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ORIGINAL PAPER

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Transitions between two different motor patterns in *Xenopus* embryos

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Abstract The *Xenopus* embryo is a valuable system in which to study the poorly understood mechanisms underlying vertebrate motor pattern switching. Here, we present a combined kinematic and electrophysiological description of the changes that occur during a switch between two rhythmic behaviours: struggling and swimming. Stable struggling movements evoked by sensory stimulation were followed by a transitionary period of variable duration leading to swimming. During the transition, cycle period and bending strength (local bending angle) progressively decreased and longitudinal delay progressively reversed. These changes were paralleled by similar changes in cycle period, burst duration and longitudinal delay of the motor pattern in immobilised embryos. The three movement parameters and their motor pattern correlates all scaled together during struggling and transitionary patterns. Our results indicate that transitions can be gradual (consistent with an earlier conclusion that a single set of pattern generating circuitry is involved); that the transitional movements are centrally programmed; and that they form a continuum with struggling movements. The correlated change of motor pattern parameters suggests that either a single mechanism underlies the components of the switch from struggling to swimming, or that separate mechanisms are closely linked.

Key words *Xenopus* · Embryo · Behavioural switching · Swimming · Kinematics

Introduction

Many animals are able to perform multiple rhythmic behaviours each of which must be driven by a separate and distinct pattern of motor output to the muscles. In order to achieve economy of function they often use the same set of multifunctional muscles to express different rhythmic behaviours (Getting and Dekin 1985; Robertson et al. 1985; Gelfand et al. 1988; Hennig 1990). This poses the question of how the underlying neural drive to the muscles switches appropriately between the different motor programmes to drive each behaviour. In invertebrate systems we are starting to gain a quite detailed understanding of how such processes can occur (eg. see Selverston 1995). The situation in vertebrates however is less clear.

The *Xenopus laevis* embryo is a simple vertebrate system which performs two rhythmic locomotory behaviours termed struggling and swimming (Kahn and Roberts 1982b; Kahn et al. 1982). Both of these are expressed using the same set of muscles, the axial myotomes. Much is already known about the neural circuitry underlying swimming (Roberts et al. 1986; Soffe 1991c) and it has recently been established that essentially the same neural circuitry underlies struggling (Soffe 1993a). It is also proposed that struggling rather than swimming can be evoked by higher levels of excitation within the spinal cord. This is normally achieved by sustained sensory input and can be experimentally mimicked by higher levels of bath applied excitatory amino acid agonists (Soffe 1993b). The *Xenopus* system thus presents itself as a suitable model system in which to study the general problem of motor programme switching. The movements and the underlying motor patterns for both struggling and swimming have been documented previously (Kahn and Roberts, 1982b; Kahn et al. 1982; Soffe 1993a). What is not clear are the details of the changes in the movements and underlying motor patterns that occur when the embryo switches behaviour. If we are to gain an understanding of the changes that occur at the neural level to bring about a switch in behaviour it is important to start with a detailed description of what happens during the switch at the levels of the behaviour and the underlying motor output.

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Swimming movements in *Xenopus* embryos have previously been described as lateral undulations of low amplitude which pass with a rostro-caudal longitudinal delay down the body of the animal with a cycle period of 40–100 ms (Kahn et al. 1982). Struggling is described as movements of larger amplitude and longer cycle period (112–224 ms) than in swimming in which waves of bending propogate caudo-rostrally (Kahn and Roberts 1982b). In this paper we present an analysis of both struggling and swimming behaviour and a description of the movements that occur when an embryo switches from struggling to swimming. Rather than attempt a detailed kinematic analysis, we have used a relatively simple procedure in which plots of local bending angles for a series of points along the body are approximated by a set of fitted sine functions. These functions are used to provide measures of cycle period, strength of bending and the longitudinal spread of bending waves: features that may be most readily related to the underlying neural drive. This procedure has been used previously on stable swimming movements in *Rana temporaria* embryos (Soffe 1991a). We have now explored the robustness of the method and shown that it can perform well with short sequences of data, allowing us to examine changing movements that occur during transitions between behaviours. We then describe the patterns of motor activity in immobilised embryos that underlie the movements associated with switches from struggling to swimming. As well as addressing the question of what happens during switching, the results have allowed us to reassess the distinction between the struggling and swimming patterns themselves.

Materials and methods

Stage 37/38 (Nieuwkoop and Faber 1956) embryos of the clawed toad *Xenopus laevis* were obtained by induced pairing. All experiments were carried out at temperatures of between 18° and 22°C.

Kinematic procedures

Embryos were placed in a petri dish (diameter 90 mm) containing dechlorinated tap water and movements recorded from above using a high speed video recorder (NAC 400) at 200 frames/s. Illumination was from below using reflected stroboscopic light synchronised to the recorder frame rate.

Swimming movements were evoked by a light tactile stimulus to one side of the embryo using a fine mounted gerbil hair (cf Clarke et al. 1984). Stronger struggling movements were evoked by applying pressure around the head/rostral trunk with fine forceps (cf Kahn and Roberts 1982b). Restraint was applied for a time just sufficient to evoke a rhythmic response (typically 1–2 s) after which time embryos were released and allowed to move freely. Embryos were recorded from the time they were grasped until they swam out of the field of view. Movements were also recorded in embryos held restrained between etched tungsten pins placed vertically on either side of the neck. Of over 1,000 video sequences of embryo movements recorded, 28 were analysed in detail.

Analysis of video sequences was performed using a relatively simple procedure (see Soffe 1991a) which was briefly as follows. Tracings of body outlines were made for individual frames using 35 mm photographic negatives taken of a flat screen monitor. The midline of the body (body length = L) was described using 11 equidistant points from snout (0.0L) to tail tip (1.0L) (Fig. 2). Frame number and the (x, y) coordinates of each point for a single frame were assigned using a grid with definition of approximately 0.005L (approx 30 μm). Angles of bending (α) at each point from 0.1L–0.9L were calculated and plotted against time (e.g Fig. 1). Sine functions were fitted to these plots of local bending angle against time for body points 0.1L to 0.9L using a least squares method and the simplex algorithm (Nelder and Mead 1965). Fitting was performed in two stages. A first fit was determined for each body point. Cycle periods for these curves usually differed slightly between body points. A second set of 'coerced' sine curves was then fitted, all having the same cycle period. This coerced cycle period was fixed as the mean of values from the first fitted curves. In practise, this second stage made very little difference to the fits but setting identical cycle periods was necessary for making measurements of local phase delay. Equations of the sine curves had the form:

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

where y = angle α; x = time in ms; a represents the maximum bending angle (max α); b provides the cycle period (cycle period = $360 \cdot b^{-1}$); c represents the magnitude of the longitudinal time delay; d represents the asymmetry of the bending between the two sides of the animal. The equations of these curves were used to calculate the maximum local bending angle (max α) and local phase delay (time delay · cycle period^{-1}) at each point, as well as the overall cycle period.

Testing the robustness of the method for movement analysis

Analysis was ideally carried out with sequences of several cycles of stable rhythmic movements. However, this was not always possible. Firstly, during transitions between movements, the pattern changed on a cycle by cycle basis, This required that we analyse sequences as short as a single cycle. Secondly, data for some frames within a sequence sometimes had to be omitted, usually because of movement of the embryo out of the plane of focus. We therefore explored the robustness of the curve fitting employed in our analysis method under these conditions by removing frames progressively from longer data sets. We used three sequences of steady swimming, obtained in a previous study using *Rana temporaria* embryos (Soffe 1991a). These sequences had cycle periods of 88, 114 and 146 ms (8.8, 11.4 and 14.6 data points per cycle) respectively.

