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Mutual Inhibition Between Olivary Cell Groups Projecting to Different Cerebellar Microzones in the Cat

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Summary. This study was carried out predominantly on the b zone in the lateral vermis of the cerebellar anterior lobe. This zone is divided into sagittally oriented microzones which receive a somatotopically organized climbing fibre input.

1. It was shown that the climbing fibre input to one microzone is inhibited by stimulation of a nerve that projects to an adjacent microzone.

2. The degree of inhibition was related to the proximity of the microzones involved.

3. The latency of the inhibition was short and the duration 70–110 ms.

4. The inhibition of climbing fibre responses occurred in the inferior olive and was presumably due to post-synaptic inhibition of the olivo-cerebellar neurones.

5. The mutual inhibition could be produced by antidromic activation of olivo-cerebellar neurones.

6. An inhibition with similar properties as in the b zone, but weaker, was observed between forelimb and hindlimb inputs to the c1 and c3 zones in the pars intermedia. In the c3 zone, an inhibition between adjacent forelimb microzones also occurred.

Key words: Cerebellum – Inferior olive – Climbing fibre microzones – Mutual inhibition

Introduction

A number of studies have been dedicated to the functional organization of the spino-olivo-cerebellar pathways (SOCPs), their origin and course in the spinal cord as well as their termination areas in narrow sagittal strips in the cortex of the cerebellar anterior lobe (for references see Ekerot et al. 1979; Armstrong and Schild 1980; Brodal and Kawamura 1980; Oscarsson 1980). However, very few studies have been concerned with the synaptic actions on the olivary cells of the spino-olivary pathways (Armstrong et al. 1968) or of other inputs (Armstrong and Harvey 1966 and 1968; Crill and Kennedy 1967; Crill 1970) and with information processing in the inferior olive.

Inhibitory mechanisms in spino-olivo-cerebellar pathways have been described (Leicht et al. 1972 and 1973; Rowe 1977). This inhibition could be produced by stimulation of the sensorimotor cortex as well as of cutaneous areas outside the receptive fields of the Purkinje cells studied. It was demonstrated that this inhibition occurred at a precerebellar level (Leicht et al. 1973), but it was not shown whether it occurred in the inferior olive or at a preolivary level.

In the spinal receiving part of the inferior olive, recurrent inhibition has been described (Armstrong et al. 1968; Armstrong and Harvey 1966 and 1968; Crill 1970; Llinás et al. 1974). However, the inhibition mentioned above occurred without first activating the olivo-cerebellar neurones under study and can thus not be explained by this recurrent inhibition. Recently, inhibitory interactions were described in the dorsal cap of the inferior olive (Takeda and Maekawa 1980). This inhibition was shown to be unidirectional, i.e. activity in the pathway from the contralateral eye inhibited dorsal cap neurones receiving an input from the ipsilateral eye but not vice versa.

The present study was undertaken to investigate possible interactions between different inputs to the spinal receiving part of the inferior olive. Most of the experiments were concerned with the climbing fibre input to the b zone in the lateral vermis of the anterior lobe. This zone was chosen for two reasons: 1. The b zone is divided into sagittally oriented microzones with a width of 100–200 μm (Andersson and Oscarsson 1978; Andersson and Eriksson 1981).

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The most medial microzone receives an input from the trigeminal nerves while the microzones further laterally receive inputs from successively more caudal parts of the neuraxis. 2. The neurones of the caudal part of the dorsal accessory olive, projecting to the b zone (Groenewegen and Voogd 1977), are excited monosynaptically by spino-olivary fibres ascending through the ventral funiculus (Oscarsson and Sjölund 1977b). This fact was used to demonstrate that the inhibition described in the present report actually takes place in the inferior olive.

Methods

The experiments were performed on 18 adult cats (2.0–3.1 kg), 15 under pentobarbitone (initial dose 40 mg/kg intraperitoneally) and 3 under chloralose anaesthesia (initial dose 80–100 mg/kg intravenously). All animals were given supplementary doses of the respective anaesthetic as required. The anaesthesia was maintained at such a level that the size of the pupils was always minimal. The animals were paralyzed with gallamine triethiodide and artificially ventilated. The arterial blood pressure, end-expiratory CO_2 concentration and rectal temperature were continuously monitored and kept within physiological limits.

The sciatic and ulnar nerves were dissected bilaterally and mounted for stimulation. In some experiments, the left medial cutaneous nerve was dissected and mounted and the radial side of the second digit (SR 1) was stimulated with a pair of percutaneous needle electrodes. The infraorbital branches of the trigeminal nerves were stimulated bilaterally with needle electrodes inserted through the gingiva, and thoracic nerves (in most experiments) with similar electrodes inserted percutaneously (Andersson and Eriksson 1981). All nerves were stimulated at 20 times nerve threshold, with square pulses of 100 μs duration and with a frequency of 1 Hz.

A laminectomy of the C2 vertebra was made and the spinal cord transected in the C3 segment sparing only the right ventral funiculus (4 cats) or the dorsal funiculi and the right ventral funiculus (14 cats). The right ventral funiculus was stimulated 2–3 mm caudal to the lesion with a monopolar electrode (cathode) touching the surface. The cord was covered with a high density insulating carbonfluor liquid (FC 40, 3M Company) to prevent drying of the cord and shunting of stimulation currents. The cerebellar anterior lobe was exposed on the left side and was covered with warm mineral oil. The cerebellar surface was stimulated with monopolar silver ball electrodes which could be used either as anodes or cathodes, the indifferent electrode being placed in the temporal muscle. To increase recording stability, a bilateral pneumothorax was performed.

The climbing fibre responses of single Purkinje cells were recorded with glass capillary microelectrodes filled with a 3 M KCl solution and having a resistance of 5.0–9.0 MΩ. In most experiments, the responses were also recorded as negative field potentials from the molecular layer or as positive surface potentials recorded with silver ball electrodes.

