Electrophysiological Properties of Dendrites and Somata in Alligator Purkinje Cells

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DURING THE LAST hundred years, some of the most fascinating and controversial questions in neurobiology have concerned the functional significance of neuronal dendrites in the central nervous system. The problems first became evident when morphologists such as Kölliker (35), Retzius (61), Dogiel (7), Held (24), and especially Ramón y Cajal (58) described, with a wealth of detail, the enormous complexity and extent of the dendritic arbor in neurons such as the Purkinje cell. Adding to the interest generated by those discoveries was the almost simultaneous realization that the neuronal dendrites represent most of the postsynaptic neuropil in the vertebrate central nervous system (58).

From the physiological point of view, on the other hand, it is now tacitly agreed that a proper understanding of the functional role of dendrites in neuronal integration is of central importance in gaining further insight into the collective operations of nerve nets.

This paper presents electrophysiological results, obtained by intra- and extracellular recording techniques, regarding the generation and conduction of dendritic spikes in the alligator Purkinje cell and their action at somatic level. Certain corollaries to findings reported here and in the preceding paper (53)—such as partial independence of spike activation in dendrites, dendritic inhibition, somatopetal tendency for dendritic spike conduction—and their implications concerning neuronal integration will be elaborated on in the DISCUSSION.

A few records from a previous publication (41) have been reintroduced here to provide a more coherent presentation.

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MATERIALS AND METHODS

The alligator, *Caiman sclerops,* was used in the present research. The basic methods have been described in previous papers (41, 42, 53).

Intracellular recordings from the somata and dendrites of Purkinje cells were obtained using micropipettes filled with 3 M KCl and K citrate (average d-c resistance 10–20 megohms), or with an aqueous solution of 10% Procion yellow dye (average d-c resistance 30–40 megohms). In order to localize as precisely as possible the place of electrode penetration, Procion dye was electrophoretically injected intracellularly (2, 32, 52, 56, 60, 63) using brief current pulses of the order of 10^{-8} to 10^{-7} amp across the cell membrane. The animals were sacrificed within 30 min after dye injection, and the tissue was fixed in a formalin and cacodylate mixture and imbedded using standard paraffin technique. Sections 10 μ thick were examined using a Zeiss fluorescence microscope, and photomicrographs were made on Ektachrome 125 ASA and Anscochrome 500 ASA film. Current was also applied through the impaled neurons by means of a "bootstrap" Wheatstone bridge (30).

RESULTS

Extracellular and Intracellular Potentials Recorded at Different Levels in Molecular Layer

A detailed analysis of the field potentials generated in the molecular layer by cerebellar surface (Loc) and white matter (WM) stimulation has been presented in previous papers (41, 53). In this section we shall confine ourselves to the study of generation and interaction of unitary potentials in the molecular layer.

Extracellular unitary potentials

Local stimulation of the cerebellar cortex evokes a parallel fiber potential transient (PFP) (53) followed by a prolonged nega-

tivity. The latter component can be recorded in the molecular layer from the surface to 400 μ depth, directly beneath the activated parallel fiber bundle. It has been postulated that this negative potential is produced by the sum of discrete all-or-none action currents generated by the electroresponsive properties of Purkinje cell dendrites (41, 42, 53). An example of the discrete nature of these potentials is illustrated in Fig. 1. The various all-or-none components were demonstrated by means of threshold separation, as the strength of the Loc stimulating current was varied in small steps. In this example a minimum of five different all-or-none negative spike components could be observed following small increments of the Loc stimulus amplitude (Fig. 1*A–E*). Although the potentials in Fig. 1 were recorded at 250 μ depth (i.e., about middendritic level), similar results were easily obtained at most levels in the molecular layer. In accord with the assumption of electroresponsive origin for these negativities, the stepwise increments in the amplitude of the Loc stimulus produced a distinct reduction in their latency.

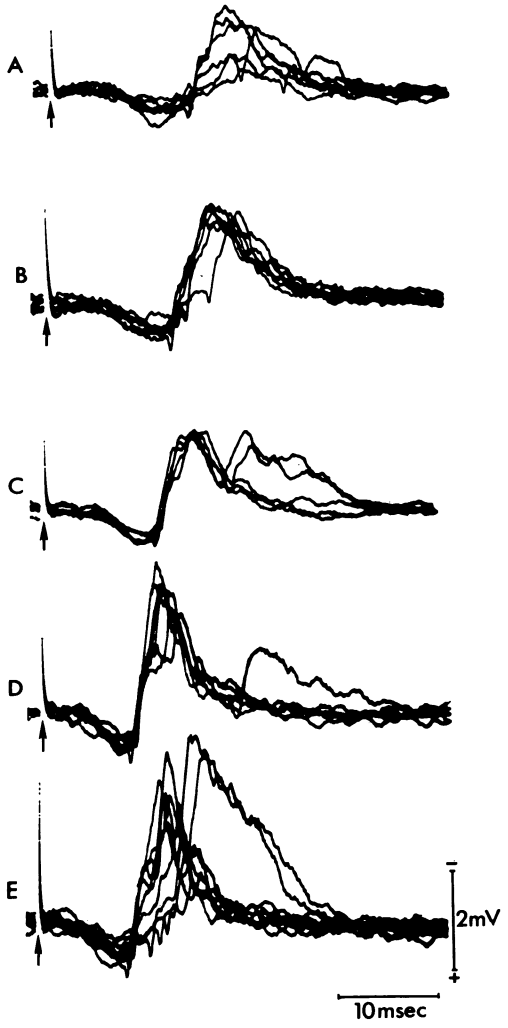

FIG. 1. Extracellular recordings in the molecular layer following surface stimulation of the cerebellar cortex. Potentials were recorded at 250 μ depth. Local (Loc) stimulation (arrows) was increased from 1.2× threshold in *A* to 2× threshold in *E*, which produced an increase in the amplitude of the negative field and a simultaneous reduction of its latency. Note that the response increased in all-or-none steps as the stimulus amplitude was raised. In this and all subsequent figures, amplitude, polarity, and time scales are indicated in illustration.

In most cases, large unitary all-or-none negative potentials could be separated from the aforementioned negativity (41, 42). Characteristically, these spikes consisted of a smooth, rapid rising phase and a long-lasting, notched decay (Fig. 2*A*). They could be recorded from 50 to 350 μ depth, had an average duration of 10 msec, and their latency was inversely related to the Loc stimulus strength (41, 53).

These special types of negative potentials, which have been ascribed to the spike generation in Purkinje cell dendritic trees (41, 42), were never evoked by the antidromic invasion of the Purkinje cells. They were often generated, however, by mossy fiber activation following WM stimulation. In addition, on most occasions tested, these potentials could be produced by physiological stimulation (joint and tactile), which demonstrated that they were not electrophysiological anomalies due to electrical stimulation.

Interaction of extracellular unitary potentials

Double stimulation of the alligator cerebellar surface via a Loc electrode evokes a clear inhibition of the negative field potential generated by the second stimulus (41, 42, 53). This inhibition, which is assumed to be mediated via the stellate cell (see DISCUSSION) can also be demonstrated at the

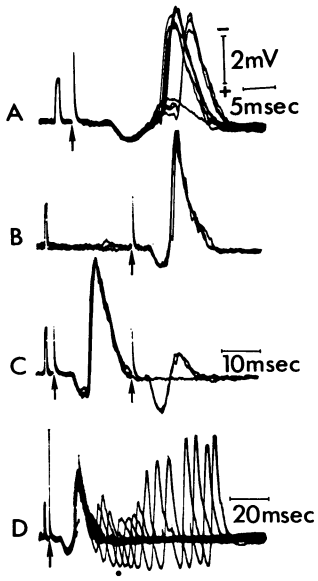

FIG. 2. Unitary extracellular dendritic spikes and their interaction following double Loc stimulation. Records were taken at 100 μ depth in the molecular layer. In *A* the Loc stimulus (arrow) generated a large all-or-none negative spike. In *C* a conditioning Loc stimulus produced a blockage of the test response (*B*). In *D* the conditioning stimulus was reduced and the superimposed test stimulus presented at different times after the conditioning stimulus. Note that maximum reduction of the amplitude of the negative spike occurred at about 15 msec following conditioning stimulus (dot).

unitary level as shown in Fig. 2. Thus, the all-or-none negative spike (Fig. 2*A*) was blocked when the test stimulus (Fig. 2*B*) was preceded by a conditioning Loc stimulus at a fixed time interval (Fig. 2*C*). If the interval between the two Loc stimuli was continuously varied (Fig. 2*D*), the maximum reduction of the test response occurred with an interval of about 15–30 msec which, as will be seen below, corresponds to the maximum peak of the inhibitory postsynaptic potential (IPSP) generated in the Purkinje cell by a Loc stimulation. Furthermore, the time course of this reduction and its duration (40–50 msec) agrees with that observed at the field potential level under similar conditions (53). Note that, as shown in Fig. 2*D*, the conditioning Loc stimulus produced a graded reduction of the amplitude and duration of the test all-or-none spike. This suggests that while the generation of spikes in a dendrite occurs in an all-or-none manner, inhibition may prevent the activation of particular dendritic patches. The dissection of dendritic spikes into multiple-spike components generated by "hot spots" (17) will be demonstrated more clearly below by means of intradendritic recording.

