J. exp. Biol. **113**, 493–497 (1984)
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DO THE MOTONEURONES CONSTITUTE A PART OF THE SPINAL NETWORK GENERATING THE SWIMMING RHYTHM IN THE LAMPREY?

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Accepted 29 March 1984

In the study of the neural control of rhythmic motor acts, the operation of the central neuronal networks that produce the motor pattern is an area of considerable interest (cf. Delcomyn, 1980; Grillner, 1981). The motor pattern underlying swimming in the lamprey, a primitive vertebrate, can be produced in the isolated spinal cord *in vitro* (Cohen & Wallén, 1980; Wallén & Williams, 1984). The capacity to generate the rhythmic pattern is distributed throughout the spinal cord, and short pieces of just a few segments may produce a coordinated pattern (Cohen & Wallén, 1980; Grillner & Sigvardt, in Grillner *et al.* 1982).

One question to consider is whether the motoneurones themselves constitute a part of the rhythm-generating circuitry, or merely represent an output stage, phasically driven by signals from an interneuronal network (cf. Wallén, 1982; Russell & Wallén, 1983). Motoneurones could possibly influence other spinal neurones *via* axon collaterals, gap junctions or even output synapses on the soma or dendrites. While for vertebrate systems the motoneurones are generally assumed to be separate from the pattern-generating network, it has been shown in some invertebrates that motoneurones may be part of the circuitry (Selverston, Russell, Miller & King, 1976; Heitler, 1978; Simmers & Bush, 1983). We have addressed this question for the swim rhythm-generating network of the lamprey spinal cord, by activating motoneurones antidromically by means of electrical stimulation of ventral roots in the *in vitro* preparation during fictive swimming, i.e. the motor pattern underlying locomotion recorded in the absence of movements. If the motoneurones were part of the generator network, such a stimulation would be expected to modify the rhythmic activity, recorded in nearby ventral roots. The results have been presented in abstract form (Wallén & Lansner, 1983).

The lamprey spinal cord is flattened and thin (about 0·3 mm) and survives well under *in vitro* conditions for several days. Preparations from *Ichthyomyzon unicuspis* or *Lampetra fluviatilis* were used with similar results. Animals were anaesthetized with tricaine methane sulphonate (MS-222, Sandoz) in cold tap water. Spinal cord-notochord pieces (11–29 segments long) were dissected out from a level between the last gill opening and the anus and pinned down in a Sylgard dish, containing cooled physiological solution (composition, see Wickelgren, 1977). Fictive swimming activity

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Key words: Fictive locomotion, motoneurones, central pattern generation, spinal cord, lamprey.

(recorded from ventral roots with glass suction electrodes) was induced pharmacologically (Cohen & Wallén, 1980; Poon, 1980) by addition of the aspartate analogue *N*-methyl-aspartate to the bath (0·07–0·33 mM; Grillner *et al.* 1981). Electrical stimulation was given to one ventral root or, in two experiments, to two or three ventral roots simultaneously, *via* suction electrodes connected to constant current stimulators (Neurolog System, Digitimer Inc.). To avoid the possibility of current spread to the spinal cord, the ventral root was in some cases dissected free outside the vertebral canal for stimulation. To verify the antidromic activation of the motoneurone soma, intracellular records were taken from 10 motoneurones during fictive swimming, using conventional microelectrodes filled with 2 M K-citrate. As previously described (Russell & Wallén, 1983), a motoneurone was identified by the appearance of a unit spike in the ventral root, time-locked to an action potential in the cell. The same ventral root was then stimulated for antidromic activation.

