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SUSTAINED RESPONSES TO BRIEF STIMULI: SWIMMING IN *XENOPUS* EMBRYOS

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SUMMARY

Prolonged responses to brief triggering or releasing stimuli are commonplace in animal behaviour. The initiation of locomotion is an example but hypotheses for the central nervous origin of locomotory rhythms generally do not explain how activity is sustained. After a brief review, which suggests that positive feedback excitation could be involved, evidence from *Xenopus* embryos is considered. Here a brief skin stimulus can evoke long episodes of swimming even in curarized embryos. Feedback excitation provides a possible explanation for sustaining fictive swimming. This hypothesis is evaluated by simulation of simple neuronal networks using a physiologically realistic digital computer modelling programme. The results from simulations suggest that: (1) positive feedback excitation could sustain activity either in central pattern generators for locomotion or in postural motor systems and (2) that the model networks tested here are not appropriate to produce the pattern of motor output from the *Xenopus* embryo spinal cord.

INTRODUCTION

In whole animals brief stimuli often lead to responses which far outlast the stimulus. When surprised, an insect flies off, a fish swims away, a crab adopts a threat posture. The stimulus in these cases *triggers* a stereotyped sequence of behaviour or a fixed action pattern (Tinbergen, 1951). If the fixed action pattern is brief (1–2 s) then it is easy to explain on the basis of our knowledge of the properties of neurones and their synaptic interactions. However, for more sustained responses (over 30 s), it becomes difficult to find examples of suitable neuronal mechanisms in the literature. Now this does not mean that whenever a sustained response occurs a single mechanism is in operation. A variety of factors could be involved. If an animal starts to walk, then sensory feedback changes dramatically. If surprised, an animal may have a humoral change which lasts for many minutes. However, there is a real problem here which is particularly clear for rhythmic locomotory movements triggered by a brief stimulus. In many cases it is now known that sensory feedback is not necessary for the generation of a basic locomotory pattern of motor discharge (Delcomyn, 1980; Roberts & Roberts, 1983). In the absence of such feedback, what is it that sustains the swimming of a leech, or a sea-slug, or a clawed toad embryo (Kristan & Weeks, 1983; Getting, 1983*a*,*b*,*c*; Roberts, Soffe, Clarke & Dale, 1983)?

A similar problem exists for changes in posture triggered by brief extrinsic stimuli.

Key words: Locomotion, *Xenopus*, swimming.

Change in the posture of a limb, or of an eye fixating a new target, must involve change in the firing of tonically active motor neurones controlling postural muscles. There is no difficulty in explaining such tonic discharge which can depend on membrane properties of the motor neurones or premotor interneurones. There is, however, a problem in explaining a step change in firing frequency triggered by a brief stimulus (but see Russell & Hartline, 1978). This problem exists even if there is sensory feedback (which may be absent, for example in the mammal eye). The problem is equivalent to that of explaining a sustained locomotory rhythm after a brief stimulus. In both we need a mechanism to give a step function response to a single, brief, triggering stimulus (Fig. 1). This may seem a very obvious problem, and in most man-made systems it is trivial, like moving a lever, switching on a light or adjusting a volume control. Perhaps it is this apparent triviality which has prevented more than a few investigations of possible neuronal mechanisms.

BACKGROUND

Since the problem of how responses are maintained *after* stimulation has emerged from our studies on the central nervous generation of the swimming motor pattern in clawed toad embryos, we can ask what hypotheses exist for sustaining central pattern generation. Recent reviews indicate that most hypotheses for rhythm generation depend on an unspecified source of extrinsic tonic drive (Friesen & Stent, 1978; Selverston, 1980; Selverston, Miller & Wadepuhl, 1983). Such a proposal fits well with the activation of locomotory rhythms by high frequency stimulation of brain motor areas in mammals (Shik & Orlovsky, 1976; Mori, Kawahara & Sakamoto, 1983) and by the application of excitatory chemicals to spinal rabbits, cats or lampreys (Viala & Buser, 1971; Grillner & Zangger, 1974; Grillner *et al.* 1983). The mechanism for production of a steady tonic drive is generally assumed to be separate from the

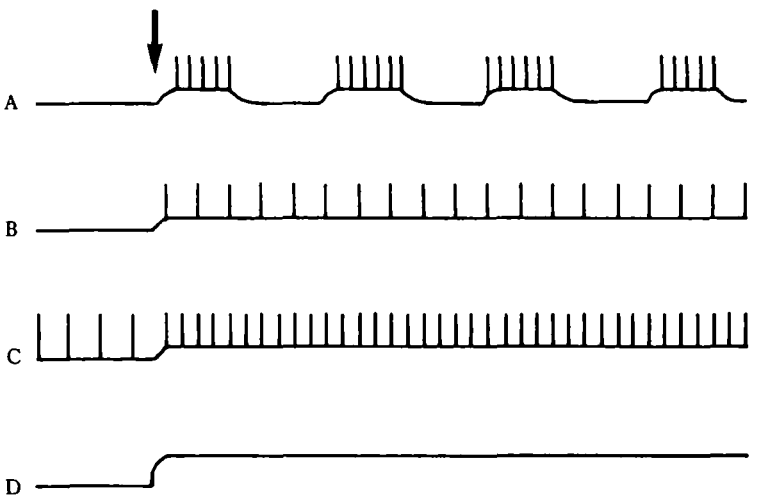

Fig. 1. Step function responses to a brief triggering stimulus (at arrow): (A) repetitive bursting is triggered as in the locomotory systems of many animals, (B) a regular discharge is triggered, as in *Xenopus* embryo swimming, (C) an ongoing discharge is changed in frequency, as in a postural adjustment, (D) the step function of excitation required for (A) to (C).

