Patch-Clamp Investigations and Compartmental Modeling of Rod Bipolar Axon Terminals in an In Vitro Thin-Slice Preparation of the Mammalian Retina

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Oltedal L, Mørkve SH, Veruki ML, Hartveit E. Patch-clamp investigations and compartmental modeling of rod bipolar axon terminals in an in vitro thin-slice preparation of the mammalian retina. J Neurophysiol 97: 1171–1187, 2007. First published December 13, 2006; doi:10.1152/jn.01010.2006. To extend the usefulness of rod bipolar cells for studies of chemical synaptic transmission, we have performed electrophysiological recordings from rod bipolar axon terminals in an in vitro slice preparation of the rat retina. Whole cell recordings from axon terminals and cell bodies were used to investigate the passive membrane properties of rod bipolar cells and analyzed with a two-compartment equivalent electrical circuit model developed by Mennerick et al. For both terminal- and soma-end recordings, capacitive current decays were well fitted by biexponential functions. Computer simulations of simplified models of rod bipolar cells demonstrated that estimates of the capacitance of the axon terminal compartment can depend critically on the recording location, with terminal-end recordings giving the best estimates. Computer simulations and whole cell recordings demonstrated that terminal-end recordings can yield more accurate estimates of the peak amplitude and kinetic properties of postsynaptic currents generated at the axon terminals due to increased electrotonic filtering of these currents when recorded at the soma. Finally, we present whole cell and outside-out patch recordings from axon terminals with responses evoked by GABA and glycine, spontaneous inhibitory postsynaptic currents, voltage-gated Ca$^{2+}$ currents, and depolarization-evoked reciprocal synaptic responses, verifying that the recorded axon terminals are involved in normal pre- and postsynaptic relationships. These results demonstrate that axon terminals of rod bipolar cells are directly accessible to whole cell and outside-out patch recordings, extending the usefulness of this preparation for detailed studies of pre- and postsynaptic mechanisms of synaptic transmission in the CNS.

INTRODUCTION

Chemical synaptic transmission is arguably the most important mode of communication between neurons in the CNS. Our knowledge of the fundamental mechanisms of synaptic transmission is, to a large extent, based on information obtained from a relatively small number of experimental model systems with the neuromuscular junction being the classically studied system (Kushmerick and von Gersdorff 2003). During the last 20 yr, model systems for investigating synaptic transmission at the same level of mechanistic detail in the CNS have been developed. Although a model system should ideally be accessible to electrophysiological investigations both at the pre- and postsynaptic level, several systems have been limited to investigations at the postsynaptic side. Over the past decade, a few model systems with large (or even giant) presynaptic terminals have become accessible to direct electrophysiological investigations in situ, such as combined pre- and postsynaptic recording and measurement of changes in presynaptic membrane capacitance as an index of exo- and endocytosis. These model systems have greatly expanded our understanding of basic mechanisms of synaptic transmission and include hair cells in the inner ear (Moser and Beutner 2000), photoreceptors (Thoreson et al. 2004) and isolated bipolar cell axon terminals (Palmer et al. 2003) in the retina, presynaptic terminals of basket cells in the cerebellum (Southan and Robertson 1998), the calyx of Held in the auditory brain stem (e.g., Borst and Sakmann 1996; Borst et al. 1995; Forsythe 1994; Sun and Wu 2001; Tasschenberger et al. 2002; Wölfel and Schneggenburger 2003), and mossy fiber terminals in the hippocampus (Geiger and Jonas 2000; Hallermann et al. 2003). Most importantly, for the calyx of Held synapse, it is possible to combine pre- and postsynaptic measurements. It has also been possible to perform dual recordings from pairs of pre- and postsynaptic cells in the retina: the photoreceptor → bipolar cell synapse (DeVries and Schwartz 1999; DeVries et al. 2006; Thoreson et al. 2004) and the rod bipolar cell → AII amacrine cell synapse (Singer and Diamond 2003; Veruki et al. 2003, 2006). By delivering physiological stimuli to the presynaptic cell, one can directly observe the input-output relationship of the synaptic connection.

An important goal is to extend the rod bipolar cell → AII amacrine cell model system by being able to routinely record not only from the soma of the rod bipolar cell but directly from the axon terminal endings. First, such recordings can potentially allow improved voltage clamp of voltage- and ligand-gated ion currents generated at the axon terminal compared with recordings at the cell body. Second, by establishing whole cell recordings at the axon terminal, it should be possible to isolate outside-out patches and thereby directly study ion channels located in the terminal. Third, it might be possible to perform time-resolved capacitance measurements of exocytosis (Hallermann et al. 2003; Mennerick et al. 1997; Palmer et al. 2003; Pan et al. 2001; Zhou et al. 2006). Recordings from axon terminals of acutely isolated rod bipolar cells in culture have been reported (Pan 2001; Pan et al. 2001; Zhou et al. 2006), but recordings from axon terminals of rod bipolar cells in situ have only been reported in one previous study with an apparently low success rate (Protti and Llano 1998).
Here we report detailed methods and results from electrophysiological recordings from axon terminals of rod bipolar cells in situ using an in vitro thin slice preparation of the rat retina. These recordings, together with recordings from the soma of rod bipolar cells, were used to investigate the passive membrane properties of rod bipolar cells and analyzed with a two-compartment model of bipolar cells (Mennerick et al. 1997). The results were compared with results obtained from computer simulations of idealized models of rod bipolar cells to systematically investigate the influence of recording location and series resistance on estimates of model parameters and synaptic current kinetics. We also present physiological recordings from rod bipolar axon terminals that verify the integrity of the synaptic circuits in which they are involved. Finally, we present recordings of transmitter-evoked responses that verify the feasibility of isolating outside-out patches from rod bipolar axon terminals.

METHODS

Preparation of slices

General aspects of the methods have previously been described in detail (Hartveit 1996; Veruki et al. 2003). Albino rats (4–7 wk postnatal) were deeply anesthetized with halothane in oxygen and killed by cervical dislocation (procedure approved under the surveillance of the Norwegian Animal Research Authority). Retinal slices (working distance: 1.9 mm; Zeiss) for recordings from rod bipolar cell somata, we used a • VOL 97 • FEBRUARY 2007 • www.jn.org

> 125 NaCl, 8 NaCl, 10 HEPES, 1 CaCl2, 5 EGTA, 15 TEA-Cl, and 4 MgATP. For some whole cell recordings we replaced KCl by 20 mM NaCl with the equivalent amount of tetraethylammonium chloride (TEA-Cl) and increased the concentration of CaCl2 to 5 mM. For whole cell recordings from cell bodies of rod bipolar cells, recording pipettes were filled with solution A, which contained (in mM): 125 CsCl, 8 NaCl, 10 HEPES, 1 CaCl2, 5 EGTA, 15 TEA-Cl, and 4 MgATP. For some whole cell recordings of synaptic currents (from cell bodies or axon terminals), recording pipettes were filled with solution C, which contained (in mM): 130 KCl, 8 NaCl, 10 HEPES, 1 CaCl2, 5 EGTA, and 4 MgATP. For all intracellular solutions, pH was adjusted to 7.3 (with CsOH or KOH) and Lucifer yellow (1 mg/ml) was added for visualization of cells with fluorescence microscopy after the electrophysiological recording. Cells were not exposed to UV-light during recording. All recordings were performed at room temperature (20–25°C).

Electrophysiological recording and data acquisition

Patch pipettes were pulled from borosilicate glass (GC150-11; Harvard Apparatus, Edenbridge, UK) on a two-stage vertical puller (PP-83, Narishige, Japan) and heat-polished before use. For cell body recordings, and some axon terminal recordings, electrodes were coated with Dental wax (Kerr’s sticky wax) to reduce their capacitance. Pipettes for soma recordings had resistances of 5–6.5 MΩ (when filled with intracellular solution A). Pipettes for axon terminal recordings had resistances of 6.5–8.5 MΩ (when filled with intracellular solution B). When establishing outside-out patch recordings, the pressure applied to the recording pipettes was continuously monitored with a Manual-Seal-Sucker (Sigmam Elektronik, Hüttenhardt, Germany). Theoretical liquid junction potentials of extracellular solutions against internal solutions were calculated with JCalcW (Molecular Devices, Sunnyvale, CA). Holding potentials were automatically corrected for the liquid junction potentials on-line. Liquid junction potentials were +3.9, +3.3, and +3.4 mV for solutions A–C, respectively.

Voltage-clamp recordings were made with EPC9-dual amplifiers (HEKA Elektronik, Lambrecht, Germany) controlled by Pulse software (HEKA Elektronik). Cells and patches were generally held at a membrane potential of ~−60 mV. Application of voltage protocols and digital sampling of the analog signals were performed via an ITC-16 interface (Instrutech, Port Washington, NY) built into the amplifier. Before sampling, signals were low-pass filtered (analog 3- and 4-pole Bessel filters in series) with a corner frequency (~3 dB) automatically adjusted to 1/5–1/3 of the inverse of the sampling interval (20–250 µs depending on the protocol). Currents caused by the residual recording pipette capacitance (Cfass) and the cell membrane capacitance (Cmslow) were measured with the automatic capacitance neutralization network feature of the EPC9 amplifiers that also provided on-line estimates of the series resistance (Rseries). Compared with our off-line estimates, the EPC9 typically overestimated Rseries, most notably for terminal-end recordings. For measurements of capacitive transients, the Cmslow capacitance neutralization circuitry of the amplifier was transiently disabled and the time constant of the internal stimulus filter was reduced from 20 to 2 µs. These responses were acquired with a sampling interval of 10 µs (low-pass filter set to 30 kHz). For a few axon terminal recordings, the sampling interval was reduced to 5 µs (low-pass filter set to 100 kHz).