Results

I. Mutual Inhibition in the b Zone

Some of the experiments, which will be described first, were performed on cats under pentobarbitone anaesthesia and with the spinal cord transected in the third cervical segment sparing only the right ventral funiculus. Thus, all known pathways from the spinal cord, except the Ventral Funiculus Spino-Olivo-Cerebellar Pathways (VF-SOCPs), were interrupted (Oscarsson and Sjölund 1977a). Climbing fibre responses were evoked in the left b zone in the lateral vermis of the anterior lobe on stimulation of peripheral nerves or on direct stimulation of the spino-olivary fibres immediately below the spinal cord lesion. Figure 1A shows records from a Purkinje cell, encountered in the lateral part of the b zone, which was activated on stimulation of both hindlimb nerves (iSci and cSci) and on stimulation of the ipsilateral ulnar nerve (iUln). Stimulation of the ventral funiculus (cVF) activated the spino-olivary fibres and evoked a response at a latency of 7 ms. The olivo-cerebellar neurones are activated monosynaptically by such a stimulus (Oscarsson and Sjölund 1977b).

Purkinje cells in the lateral and intermediate microzones, i.e. cells with peripheral receptive fields including the hindlimbs and/or the thorax (Andersson and Eriksson 1981) responded typically with this short latency to stimulation of the ventral funiculus. On the other hand, Purkinje cells activated exclusively from forelimb nerves and/or trigeminal nerves were very seldom activated by a ventral funiculus stimulation. Increasing the stimulus strength and/or the number of shocks to well above supramaximal stimulation, as judged from the climbing fibre responses recorded from the surface of the lateral b zone, did not increase the response probability of these Purkinje cells to ventral funiculus stimulation.

Climbing fibre responses could be evoked on ventral funiculus stimulation in only 9 out of 86 Purkinje cells which were activated either exclusively from forelimb nerves or from forelimb nerves and weakly from thoracic nerves. The response latencies were 9–13 ms (mean 10.5 ms) as compared to 7–9 ms (mean 7.7 ms) in the hindlimb activated cells. Such a cell is illustrated in Fig. 3A. It responded to ulnar but not to sciatic nerve stimulation (thoracic nerves were not stimulated in this experiment). The cell responded to ventral funiculus stimulation with a latency of 9 ms. The reason why ventral funiculus stimulation usually did not activate the forelimb cells is considered in Discussion.

In order to test if the absence of a response to ventral funiculus stimulation was due to a failure to activate the spino-olivary fibres which mediated the forelimb responses or to an inhibition produced by the ventral funiculus stimulation, forelimb nerve stimulation was conditioned with a stimulus to the ventral funiculus. Figure 1B shows records from a Purkinje cell activated from the ipsilateral forelimb (iUln) but not from the ventral funiculus (cVF). When ulnar nerve stimulation was preceded by a

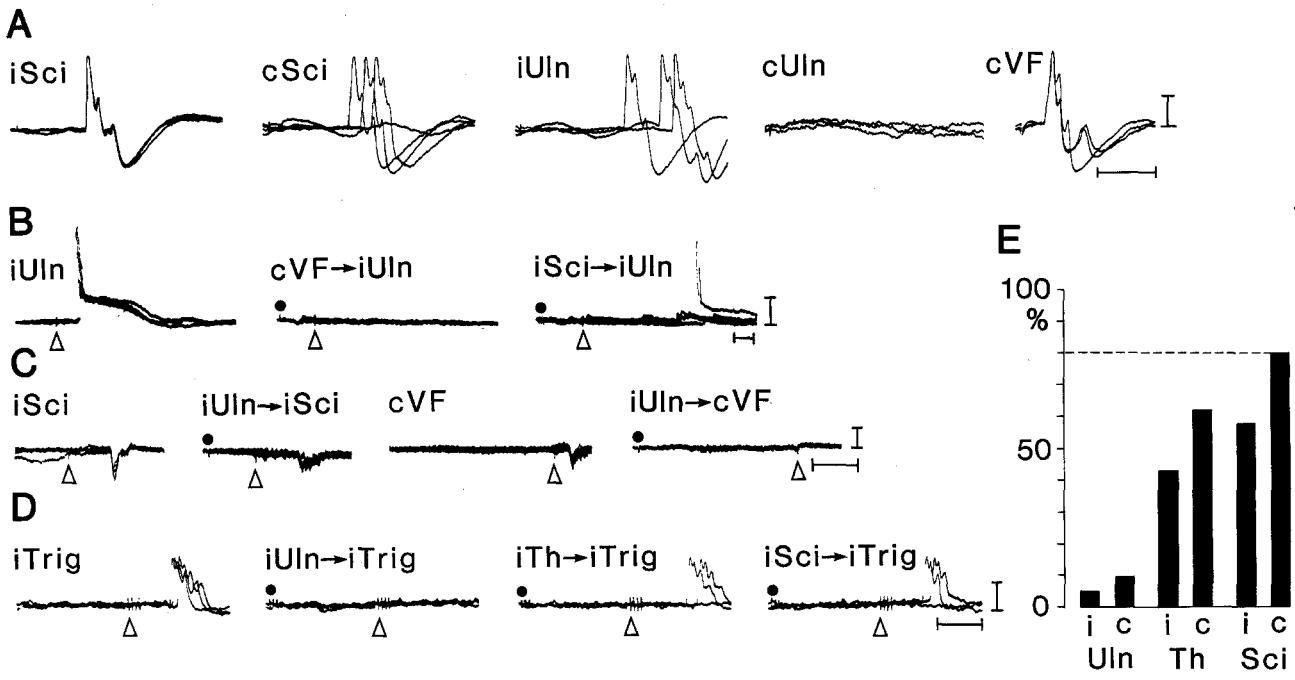

Fig. 1A. Intracellular recording of climbing fibre responses in a Purkinje cell in the lateral b zone. **B–D** Inhibition of climbing fibre responses – recorded in single Purkinje cells **B** and **D** or as molecular field potentials **C** – with conditioning stimulation of peripheral nerves or ventral funiculus. Filled circle indicates conditioning stimulus and triangle, test stimulus. Horizontal bars: 20 ms. Vertical bars: 5 mV in **A, B** and **D;** 1 mV in **C. E** Data from the cell shown in **D**. Vertical bars indicate the percentage of tested cases in which the cell responded to ipsilateral trigeminal nerve stimulation – with a latency of 30 ms or less – when it was conditioned with stimuli to the nerves indicated below the bars. Each conditioning was performed 20–40 times. Dashed horizontal line: response probability to control stimulation of the ipsilateral trigeminal nerve. Positivity in this and all other figures upwards

