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Sodium action potentials in the dendrites of cerebellar Purkinje cells

(dendritic spikes/ion channel distribution)

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ABSTRACT **We report here that in cerebellar Purkinje cells from which the axon has been removed, positive voltage steps applied to the voltage-clamped soma produce spikes of active current. The spikes are inward, are all-or-none, have a duration of ≈1 ms, and are reversibly eliminated by tetrodotoxin, a Na channel poison. From cell to cell, the amplitude of the spikes ranges from 4 to 20 nA. Spike latency decreases as the depolarizing step is made larger. These spikes clearly arise at a site where the voltage is not controlled, remote from the soma. From these facts we conclude that Purkinje cell dendrites contain a sufficient density of Na channels to generate action potentials. Activation by either parallel fiber or climbing fiber synapses produces similar spikes, suggesting that normal input elicits Na action potentials in the dendrites. These findings greatly alter current views of how dendrites in these cells respond to synaptic input.**

Information in the nervous system passes from cell to cell mainly through synaptic connections on dendrites. Although dendritic properties are obviously important, experimental techniques for examining them have been slow to develop, and little compelling information exists regarding their electrical behavior. An early and quite elegant hypothesis held that dendrites are passive cables that weight synaptic input in proportion to the proximity of synapse to soma (for review, see ref. 1). More recent evidence shows that at least some dendrites are not passive but instead can generate active spikes (2–5). In experiments on isolated neocortical pyramidal cells from newborn rats, differential microperfusion was used to show the presence of Na channels in dendritic stumps that stretched as far as 100 μm from the soma (6).

For neurons *in situ*, the distribution of spike-generating ion channels has been inferred primarily from intradendritic recordings (3–5). Such measurements suggested that Purkinje cell dendrites could generate Ca action potentials but were devoid of Na channels (2, 3). Dendrites of hippocampal pyramidal cells have action potentials produced by both Na channels and Ca channels (4, 5). More recently, microfluorometric imaging techniques have also provided some evidence regarding channel distribution (7–9).

The recent application of whole-cell patch-clamp techniques to brain slices holds promise for helping with these questions. Here we have used the voltage clamp in an unconventional way to show the presence of Na spikes in the dendrites of cerebellar Purkinje cells.

MATERIALS AND METHODS

Experiments were done on 200-μm sagittal slices of cerebellar vermis from 22- to 55-day rats, by using methods similar to those described (10, 11). In brief, the cerebellum was quickly removed from the animal and placed in ice-cold saline for 1–2 min. A Vibratome was used to cut slices, which were then allowed to incubate for 1–5 hr at 35°C. A slice was placed in a simple chamber on a Zeiss Axioskop and viewed with a 40× water immersion objective. We elected to view the preparation with bright-field rather than Nomarski optics; it was possible to resolve individual Purkinje cells, their dendrites, and, in many cases, the axon. Cells near the surface of the slice were selected for recording and were gently cleaned as described by Edwards *et al.* (10).

In cases where axotomy was done, a well-defined procedure was adopted to ensure removal of the axon. Two investigators carefully inspected the cells at various stages throughout the procedure to minimize the possibility of error. We selected a Purkinje cell with a clearly visible axon. Pressure was applied to the cleaning pipette to produce a forceful blowing action; this pressure was increased until the cell body was pushed up from the surface of the slice, remaining tethered only by its axon and dendrites. Still more pressure severed the axon, which is smaller and mechanically weaker than the dendrite. The Purkinje cell then consisted of the dendrites, which appeared unaffected by this procedure; the soma, which was above the surface of the slice and could be easily moved by gentle blowing; and the axon stump. By changing the plane of focus, we could estimate the length of the axon stump. Recordings were obtained from cells for which two investigators independently estimated the axon stump at <10 μm in length. About one-third of the cells were damaged by this cleaning procedure and were unsuitable for recordings; for such cells the soma appeared very granular.

