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EXPERIMENTS ON THE CENTRAL PATTERN GENERATOR FOR SWIMMING IN AMPHIBIAN EMBRYOS

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[Plate 1]

CONTENTS

The central nervous system of paralysed *Xenopus laevis* embryos can generate a motor output pattern suitable for swimming locomotion. By recording motor root activity in paralysed embryos with transected nervous systems we have shown that: (*a*) the spinal cord is capable of swimming pattern generation; (*b*) swimming pattern generator capability in the hindbrain and spinal cord is distributed; (*c*) caudal hindbrain is necessary for sustained swimming output after discrete stimulation. By recording similarly from embryos whose central nervous system was divided longitudinally into left and right sides, we have shown that: (*a*) each side can generate rhythmic motor output with cycle periods like those in swimming; (*b*) during this activity cycle period increases within an episode, and there is the usual rostrocaudal delay found in swimming; (*c*) this activity is influenced by sensory stimuli in the same way as swimming activity; (*d*) normal phase coupling of the left and right sides can be established by the ventral commissure in the spinal cord.

We conclude that interactions between the antagonistic (left and right) motor systems are not necessary for swimming rhythm generation and present a model for swimming pattern generation where autonomous rhythm generators on each side of the nervous system drive the motoneurons. Alternation is achieved by reciprocal inhibition, and activity is initiated and maintained by tonic excitation from the hindbrain.

Introduction

In the undulatory swimming of *Xenopus* embryos there is a rhythmic alternation in the activity of myotomes on opposite sides of a segment of the body (Kahn *et al.* 1982). As in other animals, the swimming motor pattern can be generated by the central nervous system without sensory feedback (Grillner 1975; Kahn & Roberts 1982*a*). Here we investigate whether generation of the swimming rhythm is dependent upon reciprocal interactions between antagonistic motor systems, as would be expected from Brown's hypothesis (Brown 1911, 1912, 1914; Friesen & Stent 1978; Miller & Scott 1977) or whether reciprocal interactions are mainly concerned with attaining appropriate phase-coupling between two inherently rhythmic motor systems (von Holst 1939; Wilson 1966, 1968; Waldron 1967; Wilson & Waldron 1968).

In swimming the antagonistic muscles are those on the left and right sides of a body segment. These are innervated by motoneurons lying respectively on the left and right sides of the spinal cord (Hughes 1959; Blight 1978; Roberts & Clarke 1982). The neurocoel, running along the middle of the hindbrain and spinal cord (see plate 1*a*), separates the left and right sides. The main connections between the sides are in the ventral commissure (Roberts & Clarke 1982). In the experiments reported here we cut these commissural connections, and in this way it was possible to determine whether reciprocal connections were necessary for rhythm generation. Experiments are also reported in which the pattern-generating capabilities of different regions of the central nervous systems were examined.

Materials and methods

All the experiments were on *Xenopus* embryos at developmental stage 37/38, which were removed from their egg membranes just before an experiment. For treatment of embryos and recording methods see the preceding paper (Roberts & Kahn 1982). Experiments were carried out at temperatures of 18–24 °C.

Transection of the nervous system

Embryos were cut into two pieces in saline by means of a mounted piece of razor blade. After transection, embryos were tested for responses to tactile stimulation with use of a fine mounted hair to stroke the skin (Roberts & Smyth 1974). Responses usually reappeared within 10 min of the operation, and observations were continued for about an hour. Movement records (Kahn *et al.* 1982) were made from two preparations. In curarized preparations skin and tissue overlying the nervous system was removed in the area in which the transection of the nervous system was to be made. A region of the nervous system was transected by means of fine pins, and, in many cases, a small amount of nervous tissue rostral to the cut was removed to make sure of a completely successful separation. Twenty-nine embryos were used in these transection experiments.

Longitudinal division of the nervous system

Experiments in which the nervous system was divided longitudinally were carried out on curarized animals. The tissue lying dorsally over the nervous system was cleared with fine pins. With the embryo viewed from the dorsal aspect, the point of a fine pin was repeatedly drawn gently along the dorsal midline of the spinal cord and hindbrain. When the dorsal roof of the neurocoel had been completely cut, the two sides of the spinal cord and hindbrain opened

outwards a little. Depending upon the region to be cut, the ventral floor of the hindbrain and/or spinal cord was cut in a similar way. It was possible to determine when the two sides were completely separated because the transparent notocord could be seen ventrally between the two sides of the divided nervous system.

The spinal cord tapers and becomes very narrow caudally. To avoid having to cut commissures in this region, a portion of the spinal cord of about two segments length (about 300–400 μm) was removed, and experiments were carried out on the brain and spinal cord rostral to this division. In different experiments the piece of spinal cord was removed caudal to the 4th to 8th post-otic myotome. In these preparations with reduced spinal cord, normal rhythmic 'swimming' activity (Kahn & Roberts 1982*a*) was recorded in curarized preparations. Activity in the motor nerves on the left and right sides of a segment of the body alternated (figure 2*a*), and the rhythm had a period of about 40–125 ms, the same as for swimming in the intact curarized preparation (Kahn & Roberts 1982*a*).

The success of the operations in which the nervous system was longitudinally divided was checked histologically in two preparations after recordings had been made, by examining light microscope sections of the embryos, fixed in formaldehyde and stained with haemotoxylin and eosin.

Results

Pattern generating capabilities of different regions of the central nervous system

Previous observations on *Xenopus* embryos (Hughes 1959) indicated that there is a capability for the generation of the swimming rhythm in the central nervous system caudal to the level of the otic vesicles. This was confirmed in the present experiments. After embryos had been cut into two with the cut passing across the hindbrain, caudal to the otic vesicles, the region caudal to the cut responded to touch with swimming movements which carried the embryo around the dish. Episodes of swimming were usually prolonged, often lasting 1–5 min.

To determine more fully the pattern of motor output in animals in which the central nervous system had been transected, recordings were made of motor nerve activity in curarized animals. Unoperated curarized embryos can generate the swimming motor pattern (Kahn & Roberts 1982*a*). A similar rhythmic, alternating motor output was recorded in operated animals with the central nervous system transected through the hindbrain (figure 1*a*). The rhythm period in such operated embryos was 50–120 ms, within the range for swimming (Kahn & Roberts 1982*a*). Episodes were often prolonged, lasting several minutes

The spinal cord alone is able to generate swimming-like activity. After the embryos had been cut into two, with the cut passing through the rostral spinal cord, episodes of rhythmic lateral movements appeared in the region caudal to the cut when the skin was stroked (figure 1*b*). Responses were usually only a brief flutter lasting up to 1 or 2 s, which carried the body a short distance forward. The movements involved a lateral oscillation (figure 1*b*) with cycle period 45–90 ms, within the range for the swimming rhythm of intact embryos. Brief flutters of activity in response to touch were seen in caudal halves of embryos transected as far caudally as the 7th or 8th post-otic myotomes. After more caudal cuts, no oscillatory responses were seen. When embryos were cut into two with the cut passing through the spinal cord about one or two segments caudal to the hindbrain, both halves responded independently to touch with rhythmic movements.

Confirmation that the spinal cord can generate the swimming pattern was obtained in

curarized embryos. After transection of the rostral spinal cord rhythmic, alternating motor nerve activity was recorded (figure 1*c*) in response to touch. The rhythm had a period of 45–90 ms, within the range for swimming. As in uncurarized embryos, episodes were usually short.