Abbreviations. Sci sciatic, Uln ulnar, Trig (infraorbital branch of) trigeminal, Th thoracic nerves, respectively; VF ventral funiculus; i ipsilateral; c contralateral

ventral funiculus stimulation, the response was inhibited. The response to ulnar nerve stimulation could also be inhibited with a conditioning stimulus to the ipsilateral sciatic nerve (iSci). In this case, a climbing fibre response occurred about 150 ms after the conditioning stimulation. Such rebound responses are described below (cf. Fig. 3B).

Figure 1C illustrates that the inhibition is reciprocal, i.e. forelimb input can also inhibit hindlimb input. Molecular layer field potentials were recorded in the lateral part of the b zone. Large responses were evoked on hindlimb nerve stimulation (iSci) but none on stimulation of the forelimb nerve (iUln). The amplitude of the response to sciatic nerve stimulation was markedly reduced when preceded by a conditioning ulnar nerve stimulation. The response on ventral funiculus stimulation (cVF) was also inhibited by a conditioning ulnar nerve stimulation.

The degree of inhibition was related to the proximity of the microzones responding to the conditioning and test stimuli. The Purkinje cell illustrated in Fig. 1D was encountered in the most medial microzone and was activated exclusively by stimulation of the trigeminal nerves. On ipsilateral trigeminal nerve stimulation (iTrig), the cell responded with a latency of 20–23 ms. When this stimulation was conditioned with stimuli to other nerves, the responses were inhibited. The graph (E) shows in what percent of tested cases the cell responded to trigeminal nerve stimulation with a latency of 30 ms or less when it was preceded by a conditioning stimulus, the interval being 50 ms. Each conditioning

Table 1. Summary of observations on inhibitory interactions in the b zone. Left column: conditioning (Cond.) and Test stimuli given. Middle column (Units): Number of Purkinje cells showing mutual inhibition. Numbers in brackets indicate total number of units tested. Right column: Number of experiments in which mutual inhibition was observed in responses recorded as surface potentials or molecular layer field potentials. Numbers within brackets indicate the number of experiments in which inhibition was tested

Cond.	Test	Units	Surface or molecular layer field potentials
Sci	– Uln	15 (16)	5 (5)
Uln	– Sci	2 (3)	9 (9)
Uln	– Trig	20 (20)	–

was performed 20–40 times. When trigeminal stimulation was preceded by stimulation of the ipsilateral ulnar nerve, the Purkinje cell responded in only 5% of the tested cases. When the conditioning stimulus was applied to the ipsilateral thoracic or sciatic nerve, the cell responded in 43% and 58% of the cases, respectively. Under control conditions, the cell responded in 80% of the cases to stimulation of the ipsilateral trigeminal nerve (dotted line). The contralateral nerves were less effective in producing inhibition than were the corresponding ipsilateral nerves.

Inhibition of climbing fibre responses, recorded either from single Purkinje cells or as surface potentials or molecular layer field potentials, was observed in all the experiments (Table 1). The amount of inhibition varied from a complete inhibition to a small reduction of response amplitude or response probability or to a prolongation of response latency.

The time course of the inhibition was studied on both molecular field potentials and on single Purkinje cells. Figure 2A illustrates an experiment in which the responses of a Purkinje cell to ipsilateral trigeminal nerve stimulation were recorded when a conditioning ventral funiculus stimulus was applied. The records to the right show extracellular recordings of the cell's responses to control stimulation (CON.) and to test stimulation at different conditioning-test intervals as indicated (in ms). In the graph, the ordinate indicates the percentage of responses with a latency of 30 ms or less to a test stimulus. The abscissa indicates the conditioning-test interval. Negative time means that the test stimulus was applied before the conditioning stimulus. The latency of the inhibition was very short. In fact, the test stimulus to the trigeminal nerve could precede the conditioning ventral funiculus stimulus with as much as 14 ms and the responses to the test stimulus were still inhibited to some extent.

The total duration of the inhibition in the case illustrated in Fig. 2A was about 85 ms. In most cases tested, the inhibition lasted between 70 and 110 ms. As a comparison, the time course of a typical recurrent inhibition is shown in Fig. 2B. The amplitudes of surface climbing fibre responses to contralateral sciatic nerve stimulation (cSci), recorded from the lateral b zone, are plotted against the interval between the test stimulus and a preceding stimulus to the same nerve. There was a complete inhibition of the test response at intervals less than 55 ms and a partial inhibition at intervals up to 100 ms. In most experiments where the recurrent inhibition was studied, the total duration was between 75 and 110 ms, which is in agreement with previous findings (Armstrong and Harvey 1966 and 1968; Armstrong et al. 1968). The duration of the recurrent and the mutual inhibition was thus similar.

In some cases, two components were observed in the time course of the mutual inhibition. This is illustrated in Fig. 2C. The molecular layer field potentials evoked in the lateral b zone on ventral funiculus stimulation were conditioned with stimulation of the ipsilateral ulnar nerve (same experiment as in Fig. 1C). The inhibition started at a conditioning interval of about 15 ms. After about 40 ms, a second and more powerful inhibition began which, at intervals between 55 and 80 ms, cancelled the test response completely. (Two components can also be discerned in Fig. 3C). Occasionally, only a short-lasting inhibition was seen. The short duration (30–40 ms) was not due to a weaker inhibition, since, in these cases also, the inhibition of the test response could be complete or almost complete at the optimal intervals. The underlying mechanisms of the two inhibitory components were not studied further.

