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THE INHIBITORY EFFECT OF THE OLIVOCEREBELLAR INPUT ON THE CEREBELLAR PURKINJE CELLS IN THE RAT†

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SUMMARY

1. In rats under Nembutal anaesthesia the inferior olive region has been reversibly inactivated by applying a cooling probe to the ventral surface of the medulla. Simple and complex spike activity has been recorded from Purkinje cells of the cerebellar cortex.

2. Following cooling of the inferior olive of one side we have observed a remarkable increase of the simple spike activity in all the twenty-two Purkinje cells, showing a disappearance of the complex spike activity.

3. In some rats two Purkinje cells were recorded simultaneously from each side of the cerebellar cortex. Following cooling of the left inferior olive the effect on the Purkinje cell was observed only or predominantly on the contralateral cerebellar cortex.

4. In a group of animals the inferior olive has been destroyed by 3-acetylpyridine 4–221 days before the recording session. Cooling of the inferior olive region was not accompanied by any significant and consistent increase in the spike activity of presumed Purkinje cells of the contralateral cerebellar cortex.

5. These results indicate that the remarkable increase of the simple spike frequency following cooling of the inferior olive region is due specifically to the suppression of the activity of the olivocerebellar neurones.

6. Only a small amount of the simple spike frequency increase is attributable to the removal of the post-climbing fibre pause.

7. In some lesioned rats recording was made from Purkinje cells, which showed complex spikes due to the few surviving inferior olive cells. In these Purkinje cells cooling of the inferior olive region was accompanied by a disappearance of the complex spike and by a small increase of the simple spike frequency of discharge. Such an increase is mainly attributable to the removal of the post-climbing fibre pause.

8. These results suggest that a given Purkinje cell is not only under the inhibitory influence of its own climbing fibre, but also of other olivocerebellar neurones, probably through climbing fibre collaterals to the cerebellar cortical interneurones.

9. It is suggested that one role of the olivocerebellar system is to exert a powerful *tonic* inhibitory action on the Purkinje cells and consequently to exert a significant control on the excitability of the subcerebellar centres.

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† This paper is dedicated to Professor Giuseppe Monizzi.

INTRODUCTION

The Purkinje cell of the cerebellar cortex receives information through two main distinct afferent channels, the mossy and the climbing fibres, whose morphological and functional organization has been extensively investigated (Eccles, Ito & Szentágothai, 1967). However, the functional role of the climbing fibres is still far from being understood. This afferent channel has peculiar characteristics, which are very different from those shown by the mossy fibre input and from the generality of other brain neurones (Bloedel, 1973; Armstrong, 1974, 1978; Strata, 1976). One striking feature of the climbing fibre input is its pattern of discharge. This consists of a burst of action potentials, the complex spike, which, in resting conditions, occurs at a frequency close to 1/s. The frequency cannot be driven over 8–10/s, even in the case of strong excitation such as caloric or galvanic stimulation of the labyrinth (Ferin, Grigorian & Strata, 1970, 1971) or following harmaline administration (Lamarre & Mercier, 1971; Llinás & Volkind, 1973; Lamarre & Weiss, 1973). It therefore seems unlikely that the climbing fibre excitatory effect on Purkinje cell output can have a main role in cerebellar operation if one considers that the Purkinje cell fires a much high number of spikes, the simple spikes, under the drive of the mossy fibre input, or as an expression of its intrinsic (pace-maker) activity. Attention has therefore been repeatedly focused on the pause which follows the complex spikes, whose duration is from 20 to several hundreds of milliseconds (Granit & Phillips, 1956; Ferin *et al.* 1970, 1971; Bloedel & Roberts, 1971; Murphy & Sabah, 1971; Burg & Rubia, 1972). Indeed, in several instances an increase in the complex spike activity mirrors a reduction in the simple spike (Ferin *et al.* 1970, 1971; Ghelarducci, Ito & Yagi, 1975). When the frequency reaches 8–9/s a complete suppression of the simple spike can occur.

A beautiful demonstration of the importance of the inhibitory action of the climbing fibres has recently been provided by Colin, Manil & Desclin (1980). These authors have chemically poisoned the inferior olive, the source of the climbing fibres, with 3-acetylpyridine in order to kill its cells. They have shown that a few hours after the injection, when the complex spikes were failing, the background activity of the cerebellar cortex was increasing. They were able to record from one single Purkinje cell during this period and have shown a strict temporal relationship between the complex spike failure and the simple spike increase, during which the firing appeared very regular. According to these authors the increase is not only due to the suppression of the post-climbing fibre pause, but also to a reduction in an inhibitory action of the complex spike which outlasts the duration of the pause. The inhibition has been attributed to a long-lasting change in the excitability of the post-synaptic membrane, due to the withdrawal of synaptic activity between the climbing fibre and the Purkinje cell. They have ruled out the possibility that the increase in the simple spike frequency may be due to a concomitant failure of other inferior olivary neurones, which may exert an inhibitory action through climbing fibre collaterals impinging upon the cerebellar cortical inhibitory interneurones.

In line with this interpretation Rawson & Tilokskulchai (1981) have shown that repetitive stimulation of the climbing fibres may suppress the simple spike activity, and have provided evidence that such an inhibition is not mediated by the cerebellar cortical interneurones.

More recently Ekerot & Oscarsson (1980, 1981) have shown prolonged depolarization (up to more than 600 ms) elicited in the dendrites of the Purkinje cell by climbing fibre stimulation. It has been suggested that this depolarization, by shunting the dendritic membrane, may be responsible for the decreased probability of firing which follows each complex spike.

Our experiments have been aimed at studying the effect of the suppression of the climbing fibre activity with a different technique, where the inferior olive has been inactivated in a reversible manner by cooling. This technique avoids possible criticisms of toxic and unspecific effects due to the 3-acetylpyridine and enabled us to examine reversible effects on many Purkinje cells. Our results give full support to the findings by Colin *et al.* (1980), concerning the inhibitory effect of the olivocerebellar system. However, in addition to a possible phasic inhibitory mechanism associated with a direct action of a climbing fibre on a Purkinje cell, the data also show that the olivocerebellar pathway exerts a strong tonic inhibition probably mediated by the cerebellar interneurones.

Some of these results have been published as an abstract (Montarolo, Palestini & Strata, 1981).