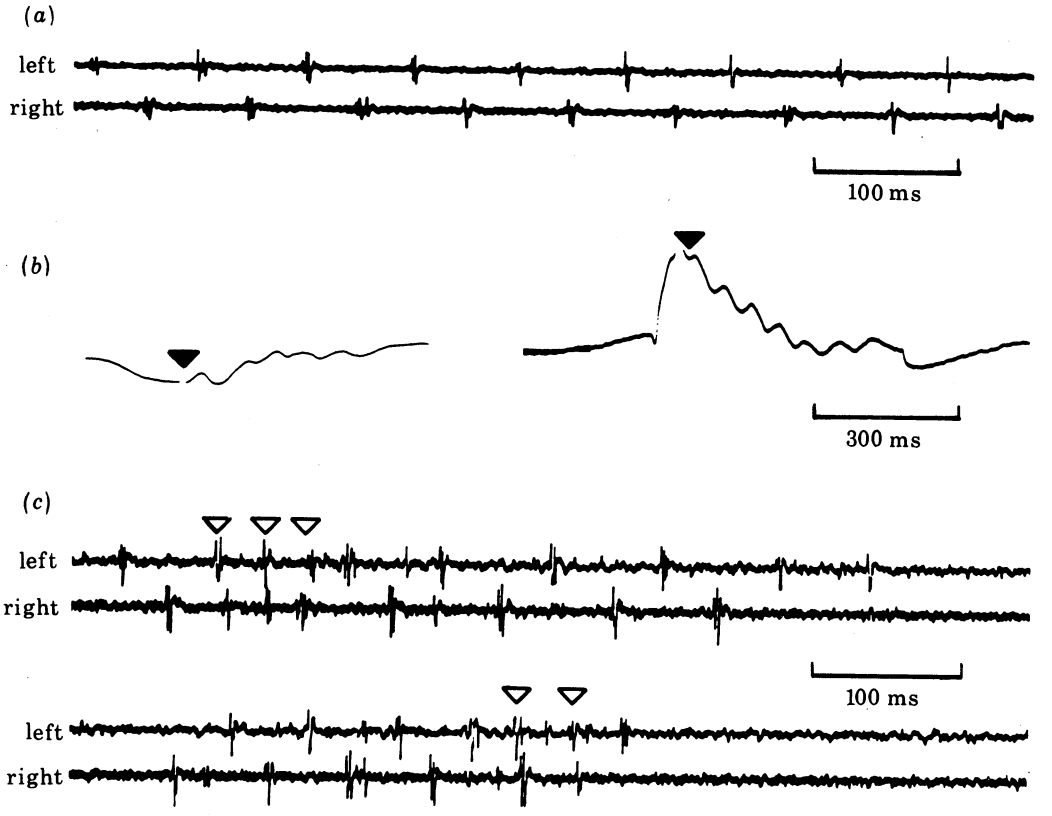

FIGURE 1. Pattern generation by different central nervous system regions in *Xenopus* embryos.
(*a*) Alternating swimming motor nerve activity on the two sides of the body in response to touch, after transection through the caudal hindbrain in a curarized preparation.
(*b*) Lateral oscillation with swimming period beginning at triangle (movement of body to left moves trace upward) in response to stroking skin in spinal embryos, cut through at level of 4th and 5th post-otic myotome. The slow change of position was caused by the stroke to the body.
(*c*) Two brief episodes of alternating motor nerve activity in a curarized, spinal embryo, cut through at level of 4th post-otic myotome. Activity evoked by stroking skin. Electrodes over clefts between 12th and 13th post-otic myotomes. Open triangles indicate 'synchronous' pattern.

Interactions between rhythm generators on either side of the nervous system

We report here the results of longitudinal cuts along the midline of the central nervous system, which sever the commissural connections between the two sides. Commissural connections were cut in the hindbrain and spinal cord of ten embryos (figure 2). Within 10 min of this operation curarized embryos responded to stimuli that evoke swimming (see below) with rhythmic motor nerve activity. The features of this rhythmical activity in divided preparations will now be described, and compared with the swimming rhythm of undivided preparations (Kahn *et al.* 1982).

In swimming, motor nerve bursts on either side of a segment alternate (figure 2*a*), with a phase relation of 0.5 (figure 3*a*). After cutting commissural connections between the two

sides the phase coupling of the rhythms across the body was lost (figures 2*b*, 3*b*) and drifted continuously. The period of the swimming rhythm in undivided embryos was 40–125 ms (Kahn *et al.* 1982). After separating the two sides of the nervous system the rhythmic motor nerve activity often had a shorter cycle period, though the range of 20–100 ms overlaps considerably with the swimming rhythm. (In many operated animals there was greater cycle to cycle variation in the rhythm period, evident from the scatter of points in figure 4, than during

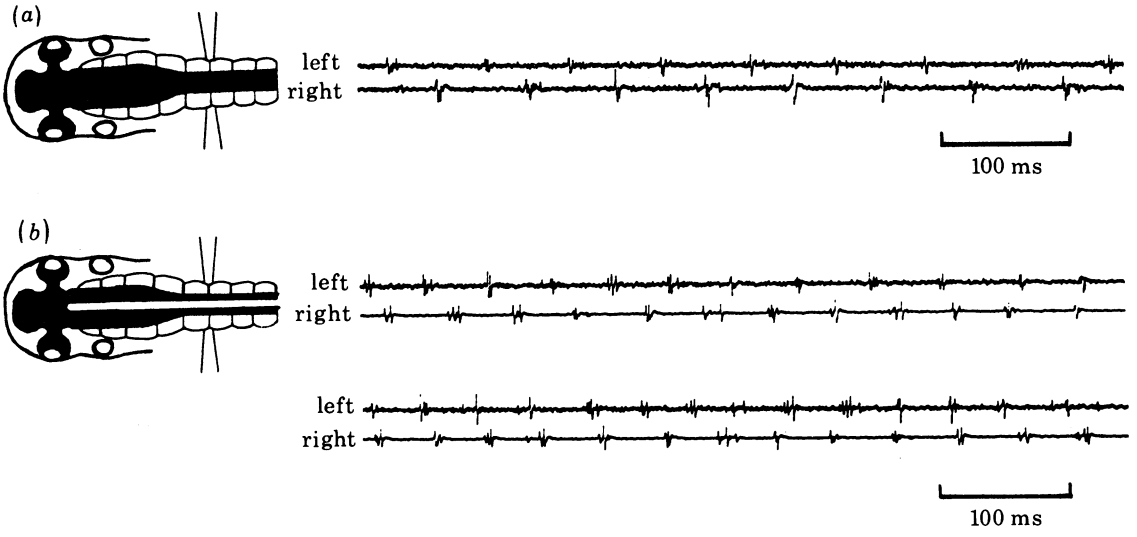

FIGURE 2. Independent rhythms of motor nerve activity on the left and right sides of the body of a curarized embryo after longitudinal division of the hindbrain and spinal cord.

(*a*) Before dividing the nervous system, recording of motor nerve activity on the left and right sides of a segment (as indicated). A region of the spinal cord had been removed caudal to the 8th post-otic myotome.

(*b*) The same preparation as (*a*) after separating the two sides of the hindbrain and spinal cord dorsally and ventrally along the neurocoel. Recording of motor nerve activity on left and right sides of the body (as indicated). The two traces are continuous.

the 'swimming' rhythm of the intact preparation; however, this was not examined quantitatively.) Episodes of rhythmic activity were evoked in divided preparations by phasic stimulation (see below) and episodes often continued long after stimulation ceased, occasionally lasting 30 s or more. During a single episode there was usually an increase in the rhythm period (figure 4), a feature also usually observed in swimming episodes in undivided embryos.

The motor nerve bursts recorded on each cycle of the rhythmic activity in the divided preparations appeared to be the summed activity of several different motoneurones spiking close together but not exactly synchronously. This was indicated by the variable amplitude and duration of bursts on successive cycles at a single electrode (figure 2*b*). These features are similar to those of bursts in the 'swimming' rhythm of undivided animals (figure 2*a*).

In undivided embryos in the swimming rhythm there is usually a rostrocaudal sequence in the motor nerve bursts on each cycle (Kahn & Roberts 1982*a*). In the divided embryos the activity in different motor nerves on the same side of the body was also closely coupled, and there was often a slight delay in burst onset at the caudal electrode (figure 5). However, as in undivided preparations, the bursts at the caudal electrode could sometimes lead (figure 5) or bursts could begin simultaneously at both electrodes.

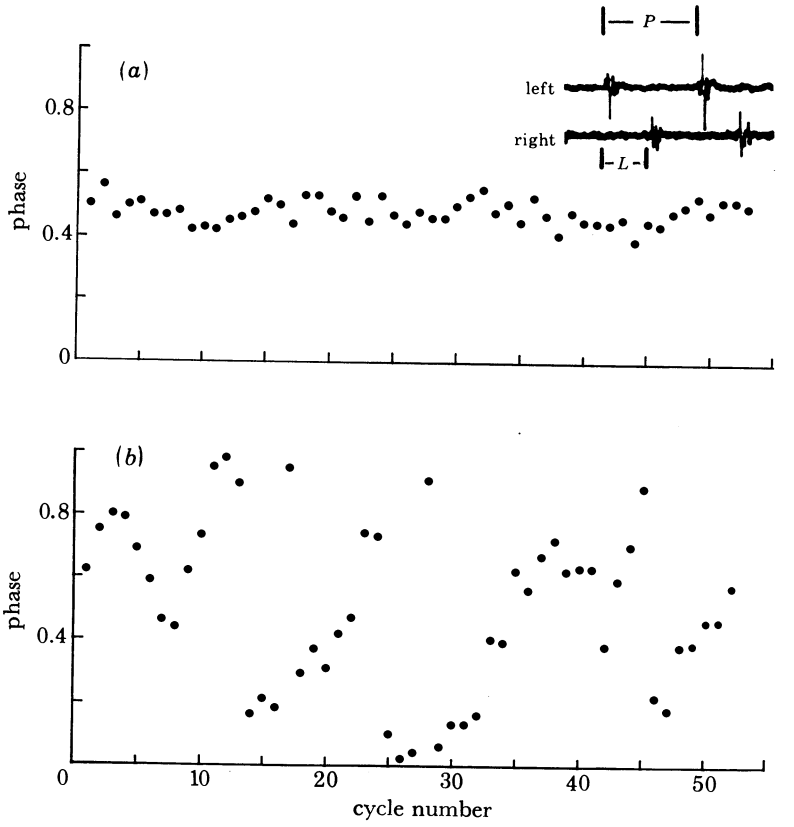

FIGURE 3. Phase relations (latency, L, expressed as a fraction of period, P, see inset) of motor discharge on left and right sides. Plots of phase on successive cycles in an episode of activity.

