before the opposite flexion commenced. Once again, these movements could be seen to occur apparently spontaneously while the animals were still in their egg membranes, but could also be evoked in released animals. By the time of hatching, a clear head-to-tail progression could be seen and the extremes of flexion were held less long, leading to clearly alternating lashing movements.

At around the time of hatching, at total lengths of 8–9 mm, embryos became capable of rhythmic swimming.

Sustained rhythmic movements

Episodes of sustained rhythmic movements occurred both spontaneously and in response to a light touch on the skin of the trunk or head. These movements fell into a range of forms whose extremes were typified by slow alternating flexions at 1–6 Hz (cycle period 170–1000 ms) and higher-frequency movements of up to 10–12 Hz (cycle period 80–100 ms). At lower frequencies, the amplitude of movements was greatest (see below) and there was no clear net forward progression. Movements at this extreme of the range are here termed 'lashing' (Fig. 2) to distinguish them from higher-frequency movements producing clear forward 'swimming' (Fig. 3). Other rhythmic movements fell between these extremes without obvious transitions from one form to another.

In response to a light touch, most episodes of rhythmic movement consisted purely of swimming; that is, movements in the higher frequency range and with a clear forward motion of the body. Some episodes, however, started with one or more cycles of lashing, which appeared to progress smoothly into swimming. Occasionally, a response consisted entirely of lashing. During sustained swimming, the cycle period usually remained fairly constant. Long episodes of unhindered swimming could not be examined because of the restrictions imposed by the dish (see Materials and methods). However, a few measurements made on animals that continued to swim around the edge of the dish showed that the cycle period remained fairly constant throughout longer episodes.

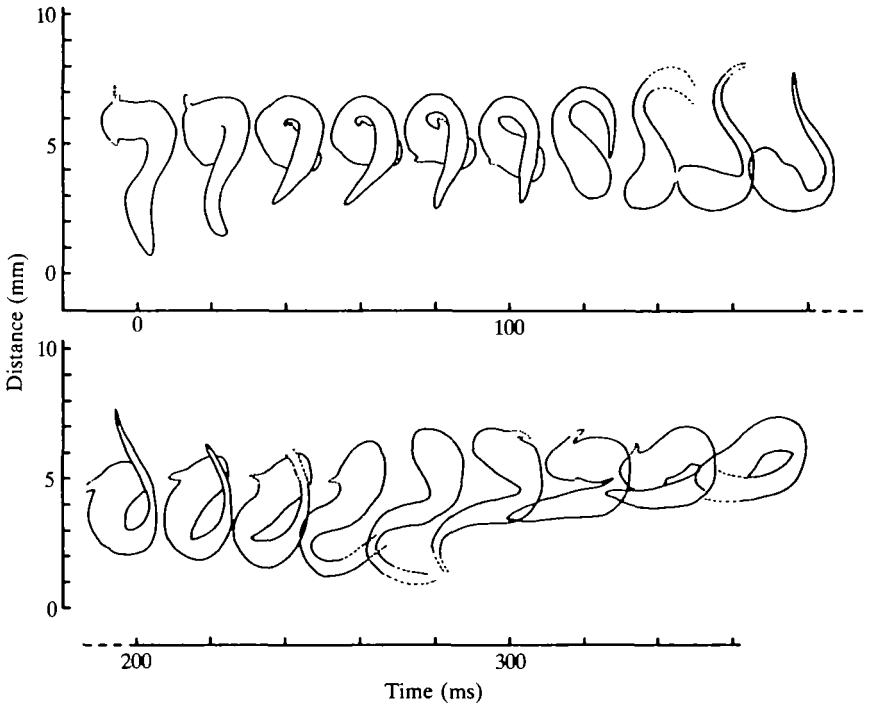


Fig. 2. Rhythmic lashing recorded at 50 frames s^{-1}, evoked by pinching the tail fin. Consecutive frames are drawn from approximately one cycle, each displaced by the distance indicated on the time scale.

The nature of alternating body movements

Analysis of sequences of sustained rhythmic movements showed a remarkable homogeneity of form over the whole frequency range, the movements differing largely in magnitude. Rhythmic movements, from slow lashing to fast swimming, all involved a travelling wave of bending that passed along the body from head to tail. By fitting sine functions to plots of angles of bending at intervals along the body (Fig. 4) several features were determined for sequences of movement analysed in detail. As with any curve fitting, results must be interpreted with caution; however, use of this method appears justified by the closeness of the fit of the theoretical curves to the data.