General data analysis

Data were analyzed with FitMaster, PulseFit and PulseTools (HEKA Elektronik), Igor Pro (WaveMetrics, Lake Oswego, OR), and AxoGraph (Molecular Devices). Capacitive transients were analyzed off-line by averaging consecutive responses (typically 20–60) and fitting the decay with exponential functions after baseline zero subtraction. For monoeponential functions we used the function

\[ I(t) = A \exp(-t/\tau) + I_{\text{ss}} \]

(1)

where \( I(t) \) is the current as a function of time, \( A \) is the amplitude, \( \tau \) is the time constant, and \( I_{\text{ss}} \) is the steady-state current amplitude. The Drugs were either added directly to the extracellular solution used to perfuse the slices or were locally applied by pressure from a multi-barreled pipette complex. The concentrations of drugs added to the perfusing solution were as follows (µM; supplied by Tocris Bioscience, Avonmouth, UK, unless otherwise noted): 10 bicuculline methochloride, 6-cyanocinnoline-2,3-dione (CNQX), 1 strychnine (Research Biochemicals, Natick, MA), 50 (1,2,5,6-tetrahydro-2H-pyridin-4-yl)methylphosphinic acid (TPMPA), 50 DM-threo-β-benzoyloxyaspartic acid (TBOA). The concentrations (nominal) of drugs added by pressure application were as follows (in µM): 200 γ-aminobutyric acid (GABA; Sigma), 200 glycine (May and Baker, Dagenham, UK). Solutions were either made up freshly for each experiment or were prepared from concentrated aliquots stored at ~−20°C.
sum of $A$ and $I_{ss}$ represents $I(t)$ at time 0 (the instant of the voltage change). For biexponential functions we used the function

$$I(t) = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2) + I_{ss},$$

(2)

where $I(t)$ is the current as a function of time, $A_1$ and $A_2$ are the amplitudes of the first and second exponential components, $\tau_1$ and $\tau_2$ are the time constants of the first (fast) and second (slow) exponential components. The sum of $A_1$, $A_2$, and $I_{ss}$ represents $I(t)$ at time 0. For triexponential fits, a third exponential term was added. Fitting was started 30–60 $\mu$s after onset of the voltage step to reduce contamination of the fit by input and output filtering (Mennerick et al. 1997). For simulated data, fitting was started 50 $\mu$s after the voltage step, except where otherwise stated.

For estimating the circuit parameters of a two-compartment electrical equivalent circuit (results; Fig. 4A), we followed the approach of Mennerick et al. (1997), including the assumption of infinite membrane resistance, and used the following equations developed in their study

$$R_1 = \frac{\Delta V}{A_1 + A_2},$$

(3)

$$R_2 = \frac{(A_2 \tau_1 + A_1 \tau_2) \Delta V}{A_2 \tau_1 (A_1 + A_2) (\tau_1 - \tau_2)}$$

(4)

$$C_1 = \frac{(A_1 + A_2)^2 \tau_1 \tau_2}{(A_2 \tau_1 + A_1 \tau_2) \Delta V}$$

(5)

$$C_2 = \frac{A_1 A_2 (\tau_1 - \tau_2)^2}{(A_2 \tau_1 + A_1 \tau_2) \Delta V}$$

(6)

Spontaneous postsynaptic currents (PSCs) were detected with a threshold of 5 pA (MiniAnalysis; Synaptosoft, Decatur, GA) and verified by eye. For calculating event ensemble averages, events were aligned by the point in time in which each event reached 50% of the peak amplitude. Only well-separated (inter-event intervals $\geq 50$ ms), monophasic PSCs that appeared to rise in a monotonic fashion without visible deviation of the rising phase and that appeared to decay with an exponential time course were included in the ensemble averages. Any events with 10–90% rise time $\geq 2$ ms were also excluded from the ensemble averages.

For analysis of current-voltage ($I$-$V$) relationships evoked by ramp depolarizations, the linear leak current was subtracted by fitting a line to the initial linear segment of the current trace (typically from 0 to 0.3 s). After extrapolation, the linear curve was subtracted from the raw current trace, and the difference current was plotted against the corresponding voltage for each point in time.

Data are presented as means $\pm$ SD ($n = number$ of cells), and percentages are presented as percentage of control. Statistical analyses were performed using Student’s two-tailed t-test (unpaired), and differences were considered statistically significant at the $P < 0.05$ level. For illustration purposes, data traces from physiological recordings were typically low-pass filtered (digital nonlagging Gaussian filter; $-3$ dB at 0.5–2 kHz). Data records displaying capacitive transients are displayed without additional off-line filtering. Unless otherwise noted, current responses in figures represent single traces.

**Computer simulations**

Computer simulations were performed with NEURON (version 5.8) running under Mac OS X (10.4) (Carnevale and Hines 2006; Hines and Carnevale 1997). We constructed idealized morphological models of rod bipolar cells based on published morphological data, including values for the length and diameter of cell bodies, axons, and axon terminal systems (see RESULTS). All simulations were run with a time step of 1 $\mu$s. For analysis, data were decimated to give a sampling interval of 5 $\mu$s. A single electrode voltage clamp with user-specified series resistance was connected to either the soma or the terminal compartment. Before each simulation run, the model was initialized to steady state. For measurement of charging transients, 10-mV hyperpolarizing pulses were applied from a holding potential of $-60$ mV. For our default model of a rod bipolar cell (see RESULTS), the cytoplasmic (internal) resistivity ($R_i$) was set to 160 $\Omega$cm, the specific membrane capacitance ($C_m$) was set to the standard value of 1 $\mu$F/cm$^2$, and the specific membrane resistance ($R_m$) was set to 14 $k\Omega$cm$^2$, corresponding to a specific membrane conductance ($G_m$) of $7.14 \times 10^{-5}$ S/cm$^2$ (Mennerick et al. 1997).

Synaptic conductance waveforms injected into the simulated rod bipolar cell model were calculated according to the equation

$$G(t) = A \exp(-t/\tau) \quad for \quad t \geq \delta$$

$$G(t) = 0 \quad for \quad t < \delta$$

(7)

where $A$ is the peak amplitude at onset, $\tau$ is the decay time constant and $\delta$ is the delay to onset.

**Results**

**Targeting and identification of rod bipolar cells in retinal slices**

Cell bodies from rod bipolar cells were targeted according to their relatively large size (cf. Hartveit 1996, 1999) and typical location in the distal part of the inner nuclear layer, immediately apposed to the outer plexiform layer (Fig. 1, A and B). Axon terminals of rod bipolar cells were targeted according to the large size of their round or pear-shaped knob-like terminal swellings in stratum 5 of the inner plexiform layer (Fig. 1, C and D). All cells were filled with Lucifer yellow, and fluorescence microscopy allowed visualization of each cell’s complete morphology at the end of the recording. This included dendrites ascending from the cell body into the outer plexiform layer and an axon descending through the inner plexiform layer with one or more characteristic large, knob-like swellings at the terminal in the proximal part of the inner plexiform layer (Fig. 1, A and B).

**Establishing $\Omega$-seals and whole cell recordings from cell bodies and axon terminals of rod bipolar cells**

For recordings from rod bipolar cell bodies, we established $\Omega$-seals in the conventional way according to published procedures (Hamill et al. 1981; Sakmann and Stuart 1995; Stuart et al. 1993). No extra cleaning of cell surfaces was attempted, apart from the action of the stream of fluid coming from the pipette tip after applying positive pressure before entering the bath (25–40 mbar) (cf. Stuart et al. 1993). Seal resistances in the $\Omega$ range ($\geq 10 \Omega$) for electrodes with heat polishing) between the electrode and cell membrane were obtained by gently positioning the electrode tip onto the cell, releasing positive pressure and applying gentle suction by mouth ($\sim 10$ to $\sim 30$ mbar). The whole cell configuration was established manually with suction by mouth ($\sim 80$ to $\sim 120$ mbar) in combination with brief voltage transients applied to the electrode (“zap,” typically $+400$ mV for 400 $\mu$s).