METHODS

The experiments were performed on twenty-nine Wistar rats (body weight 200–360 g). In seventeen of them a lesion to the inferior olive was performed with 3-acetylpyridine (68 mg/kg), harmaline (15 mg/kg) and niacinamide (300 mg/kg), administered intraperitoneally, according to the temporal sequence suggested by Llinás, Walton, Hillmann & Sotelo (1975). The lesions were performed between 4 and 221 days before the recording. All the animals were anaesthetized with Nembutal (40 mg/kg I.P.). As a first surgical step the femoral vein and the trachea were cannulated. Additional doses of anaesthetic were given intravenously when required. The ventral part of the occipital bone, close to the occipital foramen, was exposed. A hole 1·5 mm in diameter was made with a dental drill at the level of the inferior olivary nucleus of the left side. The dura mater was left intact. A cooling probe of 1·2 mm in diameter was then inserted into the hole in contact with the dura over the lateral part of the corticospinal tract and fixed with dental cement. A very small screw was attached to the bone near the hole in order to give a better holding capacity to the cement. The system of cooling was a Freon-operated cooling probe described elsewhere (Berlucchi, Maffei, Moruzzi & Strata, 1964). The rat was then mounted on a stereotaxic apparatus. The body temperature was kept constant automatically at 37·2 °C, by means of a heating pad. The electrocardiogram was continuously monitored and the experiment performed only if the basic heart frequency was 300–450 beats/min. Under continuous irrigation with warm Ringer solution we exposed the dorsal area of the cerebellum and then covered it with 1 % agar in Ringer solution. Glass micropipettes filled with a 4 M-NaCl solution were used to record the single-unit activity. These data together with the electrocardiogram and the signal related to the flow of Freon were recorded on a magnetic tape (Philips Analog 7) to be analysed later. The animal was artificially ventilated and sometimes paralysed with pancuronium bromide (Pavulon, Organon) given intravenously (500 μg/kg). The duration of the inferior olive cooling lasted up to 90 s. The unitary activity of the Purkinje cells consists of simple and complex spikes. When possible they were separated by a WP-I window discriminator and sent to an integrator with a time constant of 800 and 4000 ms respectively. The integrated activity was then written on an ink-writer, together with the electrocardiogram and the cooling monitor. The activity was also photographed on film for visual inspection.

At the end of the experiment we checked that the position of the cooling probe was correct. The brain of the lesioned rats was fixed in 10 % formalin for a later histological control of the inferior olive lesion.

RESULTS

When a micro-electrode is inserted into the depth of the cerebellum in such a way as to explore the cortical region, one records a sequence of low- and high-noise activity. The high-noise consists of several small negative spikes. Here, it is rather easy to isolate a single unit, which in 96·7 % of the cases (Benedetti, Montarolo, Strata & Tosi, 1982*b*) shows two types of activity, typical of the Purkinje cell: the complex spike due to the climbing fibre input, and the simple spike due to the mossy fibre input or to the intrinsic activity of the cell.

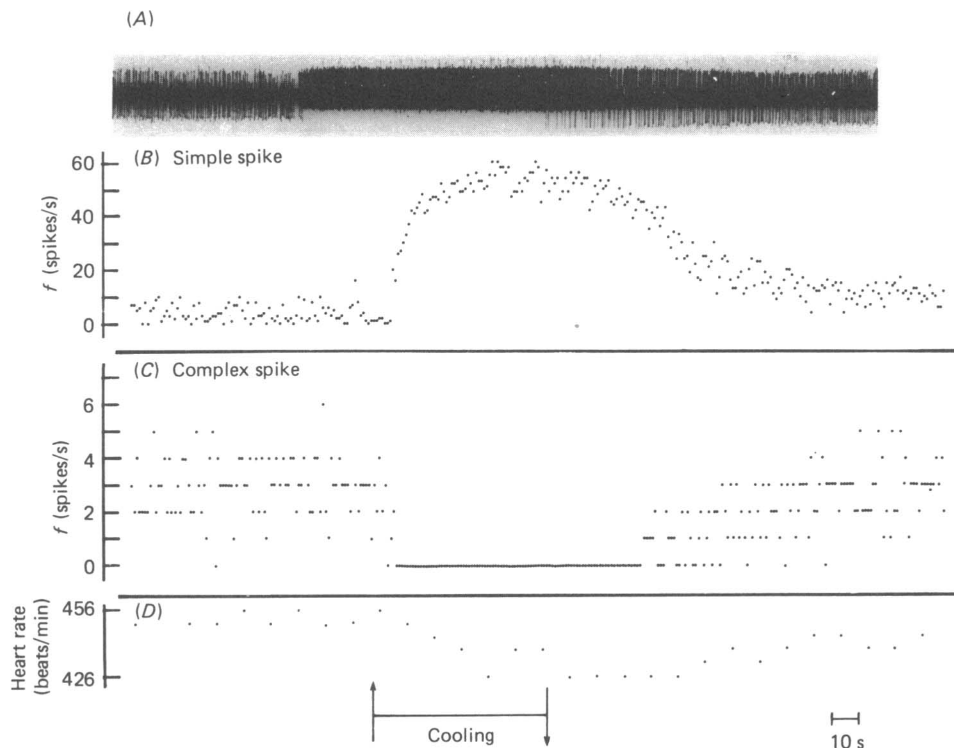

Fig. 1. Effect of cooling the left inferior olive on the Purkinje cell activity of the cerebellar cortex of the right side. *A*, specimen of Purkinje cell activity. Simple spikes have a mainly upward (negative) direction and complex spikes a mainly downward direction. *B*, the frequency (*f*) of the simple spikes has been counted every second on a specimen recorded at a more expanded time base. *C*, similar counts for the complex spikes. *D*, heart rate, in beats/min. Each dot represents the average rate calculated over a period of 10 sec. Period of cooling is indicated by the horizontal bar.

Fig. 1 shows the effect of cooling the left inferior olive on the simple and complex spike activity of a Purkinje cell located in the contralateral cerebellar cortex. Before the cooling the frequency of simple-spike discharge (Fig. 1 *B*) is around 5/s, whereas that of the complex spike is near 3/s (Fig. 1 *C*). A few seconds after the beginning of the cooling the complex spikes disappear completely, whereas the simple spike activity slowly rises to reach in about 10 s a steady-frequency level around 50–55/s. At the end of the cooling, there is at first a slight tendency for the simple spike

discharge to decline in frequency, then a gradual appearance of the complex spikes, which slowly reassume a frequency level similar to that of the control. Also the simple spike activity tends towards the initial control value. This remarkable increase of the simple spike activity accompanying the disappearance of the complex spike has been consistently found in all the twenty-two Purkinje cells tested. The increase lasted at least for the entire period of cooling, which was up to about 90 s.