Intradendritic recording from Purkinje cells

IDENTIFICATION OF SITE OF DENDRITIC PENETRATION. Intradendritic recordings from Purkinje cells in alligators can be obtained with high-resistance microelectrodes (see METHODS). Although the diameter of the dendrite at the site of penetration may be as small as 4–6 μ, penetration can be readily achieved—especially at the site of a dendritic bifurcation.

In order to demonstrate that particular potentials are generated in Purkinje cell dendrites, it must be shown that *a*) these potentials are recorded in dendrites, and *b*) the dendrites belong to Purkinje cells.

The first point was established by intracellular injection of dye at the place of recording. Thus, Fig. 3*A–C* shows a series of micrographs of dendrites filled with Procion yellow dye (see METHODS) following intracellular recording. This dye was selected because it does not cross membranes and thus gives a direct demonstration of intracellular recording. The dye is assumed to bind strongly with intracellular protein, and migration from the vicinity of the

FIG. 3. Photomicrographs of Procion yellow dye-filled dendrites and somata of Purkinje cells. *A:* typical fluorescence of a dye-injected dendrite at midmolecular layer level. Arrows indicate cerebellar surface and Purkinje cell layer. *B:* high magnification of the dendritic segment shown in *A*. Note that the site of injection corresponded approximately to the place of bifurcation of the dendritic branches. Clearly visible are dendritic spines arising from the spiny branchlets. *C:* dendritic branchlets of another Purkinje cell following intradendritic dye injection. The extent of dye penetration into dendritic branchlets was related to the time between the intracellular injection and the fixation of the tissue. *D* and *E:* dye injection of two Purkinje cell somata sequentially penetrated. In this case the rapid fixation of the specimen prevented a large spread of the dye toward the dendrites or axons. Arrows as in *A*. Calibration bars: *A* and *D*, 50 μ; *B*, *C*, and *E*, 10 μ.

injection is slow (56, 60, 63). Consequently, the animals were sacrificed within 30 min after injection in order to locate the point of intracellular recording at the place of maximum concentration of dye (maximum fluorescence). This technique allowed us to ascertain beyond doubt the site of intracellular recording in five cases, which confirmed the intradendritic origin of the particular class of electrophysiological response to be described below. These results were corroborated by the fact that the site of maximum dye concentration was always in agreement with the depth at which the dendrite was penetrated, as measured from the surface of the cerebellar cortex with the micrometer drive on the micromanipulator. Although the identification of the dye-filled dendrite as belonging to a Purkinje cell was based on electrophysiological criteria (see description below), they could also have been identified on purely morphological grounds (i.e., the large number of dendritic spines per unit length and the general organization of the dendritic branchlets). The dye-stained dendrites closely resemble those obtained with the Golgi technique (28).

It should be mentioned that a constant finding was the apparent centrifugal tendency of dye flow following injection. Thus, portions of the dendritic tree distal to the point of penetration were filled with dye to a greater extent than were the proximal regions, as if a somatofugal flow of cytoplasm toward the tips of the dendrites were taking place. Another interesting finding was the clearly visible filling of the dendritic spines with dye, often in cases where the dendritic shaft itself was only weakly fluorescent. This was surprising since the necks of these spines are about 2,000 A in diameter (40). Such filling of dendritic spines may indicate a rapid protein turnover at those sites.

INTRADENDRITICALLY RECORDED POTENTIALS. *Parallel fiber activation.* A typical intracellular recording from a dendrite at 200 μ depth from the cerebellar surface is shown in Fig. 4. The lower trace of *A* shows the negative extracellular potential following Loc stimulation (arrow); a simultaneous record taken with a dc-coupled amplifier at lower gain is shown in the upper trace. In *B* the dendrite was abruptly penetrated and a resting potential of −60 mv was obtained. The action potential evoked by the parallel fiber volley was approximately 70 mv in amplitude and had a duration of about 10 msec, followed by a long-lasting afterhyperpolarization. A characteristic of all the dendritic spikes recorded intracellularly (more than 50) was the multinotched character of the rising phase. In *B* several components can be observed following the rather small synaptic potential. The presence of several inflections in the rising phase of this recording implies that the activation of the entire dendritic tree does not occur in a synchronous manner, but rather that different parts of the dendritic

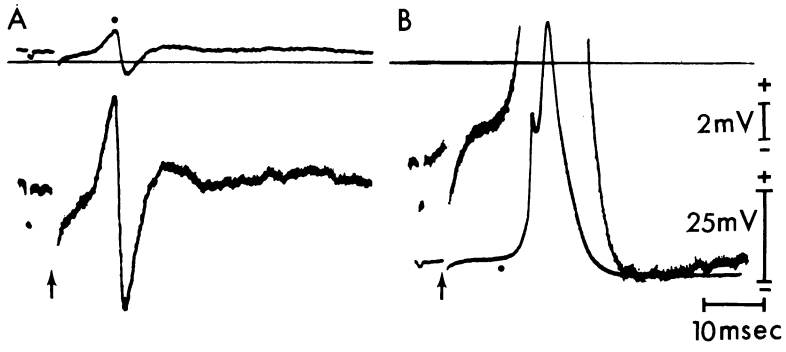

FIG. 4. Intradentritically recorded action potential. In *A* upper and lower traces were dc- and RC-coupled recordings of a dendritic spike in the molecular layer following Loc stimulation. *B:* intracellular recording immediately after dendritic impalement (note resting potential shown by d-c shift from reference line). Time of onset of the parallel fiber EPSP is marked with a dot and corresponds to the peak of the extracellular positivity recorded in *A* (dot).

tree fire at slightly different times. These findings suggest that the action potentials were being conducted in a noncontinuous manner, a point which is better illustrated in Figs. 5–7.

The relation between the strength of Loc stimulus and the generation of dendritic spikes is shown in Fig. 5; two intradendritic recordings were made at depths of 200 μ (*A–D*) and 150 μ (*E–I*). The Loc stimulus was gradually increased from near threshold for parallel fiber action in *A* to approximately 2× threshold in *D*. In *A* the stimulus evoked a small depolarization immediately after the excitatory postsynaptic potential (EPSP). Increase in Loc stimulation strength produced a large spike (*B*) and a later spike with a clear inflection in the rising phase (*C* and *D*). Records in *E–J* were taken from another cell. An extracellular dendritic spike was recorded immediately prior to (*E*) and immediately after (*J*) dendritic impalement. The dendrite was penetrated and the Loc stimulus strength was reduced to close to threshold for parallel fiber activation (*F*). As in the previous example, when the Loc stimulus was increased, the intradendritic potential demonstrated several all-or-none components (*G–I*).

