Short Communications

Parallel fiber and white matter activation of Purkinje cells in a reptilian cerebellum *(Lacerta viridis)*

Extrinsic inputs to the cerebellar cortex are of two types, namely the climbing and mossy fiber inputs. Among the various species studied, differences have been observed in the unitary behavior of Purkinje cells activated by these two inputs. Climbing fiber activation results in all or nothing multiple spikes while mossy fiber activation evokes a graded response varying with the intensity of stimulation.

We have studied mossy and climbing fiber responses of Purkinje cells following local surface parallel fiber (Loc) and white matter peduncular (WM) stimulation in nembutalized (35 mg/kg) lizards (*Lacerta viridis*). Extracellular and intracellular

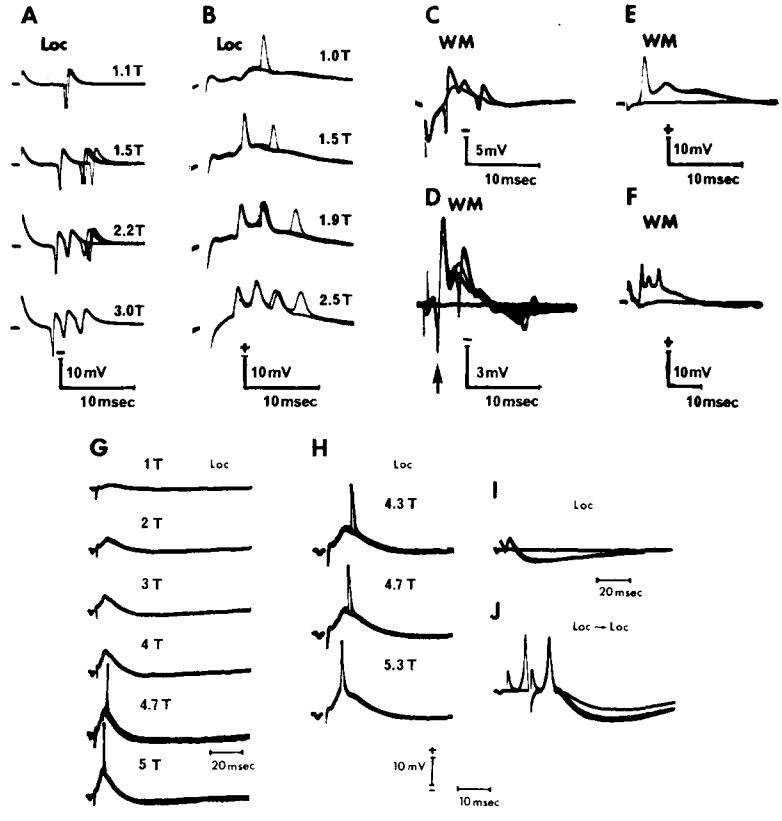

Fig. 1. Parallel fiber (A, B, and G–J) and climbing fiber (C–F) responses of Purkinje cells following parallel fiber (Loc) or peduncle (WM) stimulation. Arrow in D indicates an antidromic spike. Traces in A, C and D are extracellular, while those in B, E, F, and G–J are intracellular recordings. Numbers above traces in A, B, G and H refer to stimulus strength in multiples of the threshold. The 10 mV calibration bar at the bottom of column H applies to all records in G through J. Further discussion in text.

potentials were recorded through 4 *M* NaCl or 3 *M* KCl filled glass microelectrodes with DC resistances of 3–15 MΩ. A total of 61 units were studied. All were located at the depth of the Purkinje cell layer. A detailed description of the method and procedure for recording and stimulation is described elsewhere[10].

Typical Purkinje cell unitary responses to Loc stimulation are shown in Fig. 1A and B. They were graded (as many as 4 spikes), and the latency of the first spike was shorter at higher intensities. The average latency (n = 52) was 6.4 msec with a range of 4–8.5 msec (Fig. 2). Examples of climbing fiber responses of Purkinje cells following WM stimulation can be seen in Fig. 1C–F. When recorded extracellularly (C and D), all-or-none bursts of 3 or 4 spikes of decreasing size were observed. When recorded intracellularly these bursts were seen to be riding on an all-or-nothing compound depolarization (E and F). A fixed latency in each unit studied, regardless of the stimulus intensity, averaged 2.6 msec with a range of 1.5–3 msec (Fig. 2).

