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PATTERNS OF SYNAPTIC DRIVE TO VENTRALLY LOCATED SPINAL NEURONES IN *RANA TEMPORARIA* EMBRYOS DURING RHYTHMIC AND NON-RHYTHMIC MOTOR RESPONSES

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Summary

1. Intracellular recordings have been made from ventrally located neurones in the spinal cord of *Rana temporaria* embryos at around the time of hatching. Both short-latency 'reflex' and more prolonged rhythmic motor responses can be elicited by stimulation of the skin in immobilized embryos. Initial responses to single-sided skin stimuli usually involve excitation of neurones on the opposite side and strychnine-sensitive inhibition of neurones on the same side. Less reliable responses to dimming the lights also involve initial excitation on one side associated with inhibition on the opposite side.

2. Intracellular recordings from single neurones during rhythmic activity show that on each cycle the same neurone can fire one or many spikes during the course of a single evoked or spontaneous episode. Bursts occur at longer cycle periods, generally at the start of episodes; single spikes occur at shorter cycle periods, generally later in episodes.

3. During sustained rhythmic responses, neuronal membrane potential is generally depolarised and returns gradually to its resting level at the end of the episode. During the episode, relatively depolarising phases of synaptic excitation alternate with relatively hyperpolarising phases of chloride-dependent synaptic inhibition. Cell input resistance is reduced by around 50% throughout each episode. Within each cycle, input resistance is reduced further during the hyperpolarising phase than during the depolarising phase.

4. Rhythmic excitation and inhibition of ventrally located neurones appears to be similar throughout the whole range of cycle periods, supporting the suggestion that a single rhythm-generating system with a wide 'permissive' range drives rhythmic movements in *R. temporaria* embryos.

Introduction

By the time of hatching, embryos of the frog *Rana temporaria* L. have

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developed the necessary neural circuitry to produce patterns of motor discharge suitable to drive coordinated rhythmic and non-rhythmic behaviour (Soffe, 1991). Sustained rhythmic movements range continuously between slow, high-amplitude 'lashing' (in which there is no net movement of the body in any particular direction) and faster, lower-amplitude swimming (in which the animal moves forward). The main features of these rhythmic movements appear to be generated centrally within the nervous system since, as discussed in the preceding paper (Soffe, 1991), appropriate rhythmic ventral root activity can be recorded in paralysed embryos. This rhythmic activity often involves bursts of ventral root discharge, typical of vertebrate locomotor patterns in general but differing from the patterns that drive swimming in embryos of *Xenopus laevis* (Roberts and Kahn, 1982) and the newt *Triturus vulgaris* (Soffe *et al.* 1983). In these embryos, motoneurone activity normally consists of a single impulse per cycle.

We have now made intracellular recordings from ventrally located spinal cord neurones during the initiation and execution of both rhythmic and non-rhythmic 'fictive' motor responses. The aim was to start to examine the synaptic drive underlying these responses in *R. temporaria* embryos and to determine whether they are turned on and maintained by essentially similar or fundamentally different mechanisms from those driving related behaviour investigated in *X. laevis* embryos. In particular, we wished to know how the synaptic drive during rhythmic burst activity compared with that during rhythmic single spikes in order eventually to explore parallels with patterns in adult vertebrates, where rhythmic bursts of discharge are the norm, and in the embryos of other amphibians, where single spikes occur. Understanding non-rhythmic responses, besides their intrinsic interest, can give insights into organisational principles involved both in the rhythm-generating circuitry and in its sensory activation. Some of the results have been presented previously in abstract form (Sillar and Soffe, 1989).

Materials and methods

Rana temporaria embryos were used around the time of hatching (equivalent to stage 20: Shumway, 1942; see Soffe, 1991) according to methods outlined in the preceding paper (Soffe, 1991). Embryos were immobilized in MS222 while preparing them for recording and then in 70 or 100 μmol l^{-1} *d*-tubocurarine chloride for the duration of each experiment. For intracellular recording, an area of rostral spinal cord approximately five segments in length was exposed by removing the dorsal portion of the overlying myotomes on the right side. The preparation for making intracellular recordings, while also recording ventral root discharge from intermyotome clefts with suction electrodes, is illustrated in Fig. 1. Intracellular penetrations were made using glass microelectrodes filled with either 3 mol l^{-1} potassium acetate or 2 mol l^{-1} potassium chloride. Successful penetrations, stable for up to several hours, required the use of electrodes with d.c. resistances of around 200 MΩ (potassium acetate) and 100 MΩ (potassium chloride), respectively. Impalements were made using a piezoelectric jolting

device (Weevers, 1972), capacitance overcompensation, or a combination of the two. Data storage, analysis and plotting methods were all conventional.

The experimental bath, perfusion system and saline were as described previously (Soffe, 1991). To aid the stability of intracellular recordings, the Ca^{2+} concentration in the saline was raised from 2 to 4 or 5 mmol l^{-1} (as previously employed, Soffe and Roberts, 1989). The saline contained (in mmol l^{-1}): NaCl, 105; KCl, 2.5; $CaCl_2$, 4 or 5; and $NaHCO_3$, 15; equilibrated to pH 7.2 by bubbling continuously with 5 % CO_2, 95 % O_2. Successful recordings were made from 60 ventrally located neurones in 43 embryos at temperatures between 18 and 22 °C. In nine of these embryos, 1 mmol l^{-1} Mg^{2+} was added to the saline after initial recordings had been made. Results are described prior to addition of Mg^{2+} except where stated.

Results

Initial responses to stimulation

The methods by which both the behavioural responses of *R. temporaria* embryos and their underlying patterns of motor discharge can be evoked have already been described (Soffe, 1991). In embryos prepared for intracellular recording, the same two broad methods were used to evoke responses. The most reliable was electrical or mechanical stimulation of the skin. Less reliable was transient dimming of the light, presumably operating through pineal eye photoreceptors (Foster and Roberts, 1982). On some occasions, motor activity occurred spontaneously.