Tight-seal whole-cell recordings were obtained with fire-polished glass electrodes (Kimble Kimax-51 glass; Toledo, OH). Electrode resistances were in the 0.5–2.6 MΩ range. The access resistance was measured every few minutes throughout the experiment, from the instantaneous current response to a −50-mV step. Access resistance was typically two to four times the value of electrode resistance. We could compensate for ≈70% of this resistance. The adequacy of the clamp is important, and for this reason we calculated the series-resistance error from the product of current through the electrode and the uncompensated fraction of the series resistance. From these calculations we could establish the actual membrane potential in the soma at the time of spike initiation. This voltage was well below our estimates for the threshold of somatic spikes, as shown specifically for the spikes in Fig. 4.

Parallel fibers and climbing fibers were stimulated with the same pipette that was used to clean the cells. This electrode was placed in contact with the slice surface in the granule cell layer ≈50 μm from the Purkinje cell, unless otherwise indicated. Recordings were filtered at 10 kHz (Frequency

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Abbreviations: TTX, tetrodotoxin; FIS, internal solution with fluoride.

Devices, Haverhill, MA, 8-pole filter, model 902LPF) and digitized at sampling intervals of 20 or 50 μsec per point.

The external saline consisted of 125 mM NaCl, 2.5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 26 mM $NaHCO_3$, 1.25 mM NaH_2PO_4, and 25 mM glucose at pH 7.4. The saline solution was bubbled with 95% O_2/5% CO_2. To eliminate inhibitory synaptic conductances (11) the γ-aminobutyric acid type A antagonist bicuculline was included in the external saline (20 μM, Sigma). Standard internal solution consisted of 140 mM KCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 2 mM Na-ATP, 10 mM K-Hepes, and 10 mM K-EGTA at pH 7.3. Internal solution with fluoride (FIS) was prepared by mixing five parts standard internal solution with two parts 300 mosM KF. The results obtained with the two types of intracellular solutions were similar, but cells were much more stable (lasting for hours) with FIS, and larger recording electrodes could be used. Experiments were done at room temperature (19–23°C).

RESULTS

Whole-cell recordings were obtained from Purkinje cells by using an electrode placed on the soma, as shown schematically in Fig. 1*A*. Cells from the 22- to 55-day animals used in these studies had extensive dendritic trees, and, in some cases, intact axons, which made it impossible to accurately voltage-clamp all parts of the cell with a single electrode.

The expected voltage distribution for a greatly simplified cell (Fig. 1*B*) is illustrated by the thin line in Fig. 1*C*. The soma is assumed to be held at a command voltage below the resting potential. At sites electrically distant from the soma the membrane potential deviates from the command voltage: neither the axon nor the dendrites are under good voltage control. After an excitatory synaptic input to the dendrites, the soma remains at the command voltage, but the voltage in the dendrites deviates from the steady-state voltage as shown qualitatively in Fig. 1*C* (thick line), possibly reaching threshold for spike generation in the dendrites. Because excitatory synapses are located exclusively on soma and dendrites, the voltage in the axon will not change: it is effectively isolated from the synapses by the clamp.

Active dendritic responses might also be elicited by positive voltage steps applied to the soma. In this case, the voltage in the soma would remain fixed while remote parts of the dendritic tree escaped control and generated actions potentials. Fig. 2 illustrates such an experiment for an axotomized cell (see *Materials and Methods*). Small positive and negative voltage steps produced the similar but oppositely directed passive responses shown in Fig. 2*A*. Steps of +18 mV were just at or above threshold for the generation of active spikes of current, which displayed a long, somewhat variable latency (Fig. 2*B*). Slightly larger steps produced active spikes with an identical time course but shorter latency (Fig. 2*B*).

