Action Potentials in the Dendrites of Retinal Ganglion Cells

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Velte, Toby J. and Richard H. Masland. Action potentials in the dendrites of retinal ganglion cells. J. Neurophysiol. 81: 1412–1417; 1999. The somas and dendrites of intact retinal ganglion cells were exposed by enzymatic removal of the overlying endfoot of the Müller glia. Simultaneous whole cell patch recordings were made from a ganglion cell’s dendrite and the cell’s soma. When a dendrite was stimulated with depolarizing current, impulses often propagated to the soma, where they appeared as a mixture of small depolarizations and action potentials. When the soma was stimulated, action potentials always propagated back through the dendrite. The site of initiation of action potentials, as judged by their timing, could be shifted between soma and dendrite by changing the site of stimulation. Applying QX-314 to the soma could eliminate somatic action potentials while leaving dendritic impulses intact. The absolute amplitudes of the dendritic action potentials varied somewhat at different distances from the soma, and it is not clear whether these variations are real or technical. Nonetheless, the qualitative experiments clearly suggest that the dendrites of retinal ganglion cells generate regenerative Na$^+$ action potentials, at least in response to large direct depolarizations.

INTRODUCTION

The dendrites of some neurons of the mammalian CNS contain channels capable of causing regenerative electrophysiological events to occur, although the site of action-potential initiation has been somewhat controversial and may differ from neuron to neuron (reviews, Stuart et al. 1997; Yuste and Tank 1996). In retinal ganglion cells, regenerative dendritic potentials would have consequences for the cells’ overall functions. For example, the integration of inputs from bipolar and amacrine cells would potentially be nonlinear, a possibility important for subtle functions such as motion detection but also for the creation of the simpler, center-surround receptive field (Kier et al. 1995). In addition, back-propagation is probably necessary to create correlated firing between ganglion cells (Brivanlou et al. 1998; DeVries and Baylor 1996; Meister et al. 1995), much of which is mediated by gap junctions located on the cells’ distal dendrites, far from the site of initiation of somatic action potentials. We therefore made simultaneous whole cell recordings from the somas and dendrites of ganglion cells.

To record from ganglion cell dendrites, it was necessary to expose them. Although retinal slices have many advantages, some of the dendrites are cut and an adequate length of dendrite is exposed only in fortuitous cases (Cohen et al. 1994; Diamond and Copenhagen 1993; Werblin 1978). We chose instead to remove the surface layer from otherwise intact retinas maintained as whole-mounds. The major difficulty was...

METHODS

Preparation of the retina

The methods of isolating and maintaining the retina of the rabbit have been described in detail previously (Ames and Nesbett 1981; Peters and Masland 1996). In previous work, the somas of ganglion cells were exposed by mechanical disruption of the retina’s surface. Here we eliminated the Müller cell endfeet and the inner limiting membrane by treating the retina with papain and then washing its surface, which exposed both the somas and their dendrites.

Retinas were from adult (2.5 kg) New Zealand White rabbits. The retina was isolated from the pigment epithelium and cut into pieces ~0.7 cm$^2$, which were placed on filter paper, photoreceptor cells down, (Millipore cat. No. TMT01300) using slight suction to make them adhere. They were placed in 2-mL wells of a multiwell plate with the following activated enzyme solution: 1 mM l-cysteine, 0.5 mM EDTA, 0.005% DNase, and 30 U/ml papain (Worthington cat. No. LS03126) in Earl’s Balanced Salt Solution (BSS). The multiwell plate was incubated at 37°C, for 45–60 min. Next, the wells were slowly rocked (to superfuse the tissue’s surface) and brought to room temperature in 30 min. To stop the enzymatic digestion, an ovomucoid (10 mg/ml), BSA (10 mg/ml) solution was applied for 5 min in Earl’s BSS (all Sigma). The tissues were transferred to Ames’ medium (which is buffered with 22.6 mM bicarbonate), placed in a superfusion chamber, and maintained at 37°C. The tissue was perfused at 1 ml/min with filtered Ames’ medium (Sigma), equilibrated with 95% O$_2$-5% CO$_2$. Because the dissection and enzymatic treatment were done under ordinary room illumination, no response to light was recorded from the ganglion cells. Scanning electron microscopy (Fig. 1) was carried out after fixation with glutaraldehyde followed by osmium tetroxide, using conventional techniques of shadowing and examination.

