Exp. Brain Res. 18, 69—87 (1973)

Printed in Germany

The Olivo-Cerebellar System: Functional Properties as Revealed by Harmaline-Induced Tremor

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Received September 6, 1972

Summary. Intracellular recording from Purkinje cells in cat cerebellar cortex demonstrated an 8—10/sec burst activity following intravenous administration of harmaline (10 mg/kg), a drug known to produce tremor at the same frequency. The burst activation of Purkinje cells was generated by large all-or-none depolarizations similar to climbing fiber (CF) excitatory postsynaptic potentials (EPSPs). Polarization of the cell membrane through the recording electrode (via a Wheatstone bridge) revealed that the all-or-none depolarization had an equilibrium potential and time course identical to the electrically evoked CF-EPSP, demonstrating directly that tremor is associated with specific activation of the CF afferent system.

Interspike frequency histograms of the burst responses of Purkinje cells show that the rhythmic CF activity may continue for several hours with approximately 10% frequency scatter, the actual frequency depending on the level of anesthesia. Simultaneous extracellular recordings from Purkinje cells near the midline vermis indicated that CFs projecting to this area fire in a synchronous manner, while simultaneous recording from three Purkinje cells at different lateralities from the midline showed that the rhythmic activity is reduced in the lateral vermis and may be absent in the cerebellar hemispheres.

Intra- and extracellular recordings from cerebellar nuclear cells (fastigial) disclosed a bursting type of activation following harmaline; a similar type of activity could be recorded in the reticular formation neurons and at inferior olive level. At spinal cord level, harmaline induced a repetitive and rhythmic activation of motoneurons which was not modified by dorsal root section. Cooling of the cerebellar cortex produced a definite desynchronization of the rhythmic motoneuronal firing. However, the basic 10/sec firing of the spinal cord motoneurons could still be observed. Following lesion of the inferior peduncles which interrupted the olivo-cerebellar pathway, the rhythmic activation of Purkinje cells, nuclear cells, vestibular and reticular cells and motoneurons disappeared. However, the rhythmic activity was maintained at inferior olivary level. It is suggested that harmaline acts directly on the inferior olive since in animals with low decerebration, cerebellectomy and spinal transection, rhythmic activity of the inferior olive could still be observed.

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The results of these experiments strongly suggest that the inferior olive is able to generate the activation of motoneurons and that such influence can only take place through the activation of the cerebellar nuclei. Possible functions of the inferior olive as a generator of fast muscular transients are discussed.

Key words: Climbing fiber — Inferior olive — Tremor — Harmaline — Cerebellar nuclei

Introduction

Harmaline, an alkaloid of *Peganum harmala*, has been known since 1894 to produce a high frequency tremor in mammals (Neuner and Tappeiner, 1894). The pharmacological properties of this drug have subsequently been amply studied (Gunn, 1911—1912; Hara and Kawamori, 1954; Ahmed and Taylor, 1959; Sigg *et al.*, 1964; Poirier *et al.*, 1966) and its site of action related functionally to the basal ganglia (Poirier *et al.*, 1968; Kim *et al.*, 1970). Recent experiments by Villablanca and Riobo (1970) and by Lamarre *et al.* (1971a, b) demonstrated, however, that harmaline is able to produce a 10—12/sec tremor in decerebrate cats, thus excluding all suprategmental structures as necessary for the genesis of this motor syndrome. The latter authors associated the harmaline tremor with a 10/sec activation of the inferior olive and a similar rhythmic firing of Purkinje cells, fastigial nucleus cells, vestibular and bulbo-reticular units, and motoneurons. They concluded that the tremor was most probably relayed from the brain stem, including the inferior olive and probably the nucleus reticularis tegmentis pontis, and reached the spinal cord level via reticulo-spinal and vestibulo-spinal pathways.

Given the important functional significance of this finding we have studied in further detail some of the mechanisms proposed by Lamarre *et al.* for the generation of the tremor. Our results directly demonstrate that following harmaline administration, Purkinje cells are activated at 8—10/sec via climbing fiber afferents from the inferior olive through the olivo-cerebellar system. Furthermore, collaterals of the olivo-cerebellar system to the fastigial nucleus were shown to constitute an input which is both necessary and sufficient to generate this tremor. This demonstration has led us to postulate that the inferior olive has a direct motor action on the skeletal musculature through the cerebellar nuclei. It is further postulated that the motor involvement of the inferior olive, which can be exclusively exercised via the cerebellar nuclei, may be utilized as a command system capable of exercising a very rapid and powerful activation of motoneurons. This form of command motor response is in general agreement with previous statements regarding the phasic control of movement via the climbing fiber system (Llinás *et al.*, 1969; Llinás, 1970). Two preliminary communications on this research have been published by Llinás and Volkind (1972a, b).

Methods

A total of 30 cats was utilized for this research. Two basic types of preparation were employed: a) decerebrate and b) anesthetized with sodium pentobarbital. The animals in the first group were anesthetized with ether and placed in a stereo taxic apparatus, and the neuraxis was transected at transcollicular or pretrigeminal level. Animals in the second group were anesthetized with sodium pentobarbital (initial dose of 35 mg/kg) and a supporting dose

of 5 mg/kg was administered every 2 hours. Since the frequency of harmaline tremor is related to the level of barbiturate anesthesia (Lamarre, personal communication), in most animals studied the level of anesthesia was allowed to become slightly shallow; however, necessary precautions were taken to prevent the animals from suffering.

The vermis of the cerebellar cortex was exposed by removing the squamose portion of the occipital bone and the craniectomy was continued to the level of the tentorium. The cerebellum was then covered with agar to minimize circulatory pulsations (Eccles *et al.*, 1966a).

The inferior olive was approached from the ventral side following the removal of trachea and esophagus. The trachea was cannulated at slightly above the superior edge of the sternum. The basilar process of the occipital bone was removed rostrally to the upper pons and laterally to the tympanic bulla. Removal of the dura following this craniotomy allowed a visualization of the bulbar region slightly lateral to the exit of the 12th nerve on both sides, and of the caudal two-thirds of the pons.