For recordings from rod bipolar cell axon terminals, we found that using a $\times 60$ objective with high numerical aperture greatly increased the rate of successful recordings (see METHODS). We regularly used lightly fire-polished pipettes and in our experience this is critical for obtaining high-quality $\Omega$ seals. Pipette resistances from $\sim 6.5$ to $\sim 8.5$ $M\Omega$ (with the standard
and D swelling as visualized with IR-DIC videomicroscopy (Fig. 1, sponds to a tip diameter almost as large as an axon terminal series resistance. A pipette resistance of this magnitude corre-
in the inner plexiform layer. Scale bar: 5
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infrared videomicrograph of axon terminal swelling (Fig. 1, C)
ner layers (ONL, outer nuclear layer; GCL, ganglion cell layer) and the borders between them are indicated by horizontal lines. Scale bar: 10
m. (10- to 15-ms duration). Figure 2A shows an example of the current transients evoked in a soma-end recording of a rod bipolar cell. We fitted the current relaxation from the initial peak to the end of the voltage pulse with either a mono- or biexponential function. The relaxation was well fitted by a biexponential function, whereas the fit obtained by a monoexponential function was clearly inadequate (Fig. 2B) (Mennerick et al. 1997). The difference between the bi- and monoexponential fits is illustrated by the curve fit residuals (Fig. 2B). Adding a third exponential component only marginally improved the fit (not shown). For soma-end recordings, we obtained a fast time constant ($t_{fast}$) of $39 \pm 12 \mu s$ ($n = 22$) and a slow time constant ($t_{slow}$) of $349 \pm 164 \mu s$ (Table 1). The
intracellular solutions) gave the best results with respect to successfully forming a GΩ seal and obtaining an acceptable series resistance. A pipette resistance of this magnitude corresponds to a tip diameter almost as large as an axon terminal swelling as visualized with IR-DIC videomicroscopy (Fig. 1, C and D). Monitoring the manually applied pressure in the recording pipette was very useful feedback and aided reproducibility of the pressure applied. We approached the targeted axon terminal with a slight positive pressure (5–15 mbar) in the recording pipette. When the pipette tip made contact with the terminal, we formed a GΩ seal by removing the pressure, assisted by gentle manual suction (–10 to –20 mbar). With this approach, we have regularly been able to establish GΩ seals with resistances $\geq 10$ GΩ. In our experience, the most challenging part of recording from rod bipolar cell axon terminals is to successfully establish the whole cell recording configuration after GΩ-seal formation. If too much suction is applied to a terminal in the cell-attached mode, it will be sucked up into the recording pipette. We have found that a combination of brief positive pressure pulses (40–80 mbar; applied by mouth) and brief depolarizing pulses (zap; +500 mV for 100 μs) gives the highest success rate in breaking through the membrane. One aspect of terminal-end recordings that we have not been able to control is an increase in the series resistance over the first 2–3 min after establishing the whole cell recording configuration.

Current transients in soma- and terminal-end recordings

We examined the passive membrane properties of rod bipolar cells by applying 10-mV hyperpolarizing voltage pulses (10- to 15-ms duration). Figure 2A shows an example of the current transients evoked in a soma-end recording of a rod bipolar cell. We fitted the current relaxation from the initial peak to the end of the voltage pulse with either a mono- or biexponential function. The relaxation was well fitted by a biexponential function, whereas the fit obtained by a monoexponential function was clearly inadequate (Fig. 2B) (Mennerick et al. 1997). The difference between the bi- and monoexponential fits is illustrated by the curve fit residuals (Fig. 2B). Adding a third exponential component only marginally improved the fit (not shown). For soma-end recordings, we obtained a fast time constant ($t_{fast}$) of $39 \pm 12 \mu s$ ($n = 22$) and a slow time constant ($t_{slow}$) of $349 \pm 164 \mu s$ (Table 1). The

![FIG. 1. Rod bipolar cells recorded with patch pipettes at the soma (A) or axon terminal (B–D). A: composite fluorescence photomicrograph of cell filled with Lucifer yellow from a recording pipette at the cell body (image overlaid on an infrared videomicrograph showing the position of the cell in the retinal slice). The photomicrograph was generated by scanning and assembling a series of negatives taken with epifluorescent illumination at different focal planes. The retinal layers are indicated by abbreviations (ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer) and the borders between them are indicated by horizontal lines. Scale bar: 10 μm. B: composite fluorescence videomicrograph of a rod bipolar cell filled with Lucifer yellow from a recording pipette at the axon terminal, overlaid on an infrared videomicrograph showing the position of the cell in the retinal slice. Scale bar: 10 μm. C: infrared videomicrograph of axon terminal swelling ($\rightarrow$) of a rod bipolar cell in the inner plexiform layer. Scale bar: 5 μm (C and D). D: infrared videomicrograph of same axon terminal swelling as in C (at a slightly different focal plane), tip of patch pipette can be seen approaching the cell before establishing the cell-attached configuration.](http://jn.physiology.org/)

![FIG. 2. Current transients in soma- and terminal-end recordings of rod bipolar cells in situ. A: current response (bottom; average of 20 trials) of rod bipolar cell to 10-mV hyperpolarizing voltage pulse (10 ms; top) applied in soma-end whole cell recording. Diagram (left) indicates recording configuration. B: same response as in A, time scale expanded to display onset and initial decay of current transient ($\rightarrow$) at higher temporal resolution with overlaid biexponential fit to decay phase ($\rightarrow$). Top: corresponding part of voltage stimulus. Bottom: curve fit residuals for monoexponential and biexponential fits. Notice nonrandom deviation of residual after monoexponential fit. C: current response (bottom; average of 20 trials) of rod bipolar cell to 10-mV hyperpolarizing voltage pulse (15 ms; top) applied in terminal-end whole cell recording. Diagram (left) indicates recording configuration. D: same response as in C, time scale expanded to display onset and initial decay of current transient ($\rightarrow$) at higher temporal resolution with overlaid biexponential fit to decay phase ($\rightarrow$). Top: corresponding part of voltage stimulus. Bottom: curve fit residuals for mono- and biexponential fits. Notice nonrandom deviation of residual after monoexponential fit.](http://jn.physiology.org/)
amplitude contribution of the fast component was 91 \pm 5\%.

The input resistance, calculated from the steady-state current component of the fitted curve and the amplitude of the hyperpolarizing voltage pulse, was 3.4 \pm 1.7 \ G\Omega.

Figure 2C shows an example of the current transients evoked in a terminal-end recording of another rod bipolar cell by applying 10-mV hyperpolarizing voltage pulses. As for the soma-end recordings, the relaxation of the evoked current transients was best fitted by a biexponential function (Fig. 2D) (Mennerick et al. 1997; Palmer et al. 2003), with a polarizing voltage pulse, was 3.4

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Figure 2C shows an example of the current transients evoked in a terminal-end recording of another rod bipolar cell by applying 10-mV hyperpolarizing voltage pulses. As for the soma-end recordings, the relaxation of the evoked current transients was best fitted by a biexponential function (Fig. 2D) (Mennerick et al. 1997; Palmer et al. 2003), with a polarizing voltage pulse, was 3.4 \pm 1.7 \ G\Omega.
approach of Mennerick et al. (1997) and used the equations derived from the two-compartment circuit to calculate circuit parameters for both soma- and terminal-end recordings from rod bipolar cells in situ. We then performed computer simulations with idealized models of rod bipolar cells and subjected them to the same voltage protocols as the physiologically tested cells.

Using Eqs. 3–6 to analyze the current transients obtained in our soma-end recordings, the capacitance of C1 was estimated to be $3.4 \pm 0.6 \, \text{pF}$ and the capacitance of C2 was estimated to be $1.6 \pm 0.2 \, \text{pF}$ ($n = 22$; Table 2). The resistance of R1 (series resistance) was estimated to be $12.7 \pm 4.4 \, \text{M} \Omega$, and the resistance of R2 (axonal resistance) was estimated to be $208 \pm 96 \, \text{M} \Omega$. C1 was larger than C2, suggesting that for soma-end recordings, C1 corresponds to the soma/dendritic compartment and C2 corresponds to the axon terminal compartment. When we used the same equations to analyze terminal-end recordings, the capacitance of C1 was estimated to be $1.5 \pm 0.2 \, \text{pF}$ and the capacitance of C2 was estimated to be $4.3 \pm 0.2 \, \text{pF}$ ($n = 18$). The resistance of R1 (series resistance) was estimated to be $44 \pm 22 \, \text{M} \Omega$ and the resistance of R2 (axonal resistance) was estimated to be $185 \pm 51 \, \text{M} \Omega$. C1 was smaller than C2, suggesting that for terminal-end recordings C1 corresponds to the axon terminal compartment and C2 corresponds to the soma/dendritic compartment. These results indicate that the estimate for the capacitance of the soma/dendritic compartment (C1 in soma-end recordings, C2 in terminal-end recordings) is larger for terminal-end recordings than for soma-end recordings ($P < 0.0001$). It is likely, as suggested by Mennerick et al. (1997), that this is due to a selection bias with respect to the cells recorded. On average, the terminals that are targeted and successfully recorded from are expected to be larger (with correspondingly larger membrane capacitance) than terminals not targeted or not successfully recorded from, and it is reasonable to assume that larger terminals belong to larger cells. The estimate of R1 (series resistance) was lower for soma- than terminal-end recordings. The difference in resistance between the electrodes used for soma- and terminal-end recordings (see METHODS) did not seem large enough to account for this difference. Instead we speculated that the restricted range of curve fitting (starting 30–60 $\mu$s after the onset of the voltage pulse), imposed by residual input and output filtering, was insufficient to adequately resolve the fast initial decay of the current transient in the terminal-end recordings. Even with coated pipettes and the low-pass filter set to 100 kHz (sampling interval 5 $\mu$s), curve fitting could not start earlier than 30 $\mu$s after onset of the voltage pulse (as judged by eye). When we analyzed simulated responses generated with a computer model of a rod bipolar cell, we verified that the restricted range of curve fitting can lead to overestimation of series resistance (see following text). In contrast to Mennerick et al. (1997), we found no difference between the estimates of the capacitance of the axon terminal compartment in the two recording configurations ($P = 0.16$).

