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Release of cerebellar inhibition by climbing fiber deafferentation

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Summary. Cerebellar units were recorded extracellularly in rats before and after an intraveinous injection of 3-acetylpyridine destroying selectively the IO. All the Purkinje cells show a loss of the complex discharge between 2 h 15 min and 2 h 45 min after treatment. This time, called the "critical period" corresponds to the degeneration of the neurons of the inferior olive as revealed by the decrease of their metabolic activity. The simple spikes of the Purkinje cells increase their discharge frequency soon after the climbing fibers cease firing. On the contrary the firing frequency of the inhibitory interneurons does not show significant changes after degeneration of the inferior olive. The efferent cerebellar neurons, including cells of the cerebellar and vestibular nuclei receiving the axon terminals of the Purkinje cells, decrease their discharge rate up to thirty times during and after the critical period. It is demonstrated that this effect is due to the increased inhibitory activity of the Purkinje cells deafferented from the climbing fibers, whereas the deafferentation of the efferent cerebellar neurones from the collaterals of the olivary cells has little impact.

Key words: Inferior olive – Purkinje cells – Efferent cerebellar neurones

Introduction

There is no doubt that the inhibitory activity of the Purkinje cells (PC) discovered (Ito et al. 1964) and studied in the 1960s (see Eccles et al. 1967) mostly depends on its double excitatory input from the climbing fibers (CF) and mossy fiber-granule cells. Although already described by previous authors (Buser and Rougeul 1954; Granit and Phillips 1956), it has been only recently established that the excitatory action of the climbing fibers also produces a long-lasting suppression of the granule cell excitation of the PC (Colin et al. 1980). In the absence of CF discharges, following the total and permanent destruction of the inferior olive (IO) with the neurotoxin 3-acetyl-pyridine (3AP), simple spikes show a greatly increased frequency of discharge (Colin et al. 1980; Montarolo et al. 1982; Batini et al. 1984). Also in this condition, two other observations have been reported concerning the intracerebellar and vestibular nuclei which contain the target neurones of the P.C.: a) an increased metabolic activity at the P.C. terminals attributed to the intense discharge of the simple spikes (Bardin et al. 1982); b) a reduction of monosynaptic IPSPs at the lateral vestibular nucleus by the PC activation (Ito et al. 1978).

These findings suggest that following destruction of the IO, the target cells receive opposite influence from the PC, being in the same time more inhibited due to an increased presynaptic activity, and less inhibited, due to a decreased efficiency of the IPSPs. We have done experiments to try to resolve this question at the level of the overall discharge frequency of the target cells. Understanding the mechanisms of IO influences on PC output to the intracerebellar and vestibular nuclei will further our knowledge of this system which is the basis for the control of motor function.

The experiments reported here will definitely demonstrate an increased inhibitory capability of the PC recently deprived of the CF discharges, leading to a reduced cerebellar output[1].

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1 A preliminary note of this work had been published (Batini and Billard 1984)

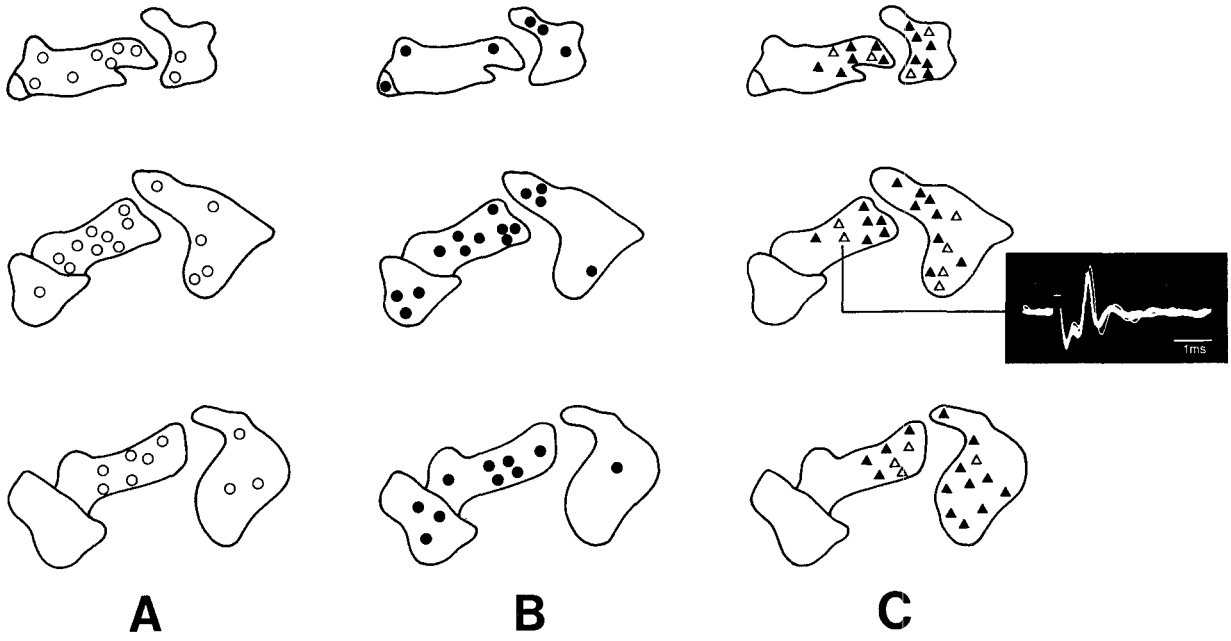

Fig. 1A–C. Schematic localization of the ECNs in the cerebellar nuclei from anterior (top) to posterior (bottom). **A** Controls; **B** Animals treated with 3AP; **C** animals with only cryocoagulation of the cerebellar cortex, open triangle, or a combined cryocoagulation of the cortex and 3AP treatment, filled triangles. The inset illustrates the antidromic identification of one neurone in the interpositus nucleus

Methods

The experiments were performed on 74 albino rats (weighing 250–350 g) anesthetized with sodium pentobarbital (40 mg/kg) during surgery.