In experiments where ventral roots were studied, a lumbar laminectomy (L6 to S1) was performed and ventral roots were sectioned. Single ventral root filaments were dissected with watchmaker's tweezers using a dissecting scope. Action potentials were recorded with silver-silver chloride hook electrodes. A similar recording system was utilized to record from peripheral nerves. Intracellular recordings were obtained from Purkinje cells and from cerebellar nuclear cells by means of KCl-filled microelectrodes with an average resistance of 10—20 MΩ. Extracellular action potentials were gathered from cerebellar cortex, cerebellar nuclei, inferior olive and reticular formation by means of NaCl-filled micropipettes with an average D.C. resistance of 2—4 MΩ. On several occasions where two and three Purkinje cells were simultaneously recorded, NaCl micropipettes were positioned in a special multielectrode Narishige holder 100 μ to 2 mm apart. The cells were simultaneously recorded with independent head stage amplifiers. Experiments in which Purkinje and inferior olive cells were simultaneously recorded necessitated simultaneous approach for ventral and dorsal regions of the central nervous system. In this case two micromanipulators were mounted in the frame and the animal positioned on its side to allow visualization of the cerebellum and of the ventral surface of the bulbar region. Intra- and extracellular records were averaged by means of a Fabritek instrument computer, and histograms were obtained with a Nuclear Chicago data retrieval system. Photographic records were taken with a Grass kymograph camera. In all experiments harmaline was injected intravenously with an initial dose of 5 or 10 mg/kg. In most experiments a similar dose was repeated after 5 or 6 hours of recording. In experiments where intracellular injection of current was performed, a bootstrap Wheatstone bridge was utilized (Araki and Otani, 1955). Bipolar stimulating electrodes were placed in the cerebellar white matter in order to activate directly the cerebellar afferent systems. In the six cats where chronic inferior cerebellar transection was performed, the surgical approach was the same utilized in previous experiments (Eccles, Llinás and Sasaki, 1966d). However, instead of a complete peduncular lesion, the medial portion of the inferior peduncle was transected bilaterally. The animals were studied 5—8 days after the lesion. The lesions were studied histologically at light microscopical level after paraffin embedding.

Results

Activation of Purkinje Cells Following Harmaline Administration

As reported by Lamarre *et al.* (1971a, b) and by Montigny and Lamarre (in press), a single intravenous administration of harmaline produces a synchronous activation of Purkinje cells in the cerebellar vermis of cats. Extracellularly the action potentials seen resemble the burst responses generated by the activation of the climbing fiber (CF) system (Eccles *et al.*, 1966a; Llinás, 1970). In order to demonstrate directly whether these burst responses were produced by the CF system or by a synchronized mossy fiber volley (see Bloedel and Burton, 1970), intracellular recordings from the Purkinje cells were obtained.

As previously shown for the cat (Eccles *et al.*, 1966a) and for other vertebrates (Llinás and Hillman, 1969), CF activation of Purkinje cells is characterized intra-

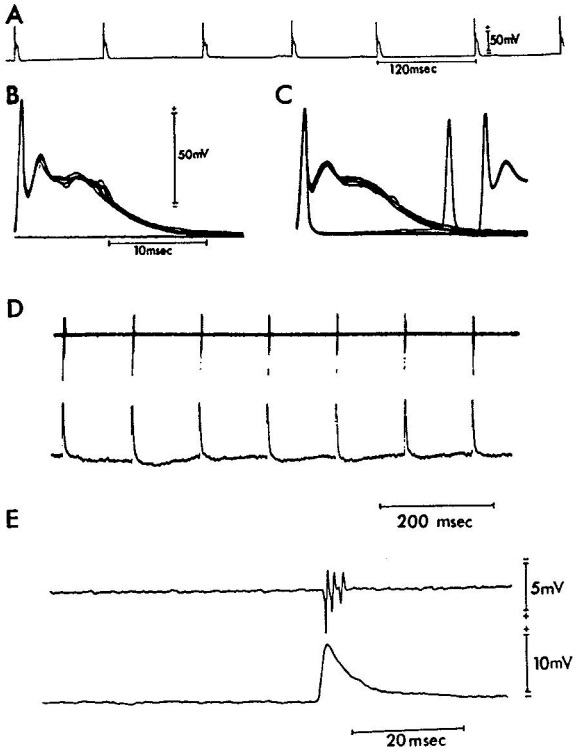

Fig. 1. *Intracellular recordings from a Purkinje cell following administration of harmaline.* A: Continuous recording from a Purkinje cell to illustrate the rhythmic nature of the intracellular bursts. Note the membrane potential fluctuations preceding the fourth, fifth and seventh spike bursts. In B the sweep was triggered by the onset of the intracellular action potential. The full duration and the stereotyped character of these potentials is demonstrated by superimposition of six sweeps. C: As in B, the presence of short lasting action potentials allows a direct comparison between "unitary" and "burst" spikes. In D and E, sequential intra- and extracellular recordings from another Purkinje cell after harmaline. Upper trace is extracellular burst spike, lower trace intracellularly recorded depolarization. The strict rhythmic nature of the Purkinje cell activation before and after penetration is illustrated in D. The record in E is taken at higher sweep speed to give further details on the extra- and intracellular waveform of the Purkinje cell response. In this and subsequent figures, time and voltage calibration are shown in the illustration

cellularly by an action potential followed by a prolonged depolarization. A typical intracellular recording in a Purkinje cell following harmaline administration is illustrated in Fig. 1A. The interval between action potentials was approximately 120 msec. A closer examination of these action potentials at higher gain and sweep speed is shown in Fig. 1B and C. In B the oscilloscope sweep was triggered by the intracellular potential. This type of prolonged "burst" spike activation is in every way similar to the fairly distinct all-or-none activation of Purkinje cells generated by the CF afferent. Characteristic of this form of Purkinje cell activation is the fast action potential, having a duration of approximately 1 msec and 80 mV

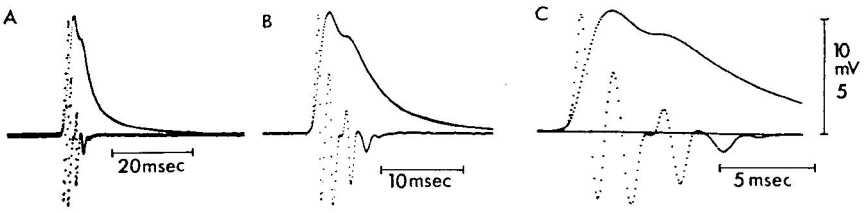

Fig. 2. *Temporal relationship between extracellular Purkinje cell burst and the intracellular EPSP which generates the burst.* A to C are superimposed averages (200) at three different sweep speeds. Since the intracellular action potential arises directly from the baseline (Fig. 1B), the onset of the EPSP and spike bursts are shown as occurring at approximately the same time

amplitude, followed by a prolonged depolarization showing a minimum amount of variability in some of the later peaks[1].