Short-latency responses to trunk skin stimulation

Electrical stimulation of one side of the trunk skin consistently evoked a depolarising excitatory postsynaptic potential (EPSP) in ventrally located spinal cord neurones. In the majority of cases, this EPSP occurred in cells on the opposite

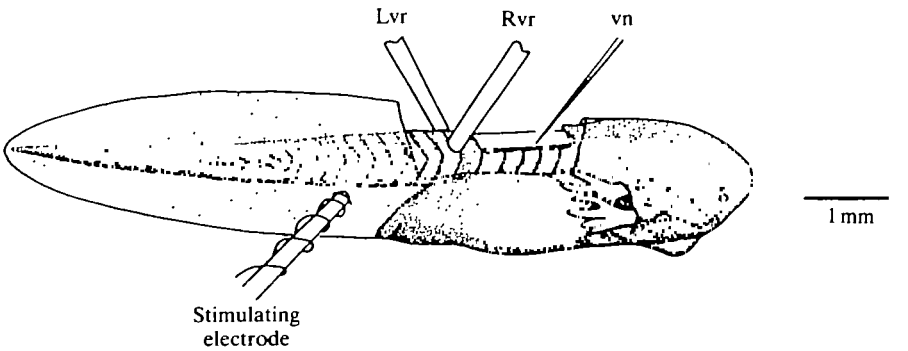

Fig. 1. *Rana temporaria* embryo (approx. stage 20, Shumway, 1942; see Soffe, 1991) prepared for intracellular recording and viewed from the right side, showing placement of ventral root recording electrodes between myotome clefts on left (Lvr) and right (Rvr) sides and a skin-stimulating electrode against the tail fin. Rostral myotomes are removed to allow placement of a microelectrode in a ventral neurone (vn) in the spinal cord.

side to the stimulus (Fig. 2A). The EPSP could be graded in amplitude with stimulus intensity and, if the stimulus was large enough, could drive a (reflex) impulse (Fig. 2B). When stimulus intensity was raised above this level, the initial response was then followed by more sustained, often rhythmic, activity. Initial EPSPs occurred with a latency in the range of 12–25 ms. Their duration was invariably rather long (greater than 200 ms). The time to peak was variable from only a few up to tens of milliseconds. In most cases, it was difficult to alter the amplitude of the EPSPs by current injection (not shown).

Where electrical stimulation of the trunk skin on one side evoked a short-latency EPSP in a ventral neurone, stimulation on the other side produced an inhibitory postsynaptic potential (IPSP). Usually the IPSP was evoked by stimulation of the skin on the same side as the neurone (Fig. 2C,D). The IPSP

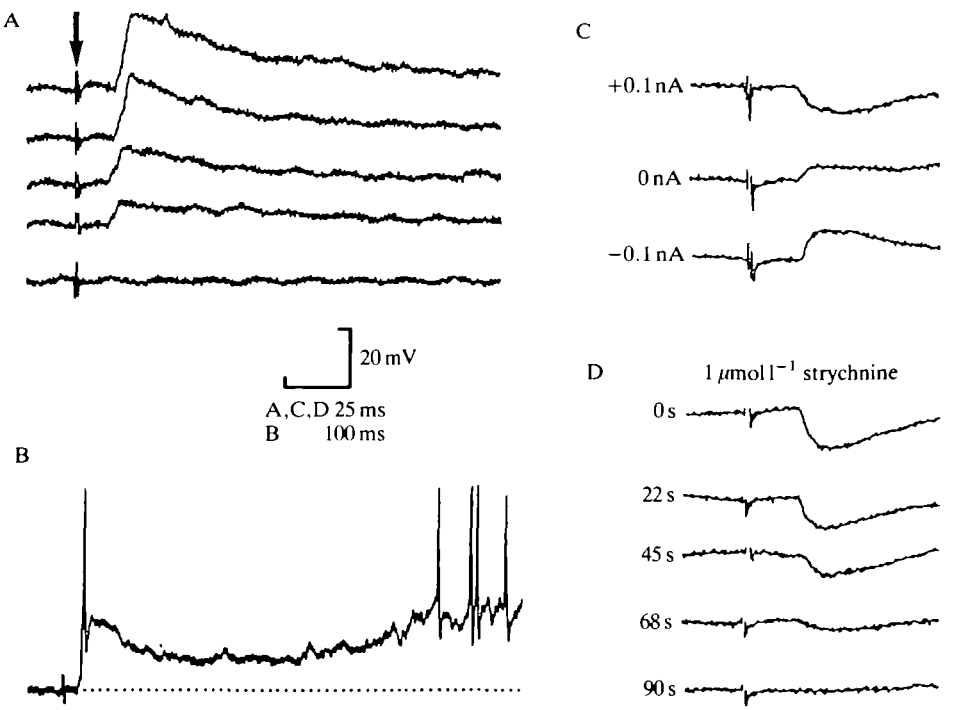

Fig. 2. Short-latency responses in ventral neurones to electrical stimulation of the skin. Responses to stimulation of the rostral tail skin on one side with brief (1 ms) current pulses. (A) Stimulation on the opposite side evokes depolarising potentials that increase in amplitude with increasing stimulus intensity. Responses to progressively stronger stimuli are displaced upwards for clarity. (B) At stronger stimulus levels, neurones can fire an impulse at short latency. Stimulation at this strength or above also evokes more sustained discharge at longer latency (see text). (C) Stimulation of the skin on the same side evokes a short-latency IPSP. Some IPSPs are slightly depolarising at rest but can be made strongly hyperpolarising by depolarisation of the neurone and strongly depolarising by hyperpolarisation. (D) Short-latency IPSPs evoked by stimulation on the same side are progressively blocked by 1 μmol l^{-1} strychnine.

could be evoked at stimulus intensities below those required to initiate rhythmic activity and sometimes increased in amplitude with increasing stimulus intensity. The latency to onset of the IPSP was generally slightly longer than that for the EPSP on the opposite side (>17 ms). At the resting potential, the IPSP could be hyperpolarising or slightly depolarising (Fig. 2C) but was always made strongly depolarising by hyperpolarising the neurone (by current injection) and strongly hyperpolarising by depolarising the neurone. Bath application of the antagonist strychnine at 1–5 μmol l^{-1} rapidly abolished the IPSP (Fig. 2D), suggesting that glycine may be the neurotransmitter involved.

These responses to stimulation of one side of the trunk, consisting usually of a contralateral excitation and an accompanying ipsilateral inhibition, were very similar in animals that had been spinalized just caudal to the hindbrain. This indicated that they can be produced by networks of neurones within the spinal cord.

Responses to stronger stimulation of the skin

Higher-amplitude mechanical or electrical stimulation of the skin produced stronger responses and more pronounced motor root discharge than those described above. Activity again only occurred at any one time in ventral neurones on one side of the cord and coincided with ventral root discharge on the same side. It was driven in each neurone by a strong compound excitatory potential that could be revealed by hyperpolarisation (Fig. 3A). At the same time as a burst of motor root discharge on one side, neurones on the opposite side received strong inhibition that could be increased in amplitude by depolarisation of the neurone and reversed by hyperpolarisation (Fig. 3B).

Unlike most low-level electrical stimulation, stronger stimulation of the skin of the trunk or tail fin frequently evoked a strong response on the side stimulated. This may be because such stimulation can activate sensory endings on both sides, particularly in the case of the tail fin where the inner edges of the skin on the two sides are closely apposed.