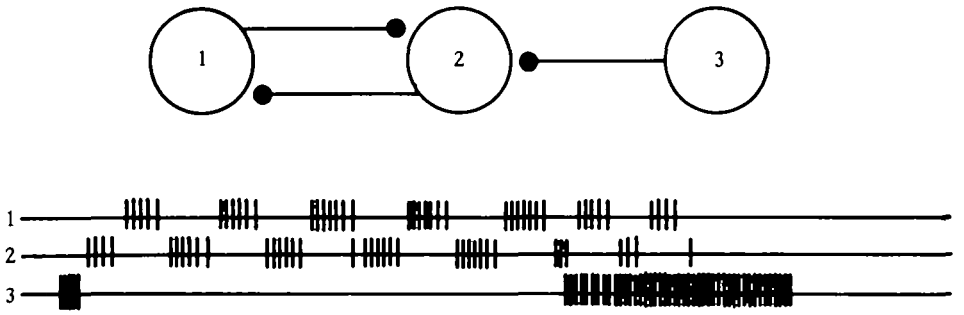

Fig. 2. Prolonged response triggered in a pair of reciprocally inhibiting model neurones with post-inhibitory rebound. A brief group of small IPSPs from neurone 3 to neurone 2 triggers an alternating burst pattern which is only stopped by a longer barrage of IPSPs with diminishing amplitude from neurone 3. (Redrawn from Perkel & Mulloney, 1974.)

mechanism for locomotory pattern generation. However, very little attention has been directed to it.

The problem of sustaining activity after a brief stimulus has received some consideration, mainly from theorists or model builders. Thirty years ago self-re-exciting 'reverbatory circuits' were proposed to explain sustained activity at synapses during the establishment of 'long-term' memory traces (Hebb, 1949; Young, 1951). This very widespread idea was never substantiated by any physiological evidence. It therefore became buried until two more recent studies on small model networks revived the idea of neuronal networks with self-sustaining activity. In 1974 Perkel & Mulloney, using a computer neurone-simulation programme, investigated the properties of a pair of mutually inhibitory neurones which exhibited post-inhibitory rebound. They found that the neurones could show stable alternating bursts of impulses and that this pattern could be self-sustaining after a brief triggering stimulus (Fig. 2). It is, in fact, a circuit based on self-re-excitation, or positive feedback. This is because, in neurones with post-inhibitory rebound, an inhibitory input effectively produces an excitation delayed until after the inhibition. This was made explicit by Dagan, Vernon & Hoyle (1975) who showed that small networks of model electronic neurones, with mutual or feedback excitation as well as inhibition, could be triggered into sustained, alternating burst generation.

There is some evidence from animal experiments that feedback excitation could be involved in sustaining rhythmic activity patterns in molluscs. In *Pleurobranchaea*, Gillette, Kovac & Davis (1978) have shown that brain neurones whose firing can initiate feeding activity, are themselves excited by neurones in the buccal ganglion which are rhythmically active during feeding. In the *Tritonia* swimming system, Getting (1983*a*,*b*,*c*) has shown that within both dorsal and ventral groups of swim interneurones there is positive feedback excitation. In neither of these cases is the exact role of the positive feedback in sustaining rhythmic activity clear. In some groups of neurosecretory cells brief stimuli evoke prolonged and synchronized discharge throughout the whole group. Electrical coupling between neurones has been found or implicated but in none is the mechanism of prolonged activity explained (Benjamin & Rose, 1984; Kupferman & Kandel, 1970; de Vlieger, Kits, Ter Maat & Lodder, 1980). However, they suggest that positive feedback could be present in

animals as well as model systems. We have therefore examined the possibility that may be present in the spinal cord networks which control swimming in clawed toad (*Xenopus*) embryos.

SUSTAINED SWIMMING IN *XENOPUS* EMBRYOS

Just before hatching *Xenopus* embryos freed from their egg membranes will swim when touched on their flank. Swimming can then last for a matter of seconds or even minutes (Kahn, Roberts & Kashin, 1982). If neuromuscular transmission is blocked, activity can be recorded in the ventral roots supplying swimming muscles. When the skin is touched a rhythmic pattern of ventral root activity can be evoked. We have concluded that this pattern must be very similar to that produced by the CNS during normal swimming (Kahn & Roberts, 1982*b*); so we call it fictive swimming (referred to here as '*swimming*'). Like normal swimming, an episode of fictive '*swimming*' can last for many seconds following a brief stimulus to the skin. In the absence of any movement-related feedback how can the nervous system sustain this activity?

A first possibility was that discharge in sensory neurones was maintained for a long period after stimulation. For stimulation to the trunk skin this has been shown not to be the case. Extracellular and intracellular recordings from primary sensory Rohon-Beard cells, whose peripheral neurites innervate the trunk skin (Fig. 3), have shown that they only fire impulses during stimulation (Roberts & Hayes, 1977; Clarke, Hayes, Hunt & Roberts, 1984). These can be sufficient to initiate '*swimming*' during which Rohon-Beard cells do not fire impulses and receive no synaptic input.

Having ruled out this first possibility we have used intracellular recording from spinal cord neurones to examine their normal activity pattern during '*swimming*' (reviewed in Roberts *et al.* 1983). In the dorsal part of the spinal cord, just ventral to the Rohon-Beard cells, recordings show superficial cells which are inhibited during '*swimming*' but are excited at short latency following skin stimulation (J. D. W. Clarke & A. Roberts, in preparation). These cells are probably the dorsolateral interneurones (Fig. 3). Like the Rohon-Beard cells they only fire briefly following skin stimulation (firing ends in less than 30 ms). More ventrally in the cord we have recorded from