Twenty-one were left intact and served as controls. Fourty-six were given an intraveinous injection of 75 mg/kg of 3-AP during the acute experiments in order to destroy the inferior olive. Discrete degeneration of neurones and their fibers in other brain structures not connected in series with the IO have been described (see Bardin et al. 1983 for literature); presumably they will not interfere with the present research. Seven rats had a large cryocoagulation of the cerebellar cortex using surface application of a piece of solid carbon dioxide for 10 to 20 s. Of these, 4 were treated with a neurotoxic dose of 3AP and 3 served as controls. A posterior craniotomy was made to expose the cerebellar surface where the microelectrodes could be introduced for exploration of either the cerebellar cortex or the intracerebellar nuclei. The exposed cerebellar cortex was covered with a gel of agar-agar 4% in Ringer solution. The animal was immobilized with flaxedil and artificially ventilated during all the experiments. The heart rate was continuously monitored during surgery and during the experiment.

Glass micropipettes filled with pontamine blue (4% in 5% NaCl solution) of 6–10 MΩ were used to record units extracellularly in the cerebellar cortex and in the cerebellar nuclei. The neurones of the cerebellar nuclei were recorded along stereotaxically oriented tracks and localized by iontophoretic pontamine deposits.

Most neurons of the cerebellar nuclei were identified by their antidromic firing following electric stimulation of their fibers. The stimulation was applied through a pair of concentric electrodes stereotaxically placed in the red nucleus.

Conventional stimulating, amplifying and displaying equipment were used. Units were recorded for a minimum time of 10 min. The frequency of spike discharges was determined using a counter over a minimum counting time of 5 min. The spike discharges to be counted were selected and transformed into uniform pulses by a window amplifier. The complex discharge of the Purkinje cells was counted as one spike.

Five rats were administered 100 μCi of [^{14}C]2-deoxyglucose intraveinously at the end of the recording period (see results) and kept alive for 45 min before being sacrificed. The brain was then rapidly removed and frozen in isopentane precooled to –45° to 50° C. Frontal sections of the brain stem were cut at 16 μ in a cryostat at –20° C and rapidly dried at 40° C. The sections were exposed for autoradiography on X ray film (single-coated SB5 Kodak) for a few days and then stained for Nissl substance.

The brainstem and cerebellum of all the rats used were removed post mortem and the serial frontal sections were Nissl substance stained for the histological control.

Results

The results reported here are from a total of 283 units which includes the activity of the PC and interneurones of the cerebellar cortex and of the neurones of the cerebellar nuclei. The relative number of units obtained in each group for a given experimental condition as described in the methods, are shown in the table. Unit activity was recorded, in all cases, as "spontaneous" activity, that is in the absence of any intentional stimulation. This was done in order to avoid artificial increase, or forcing, of the activity in one or more selected pathways, as is usually obtained with electric stimulation. In each experimental condition, for a given cell, the activity was counted for

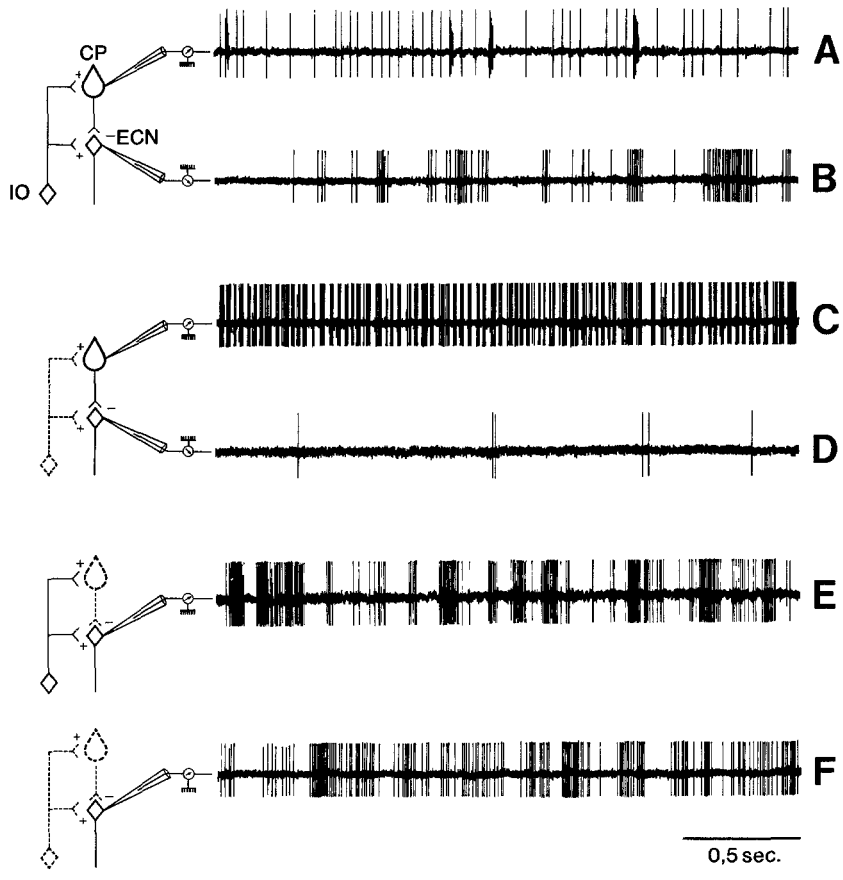

Fig. 2A–F. Spontaneous activity of the PCs (**A** and **C**) and of the ECNs (**B, D, E** and **F**) recorded in different experiments and in the experimental conditions shown schematically on the left. Solid lines in the schematic represent intact neurones, broken lines the destroyed neurones of the circuit. Controls (**A, B**); the animals treated with 3AP more than 3 h before (**C, D**); cryocoagulated animals (**E**); animals with cryocoagulation of the cerebellar cortex and treated with 3AP more than 3 h before (**F**)

variable periods of time but no data is included for counting periods of less than 5 min. The average firing rates given in the table are therefore those obtained over at least 5 min of recording. A few PC and cerebellar nuclei neurones were recorded for longer times, up to about 3 h.