Responses to head skin stimulation

Touching one side of the head of an embryo produces a flexion towards the opposite side and in the paralysed preparation a burst of motor discharge is evoked in the trunk on the opposite side (Soffe, 1991). During such bursts, evoked by touching the opposite side of the head, spikes in ventral neurones were driven by strong depolarising potentials (Fig. 4B). Touching the opposite side of the head more gently, at levels insufficient to evoke a motor root response, produced a series of smaller depolarising potentials (Fig. 4B). Similar gentle stimulation on the same side of the head as the neurone also evoked a series of depolarising potentials (Fig. 4A). However, if the stimulus strength was increased on the same side, sufficiently to evoke a strong motor root burst on the opposite side, the neurone received strong inhibition. This inhibition lasted for as long as the contralateral motor root was active and effectively shunted out an underlying

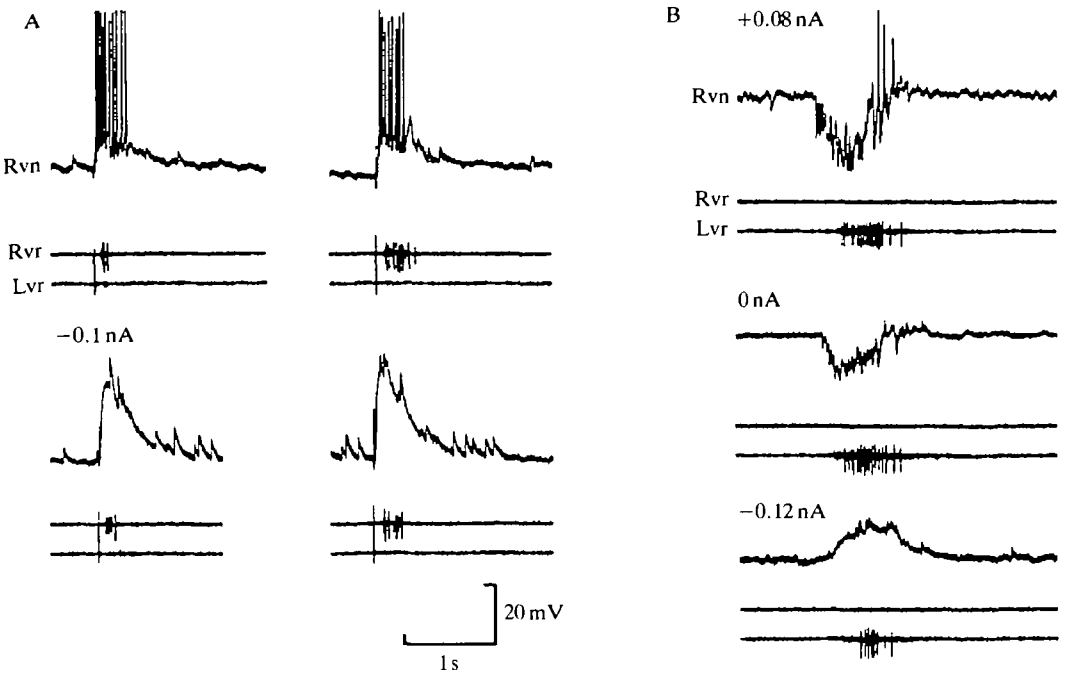

Fig. 3. Reciprocal organisation of non-rhythmic responses. (A) Brief electrical stimulation of the rostral tail skin evokes a ventral root (vr) 'flexion' burst (two examples, in this case on the same side as the stimulus). At the same time an intracellularly recorded neurone fires a burst of impulses superimposed on a prolonged depolarisation. Hyperpolarisation of the neurone by 0.1 nA reveals the underlying EPSP. Note also other spontaneously occurring depolarising potentials. (B) Inhibition of the response of a neurone on the opposite side to a spontaneously occurring 'flexion' burst. The hyperpolarising potential in a ventral neurone (vn) coincides with a motor root burst recorded on the opposite ventral root. The potential is increased in amplitude by depolarisation of the cell and reversed by hyperpolarisation. At depolarised levels, the neurone fires a few 'rebound' spikes on recovery from the potential.

excitation (Fig. 4A). This inhibition was increased in amplitude by depolarisation and reversed, to become depolarising, by hyperpolarisation (Fig. 5A). Similar responses were evoked by electrical stimulation of the head skin. As with short-latency responses to trunk skin stimulation, application of strychnine blocked the IPSP on the stimulated side (Fig. 5B).

Like the responses to trunk skin stimulation, stimulation of the head skin on one side at sufficient intensity to produce a motor root response generally evoked excitation on the opposite side accompanied by strychnine-sensitive inhibition on the stimulated side.

Responses to dimming the illumination

Motor responses to dimming the lights in *R. temporaria* were much less reliable

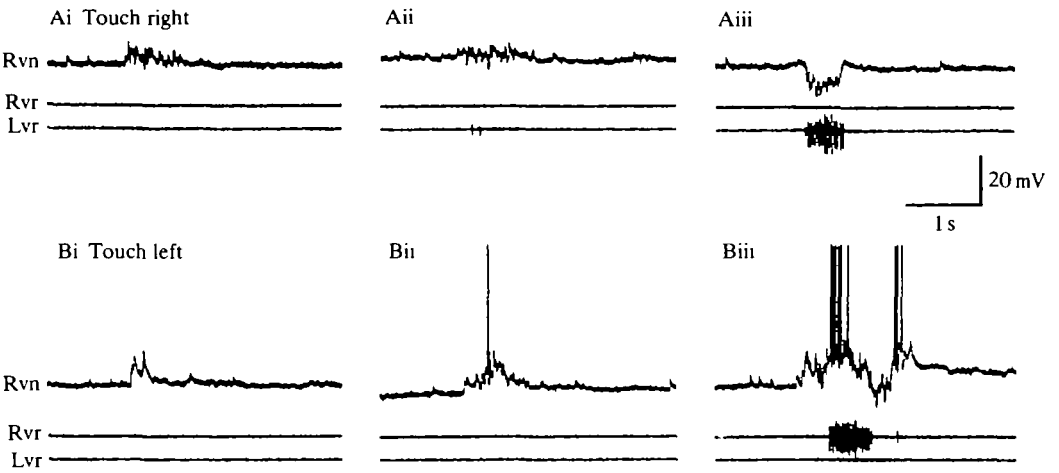

Fig. 4. Responses to one-sided head skin stimulation. All stimuli were brief pokes to the skin with a fine mounted needle, delivered by hand immediately prior to each response. (A) Touching the head skin progressively more strongly on the same side as a ventral neurone (vn) evokes: (i) small depolarising potentials, (ii) small depolarising potentials plus a weak ventral root (vr) response on the opposite side, and (iii) a strong hyperpolarising potential plus a strong ventral root burst on the opposite side (opposite the stimulus). (B) Touching the head skin progressively more strongly on the opposite side to a ventral neurone evokes: (i) subthreshold depolarising potentials, (ii) stronger potentials leading to a spike, and (iii) a burst of spikes plus a ventral root burst on the same side (again opposite the stimulus).

than those documented in *X. laevis* embryos (Foster and Roberts, 1982). They did not occur in all animals and occurred at a relatively long and variable latency. If present, the first response of rhythmic ventral neurones to dimming the lights was a depolarisation at a latency of 1–2 s (Fig. 6). On one side, the excitation produced a long train of impulses associated with a burst of motor root discharge suitable to drive a strong flexion on that side. On the opposite side, initial depolarisation was blocked by a hyperpolarising potential. In some cases, this initial response preceded a sustained episode of rhythmic activity (Fig. 6 and see below).

