Electrical Coupling and Passive Membrane Properties of AII Amacrine Cells

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Veruki ML, Oltedal L, Hartveit E. Electrical coupling and passive membrane properties of AII amacrine cells. J Neurophysiol 103: 1456–1466, 2010. First published January 20, 2010; doi:10.1152/jn.01105.2009. All amacrine cells in the mammalian retina are connected via electrical synapses to ON-cone bipolar cells and to other AII amacrine cells. To understand synaptic integration in these interneurons, we need information about the junctional conductance (g_j), the membrane resistance (R_m), the membrane capacitance (C_m), and the cytoplasmic resistivity (R_i). Due to the extensive electrical coupling, it is difficult to obtain estimates of R_m as well as the relative contribution of the junctional and nonjunctional conductances to the total input resistance of an AII amacrine cell. Here we used dual voltage-clamp recording of pairs of electrically coupled AII amacrine cells in an in vitro slice preparation from rat retina and applied meclofenamic acid (MFA) to block the electrical coupling and isolate single AII amacrine cells. In the control condition, the input resistance (R_in) was ~620 MΩ and the apparent R_m was ~760 MΩ. After block of electrical coupling, determined by estimating g_j in the dual recordings, R_m and R_m were ~4,400 MΩ, suggesting that the nongap junctional conductance of an AII amacrine cell is ~16% of the total input conductance. Control experiments with nucleated patches from AII amacrine cells suggested that MFA had no effect on the nongap junctional membrane of these cells. From morphological reconstructions of all AII amacrine cells filled with biocytin, we obtained a surface area of ~900 μm² which, with a standard value for C_m of 0.01 pF/μm², corresponds to an average capacitance of ~9 pF and a specific membrane resistance of ~41 kΩ cm². Together with information concerning synaptic connectivity, these data will be important for developing realistic compartmental models of the network of all AII amacrine cells.

INTRODUCTION

All amacrine cells play a central role for transmission and integration of signals in the network of neurons that constitute the rod pathway (reviewed by Bloomfield and Dacheux 2001), and recent results suggest important roles for all amacrine cells in photopic vision as well (Manookin et al. 2008; Münch et al. 2009). All amacrine cells are postsynaptic to and receive glutamatergic input from the axon terminals of rod bipolar cells and some OFF-cone bipolar cells (Singer and Diamond 2003; Strettoi et al. 1992, 1994; Veruki et al. 2003). They are presynaptic to and transmit their signals to OFF-cone bipolar cells via glycineergic, inhibitory synapses (Pourcho and Goebel 1985; Sassoë-Pognetto et al. 1994; Strettoi et al. 1992, 1994). In addition, all amacrine cells are connected via gap junctions to ON-cone bipolar cells (heterologous connections) and to other AII amacrine cells (homologous connections) (Chun et al. 1993; Kolb 1979; Kolb and Famiglietti 1974; McGuire et al. 1984; Strettoi et al. 1992, 1994). Functionally, both types of gap junctions correspond to electrical synapses (Trexler et al. 2005; Veruki and Hartveit 2002a,b).