(*a*) Intact: rhythms on the two sides are tightly phase-coupled in alternation, and phase relation remains almost constant with a phase difference of 0.5 of a cycle.

(*b*) With left and right sides of the brain and spinal cord divided: the strict alternation is lost and phase relation drifts.

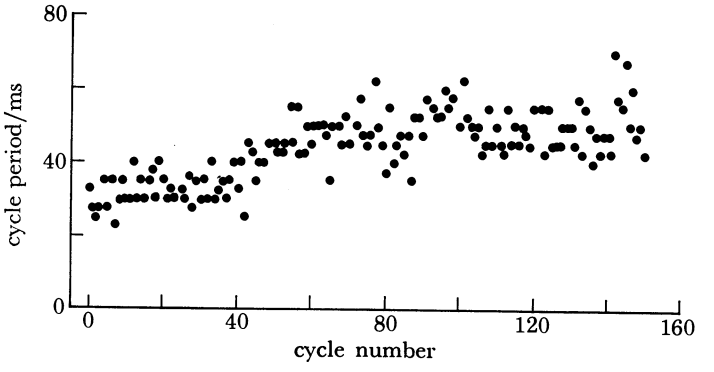

FIGURE 4. Rhythm period on successive cycles in an episode of rhythmic activity from one side after longitudinal division of hindbrain and spinal cord. Period measured from onset of one burst to onset of the next burst at the same electrode (P in inset of figure 3); measurements made in units of 2.5 ms.

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Dimming the light evokes the swimming rhythm (Roberts 1978) and this same stimulus-evoked rhythmic motor nerve activity on both sides of the body in divided embryos (figure 6*a*). In addition, stroking the skin on the side of the head will also evoke swimming (Roberts 1980). However, in divided preparations rather than evoking motor nerve activity on both sides of the body (as in undivided embryos), stroking the side of the head only evoked rhythmic activity on the side stimulated (figure 6*b*, *c*).

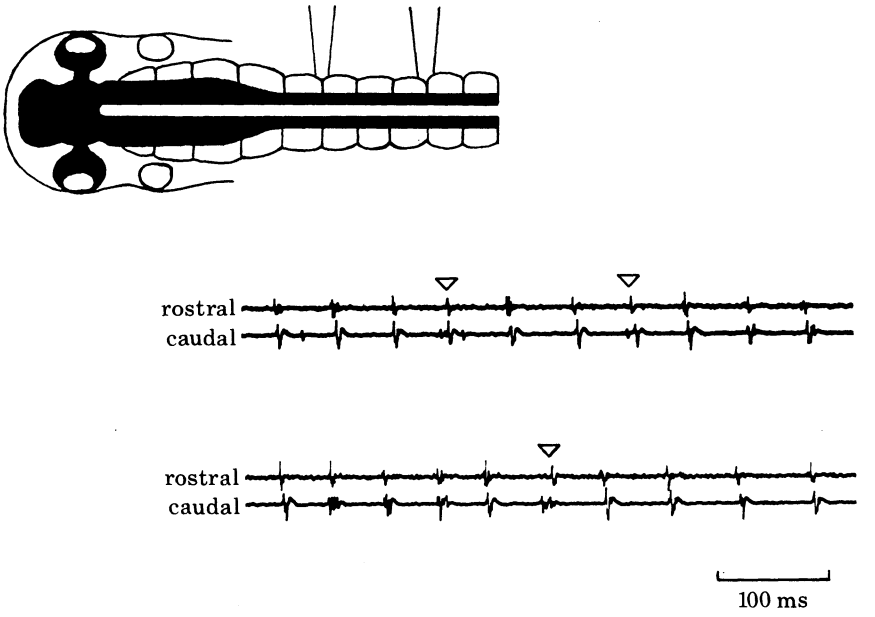

FIGURE 5. Rostrocaudal delay in the bursts of motornerve activity in different segments on the same sid body, after longitudinal division of the hindbrain and spinal cord. Some cycles are simultaneous at the two electrodes, and occasionally cycles begin first caudally (triangles). Two sequences are shown from different episodes in the same preparation. The electrode positions were as indicated, separated by about 400 μm.

These results have shown that, when left and right sides of the hindbrain and spinal cord are separated from each other, each side can generate its own rhythmic motor discharge. As described above this rhythm is similar to the swimming rhythm of the undivided embryo. That each side contains its own rhythm generator was confirmed by separating one side of the hindbrain and spinal cord completely from the rest of the c.n.s. (as shown in figure 7, inset). (Experiments were done on 13 preparations, one checked histologically (plate 1*a*, *b*).) Following a gentle stroke to the head skin, prolonged episodes of rhythmic motor nerve activity appeared (figure 7), lasting for up to 2½ min. During an episode there was usually a gradual increase in rhythm period. The period of the rhythm was similar to that recorded previously after longitudinal division of the hindbrain and spinal cord. In intact embryos, swimming is inhibited by cement gland stimulation (Roberts & Blight 1975). Similarly rhythmic motor nerve activity in these divided preparations was inhibited by cement gland stimulation. A capacity to generate long episodes of rhythmic motor activity similar to the swimming rhythm is therefore contained within each side of the nervous system.

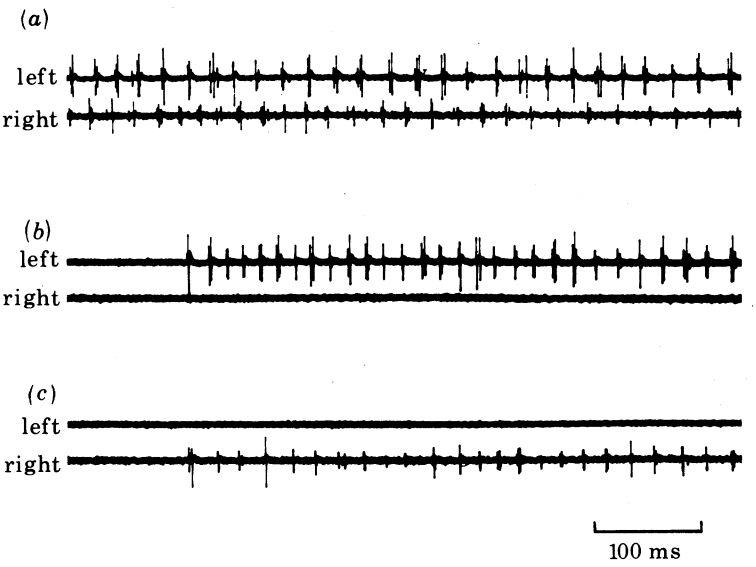

FIGURE 6. Rhythmic activity in response to stimulation in a preparation divided longitudinally in the hindbrain and spinal cord. Recordings from motor nerves on the left and right sides of the body.

(*a*) Both sides become rhythmically active when the lights are dimmed (lights dimmed several seconds before the start of the record).

(*b*, *c*) A stroke to the left (*b*) or the right side (*c*) of the head with a fine hair evokes rhythmic activity only on the side stimulated.

Phase-coupling by spinal cord ventral commissure

Experiments were done in which smaller lesions were made in the central nervous system, to determine where the commissural connections lie for phase-coupling the rhythms on the two sides into an alternating swimming pattern.

(i) When the midline lesion included the spinal cord and post-otic hindbrain (figure 8*a*) (five animals, one checked histologically, plate 1*c*), the rhythms on the two sides were not phase-coupled. This indicates that the commissures responsible for phase-coupling lie caudal to the otic vesicles.

(ii) When the hindbrain and most rostral region of the spinal cord were divided, but a short (about 150 μm) region of the spinal cord was left intact at the level of the fourth post-otic myotome, the rhythmical motor nerve activity in response to stimulation alternated strictly on

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DESCRIPTION OF PLATE 1

Histological examination of normal and operated preparations in which the central nervous system was divided longitudinally (as indicated in the diagrams). The preparations were used for electrical recordings, and subsequently fixed and sectioned. The approximate levels of the transverse sections are indicated. Scale bar applies to all sections.