On rare occasions following harmaline administration, the mossy fiber input was momentarily able to generate action potentials via the parallel fibers. One such example is shown in Fig. 1C and allows a direct comparison between the duration of the parallel fiber-generated spike (unitary spike) and that of the CF-generated burst (burst spike). For the most part, the duration and time course of the first spike of the CF response was superimposable on the "unitary" spikes generated by the parallel fiber activation (Fig. 1C). In one of the traces in Fig. 1C, the CF-generated burst was observed in the later part of the oscilloscope sweep. In this instance the mossy fiber activation of the Purkinje cell triggered the oscilloscope and captured a single CF activation which appears for this reason to be out of synchrony with the rest of the CF responses. Note the slight difference in amplitude between unitary action potentials and CF-generated responses. On several occasions, graded synaptic depolarization of Purkinje cells was encountered (Fig. 1A). That form of depolarization, which is mediated via parallel fiber activation, did not reach firing level in most instances.

A number of intracellular recordings were obtained from Purkinje cells extensively studied extracellularly prior to impalement; an example is shown in Fig. 1D and E. The extracellular action potentials from a Purkinje cell are illustrated in the upper trace while the lower trace shows the intracellular recording from the same neuron. The highly synchronous character of the occurrence of Purkinje cell burst spikes following harmaline injection can be seen in Fig. 1D. Subsequent recordings taken at higher sweep speed show the details of the extracellular burst spikes and the intracellular all-or-none synaptic depolarization (Fig. 1E). The rhythmic firing of a Purkinje cell could be followed in some cases for periods of up to 1 hour with very little variation in its firing frequency. The exact time relation between the duration of the burst and that of the intracellular depolarization can be seen in Fig. 2. These records represent 200 averaged extra- and intracellular potentials and clearly indicate the time and voltage relationship between the EPSP and the burst spikes reported extracellularly.

1 Since later in this paper a direct demonstration will be given of the CF nature of these harmaline bursts, we shall refer to them as CF activation of Purkinje cells and to the all-or-none depolarizations which generate these bursts as CF-generated excitatory postsynaptic potentials (CF-EPSPs).

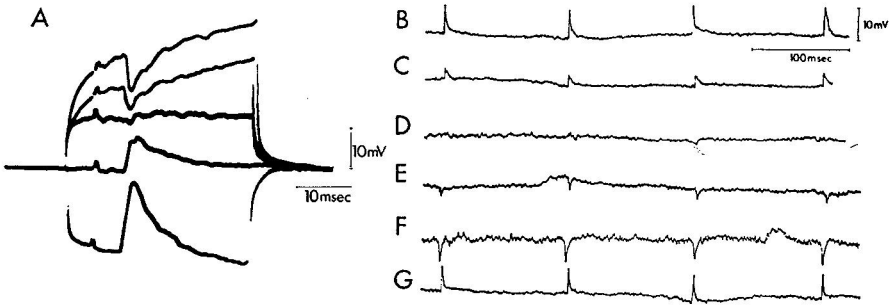

Fig. 3. *Reversal of intracellularly recorded climbing fiber EPSP by depolarizing currents.* In A the EPSP was evoked by electrical stimulation of the cerebellar white matter. Membrane hyperpolarization causes an increase and membrane depolarization a decrease and finally a reversal of the EPSP. In B to F, reversal of EPSP after harmaline, by D.C. current. B: Control of rhythmically occurring EPSP after harmaline administration. In C to F the membrane potential was depolarized in steps and shows a reduction (C) and actual disappearance of the EPSP in D (E_{EPSP}). Further depolarization (E and F) produced a reversal of this naturally occurring EPSP. G: Control after the cessation of the current injection. The current levels in C to F were calculated at 10, 20, 30 and 40 nA respectively. The all-or-none nature of this chemically mediated Purkinje cell EPSP demonstrates its climbing fiber origin

The second peak generated in the falling phase of the intracellular depolarization shown in Fig. 2 was produced by a second synaptic potential which was occasionally evoked 2—3 msec after the first and is typical of the repetitive activation of the CF system (Eccles *et al.*, 1966a). However, since this double CF activation did not occur every time, the amplitude of the averaged secondary depolarization is smaller. Note that in the extracellular action potential a break in the waveform is seen between the first and second depolarization, implying that the second depolarization may have changed the shape of the extracellularly recorded Purkinje cell burst spike. Since the intra- and extracellular recordings were not obtained simultaneously, it is impossible to ascertain in this Purkinje cell the number of action potentials produced by a single, as opposed to those produced by a double, CF-EPSP.

Demonstration of the Synaptic Nature of Harmaline-evoked Purkinje Cell Burst Spikes

That CFs produce chemically mediated all-or-none EPSPs was first reported by Eccles *et al.* (1966a). In order to ascertain whether the intracellular potentials recorded in Purkinje cells following harmaline administration were indeed CF EPSPs, electrical stimulation was given at white matter level after intracellular penetration of a Purkinje cell. Since the alleged EPSP had the same course whether it was spontaneously occurring or following electrical stimulation, it was assumed to be generated by the same afferent, i. e. the CF belonging to that particular Purkinje cell. Following electrical stimulation of the cerebellar white matter (WM), an EPSP was recorded intracellularly with a latency of 5 msec (Fig. 3A). During transmembrane current injection through the recording microelectrode the EPSP was increased in amplitude when the membrane was hyperpolarized, and decreased and ultimately reversed in polarity when the membrane potential

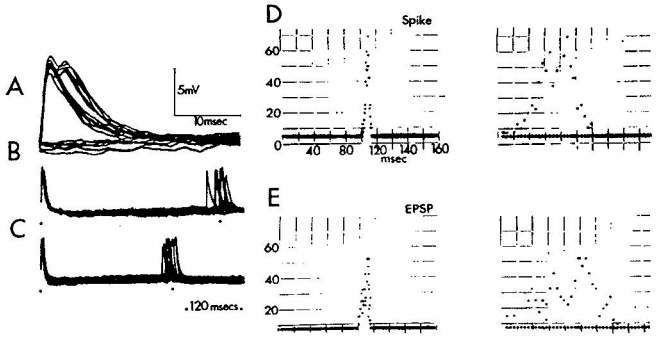

Fig. 4. *Rhythmic nature of the CF EPSP generation after harmaline.* A: The oscilloscope was triggered by the onset of the EPSP. The response consisted of one or two all-or-none synaptic potentials. B and C: Time interval between the generation of successive CF EPSPs. B, 10 sweeps; C, 20 sweeps. Time interval, 120 msec between dots. In D and E, time histograms for extracellular spike burst and EPSP in the same cell. D: Interburst frequency histogram. Ordinate, number of counts; abscissa, time in msec. To the right the abscissa is expanded to show time dispersion of histogram. E: As in D, for the EPSP recorded in the same cell

was moved in the depolarizing direction, thus demonstrating directly that the EPSP was indeed a chemically mediated phenomenon (Coombs *et al.*, 1955).