The patterns of phase delay along the body, obtained from parameter c, were similar at different cycle periods (Fig. 5A). Phase delay between points 0.1 L and 0.9 L for cycle periods between 88 and 197 ms was 0.54. This would correspond to a total phase delay of 0.68. However, phase delay computed from bending angles was not uniform along the body (Fig. 5A). Delay was largest between points along the trunk and rostral tail and lowest towards the head and caudal part of the tail. Extrapolating from phase delays between 0.4 L and 0.6 L gives a total phase delay of 0.96. During swimming it took approximately one cycle for a wave crest to pass along the body from snout to tail tip (Fig. 3), i.e. a phase delay of 1.0. The body

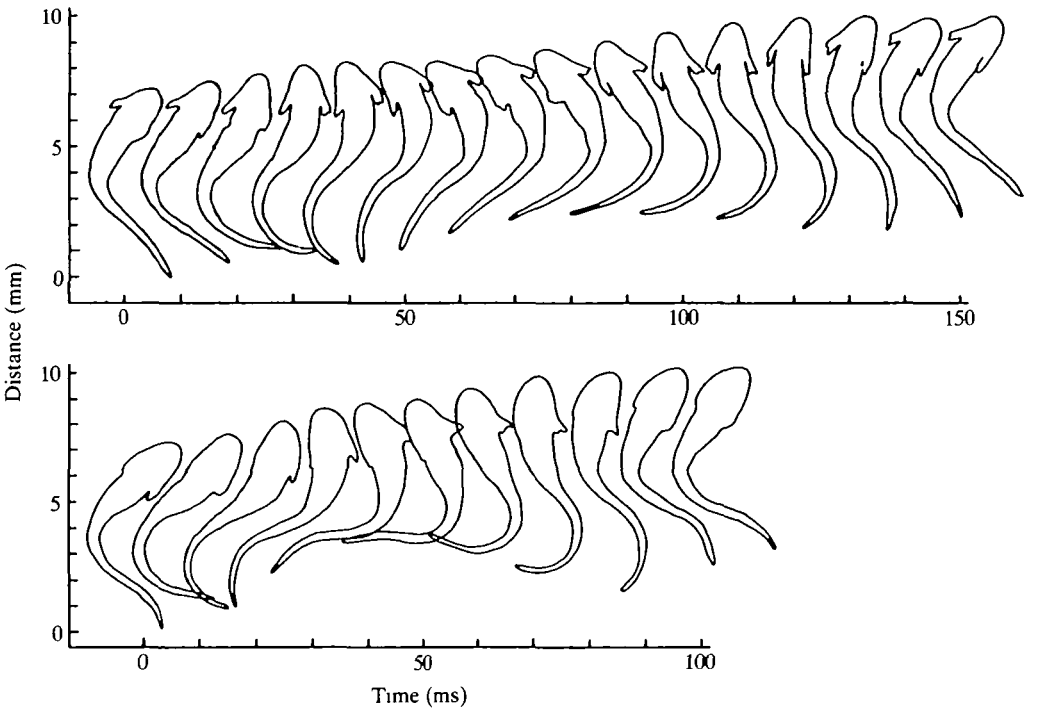


Fig. 3. Sequences of swimming from episodes recorded in two different embryos at 200 frames s^{-1}, evoked by touching the trunk. Every second frame (10 ms apart) is drawn for a single cycle, each displaced by the distance indicated on the time scale.

therefore formed approximately one wavelength at all times, with the snout and tail tip, for example, showing equivalent degrees of lateral excursion at the same point in a cycle (see Fig. 5D).

During rhythmic movements, the maximum bending angle for each point was lowest rostrally and highest at about mid-animal, decreasing again towards the tip of the tail (Fig. 5B). During lashing, the maximum bending angle was generally higher than during swimming, though for the caudal part of the tail angles were similar.

Throughout the whole frequency range of rhythmic alternating body movements, lateral displacement of the body was smallest at the rostral end of the trunk, 0.2 body lengths from the snout (Fig. 5C). It increased both rostral and caudal to this level, reaching a maximum at the tip of the tail. Once again, the only clear difference between swimming and lashing was the much greater degree of lateral displacement during lashing, reaching nearly 1 body length at the tip of the tail.

For most points along the body, motion was forward throughout each cycle, despite lateral movement. The track followed by more-caudal points along the body, however, frequently had a component of backward movement (Fig. 5D). This was particularly noticeable at the tip of the tail. Specific forward velocity