In some cells active responses were observed over a wide range of holding potentials, as in Fig. 2*C*. The primary effect of altering this potential from −30 mV to −60 mV was to change the latency of the active current spike without much affecting its magnitude or duration. When holding at −30 mV, essentially all of the Na channels located in the soma would be inactivated and could not contribute to this active response. Active currents similar to the ones shown were seen in all cells studied (n = 20). The amplitude ranged from 4–20 nA. Because the axon has been removed and the soma is in good voltage control, the origin of these active currents can only be in the dendrites.

The rapid time course of the active currents suggests that they are caused by Na action potentials in poorly voltage-clamped regions of the cell. This hypothesis was confirmed by applying tetrodotoxin (TTX) to axotomized cells, as shown in Fig. 3. Stable current spikes were obtained in response to positive voltage steps applied at the soma (Fig. 3*A*). Then a solution containing 50 nM TTX was washed in, and the currents were eliminated (Fig. 3*B*). After washout of TTX the current spikes returned, although the recovery was not perfect (Fig. 3*C*).

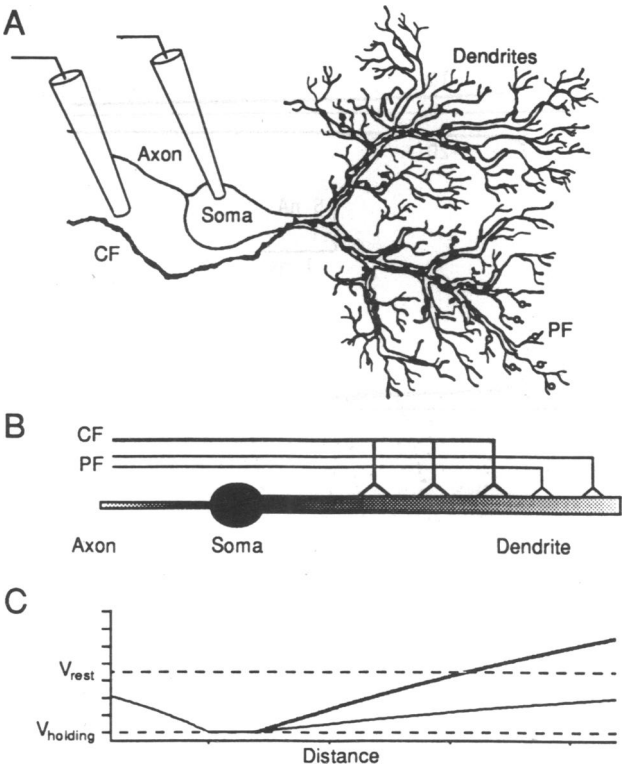

FIG. 1. (*A*) Schematic drawing of a Purkinje cell and its excitatory inputs (cf., refs. 12 and 13). A single climbing fiber (CF) makes many synaptic contacts with the large dendritic branches. Granule cells located below the Purkinje cell send their axons, known as parallel fibers, into the molecular layer, where they contact fine dendritic branches; the location of a few such contacts is shown by small circles and labeled PF. A stimulus electrode placed ≈50 μm below the Purkinje cell in the granule cell layer was used to stimulate CF and PF inputs, as well as to directly activate the Purkinje cell. (*B*) Simplified drawing of a Purkinje cell, in which the dendritic arbor is reduced to a cylinder, a crude electrical equivalent of the dendrite. (*C*) A low-resistance clamp electrode provides good voltage control of the soma, but distant regions of the axon and the dendrites are not well voltage clamped. The voltage distribution in the steady state is shown schematically for the simplified cell, in which the soma is held below the resting membrane potential (thin line). Excitatory synaptic activation depolarizes the dendrites but does not depolarize the soma or axon (thick line).

These results clearly show that Na action potentials are generated in Purkinje cell dendrites after voltage steps applied at the soma. Can similar action potentials be activated by synaptic inputs?