In Fig. 2*A* the height of each column represents the simple spike frequency of each of the twenty-two cells calculated over the 20 s preceding the cooling. The filled circles represent the simple spike frequency reached during cooling, calculated in a period of

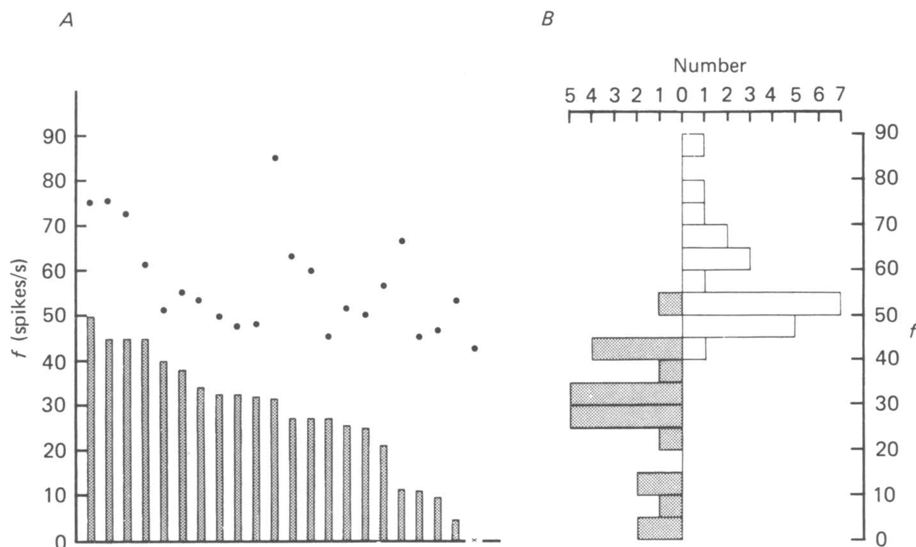

Fig. 2. Simple spike frequency (f) of Purkinje cells in relation to cooling. *A*, the height of each column represents the average simple spike frequency (spikes/s) of the twenty-two recorded Purkinje cells, calculated over a period of 20 s preceding the cooling. The filled circle above each column indicates the average frequency reached during cooling . *B*, frequency histogram of the twenty-two units before (left shaded horizontal columns) and during (right unshaded ones) cooling.

20 s, starting 10 s after the disappearance of the complex spike. Fig. 2*B* shows that before cooling (left) many units (ten out of twenty-two or 45·5 %) have a frequency of 25–35/s, whereas during cooling (right) the majority of these units (twelve out of twenty-two or 54·5 %) have a frequency of 45–55/s. Before cooling the average simple spike frequency was 28·02/s ($\pm$13·7 S.D.), whereas during the plateau phase of the cooling it was 57·62/s ($\pm$11·9 S.D.). The difference is significant (Student's t test, $P < 0{\cdot}001$). Fig. 3 shows that there is a good correlation between the amount of increase shown on the ordinate (Δf) and the simple spike frequency (f) present before cooling, shown on the abscissa, the increase being higher when the control frequency is lower.

Together with these effects on the Purkinje cells of the contralateral cerebellar cortex, we have often found a decrease in the heart rate during cooling (Fig. 1*D*). This decrease was usually in the range of 3–5 % and always below 10 %. It should also

be noted that the decline in frequency of the simple spike, which occurs in Fig. 1*B* after the end of cooling and before the appearance of the first complex spike, was not an invariable finding. In other units the first complex spike could appear very soon after the cooling and the simple spike activity could start to decline a little after this event. In other words, while there is a good correlation between the fast disappearance of the complex spike and the increase in simple spike activity at the beginning of cooling, a similar inverse relation is not strictly present on the more gradual recovery at the end of cooling.

Although this result suggests that the simple spike frequency increase is the consequence of the complex spike suppression, we cannot rule out the possibility that

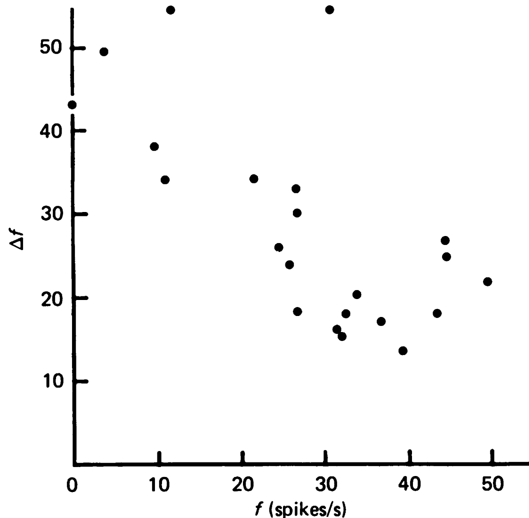

Fig. 3. Correlation between frequency increase (Δf) following cooling and control frequency (f) of the simple spikes for the twenty-two Purkinje cells shown in Fig. 2.

the increase may be due to unspecific effects of cooling. The cooling probe is very close to important vegetative centres. In fact, as noted above, a change in heart rate was often associated with cooling. Furthermore, cooling of other afferent pathways to the cerebellum may also be responsible for the effect. The results illustrated in Figs. 4 and 5, however, rule out these possible objections.

In the experiment shown in Fig. 4 we have recorded at the same time two Purkinje cells, one on the side ipsilateral to cooling (*A* and *B*) and one on the contralateral side (*C* and *D*). It can be seen that the remarkable increase in simple spike frequency (*C*) is present only in the contralateral side, where there is a complete suppression of the complex spike (*D*), whereas on the ipsilateral side the complex spike pattern of discharge (*B*) does not change appreciably and the simple-spike activity (*A*) shows a modest increase. Similar results have been obtained on nine pairs of Purkinje cells recorded simultaneously, where on the ipsilateral side the simple spike frequency increased slightly or it did not increase at all. In a few ipsilateral units a slight decrease in the complex spike rate of discharge was also observed. In others it was possible to suppress the complex spike completely with prolonged cooling. In this case

a clear-cut simple spike frequency increase was also present. Another sample of five Purkinje cells recorded only on the ipsilateral side confirmed these findings. In three of them there was no change in complex spike activity, whereas in the remaining two cells there was a slight decrease in complex spike activity. It is likely that the small decrease of complex spike activity found occasionally in the Purkinje cells of the