Fig. 3. Diagrams of the spinal cord of *Xenopus* embryos at stage 37/38 to show the neurone types defined by extracellular horseradish peroxidase staining. The view is from the right side with the embryo's head facing obliquely away (rostral to the right, dorsal up). The neural canal is formed by ependymal cells. Outside these lie differentiating cells and neurones whose central axons form lateral tracts on the outer sides of the cord. Synapses are generally made from longitudinal axons in these tracts onto dendrites which run out laterally into the tracts. (A) Dorsal and dorsolateral neurones, and ciliated ependymal cells (probably sensory). (*R-B*) Rohon-Beard neurones are primary sensory neurones with peripheral neurites forming free nerve-endings in the skin. Central axons ascend and descend in the dorso-lateral and dorsal lateral tracts. (*dc*), Dorso-lateral commissural interneurones have superficial somas, dendrites in the dorso-lateral and dorsal parts of the lateral tracts, and axons which decussate and ascend to the opposite hindbrain. (*da*), Dorsolateral interneurones are similar to *dc* but their axons ascend on the same side. (*ce*), Ciliated ependymal cells have cilia in the neural canal and ascending axons. (B) Middle to ventral cells (probably motor and all active in swimming). (*m*), Motor neurone with ventral soma, dendrites in middle and ventral lateral tract and peripheral axon(s) innervating myotomes. (*c*), Commissural interneurones with unipolar somas, dendrites in the mid-lateral tract, and an axon which decussates, T-branches and ascends and descends. (*a*), Ascending interneurones like *c* but axon ascends on same side. (*d*), Descending interneurones with dendrites throughout lateral tract and descending axon on the same side. (Based on Roberts & Clarke, 1982.)

about 200 interneurones and many motor neurones. Since there are so few anatomical types of neurone in the spinal cord (Roberts & Clarke, 1982; Fig. 3) it seems very likely that we have recorded from all the types. The main observation is that on each side of the spinal cord, all cells firing during '*swimming*' have a nearly identical pattern of activity to that which we have described for motor neurones (Soffe & Roberts, 1982*a*). Activity in cells on opposite sides of the cord alternates. Using dye- and horseradish peroxidase-filled microelectrodes we have shown that commissural and

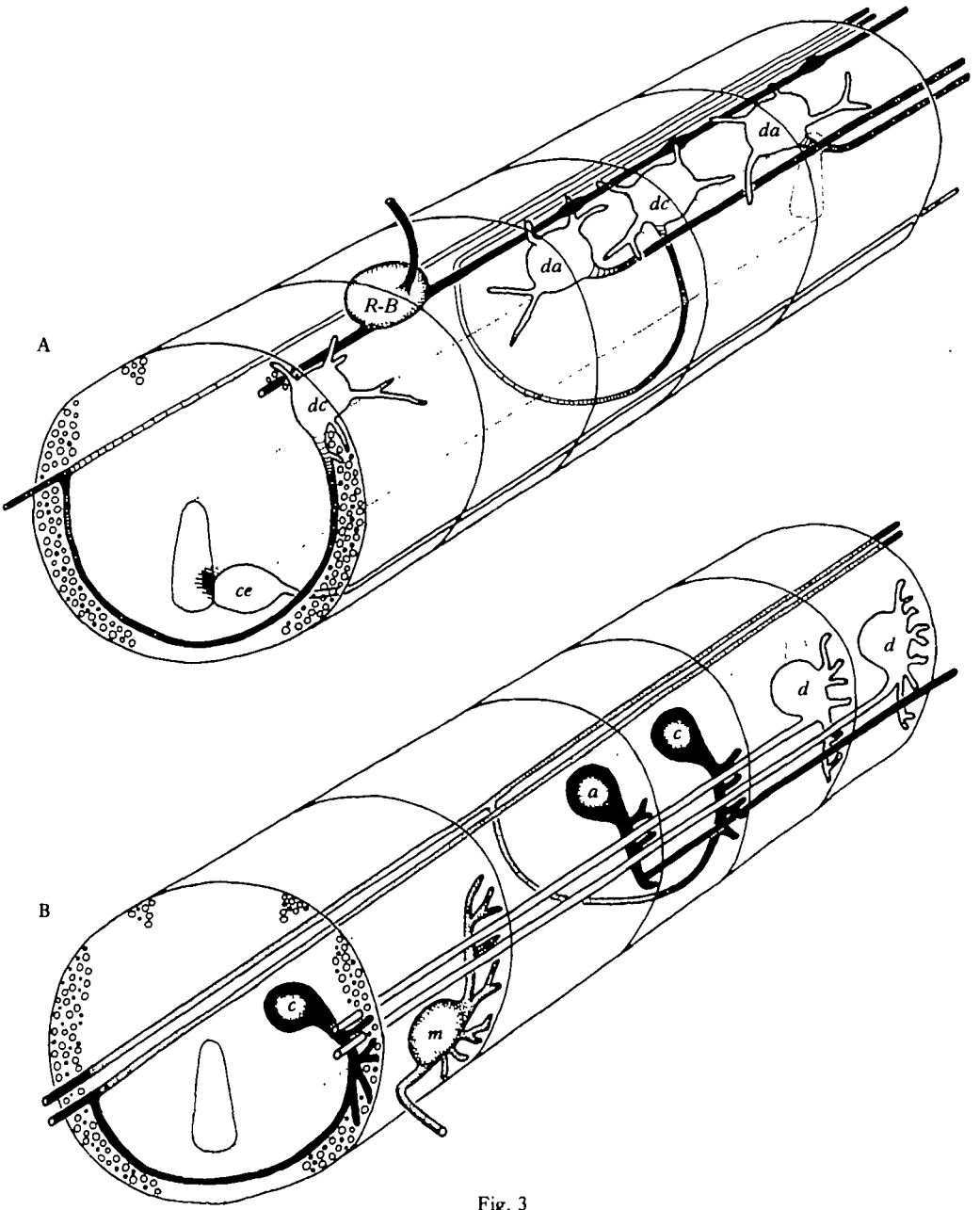

Fig. 3

descending interneurones all show motorneurone-like activity (Clarke, Roberts & Soffe, 1983; Soffe, Clarke & Roberts, 1984; J. D. W. Clarke, N. Dale & S. R. Soffe, unpublished observations). The basic motor neurone activity pattern is shown in Fig. 4 and consists of three main synaptic input components:

(a) a tonic depolarization which depends on excitatory amino acid transmitters (Dale & Roberts, 1983, 1984) and whose amplitude relates to '*swimming*' cycle period (Roberts & Kahn, 1982),

(b) a phasic excitation underlying the single impulse fired on each cycle (Roberts & Kahn, 1982; Soffe & Roberts, 1982*b*),

(c) a mid-cycle, strychnine-sensitive, inhibitory potential in phase with motor root discharge on the opposite side (Roberts & Kahn, 1982; and unpublished observations).

