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INTRACELLULAR RECORDINGS FROM SPINAL NEURONS DURING 'SWIMMING' IN PARALYSED AMPHIBIAN EMBRYOS

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CONTENTS

Intracellular microelectrode recordings have been made from probable motoneurons in the spinal cord of *Xenopus laevis* embryos during fictive 'swimming' in preparations paralysed with the neuromuscular blocking agent tubocurarine. These cells had resting potentials of −50 mV or more. During spontaneous or stimulus-evoked 'swimming' episodes: (*a*) the cells were tonically excited; the level of tonic synaptic excitation and the conductance increase underlying it were both inversely related to the 'swimming' cycle period; (*b*) the cells usually fired one spike per cycle in phase with the motor root burst on the same side; spikes did not overshoot zero and were evoked by phasic excitatory synaptic input on each cycle, superimposed on the tonic excitation; (*c*) in phase with motor root discharge on the opposite side of the body, the cells were hyperpolarized by a chloride-dependent inhibitory postsynaptic potential. The nature of synaptic potentials during 'swimming' was evaluated by means of intracellular current injections. The 'swimming' activity could be controlled by natural stimuli. The results provide clear evidence on the relation of tonic excitation to rhythmic locomotory pattern generation, and indirect evidence for reciprocal inhibitory coupling between antagonistic motor systems.

Introduction

It is now well established that in many groups of animals rhythmic activity suitable to drive basic locomotory movements can be generated within the central nervous system without any reflexes (Grillner 1975). Various hypotheses have been proposed to explain this central nervous pattern generation (Brown 1912, 1914; von Holst 1939; Szekely 1965; Perkel & Mulloney 1974; Wilson 1968; Miller & Scott 1977; Friesen & Stent 1978). These hypotheses have two important features in common. First, pattern generation is turned on and maintained by some form of tonic excitation. Secondly, neurons controlling antagonistically active muscles usually inhibit each other reciprocally. Despite their long standing there is little direct evidence on these two proposals, based on intracellular recordings from neurons active during pattern generation.

Inspired by the studies of Coghill (1929), we hope that lower vertebrate embryos may provide a simple vertebrate preparation in which to study basic principles of nervous organization and function. The preceding paper (Roberts & Clarke 1982*a*) describes the neuroanatomy of the late *Xenopus laevis* embryo. When freed from their egg membranes these animals can swim (Kahn *et al.* 1982). More significantly, paralysed embryos can generate motor nerve discharges compatible with normal swimming. These discharges can be started or stopped at will by using stimuli that normally affect swimming (Kahn & Roberts 1982*b*). We have therefore used this preparation to make intracellular recordings from rhythmically active neurons in the ventrolateral part of the spinal cord where the motoneurons lie (Roberts & Clarke 1982). Unfortunately the recorded cells cannot be categorically identified as motoneurons. However, these recordings have allowed a description of the types of synaptic drive that rhythmically active spinal cord neurons receive during swimming and the way in which this drive controls rhythmic impulse firing by these cells. The results present the first clear evidence in the vertebrates for a tonic excitation related to the generation of locomotory rhythmic activity.

Materials and methods

Experiments were carried out on embryos of the clawed toad, *Xenopus laevis*, at developmental stage 37/38 (Nieuwkoop & Faber 1956). Methods have been described previously for obtaining embryos (Kahn *et al.* 1982), for curarization in 10^{-4} M curare solution (Kahn & Roberts 1982*b*), and for obtaining extracellular motor nerve recordings (Kahn & Roberts 1982*b*). Saline composition was similar to that used before (Kahn *et al.* 1982), but on occasions the calcium concentration was raised from the standard 1.8 mM to 10 mM. This higher calcium concentration is thought to stabilize intracellular penetrations (Spitzer 1976), but penetrations were made successfully in standard calcium saline also. Unless otherwise stated in the text or figure caption the results presented refer to penetrations in the standard saline.

Electrodes

In most recordings fine glass microelectrodes were used filled with 3 M potassium acetate at pH 6.5, with resistances of 100–200 MΩ. A few penetrations were made with K_2SO_4 or potassium citrate filled electrodes, and results were similar to those with potassium acetate. Some experiments were done with 3 M KCl filled electrodes, of resistance 50–80 MΩ. For passing current through the recording electrode an amplifier with bridge circuit was used. Penetrations were made by mounting the electrode on a piezoelectric 'prodder' (Weevers 1980) which was advanced with a hydraulic drive.

Preparation

Once paralysed with curare, an embryo was pinned on its side to a Sylgard layer on a rotatable table (Kahn *et al.* 1982). Pins were pushed through the notochord, applying a slight stretch to the animal to provide a firmer base for the intracellular penetrations in the spinal cord. Skin overlying rostral myotomes was removed with fine pins, and myotome tissue over the side of the spinal cord was cleared away, thus exposing the cord between about the 4th to 8th post-otic myotomes. In most experiments, simultaneous extracellular motor nerve recordings (Kahn & Roberts 1982*b*) were made from an ipsilateral intermyotomal cleft ventral to the region of the cord penetrated with the microelectrode (figure 2, inset). If myotome tissue was taken away to near the ventral margin of the spinal cord then the motor nerve activity could still be recorded at an adjacent intermyotomal cleft. In most experiments the motor nerve recordings were made on the nearest intermyotomal cleft to the microelectrode. It is, however, not necessarily to be expected that the motor nerve electrode was recording from exactly the area in which the penetrated cells had peripheral axons. This is for two reasons. (i) Each intermyotomal cleft is approximately 500 μm in dorsal–ventral extent, while the motor nerve suction electrodes were only 50–70 μm in tip opening. The motor nerve electrode therefore probably records activity in only a small proportion of the motoneurons that pass to that cleft. (ii) Motoneurons in *Xenopus* embryos have axons that pass caudally within the ventrolateral tract of the spinal cord before leaving the cord to innervate the myotomes (Hughes 1959; Roberts & Clarke 1982). It is therefore possible that a motoneuron axon passed to intermyotomal clefts caudal to the motor nerve electrode.

Penetrations

Intracellular penetrations were obtained with the microelectrode approaching the spinal cord from the side, between the 4th and 8th post-otic myotomes. To maximize the chances of obtaining a penetration from a motorneuron the penetrations were usually made in the ventral third of the spinal cord, though a few penetrations were more dorsal, up to about the mid-lateral region. The neurons in the spinal cord have somas about 15 μm in diameter in plastic-embedded sections and they are difficult to penetrate. However, stable penetrations were obtained in 62 neurons from 33 embryos. In many of these the penetrations lasted 10 to 20 min, and in one more than 1 h. In addition to these penetrations with use of potassium acetate electrodes, 14 cells were penetrated in five embryos with electrodes filled with 3 M KCl.