These data indicate that the lizard Purkinje cell responses are, in essential features, much like those reported for frog[8,13–15,18], dogfish[6,7] and cat[2–4]. However, Loc activation of Purkinje cells in cat cerebellum rarely results in more than one spike[2] while in frog a repetitive discharge lasting 45 (ref. 15), 50 (ref. 8), or even 70 msec (ref. 14) at frequencies of 300 c/sec (ref. 14) has been reported. The dogfish Purkinje cell (under these conditions) discharges 2–3 spikes at frequencies of 200 c/sec (ref. 6). The lizard Purkinje cells, following higher intensities of Loc stimulation, fired 3 or 4 spikes at a frequency of 400 c/sec and the total response never lasted more than approximately 10 msec. The difference between species in the climbing fiber responses is that the duration of the burst response was 5–7 msec in the cat[4], 20 msec (refs. 8, 13) or longer[15] in the frog and about 10 msec in the selachian cerebellum[6] which is the usual duration observed for the lizard.

A series of intracellular records of Purkinje responses to Loc stimulation of increasing strength is presented in Fig. 1G and H. The step-wise increase in amplitude of the depolarization potential with increasing stimulus intensity is clearly shown (Fig. 1G). The same Purkinje response recorded at a faster sweep with a finer gradation of the stimulus strength (Fig. 1H) better demonstrates the typical decrease in the latency of the spike and the corresponding shortening of the rise time of the slow positive potential with increasing stimulus intensity. These data are suggestive of this depolarization being a composite EPSP which by summation produces the multiple parallel fiber responses at higher stimulus intensities and accounts for the observed latency change.

Fig. 1I shows a long lasting (90 msec) hyperpolarization which follows the EPSP after Loc stimulation. The 'cross-over' point from depolarization to hyperpolarization occurs at about 10 msec after the onset of the EPSP which fits well with the present data that all parallel fiber and climbing spikes occur within approximately a 10 msec interval. This is similar to the 'cross-over' point in the selachian[6] and alligator[17] cerebellum and at the observed frequency of 200 c/sec permits as many as 3 spikes. In frog where there is no 'cross-over'[1,14,15] or, a very late 'cross-over'[8], the parallel fiber response continues for as long as 50 (ref. 8) to 70 (ref. 13) msec.