We also rarely see an on-cycle inhibitory potential which is only clear where it is later than the repolarizing phase of the spike. On-cycle IPSPs have also been seen in interneurones which are not excited during '*swimming*' (J. D. W. Clarke & A. Roberts, in preparation; Roberts & Kahn, 1982).

All of these synaptic inputs during '*swimming*' are also present in high spinal embryos where the episodes of '*swimming*' are shorter than in intact embryos, and shorten further with more caudal spinalization (Fig. 5: A. Roberts, N. Dale, W. H. Evoy & S. R. Soffe, in preparation). Using other simple lesions of the CNS (Kahn & Roberts, 1982*a*; Soffe & Roberts, 1982*b*) we have shown that:

(a) a single side of the CNS can produce tonic excitation (S. R. Soffe, unpublished observations) and a pattern of ventral root activity similar to that in '*swimming*' but with a rather shorter cycle period,

(b) tonic excitation is a descending influence and does not cross from one side of the cord to the other,

(c) mid-cycle inhibition crosses from one side of the cord to the other (i.e. is reciprocal inhibition).

Our evidence shows that '*swimming*' is always associated with tonic excitation of firing cells and we also know that artificial tonic excitation by bath application of excitatory amino acids can produce '*swimming*' activity in spinal embryos (Dale & Roberts, 1984). Tonic excitation is therefore sufficient to produce pattern generation for '*swimming*' in the spinal cord. This is just what was expected from the earlier hypotheses on extrinsic tonic excitation. However, two lines of evidence converge to suggest that this explanation is misleading. Firstly, tonic excitation is present in spinal embryos. Secondly, recordings from cells firing during swimming show that they all appear to receive tonic excitation and all fire one spike per cycle in phase with motor neurones on the same side. Now, although interneurones in the cord are fairly similar in size, we cannot assume that they are all equally easy to penetrate and record. Also, we cannot eliminate the possibility of short axon or amacrine non-spiking neurones. However, if we do *assume* that we have *not* failed to record from a whole class of cells with different activity, then we can conclude that there must be spinal neurones firing once per swim cycle which produce tonic excitation of themselves and the other active cells. In other words, there is positive feedback within each side of the CNS which sustains the rhythmic firing underlying '*swimming*'. Clearly, to sustain excitation from cycle to cycle and to produce a relatively smooth, tonic excitation, the post-synaptic

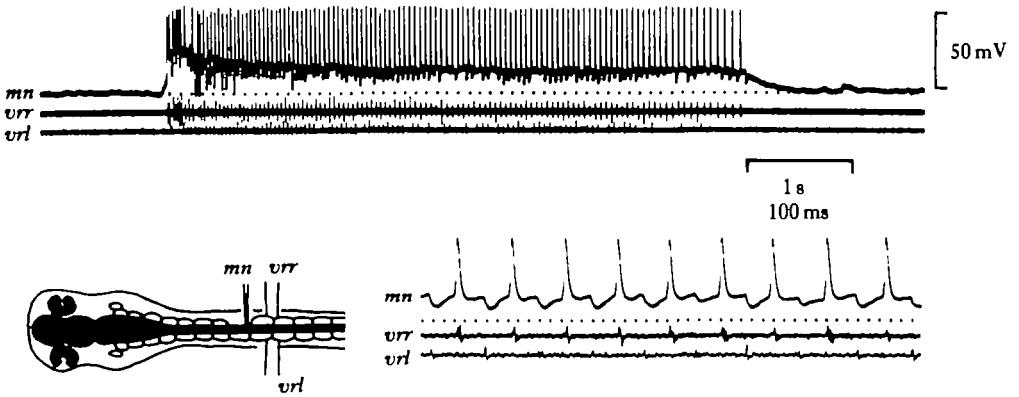

Fig. 4. Motor neurone activity during fictive '*swimming*' in a paralysed *Xenopus* embryo. Intracellular records from a motor neurone (*mn*) and ventral root (*vr*) records from right (*r*) and left (*l*) sides (as in diagram). The upper record shows a whole episode of '*swimming*'. Tonic depolarization is present throughout. At a faster time base (lower record) the single spike per cycle and mid-cycle IPSP are clear. Dots indicate resting potential between swimming episodes.

potentials involved must be long relative to the cycle period of 40–125 ms. Such long postsynaptic potentials could then produce the slow-falling potential seen at the end of '*swimming*' in both intact and spinal embryos (Figs 4, 5). We therefore propose that there could be a population of neurones, with descending axons, distributed within the spinal cord and hindbrain. (Descending interneurones, Fig. 3, fit this specification.) These should fire once per swim cycle and synapse with themselves and other active neurones including motor neurones. They should use a transmitter acting at excitatory amino acid receptor sites and produce a long, slow-falling EPSP. If enough of these neurones fire then they produce, by positive feedback, a tonic excitation which sums from cycle to cycle and sustains rhythmic activity. Spinalization reduces the number of cells and therefore the amount of positive feedback. Consequently in low spinal embryos there is insufficient feedback to sustain longer episodes of '*swimming*'.

