J. exp. Biol. (1982), 99, 197–205
With 5 figures
Printed in Great Britain

THE NEUROMUSCULAR BASIS OF RHYTHMIC STRUGGLING MOVEMENTS IN EMBRYOS OF *XENOPUS LAEVIS*

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

SUMMARY

Xenopus embryos struggle when restrained. Struggling involves rhythmic movements of large amplitude, in which waves of bending propagate from the tail to the head.

Underlying this, electrical activity in myotomal muscles occurs in rhythmic bursts that alternate on either side of a segment. Bursts in ipsilateral segments occur in a caudo-rostral sequence.

Curarized embryos can generate motor nerve activity in a struggling pattern in the absence of rhythmic sensory stimulation; the pattern is therefore produced by a central pattern generator.

INTRODUCTION

Most studies of locomotion in fish and other lower vertebrates have concentrated upon swimming movements, but fish are able to make other movement patterns also. When restrained by gripping the body firmly, the eel (von Holst, 1934) and the dogfish (Gray & Sand, 1936), for example, will make powerful writhing movements. Surprisingly, before hatching, the embryo of the clawed toad is also capable of making two different types of movement. If an embryo, released from its egg membranes, is touched, it will dart off rapidly (Roberts, 1971) with undulatory swimming movements (Kahn, Roberts & Kashin, 1982). However, if an attempt is made to grip the head or neck region between the points of forceps, the embryos behave quite differently. The movements made are powerful side to side oscillations of greater amplitude and lower frequency than in swimming, and these movements will often result in the embryo freeing itself. These movements normally occur only when the embryo is gripped, and they seem to be an attempt to escape restraint, so they will be called 'struggling' movements.

In this report we describe the form of the movements made in struggling and the underlying motor pattern of muscle activity. Experiments were carried out which indicate that the struggling pattern arises from a central nervous mechanism, but sensory input is of importance in sustaining a struggling response.

MATERIALS AND METHODS

Animals

Experiments were carried out on *Xenopus laevis* embryos at stage 37/38 (Nieuwkoop & Faber, 1956). Embryos were obtained by induced breeding. Just before observations or experiments, embryos were removed from their egg membranes with fine forceps. Temperature was 18–22 °C.

Films

Embryos were restrained by fine pins placed on either side of the head at the gill region, in tap water. This method leaves the body of the embryo free to make lateral movements, but keeps the head restrained. The restraining pins were pushed into a Sylgard layer at the bottom of the dish. Embryos were held ventral side up to prevent contact of the cement gland with the base of the dish, for stimulation of the cement gland inhibits swimming movements (Roberts & Blight, 1975). Films of struggling were made at 64 frames/s. They were viewed frame by frame, the image being projected onto a glass table, and the outline of the embryo was drawn. For an analysis of the propagation of bends in the body when struggling, the same method was used as previously in the analysis of swimming (Kahn *et al.* 1982).

Electrophysiology

The methods for making electrical recordings were the same as used previously (Kahn & Roberts, 1982; Kahn *et al.* 1982).

RESULTS

Struggling movements and their neuromuscular basis

Restrained embryos gave two different types of response to stimulation. In most cases, poking the head with the points of fine forceps evoked violent struggling (Fig. 1 *a–c*), while gently stroking the trunk skin evoked swimming (Fig. 1 *d*), (recognizable by a rhythm period of between 40 and 100 ms, and caudally propagating waves of bending (Kahn *et al.* 1982)). Struggling episodes continued after the phasic mechanical stimulus was removed; for example, if the stimulus was applied for about 250 ms, the episodes continued for up to 4 s. Struggling movements consisted mainly of slow, powerful, alternating side-to-side movements, much greater in amplitude than those in swimming. Alternating struggling movements occurred rhythmically with cycle periods of 112–224 ms (measured to nearest frame, with frame interval of 16 ms): 16–25 successive movement cycles were often seen in a single episode. Struggling embryos also sometimes made strong coiling movements to one side with the tail curved around touching the head. Such coiling was occasionally repeated several times to one side, but this was rare, and most struggling responses were of the alternating type described above.