While the electrical synapses between all amacrine cells and ON-cone bipolar cells are considered to play a role in the direct transmission of scotopic visual signals, the electrical synapses between all amacrine cells are thought to be important for removing noise from the visual signal (Smith and Vardi 1995; Vardi and Smith 1996). The magnitude of the junctional conductance (g_j) between all amacrine cells and between all amacrine and ON-cone bipolar cells is important for the functional consequences of electrical coupling (Veruki et al. 2008), and it is important to obtain experimental measurements of these parameters. However, to fully understand synaptic integration in all amacrine cells, we need knowledge of additional passive membrane parameters such as membrane resistance (R_m), membrane capacitance (C_m), and cytoplasmic resistivity (R_i). There is currently not enough quantitative information to construct compartmental models of the network of all amacrine cells with a high level of morphological and physiological realism. It is essential to construct such models that can serve as electrical skeletons onto which active, voltage-gated conductances can be added with the goal of building up a realistic computational model (Major 2001). For example, it is unknown how much of the total input conductance of a single cell the junctional and nonjunctional conductances account for. This question can be addressed experimentally for a pair of electrically coupled cells, using a two-cell circuit model (Bennett 1966, 1977), but in the case of the network of all amacrine cells, this model ignores the fact that a single cell is connected by electrical synapses to several other all amacrine cells and ON-cone bipolar cells. There are few agents that block gap junction channels, and several of these have nonspecific effects on other channel types. Recently, however, the compound meclofenamic acid (MFA) was demonstrated to block tracer coupling and electrical coupling between all amacrine cells and between all amacrine and ON-cone bipolar cells (Pan et al. 2007; Veruki and Hartveit 2009). The block is complete and reversible, but the time required for complete block is typically 20–40 min. This suggests that MFA can be used in experiments that seek to measure physiological properties of electrically isolated all amacrine cells but that the required recording durations can be technically challenging. Here we have used high-resistance pipettes to obtain long-lasting voltage-clamp recordings of electrically coupled pairs of all amacrine cells where g_j is stable over time under control conditions (Veruki and Hartveit 2009; Veruki et al. 2008). We obtained estimates of R_m and R_in at regular intervals, first in the control condition and then during block of electrical coupling with MFA. Because we performed dual recordings of electrically coupled pairs of all amacrine cells, we obtained measurements of g_j in parallel with R_m and R_in and could determine when the electrical coupling was blocked (g_j = 0). By combining the measurements of R_m under conditions of blocked coupling with mea-
measurements of membrane surface area based on quantitative morphological reconstructions of single AII amacrine cells, we obtained estimates for the specific resistance of the nongap junctional membrane. These results will be important for our ability to model the integrative properties of the network of electrically coupled AII amacrine cells and on-cone bipolar cells under conditions of changing $g_j$.

**METHODS**

General aspects of the methods have previously been described in detail (Veruki and Hartveit 2002a, 2009). Albino rats (4–7 wk postnatal) were deeply anesthetized with isoflurane in oxygen and killed by cervical dislocation (procedure approved under the surveillance of the Norwegian Animal Research Authority). Retinal slices were prepared using a Willard 840 water-immersion objective (Olympus BX51WI) and infrared gradient contrast videoscopic (Luigs and Neumann, Ratingen, Germany) (Dodt et al. 1998). Recordings were carried out at room temperature (22–25°C).

**Solutions and drugs**

The extracellular perfusing solution was continuously bubbled with 95% O$_2$/5% CO$_2$ and had the following composition (in mM): 125 NaCl, 25 NaHCO$_3$, 2.5 KCl, 2.5 CaCl$_2$, 1 MgCl$_2$, and 10 glucose, pH 7.4. Whole cell recordings were performed with pipettes pulled from thick-walled borosilicate glass (GC150-11; Harvard Apparatus, Edenbridge, UK). For paired whole cell recordings and for nucleated patch recordings, the pipettes were filled with the following solution (in mM): 125 K-glucuronate, 5 KCl, 8 NaCl, 0.2 EGTA, 10 HEPES, and 4 MgATP (pH was adjusted to 7.3 with KOH). Lucifer yellow was added at a concentration of 1 mg/ml. In single-cell recordings for morphological reconstructions with both instances running on the same computer (Mac OS X version 10.4). These amplifiers switch between current injection and potential measurement at high frequency (e.g., Halliwell et al. 1994). Because the potential is measured at a time when no current flows across the recording electrode, problems caused by voltage drops across nonzero series resistance ($R_s$) are reduced and potentially totally avoided. The switching frequency was set to 35–40 kHz, synchronized between the two amplifiers (Müller et al. 1999), and the duty cycle was set to 1/4. Before sampling, current and voltage signals were low-pass filtered (analog 4-pole Bessel filter) with corner frequency of 2 kHz (~3 dB; 1/5 of the inverse of the sampling interval of 100 μs). The voltageclamp gain and the proportional-integral controller were adjusted to give the fastest possible voltage response with minimal overshoot and ringing. The headstage output voltage signal from both amplifiers was monitored on an oscilloscope throughout each recording. For each amplifier, application of voltage protocols and digital sampling of the analog signals were performed by an LIH8+8 laboratory interface (HEKA Elektronik). The start of sampling by the slave interface was hardware triggered from the master interface via a digital line.