Sustained responses to stimulation

Patterns of rhythmic motor discharge that can be evoked or occur spontaneously in *R. temporaria* embryos have been described in the previous paper (Soffe, 1991). Episodes of rhythmic activity evoked in animals prepared for intracellular recording, in which the spinal cord was exposed and the level of extracellular Ca^{2+} was raised, were like those described previously in most respects. As before (Soffe, 1991), rhythmic discharge covered a range of cycle periods that were sufficiently different to underlie the whole range of rhythmic behaviour from slow lashing to fast swimming. The detailed structures of episodes varied somewhat in terms of cycle periods. In many cases rhythmic activity within each episode started

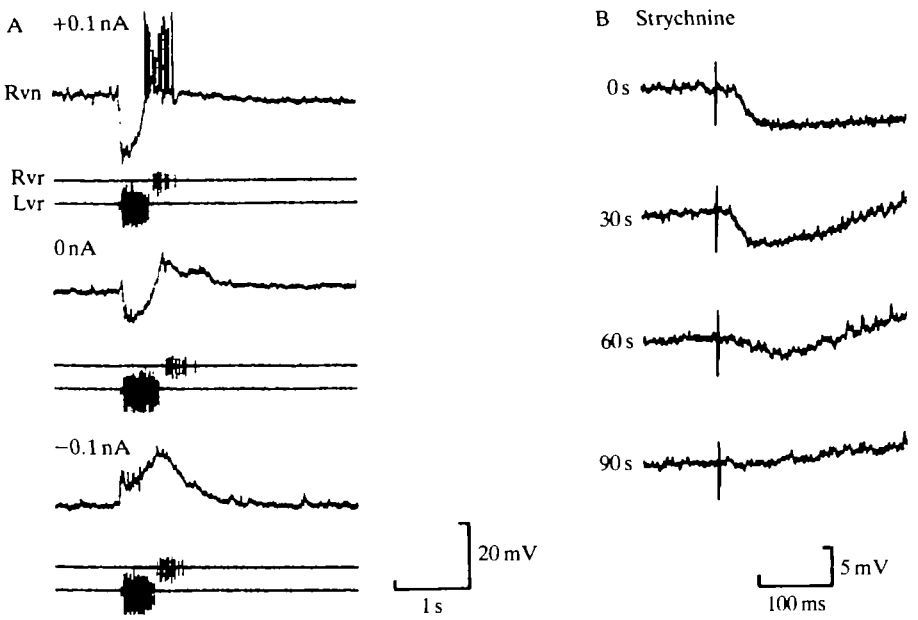

Fig. 5. Inhibitory responses to one-sided head skin stimulation. (A) Stimuli were delivered by hand as in Fig. 4. A hyperpolarising inhibitory response in a ventral neurone (vn) to touching the head skin sufficiently hard on the same side to evoke a strong ventral root (vr) burst on the opposite side. The potential is increased in amplitude by depolarisation of the neurone and reversed by hyperpolarisation. Note that, following the initial inhibition, the neurone receives a depolarising potential that increases in amplitude with hyperpolarisation and coincides with a ventral root burst on the same side. (B) The initial inhibitory response in a ventral neurone to brief electrical stimulation of the head skin on the same side is progressively blocked by 1 μmol l^{-1} strychnine.

relatively slowly and cycle periods then progressively shortened (Fig. 7). However, some episodes did occur in which cycle periods lengthened towards the end (e.g. Fig. 7C). During longer cycles, ventral neurones usually fired a burst of two or more impulses. During shorter cycles, neurones generally fired only a single impulse. In view of the variability between episodes, perhaps the most important result was that neurones were clearly able to fire one or more impulses on different cycles within the same episode, apparently with equal facility (Fig. 7). Thus, they do not show a restriction to single spiking as do neurones during swimming in *X. laevis* embryos.

Synaptic drive during sustained rhythmic locomotor activity

During episodes of rhythmic motor discharge, intracellular activity alternated between two phases of membrane potential. Neurones became relatively depolarised and could fire one or more impulses in phase with motor root discharge on the same side. They then became relatively hyperpolarised in phase with motor root

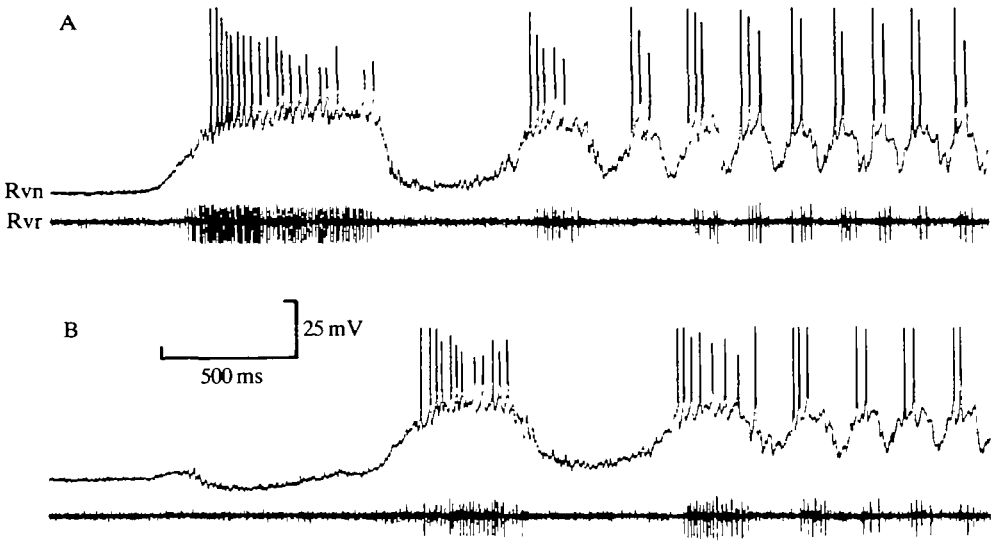

Fig. 6. Sustained rhythmic responses in a ventral neurone to dimming the lights. (A) The initial response to dimming the lights is a long burst in both the neurone (vn) and the ventral root (vr) on the same side, starting 1.9 s after dimming. This initial response is followed by sustained rhythmic discharge. (B) As for A, but after a slight initial depolarisation at a latency of 1.1 s, the episode starts with a period of relative hyperpolarisation probably corresponding to a burst of motor discharge on the opposite side.

discharge on the opposite side. Although the precise phasing of activity was not examined, no recorded neurones differed markedly from this pattern. Throughout each episode, the membrane potential appeared generally depolarised. This was particularly clear at the end of episodes, once rhythmic activity had ceased, when the membrane potential repolarised slowly over a period of 0.5–2.0 s to its resting level.