(*a*) Control, undivided, preparation: hindbrain (on left) showing narrow ventral connection between the two sides. Silver stained.

(*b*) After removal of one side of the hindbrain and spinal cord, and cutting the connections between hindbrain and midbrain (as in figure 7 though not the same preparation) it can be seen that only one half of the hindbrain and spinal cord remains. Stained with haemotoxylin and eosin.

(*c*) After longitudinal division of spinal cord and caudal hindbrain, to 200 μm caudal to the otic vesicles (the same preparation as in figure 8*a*), hindbrain and spinal cord can be seen separated into left and right halves. Abbreviations: hb., hindbrain; m., myotome; n., notocord; nc., neurocoel; o.v., otic vesicle; s., skin; s.c., spinal cord. Small arrows indicate where the nervous tissue was divided.

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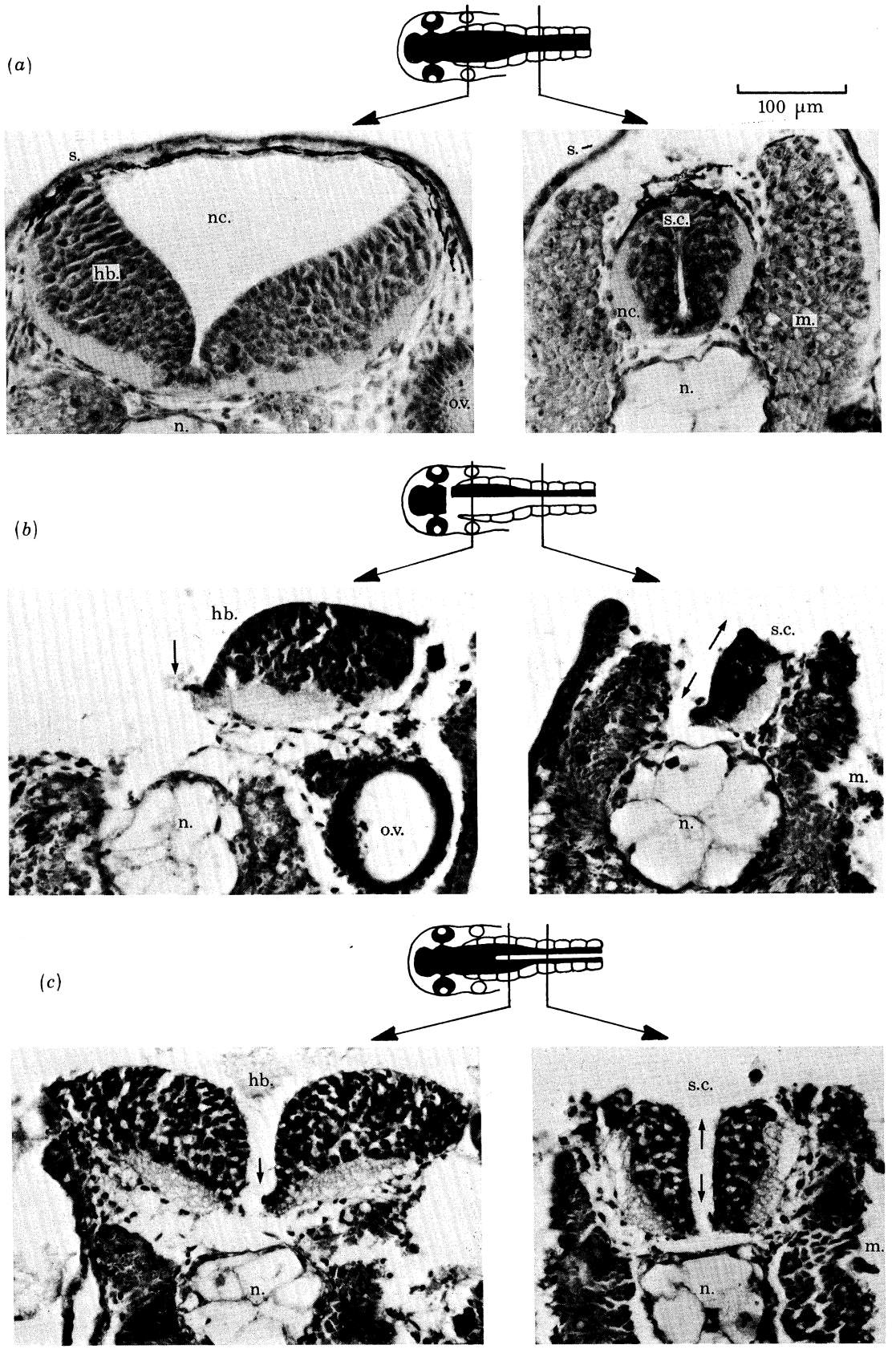

PLATE 1. For description see opposite

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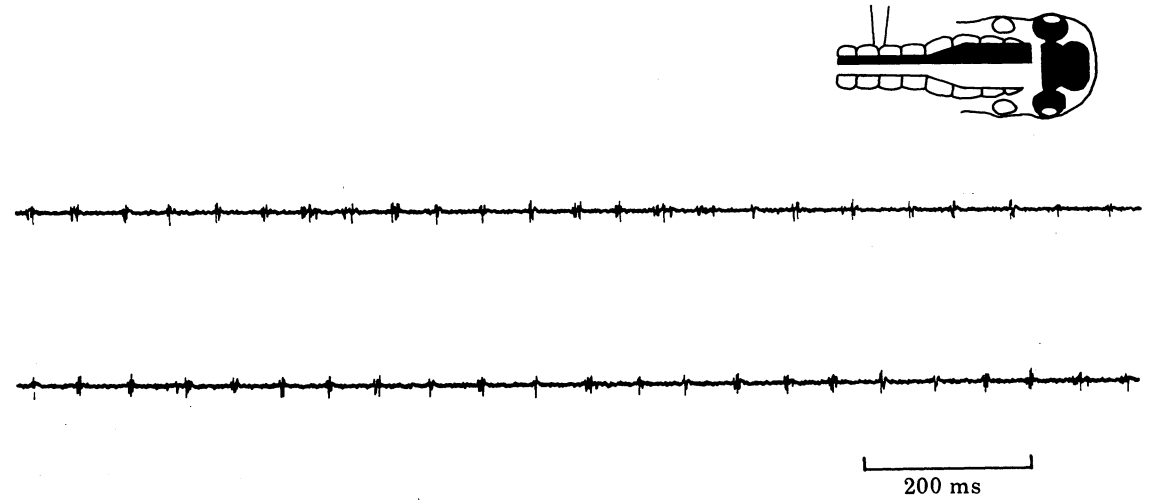

FIGURE 7. Rhythmic motor nerve activity after one side of the hindbrain and spinal cord has been removed and the connections between the remaining hindbrain and the midbrain have been cut. The two lines are continuous. Inset shows isolated part of nervous system and location of recording electrode.

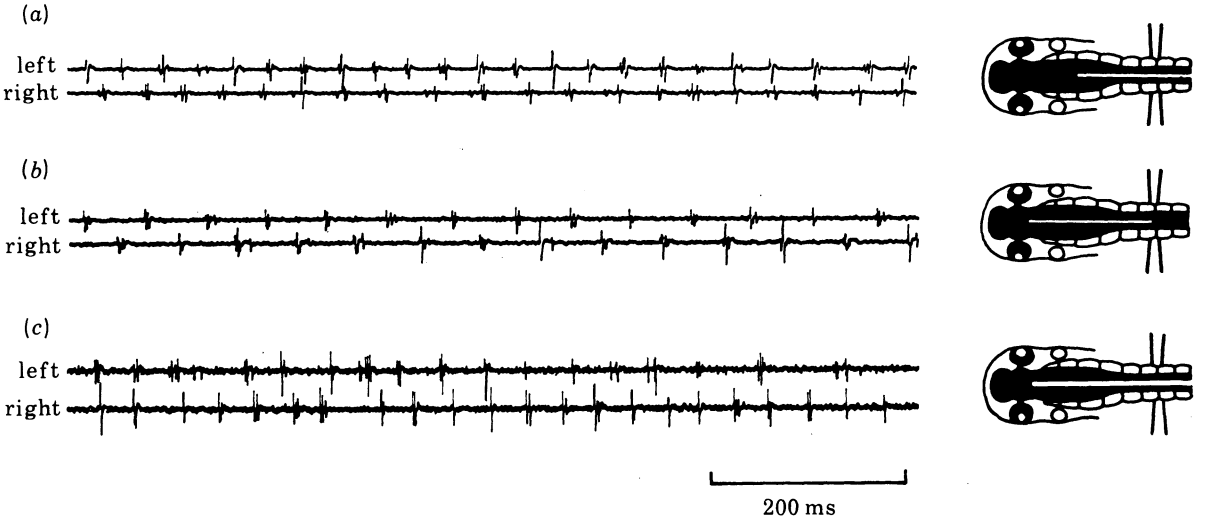

FIGURE 8. Experiments to localize the commissural connections responsible for phase-coupling motor nerve activity on the two sides.

