Frog Cerebellum: Absence of Long-Term Inhibition upon Purkinje Cells

Abstract. *Electrophysiological studies of the cerebellar cortex of the frog demonstrate a lack of long-term inhibition upon the Purkinje cells. This lack of inhibition correlates well with the absence of stellate and basket cells in the molecular layer of this cerebellum and strongly supports the idea that these interneurones are the agents responsible for the prolonged inhibition seen in the Purkinje cells of other species.*

It has been reported that the interneurones of the molecular layer of the cat cerebellum exert a long-term inhibitory action on the soma and dendrites of the Purkinje cells (*1, 2*). Since the cerebellum of the frog is lacking in these interneurones but has otherwise the same basic neural organization as that of the cat (*3, 4*), it appears an excellent preparation in which to test the validity of the above findings (*5*). In addition, it is ideal for a study of the field potentials produced at various depths by an excitatory axodendritic input (the "crossing over" synaptic junction between the parallel fibers and the dendrites of the Purkinje cells) on to an open-field arrangement, the Purkinje cell (*6*).

The experimental procedure was similar to that described by Matthews, Phillips, and Rushworth (*7*). Bullfrogs (*Rana catesbiana*) weighing from 180 to 200 g were anesthetized with pentobarbital sodium (60 mg/kg). The cerebellum was exposed by an extensive craniotomy, and the optic lobes were then sucked away, leaving the molecular layer accessible to experimentation (Fig. 1A). The molecular and granular layers were stimulated by means of bipolar electrodes (Fig. 1A). One of these electrodes was placed gently on the surface of the molecular layer (Loc) and was used to stimulate a "beam" of parallel fibers along the main axis of the cerebellum (*1, 2*). The second electrode (WM) was inserted through the lateral region of the cerebellum into the granular layer some 400 to 500 μ from the surface and was used to activate antidromically the axons of the Purkinje cells (*7*) as well as the mossy and climbing fibers which traverse this layer (*3, 4*). Surface recordings were taken by means of a ball electrode (RE) so that a suitable site for microelectrode insertion could be localized. Microelectrodes filled with 3*M* KCl or 4*M* potassium citrate were used for the intracellular recordings, while extracellular recordings were carried out with microelectrodes filled with 4*M* NaCl. Direct-current amplification was used in both instances.

Since surface recordings showed that a local stimulation of the molecular layer activated a "beam" of parallel fibers, a study of the depth profile of the field potential evoked by such stimulation was undertaken (Fig. 1, B–G). At the surface, the potential consisted of two components similar to those recorded in the cat cerebellum (*2*). The initial spike-like positive-negative potential, lasting 8 msec, was followed by a long-lasting negativity (35 msec). The electrode was then inserted to a depth of 500 μ from the surface, and potentials were recorded at different levels as the electrode was withdrawn. At 500 μ the field evoked by a single Loc stimulation consisted of three components, a small negativity followed by a longer lasting positive wave and, between these two, a negative-positive transient (Fig. 1, B–G; second arrow). As the electrode was removed, the positivity became larger, reaching a maximum at about 200 μ. Between this depth and the surface it decreased again, became negative at a depth of 150 μ, and increased further until the electrode reached the surface. The neg-

