system represents a well-defined and reliable starting material for the in vitro study of the developmental neurobiology of the cerebellum. These studies are our first steps towards establishing cultures of more purified, and better identified single cell types of the developing cerebellum.

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5 The terms perikarya and cells, while not strictly equivalent, are used interchangeably here.
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Unexpected features of the interaction between individual primary afferents and spinal motoneurones

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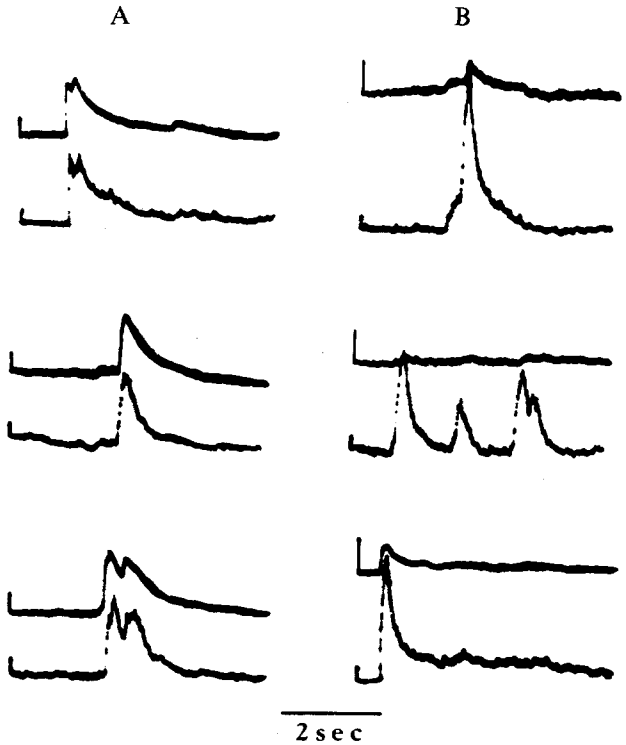
Summary. Simultaneous intracellular recording from spinal motoneurone and dorsal root fibres afferent to it in the isolated amphibian cord revealed a specific correlation between the slow spontaneous depolarizing activity in motoneurones and primary afferents.

It has recently been found in the isolated frog spinal cord preparation that in Mg^{2+}-free Ringer's solution the ventral roots exhibit high fluctuating levels of spontaneous activity and poor response stability to stimulation of the dorsal roots[2]. We developed a method which allows simultaneous microelectrode penetration of ventral horn motoneurone and dorsal root fibres afferent to it, and found that both elements reveal spontaneous graded depolarizing activity. We present here evidence that there is a specific correlation between the spontaneous activity in motoneurones and individual primary afferent fibres. This correlation may reflect an important communication mechanisms between neuronal ensembles within the spinal cord.

Isolated hemisected spinal cords from *Rana ridibunda* were prepared as described elsewhere[3] and continuously perfused with Ringer's solution of the following initial composition (standard saline) in mM: NaCl 98.0; KCl 2.0; $CaCl_2$ 1.8; $MgCl_2$ 0.01–0.05; NaH_2PO_4 1.2; Na_2HPO_4 2.0; $NaHCO_3$ 6.0; glucose 5.5; pH 7.4–7.6.

One microelectrode filled with 3 M KCl was used to impale a primary afferent fibre located in the dorsal root entry zone, and a second microelectrode filled with 3 M KCl or 2 M K-citrate was inserted into a motoneurone. A pen writer provided a continuous record of membrane potential fluctuations which were also photographed from a double-beam oscilloscope.

When the spinal cord was perfused with the standard solution, slow spontaneous potential fluctuations were waxing and waning at irregular intervals with a mean frequency of 2–4/sec. These fluctuations could be detected practically in all impaled motoneurones. The shape of the observed fluctuations was usually complex and unstable and their amplitude ranged from a few mV to over 30 mV. The maximal size of depolarizing fluctuations was observed in motoneurones with resting membrane potentials over



Examples of the spontaneous depolarizing potentials recorded from the same motoneurone (lower traces) and 2 different dorsal root fibres afferent to it (upper traces). A: records from the fibre establishing monosynaptic contact with the motoneurone. B: records from the fibre establishing polysynaptic connections with the motoneurone. Calibration pulse 1 mV precedes each record. Note different voltage gain in A and B.

−80 mV. Similar waves of depolarization could be detected in primary afferents. Direct comparison of potentials recorded from each element revealed a striking mirror-like congruity between the onset and the time course of spontaneous depolarizations in motoneurone and primary afferent fibre (figure). This congruity was especially evident in dorsal root fibres establishing direct monosynaptic contacts with motoneurones. Records of the figure, A, demonstrate such an example and indicate that the temporal profiles of depolarizing potentials in both elements are identical. Moreover, the observed waves had the same order of magnitude.