(*a*) Motor nerve recordings from left and right sides after division of the spinal cord and caudal hindbrain to about 200 μm caudal to the otic vesicles, as in diagram. The rhythmic motor nerve activity on the two sides is not phase-coupled.

(*b*) Motor nerve recordings from left and right sides of the body after division of the hindbrain and rostral spinal cord, leaving about 150 μm of the spinal cord intact at about the level of the 4th post-otic myotome. The rhythmic activity is tightly phase-coupled in alternation.

(*c*) The same preparation as in (*b*), but the spinal cord has now been completely divided, and the rhythmic motor nerve activity on left and right sides is no longer phase-coupled.

the left and right sides of the body (figure 8*b*). To see if phase-coupling was indeed due to the intact region of the spinal cord, this was then divided and the rhythmic activity on the two sides was, as expected, no longer phase-coupled (figure 8*c*).

(iii) If only the dorsal roof of the neural canal was cut along the midline in the spinal cord and hindbrain, rhythmic activity on the two sides remained in the alternating swimming

pattern. This is to be expected because there is no anatomical evidence for a dorsal commissure in the spinal cord at this developmental stage (Roberts & Clarke 1982).

In conclusion, these experiments indicate that the ventral commissure in the spinal cord is a pathway for phase-coupling the two sides in swimming.

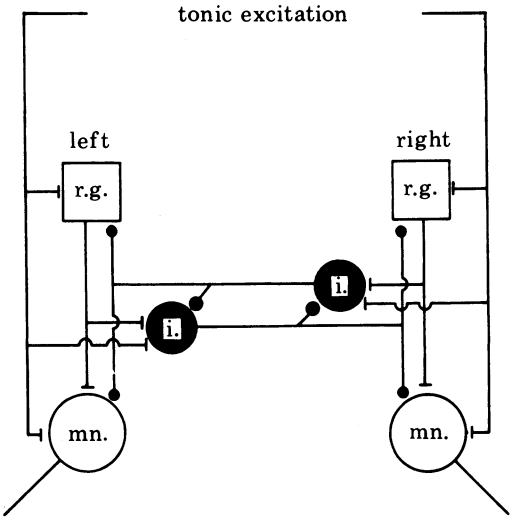

FIGURE 9. Model for the swimming pattern generator in *Xenopus* embryos (see text for details). Abbreviations: r.g., rhythm generator; i., inhibitory interneuron; mn., motoneuron. A bar represents an excitatory synapse; a dot represents an inhibitory synapse.

DISCUSSION

In accord with studies on locomotion in other vertebrates (reviewed in Grillner (1975)), the spinal cord of *Xenopus* embryos has the neuronal networks needed to generate the swimming pattern. We have also shown that both left and right sides of the nervous system have pattern generating capacity when separated from each other. Therefore models in which the locomotor rhythm arises through reciprocal interactions between two antagonistic motor systems (Miller & Scott 1977; Brown 1911, 1912, 1914) cannot account for swimming in *Xenopus* embryos. Rather the antagonistic motor systems on the left and right sides of the spinal cord each have their own rhythm generator.

A model for rhythm generation

On the basis of our studies on *Xenopus* embryos (Kahn & Roberts 1982*a*, *b*; Kahn *et al.* 1982; Roberts & Kahn 1982; Roberts & Clarke 1982) we will now consider whether the modification of Brown's model shown in figure 9 can account for our results. This scheme differs from Brown's principally in having an autonomous rhythm generator in the motor system for each antagonistic muscle (shown as a square). Before describing its operation, we will review the evidence concerning the elements in the model.

Rhythm generators

The present results have shown that a capacity to generate the swimming rhythm lies within each side of the spinal cord. Intracellular recordings from motorneurons indicated that they were not themselves generating the rhythm (Roberts & Khan 1982). This suggested that the rhythm was generated by spinal interneurons and then imposed on the motorneurons as in some other animals (Hoyle & Burrows 1973; Friesen *et al.* 1976). The mechanism of rhythm generation remains unclear and could rely on endogenously rhythmic neurons, as in the lobster pyloric system (Selverston 1977), or on a network of neurons individually lacking oscillatory properties, as proposed for the leech swimming system (Friesen *et al.* 1976).

Tonic excitation

Evidence for a tonic synaptic excitation producing a membrane conductance increase in motoneurons during swimming was presented and discussed in the preceding paper (Roberts & Khan 1982). We have reported here that the spinal cord alone cannot produce sustained swimming. However, if part of the hindbrain is attached to the spinal cord, then long episodes of swimming can be evoked by brief mechanical stimuli to the body skin. Similar long episodes of rhythmic activity can be evoked from longitudinally divided central nervous systems and from single, isolated halves. In episodes evoked by mechanical skin stimulation, the excitatory sensory neurons only fire during stimulation (Roberts & Hayes 1977; Roberts 1980); so there is no possibility that sensory discharge maintains the swimming activity. This evidence supports the proposal, already made for other vertebrates (Brown 1914; Grillner 1976; Stein 1977), that there is a system in the hindbrain of the embryo that generates a descending, tonic excitation of motoneurons and pattern-generating neurons in the spinal cord. Like the spinal rhythm generators, this hindbrain system exists autonomously on each side of the nervous system and commissural connections are not necessary for its operation. These systems can be active spontaneously or be triggered by sensory stimulation (Kahn & Roberts 1982*a*). Two related observations argue against the tonic excitatory drive depending on the release of a single long-lasting, shot of a chemical excitant. First, swimming will stop within 200 ms if the cement gland is stimulated (Roberts & Blight 1975) and, secondly, this stimulation also stops rhythmic activity in spinal neurons and leads to the abolition of their tonic depolarization (Roberts & Kahn 1982).

Reciprocal inhibition

It was suggested for the cat (Brown 1911, 1912, 1914) and the locust (Wilson 1966, 1968; Waldron 1967; Wilson & Waldron 1968) that antagonist motor systems are coordinated by reciprocal inhibition. In *Xenopus* embryos the following observations are all compatible with the presence of reciprocal inhibition between the left and right sides of the spinal cord. (*a*) When commissural connections between left and right sides of the nervous system are cut, the rhythmic activity often has a shorter period, which could result from removal, on each cycle, of a phasic inhibition from the antagonist rhythm generator. (*b*) The left and right sides normally fire alternately both in swimming (Kahn & Roberts 1982*a*) and in struggling, when motoneurons fire long bursts of impulses (Kahn & Roberts 1982*b*). (The exception to this is what we have called 'synchrony', which will be discussed below.) (*c*) Synchronous firing of left and right motor systems can occur when inhibition is reduced by picrotoxin, strychnine or low chloride

saline (Soffe & Roberts 1982). (*d*) Intracellular recordings from putative motoneurons show that they are phasically inhibited during swimming activity at the time when their antagonists on the opposite side fire (Roberts & Kahn 1982).