When we applied the two-compartment model and Eqs. 3–6 to analyze the current transients obtained from recordings of isolated cell bodies and axon terminals (after fitting with biexponential functions; see preceding text), we obtained capacitance estimates for C1 of $3.0 \pm 0.6$ and $0.94 \pm 0.55 \, \text{pF}$, respectively (Table 2). For both types of recordings, the estimate for a given compartment (soma or terminal) was lower compared with the estimate from recordings of intact cells. Similar findings were reported by Mennerick et al. (1997). In our case, two of the three isolated axon terminal compartments and four of the five isolated soma compartments also contained an axon stump of variable length. Because the current relaxations were reasonably well fitted with monoexponential functions (see preceding text), we also estimated the compartment size directly from the time constant of decay, the series resistance, and the input resistance. For isolated cell bodies, the
capacitance was $2.8 \pm 0.7 \text{ pF} \ (n = 5)$, and for isolated axon terminals, the capacitance was $1.1 \pm 0.6 \text{ pF} \ (n = 3)$. These values were not significantly different from the estimates of $C_1$ in the preceding text ($P = 0.66$ and 0.069, respectively).

*Current transients from simulated rod bipolar cells*

In the absence of accurate morphological reconstructions, our simplified models of rod bipolar cells were based on approximate morphological parameters from several different sources, including Lucifer yellow-filled cells (Billups and Attwell 2002; Attwell 1996), electron microscopic images and reconstructions of axon terminals (Chun et al. 1993), and light microscopic and confocal images of immunolabeled cells (Behrens et al. 1998; Chun et al. 1993). The axonal length can vary between ~50 and ~70 nm and the axonal diameter can vary between ~0.5 and ~1 mm. The size of axon terminal swellings was measured as the distance along the longest diameter and perpendicular to this and ranged from $2.7 \times 1.5 \mu m$ (Chun et al. 1993; their Fig. 6) to $10 \times 4 \mu m$ (Behrens et al. 1998; their Fig. 2A). The size of the cell body was measured the same way and ranged from $8 \times 5 \mu m$ (Chun et al. 1993; their Fig. 2) to $13 \times 8 \mu m$ (Attwell 1996; his Fig. 2). When we performed similar measurements on rod bipolar cells filled with Lucifer yellow during terminal-end recording ($n = 7$), the soma was $11.8 \pm 1.8 \times 7.3 \pm 0.6 \mu m$, the length of the axon was $44.8 \pm 2.5 \mu m$, and the terminal bouton attached to the recording pipette was $3.9 \pm 2.2 \times 2.0 \pm 0.8 \mu m$. These seven cells had a total of 19 terminal boutons that measured $2.8 \pm 1.7 \times 1.7 \pm 0.6 \mu m$. Our model cell was also guided by capacitance estimates for isolated rod bipolar cell terminals with small axon stumps [1.8 pF (Pan 2001), 1.46 pF (Pan et al. 2001)]. Based on these observations, we modeled our idealized rod bipolar cell with three cylindrical compartments: soma, axon and axon terminal. Dendrites arising from the soma were not modeled as explicit compartments but were considered as part of the soma. The axonal compartment resulted from collapsing two to six boutons into a single terminal compartment. From an initial set of starting values, the final size of the soma and axon terminal compartments were adjusted by slightly increasing the size of each compartment until the capacitance estimates, based on the two-compartment model, were similar to those obtained from real cells (see Tables 2 and 3). The final model of a rat rod bipolar cell had the following geometry (length × diameter): soma = $11.5 \times 11 \mu m$, axon = $50 \times 0.8 \mu m$, and axon terminal = $7 \times 5 \mu m$. Our model cell had approximately half the surface area of the goldfish bipolar cell in the study of Mennerick et al. (1997) and ~1.7 times the surface area of the rat bipolar cell analyzed by Billups and Attwell (2002). The input resistance of the model cell was 2.2 GΩ, in the lower range of values measured for our biological cells.

We first used our idealized model of a rod bipolar cell to simulate current transients evoked by 10-mV hyperpolarizing voltage pulses. For these simulations, the series resistance was set to 20 MΩ for both soma- and terminal-end recordings. We

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**Fig. 4.** Two-compartment electrical equivalent circuit model used to analyze passive membrane properties of physiological and simulated rod bipolar cells. *A*: schematic view of 2-compartment electrical equivalent circuit (adapted from Mennerick et al. 1997). R1, resistance connecting recording pipette and the proximal capacitive compartment; C1, proximal capacitive compartment. C2, distal capacitive compartment; R2, resistance between the 2 capacitive compartments (corresponding to axonal resistance). *B*: current response (*→*) of idealized model of rod bipolar cell to 10-mV hyperpolarizing voltage step (*top*) applied in soma-end recording, overlaid biexponential fit to decay phase (*→*). Bottom: curve fit residuals for mono- and biexponential fits, amplitude-scale expanded for *bottom trace* to display nonrandom deviation of residual after biexponential fit. Diagram (*right*) shows recording configuration. *C*: current response (*→*) of idealized model of rod bipolar cell to 10-mV hyperpolarizing voltage step (*top*) applied in terminal-end recording, overlaid biexponential fit to decay phase (*→*). Bottom: curve fit residuals for mono- and biexponential fits, amplitude-scale expanded for *bottom trace* to display nonrandom deviation of residual after biexponential fit. Diagram (*right*) shows recording configuration.
analyzed the resulting current transients in the same way as those from biological cells. Irrespective of whether the recording pipette was positioned at the soma or the axon terminal, the decay of the current transients was well fitted by biexponential functions (Fig. 4, B and C). The residual plots in Fig. 4, B and C, display nonrandom deviations for both mono- and biexponential fits. For the biexponential fit, the deviation is small enough that it undoubtedly would have been masked by recording noise from a biological cell. The imperfect fit with the biexponential function is probably due to the nonzero capacitance of the axonal compartment (cf. Mennerick et al. 1997). Estimates for C1 and C2 corresponded reasonably well to the theoretical (simulation input) values (Table 3). For soma- and terminal-end simulations, we obtained an axial resistance ($R_2$) of 128 and 152 MΩ, respectively (Table 3). These estimates were close to the theoretical value of $r_i$, which we calculated to be 159 MΩ according to the following equation: $r_i = (R/R_i) A/L$ ($R_i$ is the cytoplasmic resistance, 160 Ωcm; $A$ is the cross-sectional area of the axon, $5.03 \times 10^{-9}$ cm$^2$; $L$ is the length of the axon, 50 μm). We next verified that our model cell gave rise to curve-fitting parameters similar to those obtained in the physiological recordings. As detailed in Table 1, this was the case for both soma- and terminal-end recordings when the series resistance was set to 10 and 40 MΩ, respectively.

**Effect of series resistance on capacitance estimates**

Both the soma and the axon terminal of rat rod bipolar cells are smaller than those of goldfish bipolar cells (Mennerick et al. 1997), and it can be difficult to obtain recordings with very low series resistance, particularly from the axon terminals. We therefore examined the effect of varying series resistance on estimates obtained from current transients analyzed with the two-compartment equivalent circuit model. The series resistance of the recording electrode was varied between 1 MΩ and 1 GΩ, and for each condition, we simulated responses evoked by both soma- and terminal-end recordings. The capacitance of each compartment (C1 and C2), the axonal resistance ($R_2$), and the series resistance ($R_1$) were estimated after fitting the decays of current transients with biexponential functions. Based on the surface area of each compartment and a specific membrane capacitance of 1 μF/cm$^2$, the soma compartment had a capacitance of 4.0 pF, the axon terminal compartment had a capacitance of 1.1 pF, and the axon compartment had a capacitance of 1.3 pF. For the soma compartment, capacitance estimates from soma- and terminal-end recordings followed each other closely for series resistance values between ~4 MΩ and 1 GΩ (Fig. 5A). Between 4 and 160 MΩ, the estimates were within 95–107% of the theoretical value, irrespective of whether the recording electrode was located at the soma or the axon terminal (Fig. 5A). At high values of series resistance, the capacitance estimates dropped below the theoretical value (to ~50% at a series resistance of ~1 GΩ). Estimates of the soma capacitance from terminal-end recordings remained close to the theoretical value for low values of series resistance (1–3 MΩ), whereas they were below the theoretical value for soma-end recordings and dropped to <1 pF at a series resistance of 1 MΩ (Fig. 5A). We suspected that the errors for soma-end recordings with low series resistance were caused by the restricted range of curve fitting (starting 50 μs after onset of the voltage pulse for simulated data). Indeed, when we calculated the soma capacitance by starting curve fitting at the first sampling point after onset of the voltage step, the error was eliminated for soma-end recordings (Fig. 5A; full fit).

