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Electrophysiologic Characterization of Morphologically Identified Neurons in the Cerebellar Cortex of Awake Cats

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Intracellular recordings were obtained from 161 neurons of the cerebellar cortex of 20 awake cats. Seventeen neurons marked by intracellular pressure injection of horseradish peroxidase were morphologically identified as Purkinje cells. Seven other neurons were recovered with a morphology characteristic of Golgi II cells. In addition to intrinsic action potentials typical of cerebellar and other mammalian cortical recordings, both types of cells revealed a fast spike-like component that was relatively insensitive to injected current and could not be attributed to regenerative Na^+ or Ca^{2+} conductances intrinsic to the penetrated neuron. In Purkinje cells, the fast spike often appeared at the initial portion of the potential configuration normally called the climbing fiber response. In the other class of cells, the fast, current-insensitive spike followed the intrinsic cell discharge. The results demonstrate distinctive patterns of electrical activity in two different types of morphologically identified cerebellar neurons. The fast spikes could reflect electrical coupling between the two types of cells.

INTRODUCTION

Previous intracellular recordings characteristic of mammalian cerebellar cortical neurons (1) have been obtained from anesthetized or brain-damaged animals. The identification of the recorded cells was based on their pattern of electrical activity and their response to electrical

Abbreviations: HRP—horseradish peroxidase, EPSP—excitatory postsynaptic potential.

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stimulation. Other studies have been performed in a variety of species, including one, in alligator, in which some of the recorded Purkinje cells were injected with Procion, but it has not been possible to obtain information concerning the intracellular electrical activity of other, morphologically identified cerebellar neurons (2, 3, 7–11). The present study examined the electrophysiologic characteristics of morphologically identified cerebellar cortical neurons in awake cats with brains intact except for introduction of the recording electrode. Herein, we report the pattern of activity and responses to injected current obtained from two classes of cells: Purkinje and Golgi II neurons. A further report concerns electron microscopic studies of these cells (14).

METHODS

The same intracellular technique used earlier to record from the cerebral cortex and facial nucleus of awake cats proved satisfactory for the cerebellar recordings (4, 17, 18). The technique for identifying the neurons involved intracellular pressure injection of horseradish peroxidase (HRP) (15). Glass micropipets with tip sizes less than $1\ \mu\text{m}$ were filled with either 1.4 M potassium citrate (averaged resistance $31 \pm 7\ \text{M}\Omega$) or with 1 M KCl plus 4% HRP (averaged resistance $60 \pm 13\ \text{M}\Omega$). Microelectrodes were inserted through a guide tube placed in a small hole drilled on the exposed skull over the vermian area. The animal's head was held rigidly by means of four screws previously implanted in the skull under pentobarbital anesthesia. Recording of the intracellular potentials and injection of current through recording microelectrodes were done using a high-input impedance amplifier with capacitance compensation and constant current source incorporated together (Mentor N-950 intracellular probe). Depolarizing current pulses of 10-ms duration were given at 10 Hz to determine the firing threshold of the spikes and to monitor the resistance of the electrode and the cell. In addition, depolarizing ramp currents of different slopes (from 0.5 to 20 nA/s) were applied to study the change in firing rate and accommodative properties of the cell. The I–V plots obtained from these ramps were typical of simple and ceiling types [cf. (6, 18)] suggesting the absence of injury seen with minimal gradient types of plots (16). Cell penetrations were signaled by (i) a shift in the recorded steady potential (DC) level, and (ii) a change in the frequency and size of action potentials upon passing depolarizing or hyperpolarizing current through the recording electrode (Fig. 1).