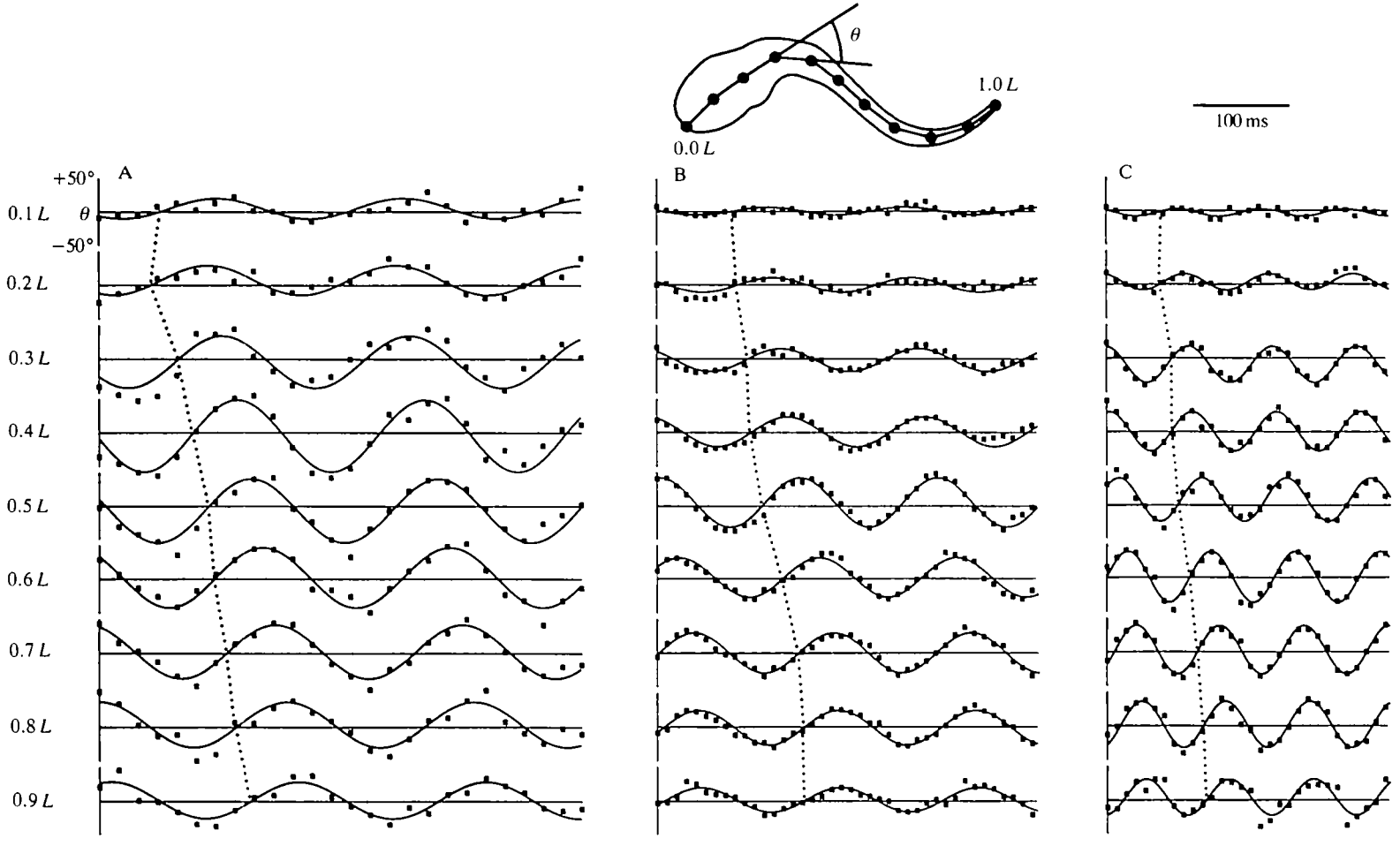


Fig. 4. Angles of bending plotted against time for three sequences of rhythmic movement. Angles (θ in degrees) measured at 0.1 L intervals along the body from the snout, where L is body length (see diagram). (A) Lashing; (B) slower swimming; and (C) faster swimming. Sine curves have been fitted to the data (see Materials and methods). Dotted lines joining values of θ=0° at consecutive points indicate a longitudinal delay in the passage of waves of bending that run from head to tail at all cycle periods (see text).