I. Identification and localization of the units

The Purkinje cells in the intact animal are identified by the presence of complex spikes (Batini and Pumain 1971). In the absence of the IO, complex spikes were no longer present. Montarolo et al. (1982) suggested to identify the PC by the intense activity typical of the cellular layer where usually only PC are encountered. Such an indirect method of identification was used here and considered accurate enough with a little practice.

The inhibitory interneurones in the superficial folia of the cerebellar cortex were identified by their depth in the cortex as read from the micromanipulator. In addition, units with large amplitude, regular frequency of discharge and a low background activity were considered as Golgi interneurones (Miles et al. 1980). Basket cells located in the inner part of the molecular layer and having a discharge similar to simple spikes of the PC (Ito et al. 1982) could not be easily identified in our work where PC were deprived of complex spikes. Although a few errors cannot be excluded, we feel confident that they would not be significant for our results (cf. also Miles et al. 1980).

The axons of the P.C. reach all the neurones of the cerebellar nuclei and part of the neurones of the vestibular nuclei. As the aim of this work was to record activity of those units under the direct control of the P.C., the three pairs of cerebellar nuclei were systematically explored. Occasionally, units in those parts of the vestibular nuclei under cortical control were also encountered. We will refer to all these cells regardless of the nucleus in which they were found, as efferent cerebellar neurones (ECN)[2]. Only those units definitely localized by the dye deposit were included in this report as is schematically illustrated in Fig. 1. Most of the neurones of the cerebellar nuclei, were also identified by their antidromic

2 Few of these neurones send axons back to the cerebellar cortex (Batini et al. 1978) but we do not know whether the projection concerns collaterals of the ECN. We, therefore, provisionally include them among the ECN

Table 1

	No. of units	Mean frequency	Standard deviation
PC – A. Controls	47	46.09	± 25.98
B.' 3AP, early recording with complex spikes	13	36.62	± 17.83
B. 3AP, late recording without complex spikes	36	100.85	± 40.02
Interneurons A. Controls	35	13.13	± 9.59
B. 3AP, recorded > 3 h	34	14.36	± 14.80
ECN Intact animals			
A. Controls	33	32.94	± 21.39
B. 3AP recorded > 2 h 45 min	15	1.03	± 1.2
3AP recorded < 2 h 45 min	16	16.84	± 13.39
B'. 3AP recorded < 2 h 15 min	13	19.45	± 13.51
ECN Cryocoagulation			
C. Controls	14	54.87	± 37.72
C". Inhibited	13	4.18	± 2.70
C'. Disinhibited	27	45.52	± 27.35

response to an electric stimulation applied around the red nucleus (Fig. 1). Short latency, less than 1 ms, persistence of the response at high stimulation frequencies (up to 300/s) and possible collision with spontaneous discharges, were taken as criteria for antidromic invasion.

II. Unit activity in the cerebellar cortex: effects of the pharmacological destruction of the inferior olive

The PC recorded in intact animals invariably displayed the typical low complex discharge rate (due to CF activity) while the simple spike discharge (due to mossy fibers-granule cell activity) had a high but variable frequency. Figure 2A illustrates a typical recording. The frequency of complex and simple discharges were counted together, each complex counting for one spike, and the average value was 46.09/s (see Table 1). A large difference in the frequency of discharge was observed between individual PC (Fig. 3), as has often been reported since their description by Brookhart et al. (1950).

In the animals intoxicated with 3AP, most of the PC were studied for short periods at different times, up to 10 h, following the intoxication. Two were continuously recorded for long periods (up to 4 h) starting before the administration of the drug. This protocol allowed a close examination of the exact time of the loss of the complex discharges and the evolution of the changes in the firing frequency of the simple spikes after deafferentation from CF.

The critical period

As shown in Fig. 3, the loss of CF discharge took place asynchronously between 2 h and 15 mn and 2 h and 45 mn. Before this time all the PC had complex spikes while nearly all the PC were deprived of complex spikes after this period. We will call these 30 min the "critical period". In 94 tracks passing through the cellular layers at more than 3 h after 3-AP administration, only two units with complex spike were observed. These were attributed to neurones exceptionally retarded or surviving the intoxication (see Chap. IV).

The mean frequencies of discharge for the PC in the group recorded after the critical time of intoxication, and therefore not having complex spikes varied more than for the control group. The average frequency for this group was more than two times higher than in the controls and the difference was highly significant as shown in Table 1 (K^2 test, $p < 0.001$).

The PC recorded after injection of 3-AP but before the critical period (complex discharges still present) were considered as a separate group, in order to observe whether modification of the discharge frequency precedes the critical period. No significant differences with the controls could be detected (K^2 test $p > 0.02$).

When the IO neurones approached disfunction, complex discharges increase in frequency, then stopped for a few seconds, started again and soon after ceased completely. At the same time the simple spikes discharged in a very capricious manner, usu-