Fig. 4*A* shows the results of stimulating the parallel fibers, which synapse on fine dendritic branches. A single stimulus seldom caused an active response in the Purkinje cell, but stimulation twice in rapid succession enhanced the second synaptic response ≈2-fold (11) and pushed the dendrite above threshold. A large "direct" response (labeled δ) followed both stimuli with a very short latency, due to excitation of either the axon (which was intact) or the dendrites. This response was followed by an inward current mediated by parallel fiber synapses (σ_{pf}). The second stimulus elicited a direct response (δ), followed by an enhanced synaptic current (σ_{pf}), which, in turn, stimulated a large inward current spike (α). Judging from its time course, the current spike is caused

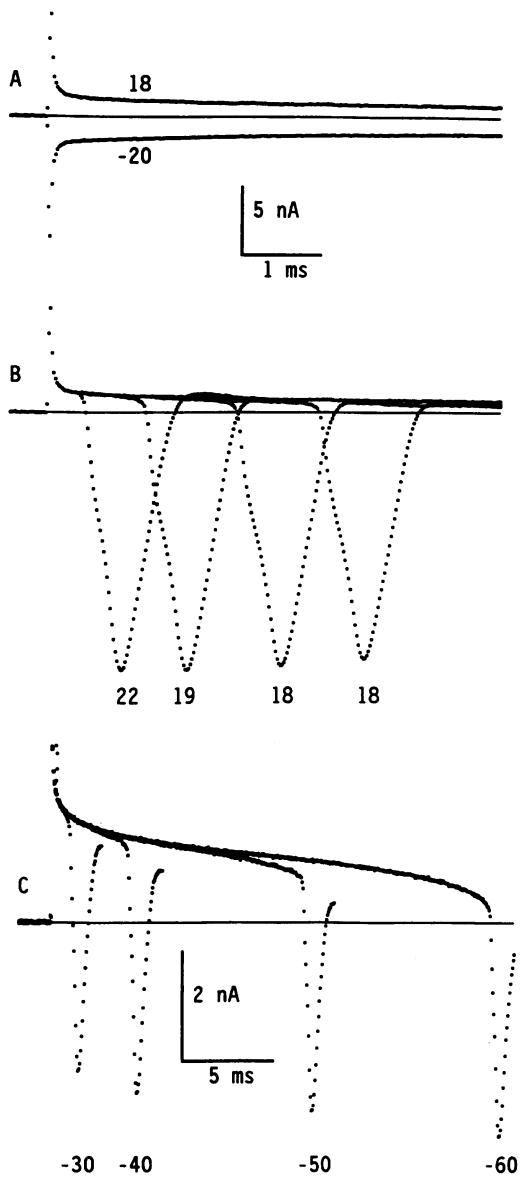

FIG. 2. In axotomized Purkinje cells, voltage clamped at the soma, depolarizing steps produce current spikes in remote dendritic regions. (*A*) For negative voltage steps and small positive ones (<18 mV) there is no active response. (*B*) Larger voltage steps trigger inward-current spikes with variable latencies. Three steps of 18 mV produced two current spikes and one failure. Slightly larger steps caused similar events with shorter latencies. In *A* and *B* cell DC121A from a 28-day-old rat was used, electrode resistance was 1.1 MΩ, series resistance was 2.6 MΩ, and the holding potential was −76 mV; FIS was used. (*C*) In another cell 50-mV steps produced active responses with a delay that depended upon the holding potential at the soma. The responses are for holding potentials of −30, −40, −50, and −60 mV. Latency increases as the holding potential becomes more negative (traces have been truncated for clarity). For *C* cell DC121B from a 28-day-old rat was used, electrode resistance was 2.6 MΩ, and series resistance was 10 MΩ; FIS was used.

by a Na action potential occurring at a site remote from the voltage-clamp electrode. The spike could be prevented by changing the holding potential from −65 to −70 mV (see figure).