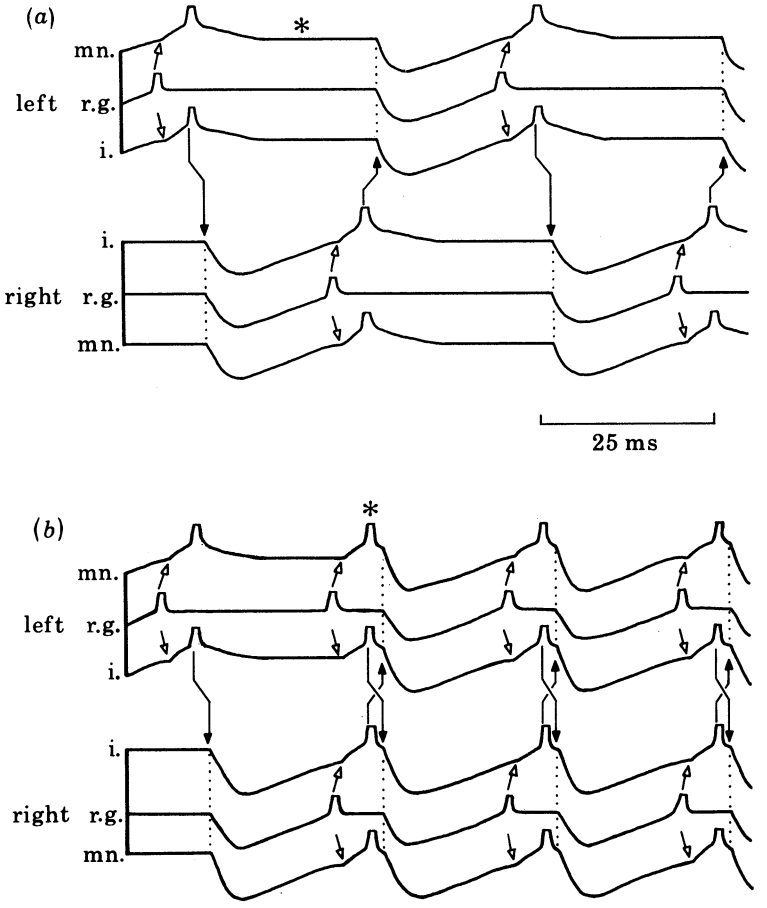

FIGURE 10. Patterns of synaptic and spike activity that could arise from the neural mechanism in figure 9. (*a*) Alternating swimming activity. (*b*) Synchronous activity starting at asterisk. Each line represents the membrane potential of a different class of neuron in the proposed mechanism. Because little is known of its mechanism the rhythm generator is made to fire rhythmically but only one inhibitory input is shown. The following values have been used: synaptic potential rise time 4 ms (Roberts & Kahn 1982); synaptic delay 0.5 ms; conduction velocity in axons 0.1 ms^{-1} (cf. Roberts & Blight 1975); spinal cord diameter 100 μm, therefore length of commissural axon about 150 μm, giving 1.5 ms conduction time; conduction time from rhythm generator to motoneuron 0.5 ms. Spikes are truncated for convenience in illustration. Open arrows indicate excitation, closed indicate inhibition. Abbreviations as figure 9.

Relation to neuroanatomy

The diagram in figure 9 shows the elements arranged crudely as they might lie in a transverse section of the spinal cord. Each element represents a population of neurons, for the rhythm generator, consisting possibly of a number of interconnected neuron classes. Since motoneuron processes are confined to one side of the cord reciprocal inhibition must be mediated by one of the two classes of commissural interneurons in the spinal cord (Roberts & Clarke 1982). It is of interest that a class of commissural inhibitory interneurons in the lamprey are rhythmically active during swimming and may serve to coordinate the two sides (Buchanan &

Cohen, personal communication). The motoneurons do not make any central synaptic contacts with other neurons and do not have recurrent collaterals. However, their axons run caudally within the spinal cord tracts for a short distance, so the possibility of such contacts exists (Roberts & Clarke 1982). Tonic excitation is thought to descend from the hindbrain in the lateral tracts. Many types of hindbrain interneuron with ipsilateral axons project in these tracts to the spinal cord (Roberts & Clarke 1982). The tonic excitation could affect most spinal cord cells with dendrites in the lateral tracts.

Like the connections of cells mediating tonic excitation those made by the rhythm generators and inhibitor interneurons could be rather non-specific within the lateral tract. Commissural inhibitory interneurons could contact all contralateral neurons with dendrites in this tract. The rhythm generators could be nearly as non-specific in their ipsilateral connections. This lack of specificity is attractive since the network could develop using a few very simple rules (e.g. for commissural inhibitory interneurons: *go to opposite side and synapse with all dendrites crossed as you ascend the lateral tract*). In general the model is compatible with spinal neuroanatomy and with the very limited number of classes of spinal interneuron (Roberts & Clarke 1982).

Operation of the model

Once activated by tonic excitation the rhythm generators on each side of the cord excite ipsilateral motoneurons and inhibitory commissural interneurons. The resulting reciprocal inhibition between rhythm generators on either side is the basis for the normal alternation of activity on left and right. By assigning values for conduction and synaptic delays, and for synaptic rise times, the type of synaptic activity expected in the motoneurons and inhibitory interneurons can be drawn out. In the example of figure 10*a* the left side fires first; so the right side is inhibited. After a period dependent on the strength of this inhibition, the right side fires and the two sides then continue to alternate. The pattern of activity in motoneurons and inhibitory interneurons is indistinguishable and very similar to that seen in our intracellular recordings from putative motoneurons (Roberts & Kahn 1982). This activity pattern is thought to underlie swimming locomotion. During struggling the motor system on one side fires rapidly for a period of 100 ms or more, then the other side becomes active and the first side silent (Kahn & Roberts 1982*b*). In this case the mechanism for alternation is unclear but the reciprocal inhibition can account for the silence on the inactive side.

The final pattern of motor activity seen in the paralysed embryo is what we call 'synchrony'. In synchrony motoneurons on left and right sides of a segment fire together at half the ongoing swimming cycle period. This pattern of activity usually occurs early in swimming episodes and after stimulation during swimming, when one expects excitatory drive to be high (Kahn & Roberts 1982*a*). Under strong drive neurons on one side could fire twice per cycle. In figure 10*a*, if this happened at the asterisk on the left side, the next firing of the right side would be delayed by inhibition. However, if the left side fired just before it was inhibited, synchronously with the right side, the right side would still fire because of the delay in onset of the inhibition. This pattern of synchronous activity could then continue for a number of cycles as shown in figure 10*b*. It has been recorded intracellularly (Roberts & Kahn 1982) and appears to be a relatively stable firing mode for this network. In a rigorous modelling study of a pair of oscillators with reciprocal inhibitory coupling, Kawato & Suzuki (1980) have found two

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stable states, one in alternation, the other in synchrony. These results support the present proposals, but more data are needed before further progress can be made.

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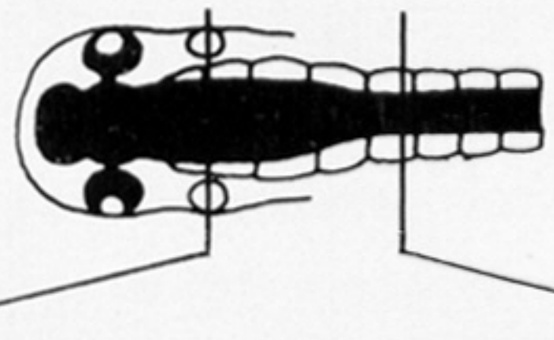
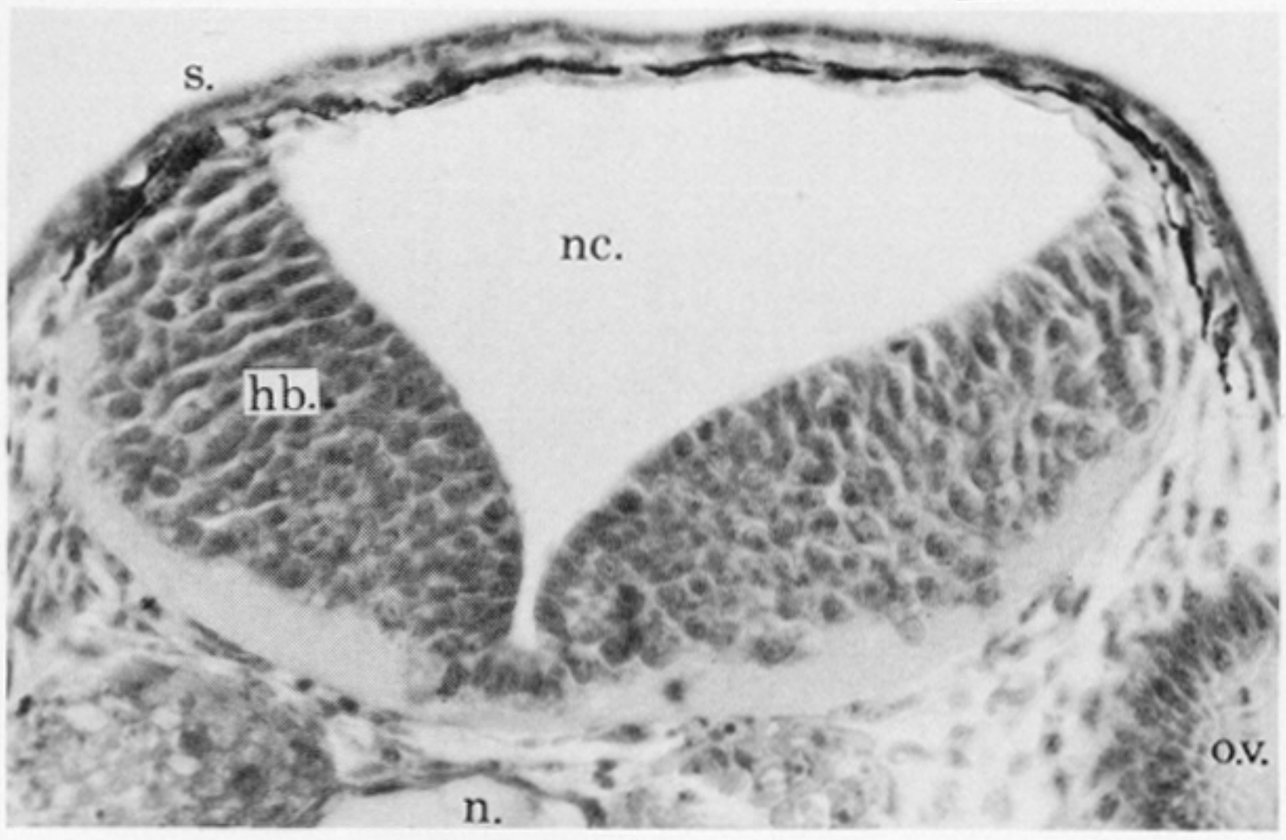
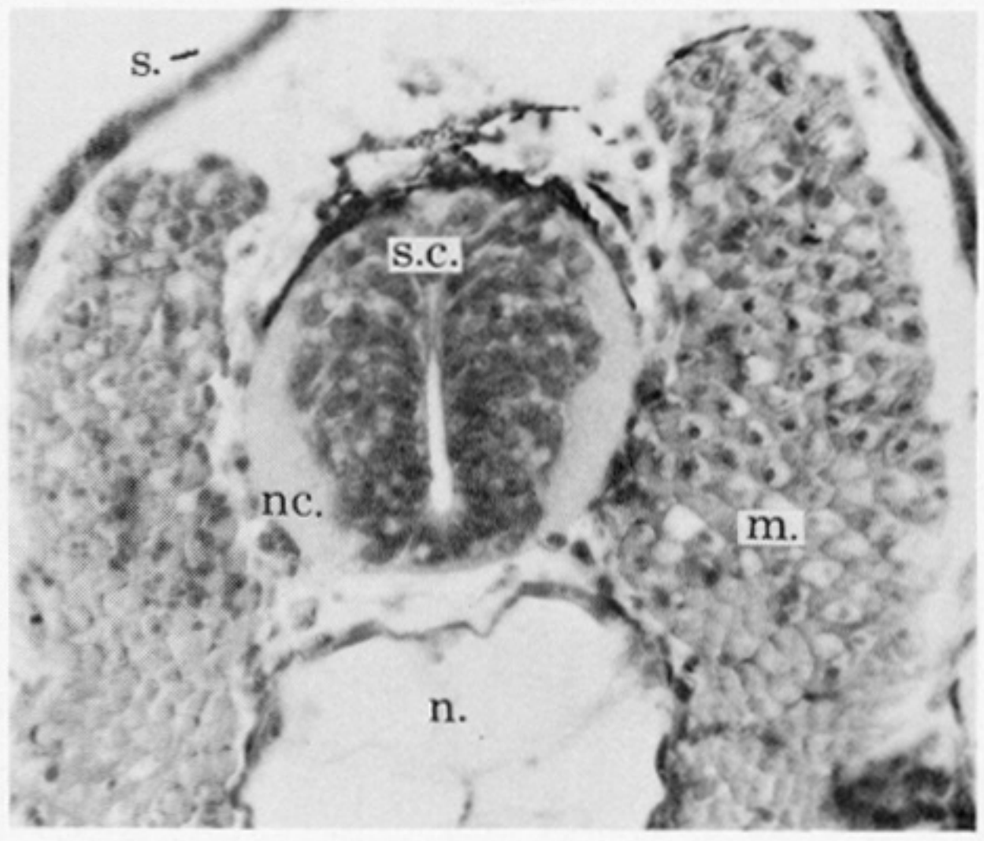
(*a*)