On several occasions, surface stimulation of near threshold strength was able to evoke small all-or-none components intradendritically. One such potential is shown

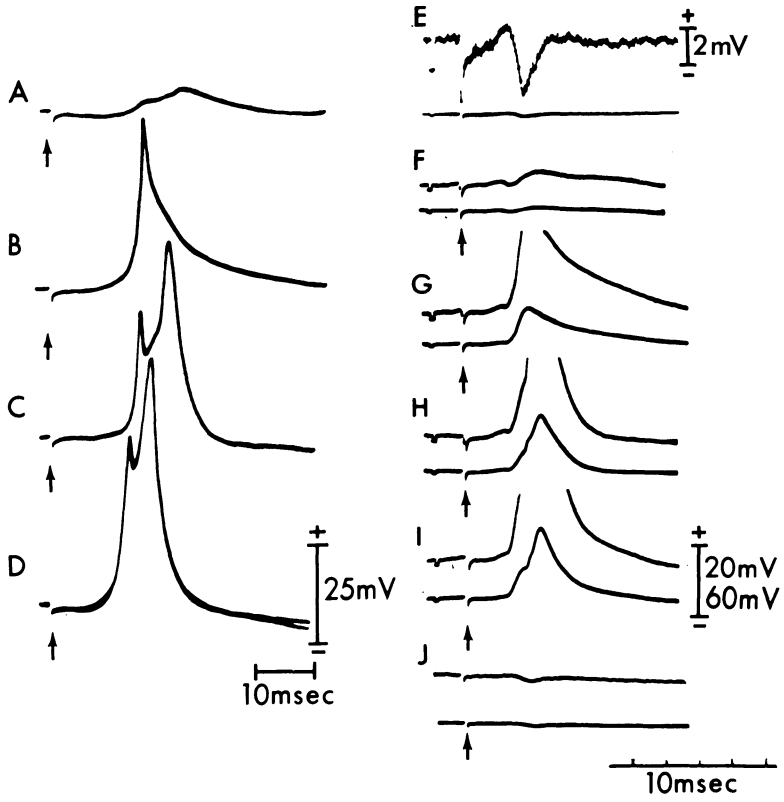

FIG. 5. Intradendritically recorded action potentials generated by Loc stimulations of different amplitude. Records *A–D* are from the cell shown in Fig. 4. As the parallel fiber stimulus increased, the number of components and the overall amplitude of action potentials increased and their duration decreased. The presence of an afterhyperpolarization was observed in *C* and *D*. In *E–J*, intracellular dendritic spikes from another Purkinje cell. In *E* extracellular field potentials were recorded simultaneously, at high gain by RC-coupled amplifier in upper trace and by dc-coupled amplifier in lower trace. In *F* the dendrite was impaled. Loc stimulation generated in *F* a subthreshold EPSP preceded by a small negative wave, the extracellular field potential. In *G*, *H*, and *I* the Loc stimulus was further increased, which generated several dendritic spikes. In *J* extracellular field potentials were evoked by Loc stimulation immediately outside the impaled dendrite.

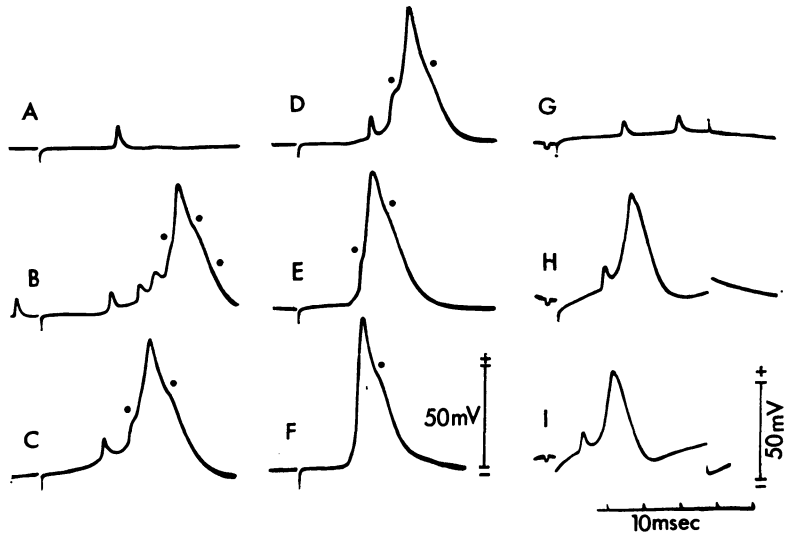

FIG. 6. Generation of dendritic spike by Loc stimulus and by direct current injection. In *A* a small all-or-none spike generated by a very weak parallel fiber stimulation. In *B–F* the Loc stimulation was increased in a graded fashion. In *B* the large intracellular depolarization was preceded by three all-or-none components. As the stimulus was increased from *C* to *F* the latency of the large depolarization and its duration decreased. In *F* the different all-or-none components (dots) fused into a sharp-rising action potential. *G–I:* intradendritic responses evoked by outward current pulses of different amplitudes.

in Fig. 6*A*; as the strength of the Loc stimulus was increased from *B* to *F*, the small spike was observed on the synaptic depolarization and was followed by a large, long-lasting notched (dots) spike with a slow rate of rise and fall. In *C* and *D* only one small spike preceded the larger depolarization which seemed to be generated from the peak of the second small action potential. Further increase in the Loc stimulus generated a full spike from the first small potential in *E*, and finally, in *F*, the spike seemed to arise directly from the synaptic potential produced by the parallel fiber volley. Note that the rate of rise of the large spike became steeper and smoother and that its latency shortened as the Loc stimulus was progressively increased.

A similar sequence of spikes could also be generated by an outward current pulse through the recording microelectrode as shown in Fig. 6*G–I*. A very moderate depolarization (*G*) generated a small spike activation similar to that observed in *A–D*. Further depolarization activated the small spike as well as a larger and longer-lasting action potential (*H* and *I*). In *I*, as current was further increased, the latency for the two spikes was reduced.

A more direct demonstration of multiple site of spike initiation is illustrated in Fig. 7. An action potential, also shown in Figs. 4*B* and 5*D*, is shown in Fig. 7*A* following a Loc stimulus. In record *B* the amplitude of the Loc stimulation was slightly decreased, so that only the first large component of the action potential was generated. At this point an inward current was passed through the microelectrode in order to hyperpolarize the cell at the site of recording (*C–E*). In *C* the fast transient peak observed in *B* was removed, leaving an underlying second component which, with further hyperpolarization, became delayed in *D*. Two components could still be observed following 25 mv hyperpolarization (*E*).

Records *F–K* in Fig. 7 show at higher amplification the potentials illustrated in Fig. 7*A–E*. The upgoing arrow indicates onset of EPSP generated in this cell by the parallel fiber activation. In *G* inward current through the micropipette produced a blockage of the largest spike component shown in *F*, and a further hyperpolarization in *H* revealed five points of inflection in the dendritic depolarization (arrows). As the hyperpolarization was increased (*I*),

the later three inflections in *H* were removed, and in *J* another step in the hyperpolarization clearly displays the inflection in the rising phase of the dendritic potential. A final increase in the hyperpolarization (*K*) removed the second component in *J* and left a depolarization with no obvious inflections in its rising phase. There may, of course, be many additional spikes in remote regions of the dendritic tree which are too attenuated to be visible as separate entities in records *F–K*. The results presented in this section strongly support the hypothesis that Purkinje cell dendrites have electroresponsive properties and that the prolonged action potentials recorded in these neurons are the result of electrotonic summation of many of these spike components.

Antidromic invasion. Electrophysiologically, intracellular recordings from Purkinje cell dendrites could be recognized by their antidromic invasion following WM stimulation. As illustrated in Fig. 8*A*, WM stimulation evoked an EPSP through the mossy fiber, granule cell, parallel fiber pathway (41). Slightly stronger stimulation generated a small all-or-none spike of approximately 15 mv amplitude (*B*), the short latency demonstrating its antidromic origin. In *C*, as the stimulus was slightly increased, a dendritic spike was recorded. Note, however, that the amplitude of the antidromic action potential remained unchanged. The absence of an active dendritic invasion to the superficial dendrites raises issues which will be treated in the DISCUSSION. For comparative purposes, an intracellular record-