The phenomenon of summation of IPSPs elicited by double Loc stimulation

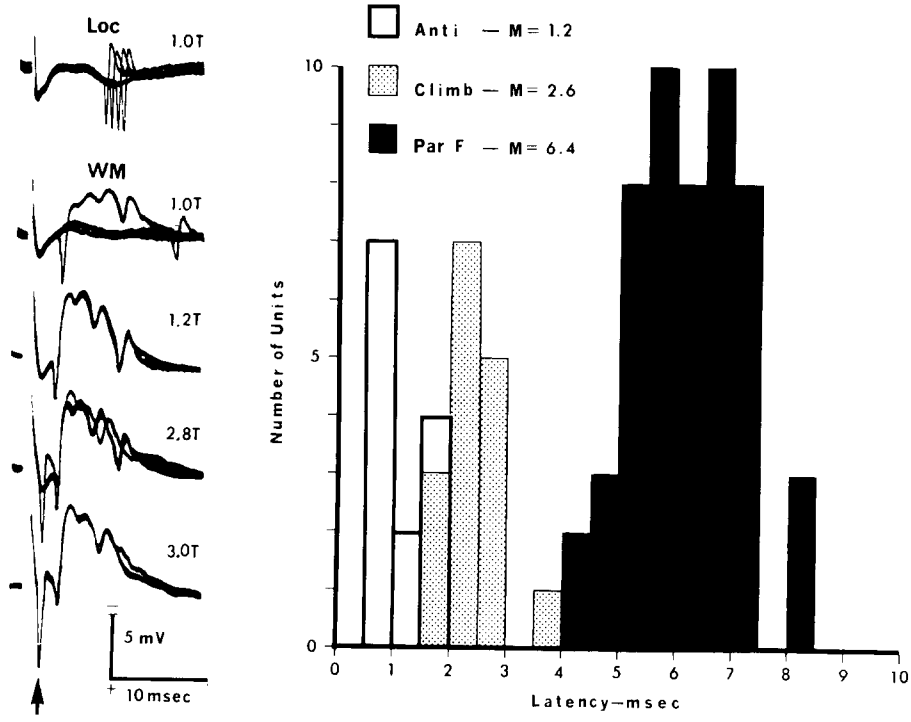

Fig. 2. Latency histogram. Examples of parallel fiber, climbing and antidromic Purkinje cell responses are shown to the left. Stimulus intensity indicated as in Fig. 1. Top record shows extracellularly recorded parallel fiber response to Loc stimulation; the next 4 records, an extracellular recording of a climbing fiber response to WM stimulation. The arrow at bottom indicates the antidromic response. Number of units included in each group in histogram: 13 antidromic, 16 climbing and 52 parallel fibers. See text for further discussion.

at an 8 msec interval is shown in Fig. 1J. Several responses to the double shock are superimposed on the single control record of the second stimulus alone. It is clearly shown that the summated IPSPs following double stimulation reach a deeper level of hyperpolarization, a phenomenon similarly observed in the cerebellum of elasmobranchs[6,19].

All of the species differences in the parallel fiber response mentioned can be related to the relative development of the inhibitory interneurons in the cerebellar cortices of the frog[1,8,9,14,23], cat[3] and dogfish[6]. The present observations fit into this scheme quite well when considered in conjunction with the available histological information[12,20]. These studies indicate that there are no true basket cells in the cerebellar cortex of the lizard, but that some of the deeper stellate cells may reach an intermediate state of development closer to a primitive basket cell than to a superficial stellate cell. It would seem highly likely that the stellate cells are responsible for the long lasting IPSP of Fig. 1I. The species differences in duration of climbing fiber responses might be accounted for on the same basis if the climbing fiber collateral input to stellate and basket cells is of a significant magnitude[21]. The role of the stellate cell in inhibition in the lizard will be established and considered in another paper[11].

Fig. 2 presents a summary of the latencies of the unit responses observed. The first column shows examples, recorded extracellularly, of the 3 types of responses recognized. The uppermost trace demonstrates the Purkinje response to threshold stimulation of parallel fibers. The next 4 traces show a Purkinje cell response to WM stimulation at increasing intensities. The first two of these traces show the typical climbing fiber response. In the last two traces the very fast spike of an antidromic response (arrow in last trace) is seen. The much higher threshold of the antidromic response in this case is probably due to the spatial relationships between the stimulating electrode and the Purkinje axon and the climbing fiber. We have observed responses in which the reverse order of apparent thresholds existed (Fig. 1D). In the histogram 13 units gave responses identified as antidromic on the basis of: (a) their very brief latency, (b) a refractory period of 1.5–3.0 msec, (c) a very stable latency for each unit even with increasing stimulus strength, and (d) a single spike response at any stimulus intensity above threshold. These responses then are similar in all respects to the antidromic responses reported for frog[8,16], dogfish[6] and cat[5]. The latency spread in this group was from 0.5 msec to 1.9 msec (1.4 msec). Climbing fiber responses were identified in 16 units by the characteristics previously mentioned. The range in latencies was from 1.5 to 3.5 msec (2.0 msec). In 52 units parallel fiber responses were identified by their variable latency and their ability to respond in a graded fashion to stimuli of increasing strength. The latency range was from 4.0 to 8.5 msec (4.5 msec), which is slow relative to other species. However, the parallel fiber conduction velocity in the lizard is slow, ranging from about 20 to 30 cm/sec respectively for the slowest, deeper fibers and the fastest, more superficial fibers[10,22]. This would account for the wide latency range, since the variation in conduction distance (stimulating to recording electrode) was largest for this group. The latencies for these 3 types of Purkinje responses are in extremely good agreement with the results of our field potential studies which will be reported later[22].

In conclusion it may be said that the inputs to the lizard cerebellum appear to be quite similar to those found in all other species studied. The variations from the general pattern of Purkinje cell responses to these inputs may be related to differences in the inhibitory mechanisms. A detailed report on our studies of the inhibition of lizard Purkinje cells will follow[11].

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