100 μm





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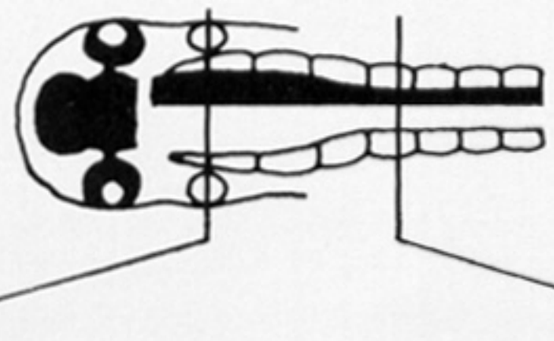
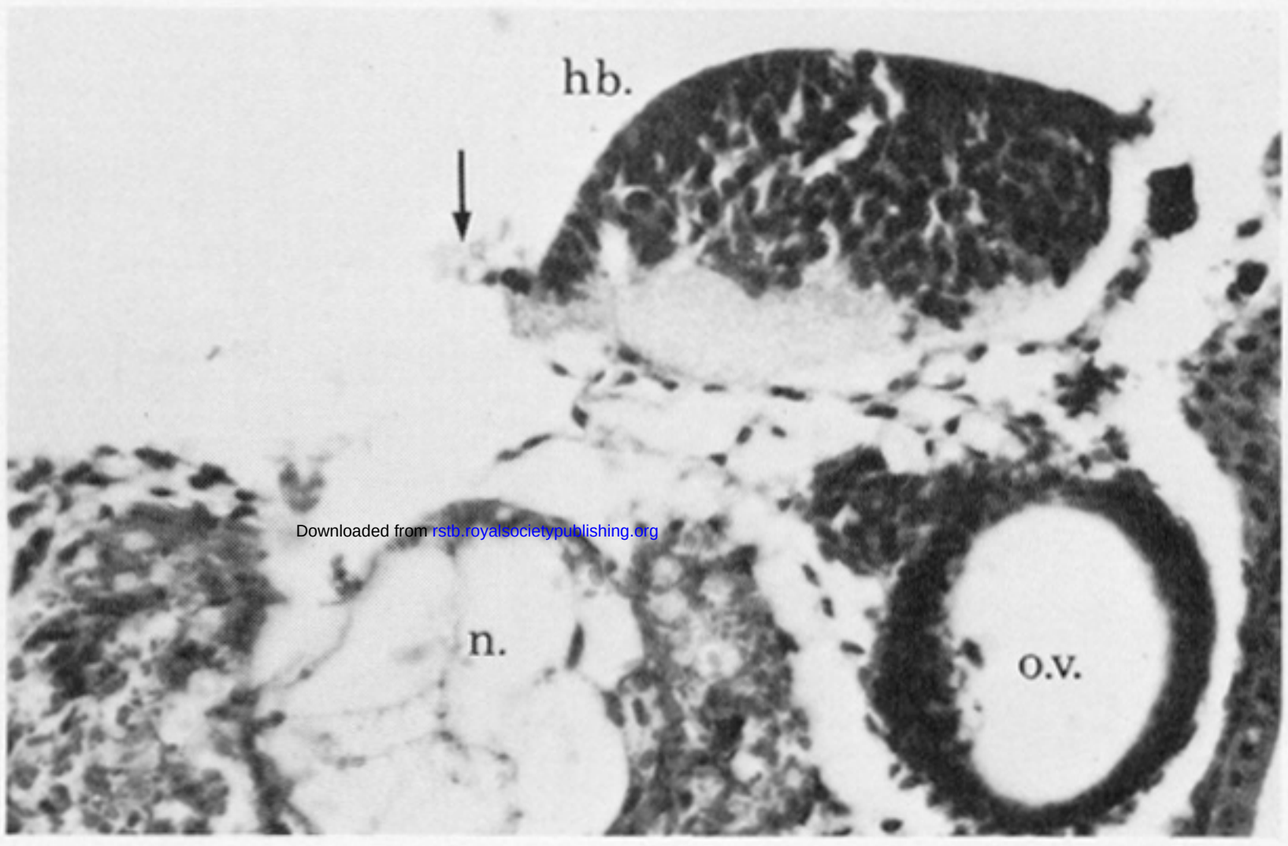
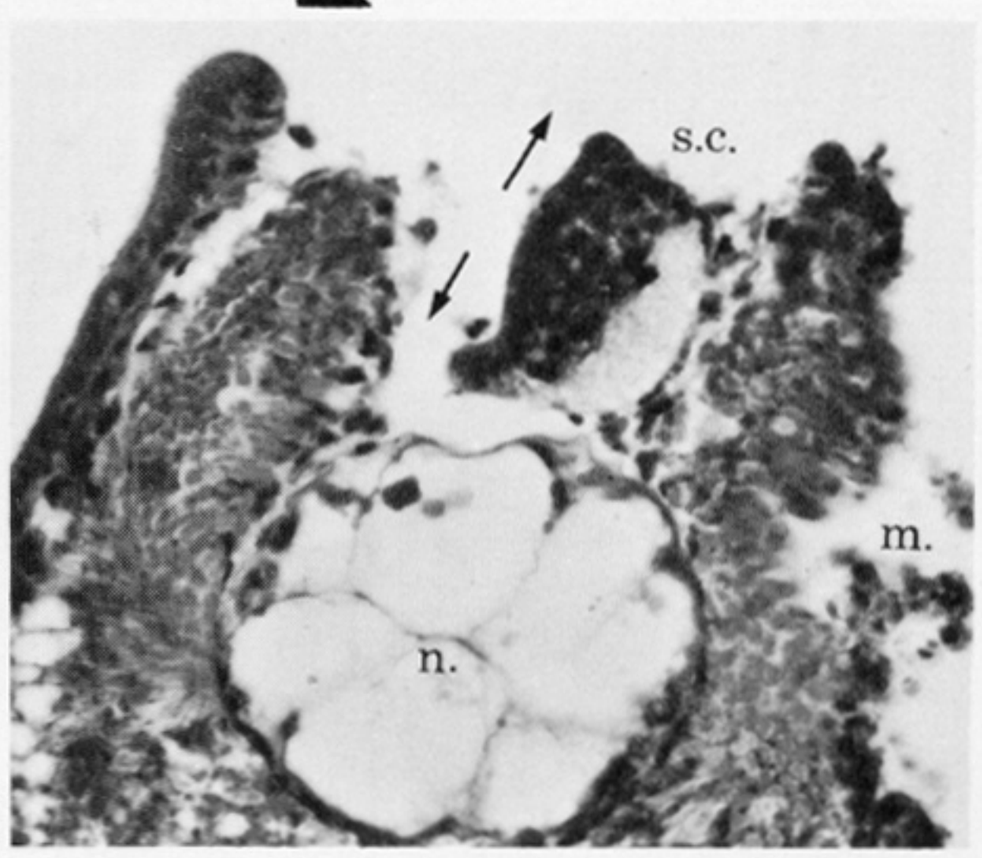