The correlation between spontaneous depolarizing potentials in motoneurones and in primary afferents, which do not synapse directly on motoneurones but can influence them polysynaptically, was less perfect. The amplitude of depolarizing potentials in such fibres usually did not exceed 10% of the depolarization recorded simultaneously from a motoneurone (figure, B). This general tendency was apparent in all 65 motoneurone-dorsal root fibre pairs investigated.

The spontaneous waves were completely and reversibly abolished by elevation of external Mg^{2+} to the final concentration of 1–2 mM or by removal of external Ca^{2+} and addition of Mn^{2+} (2 mM). In contrast, picrotoxin (0.1–0.5 mM) increased the size and frequency of depolarizing potentials. The latter remained after the isolation of the segment where the recorded elements were located suggesting that correlated fluctuations appear to result from ongoing activity within one segment.

The duration, waveform and frequency of spontaneous waves was quite distinct from those of miniature synaptic potentials detected in amphibian motoneurones[4] and in primary afferent fibres[5]. However, the waves of depolarization were occasionally associated with a burst of miniature synaptic potentials, either in motoneurones or in afferent fibres.

Although the mechanism of the observed interaction remains to be discovered, it might be of considerable physiological significance, since it may reflect the synaptic transmittability within the spinal cord, and might operate as a possible communication channel between specific neuronal ensembles. In other experiments we observed that intracellular stimulation of the individual dorsal root fibres produced quite distinct synaptic responses in target motoneurones, depending upon the phase of slow depolarizing fluctuations occurring in the latter.

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Spontaneous miniature potentials in primary afferent fibres

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Summary. Intracellular recording from primary afferent fibres of the isolated frog spinal cord revealed the existence of spontaneous synaptic activity, related probably to the firing of presynaptic inhibitory fibres.

Since the discovery of spontaneous miniature potentials at vertebrate neuromuscular junction[2], they have been recorded from the postsynaptic cells at many other peripheral and central synapses with chemical mode of transmission[3], including amphibian spinal neurones[4]. However, no spontaneous activity resembling miniature synaptic potentials could be detected at axo-axonic synapses existing between primary afferent collaterals and presynaptic inhibitory fibres, which are the axonal branches of special interneurones in the spinal cord, although these synapses exhibit the standard features such as synaptic vesicles and the active zones of contact[5]. We report here the first evidence that primary afferent fibres exhibit a spontaneous synaptic activity related probably to the functioning of presynaptic inhibitory fibres. We also report on changes in the spontaneous activity produced by picrotoxin, tetrodotoxin and manganese.

Experiments were performed on the isolated perfused spinal cord[6] of *Rana ridibunda,* using an oxygenated bathing solution of composition (mM): NaCl 98.0; KCl 2.0; $CaCl_2$ 1.8; $MgCl_2$ 0.01–0.05; NaH_2PO_4 1.2; $NaHPO_4$ 2.0; $NaHCO_3$ 6.0; glucose 5.5; pH 7.4–7.6. In order to have a higher input resistance of the fibres recorded from and a smaller distance between the site of impalement (dorsal root entry zone) and the terminal branches of primary afferents, and thus to facilitate the detection of synaptic activity, we used small specimens weighing 40–50 g. Although many fibres were slightly damaged by the microelectrode and their resting membrane potential varied between −50 and −60 mV, some had the resting membrane potential over −80 mV, and we could keep the best fibres up to 5–7 h.

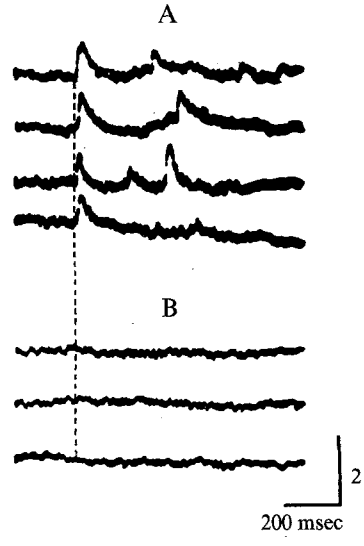

Fig. 1. Effect of picrotoxin on spontaneous miniature potentials and minimal responses on ventral root stimulation recorded from the same dorsal root fibre. A: potentials recorded in normal solution. B: 8 min following bath application of 0.3 mM picrotoxin. The dashed line indicates the beginning of minimal responses.