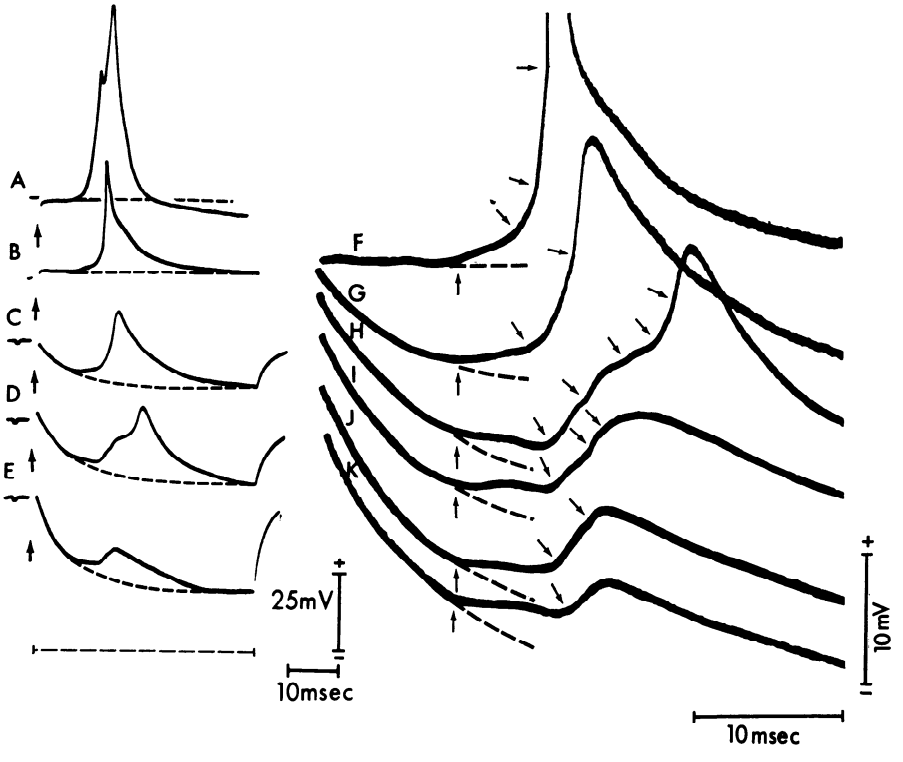

FIG. 7. Sequential blockage of different all-or-none components of an intradendritic action potential by hyperpolarizing current pulses. *A:* intradendritic potential evoked by strong Loc stimulation (arrow). In *B* the local stimulus was slightly decreased so that the spike activation was nearer to threshold. *C–E:* three different levels of hyperpolarization were applied through the recording pipette to demonstrate selective blocking of different components of the spike potential. *F–K:* similar sequence to that shown in *B–E,* but at higher amplification. Downgoing arrows indicate points of inflection in the rising phase of the action potential. Note that as the hyperpolarization was increased the different all-or-none spike components were selectively blocked. In *K* the depolarization which followed the rather remote synaptic potential (upgoing arrows) could not be further simplified by extrinsic hyperpolarization.

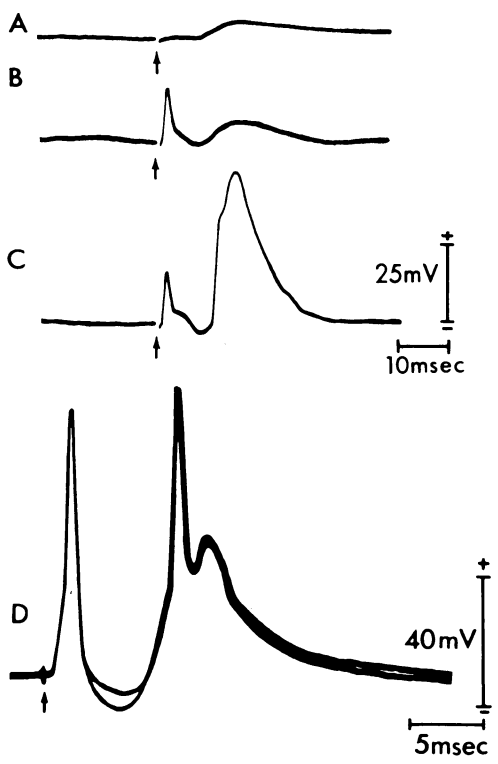

FIG. 8. Potentials evoked intradendritically in a Purkinje cell following WM stimulation. WM stimulation was increased in *A–C*. In *A* a weak WM stimulation evoked a small EPSP through the mossy fiber, granule cell, parallel fiber pathway. In *B* a slightly larger stimulus generated an antidromic action potential which was electrotonically conducted to the recording site, followed by an increase in the mossy fiber-parallel fiber EPSP. In *C* further increase evoked, following the antidromic spike, a large dendritic depolarization. *D*, intracellular recordings from the soma of a different Purkinje cell to show the typical response evoked at that level by WM stimulation. The first action potential represents antidromic invasion while the second presents an orthodromic activation through the mossy fiber, granule cell synapse; the climbing fiber system was not activated in this case.

ing from the soma of a Purkinje cell (*D*) shows typical antidromic invasion with a spike of 80 mv and an orthodromic action potential followed by a prolonged depolarization. Intracellular injection of dye under these conditions produced the type of result shown in Fig. 3*D* and *E*.

INTRADENDRITIC SYNAPTIC POTENTIALS. Three main types of synaptic potentials were observed during intradendritic recording from Purkinje cells: *a*) climbing fiber synaptic potentials; *b*) parallel fiber EPSPs; and *c*) IPSPs, most likely of stellate cell origin (41).

Synaptic potentials evoked by climbing fibers. The electrophysiological characteristics of a climbing fiber action in the Purkinje cells of alligators have been published in a recent paper (41). Basically, as in other vertebrates, the alligator's climbing fiber EPSP is characterized by its all-or-none nature and by the demonstration of an equilibrium potential by means of intracellular current injections (41). As first reported for cats (10) and confirmed in other vertebrates (38, 40), only one such all-or-none EPSP is generally evoked in one Purkinje cell following WM stimulation, which emphasizes the one-to-one relation between a climbing fiber and a Purkinje cell.[1]

Synaptic potentials produced by parallel fibers. As in other species (13, 34, 40, 41, 54), parallel fiber activation generates in Purkinje cells a graded synaptic depolarization. One such example is illustrated in Fig. 9. A large depolarization was recorded intradendritically in *A* following a Loc stimulation. The cell was hyperpolarized in order to demonstrate the graded nature of the EPSP (*B–E*). Since a direct relationship was always found between the size of the dendritic EPSP and the size of the parallel fiber volley, it is concluded that dendritic spikes such as shown in Fig. 9*A* were generated by the parallel fiber-Purkinje cell synapse. This conclusion was further tested by recording from Purkinje cells after cerebellar deafferentation (41). Under such conditions typical extracellular dendritic spikes and prolonged intracellular depolarization could also be evoked, demonstrating that such prolonged potentials can be generated in the absence of mossy or climbing fibers.

Intradendritic inhibitory potentials. Intracellular recordings from alligator Purkinje cells have demonstrated the presence of large IPSPs following the initial parallel fiber EPSP (41). Since it is known that partial activation of the neuronal membrane of Purkinje cells may produce hyperpolarizing

[1] The "one-to-one relation" refers to the fact that a Purkinje cell receives only one climbing fiber and not to the question of whether more than one climbing fiber may arise from one cell of origin (15).

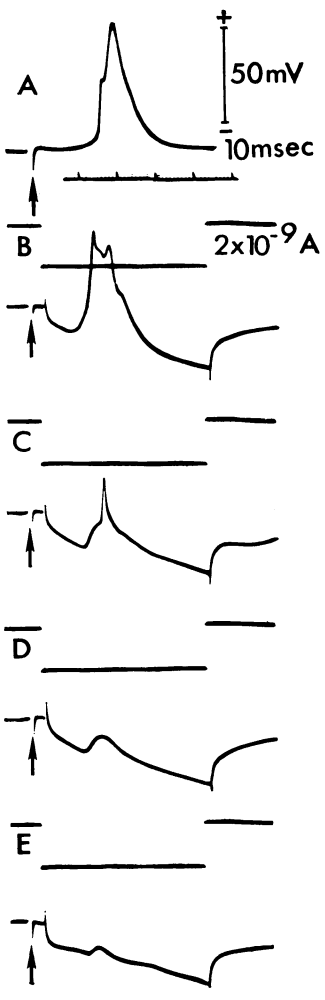

FIG. 9. Parallel fiber-evoked EPSP recorded intradendritically in a Purkinje cell. In *A*, control dendritic spike was recorded at 200 μ depth. In *B–E* a hyperpolarizing current was applied following local stimulation, in order to visualize the synaptic potential which evoked the dendritic spike. A gradual decrease of the amplitude of Loc stimulus in *B–E* demonstrated a graded EPSP, indicating its parallel fiber origin. Note that in *B* the dendritic spike showed several components and that in *C* only one component was left.