Although not studied as extensively, mutual inhibition was also observed in cats under chloralose anaesthesia. It displayed the same characteristics (described above) as those found in pentobarbitone-anaesthetized cats.

The results shown in Figs. 1C and 2C illustrate that a substantial part, or all, of the inhibition occurred in the inferior olive, since ventral funiculus stimulation activates the olivary neurones monosynaptically (Oscarsson and Sjölund 1977b). To

Fig. 2A–D. Time course of inhibition of climbing fibre responses. **A** Response probability (latency 30 ms or less) of a Purkinje cell to stimulation of the ipsilateral trigeminal nerve when conditioned with a stimulus to the contralateral ventral funiculus at different intervals. Negative time means that the test stimulus preceded the conditioning stimulus. Each dot represents the percentage of responses to 5–20 stimulations. Dashed line indicates control response probability to ipsilateral trigeminal nerve stimulation. Records to the right show extracellular recordings of control response (CON) and from conditioned stimuli at the indicated intervals (in ms). **B** Amplitude of climbing fibre responses, recorded from the surface of the lateral b zone, on stimulation of the contralateral sciatic nerve (cSci) when preceded by a stimulation of the same nerve at different intervals. Dashed line indicates control response amplitude. **C** Amplitude of molecular field potentials in the lateral b zone on stimulation of the contralateral ventral funiculus when conditioned with a stimulus to the ipsilateral ulnar nerve at different intervals. **D** Probability of olivary reflex responses (latency 9 ms) in a Purkinje cell in the medial b zone (lobule Vc) on cathodal stimulation of the surface of the b zone (lobule IV) when conditioned with a stimulus to the contralateral ventral funiculus at different intervals. (Short-latency responses (4 ms) due to axon reflex in the climbing fibre not considered, see Text.) Horizontal bars: 20 ms. Vertical bars: 100 μV **B**, 1 mV **A** and **C** and 5 mV **D**. Symbols and abbreviations as in Fig. 1

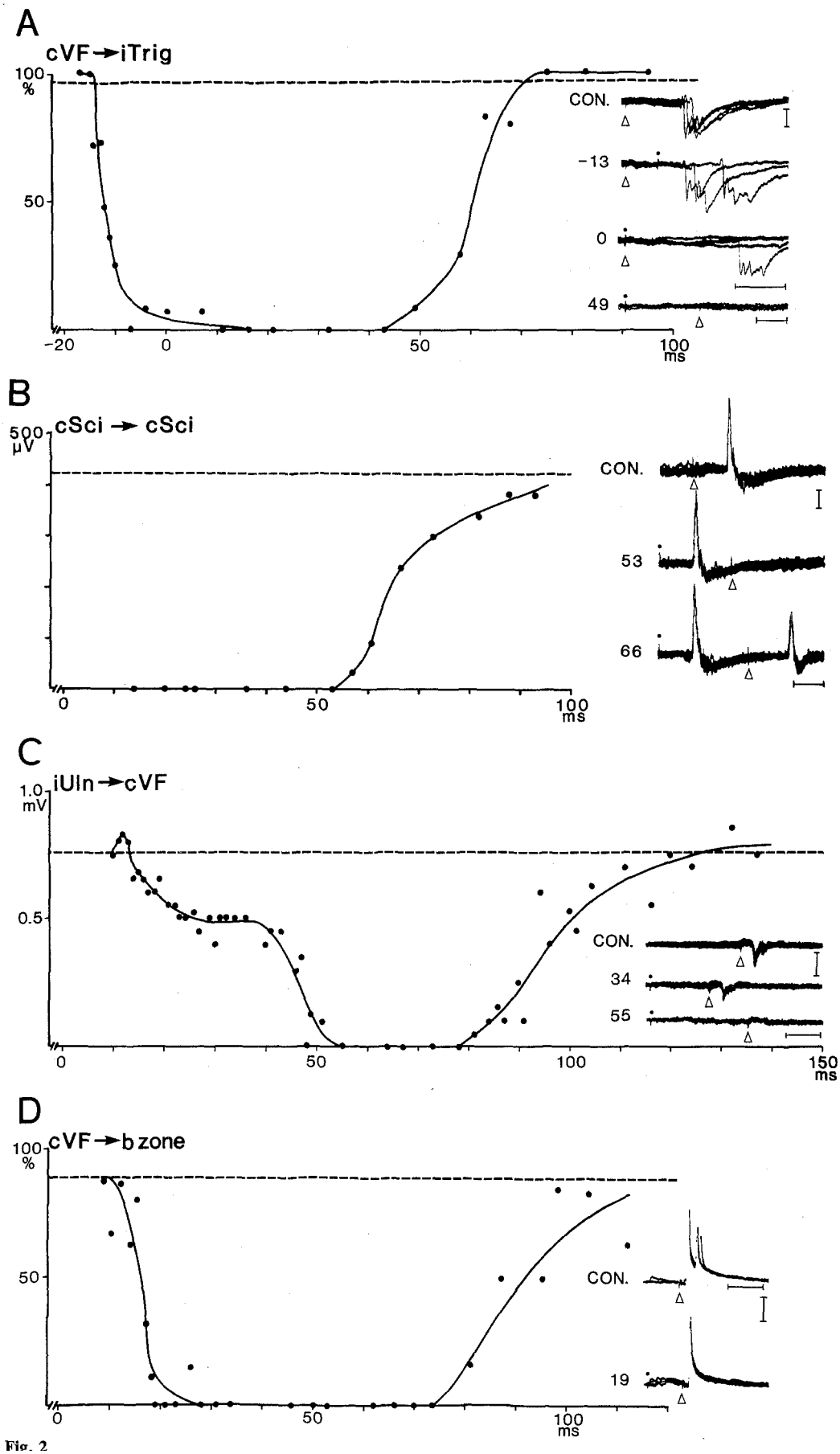
A
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Fig. 2