Results

Properties of the cells

This is the first intracellular study of ventral neurons in an amphibian embryo spinal cord, and so this report begins with some of the basic properties of these cells.

Penetrated cells usually showed resting potentials of −50 mV or more; the maximum (on emergence at the end of a recording) was −67 mV. Cells penetrated did not spike spontaneously (except during swimming episodes); however, spikes could be elicited in response to depolarizing current pulses injected through the recording microelectrode (figure 1*a*). In several cases the spikes had hyperpolarizing after potentials (figure 1*a*). On increasing the current strength the maximum responses in different penetrations ranged between cells that gave only a single spike to cells that gave repetitive spikes throughout the pulse (figure 1*a*). Where more than one spike was evoked by a depolarizing pulse, the second and subsequent spikes were sometimes

smaller than the first (figure 1*a*). The minimum interval observed between two successive spikes on a single depolarizing pulse was 5 ms, a spike frequency of 200 Hz. Spikes also occurred during episodes of swimming activity (e.g. figure 2) and the properties of the spikes were measured from these (thus avoiding problems in determining membrane potentials that result from bridge imbalance during current injection). Spikes had amplitudes of up to 63 mV. The nearest a spike approached to zero potential was −4 mV; no overshooting spikes were seen. Spikes had durations (at half amplitude) ranging from 1.2 to 5.5 ms.

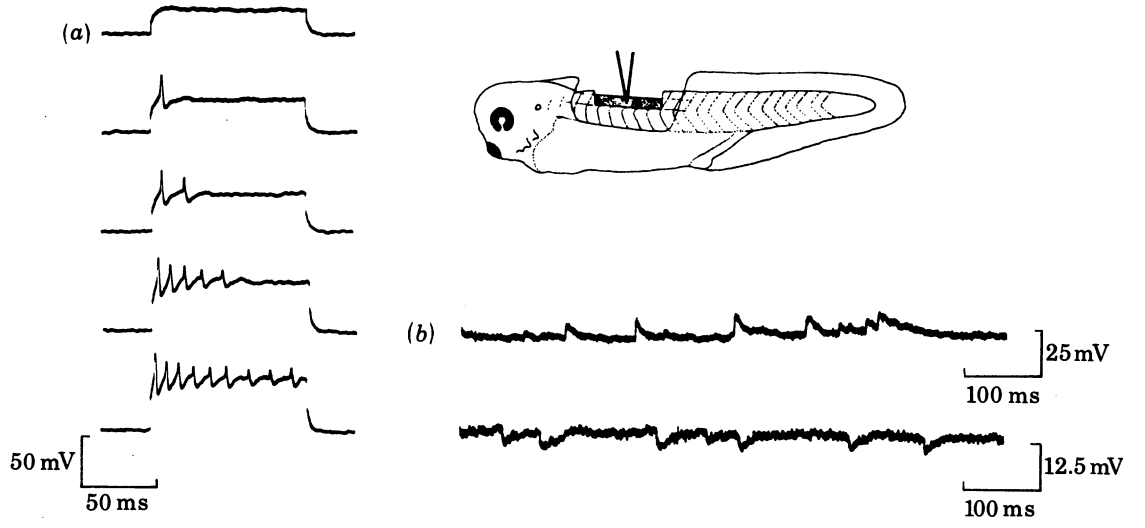

FIGURE 1. Evoked spikes, and spontaneous synaptic potentials in intracellular recordings from ventrolateral spinal cord neurons of curarized *Xenopus* embryos.

(*a*) Spikes evoked by depolarizing current pulses (passed through the recording electrode with bridge circuit unbalanced). In this example increasing the current strength evokes more spikes.

(*b*) Depolarizing and hyperpolarizing synaptic potentials from two different penetrations (upper and lower lines). These potentials occasionally appeared in quiescent preparations between swims. Approximate electrode position in this figure illustrated in the diagram.

The curarized preparation shows spontaneous episodes of 'swimming' activity separated by periods of quiescence. In the quiescent state, occasional depolarizing or hyperpolarizing potentials were seen in the penetrated cells (figure 1*b*). These had time to peak of 2–6 ms, and time to half decay of 4–12 ms. This time course is similar to that of excitatory postsynaptic potentials in adult frog motoneurons (Fadiga & Brookhart 1960). The shapes and time courses of these potentials suggest that they were excitatory and inhibitory postsynaptic potentials (e.p.s.ps and i.p.s.ps).

Synaptic and spike activity during 'swimming'

During intracellular recordings, episodes of 'swimming' occurred (figure 2), indicated by rhythmic activity on the motor nerve electrode at characteristic cycle periods of swimming (40–125 ms) (Kahn & Roberts 1982*b*). These episodes were usually evoked by dimming the lights, though sometimes they occurred spontaneously. The main components of the activity recorded in a penetrated cell during a 'swimming' episode are illustrated in figure 2*a*, *b*. A single episode of 'swimming' generally started with a gradual depolarization and terminated with a gradual repolarization (figure 2*a*). Between these lay a tonic depolarization, with the

membrane potential depolarized below resting potential. Upon the tonic depolarization was superimposed rhythmical activity with swimming periodicity. The rhythmical synaptic activity consisted of phasic excitation, usually evoking a spike, alternating with phasic inhibition (figure 2*c*). Rhythmic spikes usually occurred in phase with extracellular motor nerve bursts (figure 2*c*). The synaptic activity in a 'swimming' episode therefore consisted of both tonic and phasic components. The various components will now be described in turn.

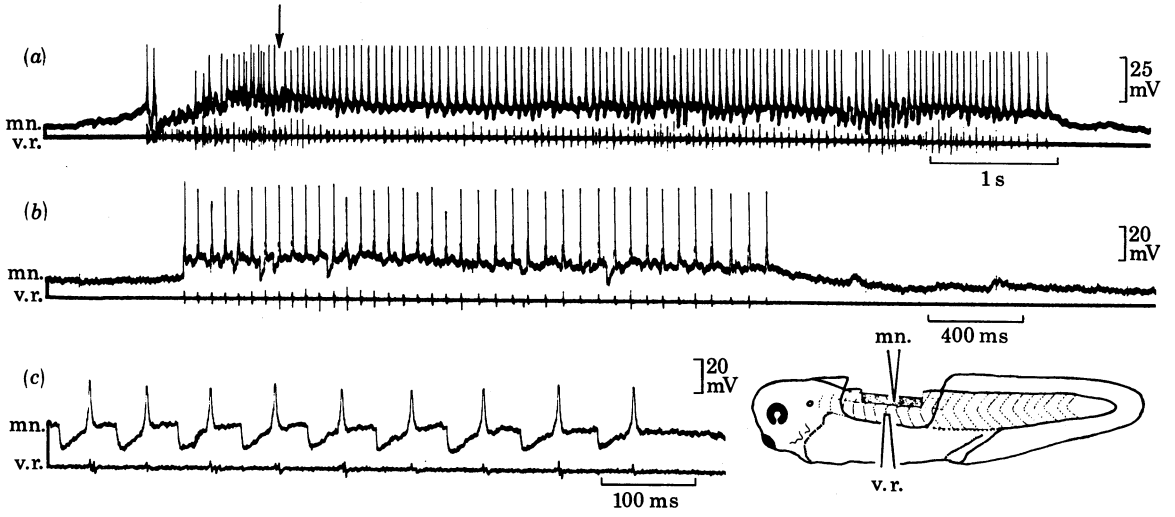

FIGURE 2. Intracellular activity in ventrolateral spinal cord neurons during 'swimming' in curarized embryos. Inset illustrates the recording arrangement.