potentials (13, 21, 38), an important test to demonstrate the synaptic origin of the hyperpolarization evoked by Loc stimulation is to show its reversibility following intracellular chloride injection (1, 5, 13, 31, 54). In Fig. 10*A–D* an IPSP was recorded approximately 350 μ below the surface from a Purkinje cell, most probably in one of the thick dendritic branches. The graded nature of the potential is shown in *A–D* for different Loc stimulus amplitudes. At this stage, chloride was injected from a KCl-filled micropipette by means of an inward d-c current pulse. *F* illustrates the synaptic potential evoked following the injection and shows that the IPSP is reversed and that its time course is much shorter than the IPSP evoked prior to chloride injection. This change in the time course, which has been observed in Purkinje cells of other species (13, 54), has also been reported in other central neurons (31) and seems to be the characteristic form of reversal of spatially distributed synaptic potentials (3; cf. 29). Figure 10*E* is a drawing of the inhibitory synaptic potential before and after chloride injection. The reversed potential could be changed back to its original hyperpolarizing form by an artificial depolarization of the cell through the recording microelectrode. Figure 10*K* shows the control reversed synaptic potential. In *J* and *I* a reduction of the later part of the synaptic potential and a reversal of this potential to its original polarity (*H* and *G*) is obtained by membrane depolarization through the recording micropipette. Record *L* shows that the hyperpolarization of the cell increased the amplitude of the reversed IPSP.

The above experiments are summarized in the graph of Fig. 10*M* which illustrates the relationship between the applied current (abscissa) and the synaptic potential (ordinate), measured at the time of peak of the IPSP in record *G*. This graph is quite characteristic for the current voltage relationships of the IPSP in central neurons. Records *N–R* illustrate an intracellular recording from another dendrite showing a graded sequence of IPSPs in response to increasing Loc stimulation. In this case the neuron was slightly out of line with respect to the activated beam of parallel fibers. Spontaneous IPSPs similar to those observed in cats (13) and elasmobranchs (51, 54) are marked by arrowheads. This particular spatial relationship between a beam of parallel fibers and surrounding inhibition is consistent with the idea of lateral inhibition produced by the axons of the molecular layer interneurons (12, 13) and is in agreement with the lateral orientation of the axons of stellate cells in alligators (28, 40).

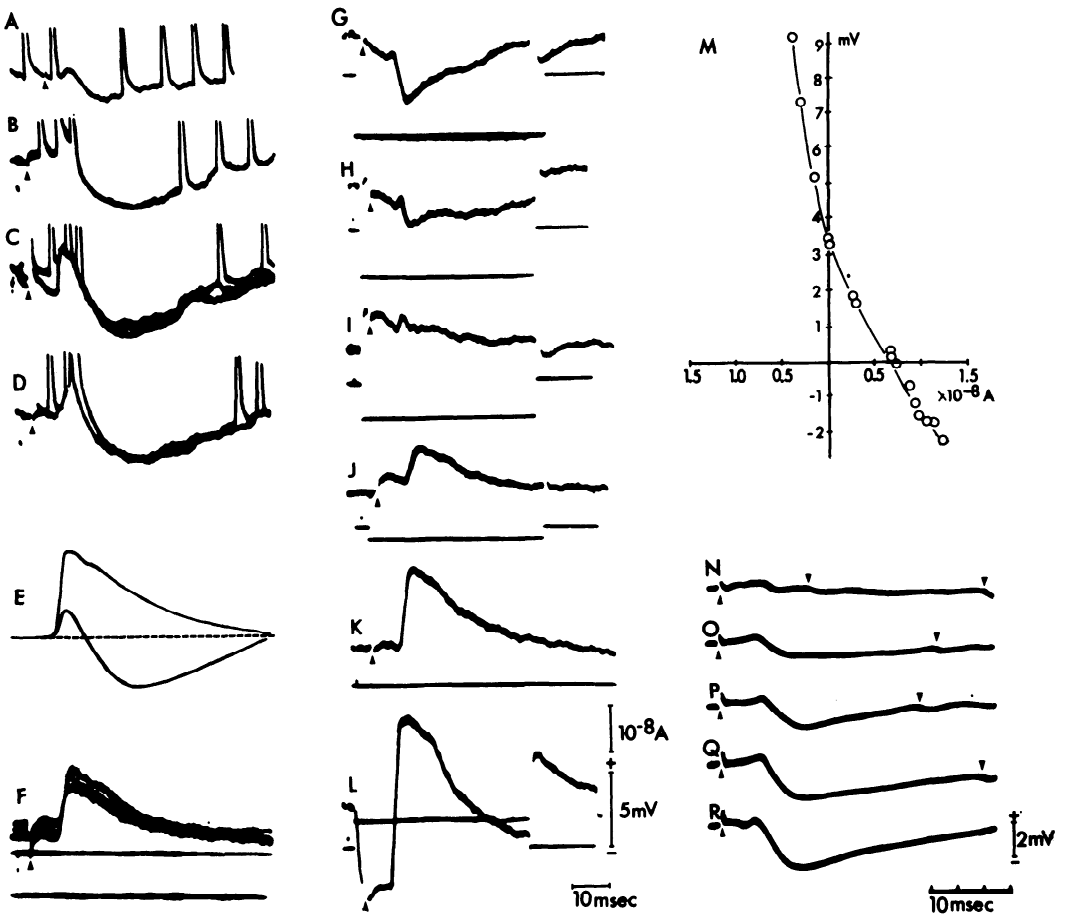

FIG. 10. Intradendritically recorded IPSP and its reversal by chloride injection. *A–D:* local stimulation of increasing amplitude generated an EPSP-IPSP sequence which produced inhibition of spontaneously active spikes. In *F* following a chloride injection, the IPSP was reversed to a depolarizing potential. In *E* a drawing of the synaptic potential evoked before and following chloride injection. In *G–L,* effects of current injection on the chloride-reversed IPSP. In *K,* control reversed IPSP. In records *G–J* the dendrite was depolarized by a current injection, and the synaptic potential was reversed to its initial hyperpolarizing direction. In *L* the hyperpolarization of the dendrite produced a large increase in the size of the reversed synaptic potential. The small size of the EPSP seen in records *G, H,* and *I* is an indication of the depolarized state of the penetrated dendrite. In *M,* a plot of the inhibitory postsynaptic potential amplitude (ordinate) against intracellularly applied current (abscissa) shown in *G–L. N–R:* a series of intradendritic recordings from a dendrite located out of line with respect to an excited beam of parallel fibers. The series shows a graded relationship between amplitude of the Loc stimulation and that of the evoked IPSP. Arrowheads indicate spontaneously occurring IPSPs.

Extracellular and Intracellular Unitary Potentials Recorded at Purkinje Cell Layer

The unitary extracellular action potentials recorded at the level of the Purkinje cell soma have been characterized by a short duration and a positive-negative polarity (23). In the alligator similar potentials can be evoked antidromically following WM stimulation (Fig. 11*A*), which confirmed their identity (11, 23, 39, 47).

As in the case of the unitary potentials recorded in the molecular layer, the somatic potentials could be evoked by parallel fiber activation (Fig. 11*B–E*). The typical positive-negative field described in previous papers (41, 53) is shown in record *B* and the effect of Loc stimulus amplitude is shown in *C–D.*

This type of electrical activity—the so-called "giant" spikes first described by Granit and Phillips (23)—is identical to the

characteristic spikes following somatic activation of Purkinje cells in other cerebella (11, 14, 34, 38, 47, 54). Their brevity indicates that the electrical properties of the soma differ from those of the dendrites. Note, however, that the burstlike response of alligator Purkinje cells following parallel fiber activation is not the usual behavior of Purkinje cells of felines (12) or anurans (38). The burst response of the alligator resembles that generated by climbing fiber activation in other vertebrates (10, 38, 40) except for the graded nature and slight variability in spike number for a given stimulus strength (Fig. 11*E, F, J*).