From inspection of films of struggling movements it was apparent that bends in the body began caudally on each cycle (Fig. 1 *a*, *b*, *c*). The rostral spread of bends along

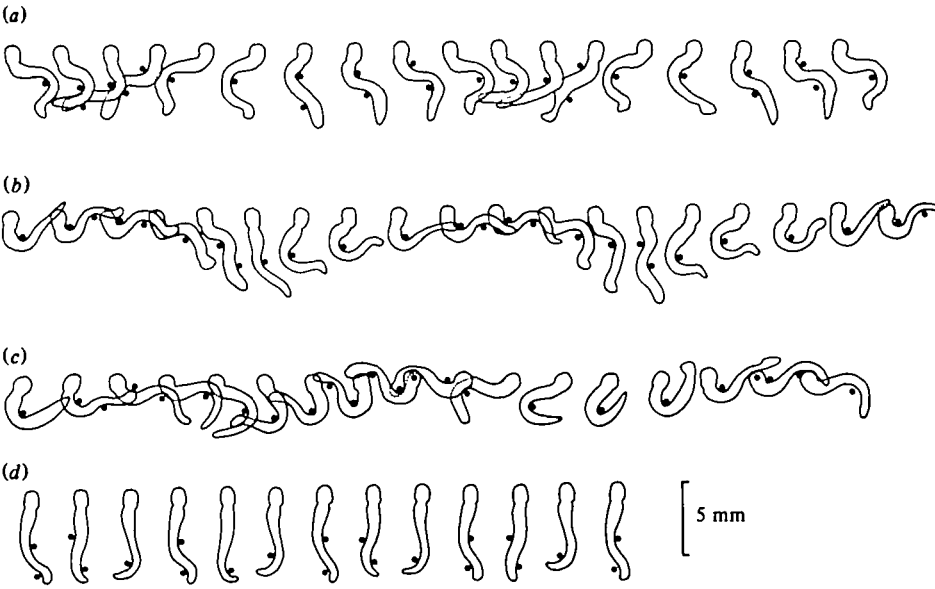

Fig. 1. Struggling movements of restrained *Xenopus* embryos (*a–c*) tethered about the gill region. (*d*) shows 'swimming' in a restrained embryo. Approximate positions of maximum bending on left and right marked ●, ○. Frames read from left to right; each frame was displaced to the right of the one before. Interval between frames is about 16 ms. Sequences (*a*), (*b*) and (*d*) from same embryo.

the body was confirmed by measurements of local angles of bending. These showed that each point on the body bends alternately to one side and the other (Fig. 2*a*), and that these cycles of bending occur in a caudo-rostral sequence, (Fig. 2*a*). The bend propagation rate can be calculated by plotting the time at which bends begin at different points along the body (Fig. 2*b*). An approximate conduction velocity can be obtained for the sequence shown in Fig. 2*a* of 40 ms per mm of the body (Fig. 2*b*). Rhythmical struggling movements varied continuously in form; the three examples shown in Fig. 1 (*a–c*) encompass most of the range seen. The movements varied in particular in whether they were symmetrical on the two sides of the body (as in Fig. 1*a*, *c*) or asymmetrical (Fig. 1*b*). During a single episode the form of the movement may change gradually over several cycles from being symmetrical to asymmetrical. However, despite these differences the basic caudo-rostral sequence was still apparent. This suggests that there is one basic neural mechanism for the range of rhythmic, alternating struggling movements, with differences in the form resulting from changes in the relative strength of motor activity on the two sides of the body.