(*a*) One brief episode of motor activity, mainly 'swimming', but beginning with irregular activity up to the arrow. The episode appeared spontaneously. Note that the membrane potential remains depolarized below the resting potential throughout 'swimming' activity.

(*b*) Another short, spontaneous episode of 'swimming' starting without a gradual depolarization.

(*c*) Activity during 'swimming' at faster time base in a different penetration (in 10 mM calcium saline). This shows the activity on nine 'swimming' cycles at the end of an episode.

Abbreviations: mn., intracellular 'motoneuron' record; v.r., extracellular ventral root record on same side as intracellular.

Tonic excitation

In a 'swimming' episode rhythmic synaptic potentials and spikes (corresponding to swimming cycles) were superimposed upon a tonically depolarized membrane potential (figure 2*a*, *b*). This tonic depolarization occurred in all 'swimming' episodes in different penetrations, and it was sustained throughout even the longest of episodes (the longest was 48 s). The presence of the tonic depolarization suggested that there was a continuous, non-rhythmic background of excitation throughout a 'swimming' episode. Two important factors to be established were, first, whether the depolarizing activity underlying an episode was actually tonic, rather than rhythmic, and, secondly, whether it was due to excitatory synaptic activity, rather than a release from inhibition. These are considered below.

The evidence that the depolarizing activity was tonic rather than fluctuating rhythmically is as follows.

(i) In that part of a 'swimming' cycle between the spike and the onset of the mid-cycle i.p.s.p., the membrane remained depolarized with respect to the resting potential (figure 3*a*). The potential here was usually steady, without any suggestion of a gradual repolarization to the

resting potential that would indicate rhythmically fluctuating background depolarization. Instead, the steady depolarization showed that depolarizing activity was tonic in the first half of a cycle.

(ii) On some 'swimming' cycles midcycle i.p.s.ps were not visible (figure 3*b*). On these cycles the membrane potential remained tonically depolarized between spikes on successive cycles, without indication of rhythmic fluctuation.

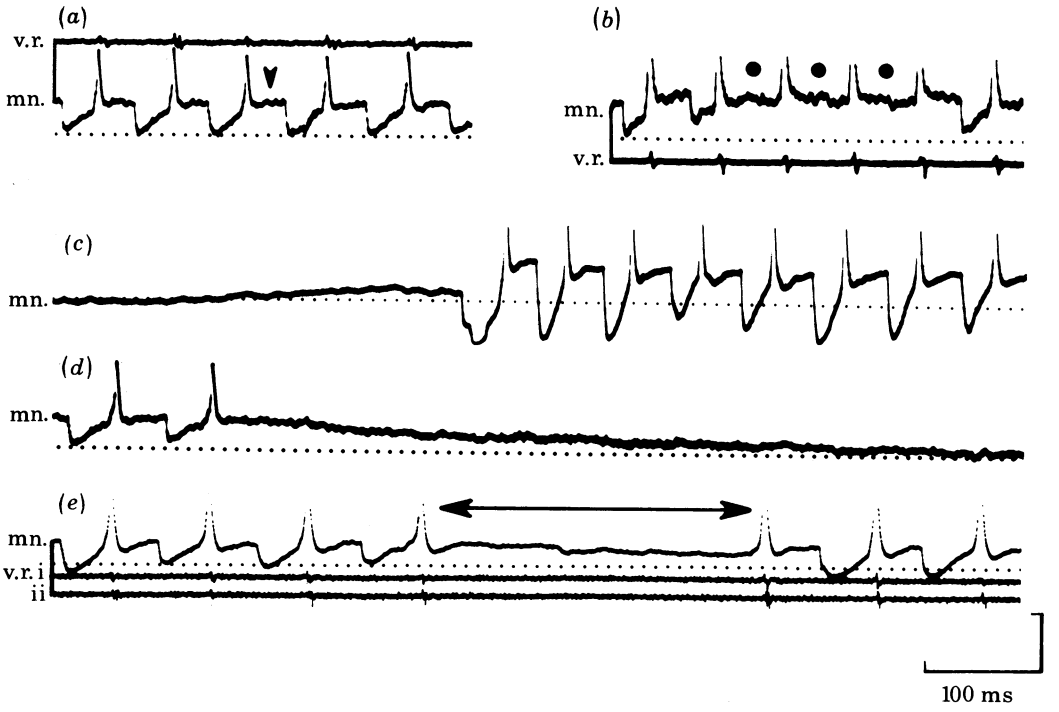

FIGURE 3. The tonic depolarization throughout swimming episodes. (Here and in subsequent figures dotted line indicates resting potential.)

(*a*) Plateau depolarization indicated (arrowhead) in first half of a 'swimming' cycle (in 10 mM calcium saline).

(*b*) I.p.s.ps not apparent on some cycles (filled circles), revealing a tonic depolarization throughout the cycle.

(*c*) Gradual depolarization before 'swimming' activity starts (in 10 mM calcium saline).

(*d*) Gradual repolarization after 'swimming' episode (in 10 mM calcium saline).

(*e*) Pause in rhythmic activity (arrows) revealing tonic depolarization (in 10 mM calcium saline).

Abbreviations: v.r. i and v.r. ii, recordings from adjacent ventral roots. Voltage calibration: 20 mV for (*a*), (*b*), (*d*), (*e*); 27 mV for (*c*).

(iii) A gradual depolarization from the resting potential often preceded the onset of an episode (figures 2*a*, 3*c*), and following an episode there was a slow repolarization (figures 2*a*, *b*, 3*d*). The time for the depolarization to decay by half after the end of an episode was 100–400 ms, complete repolarization often taking several seconds. These depolarizations before and after an episode were generally smooth, without indication of individual p.s.ps (figure 3), and they showed no indication of rhythmical fluctuations.