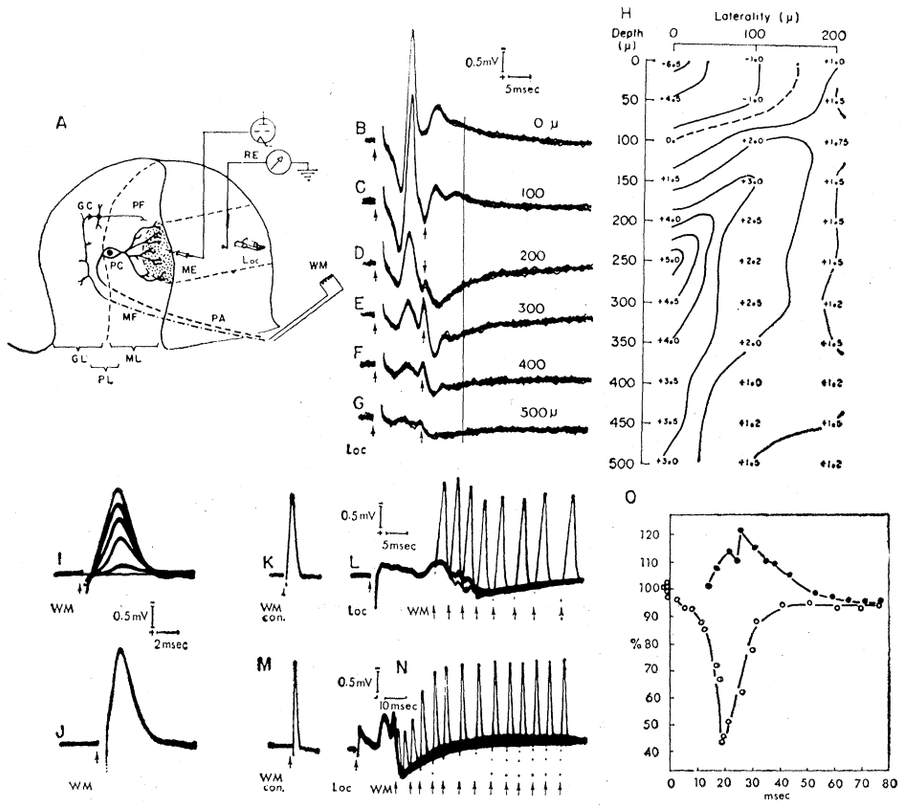

Fig. 1. (A) Diagram of frog cerebellum and experimental arrangement as described in text. *ML*, Molecular layer; *GL*, granular layer; *PL*, Purkinje-cell layer; *GC*, granule cell; *PC*, Purkinje cell; *PF*, parallel fiber; *MF*, mossy fiber; *PA*, Purkinje-cell axon; *ME*, microelectrode; *RE*, surface recording electrode; *Loc*, surface stimulating electrode; *WM*, white-matter electrode. (B–G) Field potentials evoked by Loc stimulation at indicated depths; vertical line shows latency at which potentials plotted in H were taken. (H) Isopotential contour plotting. Depth in vertical coordinates, laterality in horizontal coordinates. Values at each point are arbitrary; signs demarcate polarity of potentials. (I) Antidromic fields evoked in molecular layer by graded WM stimulation. (J) Antidromic field following WM stimulation (50 per second). (L and N) Superimposed traces showing interaction of Loc and WM stimuli at different intervals. (K and M) Control antidromic fields (note different sweep speeds). (O) Plot of records from interaction study as illustrated in L and N; interval in milliseconds, amplitude in percentage with respect to control. Closed circles, Loc stimulation at 2.5 threshold; open circles, at five times threshold. Arrows indicate stimulus artifact, except second arrows in B–G (see text).

ative-positive transient was largest at a depth of 300 μ and then reversed to a positive-negative transient at 100 μ.

Although this type of field closely resembles that recorded from the cat cerebellum (*1, 2*), one difference is immediately apparent. The positivity is maximum at 200 μ and decreases progressively from that point inwards. A more striking difference, however, is seen when the electrode is moved laterally from the beam of activated parallel fibers. The positivity recorded at 200 μ (p wave) decreases rapidly with distance, so that only a very small field is observed at 200 μ laterally. In Fig. 1H an isopotential plotting shows the profile of the field potential as recorded immediately under the beam of activated parallel fibers and in regions 100 to 200 μ lateral to that point. Thus, the potential profile is quite different from that of the cat, there being much less lateral spread of the p wave.

We also studied the interaction between potentials evoked by a stimulus applied to the white matter through the WM electrode and potentials evoked by Loc stimulation. It is possible, by careful placement of the WM electrode in the depth of the granular layer, to evoke a negativity with short latency at a depth of about 250 μ in the cerebellar cortex (Fig. 1, I and J). Figure 1, part I, shows how the amplitude of this potential increases progressively as increasing stimuli are delivered through the WM electrode. Its short latency and its ability to follow a stimulus with a relatively high rate of repetition (50 per second) (Fig. 1J) indicate that this response is produced by the antidromic invasion of Purkinje cells (*7*).

The study of the interaction between the antidromic field potential and the potentials evoked by the stimulation of parallel fibers is one of the most accurate means of demonstrating changes in the excitability of the soma and dendrites of Purkinje cells (*8*). If a stimulus to the white matter follows a Loc stimulation of moderate intensity (2.5 times the threshold for the activation of parallel fibers), there is an increase in the amplitude of the antidromic field at intervals of from 15 to 60 msec (Fig. 1L). The control amplitudes of the antidromic field are illustrated in Fig. 1, K and M. The time course of this effect is plotted for a similar set of records in Fig. 1O (closed circles). If, however, a stronger Loc stimulation is applied (five times the threshold for parallel-fiber activation), the amplitude of the antidromic field decreases (Fig. 1N). The plot in Fig. 1O (open circles) shows the time course of the depression of the antidromic field from an analogous set of records. The maximum depression occurred at a 20-msec interval; the depression was largely terminated in 30 to 40 msec. These findings suggest that a moderate stimulus to the parallel fibers will produce a facilitation of the antidromic invasion, while a stronger volley will reduce the number of Purkinje cells invaded antidromically.