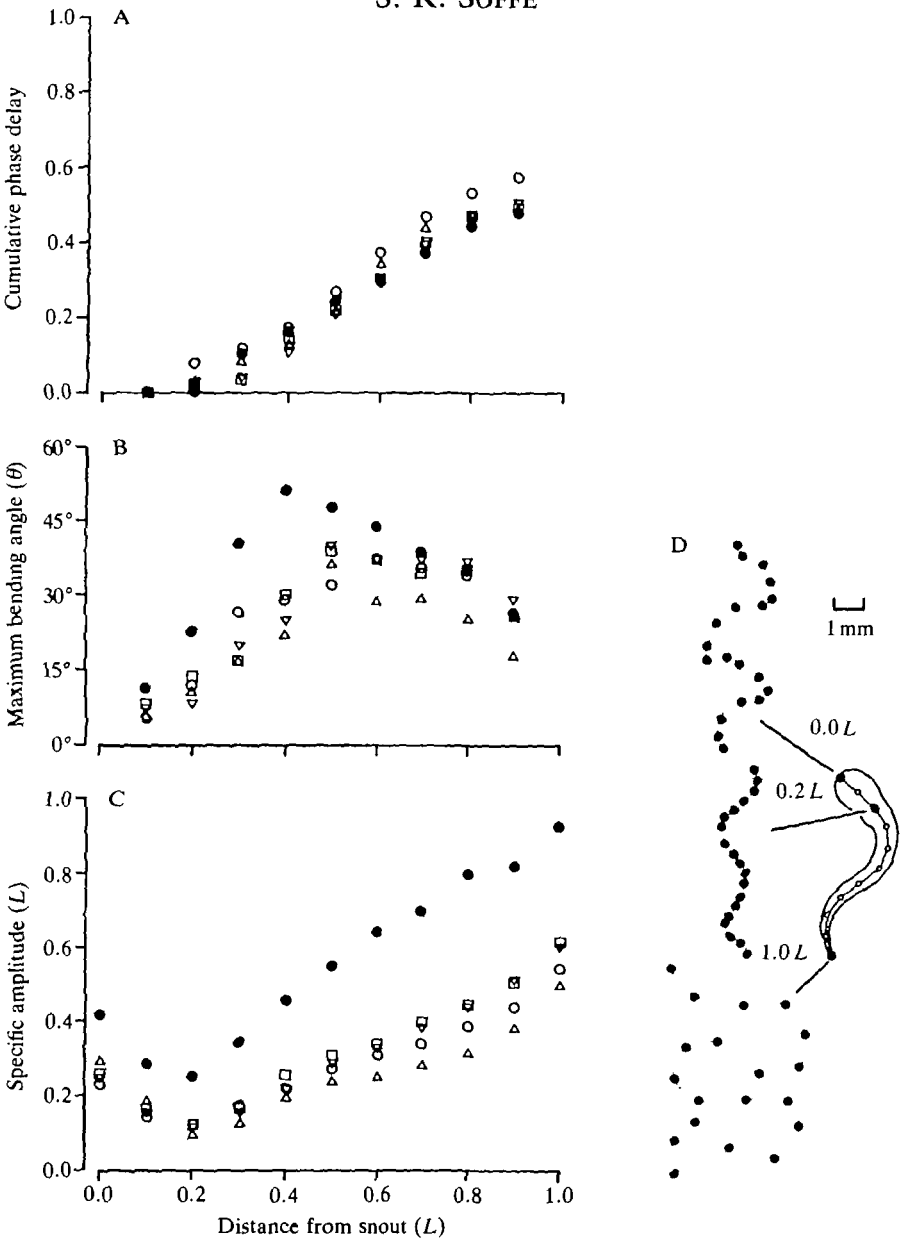


Fig. 5

during swimming, calculated for the point of minimum lateral displacement $0.2L$ from the snout, was up to $4.7 L s^{-1}$. At the other extreme, during lashing there seemed to be no net movement in any one direction and forward velocity was, therefore, effectively zero.

Electrophysiology

Motor root discharge could be recorded from the intermyotome clefts in response to the same stimuli that evoked the behavioural responses outlined in the

Fig. 5. Longitudinal phase delay, bending angles and lateral displacement during rhythmic movements. Open symbols, swimming in four different embryos (cycle periods 88–145 ms); filled symbols, lashing in a single embryo (cycle period 197 ms). (A) Cumulative phase delay (delay cycle period^{-1}) in waves of bending for points at 0.1 L intervals from the snout, derived from parameter c in fitted sine curves (see Materials and methods). (B) Maximum bending angles measured at 0.1 L intervals along the body, as in A, and taken from parameter a. (C) Lateral displacement plotted as specific amplitude (L) at 0.1 L intervals along the body, as in A. In all cases, movement is at a minimum 0.2 L from the snout. (D) The paths followed by three points on the body for two cycles of swimming (0.0 L is the snout, 0.2 L is the point of minimum lateral displacement and 1.0 L is the tip of tail, indicated by filled circles on the drawing of the midline of the embryo). Data for each point are from the same sequence of video frames 10 ms apart; direction of swimming is upwards. Cycle period is 90 ms. Note that the snout and tail tip show the same relative extent of lateral excursion at equivalent times in each cycle (see text).

previous section. Mechanical stimulation was effective, and similar responses were also obtained using brief electrical stimulation of the skin. Dimming the illumination was again a less effective stimulus, being more unreliable and having a much longer latency to response.

Simple non-rhythmic and early rhythmic responses

The simplest motor root responses made by *R. temporaria* embryos paralysed with curare to a light touch or brief electrical stimulation of the trunk skin were single spikes. Responses were graded, with progressively stronger stimulation in the same region producing longer bursts of motor root discharge of up to about 2 s in duration (Fig. 6A). Usually, bursts started at the more rostral electrode before appearing caudally. Activity was never seen synchronously on the two sides at the same level in the spinal cord. Bursts of motor root discharge could also be evoked by a light touch or electrical stimulation of the head skin. As with behavioural responses to stimulation in this region, the first response in the curarised preparation was usually contralateral. Once more, the smallest responses could be restricted to a small part of one side, recorded at a single site. Stronger responses were more widespread. In some cases the initial contralateral response was followed by an ipsilateral burst or by briefly alternating activity (Fig. 6B). The long burst duration and alternating nature of such responses would make them appropriate to drive the alternate tight flexions seen behaviourally.

Sustained rhythmic responses

Episodes of rhythmic motor root discharge were recorded in response to a light touch to the skin of the trunk, tail or head or to electrical stimulation of any of these regions (Fig. 7). On a very few occasions, they were recorded in response to dimming the lights. These episodes could last for approximately 4 s (about 30 cycles), but more often lasted about 2 s.

Episodes showed a range of forms (Figs 7, 8): cycle period could be relatively short and relatively constant throughout most of an episode; it could start