(iv) On rare occasions rhythmic activity stopped during a 'swimming' episode for a few hundred milliseconds. In the absence of the 'swimming' rhythm, an underlying tonic depolarization was seen (figure 3*e*).

This evidence indicates that the background depolarizing activity throughout a swimming episode was tonic. It preceded the onset of swimming episodes and decayed slowly after the episode ceased.

During 'swimming' episodes there were changes in the period of the 'swimming' rhythm, and also in the amplitude of the tonic depolarization (measured at the end of the plateau during the first half of a swimming cycle; arrowhead figure 3*a*). Measurements of rhythm period at different tonic depolarization amplitudes are shown for two penetrations in figure 4*a*, *b*. These show that as the tonic depolarization amplitude increased the cycle period of the 'swimming' rhythm shortened.

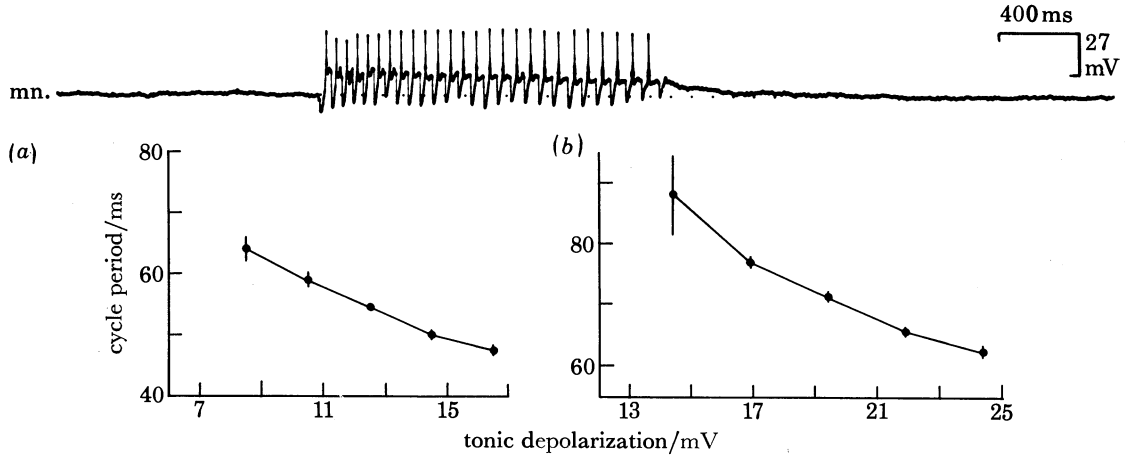

FIGURE 4. The period of the 'swimming' rhythm is related to the amplitude of the tonic depolarization, measured from resting potential. Record shows a brief 'swimming' episode in which this is clear (10 mM calcium saline). Period is measured between the onset of a burst at a motor nerve, and the next burst at the same motor nerve. (*a*, *b*) Plots of mean ± s.e. from two different penetrations. In (*a*) the results are pooled from four episodes (n = 209 'swimming' cycles); (*b*) is from one episode (n = 215 'swimming' cycles). The amplitude of the tonic depolarization at a particular rhythm period varied in different penetrations.

The tonic depolarization during a 'swimming' episode might have been due to tonic excitatory synaptic activity, or, alternatively, to a release from continuous inhibition in the quiescent state of the preparation. If it were due to excitatory chemical synaptic activity then the membrane conductance during a swimming episode should be greater than before or after an episode. To determine whether there was a conductance increase, long (240 ms) pulses of hyperpolarizing current were passed at regular intervals (560 ms) across the membrane, both during a 'swim' and in the resting state (figure 5*a*). Pulses were of constant current, and so, if there was an increase in membrane conductance during a 'swim', pulse amplitude should be smaller than in controls before and after the swimming episode. In one experiment the control pulses produced voltage deflexions of 28.5–30.5 mV, and of 49 pulses during 'swimming' none were greater in amplitude than controls. Four of the pulses fell within the control range, and the rest were smaller, pulses falling as low as 16.5 mV (the amplitude range of the pulses is discussed below). These results indicate that there was a conductance increase during a 'swimming' episode. This shows that the tonic depolarization was not due to a release from inhibition (which would produce a decrease in conductance), and suggests that it was due to excitatory synaptic activity.

The relationship (described above) that was found between the amplitude of tonic depolarization and rhythm period was also reflected in the membrane conductance measurements. When the fall in pulse amplitude was greater (indicating a greater conductance increase), the rhythm period was shorter (figure 5*b*).

Slow deformation of the cement gland normally terminates swimming activity (Roberts & Blight 1975). When applied during intracellular recording this stimulation terminated rhythmic activity and abolished the tonic depolarization.

In summary, the penetrated cells were tonically excited during 'swimming' episodes. The tonic excitation and the conductance increase underlying it were both inversely related to the 'swimming' cycle period.

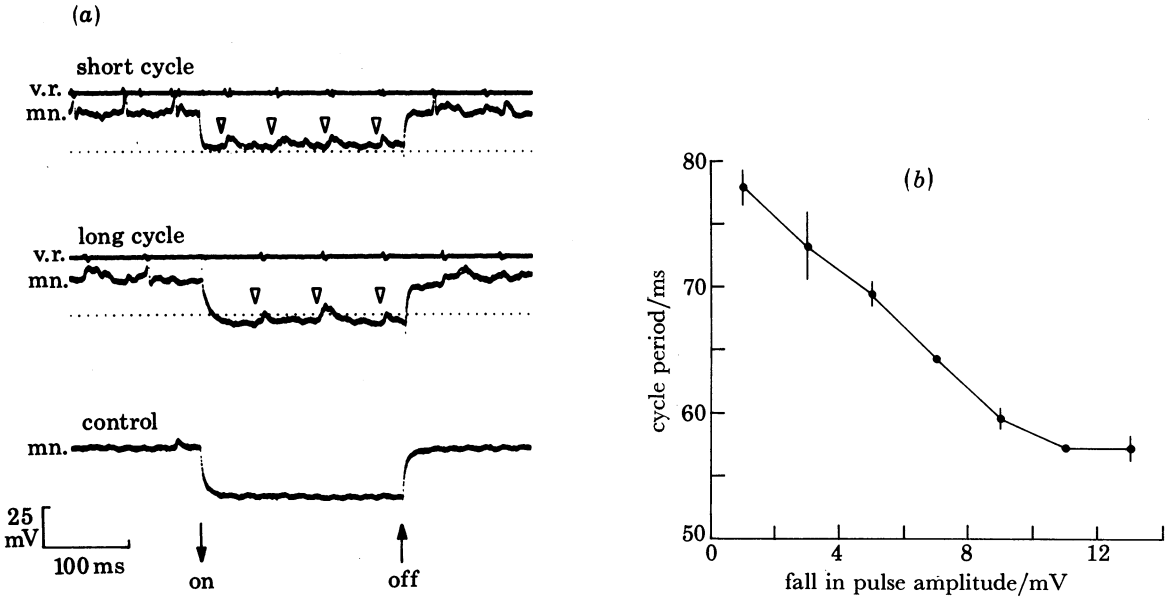

FIGURE 5. Increase in the membrane conductance during 'swimming' activity is associated with tonic depolarization.