Progressive removal of frames from the end of a sequence of data lasting about three cycles of swimming led first to an increase in the spread of values for cycle period obtained from the initial fit of sine curves (dashed, Fig. 1A). However, the mean value was not affected by more than 5% until less than one cycle (9 to 15 points here) remained. (arrowed, Fig. 1A). Phase delay measured over the whole body (0.1L to 0.9L) or the middle part (0.3L to 0.6L) similarly remained within 5% of the cycle period until the number of frames fell to below one cycle (Fig. 1B). Similar results were obtained when increasing numbers of consecutive frames were removed from within the same sequences. Cycle period and phase delay measurements were extremely robust, though for smaller data sets it was necessary to provide a realistic initial estimate for cycle period. In both cases, measurements remained within 5% until less than a total of one cycle worth of points remained. We therefore felt confident both in analysing short sequences down to around a single cycle, and of using data sets containing sequences with missing frames (e.g. Fig. 5A). In practise, sine curves were fitted to several cycles during stable patterns but typically to 1 to 1.5 cycles during transitionary sequences.

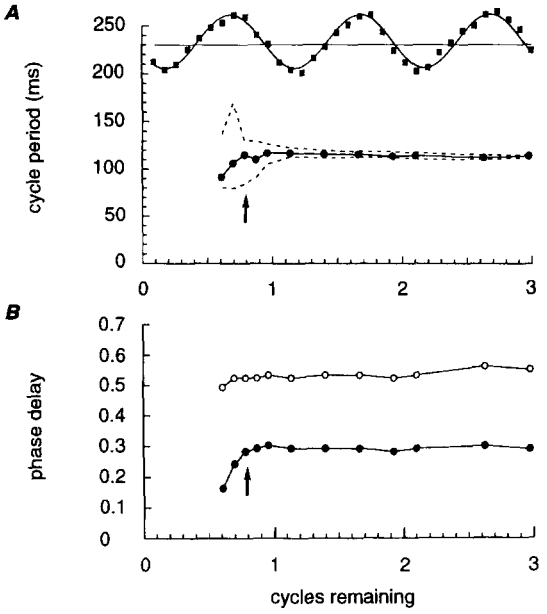

Fig. 1 A, B Testing the robustness of curve fitting. **A** Starting with three cycles of data (shown as *inset top trace*: local bending angles for one position on the body plotted as in Fig. 3, with fitted sine curve), the effect of progressive shortening of data on cycle period. *Dashed lines* indicate range for values from initial "free" curve fitting. **B** Phase delay measured between 0.1 and 0.9L (○) and between 0.3 and 0.6L (●). *Arrows* indicate minimum data subsets for which values differ by less than 5% from those for the intact data set

Electrophysiology

For electrophysiological recording, embryos were anaesthetised in MS222 (tricaine, Sigma, 0.5 mg·ml^{-1}) and their dorsal fins slit to allow access of the neuromuscular blocker α-bungarotoxin. After recovery from anaesthesia the embryos were immobilised in 1 ml of α-bungarotoxin (Sigma, 77 μg·ml^{-1}) until they could no longer swim to normal stimuli (typically about 10–20 min). The embryos were then pinned to a sylgard table with etched tungsten wire pins through the notochord and continuously perfused in a 1.5 ml bath with saline of composition (in m*M*): NaCl, 115; KCl, 3; $CaCl_2$, 2; $MgCl_2$, 1; N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulphonic acid] (HEPES), 10; at pH 7.4. Extracellular recordings were made by means of glass suction pipettes (30–40 μm in diameter) applied to the intermyotome clefts (cf Kahn and Roberts 1982a). For mechanical stimulation, pressure was applied to the head/rostral trunk of embryos using fine forceps mounted on a micromanipulator. Embryos were stimulated electrically using a glass suction electrode (diameter 60 μm) applied to the rostral trunk skin. Pulse widths of approximatley 0.5 ms were used, up to a maximum current of approximately 50 μA. Single pulses were used to evoke swimming; 30–40 pulses at a frequency of 33 Hz were applied to evoke struggling.

Extracellular recordings were stored and analysed using the Digitimer Digistore system. Measurements were taken of cycle period, burst duration and delay in burst activity. Cycle period measurements were made between the midpoints of consecutive bursts of activity (cf Wallén and Williams 1984). Measurements of delay were taken as the time difference between the midpoints of rostral and caudal bursts of activity. Our choice of burst midpoint as a reference for measurements was made to reflect best the underlying neural activity rather than, for example, the relationship to the resulting movement. The membrane potential trajectories of individual rhythmically active neurons are approximately sinusoidal during struggling with the peak corresponding to around the mid-burst position (see Soffe 1993a). In practice, measurements made with reference to burst onset produced very similar results (see legend to Fig. 8).

Results

Analysis of movements

Initial analysis of behavioural responses

When restrained by forceps, *Xenopus laevis* embryos made strong rhythmic movements. If immediately released, these strong movements continued for a number of cycles before the embryos usually then swam off. Initial observation of video sequences showed that the first movements occurring after release were of large amplitude and relatively low frequency (4–10 Hz). (Fig. 2A). They were clearly different to the subsequent rhythmic swimming movements (Fig. 2B). However, details of the different movements, particularly during the period of transition to swimming (Fig. 2C), could not be determined by simple visual inspection and so a more detailed analysis was used. The movements made by embryos while restrained were asssumed to be the struggling movements described by Kahn and Roberts (1982b). However, to avoid prejudging our characterisation of different behaviours, we have referred to all first strong responses as 'initial strong rhythmic movements'. The relationship of these responses to struggling is considered later in the discussion.

Movements in embryos released from a maintained stimulus

We firstly examined eight sequences of the movements immediately following release from the stimulus and seven during swimming. The movements were described by fitting single sine functions to approximately one to five cycles (Fig. 3). The amplitudes of these curves gave the maximum local bending angles, and the phase shifts gave the longitudinal timing of bends in terms of local phase delay (see Materials and methods). The initial strong rhythmic movements following release from a stimulus showed relatively long cycle periods (range: 86 to 187 ms, $n = 8$ embryos). Plots of local phase delay showed that bends on each cycle started at about 0.6L to 0.7L, about the level of the anus (Fig. 4A). This meant that over the trunk, the progress of the bending wave was caudo-rostral, while in the tail it was rostro-caudal. The pattern during swimming was quite different (Fig 3B). Cycle period was shorter (range: 38 to 73 ms, $n = 7$ embryos). Also the progress in the bending wave was rostro-caudal over the whole length of the animal (Fig. 4A).