After completing the electrophysiologic study, HRP solution was injected inside the cell (15). A small biopsy core of cerebellar tissue containing the HRP-injected cell was subsequently removed using a

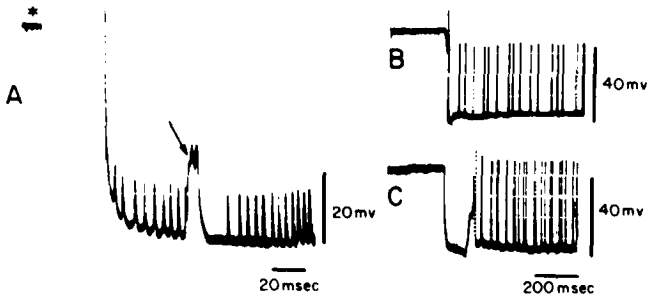


FIG. 1. A—penetration of a morphologically identified Purkinje cell with action potential significantly less than transmembrane potential (undershoot). The baseline potential prior to penetration is shown by an asterisk. A potential configuration containing a fast spike, a postsynaptic potential, and two inactivated cell spikes occurs on the falling edge of a cell spike about 50 ms after penetration (arrow). B—penetration of a cell with the action potential amplitude near that of the DC shift at time of penetration. C—penetration of another cell with action potential larger than the transmembrane potential (overshoot). Fast spikes are seen in overshoot penetrations, but are most easily seen in those of the type shown in A. The latter are thought to reflect penetrations of passive dendritic elements, as in the cerebral cortex (6, 18), or the electrical equivalent.

12-gauge needle. Histologic procedures for HRP demonstration utilized the DAB method (5) or in some cases, the Hanker–Yates reagent. Complete details may be found elsewhere (14, 15).

RESULTS

The shifts in steady potential seen on penetrating cerebellar cells (a total of 161 units) averaged 48 ± 9 mV (SD) with action potentials from 10 to 60 mV. The average DC shifts and action potentials from the 17 morphologically identified Purkinje cells were 53 ± 9 and 26 ± 10 mV, respectively. The corresponding values for seven Golgi cells were 49 ± 4 and 26 ± 8 mV. Action potentials smaller than baseline shifts (undershoot) were most commonly encountered (Figs. 1A and B), although the reverse could also be seen (Fig. 1C). Similar observations were reported in recordings from the cerebral cortex of awake animals and their cause was investigated (18). The spike undershoot reflects the penetration of passive (no regenerative spiking) dendritic regions.

Intracellular recordings from penetration of morphologically identified Purkinje cells [See Figs. 1 and 3 and (14), Fig. 2] were typical of those found by other investigators in Purkinje cells identified by other means (1–3, 7–11). In addition to the regular discharge of the cell (called the cell spike), a potential configuration was observed similar to that identified in the anesthetized preparation as the climbing fiber response. This potential

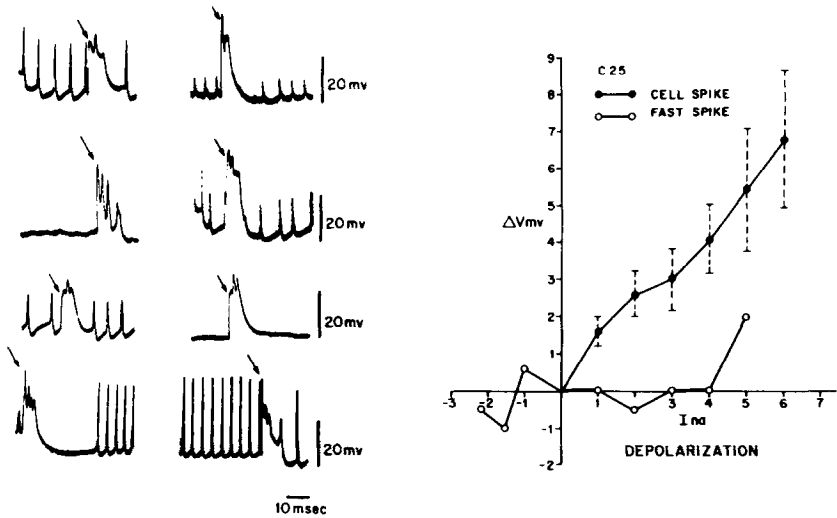


FIG. 2. Left—examples of comparable potential configurations containing the fast spike (arrow) recorded from eight different morphologically identified Purkinje cells. Right—current-voltage plot of fast spike and cell spike typical of Purkinje cells. The current was injected in the form of ramps with different slopes. Action potential amplitudes of fast and cell spikes were 42 and 20 mV, respectively, in the absence of injected current. The vertical dotted bars indicate standard deviations of averaged measurements.

configuration of 10 began with an initial, fast, spike-like component (called the fast spike). It was followed by several inactivated, partial spikes, riding on the large, long-lasting depolarizing potential. Examples of this potential from eight other morphologically identified Purkinje cells are shown in Fig. 2.