(*a*) Constant current hyperpolarizing pulses 240 ms long applied every 560 ms produce a smaller membrane potential change during 'swimming' than control pulses before or after. Pulse on and off indicated. When hyperpolarized, depolarizing potentials (indicated) are seen in phase with motor nerve bursts.

(*b*) During 'swimming', the fall in pulse height compared to controls was related to the period of the rhythm. When the fall in pulse height was greater (indicating a greater membrane conductance), the rhythm period was shorter. Control pulse height was 29.4 mV (mean; range 28.5–30.5 mV). Points given as mean ± s.e. (total 49 pulses). Results from three successive 'swimming' episodes. The pulse amplitude in 'swimming' was the difference between the mean potential before and after a pulse, and the mean potential during the pulse. (The membrane potential was measured midway through each 'swimming' cycle.) Fall in pulse height is control pulse (29.4 mV) minus the pulse amplitude. The first 50 ms after the 'on' and 'off' of the current pulse were not included because the membrane was still polarizing.

Spikes

During 'swimming' episodes penetrated cells generally spiked, with one spike on each cycle of the swimming rhythm, over the full range of cycle periods (figure 2). Exceptionally, on some cycles no spikes occurred, and, rarely, two or three spikes were seen on one cycle. The spikes occurred at about the same time as the extracellular motor nerve bursts (figure 2), recorded from an adjacent intermyotomal cleft on the same side of the body.

Rhythmic excitation

The rhythmic intracellular spikes in a 'swimming' episode often had depolarizing prepotentials (figure 6*a*), which suggested that the spikes were evoked by phasic excitation. The evidence in support of the presence of underlying phasic excitation is as follows. (i) On some cycles spikes failed to occur, and on these there was generally a phasic depolarizing potential at

the time that the spike would have been expected to occur (figure 6*a*). These potentials usually had rise times of 4–7 ms, and a slower decay. The rise time is similar to the rise times of spontaneous e.p.s.ps reported above. (ii) An underlying depolarizing potential was seen when the spikes were blocked or delayed by hyperpolarizing current (figures 5*a*, 6*b*, *c*). (iii) The underlying depolarizing potentials increased in amplitude with increased hyperpolarization (figure 6*b*). This evidence therefore indicates that the cells receive phasic excitatory synaptic input on 'swimming' cycles, and these are superimposed upon the background of tonic excitation.

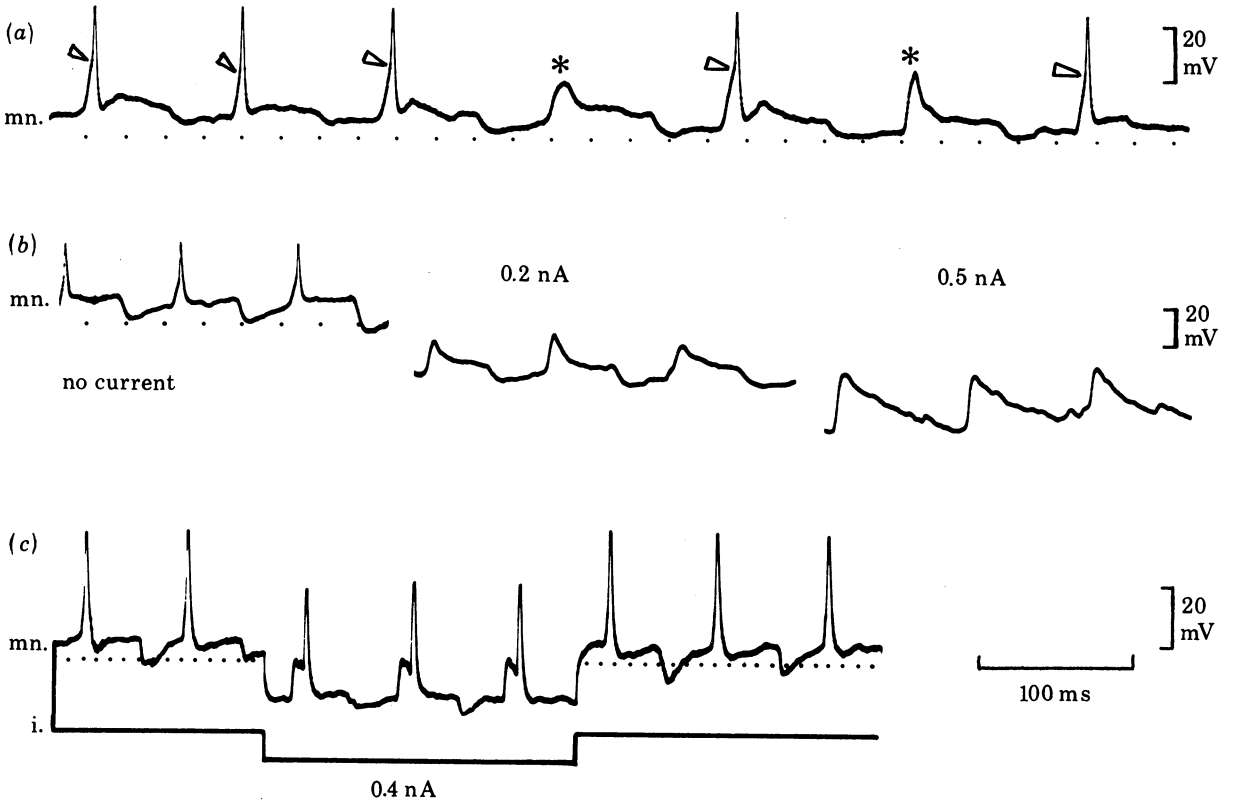

FIGURE 6. Phasic e.p.s.ps underlie the spikes on swimming cycles. In (*a*) prepotentials are apparent before spikes (arrowheads), and a phasic depolarizing potential is apparent when spikes fail to occur on swimming cycles (*).

(*b*) Effect of hyperpolarizing current injected through the recording electrode (bridge unbalanced). At 0.2 nA spikes are blocked to reveal depolarizing potentials. At 0.5 nA depolarizing potentials are larger while i.p.s.ps are no longer visible. (The three records are not sequential.) Same cell as (*a*).

(*c*) In another cell hyperpolarizing current delays but does not block the spike. A depolarizing potential is uncovered and i.p.s.ps are reduced.