Electrical recordings were made from the myotomal muscles to determine the pattern of activity which underlies struggling movements. Because of the small size of the embryo and the violent nature of struggling movements, it was necessary to use a more strongly restrained preparation than that used previously for filming, in order to make these recordings. As with the filmed embryos, these restrained embryos could be seen by eye to make two distinct patterns of activity: a fast alternating rhythm of low amplitude, and a slower alternating rhythm of larger amplitude. Fig. 2(*a*) shows muscle recordings from an episode that began with the slower rhythm and

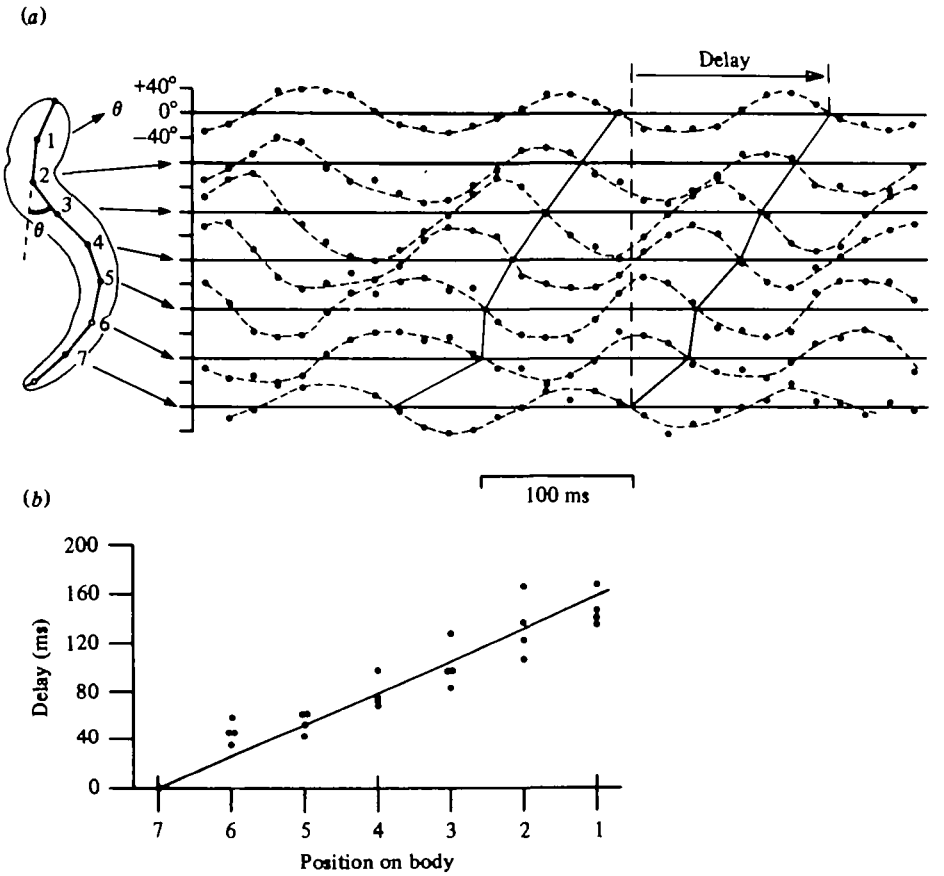

Fig. 2. Propagation of waves of bending in struggling. (*a*) Angles of bending (θ) for different points on the body, plotted against time. The time that bends begin (θ = zero) at different points on the body, are joined by continuous lines to indicate the caudo-rostral sequence in bending. Points on the body separated by 0·67 mm. (*b*) The delay in onset of bends rostrally (delay indicated in *a*). Line is fitted by eye. Measurements for (*b*) were made on each half cycle in sequence in (*a*).

ended with several cycles of the faster rhythm. Measured from the recordings, the faster rhythm had cycle periods within the range for swimming (Kahn *et al.* 1982). The slower rhythm had cycle periods of 190–575 ms, which overlaps with the range for struggling in films.