Interaction of extracellular unitary potentials at Purkinje cell layer

As reported above for the dendritic potential at the molecular layer, the use of

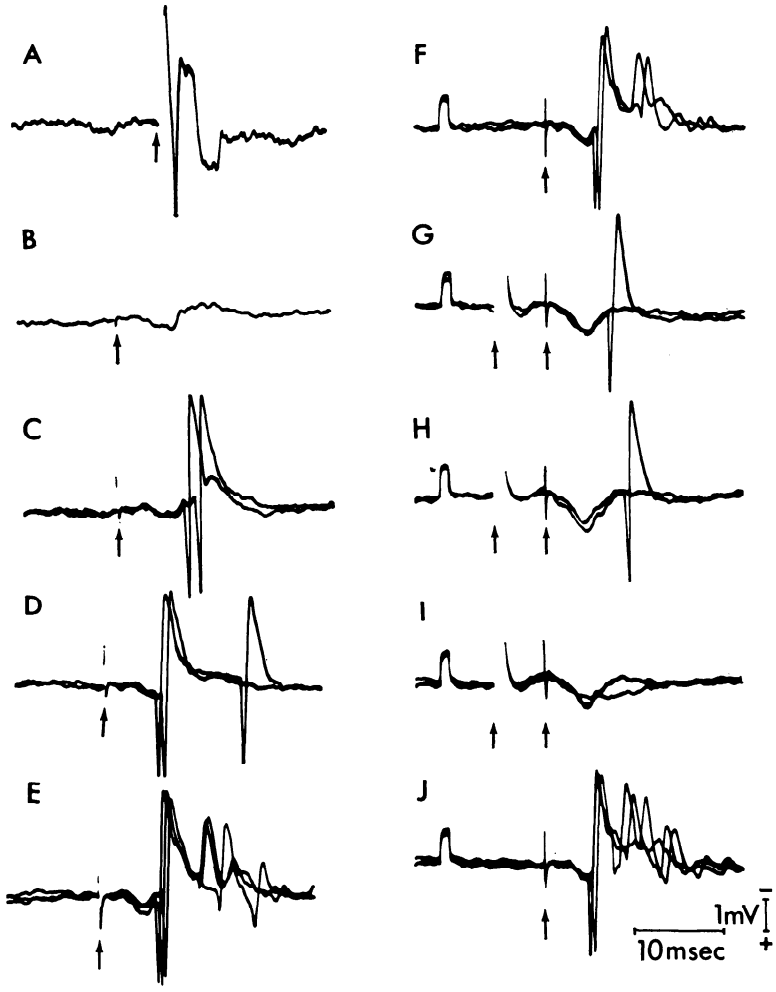

FIG. 11. Giant extracellular action potentials recorded at the Purkinje cell layer following anti- and orthodromic activation of a Purkinje cell and their interaction by double Loc stimulation. *A:* typical positive-negative action potential evoked by antidromic invasion of Purkinje cell, for purposes of identification. Increasing Loc stimulation generated in *B* a prolonged positive-negative field potential, and in *C–D* giant spikes were superimposed on the negative field potential. Note that the number of spikes increased and their latency decreased as Loc stimulation was augmented from *C* to *E*. In *G–I* a test Loc stimulation was preceded by an out-of-line conditioning Loc stimulation of increasing strength. In *G* and *H* the conditioning stimulation reduced the number of spikes generated by the test stimulation. In *I* the preceding Loc stimulation produced first a reduction in the number of spikes and then a complete inhibition of the spike generation without itself generating action potentials. *F* and *J* are control test stimuli.

paired Loc stimuli revealed that a conditioning Loc stimulus may eliminate in a progressive manner all the Purkinje cell spike potentials evoked by a test parallel fiber volley (Fig. 11). The control test volleys elicited a burstlike response from the Purkinje cell (*F* and *J*), while in *G–I* another Loc stimulus (out of line) of increasing strength was utilized for the conditioning volley and produced inhibition of the test response (*F* and *J*). The inhibition was obtained without spike generation by the conditioning stimulus, thus excluding a refractory factor (8) in the blockage of the test spike potential, and corroborating the hypothesis of a direct inhibitory action on alligator Purkinje cells (42).

Although inhibition is clearly present, the degree of inhibition observed—especially of the "somatic" spikes—is not as large as that found in cats (12). This difference accords with the lack of true basket cells in alligators (28).

Intracellular recording from Purkinje cell soma

Intracellular recordings from the somata of Purkinje cells are characterized by brief action potentials which can be generated at relatively high frequencies. Typical spontaneous action potentials are shown in Fig. 12*A*. Parallel fiber stimulation in *B–D* first produced an increase in the number of action potentials and then inhibition of spikes and a prolonged inhibitory potential. In *C* and *D* a further increase in parallel fiber stimulation generated a long-lasting depolarization. This depolarization, although in some ways similar to the climbing fiber response evoked in Purkinje cells of other vertebrates, is probably produced in these cells by the electrotonic conduction of prolonged dendritic spikes toward the Purkinje cell soma.

Stimulation of the cerebellar white matter evoked, in addition to antidromic invasion, an orthodromic action potential and a prolonged depolarization via the mossy fiber input. Following the prolonged depolarization which lasted on occasions for as long as 20 msec (*F* and *H*), a large hyperpolarization could be observed. When a preceding WM stimulus was given, as in *G*, a total blockage of both the antidromic and the orthodromic activation occurred. This was due partly to refractoriness and collision, and partly to the strong Golgi and stellate cell inhibition which is found in alligator cerebellum (41). In Fig. 12*H* the amplitude of the WM stimulus was slightly diminished, producing a marked reduction of the orthodromic depolarization. This reduction occurred in discrete steps and is assumed to represent dendritic spikes electrotonically conducted to the soma (57). Similar results have been reported in chromatolyzed motoneurons (9, 36).

DISCUSSION

As in other vertebrates, the cerebellar cortex of alligators has two main afferent systems (the mossy and climbing fibers) and one efferent system (the axons of Purkinje cells) which have been described in detail (28, 67). The main electrophysiological differences between the cerebellar circuits in alligators and those in higher vertebrates are related to the properties of Purkinje cells and the lack of basket cell inhibition. Given that successful intradendritic recordings have been obtained in the cells, the whole question of dendritic action potentials must be discussed. In particular, the existence of neurons with multiple sites for spike origin (21, 26, 57, 62) raises a number of intriguing questions.

Dendritic Action Potentials and Their Functional Role in Purkinje Cell Integration

Mechanism of generation

The presence of dendritic action potentials in alligator Purkinje cells was first deduced from field potentials and unitary extracellular dendritic spikes (42). These extracellular unitary potentials, which are generated by dendritic action currents, were characterized by a prolonged negativity with a fast rising phase and a notched falling phase (41). Given that the dendritic action currents should be close to the second derivative of the intracellular potential with respect to time (44), the notched nature of the spike was taken to indicate that the earlier potentials occur near the recording site, while those in the falling phase

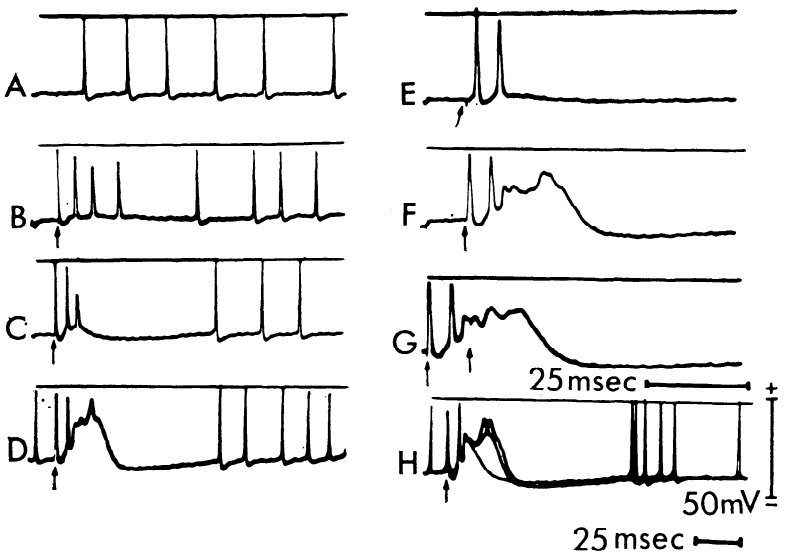

FIG. 12. Intrasomatically recorded potential from Purkinje cell. *A:* spontaneously occurring action potentials. In *B–D,* Loc stimulation of increasing amplitude generated first an increasing number of spikes (*B*) and then as the stimulus was augmented from *C* and *D*, the spike generation was followed by inhibition. In *D* a prolonged depolarization followed the Loc stimulation. Records *E–H:* intracellular potentials evoked by WM stimulation. In *E* an antidromic activation was followed by an orthodromic synaptic potential arising from a small EPSP. In *F* a further increase of the WM stimulation generated a prolonged intracellular depolarization. In *G* a preceding stimulus of the same amplitude produced a complete block of the test response. In *H*, superimposed traces demonstrate several all-or-none components of the prolonged somatic depolarizations as the strength of Loc stimulation was varied slightly.

represent spike initiation at increasing distances from that point (41). The intradendritic recordings presented here are in agreement with that view.