All in 10 mM calcium saline.

Rhythmic inhibition

On each cycle of the 'swimming' rhythm there was usually a hyperpolarizing potential (figures 2, 3, 7). These hyperpolarizing potentials began about midway between the spikes on successive 'swimming' cycles. In 'swimming' the spikes in motor nerves on the left and right sides of a segment of the body alternate (Kahn & Roberts 1982*b*); thus these hyperpolarizing potentials occurred at about the same time as the motoneurons on the opposite side of the spinal cord were active.

One question that arises is whether these hyperpolarizing potentials were i.p.s.ps, or, alternatively, the results of a fall in background tonic excitation. The evidence that indicates that they were i.p.s.ps is as follows. (i) The rise time of the potentials was short, often 4–7 ms. This is similar to the rise time of the phasic excitatory potentials described in the previous section and also to the spontaneous e.p.s.ps and i.p.s.ps seen in quiescent preparations (see above). (ii) Some penetrations were made in saline with higher calcium concentration (10 mM). In this saline the hyperpolarizing potentials were generally larger than in standard saline, and often they were

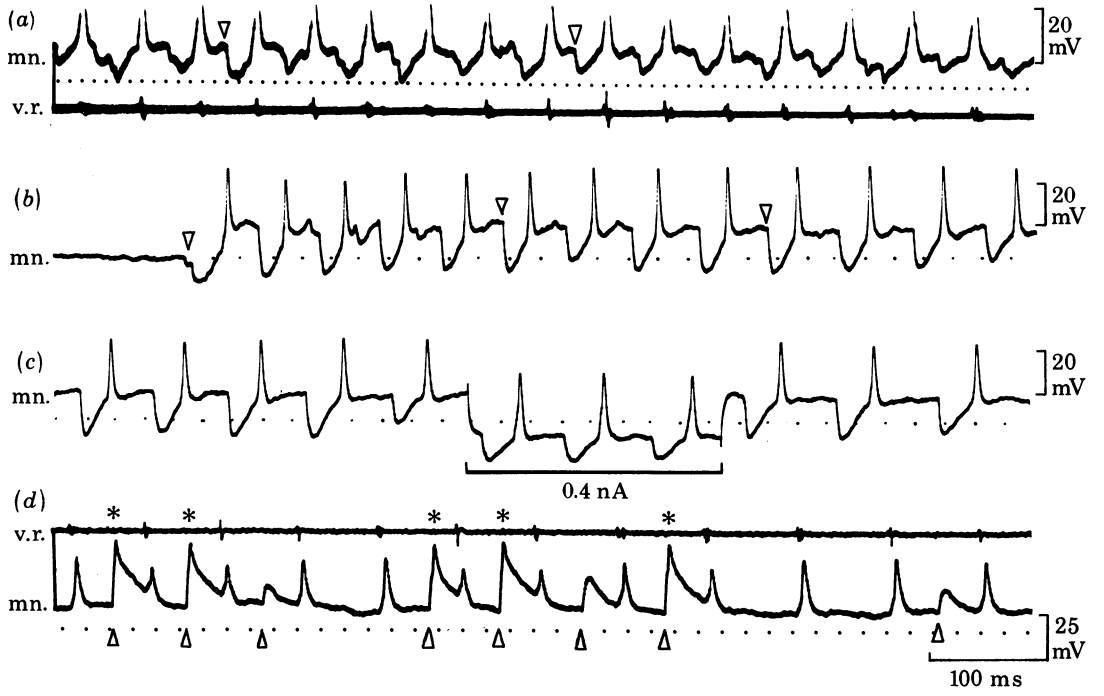

FIGURE 7. I.p.s.ps during the swimming rhythm.

(*a*) I.p.s.ps indicated: the spikes have been cut by the edge of the film. I.p.s.ps can be simple or compound. In standard saline.

(*b*) I.p.s.ps indicated; i.p.s.ps hyperpolarize membrane above resting potential (dotted line). Notice that this episode begins with an i.p.s.p. In 10 mM calcium saline.

(*c*) Effect of hyperpolarizing current on the i.p.s.p. is to reduce its amplitude. In 10 mM calcium saline.

(*d*) With 3 M KCl electrode mid-cycle depolarizing potentials are recorded (indicated) on many cycles, and are probably reversed i.p.s.ps. Some of these mid-cycle depolarizing potentials seem to evoke spikes (asterisks). Note that the cell still spikes in time with the extracellular motor nerve recording. (Motor nerve recording was made four segments rostral to intracellular recording, accounting for the slight lead in motor nerve bursts over the intracellular spikes on each cycle.) In this recording on some cycles there are no reversed i.p.s.ps. However, hyperpolarizing i.p.s.ps do not occur on all cycles in some recordings with acetate electrodes. Recording in 10 mM calcium saline.

large enough to overshoot the resting potential (figure 7*b*). This overshoot cannot be due to a fall in the background tonic depolarization as then, at most, the potential could only fall to the resting potential. (In standard saline the i.p.s.p amplitude (from take-off to peak) was up to 13 mV. In saline with 10 mM calcium, however, it was up to 32 mV. The peak of the i.p.s.p. reached a maximum membrane potential of −66 mV in standard saline, and −74 mV in higher calcium saline. Raised extracellular calcium concentration increases the size of post-synaptic potentials at a number of closely studied chemical synapses (e.g. frog neuromuscular (Dodge & Rahaminoff 1967), squid giant synapse (Katz & Miledi 1970)). A similar explanation

may apply also to the larger hyperpolarizing potentials seen in 'swimming' in the embryo in higher calcium concentrations.) (iii) During injection of hyperpolarizing current these potentials were reduced in amplitude (figures 6*b*, *c*, 7*c*). (iv) A number of intracellular penetrations were made by means of electrodes filled with 3 M KCl. These were made in the same area as those with the usual acetate electrodes but the recordings during 'swimming' episodes were consistently very different from those with acetate electrodes. With chloride electrodes there was usually a depolarizing potential in mid-cycle (figure 7*d*). This suggests that the usual mid-cycle hyperpolarization was a chloride-dependent potential and that this was rapidly reversed by an increase in intracellular chloride concentration, due to leakage from the KCl electrode (Coombs *et al.* 1955).

In summary, the mid-cycle hyperpolarizing potential has a time course typical of post-synaptic potentials, it may overshoot the resting potential, it increases in amplitude with hyperpolarization, and it is reversed in sign by intracellular chloride injection. We conclude that the potential is an inhibitory synaptic potential that is chloride-dependent.