Electrophysiological recordings

The perfusion chamber was mounted on the stage of an upright microscope (Axioskop FS; Zeiss). The tissue was viewed through a 40×/0.75NA, long-working-distance, water immersion objective (Zeiss). A modification of the “blow and seal” patch-clamp method (Stuart and Sakmann 1994) was used for whole cell recordings. The resistances of the seals on the membrane ranged from 1 to 15 GΩ and were typically 2–5 GΩ. Recording electrodes were made of borosilicate glass and were not fire-polished. Their resistances were 2–5 MΩ.
for the soma electrodes and 8–12 MΩ for the dendrite electrodes in standard electrolytes. The composition of the electrode solution was 89.4 mM K-(CH$_3$SO$_3$), 10 mM NaCl, 5 mM HEPES, 5 mM EGTA, and 0.5 mg/ml Lucifer yellow-CH; pH 7.4. (The Nernst potentials were $E_{Na} = 71$ mV, $E_{K} = -90$ mV, and $E_{Cl} = -67$ mV.) In some experiments, the intracellular sodium channel blocker lidocaine N-ethyl bromide (QX-314, Research Biochemicals International) was added to the pipette solution at 5–10 mM.

Positive pressure into a larger pipette (20 μm tip) was used to clean cells. In addition, the flow of solution could be used to help tease dendrites from the inner plexiform layer (IPL). A dendrite was first approached, under brightfield illumination. Then, the soma was patched using a Lucifer yellow–filled pipette. The dendrite, once filled, could be easily followed from the recording site on the dendrite to the soma. We targeted larger cells, because they had the largest dendrites (1–3 μm diam). We could be certain that we recorded from dendrites and not axons because they tapered and branched and were larger than the axons. Because the axon has a long, straight course and is located in a different optical plane from the soma and dendrites of the ganglion cell, the axon was easy to identify after Lucifer yellow injection. Choosing larger cell bodies probably selected for α-ganglion cells and perhaps directionally selective cells, although a mixed population likely to include other types was surely studied here (Peichl et al. 1987; Wässle and Boycott 1991). Despite variations in morphology, all cells had approximately the same electrophysiological characteristics.

Whole cell recordings were made using a patch-clamp amplifier (model PC-501A; Warner) and an intracellular recording amplifier (model IR-183; NeuroData). Either amplifier was used at the soma or dendrite with no discernible difference. Pipette capacitance and access resistance were compensated using circuits on the amplifiers. The input resistance was measured by injecting a small hyperpolarizing pulse (20–100 pA) into the soma and averaged $175 \pm 194$ MΩ (mean ± SE, $n = 12$). Recorded signals were low-pass filtered at 10 kHz (8 pole constant delay filter; Frequency Devices), sampled at 50 kHz, digitized (Digidata 1200A; Axon Instruments), and stored by computer. Stimuli consisted of short current pulses provided by external stimulators (Grass Instruments) into the amplifiers.

RESULTS

Exposure of the ganglion cells is shown in Fig. 1. The top micrograph (A) shows the surface of a retina before treatment with papain. The Müller endfeet abut each other, creating a scaly appearance. Bundles of ganglion cell axons traverse the field. The middle and bottom micrographs (B and C) illustrate the surface of the retina after treatment with papain. Most of the tufted structures are the endfeet of the Müller glia, truncated and blebbled by the enzyme treatment; some ganglion cell somas may also have been damaged and taken on this appearance. Although a few processes, usually axons, have broken, the cell bodies of many ganglion cells remained smooth and round, and their dendrites were intact. These could be followed for tens or hundreds of micrometers across the retina’s surface. Additional lengths of dendrite could be mechanically teased out from the IPL (see METHODS). The dendritic arbors were not visibly distinguishable from dendritic arbors of ganglion cells seen after injection with Lucifer yellow in untreated retinas. The exposed dendrites often had multiple branches, and dendrites distal to several branchings were accessible.

Electrodes were placed on the soma and a dendrite, usually a branch of second order or higher. The dendritic electrode was 40–320 μm (mean, 130 μm) from the soma (Fig. 2, arrow). Another patch electrode, containing Lucifer yellow, recorded responses at the soma and filled the dendritic arbor with the fluorescent dye. A total of 43 dual soma-dendritic recordings were obtained. Fourteen of these used a whole cell configuration at the soma and a cell-attached configuration on the dendrite. The other 29 were whole cell in both locations. Stimulation of the dendrites required higher currents when the dendritic electrode was cell attached, but the results were qualitatively the same as when the whole cell configuration was used at both sites. For simplicity, we present here only experiments in which both electrodes were in the whole cell configuration.

After whole cell recording was established at both the dendrite and soma, the dendrite or soma was stimulated by injecting depolarizing current (40–1,000 pA, 400 ms). When the
soma was stimulated with sufficient current to generate action potentials, an impulse was seen in the dendrite for every action potential observed at the soma \((n = 29, \text{Fig. 3, left})\). There were no failures of somatic spike invasion of the dendrites. This was seen at rates of somatic spiking up to 175 Hz.