The distribution of local bending angles along the body differed between initial strong rhythmic movements

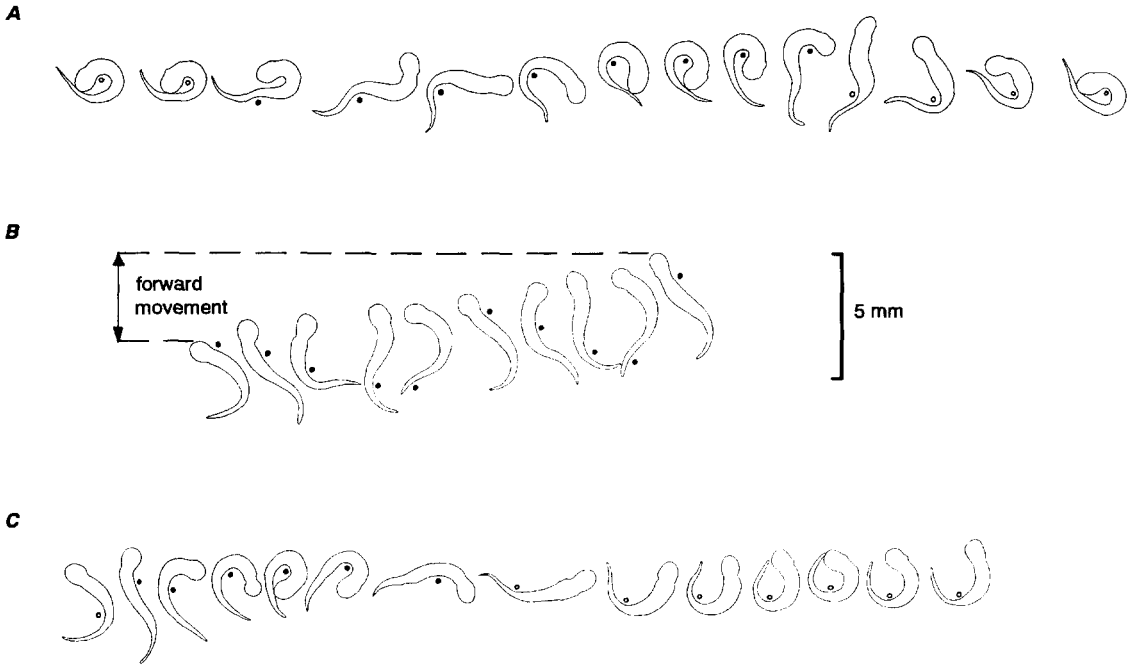

Fig. 2A–C Video frame sequences of *Xenopus* embryo movements after release from a maintained stimulus. **A** Initial strong rhythmic movements of low frequency and large amplitude occurring immediately after release. A wave of bending propogates caudo-rostrally over the trunk of the embryo (indicated by consecutive estimates of the position of maximum flexion on left ○ and right ● sides). **B** Swimming movements of characteristic high frequency and low amplitude, with a rostro-caudal delay in propagation of the wave of bending. **C** Transitionary movements following initial strong rhythmic movements and prior to swimming. Notice that it is difficult to define the direction of longitudinal delay by simple visual inspection. The interval between frames is 10 ms. Each frame is displaced to the right of the previous frame and does not reflect the overall movement of the embryo in the water

and swimming (Fig. 4B). The initial movements showed generally larger local bending angles with maximum values at the level of the rostral trunk and dropping steadily towards the tail. During swimming, local bending angles were lowest rostrally and increased steadily more caudally. Local bending angles were similar over the length of the tail for both patterns.

Movements in restrained animals

To allow us to assess what movements occur before the animal is released, movements were recorded in embryos restrained using a pair of pins placed vertically either side of the constriction just behind the head (at approximately 0.2L). This was also the method used by Kahn and Roberts (1982b) in their earlier investigation of struggling behaviour. We analysed the movements immediately after stimulation, and also during swimming,

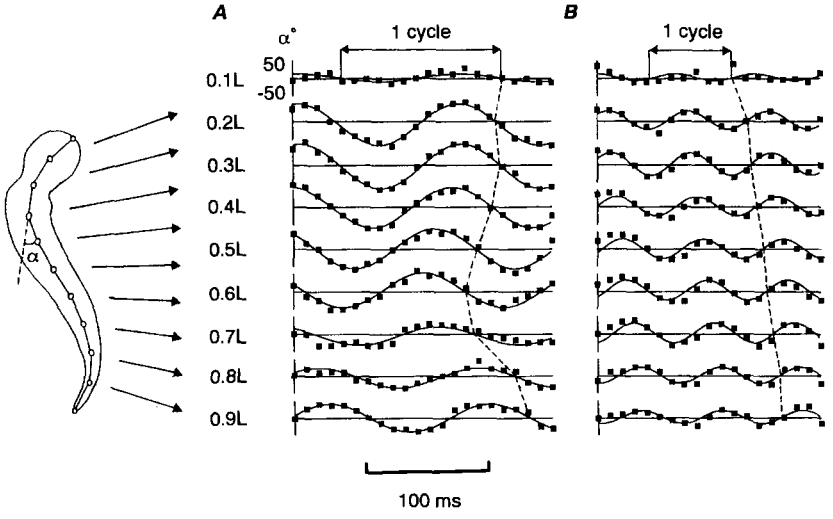

Fig. 3A, B Analysis of video sequences of *Xenopus* embryo movements (see Materials and methods for details). Local bending angles (α) at body points 0.1L to 0.9L (see *diagram*) plotted against time with fitted sine curves. **A** Initial strong rhythmic movements. The *dashed line* highlights the delay which is caudo-rostral over the trunk region (points 0.1L–0.6L) and rostro-caudal over the tail region (points 0.7L–0.9L). The wave of bending starts at about 0.6L in this sequence. **B** Swimming movements, from a different embryo. The delay in the wave of bending is rostro-caudal over the entire body length

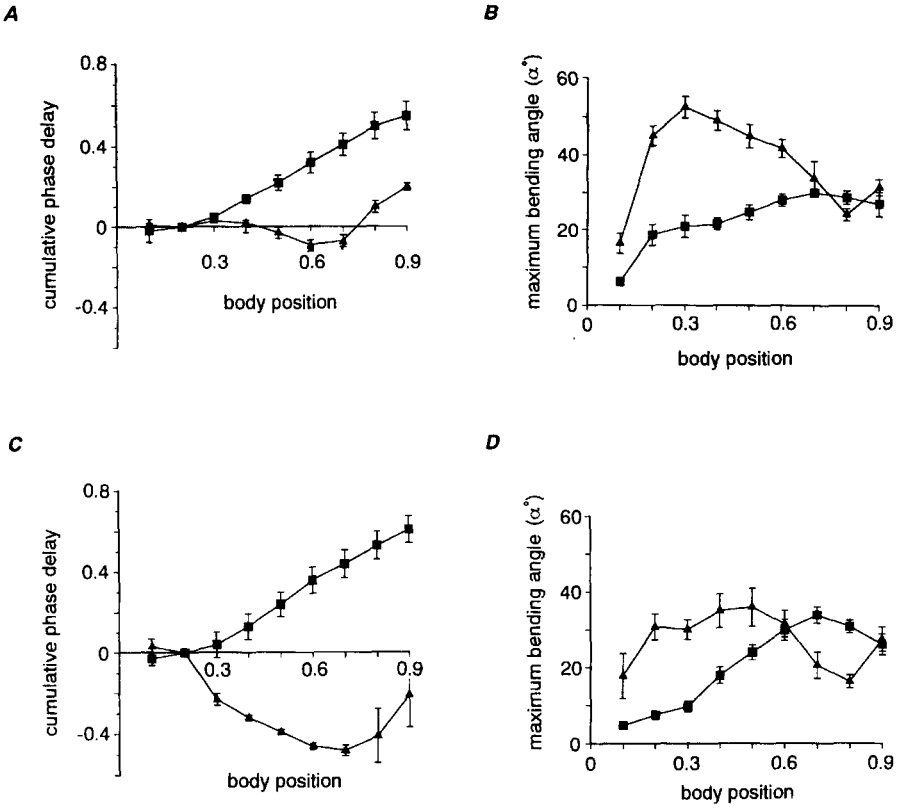

Fig. 4A–D Patterns of phase delay and maximum local bending angles during movements in freely moving and restrained embryos. All values are plotted as means ± SE against body position for initial strong movements (▲) and swimming (■). **A** Cumulative phase delay and **B** maximum bending angle (α) in freely moving embryos. **C** Cumulative phase delay and **D** maximum bending angle (α) in restrained embryos.