Using the same cell, we elicited a similar action potential by a voltage-clamp step. The cell in Fig. 4*B* was held at −65 mV and stepped to −40 mV (no active response) or to −37 mV. The latter step caused no detectable active response until ≈3 ms, when a large inward current spike developed. The inward current is followed by an outward current,

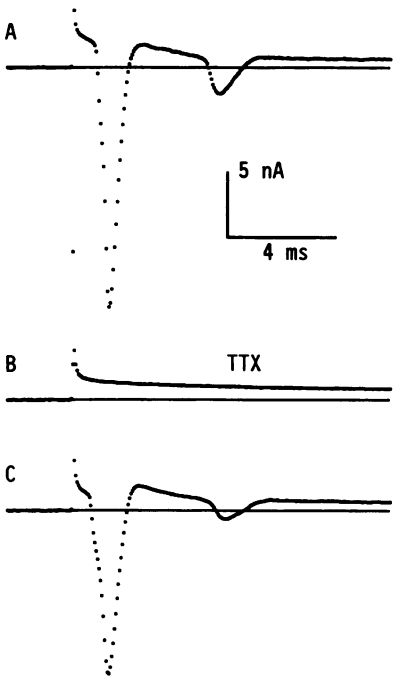

FIG. 3. TTX blocks current spikes in axotomized Purkinje cells. The response to 40-mV steps is shown immediately before adding TTX (*A*), in the presence of 50 nM TTX (*B*), and after return to control saline (*C*). For *A–C* cell DC171A from a 33-day-old rat was used; holding potential was −90 mV, electrode resistance was 1.4 MΩ, and access resistance was 5 MΩ; FIS was used.

presumably due to current through K channels. This active response resembles an inverted action potential.

What is the site of origin of the active current? As noted, it cannot arise at the soma, which is voltage clamped, nor can it arise from the axon, which is electrically isolated from the synaptic input by the clamped soma. There remains only one possibility, that the action potential is generated at a dendritic site remote from the soma. This argument would not hold were the voltage clamp of the soma of such poor quality, due to high-access resistance, that it could not prevent an action potential in the soma or axon. Close analysis shows this cannot be the case here (see Fig. 4 legend).

Experiments were conducted to determine whether climbing fiber activation could also elicit a Na action potential. For the experiment of Fig. 5, a stimulus electrode was placed in the granule cell layer. As stimulus intensity was increased, an all-or-none active response (δ) with a very short latency was observed. This current was probably generated by an antidromic action potential in the Purkinje cell axon. Further increasing the stimulus intensity produced an additional response, which consisted of a very large and rapid inward current (α) riding upon the climbing fiber synaptic current (σ_{cf}). By an argument similar to that for the parallel fiber stimulation, the response denoted α must be produced by a Na action potential in the dendrites (see Fig. 5 legend). The putative antidromic response here is much smaller than the current spike elicited by climbing fiber activation (3.1 nA compared with 6.2 nA). Judging from this amplitude difference, the direct response and the spike elicited by climbing fiber activation cannot both be produced by Na channels located exclusively in the axon, which was intact in this cell.

DISCUSSION

We have shown that axotomized Purkinje cells can generate spikes based on TTX-sensitive Na channels. Moreover, these spikes occur when the soma is voltage clamped and