A similar reversal was then attempted in spontaneously occurring EPSPs after harmaline injection. In this situation the Wheatstone bridge was balanced with a square pulse, and a D.C. current was then applied through the recording electrode. The controlled EPSP before current injection is shown in Fig. 3B. Depolarizing current was gradually applied and kept at each level for a length of time sufficient to record several spontaneously occurring depolarizations. As the membrane was depolarized by the outward current, the amplitude of spontaneously occurring PSPs showed a marked decrease (C at 10 nA), a total disappearance (E_{EPSP}) (D at 20 nA), and finally a distinct reversal of the potentials (E and F at 30 and 40 nA). This finding characterized the potential as generated by a chemically acting synapse in every way identical to that activated electrically from the WM. In F removal of the D.C. current returned the PSPs to their original polarity.

It is clear, therefore, that these intracellular all-or-none depolarizing potentials are produced by the rhythmic activation of the CF afferent, inasmuch as all of the necessary criteria for the characterization of a chemically mediated synaptic potential were met. It may be concluded, therefore, that following harmaline administration, the CF system produces a rhythmic generation of synchronous burst responses of Purkinje cells.

Synchronous Properties of Climbing Fiber Activation in Purkinje Cells by Harmaline

Intracellular recordings from another Purkinje cell are shown in Fig. 4. The all-or-none character of the EPSPs is shown in record A; the oscilloscope trace was being triggered by the EPSP and the baselines generated by a momentary change in the triggering level of the scope. The actual interval between successive EPSPs was directly observed by reducing the oscilloscope's sweep speed and superimposing many such sweeps (Fig. 4B, C). Thus, the oscilloscope was triggered by

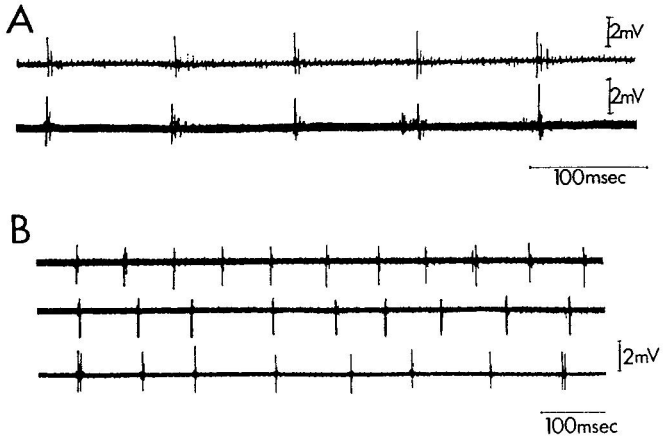

Fig. 5. *Multiple extracellular Purkinje cell bursts recorded simultaneously to demonstrate their synchronous occurrence.* A: Purkinje cell bursts recorded near the midline in the cerebellar vermis with two independent electrodes. The bursts occur in an almost synchronous manner. A second burst may be seen in the lower record in A occurring at approximately the same times. B: Three Purkinje cells simultaneously recorded at increasing distances from the midline with three independent electrodes. Upper trace, Purkinje cell near the midline; middle trace, Purkinje cell near the paravermal zone; lower trace, paravermal zone. The cells were recorded in the lobus simplex

every third EPSP; the second EPSP was displayed later on the beam and gave an immediate measure of the frequency and degree of synchrony of the CN activation. In Fig. 4B—C the dots measure a time period of 120 msec. It is evident, therefore, that the harmaline activation of the CF system is produced with a high degree of synchrony and that no intermediate activation occurs between these time intervals.

A more quantitative way of demonstrating the rhythmic nature of the CF activation of Purkinje cells is shown in Fig. 4D and E. In Fig. 4D an interburst frequency histogram illustrates the degree of synchrony of the Purkinje cell bursts; there were in fact no spontaneously occurring Purkinje cell bursts at any other time during the recording period (250 bursts were computed). In the histogram to the right, the ordinate (time) was expanded ten times to show in more detail the interval variation in occurrence of spike bursts generated by the CF repetitive activation. A histogram of the time interval of the intracellularly recorded EPSP (E) in the same cell is quite similar to that in D. The periodic nature of the activation of the CF system, as well as the rather high degree of synchrony which the system can display (histogram to the right), are demonstrated with this type of recording. Note that the frequency scatter is less than 10% of the basic frequency.

Simultaneous Multiple Purkinje Cell Recordings in the Cerebellar Cortex After Harmaline

Serial extracellular recordings from successive Purkinje cells after harmaline administration made it apparent that many of the CF activations were occurring in a simultaneous manner throughout the cerebellar vermal cortex. In order to

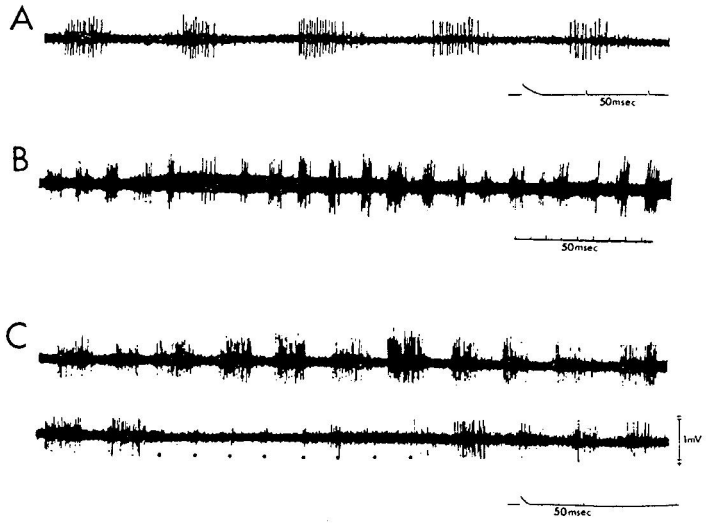

Fig. 6. *Extracellular recordings from fastigial neurons after harmaline.* A: Fastigial neuron showing typical burst of spikes at 10/sec frequency. B to C: Another two cells. In C electrical activation of the cerebellar cortex at 20/sec produced a total inhibition of the fastigial bursting (lower trace)