in these restrained embryos. Once again, cycle period was relatively long (range: 151 to 320 ms, $n = 6$ embryos) during the initial movements compared with those during swimming (range: 69 to 85 ms, $n = 6$ embryos). Differences between the two patterns of movement in restrained embryos were like those seen in freely moving embryos (Fig. 4C). The direction of phase delay in the progress of the bending wave during the initial strong rhythmic movements showed a large caudo-rostral component over the whole of the trunk. Once more, though, the wave was rostro-caudal in the tail. During swimming, the pattern of phase delay was identical to that in freely moving embryos.

The distribution of local bending angles was again different in the two movements (Fig. 4D). During the initial strong rhythmic movements, the largest bending angles were again in the trunk, though they were generally lower than in freely moving embryos. In contrast, the pattern during swimming was like that in freely moving embryos with the local bending angles again lowest at the head and increasing steadily towards the tail.

Transitions between movements

After describing the initial strong rhythmic movements following stimulation and the movements during swimming, we analysed seven sequences during which there was a transition between the two patterns. Clearly, a single sine function could not equally describe all parts of these transitions. Instead the movements were analysed by fitting several sine functions to successive portions of each sequence (see Materials and methods; Fig. 5A). Typically, sequences started and finished with relatively stable periods of initial strong rhythmic movement and swimming respectively. Between these were transitions during which cycle period shortened (Fig. 5A). The progress of the bending wave also changed between the extreme forms described above. There was a progressive change from a wave with a clear caudo-rostral component to one that was entirely rostro-caudal (Fig. 5B). In a similar way, there was a progressive change in the distribution of maximum local bending angles from one typical of the initial strong rhythmic movements, with a peak of large angles at the rostral trunk, to one typical of swimming, with a steady rostro-caudal increase in local bending angles (Fig. 5C).

Changes occurring during transitions

The changes in the form of body movements that occurred during transitions were analysed in more detail by comparing three main components: cycle period,

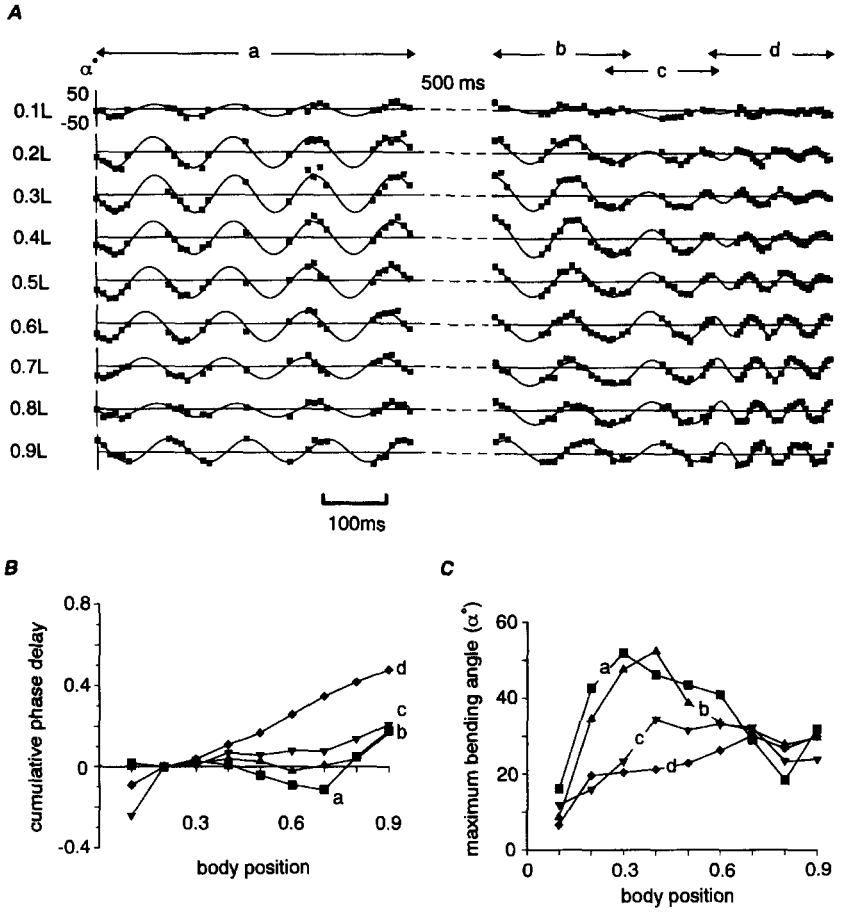

Fig. 5A–C Changes in movement pattern during transitions. **A** Changes in local bending angles (α) plotted as for Fig. 3 during a transition from initial strong movements to swimming. Separate sine curves are fitted to different sequences of the transition (*a–d*). The sequence begins with several stable cycles of initial strong rhythmic movements (*a*) and ends with several cycles of stable swimming (*d*). Between these two is a transitionary region (*b* and *c*) showing a progressive change in cycle period, maximum local bending angle and longitudinal delay. **B** Cumulative phase delay and, **C**, maximum local bending angle for sequences *a–d*, showing the progressive change in each

progress of the bending wave (expressed as phase delay), and the strength of the movement (expressed as maximum local bending angle). To make the contrast as clear as possible, analysis was restricted to the region of the body over which the major differences were clearest: from 0.3L to 0.6L. This region includes most of the trunk. All three parameters changed progressively during transitions, and changes in one parameter were paralleled by changes in the others (Fig. 6A–D). This was seen most clearly by plotting phase delay and maximum local bending angle against cycle period (Fig. 6EF). All were significantly correlated. No examples were encountered where the three parameters appeared to change independently. For example, strong bending angles were not associated with a rostro-caudal bending wave.

Electrophysiological recordings

Pattern of responses to mechanical stimulation

To examine the extent to which the different movements are driven by specific patterns of neural activity, motor root recordings were made from 18 immobilised embryos. Activity was evoked in a similar manner to that for the kinematic recordings using gentle sustained pressure in the head/rostral trunk. The stimulus was released once activity was established (usually after several cycles of strong motor bursts). As with the movements, motor responses in immobilised embryos started with relatively slow, strong rhythmic motor activity which changed over a period to faster rhythmic activity typical of swimming (Fig. 7). The initial activity showed the characteristics previously described for struggling behaviour (Kahn and Roberts 1982b; Soffe 1991b). It had a relatively long cycle period (range: 90 to 460 ms for the sequences measured here). Motor root bursts were long and showed a caudo-rostral delay. Subsequent swimming activity, in contrast, showed shorter cycle periods (range: 35 to 45 ms for the sequences measured here). Swimming motor root bursts were brief and showed a rostro-caudal delay. During the transitions between patterns, cycle period, burst duration and longitudinal delay all changed progressively.