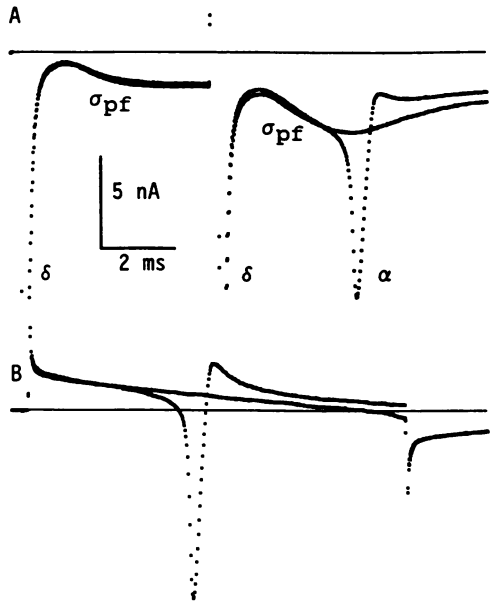

FIG. 4. Active parallel fibers elicit Na action potentials in the dendrites. (*A*) Parallel fibers were stimulated twice at an interval of 5 ms. Stimulation of the parallel fibers caused an active response when the soma was held at −65 mV but not at −70 mV. The axon of this cell was intact. (*B*) When holding potential was −65 mV, a step of +25 mV did not elicit an active response, but a +28-mV step did elicit an active response. From measurements of the series resistance (5.8 MΩ), the holding current (−0.3 nA), and the synaptic current, the threshold for the action potential elicited by parallel fibers in *A* is −45 mV, whereas the threshold for action-potential production via step depolarization in *B* is −38 mV. This fact shows that series-resistance errors are not large enough to allow an action potential in the soma or in the axon and that the action potential in *A* must be produced in the dendrites. Cell DC101A of a 26-day-old animal was used.

cannot itself generate an action potential. The only possible origin for these spikes is the dendrite. Our results thus establish that TTX-sensitive Na channels are present in Purkinje cell dendrites at a density high enough to produce Na action potentials. In addition, we find that synaptic inputs

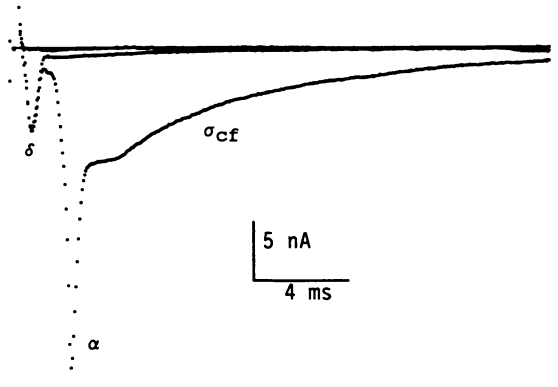

FIG. 5. A dendritic current spike in response to climbing fiber activation. The axon of this cell was intact. Progressively stronger extracellular stimulation produced (*i*) no response, (*ii*) a short latency response (δ), and (*iii*) a short-latency response followed by an additional all-or-none response with two components: the climbing fiber synaptic current (σ_{cf}) and a superimposed spike (α). The current spike was generated in a remote region, while the somatic voltage was −105 mV, taking into account series-resistance errors. Obviously this spike could not have originated in the soma or in the axon and must have been produced in the dendrites. Cell NO221A of a 30-day-old rat was used, electrode resistance was 2.6 MΩ, uncompensated access resistance was 1.8 MΩ, and holding potential was −112 mV; standard internal solution was used.

to the dendrites via either parallel fibers or climbing fibers can elicit Na action potentials in these dendrites.

The presence of Na channels in Purkinje cell dendrites is at odds with previous conclusions that Ca but not Na action potentials are generated in these dendrites (2, 3). The evidence for absence of Na channels was based largely on intradendritic microelectrode penetrations in slices from adult guinea pig cerebellum. Na action potential amplitude decreased with distance from the soma, apparently reflecting a somatic origin. In dendritic recordings, even far from the soma, slow Ca action potentials could be observed.

Is it possible to reconcile the two sets of observations regarding Na spikes? Differences in experimental conditions (such as the animal used: adult guinea pig vs. juvenile rat; or temperature) might account for these inconsistencies. It is also difficult to completely exclude the possibility of electrode damage when recording from a structure as small as a dendrite. Penetration damage might raise the resting potential sufficiently to inactivate Na channels but not to inactivate Ca channels. In this case measurement of dendritic activity with an electrode at the soma seems more appropriate than the more invasive procedure of dendritic penetration.