MODEL NETWORKS WITH POSITIVE FEEDBACK

Our results from *Xenopus* embryo spinal cord have led to the proposal of positive feedback excitation within the cord. Direct evidence on these proposals is being sought by making paired intracellular recordings, initially from interneurones and motor neurones, to see if there are interneurones with appropriate postsynaptic effects. However, in view of the difficulty and limitations of this type of experiment we have used another approach to try to evaluate these proposals. This is to make physiologically plausible neurone networks using a digital simulation system. Such networks can then be used to model the proposed synaptic interactions, to formalize and quantify hypotheses, and to see whether they are compatible with the properties of the real network in the spinal cord. The great advantage of the model network is that parameters of cell membranes or synapses can be varied systematically and their significance assessed in a way that at present is just not possible for real networks of neurones. Finally, in the embryo spinal cord, motor output is controlled by populations

of neurones. Critical cell by cell elimination experiments like those performed on some invertebrate networks (Selverston *et al.* 1983) cannot be performed. The best we can do at present is to use pharmacological agents whose specificity to a single cell

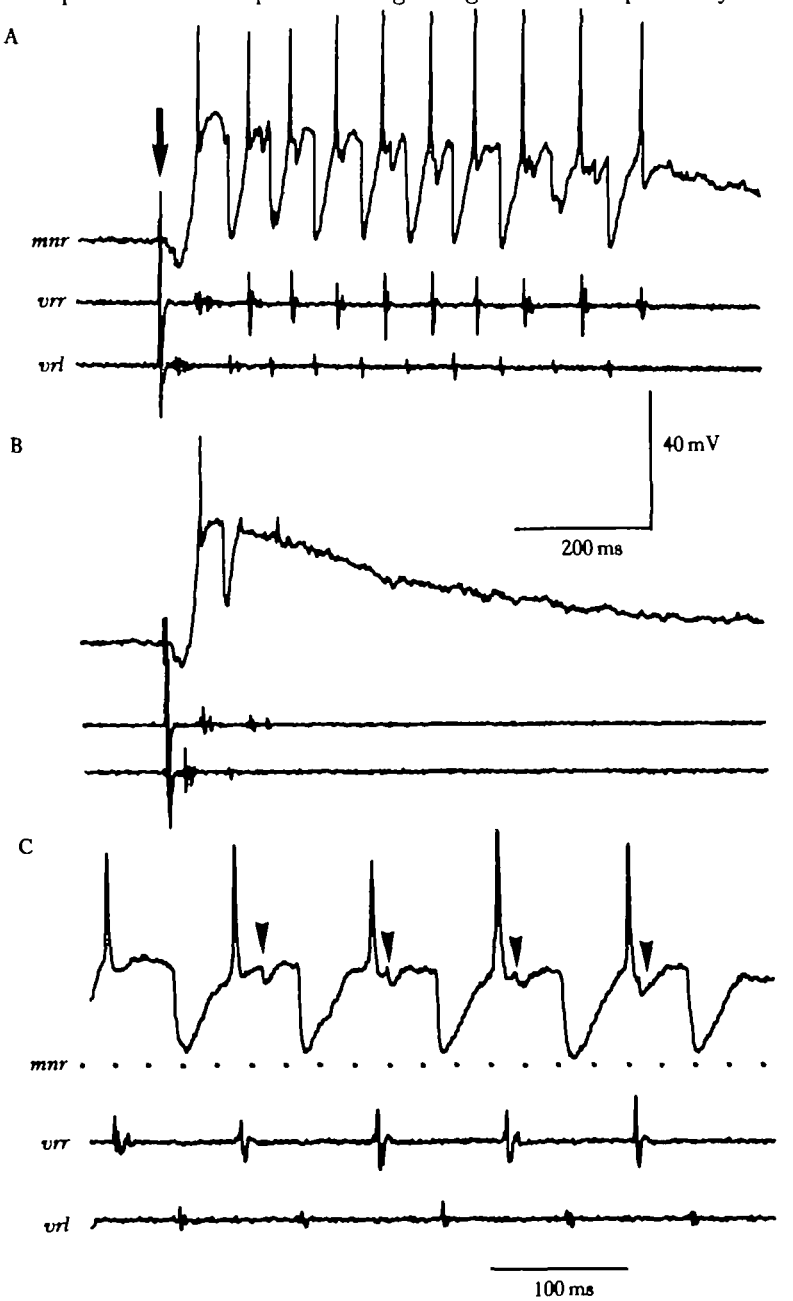

Fig. 5. Motor neurone activity in a paralysed *Xenopus* embryo made spinal at the 3rd post-otic myotome. Stimulation of the skin (at arrow) evokes swimming activity (A). Episodes of swimming can last for a few seconds but are usually shorter and can consist only of one or two cycles (B). During swimming (C) the activity of motor neurones is very similar to intact animals, with one spike per cycle, mid-cycle IPSPs and a tonic depolarization which falls away slowly when spiking stops. Note on-cycle IPSPs in this recording (arrowheads). Abbreviations as in Fig. 4.