Injection of constant-current hyperpolarising pulses into ventral neurones produced changes in membrane potential that were reduced in amplitude during episodes of rhythmic activity. Episodes were therefore associated with an overall decrease in cell input resistance (Fig. 8). This decrease generally measured around 50 %. However, because the neuronal membrane time constant was long compared to the duration of current pulses used, the membrane potential did not level off during each control pulse (before and after each episode, Fig. 8A,B). This meant that absolute changes of input resistance could not be determined and that real changes were probably greater than those measured. The decrease in input resistance was always greatest at the start of an episode. It then either remained at this level or recovered slightly during the course of the episode (Fig. 8C).

Clearer than the pattern of change in input resistance during each episode as a

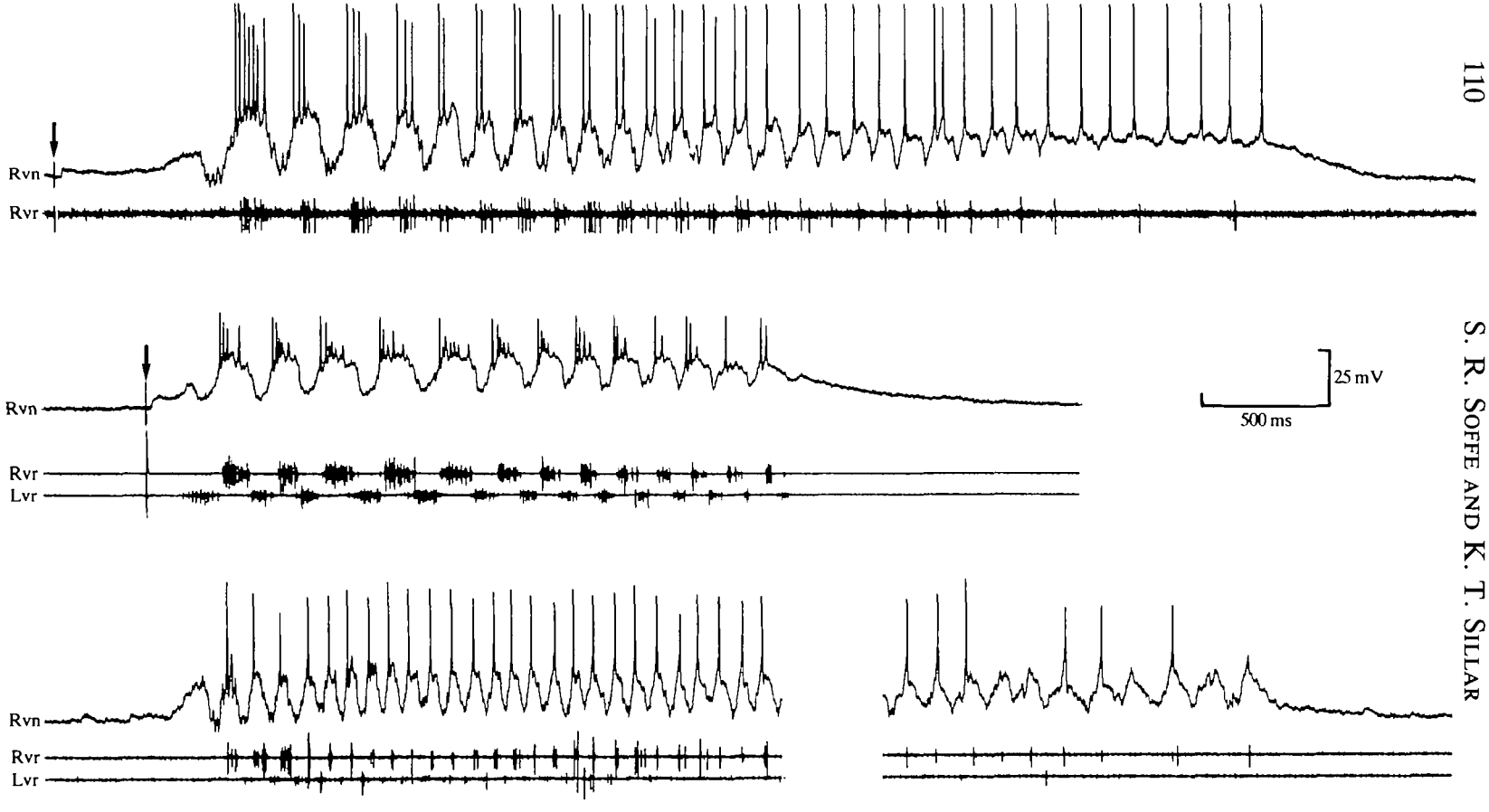

Fig. 7. Sustained episodes of rhythmic activity in ventral neurones (vn) in response to electrical stimulation of the skin. During each cycle, neurones are depolarised and fire impulses in phase with ventral root (vr) discharge. In the middle of each cycle, neurones are relatively hyperpolarised. At the end of each episode, the membrane potential slowly returns to the resting level. (A) A spectrum of activity ranging from rhythmic bursts of impulses to rhythmic single spikes within a single episode evoked by brief electrical stimulation of the skin (arrowed). (B) Episode consisting mostly of rhythmic bursts recorded in a different neurone together with ventral root records from the same and opposite sides. (C) Spontaneously occurring episode (middle part omitted) consisting predominantly of single spikes recorded in a third neurone. Note that, towards the end of the episode, the neurone does not fire on every cycle.