The observation that synaptic activation can elicit action potentials in Purkinje cell dendrites significantly changes our view of the way synaptic inputs are processed by these cells. According to the passive cable theory of the dendrite, synaptic currents are conducted to the soma with a decrement due to the cable properties; the decision of whether or not to generate an action potential is made exclusively at the soma (1). In this theory the passive properties of dendrites make a major contribution to the effectiveness of synaptic inputs by weighting proximal inputs more heavily than distal ones.

This theory was shown to be incomplete by reports of active responses in dendrites (2–6). Llinas and Sugimori (3) have given convincing evidence for Ca channels in Purkinje dendrites, which under some circumstances can generate Ca action potentials. Ca imaging experiments are also consistent with the presence of dendritic Ca channels (7, 8). Our records clearly show active responses in the dendrites but mediated by Na ions. We have, so far, no evidence for a contribution by Ca channels to the spikes that we record. Our records can, instead, be explained as the summation of synaptic currents with TTX-sensitive current spikes generated in the dendrites. This result does not exclude the possibility that Ca channels open during the spike, as they do during the Na-driven action potentials at presynaptic terminals. Also, we probably did not reach the threshold for Ca electrogenesis: Ca spikes have been shown to be elicited by an electrode at the soma only with prolonged and heavy current injection (2).

The unexpected finding of Na action potentials in the dendrites of *in situ* Purkinje cells brings these cells into conformity with other central neurons (4–6) and suggests that Na spikes may be a common property of dendrites. Clearly, the theory of dendrites as exclusively passive cables can be laid to rest. Our results suggest that the primary spike-generating zone may be in the dendrites, rather than in the soma or initial segment. Further functional speculations should probably await more evidence regarding the distribution of Na channels. At present we can only say Na channels are found at dendritic sites remote from the soma.

In passing, we would point to two techniques developed for these experiments that may be helpful when applied to the study of other cell types. (*i*) Removal of the axon, which can be accomplished easily and reliably for the Purkinje cell, can serve to eliminate ambiguity in the location of conductances. (*ii*) The use of a voltage clamp to electrically divide a cell into regions. In this case the axon and the dendrite were electrically isolated from each other by clamping the soma. In principle, this approach could find more widespread application, such as separating two dendritic regions. Isolation, of

course, can only be achieved by an effective voltage clamp, which requires a low access resistance.

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1. Rall, W. (1989) in *Methods in Neuronal Modeling*, eds. Koch, C. & Segev, I. (MIT Press, Cambridge, MA), pp. 9–62.
2. Llinás, R. & Sugimori, M. (1980) *J. Physiol. (London)* **305**, 171–195.
3. Llinás, R. & Sugimori, M. (1980) *J. Physiol. (London)* **305**, 197–213.
4. Wong, R. K. S., Prince, D. A. & Basbaum, A. I. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 986–990.
5. Bernardo, L., Masukawa, L. & Prince, D. A. (1982) *J. Neurosci.* **4**, 1614–1622.
6. Huguenard, J. R., Hamill, O. P. & Prince, D. A. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 2473–2477.
7. Ross, W. N. & Werman, R. (1987) *J. Physiol. (London)* **389**, 319–336.
8. Tank, D. W., Sugimori, M., Connor, J. A. & Llinás, R. R. (1988) *Science* **242**, 773–777.
9. Regehr, W. G., Connor, J. A. & Tank, D. W. (1989) *Nature (London)* **341**, 533–536.
10. Edwards, F. A., Konnerth, A., Takahashi, T. & Sakmann, B. (1989) *Pflügers Arch.* **414**, 600–612.
11. Konnerth, A., Llano, I. & Armstrong, C. M. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 2662–2665.
12. Palay, S. L. & Chan-Palay, V. (1974) *Cerebellar Cortex* (Springer, New York).
13. Ito, M. (1984) *The Cerebellum and Neural Control* (Raven, New York).