Synaptic and spike activity during synchrony

We have shown, using extracellular motor nerve electrodes, that in 'swimming' the rhythmic bursts on the left and right sides of a segment alternate. Under some circumstances a different pattern can occur in which the two sides produce motor nerve bursts in synchrony for short periods (Kahn & Roberts 1982*b*). The change from alternation to synchrony is accompanied by a halving in the interval between successive bursts on one side. The synchronous pattern also appeared during intracellular recordings in the present study (figure 8). In 'swimming' cycles there was a delay of about half a cycle between a spike and the onset of the mid-cycle i.p.s.p., while in synchrony there was no equivalent delay and an i.p.s.p. usually occurred immediately after each spike. There is therefore a change in position of the i.p.s.p. in the cycle (as well as a doubling of the spike frequency) accompanying the change in phase relations of the two sides. The significance of these observations will be raised in the succeeding paper (Kahn & Roberts 1982*a*).

Discussion

The results presented here describe the synaptic activity in probable motoneurons during 'swimming' in curarized embryos.

The identity and properties of the cells

The majority of penetrated cells were probably motoneurons; the evidence is as follows.

(i) Anatomical studies show that the motoneurons to the myotomal muscles lie ventrolaterally along the spinal cord in amphibian embryos (*Xenopus* (Hughes 1959; Roberts & Clarke 1982), *Triturus* (Blight 1978); *Amblystoma* (Coghill 1913)). Interneurons are present in the spinal cord but they are generally dorsal to the motoneurons (*Xenopus* (Muntz 1964; Roberts & Clarke 1982)). From the ventrolateral position of the penetrations in the present study, it is therefore probable that most cells impaled were motoneurons.

(ii) Cells penetrated were not deep in the cord like ciliated ependymal cells and some interneuron classes (Roberts & Clarke 1982).

(iii) Motoneuron somas are generally larger than interneuron somas, which makes penetration of motoneurons more likely than that of interneurons (Roberts & Clarke 1982).

(iv) During swimming episodes, the penetrated cells spiked at about the same time as the rhythmic bursts on a nearby motor nerve. This is consistent with the cells being motoneurons.

(v) The cells usually spiked just once on each cycle of the swimming rhythm. This is the spike pattern of the motoneurons indicated from previous extracellular motor nerve recordings during swimming episodes (Kahn & Roberts 1982*b*).

To obtain conclusive identification of motoneurons it would be necessary to accomplish one of the following experiments.

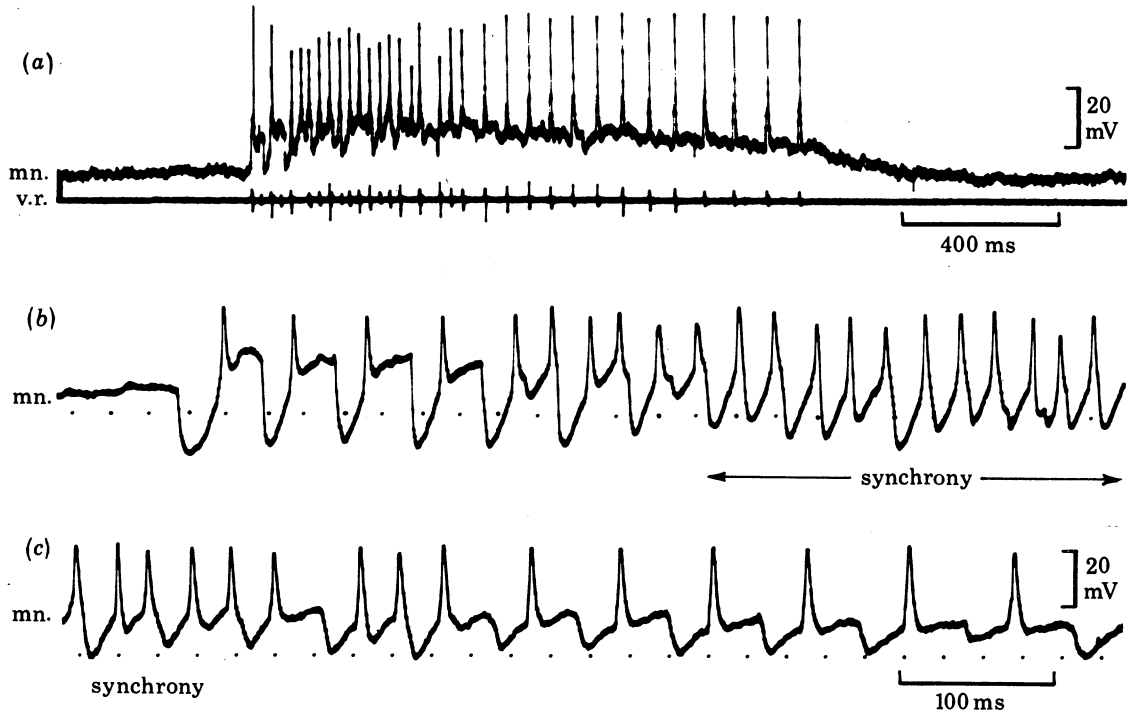

FIGURE 8. Intracellular recording during synchrony.

(*a*) A short spontaneous episode which begins with about 20 cycles of the synchronous pattern (period 18–28 ms) and then switches to 'swimming' alternation (period 56–78 ms).

(*b*, *c*) At faster time base the transition from swimming to synchrony and vice versa can be seen. In 10 mM calcium saline. At transitions the cycle period is halved or doubled.

(i) Initiate spikes in the penetrated cell by passing depolarizing current through the recording electrode, and then look for 1 :1, constant latency extracellular spikes in the peripheral motor nerves. This was unsuccessful in most cases, presumably because the extracellular electrode was not over the region of myotomal muscle innervated by the penetrated cell. However, in one penetration extracellular motor nerve spikes did follow intracellular depolarization. In this recording the synaptic activity during swimming was similar to the general pattern described above. However, the cell did not spike and the extracellular motor nerve spikes evoked were not of constant amplitude.

(ii) Initiate spikes in the peripheral motoneuron axons by electrical stimulation, and show that an intracellular antidromic spike followed each shock at a short, constant latency. This would be technically very difficult because the axons lie for most of their length within the clefts between myotomal muscle segments (Hughes 1959). Electrical stimulation of the peripheral motoneuron axons would evoke muscle contractions and dislodge the intracellular microelectrode.

(iii) Pass dye into the penetrated cell through the recording electrode, and thus stain the peripheral axon. This has now been done for some cells by means of lucifer yellow (Stewart 1978). The results indicate that motoneurons show the kinds of behaviour described in this paper (Soffe & Roberts 1982).