investigate this phenomenon, the activity of several Purkinje cells was recorded simultaneously with independent microelectrodes. The responses of two Purkinje cells recorded 200 μ apart in vermis lobule VI near the midline are shown in Fig. 5A. It is clear that the CF activation of these two Purkinje cells occurred with a rather close degree of synchrony. In the second trace a third and smaller CF Purkinje cell burst can be seen to occur about the same time as the larger one. This degree of synchrony between adjacent Purkinje cells was observed many times and will be analyzed further in a forthcoming publication. Simultaneous recordings from three different Purkinje cells were obtained at different distances from the midline (Fig. 5B); the first close to midline, second lateral in the vermis, and third in the pars intermedia of lobule V. Note that as recordings were obtained at increasing distances lateral to the center of the vermis, the synchronization of the Purkinje cell bursts became less apparent. With even further lateral recording (not shown), CF activation of the Purkinje cells was reduced in frequency and synchrony, and parallel fiber activation was often simultaneously observed. This is in agreement with ongoing experiments by Lamarre (personal communication) and by Montigny and Lamarre (in press) which demonstrate that harmaline has an action largely restricted to the medial accessory olive nucleus, which is the site of origin for the CF projection to the vermal lobules II to IX. Harmaline has considerably less effect on those portions of the inferior olive projecting to the pars intermedia or the cerebellar hemisphere (lateral accessory and main olivary nucleus) (Jansen and Brodal, 1954).

Electrical Activity Generated by Harmaline on the Fastigial Nucleus

Microelectrode recording from the fastigial nucleus (FN) is characterized by a synchronous repetitive activation of single cells (Fig. 6). Bursts of spikes lasting

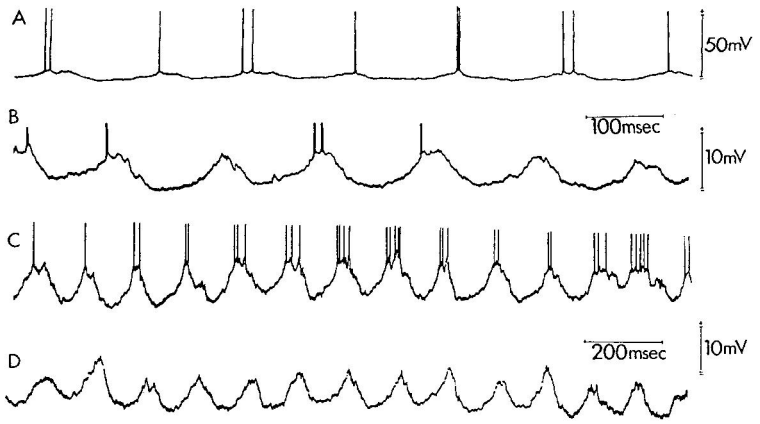

Fig. 7. *Intracellular recording from fastigial neurons after harmaline.* A: Intracellular action potentials showing characteristic rhythmic firing. B: Same cell at a higher gain to show the membrane potential fluctuations which generate the repetitive firing. C and D: Another fastigial neuron recorded at slower sweep speed. In D the cell is hyperpolarized to block spike initiation. Note the oscillatory behavior of the membrane potential

for approximately 20—30 msec and occurring every 100 msec are illustrated in Fig. 6A. A similar type of activity from another FN is illustrated in Fig. 6B at slower sweep speeds. In record C a third FN from a different experiment shows bursting behavior similar to that in A and B. In the lower trace of C a portion of the cerebellar cortex projecting to the FN (in this case the cerebellar cortex of lobulus V) was activated electrically at a frequency of 10/sec just before the spontaneous burst of spikes occurred. Note that cortical stimulation completely obliterated the firing of the FNs.

The bursting of FNs following harmaline intoxication was characterized intracellarly by a rhythmic fluctuation of their membrane potential at a frequency of 8—10/sec. Some examples of intracellular records from the FN are shown in Fig. 7. In A the intracellular recordings indicate the rhythmic variations of the membrane potential. In many cases these variations reached the firing level of the cell. In B records from the same cell were taken at higher gain to show in more detail the membrane potential fluctuations. Intracellular recordings from another FN are illustrated in C and D. These potentials were taken at slower sweep than those illustrated in A and B. In this cell the depolarization was always accompanied by action potentials of varying number (C). In D the neuron was artificially hyperpolarized by an inward D.C. current through the recording electrode. Even when the action potentials were blocked by the imposed hyperpolarization, the oscillations of membrane potentials were still present. However, these oscillatory depolarization-hyperpolarization sequences were not generated by the activation of cerebellar nuclear cells and their inhibition by Purkinje cells (Ito *et al.*, 1964, 1970). Rather, they were produced by a mixture of Purkinje cell inhibition and the disfacilitation generated by the abrupt removal of olivo-nuclear synaptic input following the synchronous activation of the olivo-cerebellar system, since hyperpolarization to E_{IPSP} did not substantially change the duration and fre-

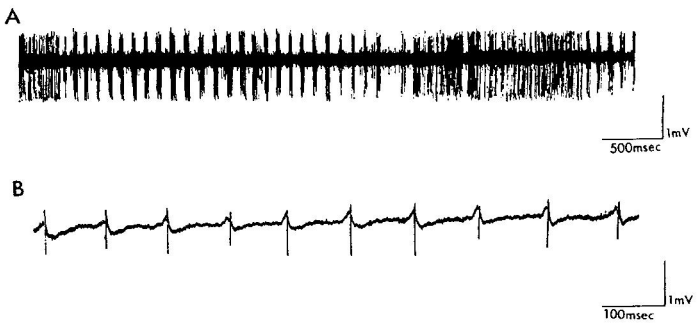

Fig. 8. *Unitary responses for bulbar reticular formation and inferior olive after harmaline.* In A, a bulbar reticular unit displays two types of firing behavior: continuous firing such as observed at the beginning of the record and near the end, and the rhythmical action potential burst occurring at 10/sec between tonic discharges. In B, the unitary action potentials of an inferior olive cell are superimposed in the peak negativity of the inferior olive field potential

quency of this rhythm. It appears that while the main rhythm is provided by the inferior olive, the Purkinje cells are almost co-activated (an average delay of 4—6 msec between olivo-nuclear and olivo-Purkinje-nuclear has been estimated) and function more as subtle modulators of the inferior olive rhythm (see below). Faster IPSPs have been recorded intracellularly from Deiters neurons after harmaline (Bruggencate *et al.*, 1972). These fast IPSPs probably occur in the cells showing maximum spatial summation of Purkinje cell IPSPs.