Analysis of transitions

As with the movements, transitions could be fast or slow, lasting several cycles. To analyse the changes

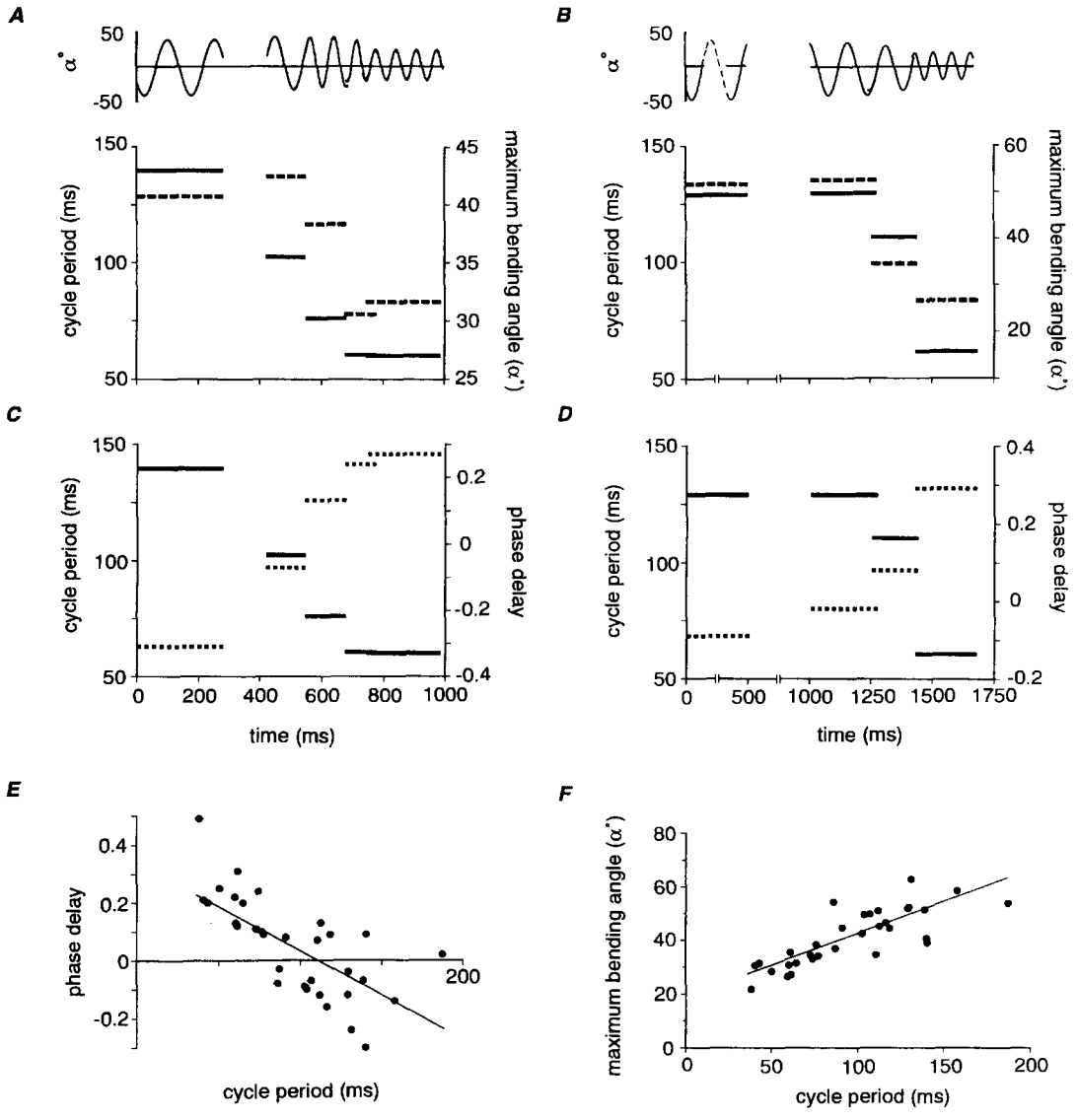

Fig. 6A–F The relationship between movement parameters during transitions. **A** and **B** Changes in cycle period (*solid lines*) and maximum bending angle (*dashed lines*) during transitions between initial strong movements and swimming in two embryos. Values were obtained from sine curves (see *insets*) fitted to sequences whose duration is indicated by the length of the lines. **C** and **D** Change in cycle period (*solid line*) and phase delay (*dotted line*) during the same transitions shown in **A** and **B**. Maximum local bending angle and cumulative phase delays are both measured over body region 0.3L to 0.6L. All three parameter values change in parallel. Notice that the transition shown in **A** and **C** is relatively slow (approx 400 ms) while that shown in **B** and **D** is relatively fast (approx 150 ms). **E** Phase delay is negatively correlated with cycle period (regression line $r = -0.71$ $n = 31$). **F** Maximum local bending angle is positively correlated with cycle period (regression line $r = 0.83$ $n = 31$)

occurring during transitions between motor patterns, three parameters were again measured in detail (Fig. 7CD). As with the movement analysis, two of these were cycle period and longitudinal delay between bursts recorded rostrally and caudally. To allow comparison with measurements of the bending wave from kinematic data, the longitudinal delay was expressed as a phase delay over the same distance: equivalent to body positions 0.3L to 0.6L (= 1.65 mm). In addition, rostral burst duration was also measured. Although burst duration was not expected to relate directly to the bending angles measured in the movement analysis, it appeared to give a more reliable measure of the "strength" of the response than, for example, burst amplitude, which varies considerably with quality of recording. Measurements for all three parameters changed progressively during a transition (Fig. 8AB). Also, as with the kinematic parameters measured, these changes occurred in parallel. Once again, the relationship between the parameters was seen most clearly by plotting values for burst duration and phase delay against cycle period (Fig. 8CD). Data were taken from three transitions in each of ten embryos. For each