While a ventral root was stimulated tonically with continuous pulse trains (most commonly 2-ms pulses at 20 Hz) during fictive swimming, the bursting activity in an adjacent and/or opposite ventral root was examined. The stimulation evoked no noticeable change in the timing of successive bursts, neither on the opposite side of the same segment, nor in the hemisegment immediately adjacent to the stimulated one (Fig. 1). A net increase or decrease of the cycle duration during stimulation was absent, as well as any transient effects related to stimulus onset or termination. The lack of effect on the rhythm, which was seen in seven out of eight tested preparations, was found also when trains (e.g. 200 ms long) of stimulus pulses were given in different parts of the cycle, as well as when the stimulus intensity was increased further than in the illustrated case (up to 300 μA or more). In one preparation, in which the ventral root was stimulated inside the vertebral canal, a rhythm disturbance

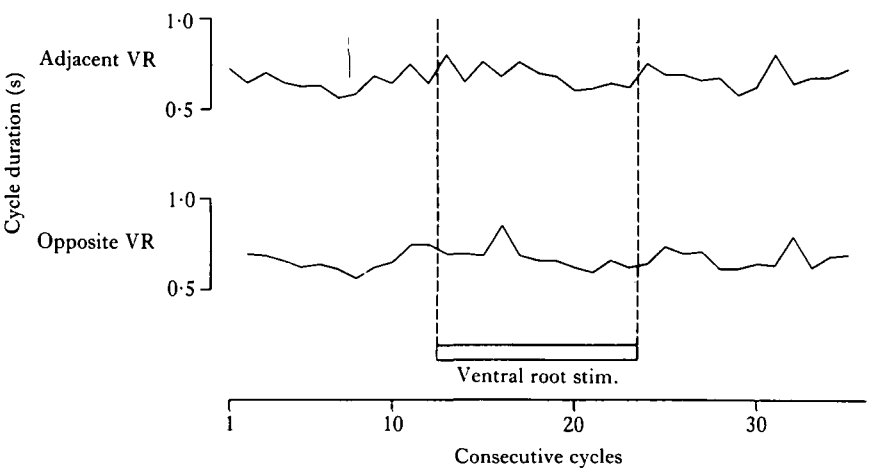

Fig. 1. Antidromic stimulation of a ventral root during fictive swimming activity. Records of rhythmic activity were taken from a ventral root (VR) immediately adjacent to the stimulated VR, as well as from the VR directly opposite to the stimulated one. The cycle durations before, during and after stimulation (horizontal bar; 2-ms pulses of 35 μA at 50-ms intervals) have been plotted for 35 consecutive cycles. Fictive swimming activity was induced by adding *N*-methyl-aspartate to the bath (final concentration 0·1 mM).