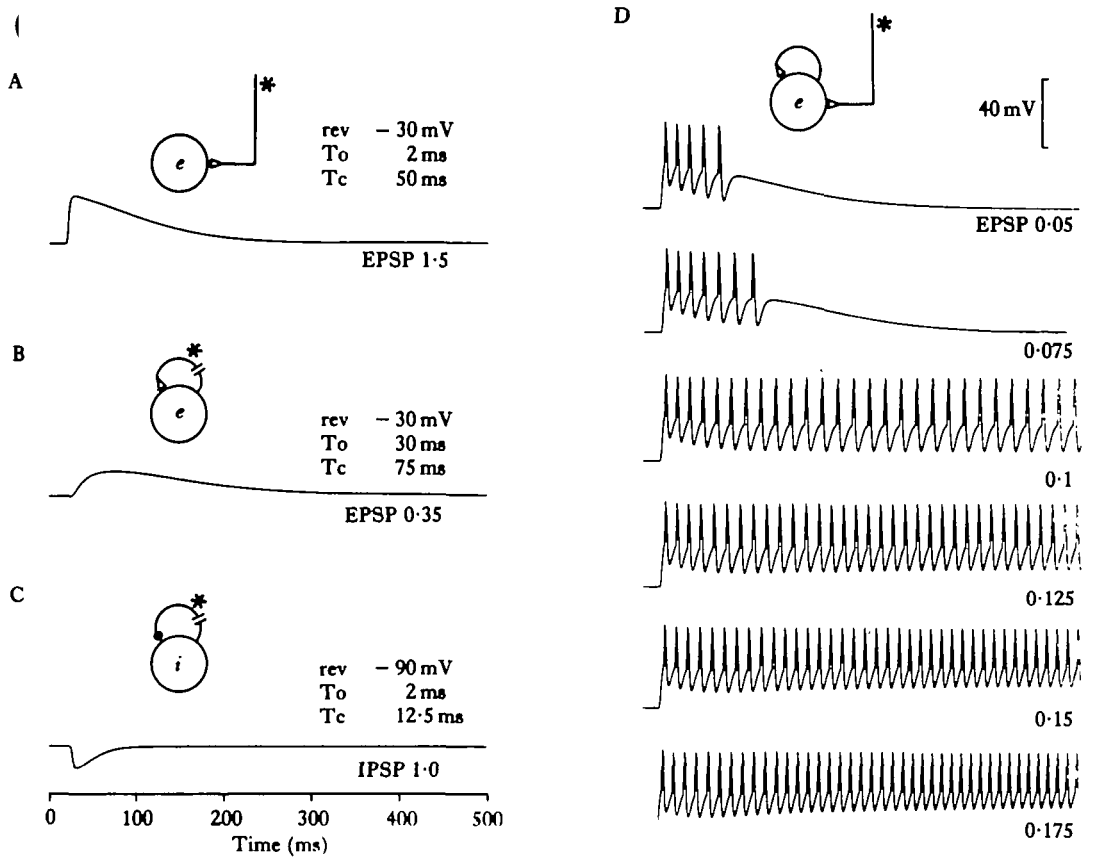

Fig. 6. Simulations to show basic synaptic potentials (A, B, C) and a cell with excitatory feedback (D). In this and subsequent figures: (1) activity is initiated by a single stimulus given simultaneously to fibres with an asterisk (*), (2) excitatory regions (neurones) are labelled *e* and inhibitory one *i*, (3) excitatory synapses are open triangles and inhibitory synapses are black circles, (4) synaptic strength is varied by changing the *conversion factor* (see text). This is given under each record at the right side. (A) The *extrinsic* EPSP used to initiate activity. (rev is *reversal potential* and To and Tc are the opening and closing *time constants* for postsynaptic transmitter receptor channels.) (B) The feedback EPSP with a Tc giving a similar repolarizing time to that seen at the end of swimming activity (see Figs 4, 5). (C) The recurrent and reciprocal IPSP with a time course (To and Tc) based on mid-cycle IPSPs. (D) Effect of increasing amounts of feedback excitation on the response of a single neurone to a single extrinsic EPSP.

class can never be completely certain. The modelling would therefore serve a useful function if it can help develop a feeling for important membrane properties and synaptic connections or perhaps even eliminate some hypotheses as unworkable.

The simulation system uses a programme called '*Mickey*' devised and described in a series of papers by Perkel & Mulloney (Perkel, 1976*a*,*b*; Perkel & Mulloney, 1974, 1978*a*,*b*; Perkel, Mulloney & Budelli, 1981; Perkel & Smith, 1976). As far as possible model parameters are based on measurements from embryo neurones. '*Mickey*' allows one to specify isopotential regions (equivalent to neurone somas in this case) with input resistance ($1\times10^8\,\Omega$), time constant (5 ms), resting potential (−70 mV), threshold (−50 mV) and absolute refractory period (6 ms). Synapses can be made onto regions from *extrinsic* fibres or from *intrinsic* fibres from other regions. Synaptic

properties are determined by parameters most of which need not be varied (see Appendix for a full listing of parameters used in the present set of '*Mickey*' simulations). The important variable parameters are the reversal potentials (−30 mV for EPSPs, −90 mV for IPSPs), the time constants for postsynaptic transmitter receptor channel opening (To) and closing (Tc), and the conversion factor for conversion of transmitter substance into postsynaptic conductance. Examples of synaptic potentials are shown in Fig. 6. Impulses are simulated using pairs of *virtual* fibres. These are activated when threshold is crossed and model the sodium and potassium conductance changes underlying impulse generation (see Appendix for detailed parameters). When crossed, threshold is raised by a constant (100 mV) and falls back exponentially (time constant 3 ms) to its initial value. The initial models had no adaptation, accommodation, antifacilitation or facilitation.

The simplest positive feedback network is a single 'cell', excited by an extrinsic fibre, which excites itself (Fig. 6D). If the amplitude of the feedback EPSP (set by