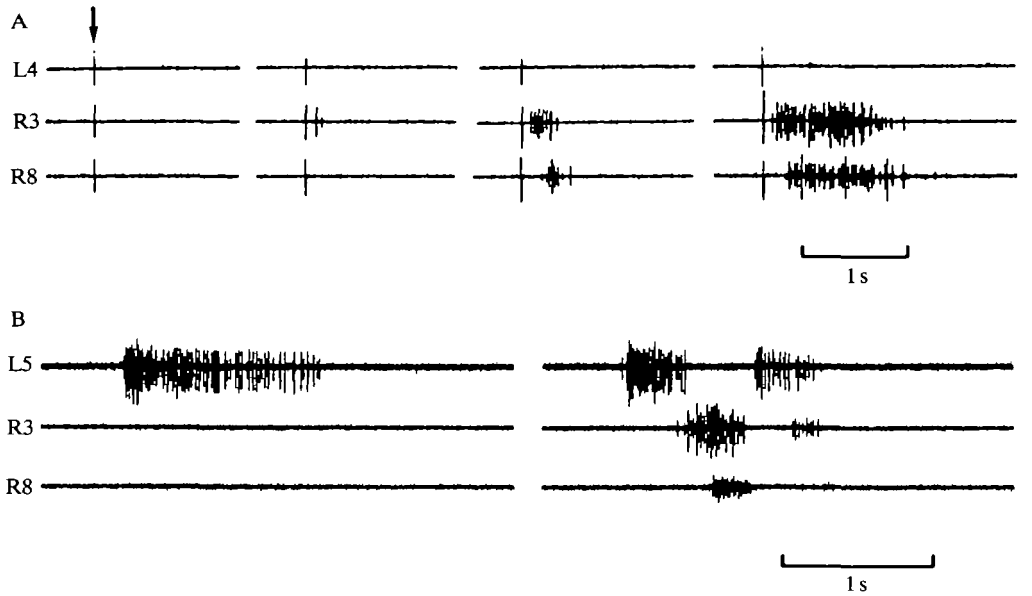
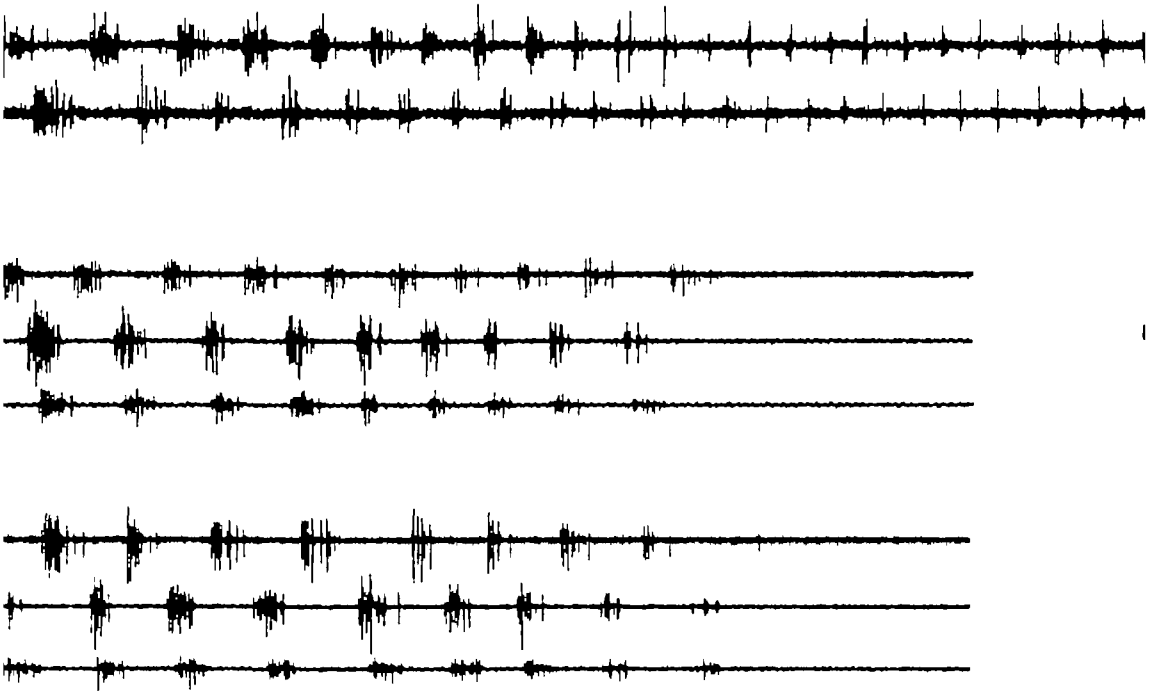


Fig. 6. Contralateral motor root responses evoked by brief electrical stimulation of the caudal trunk skin in curarised embryos. Recordings are made from the left side (L4, L5) and rostrally (R3) and caudally (R8) from the right side. In this and subsequent figures, numbers represent post-otic segments (*Xenopus laevis*: Soffe, 1989). (A) Increasing the strength of a single 0.5 ms stimulus (artefact arrowed in first trace) increased the response from a small discharge recorded at the rostal electrode to a long burst recorded rostrally and caudally. Note that bursts start rostrally. (B) Responses to touching the right side of the tail of a different embryo. Responses included both simple bursts and briefly alternating bursts. The first response was always contralateral.

relatively long and then progressively shorten, reaching a fairly constant level; it could progessively shorten without reaching a constant level by the time the episode finished; or it could remain relatively long throughout the episode. Very occasionally, progressive lengthening of cycle period was seen in the last two or three cycles of an episode (though see Soffe and Sillar, 1991). In all cases, discharge on the two sides alternated (Figs 7, 8). The phase, measured with respect to the start of each motor root burst, was around 0.5, but varied somewhat between cycles. In no case was activity seen synchronously on the two sides.