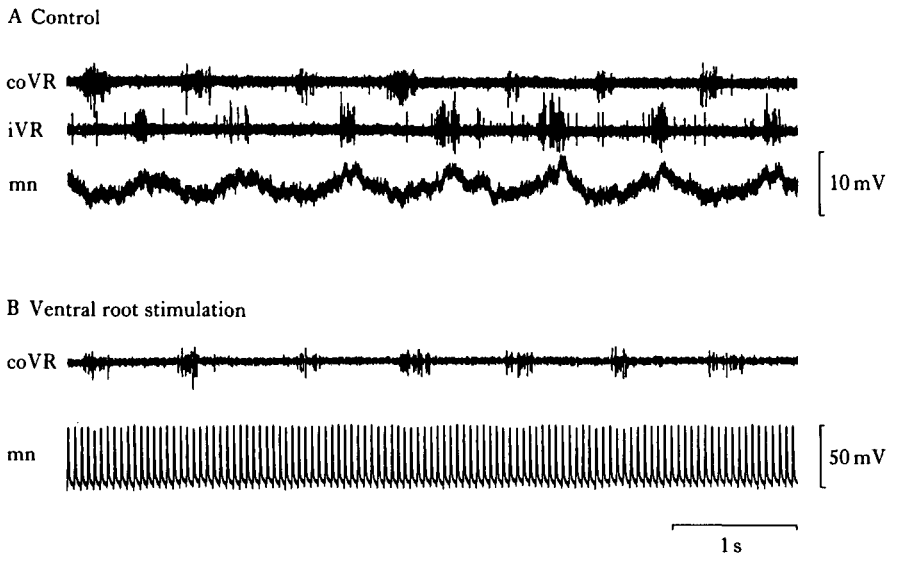

Fig. 2. Invasion of a motoneurone during fictive swimming by the antidromic action potential elicited by ventral root stimulation. (A) Control record before stimulation. The alternating bursting activity in two opposing ventral roots (VR) (i, ipsilateral; co, contralateral) of one segment can be seen, as well as the oscillating membrane potential of a motoneurone (mn) that had its axon in iVR. This particular motoneurone did not reach threshold for firing spontaneous action potentials. (B) Antidromic stimulation of iVR with a continuous train of pulses (2-ms pulses of 4·6 μA at 50-ms intervals). An antidromic action potential is elicited in the motoneurone (same as in A) with each pulse. Threshold for invasion of this motoneurone was 4·2 μA. Note that the bursting activity in coVR remains unchanged. In this VR record the stimulus artefact was removed by using an electronic artefact eliminator circuit. Note voltage calibrations for the intracellular records, and time mark in B, which applies to all traces. The solution contained 0·1 mM *N*-methyl-aspartate.

could be seen with intensities higher than 30 μA which, however, most probably was caused by current spread to the spinal cord itself.

It is thus evident that stimulation of a ventral root during fictive swimming fails to affect the efferent rhythm recorded in nearby roots. It may be argued, however, that if the motoneurones were part of the pattern-generating network, it would still be necessary to activate a larger number than those belonging to a single ventral root in order to affect the rhythm. When three of the four ventral roots belonging to two adjacent segments were stimulated simultaneously (300 μA at 20 Hz), no effect was seen on the rhythmic activity recorded in the fourth root. Furthermore, simultaneous stimulation of both ventral roots of one segment did not influence the intersegmental coordination, as judged by recordings from rostral and caudal, nearby ventral roots.

It is not evident that the motoneurone soma will be invaded with each shock, since the motoneurones are actively inhibited during the hyperpolarized phase of each cycle (Russell & Wallén, 1983). If the motoneurone somata were not invaded the antidromic stimulation would only test possible synaptic interaction between the axon of the motoneurones and other neurones. The possibility of course exists that the motoneurone soma or their extensive dendrites would interact with each other or with interneurones by gap junctions or by dendrodendritic synapses. It was therefore important to establish that the motoneurone soma was actually invaded with each

antidromic stimulus pulse. Alternating bursting activity in the two opposing ventral roots of the same segment was recorded, together with the oscillating membrane potential of a motoneurone, the axon of which exits through the ipsilateral ventral root (Fig. 2A). When this root was stimulated tonically (20 Hz) an antidromic action potential locked to each stimulus pulse was recorded in the motoneurone soma (Fig. 2B). This was seen in all of 10 motoneurones recorded in six separate experiments. The threshold for invasion was typically less than 10 μA. It should also be noted that the motoneurone soma is invaded throughout the fictive swim cycle (Fig. 2B), i.e. also during its period of hyperpolarization. In addition to the motoneurone soma, the antidromic action potential is likely to influence the majority of the dendrites, perhaps with the exception of the most distal, thinnest branches. With the stimulus intensities used, it is highly probable that all the motoneurones with their axons in the stimulated ventral roots (1–3) were invaded. Each root contains about 60–80 motoneurones (*Ichthyomyzon*; Rovainen, 1983). Not even such a massive stimulation could thus influence the swimming rhythm or the intersegmental coordination. From the present results one may therefore conclude that synaptic contacts that possibly could be located on the motoneurone axon, the soma and the main part of the dendritic tree are unlikely to take any part in the generation of the locomotor rhythm. It cannot be entirely excluded, however, that output synapses on the most distal dendritic branches, if present, could contribute.

We would like to thank Dr S. Grillner for generous support and fruitful discussions in the course of this study. We are also grateful to Mrs I. Klingebrant for help with typing the manuscript. This work was supported by the Swedish Medical Research Council (Proj. No. 3026), Karolinska Institutets fonder and M. Bergvalls stiftelse.

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