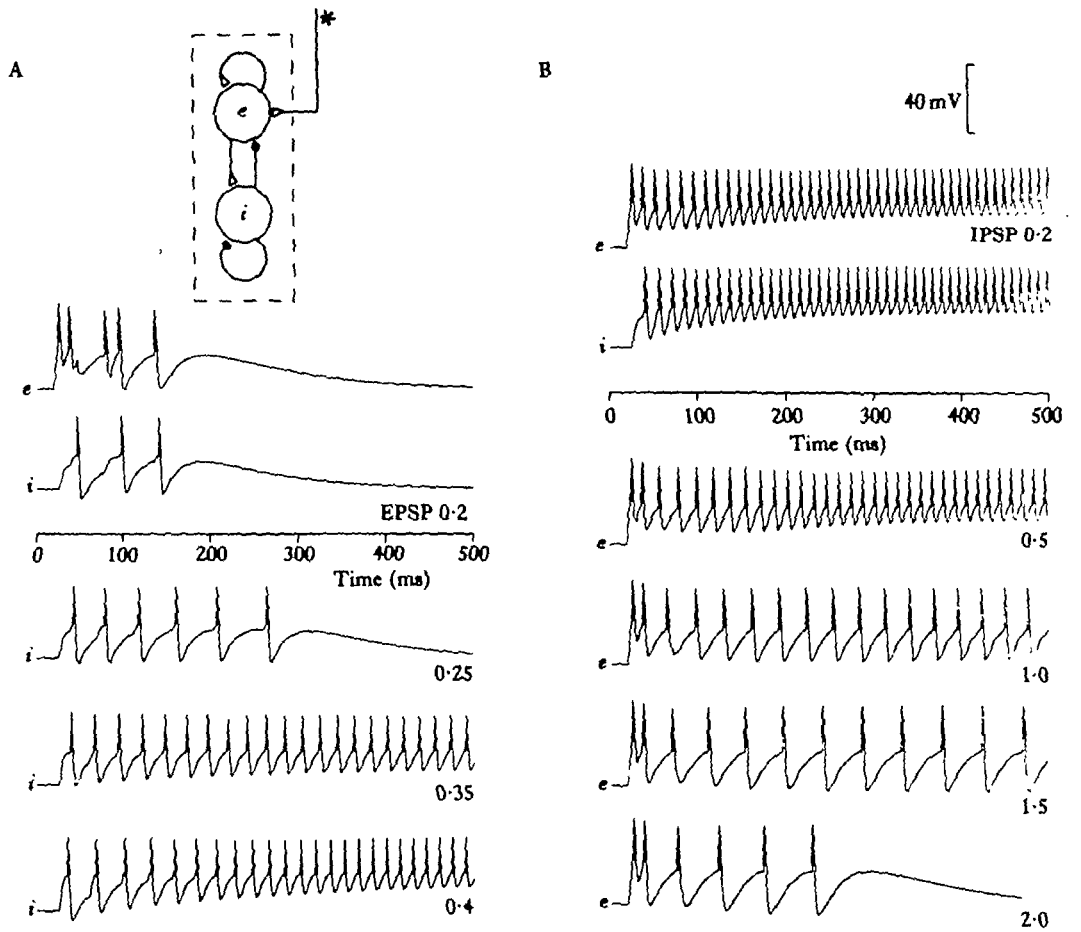

Fig. 7. Simulation of a two cell network with feedback excitation and inhibition as in the diagram. (A) Effects of increasing strength of intrinsic EPSPs, when IPSP conversion factor is 1. (B) Effects of increasing strength of intrinsic IPSPs when EPSP conversion factor is 0·3. A wide range of stable final firing frequencies can be obtained. Lettering as in Fig. 6.