During struggling activity, muscle potentials alternated on opposite sides of a segment of the body (Fig. 3*a*, *b*). The bursts at each electrode on each cycle were 70–240 ms long. Recordings were also made from two different segments on the same side. In the filming previously, it was noted that in struggling bends on the body began caudally. In electrical recordings the bursts on each cycle in the myotomal muscles usually also began caudally (Fig. 4). The caudo-rostral delays in onset of the bursts had a mean of 20 ms (range 0–40 ms) per mm. This was measured in one preparation with electrodes spaced 2 mm apart at either end of the trunk.

Fig. 3. Electrical recordings from myotomal muscle on left and right sides of a body segment in struggling embryos. In (*a*) struggling activity occurs up to the mark, and then swimming activity appears (see text). (*b*) Struggling at faster sweep speed. (*a*) and (*b*) from the same preparation, (*a*) recorded at the 5th post-otic myotomes, (*b*) at the 9th post-otic myotomes.

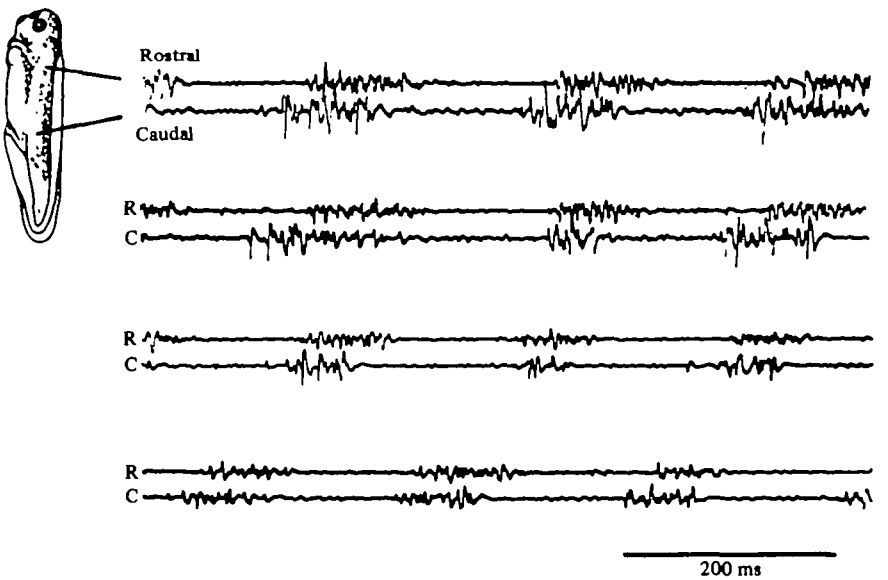

Fig. 4. Electrical recording from myotomal muscles during struggling in restrained embryos from two points on the same side. Upper traces, rostral; lower traces, caudal. On most cycles the bursts begin first at the caudal myotome. The four lines are from one episode of struggling, the first and second lines are continuous, as are the third and fourth. Approximate positions of the electrodes are indicated.

The central nervous origin of the struggling motor pattern

Experiments were carried out to determine whether the rhythmic, alternating struggling pattern is, like swimming (Kahn & Roberts, 1982), generated by a central nervous mechanism. This question was examined by paralysing embryos with curare to prevent movements and thus eliminate any phasic sensory feedback. Extracellular recordings were made of motor nerve activity in these curarized embryos.

A slow, alternating pattern of motor nerve activity appeared in curarized embryos