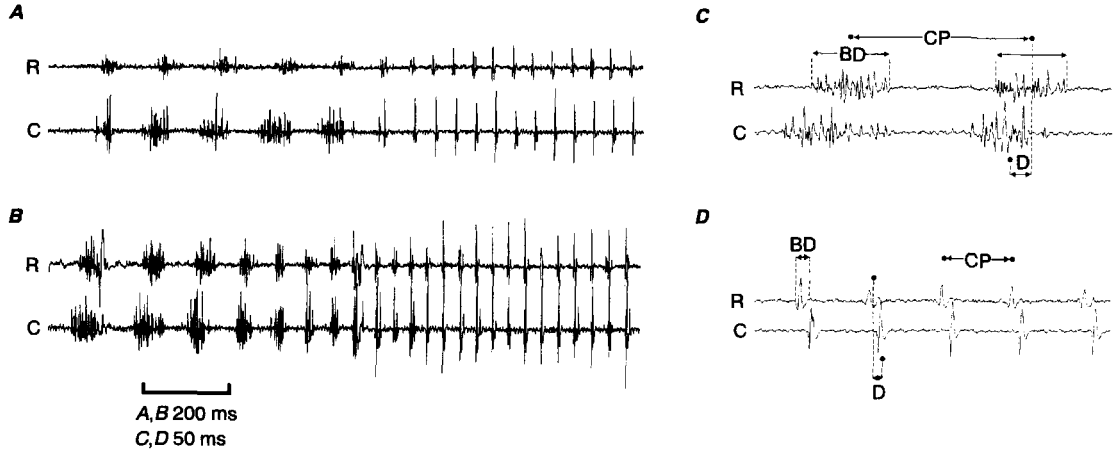

Fig. 7A–D Motor root discharge following release from a maintained stimulus. **A** and **B** Two examples of motor responses recorded rostrally and caudally on the same sides of immobilised embryos. Each shows an initial pattern of strong rhythmic bursts of relatively long cycle period and caudo-rostral longitudinal delay, typical of struggling, which then changes progressively to a faster rhythmic pattern of brief bursts with a rostro-caudal longitudinal delay, typical of swimming. The transition is fast in **A** (1–2 cycles), and slower in **B**, (approximately 6 cycles). Sequences of struggling (**C**) and swimming (**D**) discharge taken from **B**. Cycle period (*CP*), longitudinal delay (*D*) and burst duration (*BD*) all differ between the two (measured as indicated; ●: mid points of bursts)

embryo, there was a significant positive correlation between burst duration and cycle period, and a significant negative correlation between longitudinal phase delay and cycle period (Fig. 8CD). As with the kinematic measurements, there was no evidence overall for the three parameters varying independently.

Transitions following electrical stimulation

Earlier studies of swimming and struggling, particularly those involving intracellular recording, have largely used electrical rather than mechanical stimulation. To allow comparison with such previous studies (e.g. Soffe 1991b, 1993a), responses were examined following electrical stimulation of the skin.The pattern of responses was very similar to that seen following mechanical stimulation. Embryos responded to repetitive stimulation with strong rhythmic bursts and a caudo-rostral delay for a period lasting up to 1.2 s. Following stimulation, there were both fast and slow transitions to the swimming pattern, lasting 1 to 2 cycles or up to 8 cycles respectively, as found for mechanical stimulation. The longest cycle period, the maximum burst duration and the largest caudo-rostral phase delay all occurred during, and immediately after, stimulation. Subsequent transitions to swimming involved a progressive change in all three measures (Fig. 8EF), and again all were significantly correlated (Fig. 8GH).

Longitudinal pattern of phase delays

The longitudinal phase delay describing the progress of the bending wave during real movements was not linear along the body during the initial strong rhythmic movements (Figs. 3A, 4A). To investigate whether this was also a feature of the underlying pattern of motor root discharge, recordings were made using several different rostro-caudal electrode spacings in four immobilised embryos. Embryos were stimulated electrically. In each case, the rostral recording position was kept constant at the third post-otic cleft. The caudal electrode was then placed at a variety of locations along the body. For each electrode spacing, the relationship between longitudinal phase delay and cycle period before and during transitions was plotted as described above. Using the regression equations for plots for each electrode spacing, phase delays were calculated at a series of cycle periods, corresponding to the stable activity during stimulation, and various times through a transition. Additionally, a series of phase delay measurements were obtained for swimming, using mean values for 20 swimming cycles, starting 1s after stimulation ceased. Using these values, graphs of cumulative phase delay against body position were plotted (Fig. 9), equivalent to the plots for transitions obtained from the kinematic analysis (Fig. 5B). During struggling, the pattern of phase delay along the trunk (body positions 0.3 to 0.65L) was nearly linear and caudo-rostral. Caudal to 0.65L, the phase delay along the rostral part of the tail was rostro-caudal. At progressively shorter "transitional" cycle periods, the phase delay over the trunk decreased until during swimming, phase delay was approximately linear and rostro-caudal over the whole length of trunk and tail measured. Qualitatively, this pattern is very