Bulbar Reticular Formation and Inferior Olive Recording During Harmaline Intoxication

Recordings from neurons in the bulbar reticular formation, immediately dorsal to the inferior olive, indicate that these cells tend to fire in a burst manner synchronously with the activation of the cerebellar nuclei (Fig. 8A). In this case, as with some reported by Montigny and Lamarre (in press), reticular neurons showed a rhythmic firing interrupted every so often (approximately every 4 sec here) by a tonic activation of the reticular cells. Following a lesion of the inferior peduncle, however, we were unable to demonstrate rhythmic reticular activation. This suggests that these neurons are being driven from cerebellar nuclei and not directly from possible inferior olive collaterals to the reticular formation.

As the electrode was lowered into the olivary nucleus, another type of activity was recorded. Field potentials and superimposed unitary spikes were always observed (Fig. 8B). As expected, the inferior olive demonstrated a rhythmical oscillatory bursting of the cells. This was especially clear in the caudal medial accessory olive, as has been elegantly shown by Montigny and Lamarre (in press).

Action of Harmaline at Spinal Cord Level

Simultaneous recording from ventral root 7 and Purkinje cells in lobulus V showed that motoneurons were firing in a burst-like manner at a frequency quite close to that of spontaneously occurring Purkinje cells (Fig. 9, two examples shown). While it is evident that no actual relation exists between the activation of that particular Purkinje cell and the motoneuronal axon bursts in the ventral

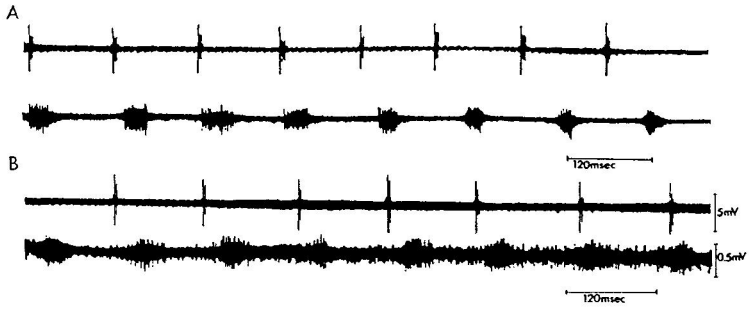

Fig. 9. *Climbing fiber activation of Purkinje cell and its frequency relationship to ventral root firing.* A: Upper trace, climbing fiber activations of Purkinje cell. Lower trace, electrical activity recorded in lumbar ventral root. The basic frequencies for the two phenomena are quite close. B: As in A, another example from a different experiment

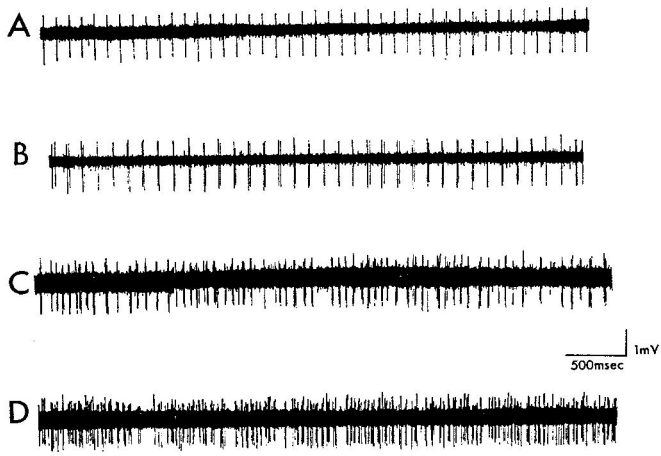

Fig. 10. *Electrical activity from small filaments in lumbar ventral roots.* A: Single unit firing regularly at approximately 10/sec. B: Two units. C: A larger filament. Note that the frequency doubles (from 10 to 20/sec) but still occurs in bursts of spikes. In D (same experiment as C) the cerebellar cortex was cooled with a freon aerosol

root, it is significant that the frequency of both electrical phenomena seems to be rather closely related. All the records were obtained following dorsal root rhizotomy from levels L5 to S3, which demonstrates that the tremor does not require the presence of the gamma loop.

Small filaments from ventral roots were isolated and showed an 8—10/sec rhythmic firing following harmaline administration (Fig. 10). In A a single motoneuron is seen firing in a repetitive fashion with a fairly constant frequency; in B another filament containing two active motoneuronal axons recorded in the same experiment. These two cells in B fired at approximately the same frequency although some variation is seen between the firing of the two axons. A larger filament was utilized for the recording in C which illustrates distinct motoneuronal spike bursts. Of interest here is the fact that the frequency appears to increase to approximately 20/sec, suggesting that different motoneuronal groups (e. g. extensor and

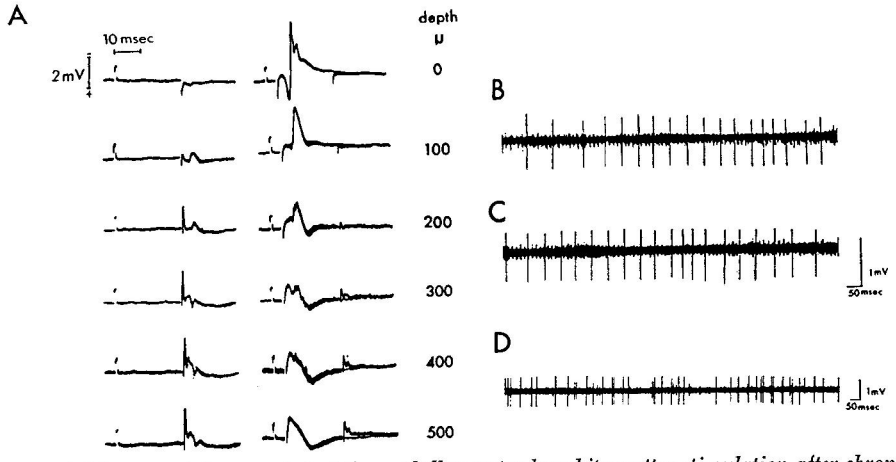

Fig. 11. *Field potentials generated at the cerebellar cortex by white matter stimulation after chronic bilateral inferior pedunculotomy, and extracellular activity of Purkinje and fastigial nucleus neurons following harmaline administration in the same preparation.* In A, tracings on the left are field potentials generated by a WM stimulus. In those on the right, the WM stimulus is preceded by a local stimulation of the cerebellar cortex. For further description see text. Depth of recording shown at right. In B and C, unitary Purkinje cell spikes. Note the lack of CF activation and the high background activity. D: Fastigial nuclear cell. Note the lack of rhythmic firing. The Purkinje cells were identified by their antidromic invasion from the WM and the fastigial neurons by their anatomical location

flexor) may be activated out of phase. In the same experiment, the vermal area at cerebellar cortex was cooled with a freon aerosol system which produced, a few minutes after onset of cooling, an obvious disorganization (D) of the rather patterned activation of motoneurons shown in record C. It must be noted, however, that although the pattern had not altogether disappeared, considerably more spontaneous motoneuronal firing was observed, implying that the integrity of the Purkinje cell system is required for the generation of sharp burst motoneuronal activation. In fact, with harmaline administration following a chronic cerebellar decortication, two cats demonstrated a transition from a fast well-defined tremor in the preoperative state to an almost tonic activation of the skeletal musculature following decortication. Although in these cases a definite tremor could be observed, it was never as clearcut as in the presence of an intact cerebellar cortex.