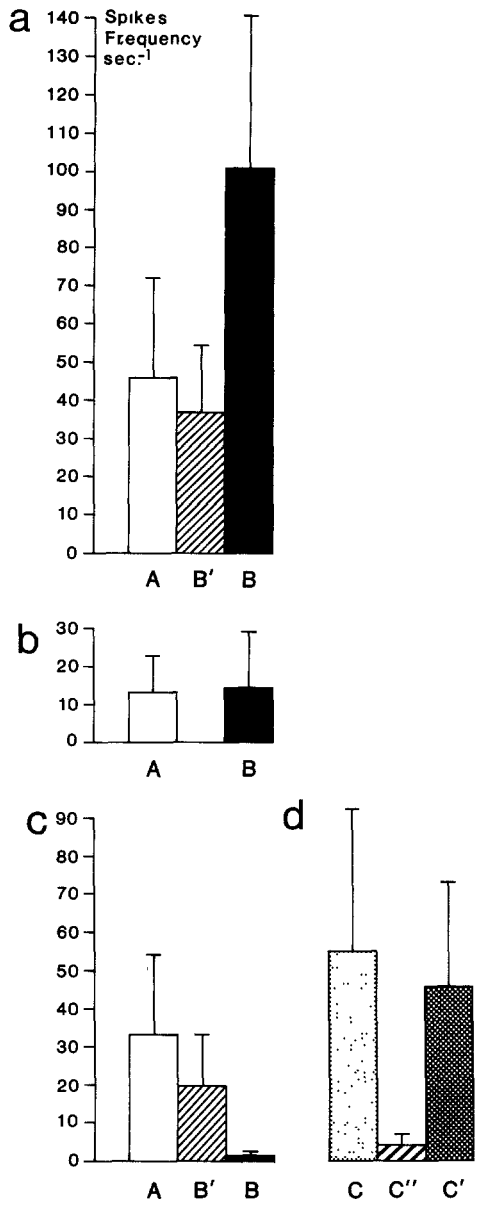

Fig. 3a–d. Histograms of the average discharge frequency of the PCs **a**, presumed interneurones **b** and ECNs **c** and **d**. A: controls, B: late and B': early recordings in animals treated with 3AP. C: units recorded in control animals with cryocoagulation of the cerebellar cortex, C': disinhibited and C'': inhibited units recorded in animals with both cryoagulation of the cerebellar cortex and 3AP treatment. See text and Table 1 for further explanation

ally in long bursts at high frequency, followed by few seconds of silence before beginning a continuous high frequency discharge several minutes after suppression of the complex spikes. The firing rate stabilized at a much higher level than that observed before administration of the drug and often became very regular (Desclin et al. 1980). Figure 4 describes one such PC recorded for 3 h after injection of 3-AP and one control PC recorded for 3 h after injection of an equal volume of ringer solution.

Presumed inhibitory interneurones were recorded in the cerebellar cortex in control animals and in those injected more than three hours before with 3-AP. The discharge rates of individual neurones varied similarly in both groups and the mean frequency of discharge was also similar (Fig. 3b). The small increase seen in the table for the treated animals showed no significant differences from controls; (K^2 test, $p > 0.5$).

III. Deep depression of the activity of the cerebellar efferent neurones following the pharmacological destruction of the inferior olive

The results obtained in the previous section demonstrate that the degeneration process affecting the neurones of the IO was completed in less than 3 h after administration of the 3AP. During the critical period, individual PC were deprived abruptly of their CF discharge, the process took place without an obvious pattern until all the PC were deprived of their complex spikes. A single ECN is innervated by terminals from several PC, therefore a graded PC influence may be expected on the ECN during the critical period.

As the microelectrode penetrated the cerebellar nuclei in the control rats, several units were usually encountered along a single track: we have recorded the activity of 33 cells over 24 tracks. On the contrary, when the penetration in the same regions was made in rats more than three hours after 3AP, we found only 15 units in 28 tracks. This result is in favor of a very low excitability of the ECN in the absence of the IO and is similar to that reported in the cat under different experimental conditions (Batini et al. 1983).

ECN discharge frequencies in the controls and after the critical period in the rats intoxicated with 3AP were very different. The units of the former group showed a sustained discharge (Fig. 2B) with large differences for individual neurones. Those of the latter had a very low firing rate and tended to be about the same for all the ECN. The average frequency (see Table 1) fell thirty fold in the treated animals compared to the controls, with a very highly significant difference (K^2 test, $P < 0.001$).

During the first 2 h and 45 mn after administration of 3-AP, the units recorded still showed a great variability in discharge rate, but the average frequency (see Table 1) was significantly lower than for the controls (K^2 test, $P < 0.01$). Since we do not know the exact moment of CF deafferentation of

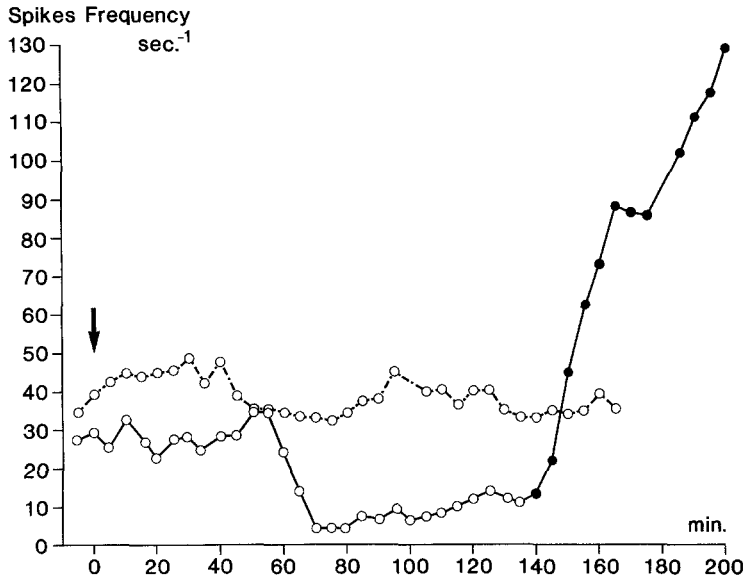

Fig. 4. Changes in discharge frequency of a PC recorded in two different experiments, a control animal (broken line) and a rat treated with 3AP (solid line). 3AP or an equal volume of saline solution were injected at the arrow. The discharge frequency intervals over a five minutes. Open circles: frequency of discharge included simple and complex discharge. Filled circles: after complex spikes had ceased firing following 3AP treatment

those PC in series with the particular ECN under examination, neurones recorded during the critical period were excluded from this group. Although a tendency to lower firing rates exists, the mean frequency in this case (see Table 1) is not significantly different from that of the controls (K^2 test, $p > 0.01$). Figure 3c shows the distribution of discharge frequencies for neurones recorded at different times after 3AP administration.

A few units had been followed for long recording periods from before drug injection and during the critical period. Figure 5 illustrates such a unit and one recorded in a control animal treated only with saline solution. It was clear that during the first hour after intoxication the firing rate was high and variable, then, during the second hour, stabilised at a lower average frequency. At nearly three hours, individual cells began to increase their firing rate before falling into the deep inhibitory state of the critical period. This last phase took place rapidly along with the increase in the excitability of the PC just deafferented of their CF, and persisted for the remaining time of recording. It will be noted that controls did not show much change in frequency during the 165 min of continuous recording.