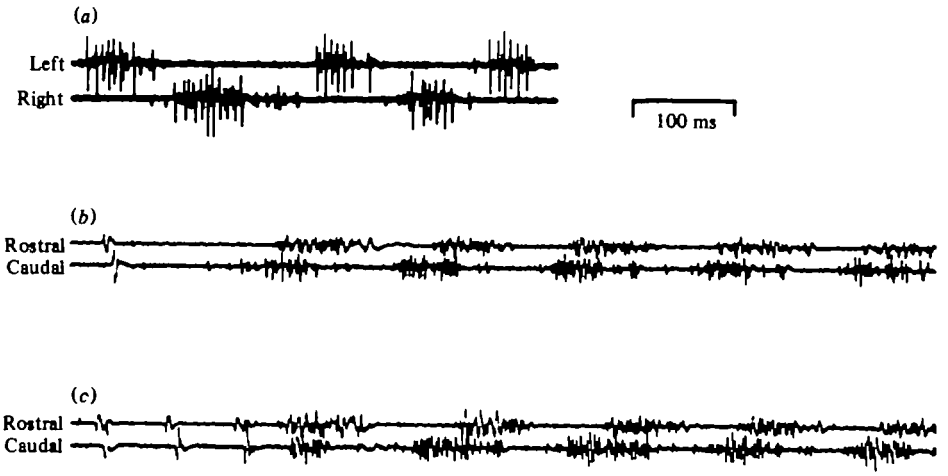

Fig. 5. Motor nerve activity in 'struggling' pattern in curarized embryos, evoked by mechanical stimulation of the head. (*a*) Recording from left and right sides of a trunk segment. (*b*), (*c*) Recording from two motor nerves on same side; upper traces, rostral; lower traces, caudal. Two episodes from the same preparation. Traces begin with 'swimming' and, at marks, change to 'struggling'. The change was caused by application of mechanical stimulation. Rostral electrode over cleft between 4th and 5th post-otic myotomes, caudal electrode over cleft between 16th and 17th post-otic myotomes. Electrodes 1·6 mm apart.

(Fig. 5*a*), which resembled the pattern of myotomal muscle activity recorded previously during struggling. The motor nerve activity in curarized embryos occurred in long bursts, 60–200 ms in duration, which appeared alternately on either side of a segment of the body (Fig. 5*a*). Cycle periods were 120–325 ms. Recordings were also made from two different motor nerves on the same side. As predicted from film evidence and muscle recordings, these showed that the motor nerve bursts usually began first, on each cycle, at the caudal motor nerve (Fig. 5*b*, *c*). As can be seen from Fig. 5(*b*, *c*), this was the reverse order to the sequence in 'swimming' (Kahn & Roberts, 1982). An exception to this caudo-rostral sequence in the slow rhythm was seen in several episodes, on the first cycle in the episode shown in Fig. 5(*c*). If these initial cycles are excluded, the caudo-rostral delay in burst onset had a mean of 20·5 ms (range 16–32 ms) per mm. This was measured in one preparation with electrodes separated by 1·6 mm at either end of the trunk. This pattern of motor nerve activity in curarized embryos is very similar to that recorded from the muscles of uncurarized embryos during struggling: in alternation of bursts on opposite sides of a segment, a caudo-rostral sequence in burst onset, and in cycle period.

In order to evoke the 'struggling' pattern in curarized embryos, strong mechanical stimulation to the head with a fine hair was usually used. In contrast to the uncurarized embryos, the episodes in curarized embryos were not maintained after stimulation was removed. Thus, stimulation needed to be continuous to evoke sustained 'struggling'.

The mechanical stimulation of the curarized embryo was usually from a hand-held hair. However, the possibility that slight shaking of the hand might provide embryos with essential phasic stimulation can be eliminated. When a probe (an insect pin),

mounted on a loudspeaker cone and driven by a slow rising voltage, was used to poke the side of the head, a very similar slow rhythm appeared. Therefore the rhythmic struggling motor pattern can be generated in curarized embryos without rhythmic sensory inputs.