For single-cell, whole cell recordings (for morphological reconstructions; see following text) and for nucleated patch recordings, we used lower-resistance pipettes (~5 MΩ for whole cell recording; ~9 MΩ for nucleated patch recording) and an EPC9-dual patch-clamp amplifier (continuous single-electrode voltage-clamp; CSEVC; HEKA Elektronik) controlled by PatchMaster software. To establish nucleated patch recordings, the pipette was slowly withdrawn after establishing the whole cell configuration while continuous light suction (50–100 mbar) was applied to the pipette. Before sampling, signals were low-pass filtered (analog 3- and 4-pole Bessel filters in series) with a corner frequency (~3 dB) of 2 kHz (1/5 of the inverse of the sampling interval of 100 μs). Currents caused by the recording pipette capacitance ($C_{pip}$) and the cell membrane capacitance ($C_{mem}$) were measured with the automatic capacitance neutralization circuitry of the amplifier.

In all experiments, cells and patches were voltage clamped at a holding potential ($V_{hold}$) of ~60 mV.

**Dynamic clamp electrophysiology**

We implemented artificial electrical synapses between pairs of electronic model cells by conductance injection (dynamic clamp) electrophysiology (Robinson and Kawai 1993; Sharp et al. 1993; reviewed by Goaillard and Marder 2006). The conductance injection was performed with real-time software (SM-2; Cambridge Conductance, Royston, UK) (Robinson 2008) running on a digital signal processing (DSP) analog board (Toro-8; Innovative Integration, Simi Valley, CA) interfaced with a host PC. A current command signal was generated from a user-specified conductance value and the instantaneous values of the time-varying membrane potentials of the cells. For an electrical synapse connecting two electronic model cells (cells $a$ and $b$) in an artificial network, the current injected into each cell [$I_{a}(t)$ in cell $a$, $I_{b}(t)$ in cell $b$] was calculated according to the following equations:

\[
I_{a}(t) = g_{j}(V_{a}(t) - V_{b}(t))
\]

\[
I_{b}(t) = g_{j}(V_{a}(t) - V_{b}(t))
\]

where $g_{j}$ is the junctional conductance and $V_{a}(t)$ and $V_{b}(t)$ are the instantaneous values of the membrane potentials of cells $a$ and $b$, respectively. The same conductance value was used for each direction of coupling (cell $a \rightarrow$ cell $b$, cell $b \rightarrow$ cell $a$). The SM-2 system was run with a sampling interval of 50 μs. The conductance injection experiments were performed with two clustered EPC10-triple amplifiers (CSEVC) in the current-clamp configuration, using 100% bridge-balance compensation (10 μs time constant).

**Histology and three-dimensional reconstruction**

In single-cell recording experiments, AII amacrine cells ($n = 6$) were filled with biocytin for staining the cells and subsequent off-line
morphological reconstruction. After 5–10 min of whole cell recording, the recording pipette was removed, and the slice was immediately fixed for 2 min in 2% paraformaldehyde (PFA; 4% PFA in 0.1 M phosphate buffer was added to an equal volume of HEPES-buffered extracellular solution). Thereafter the slice was fixed in 4% PFA in phosphate buffer at room temperature for 30 min and at 4°C overnight. To minimize problems with tissue shrinkage, we did not dehydrate or resection the slices. Development of a reaction product with avidin-biotinylated horseradish peroxidase complex (VECTORSTAIN ABC-elite kit; Vector Laboratories, CA) and diaminobenzidine was done as described in detail in Oltedal et al. (2009).

Quantitative morphological reconstruction of the labeled cells was done with a motorized microscope (Olympus BX 51) equipped with a ×100 oil-immersion objective (NA 1.25; Olympus), a digital CCD camera (MicroFire S99808, Optronics) and Neurolucida software (v7; MicroBrightField Bioscience, Williston, VT). The soma of each cell was traced as a single contour. The surface area of the three-dimensional (3D)-reconstructed cells was calculated with the help of the software program Neurolucida Explorer (MicroBrightField Bioscience). No attempt was made to correct for errors in morphological measurements due to shrinkage and consequent distortion.