IV. Release of cerebellar efferent neurones' activity following cerebellar cortex destruction

The results described above have shown that destruction of the IO produced a significant increase in the spontaneous simple spike discharge in the PC and a drastic reduction of spontaneous discharge in the ECN, the two effects appeared simultaneously. There was therefore a strong indication of an increased inhibitory influence of the PC.

However, the axons of the IO neurones, before reaching the cerebellar cortex, send collaterals to the ECN. Such collaterals, which have an excitatory function (Eccles et al. 1967) cease firing, together with the CF, when the IO is intoxicated by 3AP. They were described by some authors (Desclin and Colin 1980) as being few in number, but by others (Wiklund et al. 1984) as being numerous. If the quantity is large, the possibility exists that their target neurons decrease firing when deprived of this excitatory input. The mechanism would be, in this case, a desactivation, or disfacilitation, of the ECN, which could be effective even in the absence of the inhibitory action of the PC.

We therefore performed the following experiments: the spontaneous activity of the ECN was recorded in rats with not only a pharmacological destruction of the IO, but also a cryocoagulation of the cerebellar cortex destroying the overlying PC. Such an experiment deprives the ECN of both the presumed inhibition exerted by the PC and the facilitatory influence exerted by the collaterals of the olivary neurones. If the latter mechanisms is the only one responsible of the decreased firing of the ECN, the desactivation would persist. If, on the contrary, the inhibitory mechanism prevails, the ECN should recover a frequency of discharge at least as important as that obtained in the control animals. On the other hand the two mechanisms may cooperate in reducing the activity of the target neurones and their additive effect could therefore be responsible for the deep

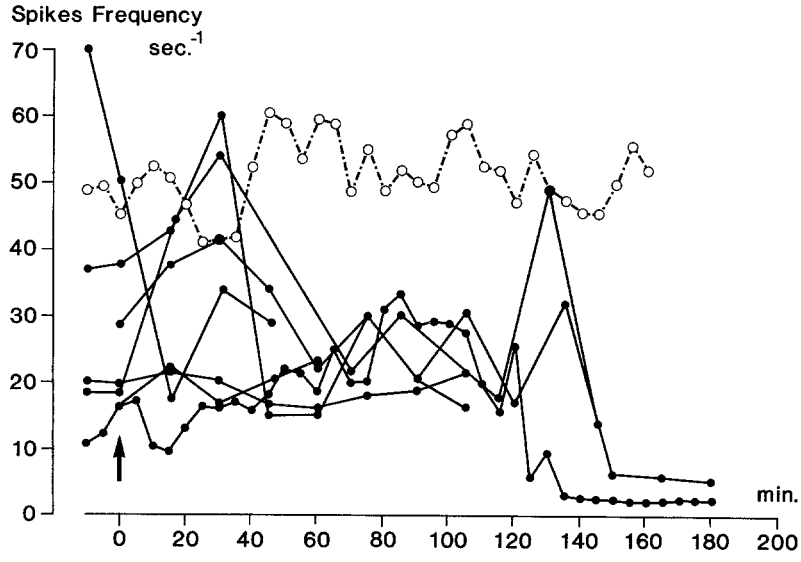

Fig. 5. Variations in ECN average discharge frequency over 5 min intervals, in rats injected with 3AP (filled circles) or with an equal volume of saline (open circles) at the arrow. Note that the data for the 3AP injected animals are not uniform in total recording time. Each unit was recorded in a different experiment

unresponsive state observed in our results. To verify this possibility, we then also recorded spontaneous activity of the ECN in a group of control rats with a cryocoagulation of the cerebellar cortex but not injected with 3AP. If the IO collaterals carry an effective facilitatory input, the ECN would have a discharge frequency somewhat higher than that of the group deprived of both the inhibitory and the facilitatory influence.

A large cryocoagulation of the cerebellar cortex was first performed in 7 rats (see below), 4 were then given a neurotoxic dose of 3AP, finally all were used for recording starting 3 h after injection, so that the critical period was over for the intoxicated animals. Only short recording sessions for counting the spike frequencies were performed in these experiments.

Units recorded in the control animals with destruction of the cerebellar cortex only, had mean discharge frequencies significantly higher than for the intact controls (K^2 test, $p < 0.02$) (see Table 1 and Fig. 3d). Since the cryocoagulation did not affect the entire cortex (see below), therefore some inhibition remained but the results demonstrated an effective disinhibition of the ECN.

The units recorded in rats with destruction of the cerebellar cortex and of the IO were found to fit two discharge rate populations (see Table 1 and Fig. 3). One population had a low mean frequency approaching that obtained in animals treated with 3AP and recorded after the critical period. Since the cryocoagulation of the cortex was incomplete (see below), these neurones to some degree remained under the inhibitory influence of the overlying intact PC. The second, more numerous population, on the contrary, was clearly released from the inhibitory influence of the destroyed PC. These neurones showed a sustained higher average frequency of discharge (see Table 1 and Fig. 2F), significantly different from the group of neurones recorded in the animals treated only with 3AP (K^2 test, $p < 0.001$). The firing rates for the ECN of this population were not very different from units recorded in the animals with destruction of the cerebellar cortex only. Their mean frequency of discharge was somewhat lower (see Table 1) but not significantly (K^2 test, $p > 0.05$). This last result indicates that the collaterals from the olivary neurones have little, if any, influence on the activity of the ECN.