The motor root activity itself displayed a range of forms. Most commonly recorded were bursts of discharge that occupied up to 40–50 % of the cycle period. This is consistent with motoneurones each firing a burst of impulses on each cycle. The duration of the motor bursts was correlated with cycle period for much of the range of periods (Fig. 9A). Towards the end of episodes, where cycle periods tended to be shorter, motoneurones were probably each able to fire only a single spike per cycle (see also Soffe and Sillar, 1991).



les of sustained rhythmic ventral root discharge recorded in three different embryos. (A,B) Discharge in resp
runk skin. (C) Discharge in response to a brief train of three 0.5 ms electrical stimulus pulses (arrowed at a
tail skin.

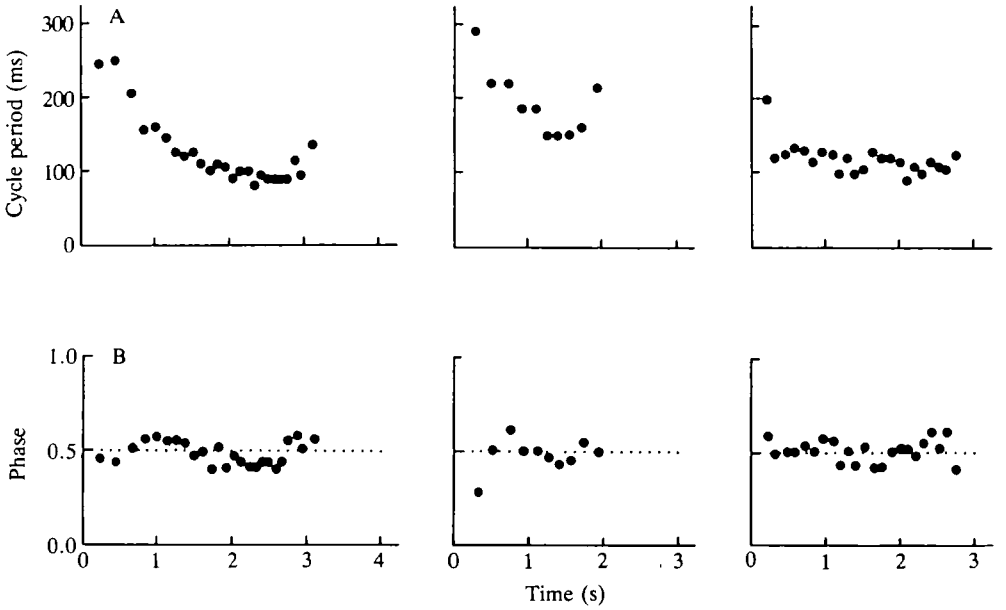


Fig. 8. Cycle periods and phase relationships for rhythmic motor discharge plotted for three episodes recorded in curarised embryos. (A) Cycle periods plotted against time into an episode. (B) Phase relationships of activity on the two sides for the episodes in A (plotted for each cycle as the delay between the onset of adjacent bursts on the two sides divided by the cycle period). These all remain close to 0.5, showing alternation of discharge between the two sides.

Motor root discharge recorded at more caudally placed electrodes always started later than that recorded at more rostral sites (Figs 7, 9). In two preparations where it was measured in detail, the longitudinal delay was correlated overall with cycle period (Fig. 9B), though it was rather variable between cycles. Values for longitudinal delay extrapolated to the length of the whole animal corresponded to mean total phase delays of 0.62 and 0.63 for these two embryos.

Responses to stimulation of the skin of the head

Stimulation of the skin on one side of the head usually evoked a large burst of discharge on the opposite side lasting 0.5–2.0 s. In some cases this response could occur alone. Often, though, the initial response was followed by an episode of rhythmic discharge (Fig. 10). Such motor root responses would be appropriate to drive the contralateral flexions shown by freely moving animals (described above) that turn them away from stimuli to the head and that can also be followed by swimming.