Action of Harmaline Following Inferior Peduncular Transection

In order to demonstrate that the activation of the olivo-cerebellar system was the input responsible for the harmaline activation of Purkinje and fastigial nuclear cells, the inferior peduncle was severed chronically in five cats, and electrical activity of Purkinje and cerebellar nuclear cells was studied following harmaline administration in two of these animals. A field potential analysis of cerebellar cortex responses evoked by cerebellar WM stimulation following chronic peduncular transection is illustrated in Fig. 11. The records to the left were made at different depths in the cerebellar cortex (from surface to 500 μ as marked at the right of the figure) following WM stimulation. As opposed to the records obtained in normal cats, where a WM stimulus activates the two afferent systems (mossy and

climbing fibers) and the Purkinje cells antidromically (Eccles *et al.*, 1966a), in this case the field potential generated by the CF system was absent. In fact, WM stimulation generated the classical early antidromic field potential (activation of Purkinje cells) and a subsequent complex potential which is negative near the surface. This latter field potential, which is in every way similar to that generated by the mossy fiber-granule cell-parallel fiber pathway (Eccles *et al.*, 1966b, 1967; Sasaki and Strata, 1967; Precht and Llinás, 1969), is totally inhibited by a preceding activation of the surface of the cerebellum via Golgi cell inhibition, demonstrating its mossy fiber origin (Eccles *et al.*, 1966c). At lower levels (300, 400 and 500 μ) the Purkinje cell responses generated on this late field potential are also inhibited via the same mechanism (Eccles *et al.*, 1966c). The inhibition of the antidromic field at depths of 200 to 500 μ is due to basket cell inhibition (Eccles *et al.*, 1966b). These results demonstrated the total transection and subsequent degeneration of the olivo-cerebellar system.

In such preparations, recordings from Purkinje cells after harmaline intoxication (Fig. 11) show only unitary spikes of the type generated via parallel fibers (Eccles *et al.*, 1966d), there being no sign of CF activity. A similar lack of rhythmic firing after harmaline administration was also encountered at the cerebellar nuclear level (Fig. 11).

Following chronic as well as acute inferior pedunculotomy, we were unable to find the 10/sec tremor after harmaline administration in doses as high as 15 mg/kg. Indeed a third of this dose was usually more than sufficient to evoke a very definite tremor in normal cats. We also tested harmaline in animals having had inferior peduncle transection 2.5 and 4 years previously. These animals also demonstrated a total lack of tremor following harmaline administration. Even when the inferior peduncle section was limited to its medial part (which is known to be the actual location of the olivo-cerebellar pathway), the animals showed no signs of tremor following adequate doses of harmaline. Acute recordings from the inferior olivary level demonstrated, however, that the 10/sec activation of inferior olivary cells continued following the removal of the cerebellum and transection of the neuraxis at the intercollicular level rostrally and at spinal C-1 caudally. Furthermore, in those cases where sections were performed below the red nucleus, the inferior olivary oscillations continued. These findings strongly suggest that harmaline probably acts directly on the inferior olive or its immediately surrounding tissue, and that its action does not require a feedback from the cerebellar nuclei or from the red nucleus or spinal cord.

Discussion

The experiments described above fully support and extend Lamarre's hypothesis that harmaline acts through the olivo-cerebellar system. However, our study of the action of harmaline was motivated more by the possibility of employing the drug as a tool for the understanding of the functional significance of the inferior olivary system, rather than of using it as a model for the study of a tremorigenous drug. From the results given above, we conclude that harmaline acts on the inferior olive and through this nucleus upon Purkinje cells via CF afferents and on cerebellar nuclear cells via collaterals of this olivo-cerebellar system. Whether the action of harmaline is directly on the cells of the inferior

olive themselves or on presynaptic structures impinging onto the inferior olive, has not been determined so far. However, several studies on the physiological properties of inferior olivary cells have demonstrated that they have an oscillatory tendency at about 10/sec (Armstrong and Harvey, 1966; Crill, 1970). We assume, therefore, that harmaline probably acts as a depolarizing agent either directly[2] or through increased synaptic bombardment which, via recurrent collaterals, will result in an activation of a large number of inferior olivary cells. That such collateral interaction may occur has been hypothesized by Eccles *et al.* (1966a) and Crill (1970); actual collaterals were demonstrated anatomically by Ramón y Cajal (1911), who showed that a large number of the collaterals return to the inferior olive of origin or else may terminate at the contralateral inferior olive. It is assumed, therefore, that recurrent facilitation of a large number of inferior olivary cells belonging to particular groups would tend to occur as a minimal number of olivary neurons (critical mass) begin to fire. Whether the 10/sec frequency of the inferior olive is produced by hyperpolarization generated by inhibitory interneurons via the axon collaterals, or whether it is generated by prolonged after-hyperpolarization due to delayed rectification or the presence of an electrogenic pump, has not been determined (see note added in proof). What does seem evident, however, is that in an isolated lower brain stem the inferior olive is able to maintain its 10/sec oscillatory behavior. The fact that, in the awake animal, a transection of the medial part of the inferior peduncle is by itself able to suppress harmaline tremor, and that this transection impedes Purkinje and nuclear cell rhythmic firing, as well as vestibular and reticular synchronization, constitutes in our view the strongest demonstration that the tremor is indeed produced via the olivo-cerebellar system. It is also a demonstration that the inferior olive must, of necessity, exert its influence on the skeletomusculature exclusively through the cerebellar nuclear system.

We consider, therefore, that this finding provides still further evidence that the function of the olivo-cerebellar system is probably related to a phasic activation of skeletal muscle groups. The fact that the inferior olivary system cannot be re-activated for 100 msec following its activation supports the view that movement may be organized in a series of continuous steps occurring at a frequency of 10/sec. That the inferior olive is able to produce a clearcut tremor at such high frequency is in itself strongly suggestive that the olivo-cerebellar system is a motor transient generating system[3]. The existence of such a system may be ex-

2 This is a distinct possibility since harmaline is known to inhibit sodium pumping in squid axon (Canessa, 1970). If the resting potential of inferior olivary neurons is partially maintained by an electrogenic sodium pump, the removal of this polarizing mechanism could be sufficient to produce the postulated depolarization.