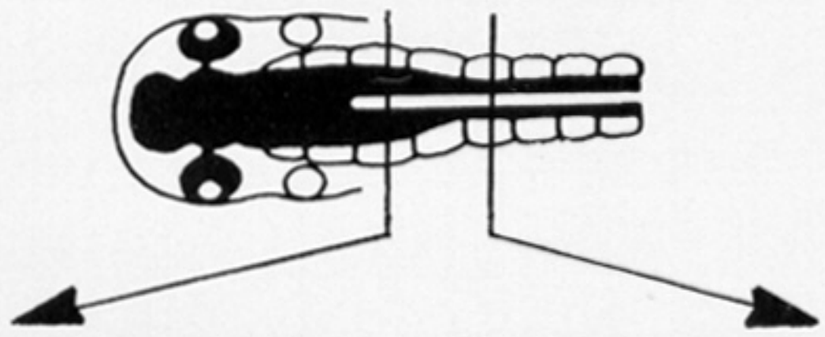
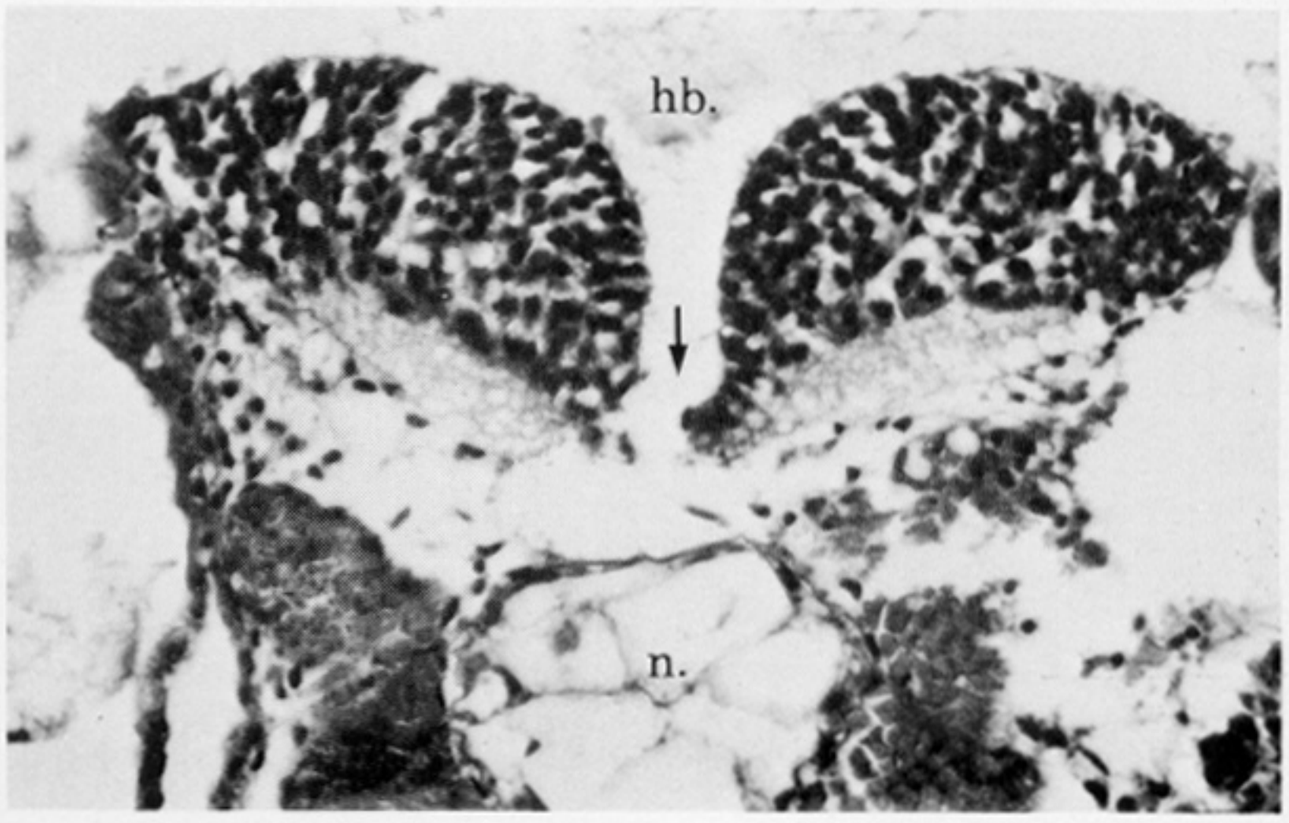






(*c*)





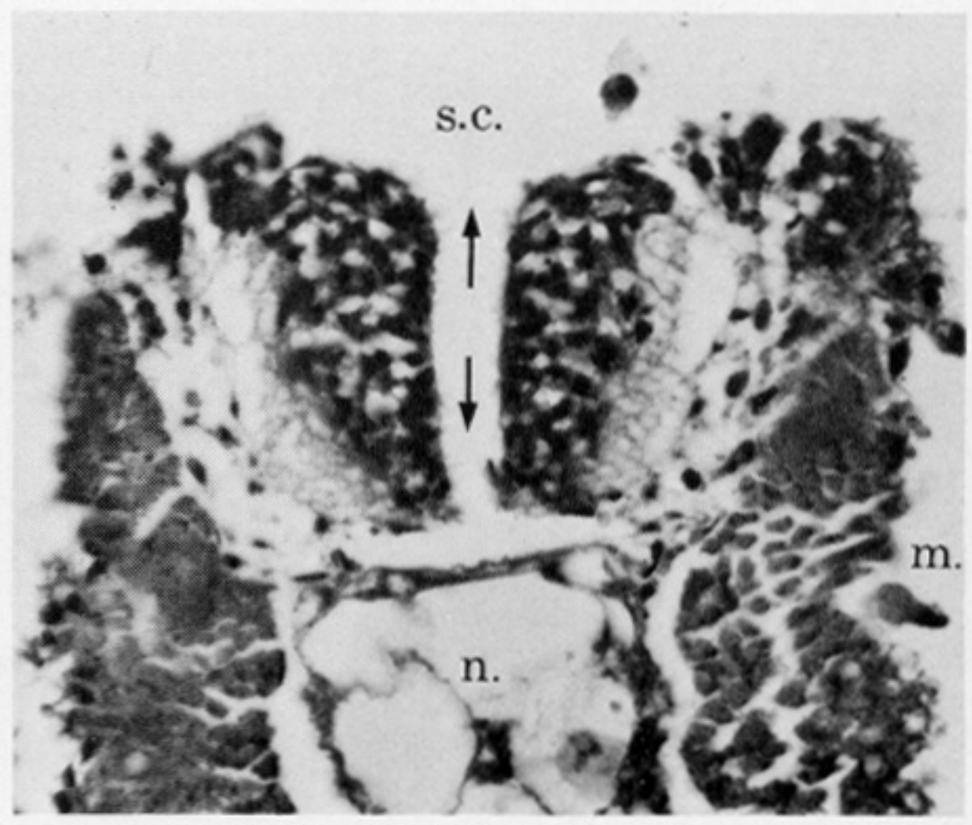


PLATE 1. For description see opposite