Discussion

In this study, video analysis and electrical recordings of motor root discharge

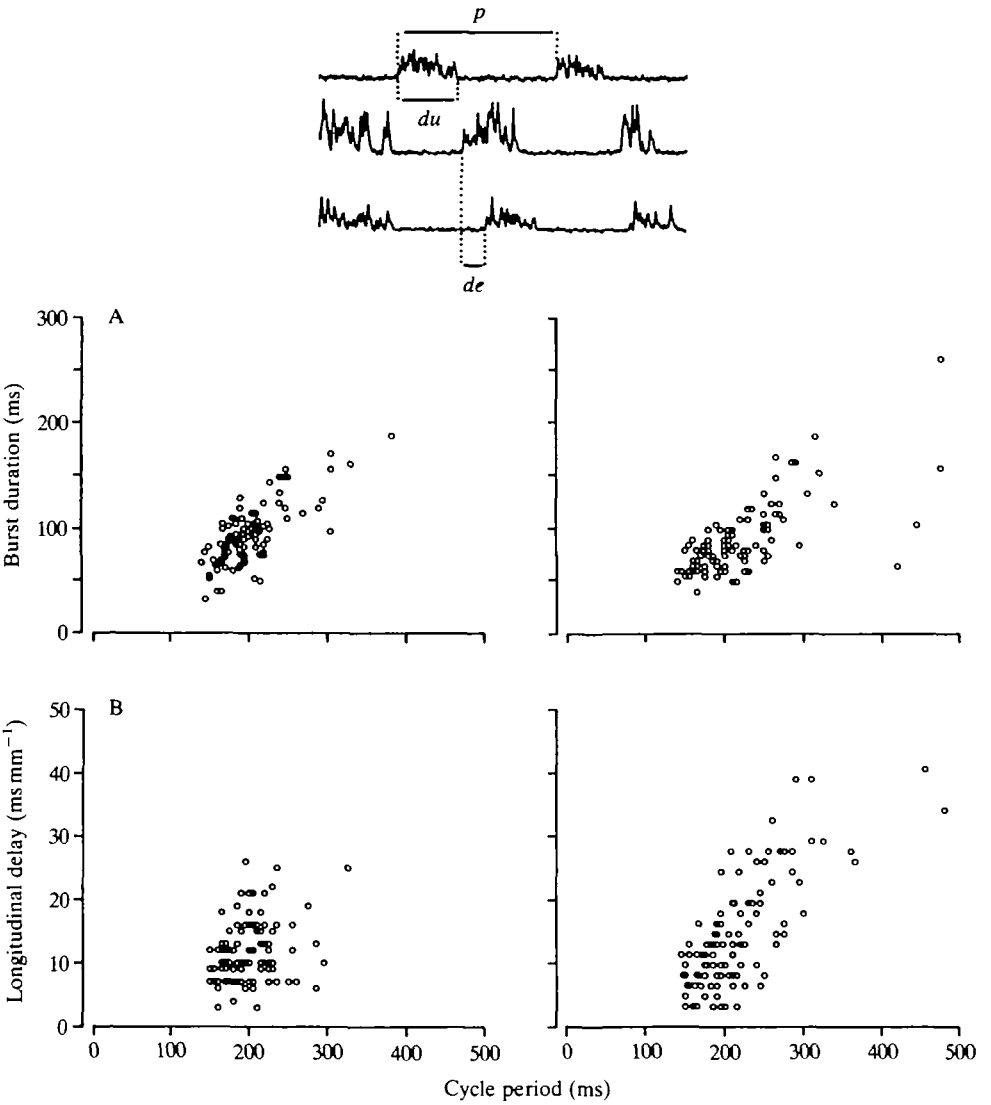


Fig. 9. Motor root burst duration and longitudinal delay during rhythmic discharge. Measured from rectified signals, filtered above 200 Hz, as shown in the top trace (*p* is cycle period, *du* is burst duration, *de* is longitudinal delay). Results are illustrated for a number of episodes from each of two embryos (108 and 102 cycles). Some points on the graph represent more than one measurement. (A) Burst duration is correlated with cycle period (correlation coefficients 0.763 and 0.691, $P<0.01$). (B) Longitudinal delay, measured between two electrodes on the same side, is also correlated with cycle period (correlation coefficients 0.300 and 0.749, $P<0.01$).

have been used to examine the movements made by *R. temporaria* embryos at around the time of hatching, and their central nervous origin. These are the movements that allow the animal to survive the period immediately after hatching

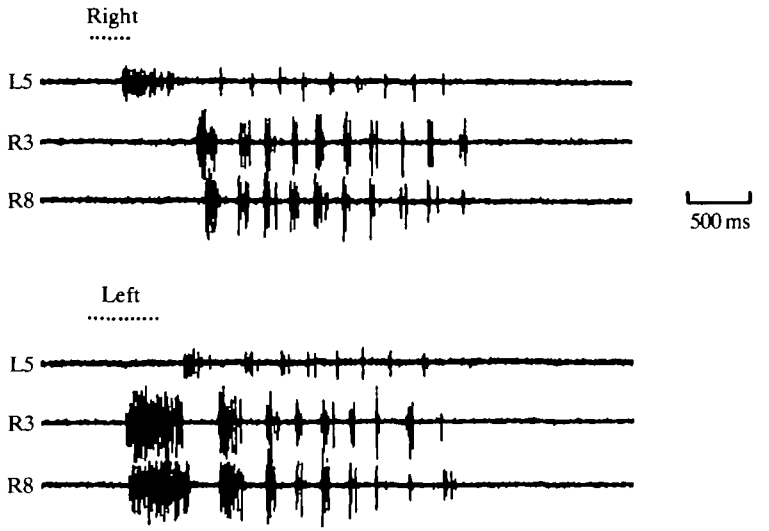


Fig. 10. Responses to stimulation of the head skin on one side. Strokes to the right or left side (approximate time indicated by dotted line) evoke a sustained burst of discharge on the opposite side. In both cases illustrated, the burst is followed by an episode of sustained rhythmic discharge.

(and possibly assist with hatching itself). Movements start as simple flexions within the egg membranes and develop in complexity so that, by the time of hatching, embryos can make a variety of responses to stimuli. These responses include flexions and also sustained lashing and swimming movements suitable for escape and locomotion.

Central programming of behavioural responses

In animals paralysed with curare, it is possible to evoke patterns of motor root discharge that correspond to all the different behavioural responses. This means that not only simple rhythmic and non-rhythmic patterns, like swimming and flexing, but also more elaborate responses, like the turning and swimming that occur in response to touching the head, are largely centrally programmed. In view of the ubiquity of central patterning of locomotor behaviour, it would have been surprising if this had not been true, to some extent at least, for swimming in *R. temporaria* embryos. However, since rhythmic motor patterns in *R. temporaria* differ somewhat from those previously described for *X. laevis* and *T. vulgaris* (Kahn and Roberts, 1982; Kahn *et al.* 1982; Soffe *et al.* 1983), it was important to establish that they too were indeed centrally programmed. The degree to which each pattern of movement is determined by central mechanisms is, of course, harder to establish.