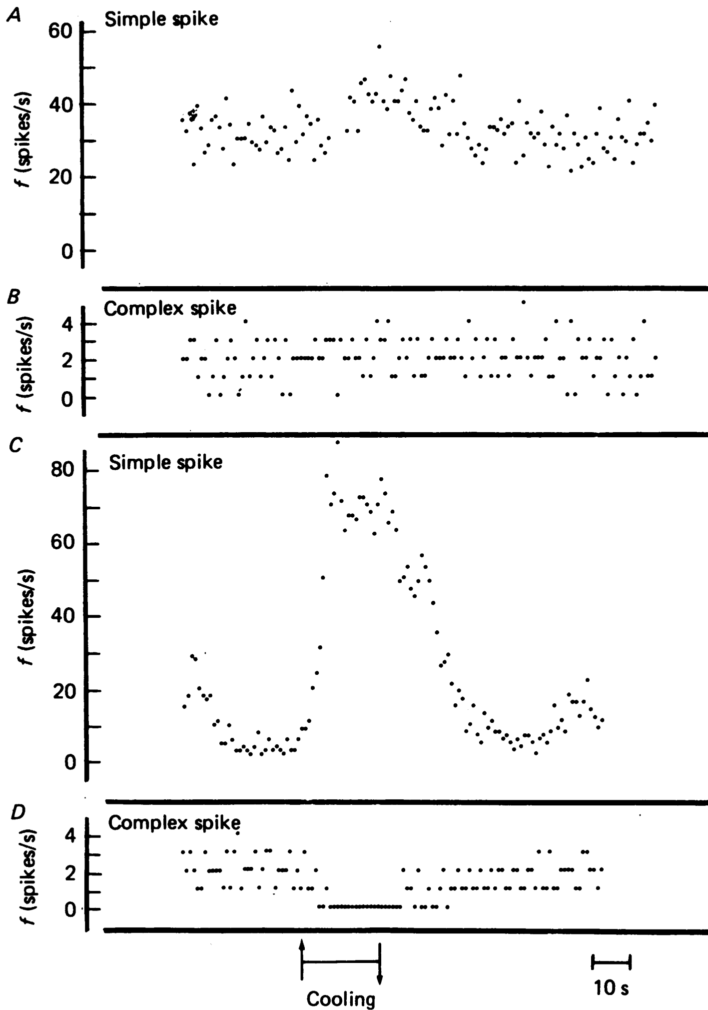

Fig. 4. Simple spike and complex spike activity of two Purkinje cells recorded simultaneously from the left (*A* and *B*) and the right (*C* and *D*) side of the cerebellar cortex, during cooling of the left inferior olive. Plots as in Fig. 1.

ipsilateral side or the total suppression obtained with prolonged cooling is due to inactivation of olivocerebellar fibres originating in the inferior olive of the opposite side. It is known that some of them cross the brain stem at the medullary level and become intermingled with the somata of the ipsilateral inferior olive (Batini, Corvisier, Destombes, Gioanni & Everet, 1976; Courville & Faraco-Cantin, 1978).

In the experiment illustrated in Fig. 5 the inferior olive of both sides was chemically destroyed as much as possible with 3-acetylpyridine 217 days before. In this experimental condition the identification of a Purkinje cell cannot be done on the basis of the presence of a complex spike, because they occur very rarely (see later). However, by applying the same criteria used in the intact rat it is possible to recognize the Purkinje layers and to isolate units which have a probability of over 95 % of being Purkinje cells (see above). Fig. 5*A* shows that before cooling the rate of discharge of this cell was between 20 and 30/s, and that during cooling of the contralateral inferior olive it sets itself at about 30/s. Clearly, cooling produced only a modest effect on the discharge of this cell, despite the fact that it was intense enough to produce a prominent effect on the heart rate. The lack of a clear frequency increase following

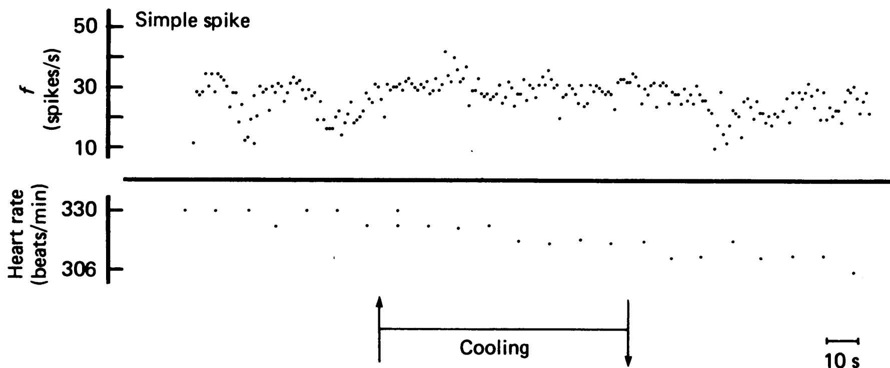

Fig. 5. Effect of cooling the left inferior olivary region, following inferior olive lesion, recorded from a presumed Purkinje cell of the right cerebellar cortex. *A*, frequency (*f*) of discharge of the unit, and *B*, heart rate plotted as in Fig. 1.

inferior olive cooling has been observed in twelve out of fifteen similarly recorded units. In some of these twelve units there was a slight decrease. In the remaining three units an increase of the simple spike frequency of discharge appeared rather late, around 20–30 s from the beginning of the cooling. In addition the increase had a very slow rise time and was always small. In fact, the simple spike frequency never reached the typical values around 50/s or more observed in the intact rat, when the complex spikes are suppressed. The average frequency of these fifteen units was 30·73/s ($\pm$9·2 S.D.) before cooling and 32·93/s ($\pm$10·5 S.D.) during cooling. It should here be noted that the average control frequency of discharge of these fifteen units is not significantly different from the simple spike frequency of the twenty-two Purkinje cells recorded in the intact rats. This means that the simple spike frequency increase following the inferior olive lesion, shown by Colin *et al.* (1980), and following inferior olive inactivation shown in our experiments undergoes a process of adaptation. The time course of such an adaptation is at present under investigation.

These results strongly support the view that the large simple spike frequency increase following the inferior olive cooling is due to the suppression of the activity of the olivo-cerebellar neurones, and that only minor and occasional variations may be attributed to the cooling of extra-olivary structures.

The question then arises whether the simple spike increase is due to the suppression

of the activity of only that olivocerebellar neurone impinging upon the Purkinje cell from which we are recording. If this is so, the increase is due to the suppression of the *phasic* pattern of excitation–inhibition of the Purkinje cell. In addition there is the problem whether the effect may also be due to the concomitant inactivation of other olivocerebellar neurones which, due to their asynchronous activity, may exert a *tonic* background inhibition possibly through the cerebellar cortical interneurones.