V. Controls: histology and metabolic activity

Recordings from the PC of the cerebellar cortex demonstrated that between 2 h 15 min and 2 h 45 min after i.v. injection of 3-AP, neurones of the IO ceased firing and modified the cerebellar output activity so that the PC increased simple spike firing and the ECN were increasingly inhibited. These results were interpreted as due to a slow intoxication leading to an asynchronous cellular death in the IO. In the histological control it was not possible to observe cellular degeneration within the first 4 h in the IO, at least not with the techniques used in the present study. However, most of our animals treated with 3-AP were sacrificed after more than 4 h and IO neuronal degeneration was found in all of these animals. In a few rats, small islands of intact tissue (10–20 neurones) or a few scattered single neurones remained intact and in only one case an important part of the principal olive was intact. Such neurones

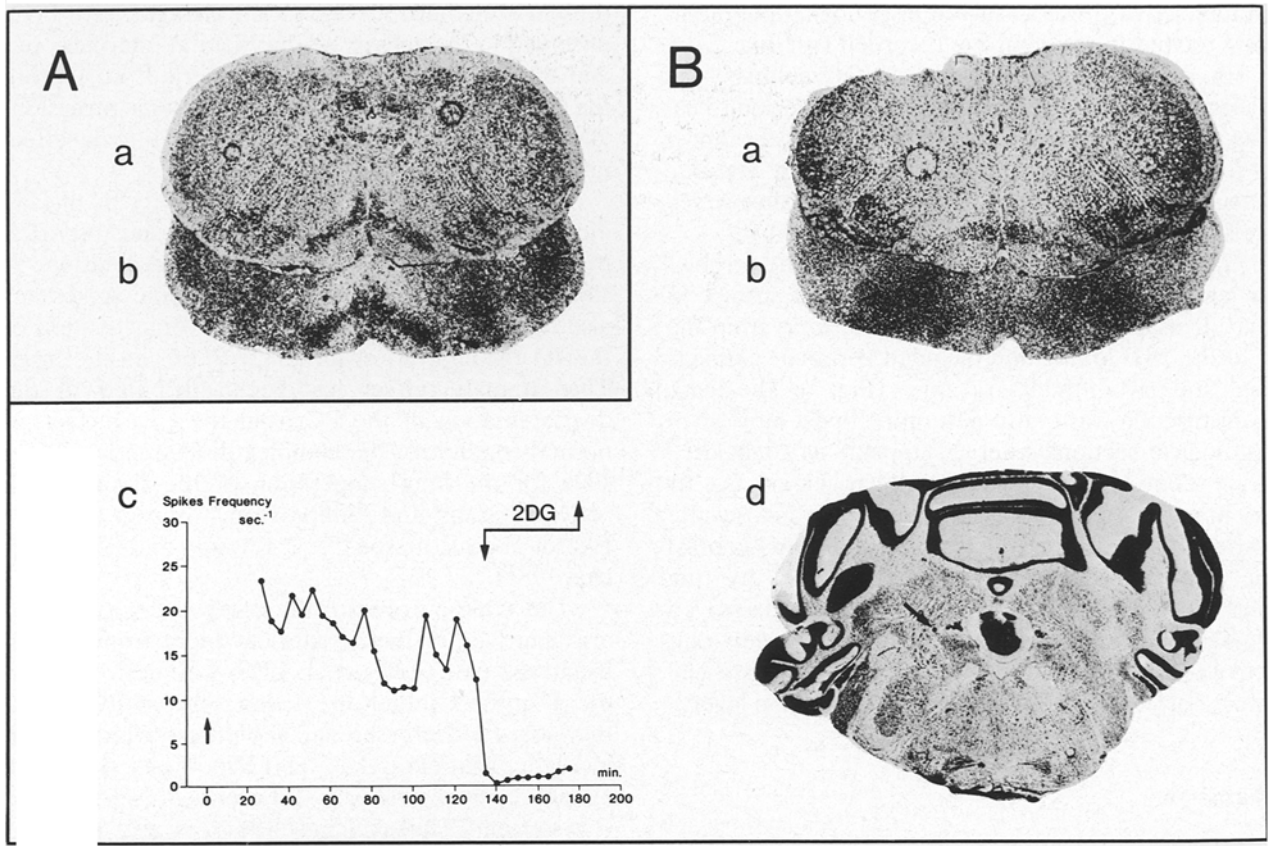

Fig. 6A and B. Metabolic activity of the IO in a control rat **A** and in another rat at 135 min after 3AP (**B**) a: Nissl stained section. b: corresponding radioautograph. Note the absence of marking in the IO in **B**b. **B**c shows the ECN discharges frequency recorded in the vestibular nuclei (**B**d, arrow) in the same rat as in **B**b. Note the extreme reduction of the average frequency of discharge at 135 min after 3AP injection (arrow). 2DG indicates the time for the [^{14}C]2-deoxyglucose marking

would explain the persistence of the few complex spikes in the cerebellar cortex of the treated animals.

In previous works (Bardin et al. 1983) dealing with the metabolic activity of the brain under the influence of 3AP injected intraperitoneally, it has been shown that the metabolic marking of the IO decreases between the second and the third hour after treatment, therefore before the critical period, but reaches its lowest level in the fourth hour after intoxication.

As a control for the metabolic effect of 3AP at less than 4 h, 4 animals were injected with 2-deoxyglucose intravenously at 2 and 3 h after 3AP treatment and one animal was used for the control. In two of the intoxicated animals, the experiments with the tracer started 2 h 15 min after 3AP treatment and therefore included the critical period. In one of the 2 animals it was made during recording of an ECN, the injection being applied at the moment when the unit decreased its firing rate (see Fig. 6). Marking of the IO was generally low compared to the control and absence of marking (equal to the back-

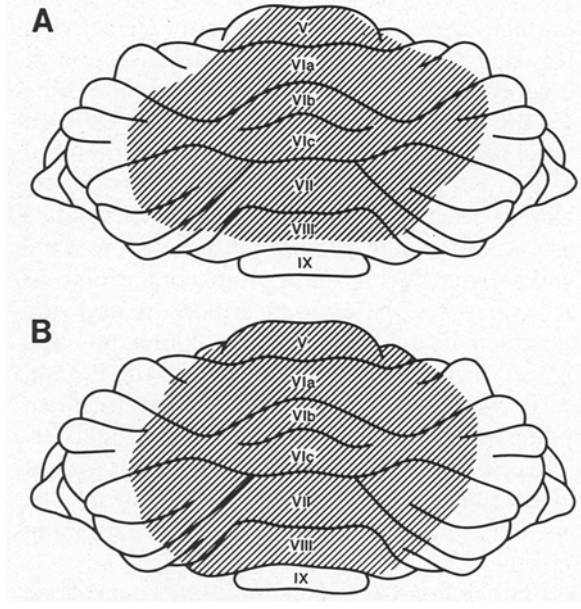

Fig. 7A and B. Schematic representation of the cerebellar cortex of the rat (lobules V to IX) illustrating the extent of the cryocoagulation (shaded area) in a control (**A**) and in a rat treated with 3AP (**B**) as determined visually

ground activity) was observed in regions topographically related to the neurone recorded (see Fig. 6).