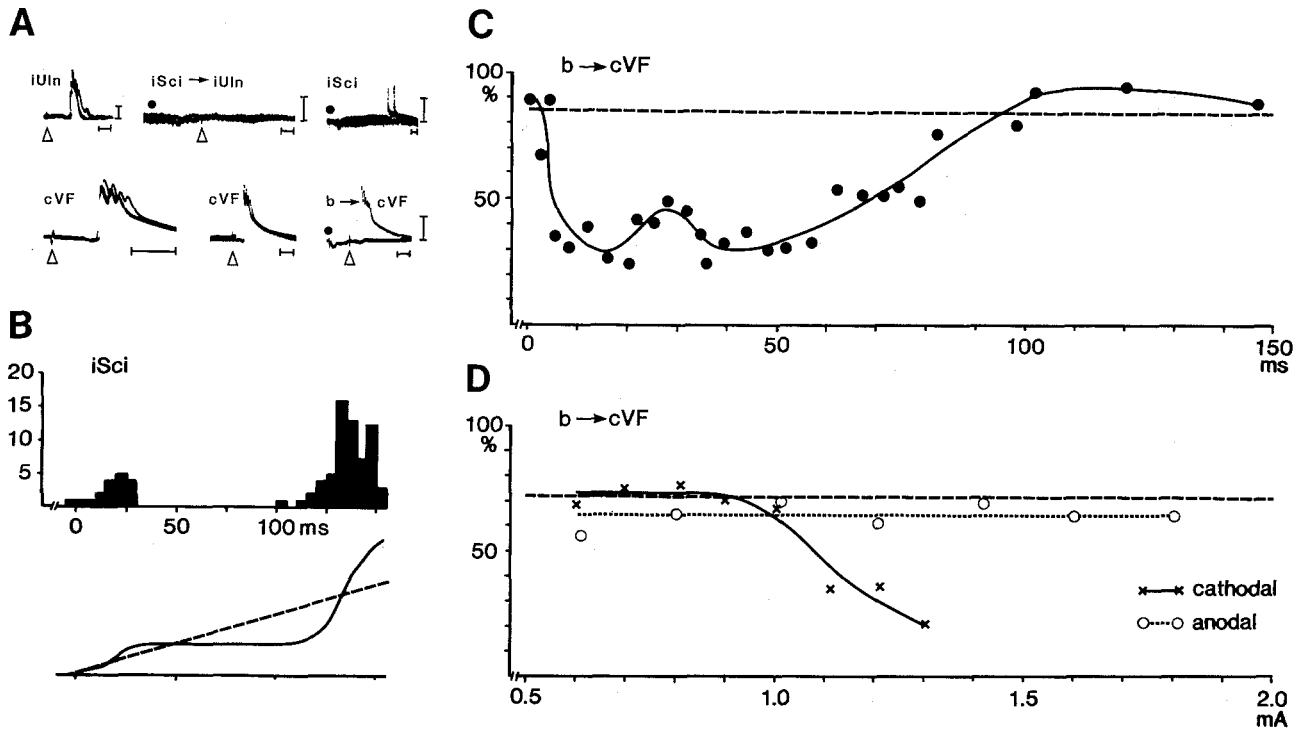

Fig. 3A–D. Climbing fibre responses in a forelimb activated Purkinje cell in the medial b zone. **A** Intracellular records of responses on stimulation of peripheral nerves, contralateral ventral funiculus and the surface of the b zone (b). Horizontal bars: 10 ms. Vertical bars: 1 mV in upper row and 4 mV in lower row. **B** Post-stimulus time histogram (upper part) and its integral (lower part) of the Purkinje cell's climbing fibre activity on stimulation of the ipsilateral sciatic nerve. Bin width 5 ms. Number of sweeps 340. Dashed line indicates a frequency of 1 Hz. **C** Time course of inhibition of climbing fibre responses to ventral funiculus stimulation when preceded by a conditioning stimulation (cathodal) of the surface of the lateral b zone. Each dot represents 10–30 measurements. Dashed line: control response probability. **D** Response probability of the Purkinje cell to ventral funiculus stimulation when preceded by a conditioning stimulus to the surface of the lateral b zone. Cathodal (continuous line) or anodal (dotted line) conditioning stimulation was used. Conditioning interval 28 ms. Dashed line: control response probability. Symbols and abbreviations as in Fig. 1

determine if it was due to a presynaptic inhibition of the olivo-petal fibres or to a postsynaptic inhibition of the inferior olivary cells, the excitability of the olivary cells was tested by evoking an olivary reflex (Armstrong and Harvey 1966; Eccles et al. 1966; Armstrong et al. 1973a). A mechanism by which an olivary reflex can be elicited was presented by Llinás and coworkers (1974), who reported an extensive electrotonic coupling between olivary cells. Through this coupling, a short-latency depolarization in an olivary neurone, due to antidromic invasion of adjacent neurones, was often sufficiently large to initiate spike generation. Thus, if an olivary reflex can be inhibited by a conditioning stimulus, the results strongly suggest that the inhibition occurs in the olivary neurones.

In Fig. 2D, the responses of a forelimb-activated Purkinje cell on stimulation of the surface of a more rostral folium in the b zone are shown. At a latency of 4 ms, the cell responded with a single EPSP due to an axon reflex in the climbing fibre. At 9 ms, a second response with a variable number of EPSPs (usually 1–2) appeared. This response was due to an olivary reflex. If stimulation of the b zone was preceded by a stimulus to the ventral funiculus, which in itself did not activate the cell, the olivary reflex response was inhibited while the short-latency axon reflex response was unaltered. Thus, at least part of the inhibition occurs in the olivary neurones.