Data analysis

To estimate the steady-state $g_j$ between the two cells of a physiologically coupled pair, we used current responses obtained with dual voltage-clamp recordings. For the calculations, we assumed an equivalent-circuit model (Fig. 1A). For recordings using DSEVC amplifiers, we assumed that $R_m$ was effectively zero. This means that the junction current ($I_j$) corresponds to the current evoked in the postsynaptic cell and $g_j$ can be calculated directly from Ohm’s law (Hartveit and Veruki 2010; Müller et al. 1999) according to Eq. 3 for voltage pulses applied to cell $a$ and according to Eq. 4 for voltage pulses applied to cell $b$.

For dynamic clamp recordings with CSEVC amplifiers, we mathematically corrected for nonzero $R_j$ and finite $r_m$, according to published procedures (van Rijen et al. 1998). Each measurement of $g_j$ was obtained by plotting $I_j$ versus the junction voltage ($V_j$) and by calculating $g_j$ as the slope of a straight line fitted to the $I_j$-$V_j$ relationship. For a given cell pair, $g_j$ was calculated as the average of the $g_j$ values obtained for both directions of coupling.

The membrane resistance (apparent or real) was estimated according to Eq. 5 when stepping cell $a$ ($r_{ma}$)
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\[ r_m = \frac{V_a - V_b}{I_a + I_b} \]  
(5)

and according to Eq. 6 when stepping cell \( b \) \( (r_{m2}) \)

\[ r_{m2} = \frac{V_a - V_b}{I_a + I_b} \]  
(6)

Each measurement of \( r_m \) was obtained by plotting the voltage versus the current and by calculating \( r_m \) as the slope of a straight line fitted to the V-I relationship.

In dual recordings of electrically coupled cells, the input resistance \( (R_w) \) of each cell was obtained indirectly by calculating it from the measurable membrane conductance \( (g_j) \) and \( r_j \) according to Eq. 7 for cell a \( (R_{m1}) \) and Eq. 8 for cell b \( (R_{m2}) \)

\[ \frac{1}{R_{m1}} = \frac{1}{r_{m1}} + \frac{1}{r_j + r_{m2}} \quad \text{or} \quad R_{m1} = \frac{r_{m1}(r_{m2} + r_j)}{r_{m1} + r_{m2} + r_j} \]  
(7)

\[ \frac{1}{R_{m2}} = \frac{1}{r_{m2}} + \frac{1}{r_j + r_{m1}} \quad \text{or} \quad R_{m2} = \frac{r_{m2}(r_{m1} + r_j)}{r_{m1} + r_{m2} + r_j} \]  
(8)

where \( r_{m1} \) is the apparent membrane resistance of cell a (estimated from Eq. 5), \( r_{m2} \) is the apparent membrane resistance of cell b (estimated from Eq. 6), and \( r_j \) is the inverse of the junctional conductance (estimated from Eqs. 3 and 4).

Off-line data analysis was performed with FitMaster (HEKA Elektronik), IGOR Pro (WaveMetrics, Lake Oswego, OR) and Excel (Microsoft, Seattle, WA). Data are presented as means \( \pm \) SE (n = number of cells or cell pairs), and percentages are presented as percentage of control. Statistical analysis was performed using Student’s two-tailed t-test (paired). For illustration purposes, most raw data-records were low-pass filtered (digital nonlagging Gaussian filter; \(-3\) dB at 0.5–1 kHz).

Results

Equivalent electrical circuits for electrical coupling

A pair of electrically coupled cells can be represented by the equivalent electrical circuit illustrated in Fig. 1A (Bennett 1966, 1977; van Rijen et al. 1998). Each cell is represented by a simple resistor-capacitor loop circuit with a single lumped resistance \( (r_{m1} \text{ or } r_{m2}) \) and capacitance \( (C_{m1} \text{ or } C_{m2}) \). The resistance of the electrical synapse is represented by \( r_j \) (junctional resistance), corresponding to a junctional conductance \( g_j \) \( (= 1/r_j) \). The parameters \( r_{m1}, r_{m2}, \) and \( g_j \) can be estimated with dual voltage-clamp recording of a pair of electrically coupled cells. When the two cells are embedded in a network of electrically coupled cells, with each of the two cells coupled to additional cells, the two-cell circuit is an obvious simplification. For a simple two-dimensional network and low to moderate values of \( g_j \), the two-cell circuit can still be used as an approximation to obtain reasonably accurate estimates of \( g_j \) between two neighboring cells (Veruki et al. 2008). However, when the two coupled cells are each coupled to other cells, we can no longer use the two-cell circuit to estimate the true membrane resistance. Specifically, when each cell is connected via electrical synapses to additional cells, the estimated values for \( r_{m1} \) and \( r_{m2} \) will represent apparent membrane conductances instead of true membrane resistances. Irrespective of these complicating factors, the two-cell circuit serves as a useful analytical model and will be used in the present study to estimate the true \( r_m \) of AII amacrine cells when \( g_j \) is reduced pharmacologically.