The timing of the fast spike did not depend on the appearance of the cell spike (see Figs. 1, 2) or necessarily on that of the excitatory postsynaptic potential (EPSP) of the climbing fiber. It could occur during the refractory period of the cell spike or in the absence of the climbing fiber EPSP, and could be obscured by coincidental occurrence of the cell spike or the depolarizing PSP.

Differences in the amplitude of the fast spike and the cell spike were studied upon injecting current through the recording microelectrode. The current-voltage ($I-V$) plots for the cell spike and the fast spike of a Purkinje cell are shown in Fig. 2. The ordinates in this plot represent changes in baseline-to-peak spike amplitudes. The amplitude of the normal cell spike (initially 20 mV) was readily reduced by injecting depolarizing current, but that of the fast spike (initially 42 mV) was less sensitive to injected current. Measuring percentage change in spike amplitude as a function of injected current, the cell spike in Fig. 2 was six times more sensitive to 5-nA depolarizing current than the fast spike. In Fig. 4, the cell

spike was again more sensitive. Differences in relative sensitivity to injected current of fast spike and intrinsic spike being in the opposite direction to their relative size would seem to rule out the possibility of a remote, intrinsic dendritic location for generation of the fast spike.

In some penetrations, the amplitude of the fast spike was larger than that of the normal cell spike; in others, it was smaller. In either case, discharge complexes characteristic of those illustrated in Fig. 2 were observed, with