In the Purkinje cell illustrated in Fig. 3, a climbing fibre response was evoked on forelimb nerve stimulation (A, iUln). This response was inhibited by a preceding stimulus to the ipsilateral sciatic nerve (iSci). Figure 3B shows a post-stimulus time histogram (upper part) and the integral (lower part) of this cell's climbing fibre responses on stimulation of the sciatic nerve. The dashed line indicates a frequency of 1 Hz, which was close to the spontaneous climbing fibre response frequency. After a weak initial facilitation, the spontaneous climbing fibre responses were completely suppressed between 30 and 100 ms. Following the inhibition, there was a strong rebound activation. Climbing fibre responses following the inhibition on ipsilateral sciatic nerve

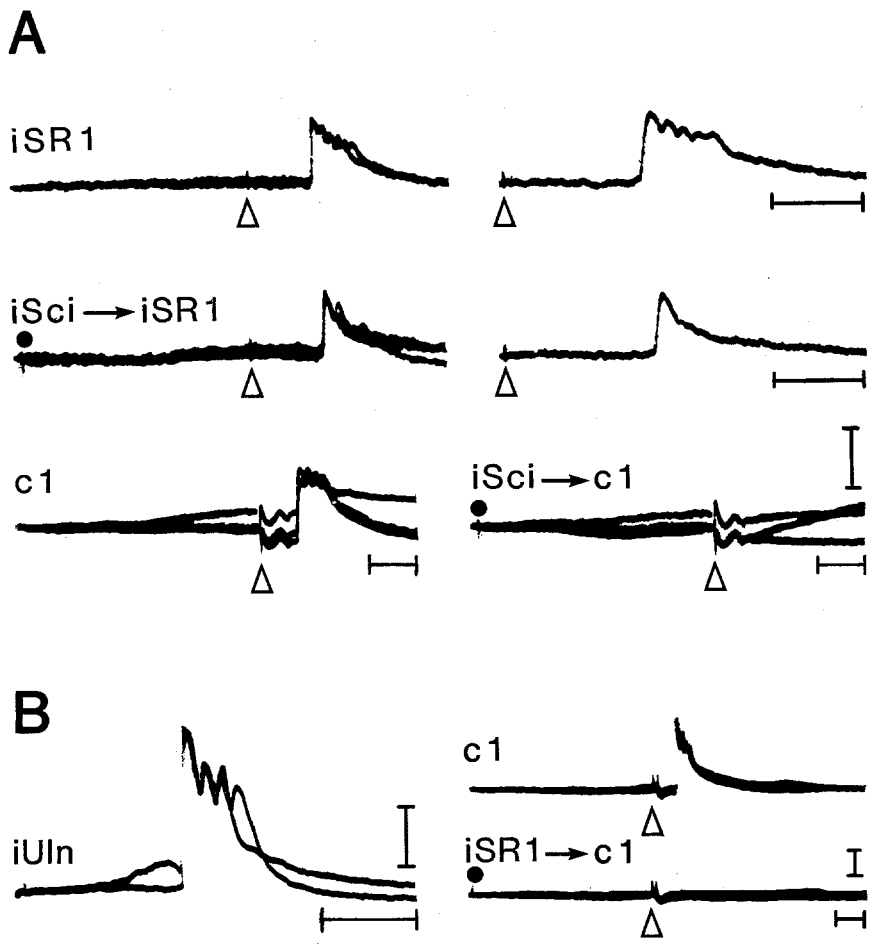

Fig. 4A. Climbing fibre responses of a Purkinje cell in lobule Va of the c3 zone to stimulation of the ipsilateral SR1 at two different sweep speeds (upper row) and when preceded by a conditioning stimulus to the ipsilateral sciatic nerve (second row). Third row: olivary reflex response on stimulation of the c1 zone and its inhibition when preceded by sciatic nerve stimulation. **B** Climbing fibre responses of a Purkinje cell in lobule Va of the c3 zone to stimulation of the ulnar nerve (left) and of the c1 zone (upper right). Lower right: inhibition of the olivary reflex response when the c1 stimulation was preceded by a conditioning stimulus to the SR1 nerve. Horizontal bars 10 ms. Vertical bars 5 mV. Symbols and abbreviations as in Fig. 1

stimulation (iSci), are illustrated in the record with slower sweep speed in Fig. 3A. Such a rebound facilitation at this latency was observed in many cells after an inhibitory stimulus. A similar rebound facilitation has also been observed after recurrent inhibition (Armstrong and Harvey 1966).

Inhibition of climbing fibre responses could also be produced by stimulation of the cerebellar surface. The Purkinje cell illustrated in Fig. 3 was activated on ventral funiculus stimulation with a latency of 9 ms. This cell was located in the medial half of the b zone in lobule Vc. Cathodal stimulation of the cerebellar surface in the lateral part of the b zone in lobule IV, which in itself did not activate this cell but those in more lateral microzones, inhibited the climbing fibre responses to ventral funiculus stimulation. The time course of this inhibition, shown in Fig. 3C, closely resembled that shown in Fig. 2.

The inhibition on cerebellar surface stimulation was easier to elicit with cathodal than with anodal stimulation. In Fig. 3D, the amount of inhibition is plotted against the stimulus strength. At stimulus strengths above 1 mA, cathodal stimulation inhibited the response to ventral funiculus stimulation. At strengths above 1.3 mA, cathodal stimulation would activate the cell through an olivary reflex due to stimulus spread into more medial microzones. Anodal stimulation did not affect the test responses even with a stimulus strength of 1.8 mA. In two more cells tested systematically, cathodal stimulation produced inhibition more effectively than did anodal stimulation. Since climbing fibres are more easily activated by cathodal than anodal surface stimuli in contrast with mossy fibres and Purkinje cells (Armstrong et al. 1973a and b; Clendenin et al. 1974), this finding suggests that the inhibition was produced by antidromic activation of olivo-cerebellar neurones.