Single Purkinje-cell action potentials were observed. Figure 2A shows a giant spike (*9*) evoked by an electrical stimulus through the WM electrode; the stimulus strength was approximately threshold. The record was taken at a depth of 200 μ and represents the all-or-nothing activation of a Purkinje cell (*7*). The same cell was activated by a Loc stimulation of moderate strength (Fig. 2B). This activation was sometimes double (Fig. 2C). If two volleys were delivered through the local electrode (Fig. 2C), a facilitation of the second was observed, the control being Fig. 2B. In this respect the Purkinje cells of the frog cerebellum definitely differ from those of the cat, since activation of parallel fibers of Purkinje cells in the latter generally produces a single spike (*1, 2*) and only rarely a double activation. In addition, the cat cerebellum never shows summation of the excitatory action of two consecutive stimuli of parallel fibers, since the first stimulus produces, after a short period of excitation, a powerful and long-lasting inhibition of the Purkinje cells through the interneurones of the molecular layer. The lack of inhibition on frog Purkinje cells is further illustrated in Fig. 2, D–F. Here a strong Loc stimulus (five times threshold) evoked a repetitive activation of such a cell for a period of up to 70 msec. In some cases there was a reduction in the amplitude of the spike, as has been observed for the interneurones of the molecular layer of the cat cerebellum (*10*). In other cases (Fig. 2E) this reduction did not occur. We recorded intracellularly excitatory postsynaptic potentials (EPSP) from Purkinje cells following WM stimulations of increas-

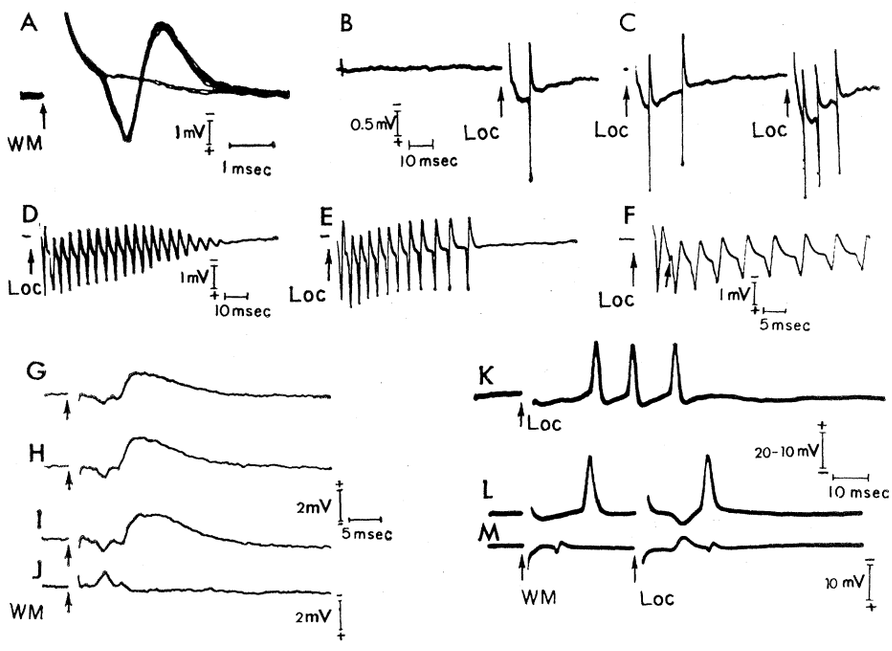

Fig. 2. (A) Extracellular record of Purkinje cell activated antidromically. (B) Control record. (C) Extracellular Purkinje-cell spikes showing facilitation of the second of two Loc stimuli when preceded by a similar stimulation. (D–F) Repetitive firing of a Purkinje cell following a strong Loc stimulation. The second arrow in F shows incomplete activation of Purkinje cell. (G–I and K–L) Intracellular records from Purkinje cells; (G–I) EPSP's evoked by graded WM stimulation. (K) Repetitive activation following Loc stimulation. (L) Interaction between WM and Loc stimulation; there is no inhibition of the Loc spike by the preceding WM stimulus. The WM spike was evoked, as in G–I, via the mossy fiber-granule cell pathway. (J and M) Extracellular records showing field potentials in the vicinity of recorded cells. Note change in polarity. Time and voltage calibrations and polarity indicated. Voltage calibration 20 mv for K and 10 mv for L. Arrows indicate stimulus artifact, except second arrow in F.