In the two remaining intoxicated animals, the tracer was injected just after the critical period (3 h). The radioautographic marking of the IO was almost as low as the background, but in one rat marking persisted in a large region of the principal olive which presumably was not degenerated.

The extent of the cryocoagulation of the cerebellar cortex was determined *de visu* at the surface in vivo. It usually extended anteroposteriorly from the V to the VIII lobule and included vermian, paravermian and part of the lateral zones (Fig. 7). The depth of destruction was better determined post mortem in histological sections where it appears as coagulated tissue deprived of stained cellular elements. Its extent was variable but did not reach the inner white matter in any of the animals. An additional control for the cryocoagulation was provided by the microelectrode as it passed through the destroyed regions before reaching the cerebellar nuclei: unit activity was never encountered in the superficial layers but persisted in some cases in the deep layers.

Discussion

The two kinds of results reported here indicated an increased cerebellar inhibition at the cerebellar nuclei in rats deprived of the IO: the PC discharge more simple spikes and the ECN discharge less. In both cases an opposite effect started at a particular time after intraveinous injection of 3AP, the critical period which corresponded to the loss of CF activity. This loss due to the asynchronous degeneration of the IO neurones resulted in greatly varying the firing rates for the individual PC and ECN. It also explains the partial loss of the metabolic activity in the IO of the present and of the previous experiments (Batini et al. 1983) since the accumulation of radioactive products was a result of a temporal summation of the neuronal activity during the 45 min uptake time of the deoxyglucose. After the critical period the metabolic activity of the IO ceased completely and the changed firing rates were found in all the PC and the ECN, with only a negligeable number of them responding to the olivary neurones escaping degeneration. In electromicroscopic studies performed at three hours after 3-AP treatment, only a few normal neurons were found in the IO (Desclin and Colin 1980).

The possibility of a general 3-AP neurotoxic effect has been excluded in recent works (Bardin et al. 1983) where the metabolic effect persisted with longer survival time after injection of the drug. In the present study also no significant changes have been observed in the activity of the PC, the interneurones and the ECN before the critical period, i.e. before the CF began to cease activity. We assume provisionally that there was no acute general toxic effect influencing the system studied here.

The increase of simple spike activity in the PC after the critical period confirms the results reported by Colin et al. (1980) in the same preparation. A similar but reversible increase of simple spikes has also been obtained in the rat by cryoinactivation of the IO (Montarolo et al. 1982; Batini et al. 1984). This dramatic effect has been attributed to the deafferentation of the PC from the CF. In fact, in normal conditions, the complex discharge is responsible for the long suppression of the simple spike activity (Granit and Philips 1956; Colin et al. 1980; Ekerot and Oscarsson 1981; Rawson and Tilokskulchai 1981).

The interneurones of the cerebellar cortex were also deprived of their excitatory input from the CF collaterals (see Eccles et al. 1967) with destruction of the IO; their inhibitory action on the PC would therefore be decreased and should contribute to the increase of simple spikes. This hypothesis, which has already been rejected by Colin et al. (1980) and by Rawson and Tilokskulchai (1981), is also not confirmed by our results showing that the discharge activity of the interneurones does not change after treatment with 3AP. Whether the CF collaterals are few in number (see Desclin and Colin 1980) or numerous (see Wiklund et al. 1984), they do not affect the firing rate of the interneurones in our experimental conditions. Nevertheless, our results do not rule out the possibility of their participation in other experimental conditions (Montarolo et al. 1982), particularly when their firing rate could be changed by the activity of the mossy fiber collaterals impinging upon them.

The deep depression observed in the ECN, starting at the critical period, is the result of an increased inhibition from the hyperperactive PC deafferented of their CF. The only alternative possibility could be a disfacilitatory process taking place at the ECN deprived of the excitatory input from the collaterals of the CF reaching the cerebellar nuclei in normal conditions. Our results demonstrate that such a disfacilitation would be negligible, at least in our experimental conditions. The degree of depression resulting from the deafferentation would be a function of the number of collaterals and their previous frequency of discharge. The firing rate of the CF is very low, around 1/s (Pellet et al. 1974). That of the collaterals is presumably not very different. Therefore, even assuming that their number is large

enough to contact all the ECN, the actual effect that they would have on the average frequency of the ECN could be unnoticeable. Nevertheless, we cannot exclude that they have other functions not revealed by the present experimental design.

The increased inhibitory efficiency of the PC observed in the ECN recorded extracellularly in our work (see also Montarolo et al. 1981) is apparently not contradictory with the reduction in size and number of the monosynaptic IPSPs evoked in the lateral vestibular nucleus of rats intoxicated with 3AP (Ito et al. 1978). The intense synaptic firing arising from the hyperactive PC is likely to produce a rather deep and continuous postsynaptic hyperpolarization of the ECN. Therefore, the size of the extracellularly recorded IPSP elicited by electric stimulation will be reduced in size and will even disappear as the membrane approaches the equilibrium potential for the Cl^- conductance. Alternately this interpretation might not hold true for the experiments of Ito et al. (1978) where 2/s continuous stimulation was applied to the cortex: inhibition of the PC could occur during the interstimuli intervals due to activation of the mossy fibers-inhibitory interneurones, therefore, leading to a decrease of the intense discharge frequency of the PC (see also Bardin et al. 1983). Moreover, the reduction of monosynaptic IPSPs in animals treated with 3AP was mostly obtained in chronic experiments and was observed to increase with time after treatment (Ito et al. 1978). The authors suggest a trophic factor affecting the PC following destruction of the IO and its CFs. And, in fact, in further experiments (Ito et al. 1979) the reduction of the PC inhibition was not obtained in acute conditions where the activity of the IO neurones was suppressed with TTX. In addition, Benedetti et al. (1983) reported reduced activity in the ECN following cooling of the IO.