During the long period of testing, there was a small excitability decrease of the olivo-cerebellar neurone projecting to this Purkinje cell. Thus, the cell responded in 85% of the cases to the control stimulus during the testing illustrated in Fig. 3C. When the testing illustrated in Fig. 3D was performed, the excitability had decreased so that the cell responded in only 72% of the control cases. In fact, there was a slight excitability decrease between the testing with cathodal and anodal conditioning stimulation which explains why the response probability on conditioning with anodal stimulation was slightly lower than the average control response probability.

Inhibition by surface stimulation could be elicited in 10 of 14 cells tested (in 6 experiments); it was rather weak and did not abolish the test response completely. This is most likely correlated with the relatively small number of olivo-cerebellar neurones activated by this kind of conditioning stimulation.

II. Mutual Inhibition in the c1 and c3 Zones

The mutual inhibition of climbing fibre responses was mainly studied in the b zone, as described above. In some of the cats in which the dorsal funiculi were left intact, the existence of mutual inhibition in olivary neurones projecting to other zones was studied. Most of this work was done on the c3 zone because of its topographically well organized climbing fibre input. Its rostral part receives hindlimb input and its caudal part (lobule V) is divided into narrow sagittal strips to which different forelimb nerves project (Oscarsson 1969; Ekerot and Larson 1979). These strips seem to correspond to the microzones in the b zone.

In Fig. 4A, records from a Purkinje cell in lobule Va of the c3 zone are shown. The cell was activated from the ipsilateral SR1 nerve at a latency of 14 ms. When a conditioning stimulus was applied to the ipsilateral sciatic nerve, the response from the SR1 nerve was delayed by about 2 ms. In addition, the control response of the Purkinje cell consisted of 4–5 EPSPs, while the conditioned response consisted of usually one EPSP. This is better seen in the two records to the right with a faster sweep speed.

Thus, a weak inhibition between hindlimb and forelimb inputs was found also in the c3 zone. The conditioned response was not abolished, as was often the case in the b zone, but only delayed and the number of spikes in the olivo-cerebellar axon were reduced (as judged from the number of EPSPs in the Purkinje cells). Similar tests were made in both the c1 and c3 zones with surface recordings. No, or only a small, reduction of the response amplitude on forelimb nerve stimulation was observed when it was conditioned with a sciatic nerve stimulation and vice versa.

To demonstrate that at least part of the inhibition was produced in the inferior olivary neurones, and not only in the cuneate nucleus, the inhibition of the olivary reflex was tested. The medial parts of the c1 and c3 zones receive collaterals of the same olivo-cerebellar axons (Ekerot and Larson 1982). Thus, stimulation of the c1 zone elicited a climbing fibre response with a latency of 9 ms in the cell illustrated in Fig. 4A, i.e. typical for the olivary reflex response. When this stimulation was conditioned with a stimulation of the ipsilateral sciatic nerve, the response disappeared. Thus, the conditioning stimulation caused an inhibition of the olivo-cerebellar neurones involved in this reflex.

Figure 4B illustrates mutual inhibition between two forelimb microzones in the c3 zone. The illustrated cell was activated from the ulnar nerve (left record) and the medial cutaneous nerve (not shown) but not from SR1. On stimulation of the c1 zone, an olivary reflex response was evoked (upper right). This response was inhibited when preceded by a conditioning stimulation of the SR1 nerve (lower right). Similar observations were made in four other cells. In addition, climbing fibre responses were recorded as molecular field potentials in one experiment. The olivary reflex response on c1 stimulation was completely inhibited by conditioning stimulation of the medial cutaneous nerve, which did not evoke any response in the microzone recorded from.

The inhibition in the c1 and c3 zones could also be produced by stimulation of the cerebellar surface. The time course was also similar to that in the b zone. Thus, the mutual inhibition in the c1 and c3 zones displayed the same characteristics as in the b zone, although the inhibition between fore- and hindlimb inputs was weaker.

Discussion

The present investigation has demonstrated mutual inhibition between neurones located in the dorsal accessory olive and projecting to adjacent microzones within a sagittal zone in the cerebellar anterior lobe. A similar, afferent inhibition of peripherally evoked climbing fibre responses has previously been described by Leicht et al. (1973) who also concluded that the inhibition did not occur in the cerebellar cortex. This is confirmed by the results shown in Fig. 2D. The Purkinje cell always responded at a short latency to the climbing fibre impulses mediated through an axon reflex on cerebellar surface stimulation, while the olivary reflex responses were inhibited.

As shown in Figs. 2D and 4, the excitability of the olivary cells is decreased when an appropriate conditioning stimulus is applied. Thus, at least part of the inhibition is mediated through a postsynaptic inhibition in the inferior olive and not through a presynaptic inhibition. This is consistent with the anatomical findings of Sotelo et al. (1974) who identified only a few axo-axonic synapses in the inferior olive. Likewise, in their study on inhibitory interactions in the dorsal cap, Takeda and Maekawa (1980) concluded that the inhibition occurred postsynaptically.

The mutual inhibition could be produced either through electrical synapses between the olivary cells or through inhibitory interneurones eliciting chemically mediated IPSPs. When olivary neurones are activated antidromically or orthodromically by the conditioning stimulus, a Ca^{++}-dependent K^{+}-permeability increase is triggered which gives rise to an afterhyperpolarizing potential that probably participates in generating the well known recurrent inhibition in the olivo-cerebellar system (Armstrong and Harvey 1966; Armstrong et al. 1968; Llinás and Yarom 1981). This hyperpolarization might be spread through electrotonic coupling (Llinás et al. 1974; Llinás and Yarom 1981) to the cells under study which were not activated by the conditioning stimulus and thus contribute to the mutual inhibition. However, for the following reason, this mechanisms can hardly be responsible for more than a small part of the inhibition. The mutual inhibition is very powerful. If it was mediated only through electrotonic coupling, one would expect an equally strong depolarization due to spread of the EPSPs, action potentials and large after-depolarizing potentials when olivary cells are activated (Llinás and Yarom 1981). Such a depolarization would be expected to lead to a marked facilitation of the conditioned cells. However, no, or only a very weak, facilitation of the spontaneously occurring climbing fibre responses was observed after the conditioning stimulation. A weak facilitation can be seen immediately before the inhibition in Figs. 2C and 3B. Since there are no sharp borders in the caudal dorsal accessory olive (DAO) between the termination areas of cervical and lumbar spino-olivary fibres (Boesten and Voogd 1975; Armstrong et al. 1982), it cannot be excluded that this weak facilitation was due to a direct activation of the cells from the olivopetal fibres activated by the conditioning stimulation. The abovementioned reason is in favour of inhibitory interneurones mediating most of the mutual inhibition.