When the dendrite was stimulated, dendritic action potentials could be generated independently of the somatic action potentials in 12 of 20 cells tested. Action potentials generated in the dendrites propagated to the soma and appeared as a mixture of subthreshold depolarizations and full-blown action potentials (Fig. 3, right). In 6 of these 12 cells, stimulation of the dendrite resulted in occasional double action potentials in the dendrite: an action potential initiated in the dendrite propagated to the soma, giving rise to a somatic action potential that propagated back, within 1–2 ms, into the dendrite (Fig. 3, *).

In 8 of the 20 cells tested, there were no subthreshold depolarizations in the soma following stimulation of the dendrite: only full-height action potentials were observed in the soma and dendrite. In five of these eight cells, the relative timing of the action potential between the soma and dendrite could be shifted, as discussed below. In these cases, action potentials were likely initiated in the dendrite, faithfully propagated to the soma and always initiated a spike there. In the other three cells, the depolarization in the dendrite apparently propagated electrotonically to the soma and there triggered an

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**FIG. 2.** Dual whole cell access to a ganglion cell. One electrode recorded responses from the dendrite, in this case 145 \(\mu\)m from the soma. A 2nd electrode, placed at the soma, contained Lucifer yellow to fluorescently label the cell. Here, the brightfield image is superimposed on the fluorescent image to allow visualization of the dendritic electrode (arrow). Scale bar, 20 \(\mu\)m.

**FIG. 3.** Simultaneous current-clamp recordings from the soma and dendrite of a ganglion cell. A schematic drawing of the recording arrangement is shown on the right. When the soma was stimulated by injecting depolarizing current, action potentials were also evident in the dendrite (left). In 12 of 20 cells, stimulation of the dendrite resulted in a mixture of small depolarizations and action potentials at the soma (right). This suggests that action potentials initiated in the dendrite propagated to the soma, where they either triggered an action potential or failed to sufficiently invade the soma. Inset: responses of the soma and dendrite in more detail. The responses from the dendrite were recorded 110 \(\mu\)m from the soma. The apparent resting potentials of the soma and dendrite were \(-70\) and \(-75\) mV, respectively.
action potential that subsequently propagated actively into the dendrites. This would happen if the threshold for generating an impulse at the dendrite were high compared with the threshold at or near the soma. Alternatively, the site of generation of action potentials in these cases could have been focal and located distal to the electrode.

Figure 4 shows the mean amplitudes of the dendritic spikes observed in the cells as a function of distance of the dendritic electrode from the soma. In addition, the ratio of the soma to dendritic spikes is shown. Although there is substantial variability, neither appears consistent with the large decrement expected were conduction from the soma to the dendrite passive. The data also argue against the possibility that the “dendritic” spikes actually originate in amacrine cells, to which the ganglion cells are coupled by gap junctions (Nedergaard and Hounsgaard 1996; Valiante et al. 1995). In that case, the dendrites should have been largest at the more distal points on the dendrite and decremented more proximally.

To study the timing of somatic and dendritic action potentials, we first had to establish the limits of dual-electrode recording. Two electrodes were placed on the same ganglion cell’s soma. Dual whole cell recordings obtained from the soma had a latency of 300 ± 86 μs (n = 8) between the peaks of the same action potential (generated by injecting current into one soma electrode or the other). If both electrodes recorded in the cell-attached configuration, the latency was 66 ± 22 μs (n = 7). If one electrode was in the whole cell configuration and the other was in the cell-attached configuration, the latency was 352 ± 158 μs (n = 4). The amplitude and latency of the recorded action potentials were sensitive to the properties of the electrode and the amplifiers’ resistance and capacitance compensations. Because of the size and variability of the timing interval, latency could not be used, in these adult cells at 37°C, to judge the location of action-potential initiation. However, the latency was nearly constant (<20 μs variation) for a particular electrode pair.

Because the relative timing of somatic and dendritic action potentials was reproducible for a given electrode pair, we could study the order of firing of dendrite and soma. In 12 of 16 cells tested in this way, depolarizing the soma caused a somatic action potential that preceded the dendritic action potential (Fig. 5, left). When the same cells were stimulated at the dendrite, the dendritic action potential was seen first (Fig. 5, right). This indicates a change in the site of action-potential generation. For the four remaining cases, the action potential occurred first at the soma, and no change in firing order could be induced by switching the location of the stimulus. We propose that in these cases the dendrites did not initiate dendritic action potentials; stimulating the dendrite depolarized the soma electrotonically and caused an action potential that subsequently propagated into the dendrites.