For the axon terminal compartment, capacitance estimates differed between soma- and terminal-end recordings for all values of series resistance tested, but both recording configurations tended to overestimate the capacitance (Fig. 5B). For soma-end recordings, the capacitance of the axon terminal was overestimated except at the highest values of series resistance tested. For terminal-end recordings, the capacitance was overestimated for series resistances between ~10 and ~400 MΩ.

### Table 2. Parameter estimates of rod bipolar cells analyzed with 2-compartment equivalent circuit model

<table>
<thead>
<tr>
<th>Emotional recordings</th>
<th>R1, MΩ</th>
<th>R2, MΩ</th>
<th>C1, pF</th>
<th>C2, pF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soma-end recordings (n=22)</td>
<td>12.7 ± 4.4</td>
<td>208 ± 96</td>
<td>3.4 ± 0.6</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>Terminal-end recordings (n=18)</td>
<td>44 ± 22</td>
<td>185 ± 51</td>
<td>1.5 ± 0.2</td>
<td>4.3 ± 0.2</td>
</tr>
<tr>
<td>Isolated soma recordings (n=5)</td>
<td>13 ± 4</td>
<td>600 ± 260</td>
<td>3.0 ± 0.6</td>
<td>0.46 ± 0.21</td>
</tr>
<tr>
<td>Isolated terminal recordings (n=3)</td>
<td>122 ± 70</td>
<td>2516 ± 1286</td>
<td>0.94 ± 0.55</td>
<td>0.32 ± 0.075</td>
</tr>
</tbody>
</table>

**Table 3. Computer model of rod bipolar cells**

<table>
<thead>
<tr>
<th>Compartment</th>
<th>$L$, μm</th>
<th>$D$, μm</th>
<th>Surface Area, μm$^2$</th>
<th>Capacitance, pF</th>
<th>$R_w$, GΩ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soma</td>
<td>11.5</td>
<td>11</td>
<td>397.4</td>
<td>3.97</td>
<td>3.5</td>
</tr>
<tr>
<td>Axon</td>
<td>50</td>
<td>0.8</td>
<td>125.7</td>
<td>1.26</td>
<td>11.1</td>
</tr>
<tr>
<td>Terminal</td>
<td>7</td>
<td>5</td>
<td>110.9</td>
<td>1.10</td>
<td>12.7</td>
</tr>
<tr>
<td>Sum</td>
<td>653</td>
<td>6.33</td>
<td>653</td>
<td>6.33</td>
<td>2.2</td>
</tr>
</tbody>
</table>

Model parameters and theoretical (directly calculated) values for the default rod bipolar cell (top). Estimates of equivalent electrical circuit parameters using a two-compartment model (see Fig. 4A; bottom). $L$, length; $D$, diameter, $R_w$, input resistance.
and in this range, the estimate was within 105–135% of the theoretical value. For lower (<10 MΩ) and higher (>400 MΩ) values of series resistance, the capacitance was underestimated compared with the theoretical value (Fig. 5B). When we calculated the terminal capacitance by starting curve fitting at the first sampling point after onset of the voltage step, the error at low values of series resistance was strongly reduced, but not completely eliminated (Fig. 5B; full fit), suggesting that even when there is no input or output filtering of the signal, a sampling interval of 5 μs can be insufficient to adequately capture a very fast initial decay of the current transient. These results indicate that whereas estimates of the soma compartment capacitance are relatively independent of the recording configuration, estimates of the axon terminal compartment capacitance can depend critically on the recording configuration with terminal recordings giving the best estimates overall.

We next considered the potential influence of series resistance on the estimates obtained from physiological recordings of rod bipolar cells based on analysis with the two-compartment model. We corrected each estimated value with respect to the series resistance calculated for the same recording. For soma-end recordings, the corrected capacitance was 3.2 ± 0.6 pF for the soma compartment and 0.92 ± 0.14 pF for the terminal compartment (n = 22). For terminal-end recordings, the corrected capacitance was 4.1 ± 0.2 pF for the soma compartment and 1.1 ± 0.2 pF for the terminal compartment (n = 18). These results support the hypothesis that there was a selection bias with respect to the cells targeted for terminal recording. In terminal-end recordings, the capacitance of the soma compartment and the terminal compartment was, on average, ~1.3 and 1.2 times larger than in soma-end recordings, respectively (P < 0.0001).

**Effect of series resistance on estimates of R1 and R2**

The time constant of decay of the capacitive charging transient of a single (RC) compartment is given approximately by the product of the series resistance and the membrane capacitance (τ = R × Cm). Thus for very small compartment sizes or for very small series resistances, the decay can become so fast that it cannot be adequately resolved because exponential curve fitting is limited by residual input and output filtering. To explore this for our rod bipolar model, we varied the series resistance between 1 MΩ and 1 GΩ and estimated R1 according to Eq. 3 for the two-compartment model. For terminal-end recordings, R1 was increasingly overestimated when the theoretical value for series resistance was less than ~20 MΩ (Fig. 6A). For soma-end recordings, R1 was increasingly overestimated when the theoretical value for series resistance was less than ~4 MΩ, but the error was generally smaller than for terminal-end recordings (Fig. 6A). As for the capacitance estimates (see preceding text), we suspected that the errors were caused by the restricted range of curve fitting (starting 50 μs after onset of the voltage pulse for simulated data). When we calculated the series resistance by starting curve fitting at the first sampling point after onset of the voltage step, the error was eliminated for soma-end recordings (Fig. 6A). For terminal-end recordings, the error was strongly reduced but not completely eliminated (Fig. 6A). When we reduced the size of the terminal compartment from 1.1 pF (5 × 7 μm) to 0.38 pF (3 × 4 μm), the overestimation of R1 for terminal-end recordings occurred for theoretical values of series resistance less than ~50 MΩ. The degree of overestimation increased and occurred for even higher theoretical values of series resistance when we removed the terminal compartment altogether (Fig. 6B). The degree of overestimation of R1 increased further when we in addition reduced the diameter of the axon (Fig. 6B).

We next examined the effect of varying the series resistance on the estimate of the axonal resistance (R2) of the two-compartment model. For soma-end recordings, R2 was underestimated when the series resistance was <320 MΩ (Fig. 6C). For terminal-end recordings, R2 was underestimated when the series resistance was <80 MΩ (Fig. 6C). In the range of series resistance values between 10 and 160 MΩ, the estimates from terminal-end recordings were closer to the theoretical value than the estimates from soma-end recordings (Fig. 6C).
Effect of terminal size and axon diameter on estimates of terminal size

Because cellular morphology is subject to natural variation, we explored the consequences of varying the morphological parameters for the circuit estimates (C1, C2, R1, and R2) obtained by the two-compartment model. We varied the size of the axon terminal, the diameter of the axon, and the cytoplasmic resistivity and estimated axon terminal capacitance for both soma- and terminal-end recordings. To compare the different conditions, we normalized estimates of capacitance to the theoretical value such that values >1 correspond to an overestimation and values <1 correspond to an underestimation. We ran simulations for four different terminal sizes. The maximum axon terminal size was equal to the size of the soma compartment (relative size 1). For this condition, there was no error in the estimate of the terminal size (Fig. 7A). Our default model corresponded to a relative terminal size of 0.28. In this case, the terminal size was overestimated at 1.67 for the soma-end recording and 1.34 for the terminal-end recording (Fig. 7A). We observed that the two-compartment model increasingly overestimated the size of the terminal compartment as its size was reduced. This was the case irrespective of whether the recording pipette was positioned on the soma or the terminal, but the error was larger for soma-end recordings than for terminal-end recordings (Fig. 7A).

We next reduced the axon diameter by 50% (from 0.8 to 0.4 μm) and ran the same series of simulations. When calculated directly from the model parameters, the reduced axon diameter decreased the value of the capacitance of the axon from 1.3 to 0.63 pF and increased the axial resistance from 159 to 636 MΩ. This condition reduced the overestimation of the size of the terminal, irrespective of whether the recording pipette was positioned on the soma or the terminal (Fig. 7B). For terminal-end recordings, the error became negligible, regardless of the size of the terminal compartment. For soma-end recordings, the error increased when the size of the terminal compartment was reduced (Fig. 7B). For soma-end recordings, the reduction of axon diameter also led to an increasing underestimation of the size of the terminal compartment when it was larger than ~0.5 times the size of the soma compartment (Fig. 7B).

To examine the influence of the axon membrane capacitance on the circuit estimates, we repeated the same series of simulations with the default axon diameter (0.8 μm) but with the specific capacitance of the axon compartment set to 10⁻⁶ μF/cm² (the lowest value allowed by the NEURON simulator). This eliminated the overestimation of the terminal capacitance when its size was reduced (Fig. 7C). This condition also reduced the underestimation of the terminal capacitance for soma-end recordings when the terminal compartment was larger than ~0.5 times the size of the soma compartment. Thus the observed errors seem to be a consequence of the fact that the two-compartment model explicitly neglects the capacitance of the axon compartment. Another assumption of the model was that of infinite membrane resistance. Accordingly, we tested the consequences of eliminating not only the capacitance of the axon segment but also the membrane conductance for the whole cell. In this condition, there were virtually no errors.