Recent results demonstrated that electrical synapses between AII amacrine cells and between AII amacrine and ON-cone bipolar cells are blocked completely, although slowly, by the drug MFA (Veruki and Hartveit 2009). This suggests that we can use MFA to electrically isolate AII amacrine cells and measure their passive membrane properties under varying degrees of coupling. With normal electrical coupling, the estimated \( r_m \) (an apparent \( r_m \)) reflects current flow across both the non-gap junctional and gap junctional membrane. As the electrical synapses are gradually blocked by MFA, the apparent \( r_m \) should gradually approach the true \( r_m \), and when \( g_j \) is zero, the two should be equal. A similar change is expected for \( R_m \), such that \( R_m \) is lower than \( r_m \) under conditions of normal electrical coupling and equal to \( r_m \) when \( g_j \) is zero.

In the following, we first report results to validate our use of the two-cell circuit model to analyze passive electrical coupling when a pair of coupled cells is embedded in a larger network. Specifically, we wanted to verify the ability of our experimental recording procedures to extract the correct membrane parameters in situations with variable strength of the electrical coupling. For this, we used patch-clamp amplifiers and model cells with known circuit parameters and implemented electrical coupling with dynamic clamp electrophysiology. Next we report results obtained with paired recordings of electrically coupled AII amacrine cells where we monitored \( g_j, r_{m1}, \) and \( R_m \) as electrical coupling was gradually blocked by MFA. Finally, we relate the measurements of \( r_m \) obtained in the absence of electrical coupling to the measurements of membrane surface area obtained for morphologically reconstructed AII amacrine cells, allowing us to estimate upper and lower limits for the specific membrane resistance of all AII amacrine cells.

Validation of two-cell circuit model to analyze passive membrane properties in a network of electrically coupled cells

We used dynamic clamp electrophysiology to implement an artificial network of electrically coupled model cell circuits (Fig. 1B). The network consisted of four model cells arranged in a linear array, and we used six patch-clamp amplifiers for controlling and recording the activity. Four amplifiers, one for each model cell, were used in the current-clamp configuration \((CC_1 \text{–} CC_4)\) with conductance injection to implement the electrical coupling (Fig. 1B). Two amplifiers were connected to cells 2 and 3 in the voltage-clamp configuration \((VC_2, VC_3)\), thus mimicking dual voltage-clamp recording of a pair of electrically coupled cells embedded in a network of coupled cells.