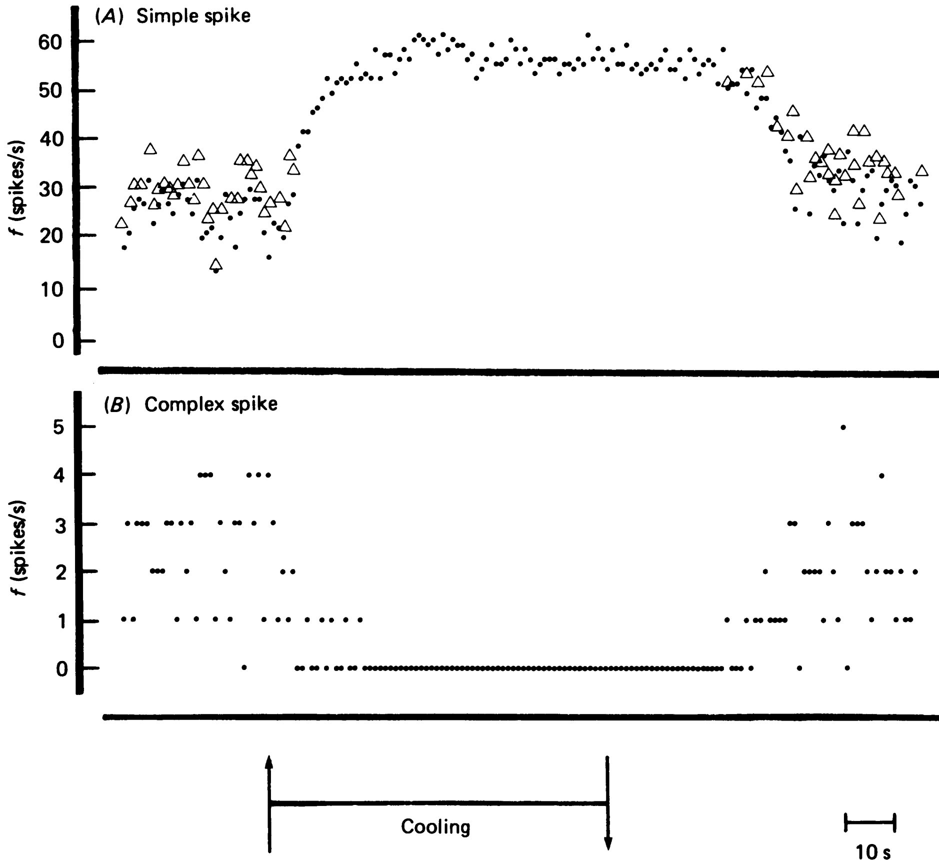

Fig. 6. Effect of inferior olive cooling on the Purkinje cell activity in an intact rat. In *A* and *B* the simple and complex spike activity have been plotted as in Fig. 1. The open triangles indicate the frequency of the simple spike calculated when the complex spikes were present, but without considering the time corresponding to the post-climbing-fibre pause.

It is known that every complex spike is followed by a pause in the simple spike discharge, whose length is in the range of 20 up to some hundreds of milliseconds. Fig. 6 provides data showing that the absence of the pause is not responsible for most of the increase in the discharge rate of the simple spike. The black dots indicate the simple spike discharge frequency, which is close to 20/s during the control period. The open triangles indicate the frequency when the time occupied by the post-climbing fibre pause is not taken into account: in each 1 s interval, when at least one complex spike was present, the time corresponding to each pause was subtracted. The

number of simple spikes present in the remaining time was expressed in terms of impulses/s. Its value in this case, is close to 30/s during the control period. During the suppression of the complex spike by cooling, the firing frequency reaches the typical value of 50–60/s. This value is far higher than the control frequency of the simple spike when computed without considering the time corresponding to the post-climbing fibre pause (open triangles). In eight of the twenty-two Purkinje cells recorded in the intact rats we have obtained corresponding data. The average simple spike frequency was 30·81/s (±6·4 S.D.). When this frequency was calculated without considering the time corresponding to the post-climbing fibre pause, the value was 36·12/s (±6·2 S.D.), whereas the average simple spike frequency reached during the plateau phase of the cooling was 58·61/s (13·4 S.D.). The difference between

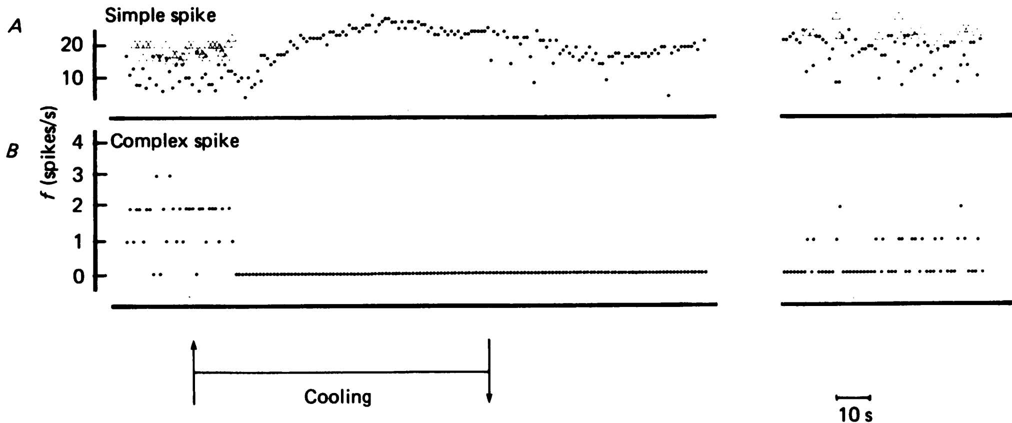

Fig. 7. Effect of cooling the few surviving cells of the left inferior olive on the simple spike and complex spike activity of a Purkinje cell of the right side. *A*, simple spike activity plotted as in Fig. 1. The open triangles indicate the frequency calculated without considering the period corresponding to the post-climbing fibre pause. *B*, complex spike activity. On the abscissa the plot interruption is of 108 s.

the two latter values ($\triangle f = 22{\cdot}49$) is statistically significant (Student's *t* test, $P < 0{\cdot}001$). These results support the view that the olivocerebellar neurones exert an inhibitory effect which cannot be accounted for only by the duration of the post-climbing fibre pause. In fact Colin *et al.* (1980) have shown that the inhibition associated with the complex spike outlasts the duration of the pause (see their fig. 2*C–D*) and it is of the order of several seconds.

In order to see whether the simple spike frequency increase can be totally attributed to the removal of the inhibition which follows the complex spike, and not to the removal of a tonic inhibition exerted also by many olivocerebellar neurones, we have studied the effect of cooling the inferior olive in rats previously submitted to an almost complete lesion of the inferior olive by means of 3-acetylpyridine. If we assume that each Purkinje cell is only under the control of its climbing fibre, its activity should not be affected by the lesion of the nearby olivary neurones. Therefore, by cooling that particular climbing fibre one should expect an effect very similar to that obtained in the intact rat.

The unit shown in Fig. 7 was the only unit over a sample of thirty-seven cells to

show a complex spike. We can assume that there was only one or very few remaining olivocerebellar neurones projecting to this cerebellar area. Therefore, cooling of the inferior olive can now suppress the activity of only a very few olivary cells. Before cooling, the frequency of discharge of the simple spike (black dots) is around 10/s and during cooling it reaches a frequency of 20–25/s. The effect shown in Fig. 7 has been repeated fifteen times in this unit and it has been confirmed in seven more units, each recorded from a lesioned rat.