3 Of interest in this context is the fact that harmaline produces a very clear tremor in newborn cats (Llinás and Volkind, unpublished observations) while in animals such as the rat, where the CF-Purkinje cell synapse matures 6—8 days after birth, harmaline tremor is not induced until the second week after birth (Henderson and Woolley, 1970). It should also be mentioned that no permanent motor abnormality was observed after a daily administration of harmaline to newborn cats. This daily injection, which was continued for up to 5 days, did not show the alteration of motor control which one would expect to result from the postulated plastic modifications of parallel fiber-Purkinje cell synapse by the prolonged activation of the CF system (Marr, 1969).

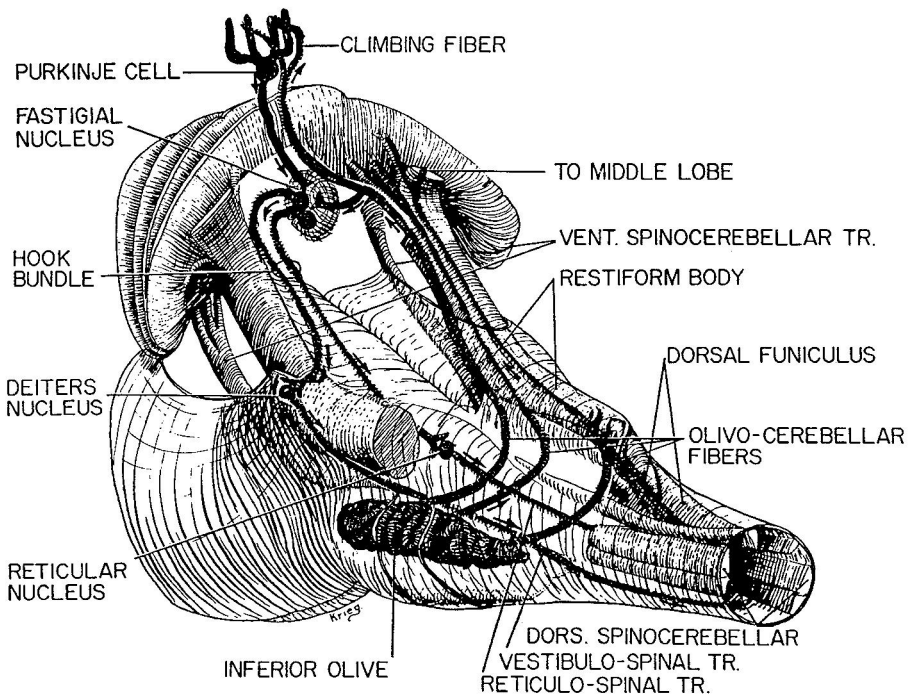

Fig. 12. *Stereogram of brain stem and cerebellar white matter, including the fastigial nucleus.* In red, the circuit activated by harmaline administration. The drug acts at inferior olive level. Through the olivo-cerebellar pathway, the olive activates fastigial nuclear cells and, via climbing fibers, the Purkinje cells. The latter project back to the cerebellar nuclei. The output of the fastigial nucleus activates alpha motoneurons through the Deiters and reticular nuclei. Arrows indicate direction of pathway conduction. Note that part of the left restiform body has been removed and that Deiters nucleus appears to be lateral to the restiform body. (Illustration kindly modified by Prof. W.J.S. Krieg, from his book *Functional Neuroanatomy*, *Blakiston*, 1953)

tremely important in either sudden initiation or sudden cessation of a motor act, given that the latter action requires not only relaxation of agonistic muscles but also activation of muscles which oppose the ongoing motion.

From this frame of reference, therefore, it may be hypothesized that the CF activation of a Purkinje cell represents part of an olivo-cerebellar command motor system with the CF-Purkinje cell junction being more powerful as a means of overriding Purkinje cell activity (Llinás *et al.*, 1969; Llinás, 1970). The function of the olivo-cerebellar system, in this analysis, would be to activate certain neurons in the cerebellar nuclear system and to utilize the Purkinje cells as a means of regulating frequency and duration of activity in the cerebellar nuclei. It is evident that since the CF activation of Purkinje cells will be modified by the background activity of the Purkinje cells, the mossy fiber system would ultimately exercise a certain degree of interaction even during the activation of this olivo-cerebellar command system.

Finally, a rather complex supraspinal action appears to be exerted onto the spinal segmental levels during harmaline intoxication; this is in apparent accord

with the view of ballistic motor control of the olivo-cerebellar system. Thus, besides the activation of motoneurons, the olivo-cerebellar system seems to momentarily "shut off" the mossy fiber input at not only the cortical but possibly also at the spinal cord level. This "switching" property of the olivo-cerebellar system, which will be analyzed in a subsequent paper, appears of central importance in the organization of a ballistic response, inasmuch as unexpected peripheral input may interfere with the final goal of a ballistically organized pattern of movement.

In Fig. 12 a schematic representation is given of the pathways involved in the generation of harmaline tremor. Note that the Purkinje cell system is organized in a feed-forward manner with respect to the cerebellar nuclei.

Although the present analysis gives only a glimpse of the subtle properties of the olivo-cerebellar interactions, we believe harmaline to be an important tool in the further understanding of this rather cryptic system.

Acknowledgment: This research was supported by U.S. Public Health Service research grant No. NS-09916-01 from the National Institute of Neurological Diseases and Stroke. Dr. Volkind is a research fellow of the Argentine National Council for Scientific and Technical Research. The authors express their gratitude to Prof. Wendell J.S. Krieg for kindly modifying Fig. 243 from his book *Functional Neuroanatomy* (Blakiston, 1953) to illustrate the pathways activated by harmaline administration, to Dr. Y. Lamarre for discussion during the preparation of the paper, and to Dr. D.E. Hillman for the anatomical verification of olivocerebellar lesion in the chronic inferior pedunculotomies.

Note added in proof: Recent functional and morphological findings by Llinás, Baker and Sotelo (Electrotonic coupling between inferior olive neurons in the cat. EEG Clin. Neurophysiol., abstract in press) demonstrate that inferior olive (IO) neurons are electrotonically coupled in the cat. In addition, antidromic activation of the olivocerebellar pathway generates, via collateral activation of inhibitory interneurons, prolonged IPSPs in the IO cells. These two mechanisms (electrotonic coupling and recurrent inhibition) are in principle sufficient to explain the oscillatory behavior of IO cells if their excitability is directly increased by harmaline.

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