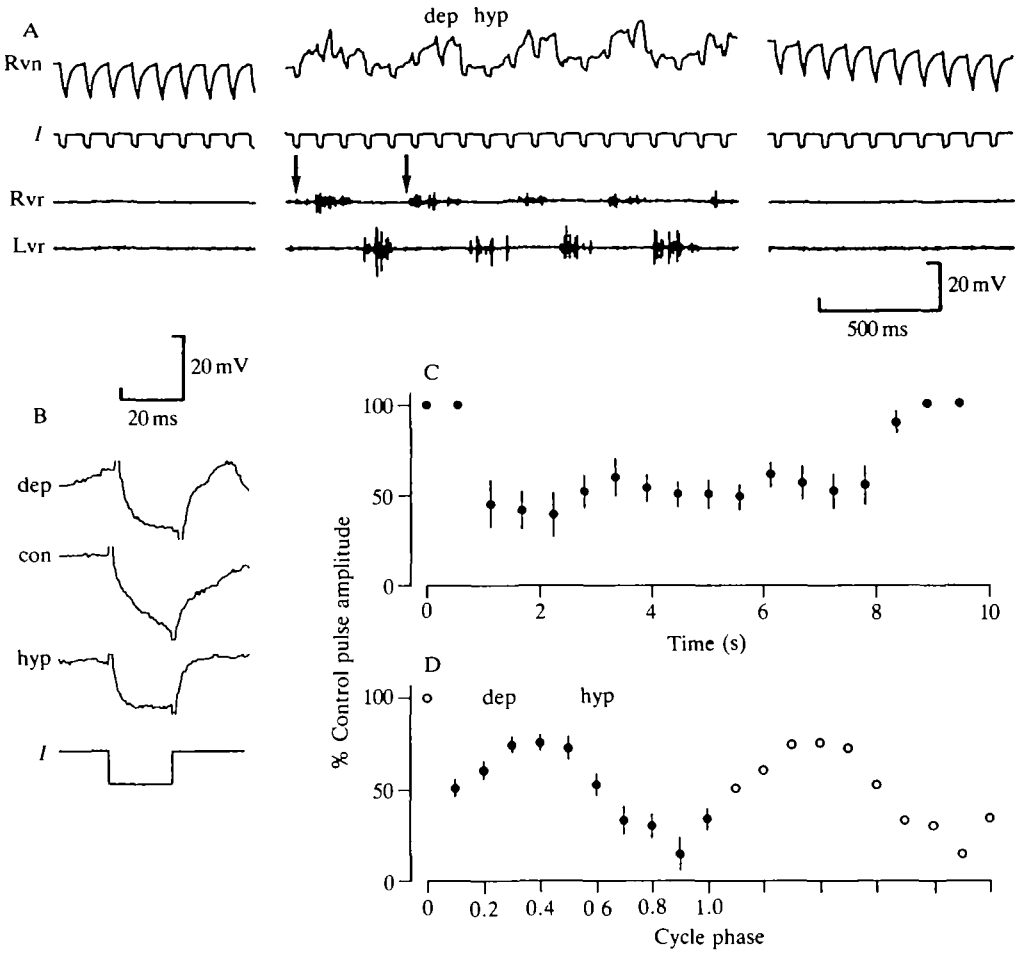

Fig. 8. Input resistance changes during rhythmic activity. (A) Short hyperpolarising current pulses (*I*) injected into a ventral neurone (Rvn) before, during and after an episode of rhythmic activity and recorded together with ventral roots on the same and opposite sides (Rvr, Lvr). Lack of firing makes the responses to current pulses easier to see here. Note that control pulses do not reach a steady level. (B) Single responses to current pulses in a different neurone. Both the amplitude and the time constant of the responses decrease during the depolarising phase (dep) and further decrease during the hyperpolarising phase (hyp) compared to the pre-episode control (con). (C) The decrease in neuronal input resistance throughout an episode of rhythmic activity, plotted as a percentage of control pulse amplitude from responses as in A and B for a third neurone. Each point is the mean and standard error for 10 consecutive responses. (D) Decrease in neuronal input resistance plotted according to cycle phase (phases 0.0 and 1.0 for one cycle are arrowed in A). The same data are plotted twice to emphasise the rhythmic decrease in input resistance which is higher during the hyperpolarising phase (hyp, phase 0.5–1.0) than during the depolarising phase (dep, phase 0.0–0.5).

whole was a phase-dependent change during each cycle. While there was a reduction in input resistance during the depolarising phase, there was a greater reduction during the hyperpolarising phase (Fig. 8B,D). This phase-dependent change in cell input resistance during rhythmic activity was also manifest as a marked cyclical change in membrane time constant. The time constant shortened during the depolarising phase and shortened further during the hyperpolarising phase. The result of this shortening was that the membrane potential was often able to reach a steady level during injected current pulses, particularly during the hyperpolarising phase (Fig. 8B).

The depolarising phase during rhythmic activity is probably a period of chemical synaptic excitation for the following reasons. First, it could lead to the firing of one or more impulses (Figs 6, 7), with individual impulses sometimes appearing to be driven by relatively quick depolarising synaptic potentials superimposed on each depolarising wave. These potentials became more apparent during injection of hyperpolarising current sufficient to prevent impulse firing (Fig. 9). The number of impulses in the depolarising phase could be increased by depolarisation (Fig. 9). Second, the amplitude of the depolarising phase relative to the membrane potential between episodes was increased by hyperpolarisation of the neurone and reduced by depolarisation (Fig. 9). Third, the depolarising phase was associated with a decrease in cell input resistance (Fig. 8; see above).

Between successive depolarising phases lay relatively hyperpolarising phases. These appeared to be due to chemical synaptic inhibition rather than to a reduction or absence of excitation for the following reasons. First, on some cycles the membrane potential could fall below that between episodes, particularly when the neurone was depolarised (Fig. 9). Second, even when neurones were held depolarised, they were generally unable to fire impulses during the hyperpolarising phase. Third, the hyperpolarising phase was associated with a decrease in input resistance (Fig. 8). Moreover, as described above, the magnitude of the decrease was even greater than during the period of excitation. Fourth, the hyperpolarising phase became strongly depolarising in neurones recorded with microelectrodes containing $2\,mol\,l^{-1}$ KCl instead of $3\,mol\,l^{-1}$ potassium acetate (Fig. 10). The concentration of intracellular Cl^- was presumably increased to the extent that a period of normally hyperpolarising inhibitory chemical synaptic input became reversed in sign, unless the neurone was strongly depolarised (Fig. 10).

Rhythmic activity in *R. temporaria* was generally not as well 'structured' as activity observed in *X. laevis* embryos. Besides the variability in cycle periods within an episode (described above), the hyperpolarising and depolarising phases were sometimes less precisely defined. This was reflected in variability in the pattern of ventral root discharge. The membrane potential did not always oscillate smoothly between phases; for example, the excitatory phase frequently showed transient hyperpolarising potentials which also increased in amplitude with depolarisation of the cell. It is most likely, therefore, that these were transient on-cycle IPSPs.