Present day intra- and extracellular techniques cannot directly reveal the detailed spatial distribution of subthreshold potentials leading to the generation of dendritic spikes. Nevertheless, all of the evidence presented here suggests that Purkinje cell dendrites have patches of excitable membrane which generate, by summation of all-or-none components, the typical prolonged intradendritic action potentials (Figs. 6, 7, and 9). Though the location of such excitable patches is unknown, it is tempting to assume, as hypothesized by Lorente de Nó and Condouris (45), that they occur at the site of dendritic bifurcation. The reasoning here is that depolarizations originating from several dendritic segments will sum at the confluence point and thus the current density per unit area of membrane may be higher at this junction than at adjacent areas of the dendritic tree. It is also possible that these loci may have special excitable properties; for instance, they may have more electroresponsive sites (the so-called sodium patches in other excitable membranes (27, 49)) per unit area than does the nonresponsive membrane. The existence of such membrane patches, or hot spots, has been shown in Aplysia neurons on the basis of voltage clamp results by Frank and Tauc (17). Although some of these potentials may represent electrotonic invasion from the axosomatic region (Fig. 8), the multiplicity, distinct waveshape, and amplitude of the spikes recorded from any given Purkinje cell dendrite are indicative of a large number of electroresponsive regions. The varying amplitude of the spikes recorded intradendritically is probably related to the distance between the activated membrane patch and the site of microelectrode impalement.

Somatopetal tendency for dendritic spike conduction

The existence of hot spots implies that dendritic spikes are conducted to the soma in a "pseudosaltatory fashion" by a mixture

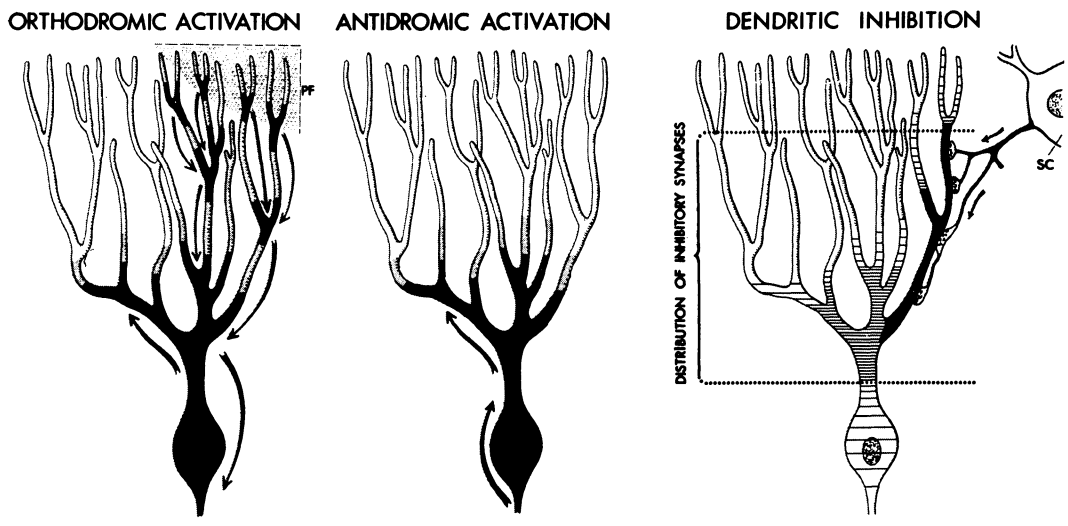

FIG. 13. Schematic representation of probable set of events following orthodromic and antidromic Purkinje cell activation and inhibition of Purkinje cell dendrites. Following a slightly out-of-line parallel fiber activation (first diagram to the left) electroresponsive patches in dendrites (black area) generate full action potentials which are conducted first in a decremental manner and then in an electrotonic fashion to the next site of spike initiation (arrows); in this pseudosaltatory fashion dendritic spikes will be conducted to the large dendritic branches and finally to the somatic level. The diagram further illustrates the thesis that dendritically evoked action potentials do not invade antidromically other neighboring dendritic branches. Thus, following orthodromic activation in one extreme of a Purkinje cell tree, a great many of the remaining dendritic branches remain free from spike invasion. In the center diagram an antidromic activation of the same Purkinje cell is shown to invade (arrow) only the lower parts of the main dendritic branches; this is assumed to be the mechanism underlying the somatopetal tendency of dendritic spike propagation orthodromically. The diagram to the right gives an idealized representation of the distribution of stellate cell (SC) inhibitory synapses on dendrites of a Purkinje cell. Although the dendritic hyperpolarization would be electrotonically conducted to the rest of the dendritic tree (density of horizontal lines), the inhibitory action would be restricted, for the most part, to those dendritic branches receiving direct inhibitory input from a given inhibitory interneuron.

of active and electrotonic conduction. Once a particular hot spot has reached the firing level, the resulting spike depolarization should be propagated electrotonically upward as well as downward into the parent dendritic branch. However, since as in other neurons (22), the electrical excitability of alligator Purkinje cell dendrites decreases with distance from the soma (41, 43), a dendritic spike would encounter a lower safety factor in the somatopetal direction and, thus, would tend to be conducted toward the soma and not toward other dendrites. Figure 13*A* shows a diagrammatic presentation of this hypothesis. In this figure, gray represents depolarization, transverse lines areas of transition between full activation and electrotonic conduction (i.e., local response), and black areas the patches for regenerative inward current activation. Since the regenerative character of spike initiation is most probably determined by the density of electroresponsive patches per unit membrane and the density of such patches should fade out gradually with distance from one such hot spot, an area of decremental membrane conduction should surround a patch having full regenerative properties (see Fig. 13).

The unidirectional properties of dendritic conduction have been postulated on the basis of two classes of experimental results. *1*) A slightly out-of-line parallel fiber activation generates a large positivity on the surface which otherwise has all the characteristics that in-line dendritic spike field potentials demonstrate (43). We interpret this as implying that as in-line superficial dendrites fire, their action potentials are conducted downward and that other dendrites of the same cell serve as current sources to those sinks. This has been confirmed with simultaneous multielectrode recordings in and out of line and with

current density analysis of the field potentials at different lateral distances from a beam of activated parallel fibers (41). 2) By reason of the decreased excitability of dendritic branches with distance from the soma, antidromic invasion cannot propagate into most of the dendritic tree. Experimental evidence for this has been presented in previous papers (41, 43, 52, 53). A similar phenomenon has been reported in invertebrate neuropil (64) and recently in fish oculomotor neurons (33). That the antidromic invasion of the Purkinje cell does not reach the peripheral dendritic tree is in accord with the view that the electric load represented by the dendritic tree and its low excitability renders the antidromic action current unable to activate the more peripherally located hot spots. Figure 13*B* illustrates this point.

Intracellular recording from dendrites, in fact, demonstrates that following antidromic invasion of the cell only an electrotonic invasion can be observed at dendritic level (Fig. 8), which is in agreement with the unidirectional tendencies for action potential conduction.

Functional independence of dendritic branches

A corollary to the demonstration of unidirectionality of dendritic spike conduction is the concept of partial functional independence of dendritic spike generation. Given that an action potential in one main branch does not necessarily invade other branches, integration in any one dendritic branch may go on undisturbed by dendritic spike invasion from the rest of the dendritic tree. This partial immunity of a given dendritic branch from the disruptive properties of antidromic invasion by an action potential in a neighboring branch has also been observed in other preparations (48). In chromatolyzed motoneurons, for instance, it has been reported (36) that dendritic spikes arising from different loci in the dendritic tree will not generate refractory interaction and thus that a certain degree of functional independence occurs under these conditions. In this latter case, however, following direct activation of the cell, or antidromic invasion, a state of refractoriness is generated in all of the dendritic tree suggesting that, in contrast to the Purkinje cell, the dendrites of chromatolyzed motoneurons can be activated antidromically by a soma-dendritic spike. In fact dual penetration of normal motoneurons (65) has already suggested that dendrites may be antidromically invaded.