We applied an intracellular sodium channel blocker (QX-314, 5 mM) into the somas of nine cells while recording from a dendrite and the soma. Whole cell recording was established first at the dendrite. Next, an electrode containing QX-314 was placed at the soma, so that we could record the effects of QX-314 as it diffused from the soma to the dendrite. Initially, the soma and dendrite generated corresponding action potentials when the dendrite was depolarized (Fig. 6, left). The first effect of QX-314 was a decrease in the amplitude and an increase in width of the impulse in the soma. After the drug diffused into the soma for 11–56 s, the sodium channels at the soma were blocked. A depolarizing stimulus in the dendrites then induced a series of action potentials that only weakly invaded the soma (Fig. 6, right). The depolarizations recorded at the soma due to these dendritic action potentials were much smaller than those when the sodium blocker was not used, again indicating that dendrites can generate action potentials independently from the soma. After continued diffusion of QX-314, impulses in the dendrites were also blocked. The complete suppression of impulses by QX-314 suggests that calcium action potentials did not occur in the soma or dendrite.

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somewhat unlikely in the present case because the gap junctions between neighboring cells coupled by gap junctions, but this seems to be due to passive dendritic conduction of action potentials from a soma (Spencer and Kandel 1961; reviewed by Stuart et al. 1997). The possibility has been raised that those potentials are indeed action potentials first recorded in the somata of hippocampal pyramidal cells (Magee and Johnston 1995; Markram et al. 1997; Stuart and Sakmann 1994). Perhaps they were seen here because the stimulating electrode was usually located beyond a dendritic branch point, where conduction failures often occur.

The three qualitative findings listed above suggest that dendrites of retinal ganglion cells contain enough Na\(^{+}\) channels to initiate action potentials, at least in response to large depolarizations, but further evidence will be needed before this can be taken as a firm conclusion. The amplitudes of the dendritic action potentials recorded at different distances from the soma (Fig. 4) were variable and can be interpreted as declining with distance from the soma. One possibility is that the amplitudes faithfully reflect the actual amplitudes of spikes along the dendrites. This would imply inhomogeneity of conduction, perhaps due to “hot spots” of Na\(^{+}\) channels along the dendrites (Chen et al. 1997). However, some other explanation would then have to be found for the other data, listed above, suggesting that the dendrites can initiate action potentials.

A more likely explanation is that both the variability and the possible decline with distance are due to the technical difficulties of recording from these cells. Among other things, better seals were probably made near the soma, where the dendrite is thickest, than farther away from it. The spatial distribution of action-potential amplitudes in these dendrites thus should remain an open question.

The preparation should allow this issue to be resolved. It also has certain advantages for the study of other questions of dendritic physiology, mostly originating from the geometry of retinal ganglion cells. The cells lie near the tissue’s surface. Like Purkinje cells, retinal ganglion cells have dendritic trees that are essentially planar. Perhaps most important, the cells branch radially, rather than emitting one or two thick processes from which finer branches emerge some distance away. This means that dendritic branch points of the ganglion cells are easily available for study.

A possible disadvantage of our technique is the use of papain in exposing the cells. A previous report (Hestrin and Korenbrot 1987) has suggested that K\(^{+}\) channels might migrate 5–10 \(\mu \text{m}\) in rod photoreceptors during dissociation of the cells using papain, but it seems unlikely that Na\(^{+}\) channels migrated in our experiments. Na\(^{+}\) channels function normally and are appropriately localized in retinal ganglion cells immediately after complete dissociation using both papain and trypsin (Barres et al. 1988; Kaplan et al. 1997), a more aggressive treatment than the one used here. Even in the unlikely event that the Na\(^{+}\) channels were completely disconnected from the cytoskeleton, a protein this large could hardly diffuse for hundreds of micrometers within the time of our experiments (Kyte 1995). The effects of papain on other channels are less well known and would have to be specifically evaluated in future work.

under our conditions, a conclusion also suggested by the short time course of the action potentials.

**DISCUSSION**

Three of our findings suggest that regenerative currents, which apparently can result in Na\(^{+}\) action potentials, occur in the dendrites of retinal ganglion cells. First, the timing of somatic and dendritic action potentials relative to each other could be shifted depending on whether depolarizing current was injected into the soma or the dendrite. Second, when QX-314 was included in the somatic electrode, action potentials were blocked in the soma before those recorded in the dendrite. Third, injecting depolarizing current into the dendrites sometimes resulted in the appearance in the soma of small depolarizations that appeared to trigger a separate, full-blown somatic spike.

These depolarizations are similar to the controversial prepotentials first recorded in the somata of hippocampal pyramidal neurons (Spencer and Kandel 1961; reviewed by Stuart et al. 1997). The possibility has been raised that those potentials are due to passive dendritic conduction of action potentials from neighboring cells coupled by gap junctions, but this seems somewhat unlikely in the present case because the gap junc-

![Figure 6](image-url)
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