In Fig. 8*A* each vertical column indicates the simple spike frequency of each cell calculated over a period of 20 s preceding the cooling. The filled circles above each column indicate the simple spike frequency reached during cooling, calculated as in

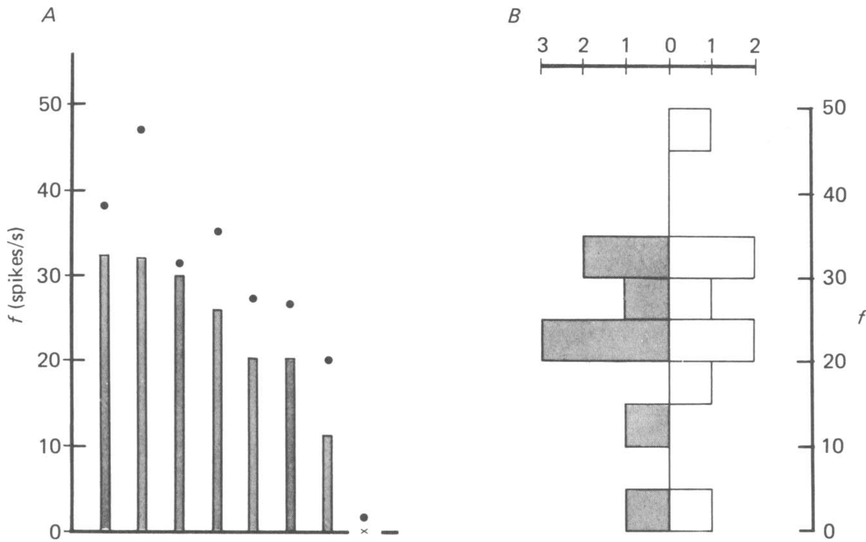

Fig. 8. Simple spike frequency (f) of Purkinje cells in relation to cooling of the lesioned inferior olive. The pattern of plotting is the same as shown in Fig. 2.

Fig. 2. Fig. 8*B* shows that before cooling six out of the eight units had a simple spike frequency of 20–35/s (left) and that during cooling (right) five out of eight remained in the same frequency range. For all eight Purkinje cells recorded in the lesioned rats the average simple spike frequency was 21·22/s ($\pm$11·2 S.D.), a value which is lower (but not significantly) than that found in the intact rats. The average value reached during cooling was 28·54/s ($\pm$14·4 S.D.).

Fig. 7 provides evidence that in these units the increase of the simple spike rate of discharge is mainly attributable to the suppression of the post-climbing fibre pause. If we now calculate in each second the simple spike firing frequency, without taking into account the time occupied by the post-climbing fibre pause, we obtain the values shown in Fig. 7 as open triangles. The increase of the simple spike discharge rate, following inferior olive inactivation, appears to be mainly attributable to the removal of the pause accompanying the complex spike. The same conclusion holds true also for the whole group of eight units. In fact their average simple spike frequency, when calculated without considering the time corresponding to the post-climbing fibre

pause, was 25·23/s ($\pm$12·4 S.D.). If we compare this value with that calculated during the plateau phase of the cooling, we obtain a Δf value of only 3·30. This value is significantly lower ($P < 0{\cdot}01$) than that calculated for eight units in intact rats, as reported above ($\Delta f = 22{\cdot}49$). This difference indicates that when we suppress the activity of many olivocerebellar neurones, the simple spike frequency of the Purkinje cell consistently reaches a much higher value than when we suppress the activity of only that olivocerebellar neurone which impinges upon the Purkinje cell under examination and possibly a few others.

Fig. 9 shows for each of the eight units from chemically lesioned rats the correlation between the amount of increase (Δf; ordinate) and the simple spike frequency before cooling (f; abscissa). From this illustration it appears that the simple spike frequency increase due to cooling of the inferior olive is not only smaller than that shown in Fig. 3, but also it does not show a clear correlation with the control frequency.

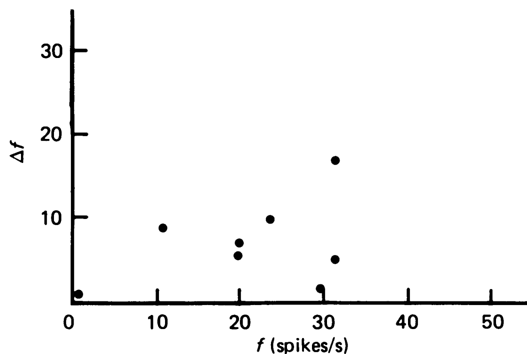

Fig. 9. Correlation between frequency increase (Δf) following cooling and control frequency (f) of the simple spikes for the eight Purkinje cells shown in Fig. 8.

Since the average complex spike frequency in the lesioned rats for the eight tested units was 1·12/s, whereas in the eight units of the intact rats it was 1·69/s, one may argue that the suppression of the complex spike activity with a higher frequency value may be responsible for the higher Δf in the second sample. We have therefore attempted to compare Δf values only on those units of the two samples which had a complex spike frequency between 1 and 2/s. In this case the average complex spike frequency calculated in five units of the lesioned rats was 1·49/s ($\pm$0·4 S.D.), whereas that calculated in six units of intact rats was 1·39/s ($\pm$0·3 S.D.). In the first sample the simple-spike frequency computed without considering the time corresponding to the post-climbing fibre pause was 29·51/s ($\pm$9·2 S.D.) before cooling and 33·94/s ($\pm$11·1 S.D.) during cooling ($\Delta f = 4{\cdot}49$), and the difference is not significant. In the second sample of six units the two values were respectively 37·35/s ($\pm$6·2 S.D.) and 53·84/s ($\pm$4·9 S.D.) ($\Delta f = 16{\cdot}31$) and the difference is highly significant ($P < 0{\cdot}001$).

DISCUSSION

The synapse between the climbing fibre and the Purkinje cell represents one of the most powerful excitatory mechanisms in the central nervous system, as shown by the large all-or-nothing depolarization always accompanied by at least one action

potential, but usually by several. A period of decreased probability of firing immediately follows this strong excitation (Granit & Phillips, 1956; Eccles, Llinás & Sasaki, 1966; Eccles *et al.* 1967; Llinás & Sugimori, 1980*a*, *b*; Crepel, Dhanjal & Garthwaite, 1981). During the first part of this period, lasting about 20 ms, the membrane of the Purkinje cell is incapable of generating action potentials. Then, the Purkinje cell is able to fire, but with a smaller probability for up to several hundreds of milliseconds (Bell & Grimm, 1969; Bloedel & Roberts, 1971; Martinez, Crill & Kennedy, 1971; see Armstrong, 1974). Very often an increase in the firing rate of the complex spike is accompanied by a reduction in firing of the simple spike (Ferin *et al.* 1970; 1971; Ghelarducci *et al.* 1975), an effect attributed at least in part to this inhibitory action. It would be expected that by removing the olivocerebellar input, the suppression of this inhibitory mechanism would lead to an increased firing rate of the simple spike. In fact, Colin *et al.* (1980) have shown that by suppressing the complex spike there is a remarkable increase of the simple spike firing and that the inhibitory effect associated with a complex spike outlasts the duration of the post-climbing fibre pause.

Part of our work has fully confirmed, by means of a different technique, the finding that by suppressing the complex spike there is an increase of the simple spike frequency. The reason for using a different technique was that in the experiment by Colin *et al.* (1980), suppression of the complex spike was mainly done by chemical lesion in the inferior olive with a substance which is also toxic, although possibly to a lesser extent, to other neurones. In addition, they recorded only one neurone, just at the moment when the inferior olive neurones were dying and the general condition of the rats was very critical (as pointed out by the authors themselves). The result was confirmed on two more neurones following surgical section of the olivocerebellar axons. The cooling technique was very suitable for inactivating the olivocerebellar neurones in a reversible manner and for confirming the existence of a long-lasting inhibitory action.