ing strength (Fig. 2, G–I). An action potential was not evoked in this cell, since the resting potential was 35 mv. The field potential was recorded in the immediate vicinity of the cell after withdrawal of the microelectrode (Fig. 2J, negative up). The EPSP is not followed by the large inhibitory postsynaptic potential (IPSP) which would be recorded under similar conditions in cat Purkinje cells (*11*). Figure 2K shows a repetitive activation of a Purkinje cell by a parallel fiber activation, and in Fig. 2M a WM stimulus precedes the Loc stimulation. Both cells had a resting potential of 40 mv. As in the previous case, there is no sign of an IPSP. The field potential was recorded in the vicinity of the cell prior to penetration (Fig. 2M). The extracellular Purkinje-cell action potentials are apparent. The negative potential following Loc stimulation is the parallel-fiber field. Most cells recorded intracellularly had resting potentials ranging from 30 to 55 mv, and thus only small action potentials could be evoked.

Our findings combine to demonstrate the absence of long-lasting inhibition upon frog Purkinje cells. The field potentials initiated by parallel-fiber activation may be interpreted as produced conjointly by the parallel-fiber action currents (initial spike-like negativity) and the dipole evoked in the Purkinje cells by the synaptic depolarization of their superficial dendrites. This synaptic impingement would establish a current sink, seen as a large negativity near the surface, and a source of current from the basal dendrites and soma responsible for the production of the deep positive potentials. The negative-positive transient observed at depths of 200 to 500 μ seemingly represents compound action potentials of Purkinje cells produced by the activation of parallel fibers, its maximum amplitude corresponding roughly to the level of the Purkinje-cell layer (300 μ). In accordance with this interpretation, the reduction of the negative-positive transient at a depth of 400 to 500 μ would be understandable, since the Purkinje-cell axons turn laterally towards the cerebellar peduncles after reaching the granular layer (*3*). The reversal of the field at 100 μ implies that the superficial Purkinje-cell dendrites cannot generate action potentials but serve as current sources to the soma and thick dendrites below. A similar lack of superficial dendritic invasion has been observed in this animal during study of the antidromic fields at approximately the same depths (*12*). The fact that there is much less lateral spread of the positivity than in the cat corroborates the suggestion that in the cat the out-of-line positivity is largely due to the inhibitory action of stellate (*2*) and basket cells (*1, 2*). Reduction of the antidromic field produced at short intervals after a strong Loc stimulation can be explained as being caused by the collision of the antidromic action potentials with the action potentials being evoked orthodromically after the Loc stimulation (Fig. 2, D–F). However, the possibility of a short-term inhibitory action by the activation of axon collaterals of Purkinje cells (*11*), which have been shown in the cat to be inhibitory to cerebellar nuclei (*13*), or by parallel-fiber activation of the small interneurones of the molecular layer (*5*) remain as possible components of this depression. On the other hand, even under conditions of low resting potential (Fig. 2, G–I), which are known to increase the electromotive force of Purkinje cell IPSP's in the cat (*11*), no IPSP's were recorded in frog Purkinje cells. This lack of inhibitory impingement was noted after graded Loc or WM stimulation or any combination of these two stimuli in over 20 Purkinje cells penetrated.

The fact that the cerebellum of the frog, which is known to have a definite regulatory action on the postural tonus of the animal (*14*), is devoid of long-lasting inhibition shows that an effective functioning of a primitive cerebellum can occur in the absence of the inhibitory regulation of Purkinje cells.

R. LLINÁS
Institute for Biomedical Research, 535 North Dearborn Street, Chicago, Illinois

J. R. BLOEDEL
Department of Physiology, University of Minnesota, Minneapolis

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Magnesium Pemoline: Lack of Facilitation in Human Learning, Memory, and Performance Tests

Abstract. *Either a placebo or 25 or 37.5 milligrams of magnesium pemoline was administered on a double-blind basis to three intelligence-matched groups of normal, adult males. Learning and 24-hour retention tests included verbal learning, motor learning, and classical conditioning. Short-term memory tests were administered through both the visual and auditory modalities. Arm-hand steadiness and visual reaction time performance tests were included. The only measures revealing significant group differences showed the performance of subjects given pemoline was inferior to that of subjects given a placebo.*

Recent animal studies with magnesium pemoline have indicated that this drug increases the amount of brain RNA (*1*), facilitates acquisition, and extends retention in shock avoidance learning (*2*). Methodological criticisms have been leveled at the interpretation of the animal retention data (*3*). Human tests of a similar drug, lacking only the magnesium constituent, have been performed in Europe over the last 5 years. In one case the findings indicated facilitation of a complex psychomotor task (*4*), while another study found the drug effect too variable to be of value in speeding the motor and auditory responses of jet pilots (*5*).

Although many stimulants have been shown capable of speeding reaction times and increasing vigilance, compounds have rarely appeared which could specifically enhance the rate of learning or the extent of retention in