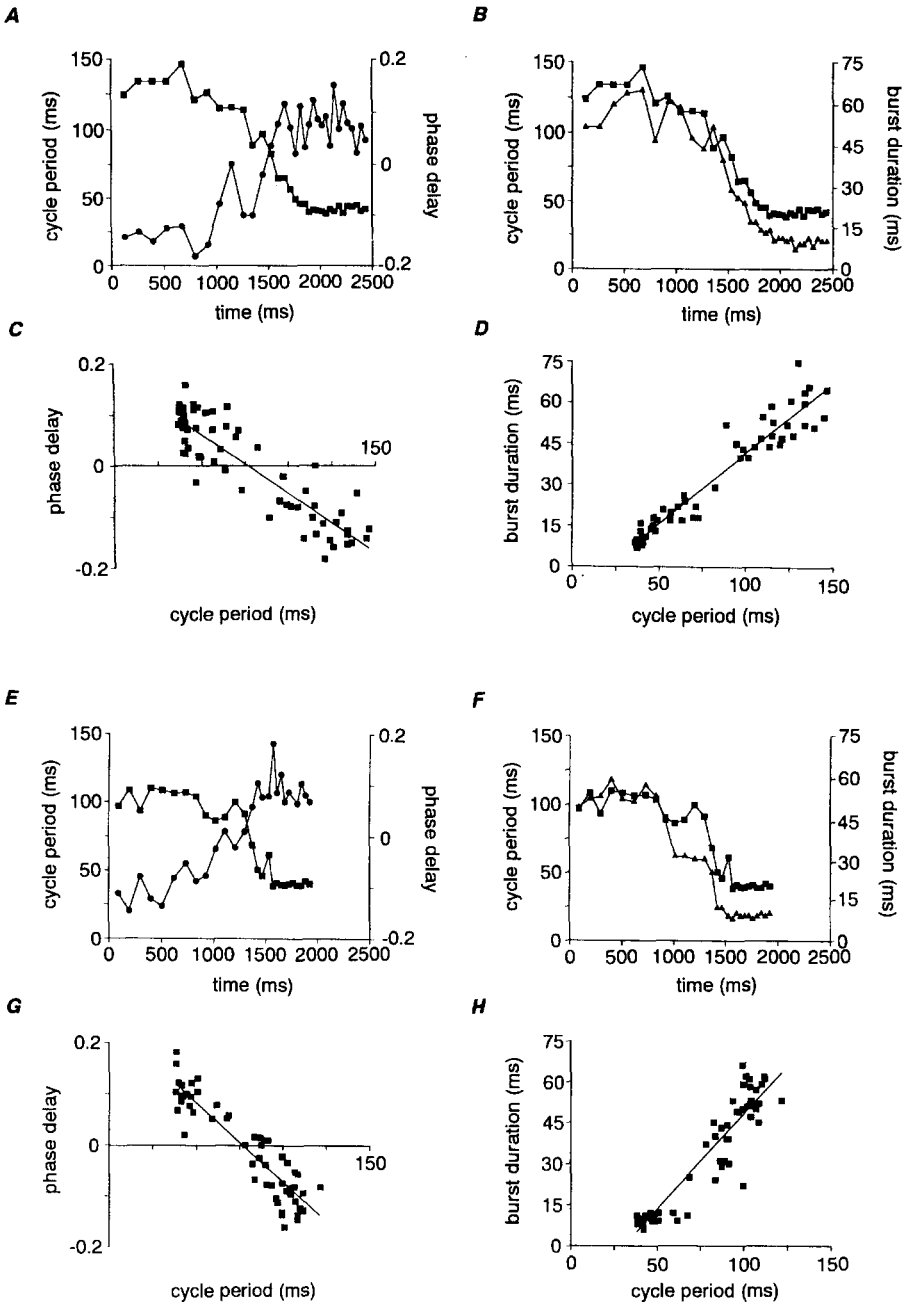

Fig. 8A–H Changes in motor root discharge during transitions. (**A–D** following mechanical stimulation; **E–H** following electrical stimulation. Changes in **A** longitudinal phase delay (●) and **B** burst duration (▲) parallel changes in cycle period (■) during a transition following mechanical stimulation. **C** Cycle period and phase delay are significantly correlated (regression line $r = -0.89$, $n = 62$). **D** Cycle period and burst duration are also significantly correlated (regression line $r = 0.97$, $n = 62$). Data in **C** and **D** are from three separate transitions. **E** and **F** Parallel changes in burst parameters for a transition in a second embryo following electrical stimulation. Plotted as for **A** and **B**. Once more, phase delay (**G**) and burst duration (**H**) were significantly correlated with cycle period (regression lines, $r = -0.92$, $n = 62$; $r = 0.93$, $n = 62$, respectively). Data in **G** and **H** from three separate transitions. (Regression coefficients for the data in **C**, **D**, **G**, and **H** measured from burst onsets rather than midpoints: $r = -0.89$, $r = 0.94$, $r = -0.90$ and $r = 0.89$ respectively)

similar to that obtained from kinematic analysis of the real movements (Fig. 5B).

Discussion

The results we have presented describe for the first time parallel kinematic and electrophysiological analyses of the transitions between two distinct rhythmic behaviours in *Xenopus* embryos. Until it becomes possible to make electrical recordings from these small embryos while they are freely moving, a truly combined study will not be possible. However, we consider that the approach we have used can give us insights into the mechanisms that underlie such a transition.

The relationship between struggling, swimming and transitional movements

Before examining the transitions themselves, it was necessary to determine the relationship between the two patterns involved. Swimming in *Xenopus* embryos has been described in some detail for both the kinematics (Kahn et al. 1982) and the electrophysiology

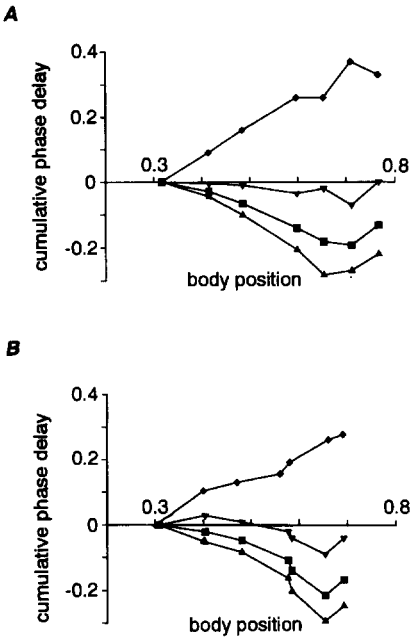

Fig. 9A, B Changes in the pattern of cumulative phase delay during two transition sequences. **A, B** Phase delays plotted for two embryos at cycle periods of 160 ms (▲), 130 ms (■) and 100 ms (▼) and during subsequent swimming ((♦)). The rostral electrode was placed at the third post-otic myotome cleft; the caudal electrode was placed at six different positions caudal to this in random order. Data were obtained from regression lines for plots of phase delay against cycle period (cf Fig. 8G) from three separate transitions at each electrode spacing (see text for details). Swimming phase delays were means of 20 cycles

(Kahn and Roberts 1982a). The stronger response to maintained stimulation, termed struggling was originally described by Kahn and Roberts (1982b) using restrained embryos. The pattern they described involved a bending wave that was caudo-rostral for most of the length of the embryo, with each wave apparently starting near the tail tip. A similar pattern was seen in the present study, though the bending waves appeared to start less caudally, near the level of the anus (0.7 L) and spread rostral and caudal from this position.

The initial strong rhythmic movements immediately following release as described here were somewhat different to the struggling pattern in restrained embryos. The progress of the bending wave along the trunk was again caudo-rostral and each wave appeared to start at about the level of the anus (0.6 to 0.7 L). However, the phase delay was smaller in the freely moving embryos than in the restrained embryos, with little delay over the rostral trunk. Two possibilites must be considered. The first is that the initial strong rhythmic movements are indeed struggling, and the difference is biomechanical, with the presence of restraint altering the pattern of reactive forces and therefore the pattern of movement. The second is that the pattern following release is no longer true struggling, but has already started the transition to swimming. This could occur because the embryo is no longer receiving stimulation. Since the stong initial movements following release from restraint could remain stable for several cycles before a further transition commenced, the second seems the less likely explanation. The electrophysiological finding that there was no sudden jump to a different pattern at the end of stimulation, but instead a progressive transition, also argues against the second explanation. If so, then struggling movements cannot be described simply as characterised by a caudo-rostral wave of bending, since the exact profile depends on whether the animal is restrained or not. In both cases, though, the progress of the bending wave along the trunk, the part of the body which should be driven most strongly by neuromuscular activity, is caudo-rostral. This remains in clear contrast to the rostro-caudal progression of the bending wave during swimming. It is interesting that the pattern of the longitudinal phase delay during swimming is not apparently altered by restraint. This may be because the lateral movement at the level of the restraint is minimal anyway (Kahn et al. 1982).

The movements that occur during a transition to swimming are not fundamentally different to the movements during struggling. They simply involve a progressive change in the main parameters: cycle period, the size and distribution of local bending angles, and the longitudinal pattern of the bending wave. For this reason, we would suggest that struggling and the transitionary movements are a continuum, in which struggling itself is one extreme. The main distinction is that the pattern during struggling remains relatively stable, while during transitions it clearly does not.

The evidence from motor root recordings in immobilised embryos supports the above suggestion. The pattern of discharge during struggling also forms a continuum with those of the transitions to swimming. Cycle period, burst duration (like maximum local bending angle) and phase delay are all correlated. Struggling is again only distinct as a pattern by being at one extreme of the range and by being relatively stable. In contrast, the motor pattern for swimming is qualitatively different. Burst duration remains short and rather constant, and does not show any clear change with cycle period. The pattern of longitudinal delay is also different. We have expressed this delay as a phase delay, for consistency with the kinematic measurements. However, the absolute longitudinal delay during struggling and transitions also changes with cycle period. In contrast, Tunstall and Roberts (1991) have shown that the rostro-caudal delay in motor root discharge during swimming does not change with cycle period.

As described above, no scaling in burst duration and longitudinal delay with cycle period is normally seen during swimming in *Xenopus* embryos. However, in early larvae, there are the first signs of the delay starting to change with cycle period (Tunstall and Sillar 1993), producing a constant phase lag like that seen in adult

fish (Grillner and Kashin 1976; Fetcho and Svoboda 1993). Tunstall and Sillar (1993) suggest that, in *Xenopus*, developing serotonergic innervation from the raphe nucleus (van Mier et al. 1986) plays a role in conferring the abilty to produce a scaled delay on larval swimming. It is clear from our results, however, that prior to establishing such innervation, the embryo is able to produce rhythmic patterns in which scaling with cycle period does occur. It simply does not usually do so during swimming (Tunstall and Roberts 1991). It is not the *Xenopus* embryo that is unusual in this respect, therefore, but *Xenopus* embryo swimming.