In their study of the inferior olive, Llinás and coworkers (1974) recorded from what they presumed to be axons of periolivary interneurones. These units responded with bursts of action potentials on stimulation of the cerebellar white matter. The number of spikes depended on the stimulation strength and the latency indicated a monosynaptic coupling. The units also responded to peripheral stimulation and are good candidates for mediating the mutual inhibition. The chloride-reversible IPSPs found in inferior olivary neurones (Llinás et al. 1974) might be produced by these putative inhibitory interneurones.

The interneurones that are likely to be responsible for the mutual inhibition would be activated from collaterals of the olivo-cerebellar axons. Thus, the mutual inhibition appears to be recurrent. The present investigation cannot exclude the possibility of an additional activation of the interneurones from the olivo-petal fibres. Llinás et al. (1974) reported from a preliminary set of investigations that these cells seemed to respond more sensitively than the olivo-cerebellar neurones to peripheral nerve stimulation, suggesting that the excitation of these cells from the periphery is not solely mediated through collaterals of the olivo-cerebellar axons.

Oscarsson and Sjölund (1977b) reported that stimulation of the ventral funiculus (VF) in the third cervical segment evoked climbing fibre responses in the medial and lateral parts of the b zone at mean latencies of 7.1 and 6.9 ms respectively. From these and other results, they suggested that the forelimb and hindlimb components of the b-VF-SOCP have similar conduction velocities and synaptic delays in the inferior olive. If this was true, it would be difficult to explain why gross stimulation of the ventral funiculus does not usually activate forelimb Purkinje cells in the b zone but only those cells which also receive inputs from more caudal body segments, i.e. thorax and hindlimbs. The most likely explanation of the present findings is that the spino-olivary fibres of cervical origin have a slower conduction velocity than those of a more caudal origin. When a stimulus is applied through an electrode on the surface of the ventral funiculus, the lumbar spino-olivary fibres will activate the hindlimb olivo-cerebellar neurones. These will in turn produce inhibition of the forelimb olivo-cerebellar neurones before these latter are activated by the more slowly conducting cervical spino-olivary fibres. This explanation is supported by the finding that those few forelimb Purkinje cells which responded to ventral funiculus stimulation did so with a longer latency than did the hindlimb cells. When Oscarsson and Sjölund (1977b) recorded climbing fibre responses with surface electrodes, they could probably not record from only one microzone (cf. Andersson and Eriksson 1981). Therefore, when they recorded climbing fibre responses in the medial b zone on both forelimb nerve and ventral funiculus stimulation, it is not certain that the two stimuli activated the same population of Purkinje cells.

As shown in Fig. 4A, a conditioning stimulus to the sciatic nerve produced only a slight inhibition of the response to SR1 stimulation. The impulses in the cuneo-olivary neurones activated on SR1 stimulation probably cause a larger synaptic activation than that caused by stimulation of the c1 zone. This is the most likely explanation why the conditioned response is only delayed in the case of SR1 stimulation and completely abolished in the case of c1 stimulation.

The inhibition between hindlimb and forelimb inputs was much stronger in the b zone than in the c1 and c3 zones. This is probably correlated with the different organizations of the inputs to the caudal and rostral divisions of the dorsal accessory olive (DAO), the origins of climbing fibres to the b zone and to the c1 and c3 zones, respectively (Armstrong et al. 1974; Groenewegen and Voogd 1977; Groenewegen et al. 1979; Kawamura and Hashikawa 1979). In the caudal DAO, the termination areas of cervical and lumbar spino-olivary fibres are very close and to a large extent overlapping (Armstrong et al. 1982; Gellman et al. 1983). In the rostral DAO, the termination areas are segmentally arranged, so that the lumbar spino-olivary and gracile-olivary fibres terminate separate from the area of forelimb input via cuneo-olivary fibres (Boesten and Voogd 1975; Armstrong et al. 1982; Gellman et al. 1983). Thus, it seems as if the proximity of two groups of olivary neurones decides the amount of inhibition occurring between them (cf. Fig. 1E).

Armstrong and collaborators (1982) have described a columnar organization in the termination of spino-olivary fibres. In the rostrolateral DAO, which receives input from the ipsilateral hindlimb and projects to the rostral parts of the c1 and c3 zones, "successively more rostral segments (of the lumbar spinal cord) projected in a precise and orderly manner to successively more rostral and medial strips or columns of olive cells". Also in the termination of spinal fibres in other parts of the olive there were signs of a columnar type of topography. Armstrong et al. (1982) suggested that "each cortical microzone receives its climbing fibres from a narrow, longitudinally oriented column of olive cells within the larger population supplying the whole Voogd zone". If so, the present findings indicate that there is a mutual inhibition between these columns of olivary neurones. Andersson and Oscarsson (1978) suggested that the cerebellar cortical microzone with its associated subcortical neurones might represent the "basic computational unit" in the cerebellum. The columnar organization of the spinal input to the inferior olive (Armstrong et al. 1982) and the mutual inhibition between olivary cell groups shown in this report might reflect a synaptic organization in the olive that corresponds to the division of the cerebellum into "basic computational units".

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