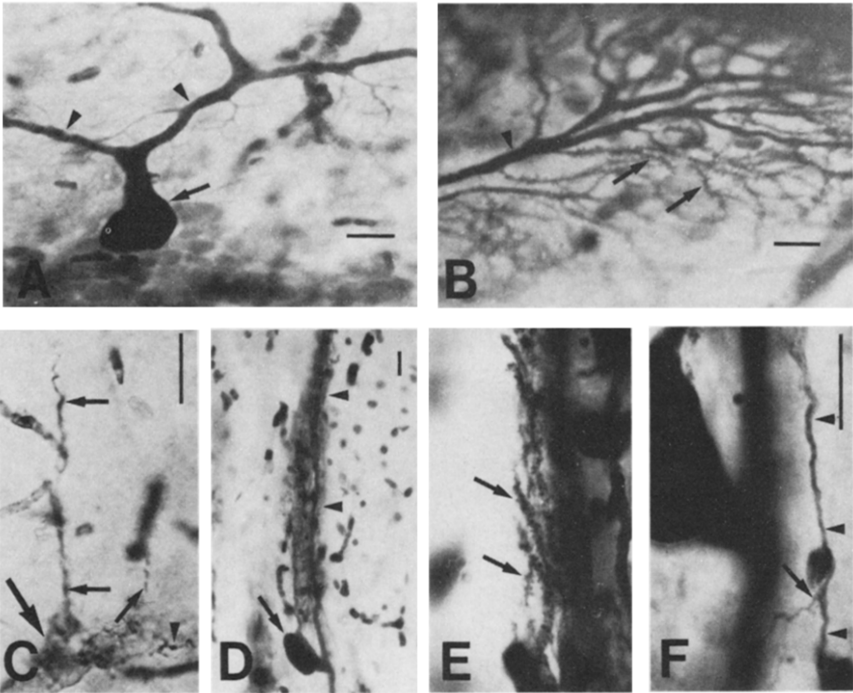


FIG. 3. Photomicrographs of horseradish peroxidase (HRP)-injected cerebellar neurons. A—HRP-filled Purkinje cell body (arrow) at the border of the Nissl-stained granule cell layer and the unstained molecular layer shows prominent primary dendrites (arrowheads). B—HRP-filled spinous dendrites (arrows) arise from a smooth secondary dendrite (arrowhead) from the Purkinje cell shown in A. C—HRP-filled Golgi II cell body (large arrow) has associated with it a beaded axon (arrowhead) and two smooth dendrites (small arrows) which ascend toward pia. Numerous red blood cells obscure some details. D—low-magnification photomicrograph of an HRP-filled Purkinje cell cut in the transverse plane shows soma (arrow) and characteristic dendritic arborization (arrowheads). E—higher magnification of the dendrites from this Purkinje cell reveals many spines (arrows). F—HRP-filled processes of another cell are shown adjacent to Purkinje cell body from D. One of these processes (arrow) in F appears axon-like and arises from the smooth dendrite (arrowheads) which is continuous with a Golgi II cell body (not shown). Calibration bars are equivalent to 20 μ m and E and F are at the same magnification.

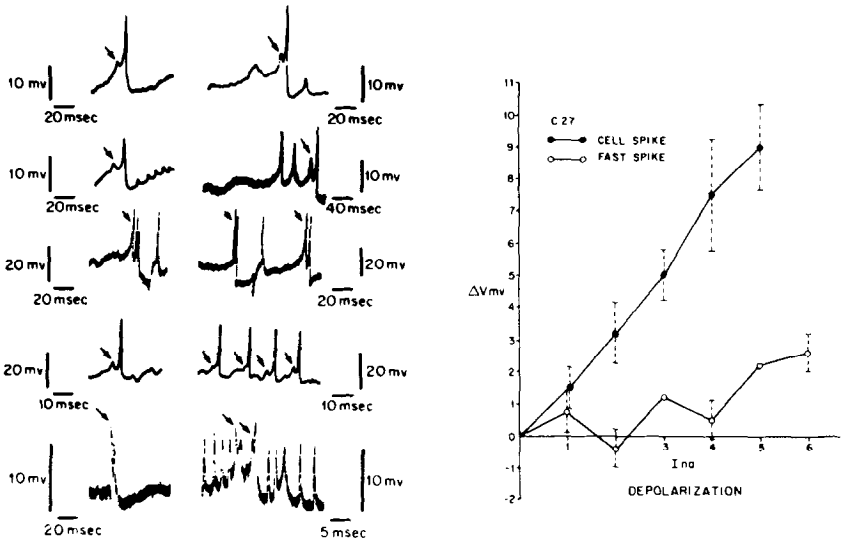


FIG. 4. Left—examples of cell spike and fast spike configurations from five different morphologically identified cells of the Golgi II type. Arrows designate cell spikes immediately succeeded by fast spikes. Right— $I-V$ plot of fast spike and cell spike typical of Golgi II cells. Action potential amplitudes were 48 and 40 mV, respectively. The fast spike was less affected by injected current than the cell spike.

the fast spike relatively insensitive to injected current and the cell spike more sensitive, as indicated. The rate of occurrence of the cell spike was increased by depolarizing current, whereas the appearance of the fast spike remained relatively insensitive to such current. Similarly, short depolarizing current pulses which elicited the cell spikes did not ordinarily elicit the fast spikes, regardless of current intensity or spike amplitude.

Examples of Purkinje cells identified by HRP injection in this study are shown in Fig. 3. The dendrites, somata, and axons could be distinguished as having characteristics of previously described Purkinje cells (11, 13). Somata were large (20 to 30 μm) and were at the border between the molecular and granule cell layers. The axons from these cells were directed into the white matter and sometimes showed collaterals. HRP-labeled dendrites radiated into the molecular layer and tertiary dendrites were studded with spines. The arborization of these dendrites were similar to those for dendrites of Purkinje cells. In electron microscopic preparations of HRP-labeled spinous dendrites, dense amounts of HRP-positive reaction product were observed within dendrites and spines without any spread of reaction product into the extracellular space (14).

In some instances, a Purkinje cell and elements of glial cells, or only glial cells, were marked by HRP injection after penetration of a cell which

showed an electrophysiology characteristic of Purkinje cells. Because of the absence of extracellular spread of HRP, these observations are attributed to the transmembrane passage of HRP between the Purkinje cell and its surrounding glial cells.