Dendritic inhibition

Parallel fiber activation of Purkinje cells evokes in addition to EPSPs, a prolonged, graded and chloride-sensitive hyperpolarization—the necessary and sufficient criterion for synaptic inhibition. Such inhibition is, indeed, to be expected from the distribution of the axons of the stellate cell, the supposed inhibitory interneurons of the molecular layer. As in other cerebella (12, 13), this inhibitory action on alligator Purkinje cells spreads laterally 500 or 600 μ from the activated parallel fibers, corroborating the relation between lateral inhibition and the distribution of stellate cell axons (28). On the other hand, the inhibitory neurons of the alligator are distributed along the dendritic tree of the Purkinje cell from 100 μ depth to just above the beginning of the soma (see Fig. 13*C*) and do not form the very specialized basket cell plexus seen around the body and axon of the Purkinje cell of higher vertebrates (28). This localization of the inhibitory terminals should, in principle, allow a selective inhibition of particular dendritic segments of a Purkinje cell. The large changes in conductance which accompany dendritic inhibition should produce, besides the hyperpolarization, a functional amputation of particular branches of the dendritic tree (40). This membrane shunt would greatly reduce the voltage-generating efficacy of outward moving currents evoked distally to the point of inhibition, whether they be produced by excitatory synapses or by dendritic action potentials. Relevant to this point is the fact that the inhibitory terminals seem to be always located proximal to some component of the excitatory input, since the most peripheral branchlets of the dendrite are almost exclusively contacted by excitatory terminals (40). On the other hand, this form of inhibition will not be as effective as basket cell inhibition at the somatic level. This

point was confirmed at unitary and field potential level (53).

Somatic Activation of Purkinje Cells

The electrical recordings obtained from the soma of alligator Purkinje cells are similar to those recorded in such neurons of other species. They can be characterized extracellularly by so-called giant action potentials. Intracellularly, a typical action potential having an IS-SD component can always be recognized following antidromic invasion. On the other hand, orthodromic invasion is characterized extracellularly by repetitive spike activation and intracellularly by a full-sized action potential (similar to that generated by antidromic invasion), followed by a prolonged depolarization produced by the summation of several all-or-none components Fig. 12*D, F, G, H*). This suggests, therefore, that it is caused in part by electrotonic spread of peripherally located dendritic spikes. Since alligator Purkinje cells usually have only one dendritic stem arising from the soma, it is likely that the funneling of action potentials would allow only the first spike to be conducted at maximum size while the others, due to the refractoriness of that dendritic segment, could only be seen at the somatic level as electrotonically conducted potentials.

The fact that only one large soma-dendritic spike is observed following strong orthodromic activation does not imply that the axon of the Purkinje cell fires only once since, as in the case of the stretch receptor in lobsters (22) and crayfish (66), it is known that a prolonged depolarization at the soma may produce a repetitive activation at the axonic level. In other neurons, however, a rapid depolarization inactivation (18) or the lack of electrogenic afterhyperpolarization, as in the case of the phasic crayfish stretch receptors (50), may decrease the number of action potentials generated at the axon by the sustained somatic depolarization. As in the case of elasmobranchs (54), a "burst response" may therefore be evoked in alligator Purkinje cells by intradendritic current injection as well as by parallel fiber or climbing fiber activation. A similar type of burst response has recently been reported in the cells of the inferior olive (6) and in Purkinje cells of the cat (46). It appears, therefore, that the burst responses of alligator Purkinje cells are produced by the prolonged somatic depolarization generated by the electrotonic conduction of dendritic spikes to its axosomatic region. They are, thus, the product of the particular electrical properties of the alligator Purkinje cells and are not necessarily characteristic of climbing fiber activation. It is possible, therefore, that the lack of typical Purkinje cell burst responses in cats and frogs following parallel fiber activation may be related to the higher threshold for dendritic spike activation in those forms as compared with that in alligators and elasmobranchs.

Finally, some consideration should be given to the unique morphology of the dendritic tree of Purkinje cells in alligators and other species. As first pointed out by Henle (25), dendrites in the Purkinje cells of higher vertebrates are approximately confined to a plane perpendicular to the parallel fibers. This surprising geometrical feature of the dendritic arbor has suggested several hypotheses concerning the functional meaning of Purkinje cell ensembles. Among the first of these was the postulate that dendritic trees of the Purkinje cells evolved for maximum divergence and maximum convergence of parallel fiber input (16, 59). Other more recent views consider the strict geometrical organization of the dendritic tree as having a special timing property for the organization of motor pattern (4, 19, 20). Anatomical studies show that this property may be exemplified in mormyrids (55) and elasmobranchs (54).

The unidirectional properties of dendritic action potentials, together with the postulated functional independence of the different dendritic branches of a Purkinje cell with respect to each other, have led to the hypothesis of vertical and transverse integrative properties (37). Basically it has been postulated that a particular spatiotemporal distribution of parallel fiber action potentials in the molecular layer may favor dendritic spike generation, and thus the bursting responses in Purkinje cells, while another input distribution may favor a more continuous form of activation. In any event it seems of consequence to stress

that neuronal integration as a simple algebraic summation of electrotonically conducted synaptic potentials to a single site of spike initiation may occur only in particular cells. A more up-to-date view of integration must take into consideration the empirical fact of dendritic spikes and the functional complexities which these spikes generate.

SUMMARY

Extracellular and intracellular potentials were recorded in the molecular layer of the alligator cerebellar cortex following surface stimulation. This form of stimulation evoked large all-or-none negative spikes which are assumed to be produced by electroresponsive properties of the Purkinje cell dendrites. Double Loc stimulation at short intervals produced inhibition of the all-or-none negativities. The inhibition had a maximum peak at 20–30 msec interval and a total duration of approximately 50 msec. Several reasons are given as to why this is a true inhibition and not refractory interaction.

Dendritic spikes in Purkinje cells were studied by intradendritic penetration. The intradendritic recording was verified by the injection of Procion yellow dye at the site of penetration. Intradendritic resting potential was found to be approximately −60 mv. Loc stimulation of different amplitudes generated prolonged intradendritic action potentials with a maximum amplitude of approximately 70 mv. Under these conditions a variation of the amplitude of the Loc stimulation demonstrated that these depolarizations were produced by the summation of many all-or-none components. The large intradendritic spikes were dissected into different all-or-none components by applying hyperpolarizing currents of different amplitudes through the recording micropipette. In several cells a minimum of seven sites for spike initiation were encountered.

In addition a study was made of the synaptic potentials evoked by parallel fiber stimulation at dendritic level. Parallel fiber activation was shown to generate graded EPSPs followed by prolonged IPSPs. Intradendritic chloride injection produced a reversal of these IPSPs to a depolarizing potential which could subsequently be reversed to a hyperpolarizing potential by the application of depolarizing current pulses.

In the Purkinje cell layer, parallel fiber stimulation generated the so-called giant Purkinje cell spikes extracellularly. These can be unitary or repetitive, depending on the strength of the Loc stimulation, and can be inhibited by an out-of-line surface stimulation. Somatic penetration of Purkinje cells was also verified with Procion yellow dye. Following parallel fiber stimulation or their activation through the mossy fiber-granule cell system, a typical synaptic potential was obtained at the somatic level. Its amplitude was related to stimulus strength. Parallel fiber stimulation also was able to evoke very prolonged depolarization following a typical orthodromic action potential. This prolonged depolarization was shown to be produced by the summation of several all-or-none components and it is assumed to be generated by electrotonic spread from dendritically evoked action potentials.

It is concluded that alligator Purkinje cells are able to generate dendritic spikes which can be inhibited at dendritic level by the stellate neurons. It is envisaged that the dendritic spikes are conducted in a noncontinuous manner and in a preferential somatopetal direction as the result of the activation of a sequence of electroresponsive sites along the length of the dendritic tree. As a synaptic depolarization reaches threshold for spike initiation in a dendrite, a full spike is generated which is then conducted, first decrementally and then electrotonically, to the next site of spike generation (hot spot). In this pseudo-saltatory fashion a dendritic spike travels down a dendrite toward the Purkinje cell soma. On this basis a discussion of the integrative properties of Purkinje cells is developed and some of the conclusions are illustrated by schematic diagrams.

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