In our experiments the increase in simple spike frequency of firing is due specifically to the suppression of the inferior olive activity and not to unspecific effects of cooling nearby structures. This was shown by the experiments illustrated in Figs. 4 and 5. In the former, a clear increase of simple spike activity is present only on the Purkinje cell of the contralateral side, synchronously with the suppression of the complex spike. A clear-cut effect on the ipsilateral side was sometimes seen but only when, during a prolonged cooling, the complex spike of the ipsilateral side was suppressed. Such an effect is probably due to inactivation of olivocerebellar fibres originating on the contralateral side. Fig. 5 shows that, when the inferior olive is lesioned, cooling the inferior olive region is ineffective in increasing the Purkinje cell activity. The identification of the cells can perhaps be challenged, since no complex spike responses were present. However, as reported above (Benedetti *et al.* 1982*b*), when a Purkinje layer is identified by the typical background noise (Montarolo, Raschi & Strata, 1980) the probability that any unit recorded is a Purkinje cell is over 95%. In the present experiments a lack of effect was observed in all the fifteen units recorded in lesioned rats, whereas a clear-cut increase was seen in every Purkinje cell recorded in intact rats. It is therefore very likely that cooling of the inferior olive is the main cause of the large increase in simple spike activity. Only small effects, either in the direction

of increasing or decreasing the Purkinje cell activity, can be ascribed to different origins.

A major conclusion of our experiments is that the increase of the simple spike frequency following inferior olive cooling is not only due to the suppression of a transient inhibitory effect related to the complex spikes. This applies even if this transient inhibition outlasts the mere duration of the post-climbing fibre pause, and even if it lasts for several seconds, as hypothesized by Colin *et al.* (1980). If this were the unique mechanism of the inhibitory action, the same effect should have been obtained by suppressing the complex spike in those few olivary cells which survived 3-acetylpyridine intoxication. In these cells the increase in simple spike frequency can be related both to the suppression of the pause following the complex spike and possibly to a more prolonged inhibitory action. Quantitatively our effect (Fig. 7) is very similar to that shown by Colin *et al.* (1980) in their Fig. 1, where presumably only one or few olivary cells became silent at the moment of their recording. The far greater increase in simple spike frequency obtained when cooling the inferior olive in normal rats must be due to the suppression of the activity of many olivary neurones rather than only a few. This leads to the conclusion that a given Purkinje cell is not only under the inhibitory action of its own climbing fibre. The most likely way in which the inferior olive neurones can influence Purkinje cells on which they do not impinge directly is through the cerebellar cortical interneurones. Therefore, it is likely that the climbing fibre collaterals have an important role in exciting these interneurones directly.

Further support for this interpretation comes from the frequent observation shown in Fig. 1. When the cooling is stopped, the simple spike frequency started to decrease before the appearance of the first complex spike (Fig. 1). It seems likely that many olivocerebellar neurones which do not project directly to that Purkinje cell are able to exert an inhibitory influence on it. Again, this effect is probably mediated by climbing fibre collaterals to the cortical interneurones. A change in responsiveness of neighbouring Purkinje cells associated with the occurrence of a complex spike in one Purkinje cell has recently been reported (Bloedel & Ebner, 1981).

The climbing fibre input to the cerebellar cortex may be seen not only as a system generating transient excitation–inhibition sequences in the Purkinje-cell discharge (Armstrong, 1974; Strata, 1976; Gilman, Bloedel & Lechtenberg, 1981). It should also be considered as a system which, by tonically exciting the cerebellar cortical interneurones can exert a very powerful tonic inhibitory action on the Purkinje cells and therefore a powerful control on the excitability of the subcerebellar centres. Preliminary investigations have shown that the suppression of the inferior olive activity not only increases the Purkinje cell discharge, but at the same time induces a drastic reduction of activity in Deiters neurones (Benedetti, Montarolo & Strata, 1982*a*).

On the basis of our investigation it is not possible to decide whether all types of interneurones may be involved in this effect. Recently the existence and the numerical importance of the climbing fibre collaterals to the basket cells and to the Golgi cells has been questioned (Desclin, 1976; Leranth & Hámori, 1978; Hámori & Szentágothai, 1980; Desclin & Colin, 1980). However, in our opinion there is enough morphological evidence that contacts exist between climbing fibre collaterals and

basket cells, though they may be very few. In addition there is ample evidence that synaptic contacts exist between climbing fibre collaterals and Golgi cells (Scheibel & Scheibel, 1954; Hámori & Szentágothai, 1966; Lemkey-Johnston & Larramendi, 1968; Chan-Palay & Palay, 1971; Palay & Chan-Palay, 1974).

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REFERENCES

ARMSTRONG, D. M. (1974). Functional significance of connections of the inferior olive. *Physiol. Rev.* **54**, 358–417.

ARMSTRONG, D. M. (1978). The mammalian cerebellum and its contribution to movement control. *Int. Rev. Physiol. Neurophysiol.* **17**, 239–294.

BATINI, C., CORVISIER, J., DESTOMBES, J., GIOANNI, H. & EVERET, J. (1976). The climbing fibers of the cerebellar cortex, their origin and pathways in cat. *Expl Brain Res.* **26**, 407–422.

BELL, C. G. & GRIMM, R. J. (1969). Discharge properties of Purkinje cells recorded on single and double micro-electrodes. *J. Neurophysiol.* **32**, 1044–1055.

BENEDETTI, F., MONTAROLO, P. G. & STRATA, P. (1982*a*). Suppression of the olivocerebellar activity decreases the excitability of the vestibulospinal neurones. *Neuroscience* (suppl.) **7**, S 22.

BENEDETTI, F., MONTAROLO, P. G., STRATA, P. & TOSI, L. (1982*b*). *Collateral Reinnervation in the Olivocerebellar Pathway in the Rat.* In GIUFFRIDA STELLA, A. M., HABER, B., HASHIM, G. & PEREZ-POLO, J. R. (eds.). New York: Alan R. Liss. (In the Press.)

BERLUCCHI, G., MAFFEI, L., MORUZZI, G. & STRATA, P. (1964). EEG and behavioral effects elicited by cooling of medulla and pons. *Archs. ital. Biol.* **102**, 372–392.

BLOEDEL, J. R. (1973). Cerebellar afferent systems: a review. *Prog. Neurobiol.* **2**, 1–68.

BLOEDEL, J. R. & EBNER, T. J. (1981). Effects of spontaneously occurring climbing fiber inputs to the Purkinje cell on the responses of neighboring cells to mossy fiber inputs. *Neurosci. Abstr.* **7**, 640.

BLOEDEL, J. R. & ROBERTS, W. J. (1971). Action of climbing fibers in cerebellar cortex in the cat. *J. Neurophysiol.* **34**, 17–31.