![Graph A](image1.png)

- terminal-end rec.
- terminal-end rec. (full fit)
- soma-end rec.
- soma-end rec. (full fit)

![Graph B](image2.png)

- terminal = 5 × 7 μm (default)
- terminal = 3 × 4 μm (34%)
- no terminal
- no terminal, axon diam. = 50%

![Graph C](image3.png)

- terminal-end rec.
- soma-end rec.
- R2_theory

**Fig. 6.** Effect of series resistance on resistance estimates with 2-compartment model. A: plot of estimated resistance R1 (R1_est) vs. theoretical value of series resistance for both terminal-end (——) and soma-end (—) recordings of simulated model of rod bipolar cell. Resistive circuit parameters were analyzed with 2-compartment model from hyperpolarization-evoked current transients and curve fitting starting 50 μs after the onset of the voltage pulse (A–C). The panel also shows values for R1 calculated with curve fitting starting at the peak of current transients (“full fit”; •, terminal-end recording; ○, soma-end recording). B: plot of estimated resistance R1 (R1_est) vs. theoretical value of series resistance for terminal-end recordings of simulated model of rod bipolar cell for default model (——) after reducing the size of the terminal swelling to 34% of default model (—), after removing the terminal swelling (— - -), and after removing the terminal swelling and reducing the axon diameter by 50% (——). C: plot of estimated resistance R2 (R2_est) vs. theoretical value of series resistance for both terminal-end (——) and soma-end (—) recordings of simulated model of rod bipolar cell. The theoretical value of R2 (R2_theory) is indicated by - - -.
The chosen value for cytoplasmic resistivity \( R_i \) but with specific membrane conductance decreased from 7.14 \( \text{S/cm}^2 \) to 0 \( \text{S/cm}^2 \). In the estimates of the terminal capacitance by the two-compartment model when the size of the terminal was varied all estimates were within ±1% of the theoretical value (Fig. 7D).

**Effect of cytoplasmic resistivity on estimate of terminal size**

In our default model of a rod bipolar cell, we used a cytoplasmic resistivity of 160 \( \Omega \text{cm} \). Cytoplasmic resistivity is in general difficult to measure accurately and estimates range from ~50 to ~500 \( \Omega \text{cm} \) (Hallermann et al. 2003; Major et al. 1994; Thurbon et al. 1994; Trevelyan and Jack 2002; Ulrich et al. 1994). The chosen value for cytoplasmic resistivity \( R_i \) is important, however, as it influences the overall degree of electrostatic compactness of a neuron (Spruston et al. 1994).

We first simulated terminal-end recordings and varied \( R_i \) between 50 and 400 \( \Omega \text{cm} \). For each condition, we estimated terminal compartment capacitance, soma compartment capacitance, axon resistance, and series resistance. With terminal-end recordings, varying the value of \( R_i \) markedly influenced the decay time course of the current transients (Fig. 8A). However, there was no effect on the estimate for series resistance (Fig. 8B), consistent with the fact that changing \( R_i \) had no effect on the peak of the current transients (Fig. 8A). For each value of \( R_i \), we directly calculated the corresponding value for the axonal resistance (see preceding text). The estimates from the two-compartment model closely followed these values over the range explored (Fig. 8B). Estimates for the capacitance of the terminal compartment were little influenced over the range of values tested for \( R_i \) (Fig. 8C), but estimates for the soma compartment decreased with increasing values of \( R_i \) (Fig. 8C).

We next simulated soma-end recordings and varied \( R_i \) between 50 and 400 \( \Omega \text{cm} \). In this case, the effect of varying \( R_i \) on
the decay time course of the current transients was much smaller (Fig. 8D). However, for increasing values of \( R_2 \), the value of \( R_2 \) was increasingly underestimated compared with the directly calculated value for axonal resistance (see preceding text; Fig. 8E). There was minimal influence of \( R_2 \) on the estimates of \( C_1 \) and \( C_2 \) (Fig. 8F).

Examples of whole cell and outside-out patch recordings from axon terminals

There is evidence that rod bipolar cells express a range of ligand-gated (e.g., Cui et al. 2003; Fletcher et al. 1998; Gillette and Dacheux 1995; Greferath et al. 1995; Karschin and Wässle 1990; Koulou et al. 1998; Pan 2001; Vaquero and de la Villa 1999) and voltage-gated (e.g., de la Villa et al. 1998; Hartveit 1999; Pan 2001; Pan and Lipton 1995; Pan et al. 2001; Protti and Llano 1998) ion channels at their axon terminals. After establishing whole cell recordings at axon terminals of rod bipolar cells, we applied GABA (200 \( \mu \)M) or glycine (200 \( \mu \)M) locally to the axon terminal using a multi-barreled pipette complex. Application of glycine evoked a strong inward current at a holding potential of \(-60\) mV (Fig. 9A; \( n = 9/9 \) cells; peak amplitude: \( 30–577 \) pA). Application of GABA also evoked an inward current at \(-60\) mV (Fig. 9B; \( n = 9/9 \) cells; peak amplitude: \( 17–488 \) pA). The tip of each multi-barreled pipette complex measured \( 20–25 \) \( \mu \)m and was displaced laterally from the axon terminal by approximately the same distance. Although it is difficult to spatially restrict the area of application with puffer pipettes, we noticed that the response amplitude was greatly reduced when we changed the position of the application pipette away from the region of the axon terminal, suggesting that glycine- and GABA-receptors located in the axon terminal region made a large contribution to the observed responses.

That currents can be evoked by exogeneous application of neurotransmitter agonists is not proof that the synaptic circuitry established by rod bipolar axon terminals is intact for the recorded cells. Importantly, however, we regularly observed spontaneous PSCs while recording from axon terminals (Fig. 9C). They appeared as transient inward currents (at a holding potential of \(-60\) mV) with very fast rise times (\(<400\) \( \mu \)s; see following text) and slower decays. The spontaneous PSCs were inward at a holding potential of \(-60\) mV and were reversibly blocked by a mixture of GABA \(_A\) (bicuculline), GABA \(_C\) (TPMPA), and glycine (strychnine) receptor antagonists (data not shown), suggesting that they are spontaneous, inhibitory PSCs (spIPSCs) generated by release of GABA and/or glycine from amacrine cell processes contacting the axon and axon terminals of rod bipolar cells (Cui et al. 2003; Eggers and Lukasiewicz 2006; Frech and Backus 2004; Ivanova et al. 2006). Similar spontaneous PSCs were seen in a total of 15 axon terminal recordings.

The responses to application of glycine and GABA, as well as the spontaneous PSCs, could potentially be generated by receptors located along the axon itself without the involvement of the axon terminal compartment. Accordingly, we tested our terminal recordings for the presence of L-type voltage-gated \( \text{Ca}^{2+} \) currents, known to be localized to the synaptic regions of the axon terminals (de la Villa et al. 1998; Hartveit 1999; Pan 2000, 2001; Protti and Llano 1998; Satoh et al. 1998). Figure 9D shows the I-V relationship for ramp-evoked \( \text{Ca}^{2+} \) currents in a terminal-end recording of a rod bipolar cell. Before the start of the ramp (\(-100\) to \(+40\) mV; \( 100\) \( \mu \)V/s), the cell was hyperpolarized from \(-60\) to \(-100\) mV for \( 50\) ms. The I-V curve displayed two separate inward current components, corresponding to a low-threshold T-type current activating at \(-70\) to \(-65\) mV and a higher-threshold L-type current activating at \(-50\) to \(-45\) mV (de la Villa et al. 1998; Hartveit 1999; Pan 2000, 2001; Protti and Llano 1998; Satoh et al. 1998). There is evidence that the T-type current is localized both in the soma (de la Villa et al. 1998; Hartveit 1999; Pan 2000; Satoh et al. 1998) and in the axon terminal compartment (Pan 2001; Pan et al. 2001). Voltage-gated \( \text{Ca}^{2+} \) currents were observed in a total of 14 terminal-end recordings.

With respect to the potential usefulness of axon terminal recordings for investigating mechanisms of signal processing, it is important to verify the presence of functionally intact reciprocal synapses in which the terminals enter into synaptic relationships with both pre- and postsynaptic functions (Dowling and Boycott 1966; Hartveit 1999). Figure 9E shows an example of a reciprocal synaptic response evoked in an axon terminal by a depolarizing voltage pulse to \(-30\) mV (from a
holding potential of \(-60 \text{ mV}\). On stepping the cell back to \(-60 \text{ mV}\), a relatively long-lasting tail current was observed. This tail-current involves several different response components, including a GABAergic component (Hartveit 1999; Singer and Diamond 2003) and a glutamate transporter-mediated component (Palmer et al. 2003; Veruki et al. 2006; Wersinger et al. 2006). In addition, several discrete IPSCs were evoked (cf. Hartveit 1999; Protti and Llano 1998; Singer and Diamond 2003). Within 10–15 min of recording in the whole cell configuration, the reciprocal response gradually disappeared, most likely due to run-down of transmitter release from the rod bipolar cell (cf. Hartveit 1999; Palmer et al. 2003). Similar reciprocal responses were evoked in a total of 13 terminal-end recordings.