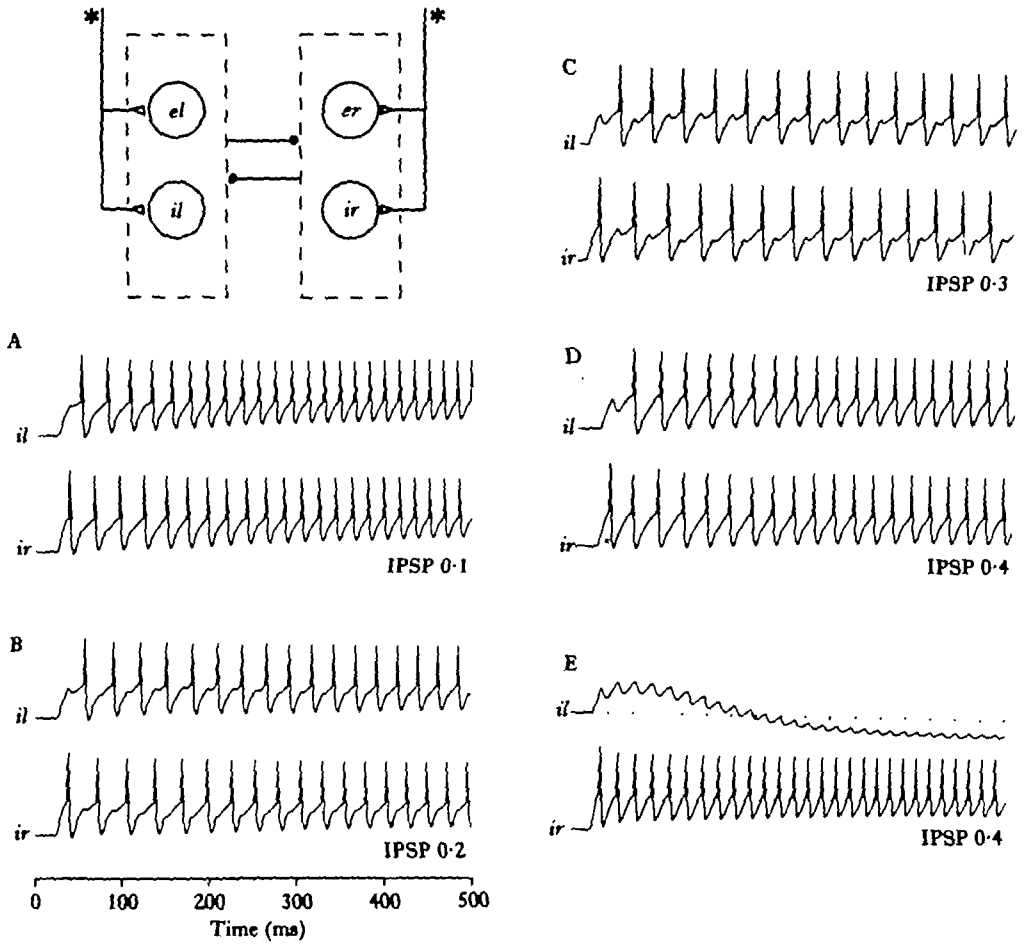

Fig. 8. Simulation of a four cell network where two networks like those in Fig. 7 (enclosed in dashed rectangles) are coupled by different *strengths* of reciprocal inhibition. In (A) to (D) the extrinsic EPSP initiating activity rose more slowly and was bigger on the left side (To = 50 ms, conversion factor = 1·5) than on the right side (To = 20 *ms*, conversion factor = 1). Without these differences alternation, as in (C) was not obtained and, with stronger IPSPs, the side which fired first turned the other side off (E). Calibrations as in Fig. 7. *Excitatory* regions, *e*; inhibitory regions, *i*; left and right sides, *l* and *r*.

the conversion factor) is sufficient, then firing continues after a triggering EPSP and is limited in frequency by refractoriness and the hyperpolarizing phase of the impulse.

Introducing inhibitory feedback from a second 'cell' considerably broadens the range of firing periods that the network can produce when either feedback excitation or inhibition is graded (Fig. 7). There is a rather narrow range of feedback EPSP amplitude which leads to sustained firing. However, by grading inhibition, stable firing at longer cycle periods can easily be obtained. Other tests showed that IPSP fall time (set by Tc) had a powerful influence on cycle period. The two cell net of Fig. 7 can therefore sustain its own activity after triggering and fire at cycle periods similar to those shown by one side of the CNS in *Xenopus* embryos (Kahn & Roberts, 1982*a*).

The next step in the simulation of swimming was to couple two of these two cell nets

by reciprocal inhibition (Fig. 8). This could lead to alternating firing at swimming frequencies provided: (1) that triggering excitation reached its peak later on one side than the other, and (2) reciprocal inhibition was carefully adjusted in amplitude. However alternation was not as stable a behaviour pattern as synchronous firing on the two sides (c.f. Kawato & Suzuki, 1980) and if inhibition became stronger, one side dominated and turned the other side off.

CONCLUSIONS

For the *Xenopus* embryo swimming system we can reach some positive and negative conclusions from the modelling. Positive feedback of long slow EPSPs can sustain activity on one side particularly if controlled by recurrent inhibition. The basic two cell net of Fig. 7 is also compatible with spinal cord anatomy where we find cells with descending and ascending ipsilateral axons (Fig. 3). On the negative side, the inhibitory effects and behaviour of the reciprocally inhibitory net (Fig. 8) are not at all like the embryo CNS in their behaviour. In the model, recurrent inhibition needs to be large and reciprocal inhibition small, the exact opposite of what we observe. Also, alternation is unstable in the model and stable, within broad limits, in the embryo. These observations suggest that in the *Xenopus* spinal cord properties are present which have not yet been put into the model. The most likely ones being accommodation coupled with post-inhibitory rebound, accumulating spike refractoriness, and antifacilitation of the positive feedback EPSPs. The latter property has in fact been tested and can easily produce the slowing in frequency usually seen as episodes of swimming progress (Kahn *et al.* 1982). As more direct measurements are obtained we hope our models can be made more realistic and we can also gain confidence that there are not other types of neurone present that we have so far overlooked. We are following a strategy in this simple vertebrate which has been developed in work on invertebrate systems. Already, the parallels that are emerging, for example with the *Tritonia* swimming system (Getting, 1983*a*; Lennard, Getting & Hume, 1980), are quite striking.

In the Introduction the problem of sustaining rhythmic activity was compared to that of sustaining a change in posture. The results from the simple two cell net of Fig. 7 offer a simple possible mechanism. A series of such nets (a, b, c, . . ., n) could be recruited in series (a, a+b, a+b+c, a+b+c+d+ . . . +n) by stronger triggering stimuli. Even if each net had the same basic firing frequency, (in which case they could share a common recurrent inhibitory system), they could converge onto a pool of motor neurones and by summation produce a series of sustained, different, firing rates. This is what is required in any postural muscle system which responds to outside stimuli.

APPENDIX

Network simulations were performed using two programmes provided on disc by Professor Donald H. Perkel, Department of Psychobiology, University of California Irvine, Irvine, California, U.S.A. The programme '*BldmicI*' was used to create files

which were then run and modified using the programme '*Mickey*'. The details given here do not attempt to explain the simulation but should allow our simulations to be repeated by others with the same programmes. The following parameters were used for each region (neurone):

Membrane resistance	100 MΩ
Membrane time constant	5 ms
Resting potential	−70 mV
Asymptotic impulse threshold	−50 mV
Threshold additive constant	100 mV
Threshold recovery time constant	3 ms
Absolute refractory period	6 ms

Synapses made by extrinsic and intrinsic fibres onto regions all had the following parameters in common:

Calcium parameters

Asymptotic concentration	100
Removal time constant	5 ms
Concentration increment	0
Dissociation constant	100
Number of ions for release	5

Neurotransmitter parameters

Maximum release fraction	0·5
Asymptotic amount	2
Replacement time constant	0·5 ms

The remaining parameters controlling postsynaptic conductance are the conversion factor, opening time constant (To), closing time constant (Tc) and reversal potential. These parameters were varied (see text and figures) to give different PSP properties.

Impulses were simulated by sodium and potassium virtual fibres with parameters which were the same for each region as follows:

	Na^+ fibre	K^+ fibre
Delay for conductance	1 ms	3 ms
Amount of transmitter	0·3	0·2
Conversion factor	0·2	0·3
Opening time constant	0·1 ms	2 ms
Closing time constant	0·75 ms	2 ms
Reversal potential	30 mV	−90 mV

Simulations were run on the Bristol University Multics system, with help from Steve Fisher and Dave Rogers.

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