In conclusion, there is good evidence, in "acute" conditions at least, for an increased inhibitory efficiency of the PC deprived of the CF discharges therefore leading to a decreased output from the ECN. As a consequence, a disfacilitation of their target neurones will take place. Since this disfacilitation originates from a release of the PC activity, the IO appears to be a modulator of the PC inhibitory capability. Inhibition is the dominant synaptic activity for cerebellar regulation of motor functions and is determined by the activity of the neurones of the IO.

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References

Bardin JM, Batini C, Billard JM (1982) Disfacilitation cérébelleuse après destruction pharmacologique de l'olive inférieure. CR Acad Sci (Paris) 295: 751–753

Bardin JM, Batini C, Billard JM, Buisseret-Delmas C, Conrath-Verrier M, Corvaja N (1983) Cerebellar output regulation by the climbing and mossy fibers with and without the inferior olive. J Comp Neurol 213: 464–477

Bardin JM, Batini C, Buisseret-Delmas C, Servière J (1983) Decrease of [^{14}C]2-deoxyglucose uptake at the intracerebellar nuclei during cerebellar cortex stimulation. Brain Res 272: 171–174

Batini C, Bernard JF, Buisseret-Delmas C, Horcholle-Bossavit G (1983) Cerebellar output response to afferent stimulation: energy consumption and unit activity in the cat fastigial nucleus. Exp Brain Res 52: 400–410

Batini C, Billard JM (1984) Dépression de l'activité efférente des noyaux cérébelleux après destruction de l'olive inférieure. CR Acad Sci (Paris) 298: 123–126

Batini C, Benedetti F, Buisseret-Delmas C, Montarolo PG, Strata P (1984) Metabolic activity of intracerebellar nuclei in the rat: Effects of inferior olive inactivation. Exp Brain Res 54: 259–265

Batini C, Buisseret-Delmas C, Corvisier T, Hardy O, Jassik-Gerschenfeld D (1978) Brain stem nuclei giving fibers to lobules VI and VII of the cerebellar vermis. Brain Res 153: 241–261

Batini C, Pumain R (1971) Données électrophysiologiques sur l'origine des fibres grimpantes. Arch Ital Biol 109: 189–209

Benedetti F, Montarolo PG, Strata P, Tempia F (1983) Inferior olive inactivation decreases the excitability of the intracerebellar and lateral vestibular nuclei in the rat. J Physiol (Lond) 340: 195–208

Brookhart JM, Moruzzi G, Snider RS (1950) Spike discharges of single units in the cerebellar cortex. J Neurophysiol 13: 465–486

Buser P, Rougeul A (1954) La réponse électrique du cervelet de pigeon à la stimulation de la voie optique et son analyse par microélectrodes. J Physiol (Paris) 46: 287–291

Colin F, Manil J, Desclin JC (1980) The olivocerebellar system. I. Delayed and slow inhibitory effects: An overlooked salient feature of cerebellar climbing fibers. Brain Res 187: 3–27

Desclin JC, Colin F (1980) The olivocerebellar system. II. Some ultrastructural correlates of inferior olive destruction in the rat. Brain Res 187: 29–46

Eccles JC, Ito M, Szentágothai J (1967) The cerebellum as a neuronal machine. Springer, Berlin Heidelberg New York, pp 1–335

Ekerot CF, Oscarsson O (1981) Prolonged depolarization elicited in Purkinje cell dendrites by climbing fibre impulses in the cat. J Physiol (Lond) 318: 207–221

Granit R, Phillips CG (1956) Excitatory and inhibitory processes acting upon individual Purkinje cells of the cerebellum in cats. J Physiol (Lond) 133: 520–547

Ito M, Orlov I, Shimoyama I (1978) Reduction of the cerebellar stimulus effect on rat Deiters neurones after chemical destruction of the inferior olive. Exp Brain Res 33: 143–145

Ito M, Nisimaru N, Shibuki K (1979) Destruction of the inferior olive induces rapid depression in synaptic action of cerebellar Purkinje cells. Nature 277: 568–569

Ito M, Sakurai M, Tongroach P (1982) Climbing fibre induced depression of both mossy fibre responsiveness and glutamate sensitivity or cerebellar Purkinje cells. J Physiol (Lond) 324: 113–134

Ito M, Yoshida M (1964) The cerebellar evoked monosynaptic inhibition of Deiters' neurones. Experientia 20: 515–516

Miles FA, Fuller SH, Braitman DJ, Dow BM (1980) Long-term adaptive changes in primate vestibuloocular reflex. III. Electrophysiological observations in flocculus of normal monkeys. J Neurophysiol 43: 1437–1476

Montarolo PG, Raschi F, Strata P (1981) Are the climbing fibres essential for the Purkinje cell inhibitory action? Exp Brain Res 42: 215–218

Montarolo PG, Palestini M, Strata P (1982) The inhibitory effect of the olivocerebellar input on the cerebellar Purkinje cells in the rat. J Physiol (Lond) 332: 187–202

Pellet J, Tardy MF, Harlay F, Dubrocard S, Gilbrodes JC (1974) Activité spontanée des cellules de Purkinje chez le chat chronique: Etude statistique des spikes complexes. Brain Res 81: 75–95

Rawson JA, Tilokskulchai K (1981) Suppression of simple spike discharges of cerebellar Purkinje cells by impulses in climbing fibre afferents. Neurosci Lett 25: 125–130

Wiklund L, Toggenburger G, Cuénod M (1984) Selective retrograde labeling of the rat olivocerebellar climbing fiber system with [^{3}H]-D-aspartate. Neuroscience (in press)

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