Recordings made from nine embryos following addition of $1\,mmol\,l^{-1}$ Mg^{2+} to

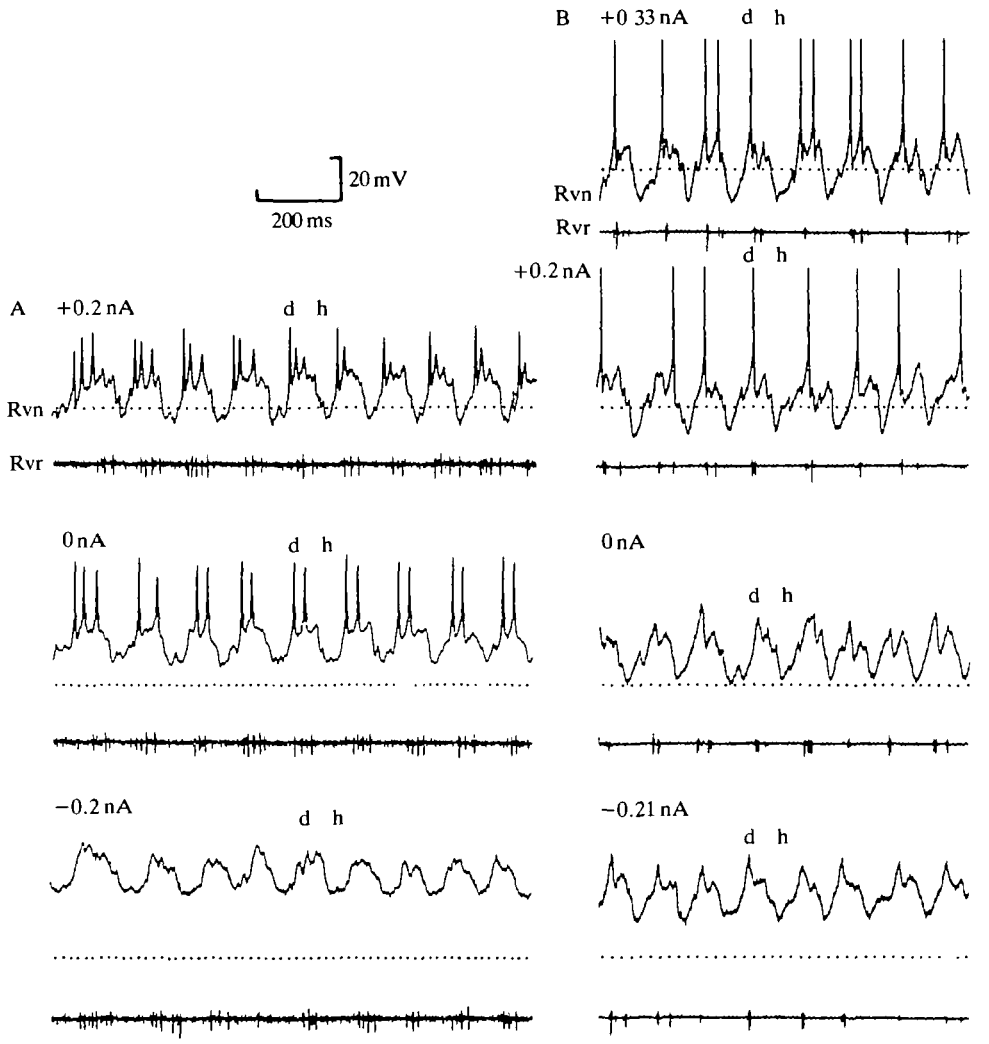

Fig. 9. Responses to current injection during rhythmic activity. Rhythmic activity recorded in two different neurones (A) firing two or more impulses per cycle and (B) not firing impulses on most cycles. Depolarising (d) and hyperpolarising (h) phases are indicated. The membrane potential between episodes, during current injection, is shown as a dotted line.

the saline (not illustrated) showed patterns of activity that could not be distinguished from the activity described above.

Discussion

This paper presents the results of intracellular recordings from neurones located ventrally in the spinal cord of *R. temporaria* embryos. Although neurones were

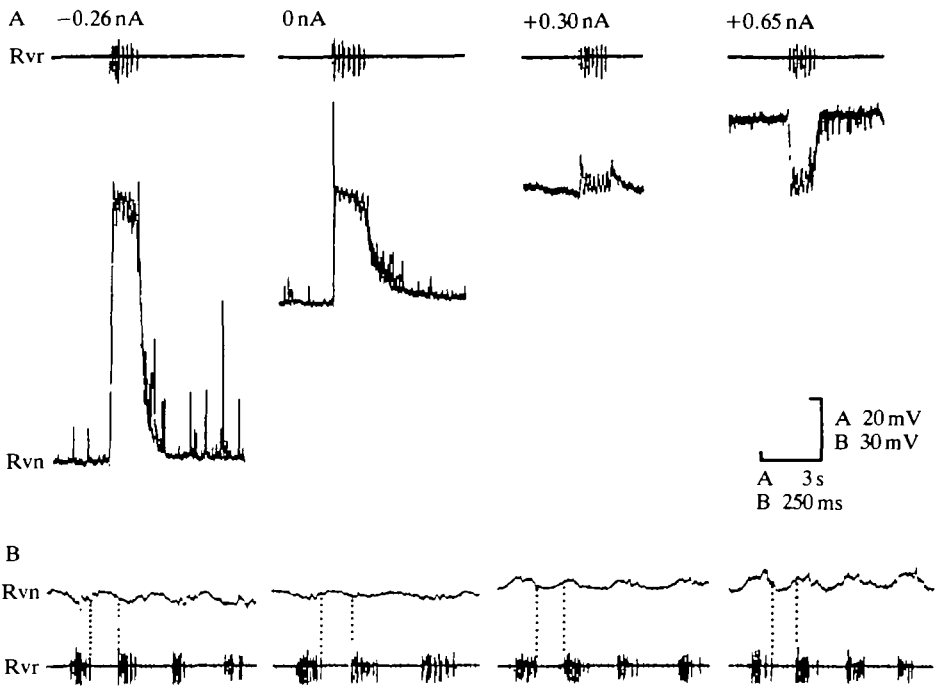

Fig. 10. The influence of a raised intracellular chloride level on rhythmic neuronal activity recorded in a ventral neurone with a microelectrode filled with 2 mol l^{-1} KCl. (A) Activity throughout an episode is strongly depolarising unless the neurone is held very depolarised. Note also reversed 'spontaneous' IPSPs before and after each episode that are only hyperpolarising when the neurone is depolarised. (B) Expanded traces from the episodes in A. Note the normal hyperpolarising phase (between dotted lines) is reversed and only becomes relatively hyperpolarising when the neurone is held very depolarised.

not characterised morphologically or physiologically, anatomical studies (C. Harper, personal communication) suggest it is likely that many of them will have been motoneurones. The common feature of all the neurones was that they were rhythmically active during episodes of rhythmic root activity and they were active in phase with ventral root discharge on the same side. The recordings provide information on the pattern of synaptic drive that underlies rhythmic and non-rhythmic behavioural responses in the embryo and also some insight into sensory pathways that initiate these movements.