BURG, D. & RUBIA, F. J. (1972). Inhibition of cerebellar Purkinje cells by climbing fiber input. *Pflügers Arch.* **337**, 367–372.

CHAN-PALAY, V. & PALAY, S. L. (1971). Tendril and glomerular collaterals of climbing fibers in the granular layer of the rat's cerebellar cortex. *Z. Anat. Ent. Gesch.* **133**, 247–273.

COLIN, F., MANIL, J. & DESCLIN, J. C. (1980). The olivocerebellar system. I. Delayed and slow inhibitory effects: an overlooked salient feature of cerebellar climbing fibers. *Brain Res.* **187**, 3–27.

COURVILLE, J. & FARACO-CANTIN, F. (1978). On the origin of the climbing fibers of the cerebellum. An experimental study in the cat with an autoradiographic tracing method. *Neuroscience* **3**, 797–809.

CREPEL, F., DHANJAL, S. S. & GARTHWAITE, J. (1981). Morphological and electrophysiological characteristics of rat cerebellar slices maintained *in vitro*. *J. Physiol.* **316**, 127–138.

DESCLIN, J. C. (1976). Early terminal degeneration of cerebellar climbing fibers after destruction of the inferior olive in the rat. Synaptic relationships in the molecular layer. *Anat. Embryol.* **149**, 87–112.

DESCLIN, J. C. & COLIN, F. (1980). The olivocerebellar system. II. Some ultrastructural correlates of inferior olive destruction in the rat. *Brain Res.* **187**, 29–46.

ECCLES, J. C., ITO, M. & SZENTÁGOTHAI, J. (1967). *The Cerebellum as a Neuronal Machine.* Berlin, Heidelberg, New York: Springer.

ECCLES, J. C., LLINÁS, R. & SASAKI, K. (1966). The excitatory synaptic action of climbing fibres on the Purkinje cells of the cerebellum. *J. Physiol.* **182**, 268–296.

EKEROT, C. -F. & OSCARSSON, O. (1980). Prolonged dendritic depolarization evoked in Purkinje cells by climbing fibre impulse. *Brain Res.* **192**, 272–275.

EKEROT, C. -F. & OSCARSSON, O. (1981). Prolonged depolarization elicited in Purkinje cell dendrites by climbing fibre impulses in the cat. *J. Physiol.* **318**, 207–221.

FERIN, M., GRIGORIAN, R. & STRATA, P. (1970). Purkinje cell activation by stimulation of the labyrinth. *Pflügers Arch.* **321**, 253–258.

FERIN, M., GRIGORIAN, R. A. & STRATA, P. (1971). Mossy and climbing fiber activation in the cat cerebellum by stimulation of the labyrinth. *Expl Brain Res.* **12**, 1–17.

GHELARDUCCI, B., ITO, M. & YAGI, N. (1975). Impulse discharge from flocculus Purkinje cells of alert rabbit during visual stimulation combined with horizontal head rotation. *Brain Res.* **87**, 62–72.

GILMAN, S., BLOEDEL, J. R. & LECHTENBERG, R. (1981). *Disorders of the Cerebellum.* Philadelphia: Davis.

GRANIT, R. & PHILLIPS, C. G. (1956). Excitatory and inhibitory processes acting upon individual Purkinje cells of the cerebellum in cats. *J. Physiol.* **135**, 520–547.

HÁMORI, J. & SZENTÁGOTHAI, J. (1966). Identification under the electron microscope of climbing fibers and their synaptic contacts. *Expl Brain Res.* **1**, 65–81.

HÁMORI, J. & SZENTÁGOTHAI, J. (1980). Lack of evidence of synaptic contacts by climbing fibre collaterals to basket and stellate cells in developing rat cerebellar cortex. *Brain Res.* **186**, 454–457.

LAMARRE, J. & MERCIER, L. A. (1971). Neurophysiological studies of harmaline-induced tremor in the cat. *Can. J. Physiol. Pharmac.* **49**, 1049–1058.

LAMARRE, Y. & WEISS, M. (1973). Harmaline-induced rhythmic activity of alpha and gamma motoneurons in the cat. *Brain Res.* **63**, 430–434.

LEMKEY-JOHNSTON, N. & LARRAMENDI, L. M. H. (1968). Types and distribution of synapses upon basket and stellate cells of the mouse cerebellum: an electron microscopic study. *J. comp. Neurol.* **134**, 73–112.

LERANTH, Cs. & HÁMORI, J. (1978). Quantitative electron microscopy of synaptic terminals onto cerebellar basket neurons. *Neurosci. Lett.* Suppl. **1**, S 148.

LLINÁS, R. & SUGIMORI, M. (1980*a*). Electrophysiological properties of *in vitro* Purkinje cell somata in mammalian cerebellar slices. *J. Physiol.* **305**, 171–195.

LLINÁS, R. & SUGIMORI, M. (1980*b*). Electrophysiological properties of *in vitro* Purkinje cell dendrites in mammalian cerebellar slices. *J. Physiol.* **305**, 197–213.

LLINÁS, R. & VOLKIND, R. A. (1973). The olivo-cerebellar system: functional properties as revealed by harmaline-induced tremor. *Expl Brain Res.* **18**, 69–87.

LLINÁS R., WALTON, K., HILLMAN, D. E. & SOTELO, C. (1975). Inferior olive: its role in motor learning. *Science, N.Y.* **190**, 1230–1231.

MARTINEZ, F. E., CRILL, W. E. & KENNEDY, T. T. (1971). Electrogenesis of cerebellar Purkinje cell responses in cats. *J. Neurophysiol.* **34**, 348–356.

MONTAROLO, P. G., PALESTINI, M. & STRATA, P. (1981). Effect of inferior olive cooling on the Purkinje cell activity. *Neurosci. Lett.* Suppl. **7**, S 120.

MONTAROLO, P. G., RASCHI, F. & STRATA, P. (1980). On the origin of the climbing fibres of the cerebellar cortex. *Pflügers Arch.* **383**, 137–142.

MURPHY, J. T. & SABAH, N. H. (1971). Cerebellar Purkinje cell responses to afferent inputs. I. Climbing fiber activation. *Brain Res.* **25**, 449–467.

PALAY, S. L. & CHAN-PALAY, V. (1974). *Cerebellar Cortex: Cytology and Organization.* Berlin: Springer.

RAWSON, J. A. & TILOKSKULCHAI, K. (1981). Suppression of simple spike discharges of cerebellar Purkinje cells by impulses in climbing fibre afferents. *Neurosci. Lett.* **25**, 125–130.

SCHEIBEL, M. E. & SCHEIBEL, A. B. (1954). Observations on the intracortical relations of the climbing fibers of the cerebellum. *J. comp. Neurol.* **101**, 733–760.

STRATA, P. (1976). A general review of the physiological function of the neuronal machine in the cerebellar cortex. *Expl Brain Res.* Suppl. **1**, 103–112.