The other readily identified class of cells was characterized by the discharge patterns shown in Fig. 4. They also contained a fast spike. The fast spike was normally *preceded* by a cell spike (often an inactivated one). This was just the opposite of the observations in the Purkinje cells (see Fig. 2). The effect on spike amplitude of injecting current through the intracellular recording microelectrodes was also studied in this class of cells. Figure 4 shows a typical I-V plot for the response of the cell spike and the fast spike. In response to injected current, the absolute change in amplitude of the fast spike, initially 48 mV, was again much less than that of the cell spike, which was initially 40 mV.

These cells, when marked by HRP and recovered, showed a morphology similar to Golgi II cells (see Fig. 3). The most characteristic feature was the absence of the profuse, well-differentiated spines seen along the dendrites of Purkinje cells. In light microscopic preparations, HRP-labeled aspiny dendrites were oriented perpendicular to the pial surface and often showed axonal-like processes arising from their shafts (see Fig. 3). These axons ramified in the granule cell layer. In addition, either a small or a large cell

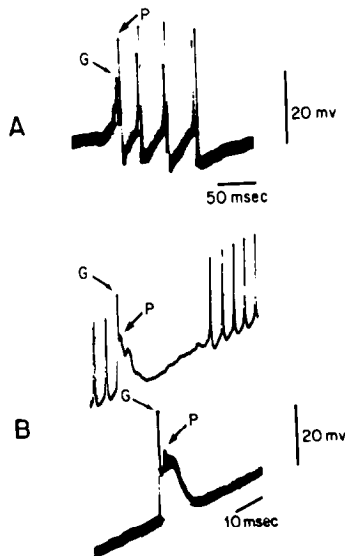


FIG. 5. Comparison of fast spike and cell spike in Golgi II (A) and Purkinje (B) cells. In Golgi II cells, the cell spike marked G precedes the fast spike (marked P). The reverse discharge pattern occurs in the Purkinje cell. Note that the fast spike is present even after inactivation of the cell spike (lower trace in B).

body in the granule cell layer could be observed to be continuous with these HRP-containing dendrites. These characteristics suggested that this neuronal type was a Golgi II cell (12, 13). Electron microscopic observations of dendrites from these neurons confirmed this identification in that parallel fiber axon terminals formed synapses with these dendritic shafts (14).

DISCUSSION

The patterns of spike activity, resting potentials, and spike amplitudes obtained from Purkinje cells in these awake cats were similar to those reported by others in a variety of species and preparations (1-3, 7-11). This includes the patterns of activity identified as climbing-fiber responses. The morphology of these cells was similar to that previously described for both noninjected Purkinje cells (12, 13) and for Purkinje cells injected with HRP (11).

A different pattern of activity was found in the cells identified morphologically as Golgi II neurons. The two types of potential configurations in Purkinje and Golgi II cells are compared in Fig. 5. In Fig. 5A (Golgi II), the cell spike is coupled closely and regularly with the fast spike and precedes it. In Fig. 5B (Purkinje), inactivated cell spikes and the depolarizing PSP succeed the fast spike, as if the position of the cell spike relative to that of the fast spike in these two types of cells were reversed.

The results indicate that two different cell types in the cerebellum have their own characteristic patterns of electrical activity. Those of the Golgi II cells have not to our knowledge been previously identified. Nor have the responses of Golgi II or Purkinje neurons to intracellularly injected current been studied in awake, intact mammals. Each pattern of discharge showed a fast spike and the comparison of patterns in Fig. 5 indicated that the times of appearance of these spikes were reciprocally related between Golgi II and Purkinje cells. In addition, the fast spikes were selectively less sensitive to injected current, regardless of whether or not they were larger or smaller than the cells spikes and whether they appeared in conjunction with the climbing fiber PSP in the Purkinje cells. Furthermore, the observation that the full fast spikes appeared during the presumed absolute refractory period of some cell spikes suggested that the fast spikes arose extrinsically to the penetrated neuron. This phenomenon could occur if there were electrical coupling between Purkinje and Golgi II neurons. A review of earlier anatomical studies indicates that gap junctions indicative of such coupling have not been reported between these two cell types. However, although the present electrophysiologic indications are indirect and the corresponding anatomic evidence from these cells preliminary (14), the possibility of electrical coupling best explains these results.

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