Responses to sensory stimulation

The short-latency responses to skin stimulation seen in *R. temporaria* embryos are very similar to those already described in *X. laevis* embryos (Roberts *et al.* 1985; Sillar and Roberts, 1988*a*). In *X. laevis*, a stimulus on one side produces an 'avoidance' response: a first contraction on the opposite side, turning the embryo

away from the stimulus. In the mechanism proposed to underlie this response, stimulation of sensory Rohon–Beard cell neurites in the trunk skin excites dorsally placed 'dorsolateral commissural' interneurones which, in turn, excite motoneurones and inhibitory interneurones on the opposite side of the spinal cord (Clarke *et al.* 1983; Clarke and Roberts, 1984; Sillar and Roberts 1988*b*). The disynaptic path to the motoneurones produces the contralateral contraction; excitation of the inhibitory interneurones mediates crossed, reciprocal inhibition of the stimulated side. In *R. temporaria*, similar circuitry may exist: physiological responses show a similar contralateral excitation and ipsilateral inhibition of motoneurones. It is not clear, however, how this mechanism alone could produce the stronger contralateral bursts of discharge that often result in *R. temporaria* from a one-sided stimulus (see also Soffe, 1991). Reflex responses to cutaneous stimulation have been known for a range of other vertebrates, in some cases for many years (e.g. lamprey: McClellan and Grillner, 1983; eel: Gray, 1936; frog and cat: Sherrington, 1906). There are some obvious parallels, such as sidedness of responses and a reciprocal organisation between antagonists reinforced by inhibition. In most cases, though, the responses are both more complex than those of amphibian embryos and the mechanisms less clear and less open to detailed study.

Sustained rhythmic motor patterns

The sustained motor responses seen in paralysed *R. temporaria* embryos in this study are very like those described in the previous paper (Soffe, 1991). The activity ranges between slow rhythmic bursts of discharge, probably responsible for driving lashing and slow swimming behaviour, and fast, rhythmic single spikes, probably underlying the faster swimming. The feature that distinguishes the rhythmic motor patterns of *R. temporaria* from those of *X. laevis* (Roberts and Kahn, 1982) or *T. vulgaris* (Soffe *et al.* 1983) is the absence of obvious switching between different motor 'programmes'. Transitions from slow lashing activity to fast swimming appear to be continuously graded in *R. temporaria* embryos. In contrast, transitions from 'struggling' (in *X. laevis*) or 'slow alternation' (in *T. vulgaris*) to swimming are distinct. Does this imply that there are fundamental differences in the nature of the spinal rhythm-generating circuitry in these different species or does it, rather, point to an underlying similarity in the neuronal mechanisms that drive the whole range of behaviour? The evidence so far would favour the latter interpretation.

Throughout the whole range of rhythmic motor patterns, from rhythmic bursts to rhythmic single spikes, the synaptic drive to ventral neurones in *R. temporaria* consists of alternate phases of excitation and inhibition. This is the typical pattern seen, for example, during swimming in *X. laevis* embryos (Soffe and Roberts, 1982), in the swimming of the adult lamprey (Kahn, 1982; Russell and Wallén, 1983) and, indeed, in locomotor rhythms of adult higher vertebrates, like cats, during walking (Jordan, 1983). Thus, there are parallels with both the locomotor rhythms of adult vertebrates and those of other amphibian embryos. It is too soon

to say how far these parallels extend. A further mechanism now implicated in vertebrate locomotor rhythm generation is that of bistable membrane properties. There is evidence for these in both lamprey (Wallén and Grillner, 1987) and cat (Hounsgaard *et al.* 1984), though their precise involvement during normal locomotion remains unclear. However, there is as yet no evidence for such oscillations in amphibian embryos. Since these oscillations rely, at least in the lamprey, on activation of *N*-methyl-D-aspartate (NMDA) receptors in the presence of extracellular Mg^{2+}, similar membrane oscillations are presumably not involved in most of the rhythmic activity described here, where Mg^{2+} was not routinely included in the saline. In cases where Mg^{2+} was added, activity appeared very similar.

It seems likely that essentially the same mechanism underlies both rhythmic single spikes and bursts in *R. temporaria*. It also seems reasonable to suggest that this mechanism will resemble the one underlying swimming in *X. laevis* embryos. However, in the mechanism currently proposed for *X. laevis*, central importance is given to the ability of neurones to fire only a single impulse when depolarised on each cycle (Roberts *et al.* 1986; Roberts and Tunstall, 1990). A mechanism that relies on neurones firing in this way is clearly not adequate to explain the less restricted activity seen in *R. temporaria*.

An important respect in which rhythmic motor discharge in *R. temporaria* resembles swimming in *X. laevis* is that both are self-sustaining following a brief stimulus. *R. temporaria* embryos, therefore, demonstrate that the relatively simple amphibian embryo nervous system can sustain a pattern of rhythmic motor bursts, driven by alternate excitation and inhibition, as well as more restricted rhythmic single spikes. In contrast, the struggling motor pattern driven by rhythmic bursts of discharge in *X. laevis* embryos requires repetitive stimulation for sustained expression (Soffe, 1989). Similarly, the rhythms of adult vertebrates generally require maintained sensory or electrical stimulation or pharmacological activation (see, however, McClellan, 1984).

Where it has been examined, excitation during rhythmic motor discharge appears to involve an excitatory amino acid neurotransmitter (*X. laevis*, Dale and Roberts, 1984, 1985; lamprey, Dale, 1986) probably acting at both NMDA and non-NMDA type receptors. The main inhibitory transmitter appears to be an inhibitory amino acid, probably glycine (Dale, 1985; Soffe, 1987). It is too soon to say whether this organisation is true for rhythmic activity in *R. temporaria*. Activation of NMDA receptors could certainly be responsible for the overall depolarisation seen during episodes of rhythmic discharge, as it is in *X. laevis*. Also, synaptic inhibition does involve a Cl^- conductance, as would be expected for mediation by glycine. Preliminary experiments with gross application of antagonists for excitatory and inhibitory amino acid neurotransmitters suggest the involvement of both in rhythm generation (S. R. Soffe, unpublished findings). However, since both are also likely to be involved in sensory transmission, such results are hard to interpret and experiments using more local application of antagonists are therefore planned.

The *R. temporaria* embryo is, therefore, a potentially valuable preparation in which to study generation of rhythmic locomotor patterns, since it combines the relative neural simplicity of other amphibian embryos with the ability to generate and self-sustain a range of motor patterns including rhythmic bursts typical of adult behaviour.

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