For high-resolution investigations of the biophysical properties of ion channels in a specific subcellular compartment, it is often necessary to isolate outside-out membrane patches (Jonas 1995; Sakmann and Stuart 1995; Stuart et al. 1993). To prepare patches, we have repeatedly been able to excise patches from axon terminals and establish outside-out patch recordings. Figure 9F shows an example of a glycine-evoked response in an outside-out patch. Glycine was applied for 1 s from a multi-barreled pipette complex, identical to the one used for the whole cell recordings. The distance from the tip of the application pipette to the outside-out patch was \(-20 \mu \text{m}\). The glycine response rose rapidly to a peak and decayed relatively rapidly after the drug application was ended. Similar responses were observed in 5 other patches (peak amplitude: 10–130 pA). Figure 9G shows an example of a GABA-evoked response in the same outside-out patch. The response rose rapidly to a peak, but at the end of the application pulse, the response decayed more slowly than the glycine-evoked response. Similar results were observed in five other patches (peak amplitude: 9–125 pA).

Differential filtering of postsynaptic currents generated at the axon terminal

An important motivation for establishing whole cell recordings at the axon terminals of rod bipolar cells is the opportunity for improving the space clamp of the cellular compartment that receives synaptic inputs, both reciprocal and nonreciprocal, from amacrine cells (Chun et al. 1993; Dowling and Boycott 1966; Freed et al. 1987; Kim et al. 1998; Strettoi et al. 1990). The effect of improved space clamp of the axon terminal region should be more pronounced for synaptic input with fast kinetics than for synaptic input with slow kinetics. On the other hand, this advantage is potentially offset by the increased difficulty of obtaining low series resistance in terminal-end recordings compared with soma-end recordings. To investigate this quantitatively, we simulated current responses generated by a series of postsynaptic conductance waveforms with instantaneous rise and monoexponential decay (Fig. 10A; calculated according to Eq. 7). The time constant of decay (\(\tau_{\text{decay}}\)) was 1, 5, or 10 ms. This covered the relevant range of values for \(\tau_{\text{decay}}\) observed in our own terminal-end recordings of spIPSCs (unpublished results), in soma-end recordings of spIPSCs from mouse rod bipolar cells (Eggers and Lukasiewicz 2006; Frech and Backus 2004; Ivanova et al. 2006), and in isolated terminal recordings of spIPSCs from goldfish bipolar cells (Palmer 2006). The conductance waveforms were injected at the axon terminal in our idealized model of a rod bipolar cell, voltage-clamped either at the soma end or the terminal end (Fig. 10A). For each conductance waveform, we repeated the simulations for a range of series resistance values of the voltage-clamp electrode and compared the amplitude and kinetics of the PSCs recorded in each location.

For \(\tau_{\text{decay}}\) of 1 ms, there was a marked difference between the attenuation in terminal- and soma-end recordings (Fig. 10B). For terminal-end recordings, the peak amplitude was reduced to 75% when the series resistance was 27 M\(\Omega\) and to 50% when it was 96 M\(\Omega\). For soma-end recordings, the peak amplitude was already reduced to 75% when the series resistance was \(<1 \text{ M}\Omega\) and to 50% when it was 41 M\(\Omega\). For \(\tau_{\text{decay}}\) of 5 ms, there was less difference in the attenuation between terminal- and soma-end recordings (Fig. 10B). For terminal-end recordings, the peak amplitude was reduced to 75% when the series resistance was 50 M\(\Omega\) and to 50% at 244 M\(\Omega\). For soma-end recordings, the peak amplitude was reduced to 75% when the series resistance was 31 M\(\Omega\) and to 50% at 234 M\(\Omega\). For \(\tau_{\text{decay}}\) of 10 ms, there was even a smaller difference between terminal- and soma-end recordings (Fig. 10B). Attenuation of the peak amplitude to 75% was reached at a series resistance of \(~75 \text{ M}\Omega\) and attenuation to 50% was reached at \(~375 \text{ M}\Omega\) for both terminal- and soma-end recordings. Only for values of series resistance less than \(~40 \text{ M}\Omega\) was there a noticeable difference between terminal- and soma-end recordings.

To analyze the temporal distortion of the PSCs recorded in the voltage-clamp simulations, we measured \(10–90\%\) rise time and half-decay time. For 10–90\% rise time, there was a marked difference between terminal- and soma-end recordings for \(\tau_{\text{decay}}\) of 1 ms, and the difference became larger with increasing values of series resistance (Fig. 10C). For \(\tau_{\text{decay}}\) of 5 and 10 ms, the 10–90\% rise was consistently larger for soma-end recordings than for terminal-end recordings, but the difference was relatively constant over a wide range of values for series resistance (Fig. 10C). With respect to half-decay time, there was little difference between terminal- and soma-end recordings for a given value of \(\tau_{\text{decay}}\) over a wide range of series resistance values (Fig. 10D).

We next used physiological recordings to confirm the expected differences in waveform characteristics by measuring 10–90\% rise time and peak amplitude of spIPSC ensemble averages in terminal-end \((n = 6)\) and soma-end recordings \((n = 4)\). Each cell included in the analysis had \(\geq 40\) spIPSCs (range: 40–720 events). In the terminal-end recordings, series resistance ranged between 76 and 125 M\(\Omega\), and in the soma-end recordings, it ranged between 11 and 27 M\(\Omega\). Despite the differences in series resistance, the terminal-end recorded spIPSCs displayed a faster 10–90\% rise time (370 ± 32 ms; range: 320–390 ms) than the soma-end recorded spIPSCs (740 ± 270 ms; range: 0.56–1.1 ms; \(P = 0.0099\)) and a larger peak amplitude (15 ± 4 pA; range: 13–22 pA) than the soma-end recorded spIPSCs (7.0 ± 1.3 pA; range: 5.8–8.7 pA; \(P = 0.0056\)). There was no difference in the half-decay time between spIPSCs recorded at the terminal (3.7 ± 1.2 ms) and at the soma (3.6 ± 1.1 ms; \(P = 0.96\)). The differences between the average spIPSC waveforms in terminal- and soma-end recordings are illustrated in Fig. 10E.
DISCUSSION

In this study, we have analyzed passive membrane properties of rod bipolar cells investigated with whole cell voltage-clamp recording in rat retinal slices. Analyses were carried out for both soma- and terminal-end recordings. We describe in detail our method for routinely performing terminal-end recordings from these cells that has allowed us to compare the passive membrane properties obtained from soma- and terminal-end recordings. For terminal-end recordings, we have verified the presence of voltage-gated Ca\(^{2+}\) currents in the axon terminal compartment as well as the integrity of synaptic circuits in which rod bipolar axon terminals are involved both pre- and postsynaptically. We also demonstrate the advantages of terminal-end recordings to accurately measure waveform characteristics of synaptic currents generated at the axon terminal compartment. Finally, we document the feasibility of isolating outside-out patches from axon terminal recordings to study the properties of ion channels and neurotransmitters located in this compartment.

Passive membrane properties of rod bipolar cells investigated with a two-compartment equivalent circuit model

We analyzed the passive membrane properties of rod bipolar cells by recording current responses evoked by 10-mV hyperpolarizing pulses from a holding potential of \(-60\) mV and fitting the capacitive current transients with mono- and biexponential functions. For both soma- and terminal-end recordings, biexponential functions provided the best fits. This is similar to the conclusions reached for goldfish bipolar cells, both for acutely isolated cells (Mennerick et al. 1997) and for cells in slices (Palmer et al. 2003). In contrast, Zhou et al. (2006) recently reported that for acutely isolated mouse rod bipolar cells, monoexponential functions provided adequate fits. This could be due to differences in preparations or, perhaps, to the stated selection of cells with short axons in the material analyzed by Zhou et al. (2006). By applying the two-compartment equivalent circuit model developed by Mennerick et al. (1997) for isolated goldfish bipolar cells, we estimated four model parameters: soma-compartment capacitance, terminal-compartment capacitance, axial resistance linking the two capacitive compartments, and the series resistance linking the recording pipette to the proximal capacitive compartment for both soma- and terminal-end recordings. A major distinguishing characteristic of the capacitive transients was the different time course of the current decay. In terminal-end recordings,

![Passive membrane properties of rod bipolar cells](http://jn.physiology.org/)