To measure the influence of the magnitude of \( g_j \) on the apparent passive membrane properties of the model cells, we applied voltage pulses alternatingly to the two cells in voltage clamp \((\text{cells 2 and 3; Fig. 1B})\) and estimated \( g_j, r_{m1}, r_{m2}, R_{m1}, \) and \( R_{m2} \) from the evoked currents. The same procedures were used to analyze the responses of the model cells and the biological cells. To correct for the effect of \( R_m \) and \( r_m \) (in the model cells), we applied the procedures developed by van Rijen et al. (1998). Dynamic clamp, implemented with the SM-2 software and the patch-clamp amplifiers in current-clamp configuration (see METHODS), was used to vary \( g_j \) of each virtual electrical synapse between 0 and 3,000 pS. For each
condition, $g_j$ was identical for all the virtual electrical synapses. When the network was completely uncoupled ($g_j = 0$), $r_m$ and $R_{in}$ were identical and equal to $-511 \text{ M} \Omega$. With increasing strength of the electrical coupling, the values of $r_m$ and $R_{in}$ gradually dropped (Fig. 1C). When $R_{in}$ for a cell drops with increasing $g_j$, it reflects the increasing flow of current to other electrically coupled cells relative to the current flowing through the cell’s real membrane resistance. When $r_m$ for a cell drops with increasing $g_j$, it reflects that the network of electrically coupled cells is larger than the simple two-cell network illustrated in Fig. 1. While $r_m$ estimated for a simple two-cell network reflects the real membrane resistance, the presence of other cells electrically coupled to the two cells in a dual recording (e.g., in the 4-cell network illustrated in Fig. 1B) means that the estimated $r_m$ will be an apparent membrane resistance. When $g_j$ increases, an increasing amount of current will flow to the electrically coupled cells not directly recorded from, appearing as a drop in $r_m$. As expected, $R_{in}$ was smaller than $r_m$ for all values of $g_j > 0$, with an increasing difference between the two parameters for increasing values of $g_j$ (Fig. 1C). The experimentally determined values for $r_m$ and $R_{in}$ corresponded very well with the values for both parameters calculated directly from the exact values of the model cell resistors and the values of the conductances injected by dynamic clamp (Fig. 1C). This indicated that our experimental methods were well suited for revealing the relevant equivalent circuit parameters.

**Passive membrane properties of electrically coupled All amacrine cells**

In dual voltage-clamp recordings of electrically coupled All amacrine cells with DSEVC amplifiers and high-resistance pipettes (Veruki et al. 2008), we tested for electrical coupling by applying voltage pulses to one cell (“presynaptic”; Fig. 2A) and recording the current responses in both the pulsed and the nonpulsed (“postsynaptic”) cell (Veruki and Hartveit 2002a). When cells were electrically coupled, the hyper- and depolarizing voltage pulses applied to the presynaptic cell evoked outward and inward currents, respectively, in the postsynaptic cell (Fig. 2, B and C). For each direction of coupling, we plotted $I_j$ versus $V_j$ and calculated $g_j$ as the slope of a straight line fitted to the $I_j$-$V_j$ relationship (Fig. 2, D and E). For each cell, we also calculated the current flowing through the apparent nongap junctional membrane ($I_m$; Eqs. 5 and 6), plotted $I_m$ versus $V_j$ and calculated the apparent $r_m$ as the slope of a straight line fitted to the $I_m$-$V_j$ relationship (Fig. 2, F and G). All current measurements for $r_m$ were obtained from responses to hyperpolarizing voltage pulses and were within the linear range without activation of voltage-activated conductances (Fig. 2, F and G). $R_{in}$ was calculated from the values of $r_m1$, $r_m2$, and $r_j$ (Eqs. 7 and 8). $r_m1$ and $R_{in}$ were calculated individually for both cells in a pair ($r_m1$, $r_m2$, $R_{in1}$, and $R_{in2}$), whereas $g_j$ for a cell pair was calculated as the average of the conductance values measured in each direction. For the All cell pair illustrated in