The relationship between kinematics and neural drive during swimming and struggling

Considering the scope for mechanical interaction between the body of the small *Xenopus* embryo and the water, it is perhaps surprising that such a good match was found between the components of the movements and their underlying neural drive. Changes of cycle period through transitions from struggling to swimming were mirrored in the motor root discharge. More importantly, the progressive changes in strength of bends and in the longitudinal phase delay were also mirrored in progressive changes in burst duration and phase delay in the motor discharge. This demonstrated that the progressive changes in the behavioural transitions did not result from mechanical constraints blurring a rapid switch of motor patterns but actually reflected a progressive, centrally programmed change in motor pattern.

The kinematic analysis and the reconstruction of the longitudinal pattern of phase delays in motor discharge during struggling agree in suggesting that the caudo-rostral bending waves start at around the level of the anus. The significance of this region is yet to be determined. It could be that a local increase in the level of excitation occurs at this longitudinal position, causing bursting here to lead on each cycle and drive more rostral regions. Alternatively, it could be that some kind of local modulation occurs, through which firing thresholds are lowered in this region (or indeed raised elsewhere).

The relationship between components of the transitionary patterns

One of the important findings of this study was the close correlation between the three main kinematic parameters and between the three electrophysiological parameters during the transitions from struggling to swimming. During sustained motor patterns, similar correlations are well known. During fish swimming burst duration, longitudinal delay and cycle period typically show a positive correlation (Grillner 1974; Grillner and Kashin 1976; Roberts 1981; Mos et al. 1990; Fetcho and Svoboda 1993). Similarly, a positive correlation with cycle period exists for some parameters in tetrapod locomotion (Grillner 1981). However, the same parameters have rarely been followed through transitions between behaviours. In crickets, delay in onset of wing opener and closer motoneuron bursts and the duration of closer bursts are positively correlated with cycle period during stridulation. These relationships are preserved during transitions between different stridulatory patterns (Hennig 1989). In the cat, extensor bursts are correlated with cycle period during transitions from stepping to scratching (Berkinblit et al. 1978). As for *Xenopus*, the mechanisms underlying these transitions remain unknown. However, the correlation between the three main kinematic and electrophysiological parameters, and the absence of any evidence for independent changes in them, suggests that in *Xenopus* the transition from struggling to swimming involves either a single mechanism or separate, closely coupled mechanisms. What mechanism might be involved?

It was originally proposed that expression of struggling rather than swimming could result from neuromodulation of the swimming circuitry (Soffe 1991b). The Rohon-Beard sensory neurones use an excitatory amino acid neurotransmitter (Sillar and Roberts 1988), but also show substance P-like immunoreactivity (Clarke et al. 1984). It seemed possible that co-release of substance P during repetitive sensory discharge could lead to modulation of the firing properties of rhythmically active neurons (Soffe 1991b). The grounds for suggesting this are now much weaker since struggling can be expressed in the absence of sensory discharge (Soffe 1993b).

A second possibility is that the struggling and transitionary patterns are driven by increased levels of excitation impinging on the motor system. This would normally come from increased sensory discharge. Expression of the struggling pattern follows sustained or repetitive sensory stimulation (Soffe 1991b). This is supported by the finding that the struggling pattern can also be evoked artificially by the use of relatively high levels of excitatory amino acid excitants (Soffe 1993b). If high maintained levels of excitation drive the struggling pattern, it seems reasonable to propose that the transition to swimming follows a decline in the level of excitation. The cycle period, burst duration and the nature of the longitudinal phase delay would each then be related to the level of excitation.

The full range of rhythmic patterns in the *Xenopus* embryo cannot be explained in terms of a single mechanism, with a simple relationship to cycle period. The swimming cycle period typically covers a range from around 40 ms to over 100 ms. As outlined above, the pattern of motor root discharge is similar over this whole range of cycle periods in that bursts are brief and

show the same rostro-caudal delay. In contrast, patterns at the same cycle periods during a transition from struggling show much longer bursts and a much smaller rostro-caudal or reversed caudo-rostral delay. At the same cycle periods, therefore, the embryo can produce two quite different patterns of motor discharge. This is analogous to the situation in mammalian, including human, locomotion where running and walking patterns can occur at the same cycle periods (Grillner et al. 1979). Also, the long cycle periods during swimming, which tend to occur later in an episode of swimming, appear to be associated with a reduced rather than a higher level of overall excitation within the motor circuitry, partly through de-recruitment of premotor interneurons (Sillar and Roberts 1993). If the full range of rhythmic motor patterns in *Xenopus* embryos is considered, therefore, the cycle period cannot be controlled in a simple graded way by the level of excitation. Similarly, the good correlation between cycle period, burst duration and phase delay seen during struggling and the transitionary period does not extend to swimming.

One question that cannot yet be resolved concerns the role of movement related feedback during struggling and the transitional behaviours. It seems reasonable to suppose that while the embryo is held or restrained, there could be rhythmic self-stimulation as it struggles. This could help to maintain the pattern. Less clear is whether the movements of the animal once released are also sufficient to generate sensory self-stimulation, or whether a component of sensory discharge remains perhaps as a result of damage during initial mechanical stimulation. Whatever the situation during the real movements, such continuing sensory stimulation appears not to be necessary during transitions, since appropriate motor responses were recorded in the immobilised embryos. This was true even in response to electrical stimulation where the timing of sensory discharge was accurately defined. If the changes occurring during the transition from struggling to swimming result from a falling level of excitation, this excitation must be generated partly within the motor circuitry, since it can continue for several cycles after sensory discharge has ceased.

Implications for motor pattern switching in Xenopus embryos from findings in other systems

It has previously been proposed that the neural circuitry underlying struggling and swimming is essentially the same, to the extent that the same classes of interneurons are active during both patterns and the same components can be recognised in the synaptic drive to motoneurons (Soffe 1993a). The present findings support this proposal. Although there are now a number of other systems in which the relationship between different patterns has been studied, there are relatively few cases where transitions between patterns have been described. Where related behaviours are thought to involve the same neural circuitry, both slow and fast transitions have been described (cat locomotor gaits: Shik et al. 1966; cat scratching and walking, Berkinblit et al. 1978; cricket call patterns: Hennig 1989). Where different pattern generators appear to be involved, however, the transitions are rarely slow, typically occurring in only one or two cycles (lamprey respiration patterns: Thompson 1990; cricket flight and stridulation: Hennig 1990; crayfish swimmeret beating: Heitler 1985). The occurrence of transitions lasting several cycles in *Xenopus* is therefore more consistent with a change in the operation of the same pattern generating circuitry. Another property associated with patterns driven by separate circuitry is the possibility of their being expressed simultaneously, even when mediated by the same multifunctional motoneurons (Ramirez and Pearson 1988; Hennig 1990). Simultaneous expression of struggling and swimming has never been observed in *Xenopus*.

Lastly it should be pointed out that the descriptions presented here for transitions between struggling and swimming in *Xenopus* embryos also set constraints on possible mechanisms that could underlie changes in the operation of the motor circuitry. Any mechanism proposed would have to be able to explain the range of intermediate patterns and maintain the relationship between cycle period, burst duration and longitudinal phase delay, as well as being able to explain swimming and struggling themselves.

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