**FIG. 10.** Differential filtering of synaptic currents generated at the axon terminal in soma-end and terminal-end whole cell voltage clamp of simulated and real rod bipolar cells. A, left: synaptic conductance waveforms \(G_{\text{syn}}\), calculated according to Eq. 7, with theoretical decay time constant \(\tau_{\text{decay (theory)}}\) of 1 (top), 5, or 10 ms (bottom). For easier visual comparison with the evoked current waveforms, the \(G_{\text{syn}}\) waveforms have been drawn inverted, corresponding to a transformation to current according to \(I_{\text{syn}}(t) = G_{\text{syn}}(t)(V_m - E_{\text{rev}})\), with \(V_m - E_{\text{rev}} = -60\) mV. **Middle:** resulting currents in terminal-end recordings (diagram; top) in response to conductance waveforms in left column for a range of series resistance values (1, 10, 20, 40, 80, 160, 320, 640, and 1,000 MΩ). Right: resulting currents in soma-end recordings (diagram; top) in response to conductance waveforms in left column for the same series resistance values. In all cases, synaptic conductance waveform injected in terminal compartment (diagrams; top) B: attenuation of peak amplitude of postsynaptic currents (PSC; generated at the terminal) in terminal-end (—) and soma-end (•••) recordings for different values of \(\tau_{\text{decay (theory)}}\) and series resistance (as in A). PSC peak amplitudes normalized to peak amplitude of theoretical \(I_{\text{syn}}\) (calculated as in A). Additional simulations performed for series resistance values of 2, 4, and 8 MΩ (B and C). C: 10–90% rise times for PSCs in peak (—) and soma-end (---) recordings for different values of \(\tau_{\text{decay (theory)}}\) and series resistance (as in A). D: half-decay times for PSCs in terminal-end (—) and soma-end (—•••) recordings for different values of \(\tau_{\text{decay (theory)}}\) and series resistance (as in A). E: average waveforms (calculated from ensemble averages for each cell) of spIPSCs in physiologic terminal-end recordings (n = 6; ---; intracellular solution C) and in soma-end recordings (n = 4; ••••; intracellular solution C). Waveforms plotted at same scale (left) and after normalization of peak amplitude (right) aligned at onset (determined by eye).
recordings, the slow exponential component had both a longer time constant and a larger amplitude contribution. This corresponds to slower charging of the soma-dendritic capacitance in a terminal-end recording compared with a soma-end recording. Overall, the two-compartment parameters estimated from soma- and terminal-end recordings are well in accordance with each other. We have not been able to perform within-cell comparisons, that is, analyzing the same cells sequentially with soma- and terminal-end recordings. Accordingly, there is a potential selection bias for larger terminals, and we believe that this bias may account, at least partially, for the larger estimate of soma-compartment capacitance obtained with terminal-end recordings (cf. Mennerick et al. 1997).

We developed a computer model of an idealized rod bipolar cell to verify that the two-compartment circuit (Mennerick et al. 1997) is an adequate model for analyzing cells with the morphological characteristics of rod bipolar cells in rat retina. We used computer simulations to analyze systematically the influence of series resistance on estimates of the parameters of the two-compartment circuit. With respect to the capacitance of the soma compartment, soma- and terminal-end recordings gave very similar results over a large range of series resistance values. However, both soma- and terminal-end recordings overestimated the capacitance of the terminal compartment with terminal-end recordings being more accurate over a larger range of series resistance values. This is similar to the results of Mennerick et al. (1997) for goldfish bipolar cells where the degree of overestimation was larger for soma-end than for terminal-end recordings. In our simulations of rod bipolar cells, the relative degree of overestimation of the capacitance of the terminal compartment was largest for small terminal sizes and was partially due to the fact that the capacitance of the axon is ignored in the two-compartment model. We also found little influence of cytoplasmic resistivity on estimates of the capacitance of the terminal compartment with terminal-end recordings. Together, these results underscore the importance of terminal-end recordings for investigating the axon terminal compartment.

For very low values of series resistance (1–10 MΩ), the capacitance of the proximal compartment (soma in soma-end recordings, terminal in terminal-end recordings) was increasingly underestimated. This was due to inadequate resolution of the very fast decay of the capacitive current transient. The same phenomenon also explained the overestimation of the series resistance. Because of the smaller capacitance of the terminal compartment, the overestimation of series resistance was larger and occurred at even higher values of series resistance for terminal-end compared with soma-end recordings. Because of residual input and output filtering, exponential curve fitting cannot start before 30–40 μs after onset of the voltage stimulus (cf. Mennerick et al. 1997). For simulated data with a sampling interval of 5 μs and with no input or output filtering, starting curve fitting at the first point after onset of the voltage stimulus largely eliminated underestimation of the capacitance of the proximal compartment. This procedure also eliminated overestimation of the series resistance for soma-end recordings and strongly reduced the overestimation for terminal-end recordings. As expected, the degree of overestimation of the series resistance increased when the size of the axon terminal was reduced.

Voltage-clamp investigations of synaptic inputs with terminal- versus soma-end recordings

An important motivation for establishing terminal-end recordings from rod bipolar axon terminals as a routine technique was the potential for increased resolution of pre- and postsynaptic currents generated in this compartment. This includes increased voltage control of voltage-gated Ca²⁺ currents (Mennerick et al. 1997; Pan 2001; Pan et al. 2001; Protti and Llano 1998). Here we used computer simulations and physiological recordings to investigate differences in attenuation and kinetics of postsynaptic currents between soma- and terminal-end voltage-clamp recordings. In the simulations, we injected conductance waveforms with different decay time constants (τ_decay 1, 5, or 10 ms) at the axon terminal and explored the degree of attenuation as a function of recording location (soma end vs. terminal end) and series resistance. For all values of series resistance, the degree of attenuation was less pronounced for terminal-end as opposed to soma-end recordings. For τ_decay of 1 and 5 ms, the advantage of terminal- over soma-end recordings occurred for realistic values of series resistance. For τ_decay of 10 ms, the difference in attenuation between the two recording locations was most pronounced for series resistance values <40 MΩ. The time course of postsynaptic currents was analyzed by measuring 10–90% rise time and half-decay time. Both parameters increased with increasing series resistance. The 10–90% rise time was always longer for soma-end recordings than for terminal-end recordings. For half-decay time, there was little difference between soma- and terminal-end recordings. In the physiological recordings, we verified that spIPSCs recorded at the terminal displayed substantially larger amplitudes and faster rise times (10–90%) than spIPSCs recorded at the soma. Unless corrected for, the stronger attenuation of peak amplitude of IPSCs in soma-end recordings will lead to an underestimation of the number of channels open at the peak of the IPSCs (see review by Silver and Farrant 1999). Our simulations and observations are supported by the fast rise times of spIPSCs recorded in isolated axon terminals of goldfish bipolar cells (0.29 ms) (Palmer 2006) compared with the slower rise times of spIPSCs in soma-end recordings from mouse bipolar cells (0.9–1.2 ms) (Eggers and Lukasiewicz 2006; Frech and Backus 2004; Ivanova et al. 2006).

The electrotonic filtering properties of the rod bipolar cells revealed in the simulations and physiological recordings of IPSCs will also impact the transmission of voltage signals between the soma and terminal compartments in situ. The extent and frequency dependence of filtering of voltage signals will be a function of the passive membrane properties, the cell morphology, and the interaction between various voltage-gated Ca²⁺ and K⁺ currents. Further work is required to incorporate such conductances in passive models such as the one presented here.

Investigating ion channels in rod bipolar axon terminals

Although there are clear advantages of terminal-end recordings over soma-end recordings, it is important to weigh these advantages against potential disadvantages, primarily the increased difficulty of obtaining very low values for series resistance. One important advantage is the possibility to isolate patches for high-resolution recording of ion channel activity.
The ability to do this for rod bipolar cells in situ avoids problems associated with the potential re-distribution of ion channels as a consequence of acutely isolating cells by enzymatic and mechanical dissociation of retinal tissue (Grefe‌rath et al. 1995).

On a few occasions, we were able to record from small, isolated compartments in the appropriate location of the inner plexiform layer, and these recordings might permit investigating isolated axon terminals of rod bipolar cells in the same way as it has been done for axon terminals of goldfish bipolar cells (Palmer et al. 2003). However, the potential uncertainty with respect to a reliable morphological identification suggests that in general recordings from intact cells are preferable. In particular, techniques similar to those used for hippocampal mossy fiber terminals by Hallermann et al. (2003) could be developed for rod bipolar cells to perform time-resolved measurements of capacitance to study exocytosis.

On occasion, we also recorded from isolated soma-dendritic compartments. Such recordings have been quite useful to indirectly identify the location and functional properties of specific ion channels in the axon terminal compartment (Euler and Masland 2000; Hartveit 1999; Pan 2000). Importantly, we have never observed a discrepancy with respect to the morphological identification (by fluorescence microscopy) of a cell as axotomized and its electrophysiological signature with reduced contribution of the slow exponential component in the decay of the capacitive transient. A potentially complicating factor is the uncertainty in reliably identifying an axotomized cell as a rod bipolar cell, as opposed to an on- or off-cone bipolar cell. Although the dendritic morphology can potentially be helpful for identification, other properties such as visual responses (Euler and Masland 2000) and responses to glutamate receptor agonists (Euler et al. 1996; Hartveit 1996, 1997) might be more reliable.

In summary, our results with physiological recordings and computer simulations suggest that recordings from axon terminals of rod bipolar cells in situ offer distinct experimental advantages for investigations of ion channels and signaling mechanisms located in this compartment. Such recordings can now be routinely performed within functionally intact synaptic circuits and will allow insight into both the presynaptic and the postsynaptic role of rod bipolar cell axon terminals. An important next step will be to further exploit the experimental opportunities offered and establish simultaneous recordings of synthetically connected rod bipolar axon terminals and all amacrine cells.

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