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**Fig. 2.** Physiological coupling between neighboring All amacrine cells analyzed with dual whole cell recording and discontinuous single-electrode voltage-clamp (DSEVC) amplifiers. A: with a pair of All amacrine cells in voltage clamp ($V_{hold} = -60 \text{ mV}$), 200-ms voltage pulses (from $-40$ to $+10 \text{ mV}$ relative to $V_{hold}$; increments of 10 mV) were applied sequentially to the cells ($V_1$, $V_2$). Right: recording configuration with dots extending laterally from resistors attached to each cell symbolizing electrical coupling to other All amacrine and on-cone bipolar cells. B and C: current responses recorded from the cells ($I_c$, $I_j$) in response to the voltage pulses in A. Hyperpolarizing voltage pulses applied to cell 1 (cell 2) result in inward currents in cell 1 (cell 2) and outward currents in cell 2 (cell 1). Depolarizing voltage pulses applied to cell 1 (cell 2) result in outward currents in cell 1 (cell 2) and inward currents in cell 2 (cell 1). Each trace is the average of 11 trials. Notice that the asymmetry of the voltage pulses relative to $V_{hold}$ helps to identify the corresponding current responses in the 2 cells. Here, and later, capacitative current transients have been truncated for clarity. D and E: junction current ($I_j$) vs. junction voltage ($V_j$) ($I_j$-$V_j$) relationships obtained with cell 1 presynaptic-cell 2 postsynaptic (D) and cell 2 presynaptic-cell 1 postsynaptic (E). For each set of measurements, the data points have been fitted with a straight line (slope = $g_j$). All data points shown included in fit. F and G: net membrane current ($I_m$) vs. relative voltage ($V_m$; identical to $V_j$) ($I_m$-$V_m$) relationships obtained with cell 1 stepped (presynaptic)-cell 2 nonstepped (postsynaptic) (F) and cell 2 stepped (presynaptic)-cell 1 nonstepped (postsynaptic) (G). $I_m$ calculated as evoked current in stepped cell plus current in nonstepped cell ($I_j$; for details see text). For each set of measurements, the data points (excluding responses to depolarizing voltage pulses) have been fitted with a straight line (slope = inverse of apparent $r_m$).
Fig. 2, $g_j$ was 390 pS, $r_{m1}$ was 1.170 MΩ, $r_{m2}$ was 1.490 MΩ, $R_{m1}$ was 910 MΩ, and $R_{m2}$ was 1.070 MΩ.

Under optimal conditions, recordings with high-resistance pipettes and DSEVC amplifiers could be maintained for $\leq 3$ h. During the recordings, we repeatedly estimated $g_j$, $r_m$, and $R_m$, with one set of measurement points obtained at intervals of $\sim 20$ s. For most cells, all three parameters were stable over time, as is illustrated in Fig. 3 for a coupled cell pair recorded for $> 120$ min. For five cell pairs, we calculated an average holding current was 2.6 $\pm$ 3.4 pA ($n = 10$) and changed little over the time course of the recordings (Fig. 3D).

Changes of passive membrane properties of AII amacrine cells during pharmacological block of electrical coupling

We next examined the changes in passive membrane properties of AII amacrine cells when the gap junction coupling between these cells, and between these cells and on-cone bipolar cells, was gradually blocked. To achieve this, we applied the gap junction blocker MFA (100 $\mu$M) during paired recordings of coupled AII amacrine. MFA blocks both tracer coupling (Pan et al. 2007) and functional electrical coupling between AII amacrine cells (Veruki and Hartveit 2009). An example of the action of MFA can be seen in Fig. 4 where the electrical coupling was completely blocked after $\sim 20$ min of application. For each measurement of $g_j$, we also calculated $r_m$ (average of $r_{m1}$ and $r_{m2}$) and $R_m$ (average of $R_{m1}$ and $R_{m2}$) and monitored $I_{hold}$ (Fig. 4). During application of MFA, $r_m$ and $R_m$ slowly increased and reached a maximum at approximately the point in time when the electrical coupling was abolished ($g_j \sim 0$). For this cell pair, $r_{m1}$ changed from $\sim 390$ MΩ in the control condition to $\sim 1,560$ MΩ after complete block of electrical coupling and $R_{m1}$ changed from $\sim 330$ MΩ in the control condition to $\sim 1,560$ MΩ after complete block by MFA (Fig. 4B).

Similar changes were observed for $r_{m2}$ and $R_{m2}$ (Fig. 4C). For six cell pairs, $r_m$ changed from 760 $\pm$ 104 MΩ (range: 390–1,490 MΩ) in the control condition to 4,430 $\pm$ 510 MΩ (range: 1,500–6,460 MΩ) after complete block of electrical coupling with MFA (monitored by repeated measurements of $g_j$: $n = 12$; $P = 8.2 \times 10^{-6}$; Fig. 4E). For $R_m$, the corresponding change was from 620 $\pm$ 76 MΩ (range: 330–1,070 MΩ) in the control condition to 4,330 $\pm$ 490 MΩ (range: 1,500–6,450 MΩ) after complete block of electrical coupling with MFA ($P = 6.2 \times 10^{-6}$; Fig. 4E). $R_m$ was 83 $\pm$ 1% of the value of $r_m$ in the control condition and 98 $\pm$ 1% of the value of $r_m$ after complete block of electrical coupling with MFA ($n = 12$). Comparing $R_m$ in the control condition with $r_m$ after complete block of electrical coupling, indicated that the nongap junctional conductance was 16 $\pm$ 2% of the total input conductance. Following application of MFA, $I_{hold}$ of both cells changed to a more positive level (Fig. 4D), reaching a peak near the point in time when $g_j = 0$. $I_{hold}$ of both cells returned to a more negative value during wash-out of MFA. For the 12 cells, $I_{hold}$ changed from $-4.5 \pm 3.4$ pA in the control condition to 10.7 $\pm 2.9$ pA in the presence of MFA ($P = 1.2 \times 10^{-4}$).