Though we cannot be certain that all the cells recorded in this study were motoneurons, it seems likely that many were and that the responses described are typical of motoneurons. It is quite possible that some classes of interneurons show very similar behaviour during 'swimming'. The electrical properties of the cells recorded were in general similar to those of adult anuran motoneurons (Araki & Otani 1955; Schwindt 1976), with resting potentials of 50–67 mV, and spikes that may have after-hyperpolarizations. In adult anuran motoneurons, spikes often overshoot zero, but overshooting spikes were not recorded in the present study of the embryo.

Mechanisms underlying motoneuron spiking in swimming

The results presented here have shown that myotomal motoneurons in *Xenopus* embryos receive at least three different types of input during 'swimming' activity. Thus, there was a tonic background of synaptic excitation, sustained throughout the whole of a 'swimming' episode. On each 'swimming' cycle, superimposed upon the tonic depolarization, there appeared to be phasic excitatory and inhibitory inputs. In 'swimming' the motoneurons spiked rhythmically, usually once on each 'swimming' cycle. This spike pattern of the motoneurons was presumably the outcome of the integration of the different types of inputs. Evidence for the synaptic nature of the tonic depolarization and phasic inhibitory input has already been considered. We will now discuss interpretations of the phasic depolarizing components, the spike and its underlying prepotential.

Two observations suggest that the spike does not actively invade the soma (the presumed recording site). (i) The spike amplitude varies considerably from cell to cell, does not reach zero, and never overshoots. (ii) The spike can occur on the falling phase of an underlying depolarizing potential. This happens rarely without current injection but is common when hyperpolarizing current is passed (figure 6*c*).

The most reasonable explanation of this second type of observation is that the spike is generated in some other part of the neuron, such as the initial segment of the axon, and then spreads passively back into the soma. (This explanation does not exclude the possibility that the spike spreading into the soma evokes some small amount of active response from the soma membrane.)

Two mechanisms, alone or together, might account for the initiation of spikes in swimming cycles:

(i) Spikes could be evoked directly by phasic synaptic excitation, seen as a depolarizing prepotential, which is superimposed upon the tonic background of excitation. In this case, tonic excitation functions to increase the excitability of the motoneurons, bringing them nearer to spike threshold and so making them more likely to spike in response to phasic excitatory inputs on swimming cycles.

(ii) Motoneuron spiking could be based on rebound from the inhibition that occurs midway through each cycle. In this case the tonic excitation would bring the cell sufficiently close to spike threshold that on rebound from the i.p.s.p. a local active response generates the prepotential which then evokes a spike. Mechanisms based on inhibitory rebound are thought to underlie rhythmic spiking in thalamic neurons in the cat (Anderson & Eccles 1962) and have been modelled by Perkel & Mulloney (1974).

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An explanation based on phasic synaptic excitation seems more likely because: (*a*) spikes still occurred at the correct time in those swimming cycles in which there was no preceding hyperpolarizing i.p.s.p. in the penetrated cell (figure 3*b*). (*b*) In recordings made with KCl-filled electrodes, i.p.s.ps were reversed to depolarizing potentials, but despite this absence of a preceding hyperpolarizing i.p.s.p., the penetrated cells still spiked at the appropriate time in the cycle (figure 7*d*). (*c*) Phasic excitatory potentials do occur on swimming cycles. Thus, when spikes failed to occur, either spontaneously or due to the application of hyperpolarizing current, underlying depolarizing potentials were seen at the time that the cell would have been expected to spike. These had time courses compatible with their being e.p.s.ps. The increase of these potentials during increased hyperpolarization argues against them being local active responses and in favour of them being excitatory postsynaptic potentials. Whether they are chemical or electrical in origin remains to be determined.

In summary, the i.p.s.ps on swimming cycles do not seem to be necessary for the initiation of spikes in the motoneurons on swimming cycles. Rather, the mechanism underlying the spikes appears to be based upon phasic synaptic excitation, superimposed upon a tonic background excitation. We suggest that phasic excitation to the soma and dendrites spreads to the axon, where an impulse is initiated on each cycle. The impulse then spreads passively back into the soma and is seen as a spike potential.

Relevance for hypotheses for pattern generation

Hypotheses for central nervous pattern generation in swimming have to account for how rhythmic activity is initiated and controlled, how rhythmic excitation of neurons arises, and how the alternating discharge of antagonists is organized. Let us consider these three issues in turn.

Tonic drive

Most hypotheses assume that rhythm generation is initiated by a tonic excitation or drive which is then maintained during rhythmic activity and can modulate rhythm period. However, in most paralysed vertebrate preparations locomotory rhythms cannot be turned on and off by natural, external stimuli. The embryo *Xenopus* when paralysed behaves in a surprisingly normal manner. 'Swimming' can be started by a light touch to the skin or by dimming the illumination and it can be stopped by pressing on the cement gland (Roberts 1978; Roberts 1980; Roberts & Blight 1975). Consequently the present results are the first from a vertebrate in which intracellular recordings have been made during short, evoked episodes of fictive locomotion. These short episodes have revealed the clear tonic excitation that rhythmically active cells receive and we have shown how the level of tonic excitation relates inversely to cycle period and directly to membrane conductance increase. Though we presently have no direct evidence, it is likely that this tonic excitation comes from hindbrain neurons with descending axons in the lateral tracts of the spinal cord (Roberts & Clarke 1982). Regardless of its source, the presence of a tonic excitation during rhythm generation is thus clearly established in a vertebrate.

Excitation

Our interpretation of the spike firing in the recorded cells was that spikes were the result of synaptic excitation. We have recorded from cells at different longitudinal levels in the cord and they all behave similarly; so it is unlikely that rostral cells provide the sole source of drive to

more caudal ones. It could be that motoneurons are electrically coupled to some extent longitudinally, but it appears that they are not the basic source of rhythmic activity. During fictive stepping in the cat, limb motoneurons also seem to be phasically excited to fire (Menzies *et al.* 1978), suggesting a similar conclusion for rhythm generation during walking in the mammal. For both groups of animals rhythmic excitation must be generated in spinal interneurons synapsing onto motoneurons.

Inhibition

Reciprocal inhibition between antagonistic motor systems is a general feature of most pattern generator hypotheses (see introduction). Intracellular recordings from antagonistic motoneurons in some other rhythmic systems have shown that when one group of motoneurons are excited their antagonists receive i.p.s.ps (mollusc feeding (Siegler 1977; Benjamin & Rose 1979), cat stepping (Edgerton *et al.* 1976)). This is also so during 'swimming' in *Xenopus* embryos. Though there is no direct evidence that this inhibition comes from the other side of the spinal cord, these observations and experiments presented elsewhere (Kahn & Roberts 1982) are most simply explained on the basis of reciprocal inhibitory connections between motor systems on the left and right sides of the cord. Our anatomical results (Roberts & Clarke 1982) have shown that there are commissural interneurons in the spinal cord that could mediate this inhibitory function.

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