As far as major components of the rhythmic patterns are concerned, the important features appear to be centrally programmed. Cyclic rhythmicity, alternation between the sides and a longitudinal delay are all present in the

paralysed animal and clearly, therefore, do not critically require sensory inflow. It has not yet proved possible to make electromyographic recordings in freely moving embryos so the temporal relationships between motor root discharge, muscle activation and movement of the body cannot yet be determined. Therefore, in the case of the longitudinal delay, for example, it is not yet possible to eliminate completely some role of sensory inflow or to determine the influence of mechanical factors. In older larval stages of *R. catesbeiana* and, presumably, at equivalent later stages of development in *R. temporaria* also, longitudinal delay requires sensory inflow through dorsal roots (Stehouwer and Farel, 1980). However, it is not known just how early in development this dependence becomes established.

Some general discrepancies suggest that the centrally programmed component of each behavioural response may not explain the whole behaviour. First, in the paralysed preparation, episodes of rhythmic activity are generally briefer than the behaviour itself, although long episodes can be evoked. Second, cycle periods may be longer in paralysed animals than in freely moving animals. Third, cycle periods are often more variable on a cycle-by-cycle basis in paralysed animals than during rhythmic behaviour. Although mechanical or other factors could be involved, these discrepancies could also point to an involvement of movement-related feedback by some as yet undiscovered route.

Motor discharge underlying rhythmic behaviour

Perhaps the most striking difference between rhythmic motor patterns in *R. temporaria* embryos and those previously described for *X. laevis* embryos is the lack of any clear discontinuity between slower and faster responses. In *X. laevis*, there is an obvious difference between the slow rhythmic bursts of struggling (Kahn and Roberts, 1982) and the faster rhythmic single spikes of swimming. Changes between the two are exaggerated by a reversal in the longitudinal delay, which is from tail to head during struggling. In *R. temporaria*, there is a smooth gradation between lashing and swimming and the same head-to-tail direction of the delay is maintained throughout. Behaviourally, lashing grades into swimming as the strength of bending (angle and lateral displacement) declines and a clear forward movement of the body is established. Underlying this, relatively slow rhythmic bursts accelerate into shorter, faster rhythmic bursts or single spikes.

The mechanisms underlying generation of rhythmic motor discharge raise a number of interesting points. First, the pattern of rhythmic bursts underlying the slower end of the range of rhythmic movements is clearly self-sustaining. Although rhythmic bursts of discharge can be generated by a wide range of other vertebrate motor systems, there are few examples where sustained rhythm generation is not driven by either maintained sensory stimulation or some other maintained excitation, such as the use of pharmacological excitants or stimulation of descending pathways. McClellan (1984) has shown a few cycles of self-sustained rhythm in the lamprey and the *in vitro* central nervous system of *R. catesbeiana* larvae can also produce spontaneous and apparently self-sustaining episodes of

rhythmic locomotor bursts (Stehouwer and Farel, 1980). Second, from the perspective of the behaviour of the animal, pre-programming of activity apparently extends beyond simple responses. For example, although relatively mild stimulation will evoke swimming, stronger stimulation evokes lashing that grades into swimming. This could allow an animal to break free from restraint (perhaps a predator) and then to swim off. Similarly, a touch to the head evokes an appropriate turn away from the stimulus and only then leads to swimming. In both cases, the whole response can be executed without further sensory input. A remarkably similar response to head skin stimulation has been descibed for *T. vulgaris* embryos (Soffe *et al.* 1983).

Possible sensory modalities involved

Motor responses in *R. temporaria* are evoked by a variety of sensory stimuli. No attempt was made here to classify the sensory systems involved beyond broad distinctions such as dimming the illumination or a light touch, either single-sided or bilateral. Responses to light dimming, which were much less reliable and of longer latency for *R. temporaria* than for *X. laevis* (Roberts, 1978), probably involve the pineal eye, as in *X. laevis*. The lateral eyes in *R. temporaria* are still developing and at this stage are covered by pigmented skin. Trunk stimulation in *X. laevis* acts through either Rohon–Beard neurones in the spinal cord (Clarke *et al.* 1984) or propagated skin impulses. Rohon–Beard neurones are present in the *Rana* spinal cord (Hughes, 1957) and the skin supports skin impulses (Roberts, 1971), so either of these possibilities also applies to *R. temporaria*. Although not clearly established, the pronounced sidedness of responses argues in favour of Rohon–Beard cell stimulation being the important route in mediating the responses to light touch described here. This is supported by the results of intracellular recording from spinal cord neurones (Soffe and Sillar, 1991). The head skin of *R. temporaria* embryos contains both rapid transient and movement type detectors (Roberts, 1980). In *X. laevis*, stimulation of the rapid transient detectors, like stimulation of the Rohon–Beard neurones of the trunk, turns on behaviour. Stimulation of the movement detectors, particularly those around the cement gland, appears to turn off behaviour. It is not yet possible to say whether this situation also holds true for *R. temporaria*.

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