DISCUSSION

Struggling in Xenopus *embryos*

The form of the movements made in struggling are of interest. First, they are large amplitude, slow movements, larger and slower than in swimming, and such powerful movements seem well adapted for prising the animal loose when trapped. Secondly, on each cycle of struggling, bends in the body begin first caudally and sweep rostrally along the body. This is the reverse of the rostro-caudal direction that bends follow during swimming (Kahn *et al.* 1982). It may be that the reversal of the direction of propagation of bends in struggling produces a force in the opposite direction to swimming, tending to drive the animal backwards away from whatever has trapped it. These movements were often effective in freeing the embryo, a strong indication that the movement pattern is an adaptation for escape when it is trapped. The pattern might be particularly useful when the animal is trapped by a predator, perhaps a small insect larva. It might also be that these violent movements play a role in hatching. However, it is thought that hatching in amphibian embryos comes about mainly through dissolution of the egg membranes by enzymes secreted from the skin (Noble, 1931), rather than through mechanical disruption of the membranes by the embryo's movements.

Struggling in other animals

Powerful rhythmic movements of frequency lower than in swimming are made by some fish when the body is gripped firmly. This has been reported in the intact eel (von Holst, 1934) and also in spinalized preparations of the eel and dogfish (von Holst, 1934; Lissmann, 1946). The great power of these movements as compared to swimming is probably due to the recruitment of white muscle (Bone, 1966). Grillner (1974) has made electrical recordings from the myotomes of the spinalized dogfish and found that stimulation of the skin at the rostral end will reverse the inter-segmental coordination and produce caudo-rostral waves. This indicates that adult fish can produce a pattern of activity that resembles the struggling of *Xenopus* embryos.

The neural basis of struggling

The results presented here show that curarized preparations (in which the neuromuscular junction has been blocked, and in which there are no rhythmic sensory inputs) can generate the rhythmical alternating pattern of motoneurone activity which produces struggling. A basic central pattern generator therefore underlies struggling. This is similar to swimming in *Xenopus* embryos, where there is also a central pattern generator underlying the rhythmic movements (Kahn & Roberts, 1982).

One marked difference was noted between struggling in restrained, uncurarized embryos and in curarized preparations. Restrained embryos responded to phasic stimulation with episodes of struggling movements that could continue for several

seconds after the applied stimulation had been withdrawn. By contrast, in the curarized preparation the struggling pattern of motor nerve activity only appeared during the application of stimulation, and ceased as soon as the stimulation was withdrawn.

The difference between the restrained and the curarized preparations is most simply explained by suggesting that tactile inputs are needed to sustain struggling activity. In the curarized preparation, this is provided only by the mechanical stimulation applied by the experimenter. In restrained, uncurarized preparations it is, we suggest, also provided by stimulation of the skin from the movements of the embryo against the restraining pins. This stimulation is likely to occur as the pins rub against the skin during the strong movements and stimulate mechanoreceptors in the skin (Roberts, 1971; Roberts & Smyth, 1974; Roberts & Hayes, 1977). Episodes of struggling in restrained, uncurarized embryos usually stopped after a few seconds, perhaps because of adaptation in the sensory receptors, or in their central pathways. This dependence of struggling responses upon sustained sensory stimulation is different from swimming in the embryo, where phasic sensory inputs, although usually needed to initiate a swimming episode, are not subsequently needed to sustain it (Kahn & Roberts, 1982).

The significance of the responses of the embryo to stimulation might be explained in the following way. (It is assumed here that the embryo had hatched, for stage 37/38 is about the time of normal hatching.) Phasic stimulation, either touch (Roberts, 1971; Roberts & Smyth, 1974) or a shadow cast over the animal, might indicate the appearance of a potential predator, and it is then best to swim away rapidly. Once swimming is underway it continues for many seconds, without need of further stimulation, until the animal reaches plants or the water surface. It then stops due to stimulation of inhibitory sensory systems of the head (Roberts & Blight, 1975; Roberts, 1980) and hangs motionless, attached by the mucus strand of the cement gland. If, however, a predator catches the animal, this would provide continuous stimulation of the skin. In this case the embryo would struggle continuously until it was free. Once free, the absence of further mechanical stimulation would bring struggling to a stop. Thus, the type of response evoked, either swimming or struggling, would depend mainly upon the type of stimulation. Brief stimulation evokes swimming, maintained mechanical stimulation, struggling.

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

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