Does MFA have a direct effect on membrane resistance of AII amacrine cells?

The increase of $r_m$ and $R_m$ for AII amacrine cells evoked by MFA is consistent with and can be explained by block of electrical coupling. From previous work we know that MFA blocks electrical coupling not only between AII amacrine cells but also between AII amacrine cells and on-cone bipolar cells (Veruki and Hartveit 2009). However, it is also known that MFA and related fenamates modulate a diversity of ion chan-
nels in addition to connexons. For example, MFA inhibits hKv2.1 potassium channels (Lee and Wang 1999), opens KCNQ2/Q3 potassium channels (Peretz et al. 2005), and stimulates BKCa channel activity (Wu et al. 2001).

To investigate the potential effect of MFA on ion channels active in AII amacrine cells at the voltages used for measurement of $g_j$, and separate such effects from the effect of MFA on electrical coupling, we isolated nucleated patches from AII amacrine cells. For each patch, we sampled electrical coupling, we isolated nucleated patches from AII amacrine cells. For each patch, we sampled $I-V$-curves repeatedly with application of voltage pulses from a $V_{\text{hold}}$ of $-60$ mV ($-30$ to $+10$ mV relative to $V_{\text{hold}}$; $10$-mV increment per sweep; Fig. 5). A complete $I-V$ curve was sampled approximately every 20 s. After a baseline period of $3$–$5$ min, we applied MFA (100 $\mu$M) for $5$–$15$ min, followed by a recovery period. As illustrated by the example in Fig. 5, MFA had no consistent effect on either $r_m$ or $I_{\text{hold}}$. Similar results were observed for six other nucleated patches. $r_m$ was $14 \pm 5$ G$\Omega$ in the control condition and $13 \pm 5$ G$\Omega$ in the presence of MFA. $I_{\text{hold}}$ was $-3.9 \pm 1.4$ pA in the control condition and $-3.6 \pm 1.6$ pA in the presence of MFA ($P > 0.3$ for both $r_m$ and $I_{\text{hold}}$; $n = 7$ patches). The period of MFA application was shorter than that required for complete block of $g_j$ but longer than the time required for onset of block of $g_j$ (Veruki and Hartveit 2009).

**Passive membrane properties of AII amacrine cells**

To obtain a simple estimate of the specific membrane resistance of AII amacrine cells, we need measurements of both the total $r_m$ and the total membrane surface area. Because our measurements of $r_m$ were obtained under conditions of blocked electrical coupling, they should reflect properties of the nongap junctional membrane. Because recordings with DSEVC amplifiers do not provide estimates of cell capacitance, we filled AII amacrine cells with biocytin to obtain morphological estimates of the surface area. To minimize diffusion of tracer from the

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**FIG. 4.** Changes in apparent $r_m$ and $R_m$ of AII amacrine cells accompany block of electrical coupling by meclofenamic acid (MFA). $A$: $g_j$ as a function of time for a pair of electrically coupled AII amacrine cells (dual whole cell voltage-clamp recording with DSEVC amplifiers). $g_j$ is calculated as the average of the conductance values measured for each direction of coupling (with voltage pulses applied to either cell 1 or 2). MFA was applied in the extracellular solution during the period indicated by the shaded area (duration $\sim 22$ min). Top: recording configuration with dots extending laterally from resistors attached to each cell symbolizing electrical coupling to other AII amacrine and on-cone bipolar cells. Same cell pair in A–D. $B$ and $C$: apparent $r_m$ and $R_m$ for cell 1 (B) and cell 2 (C) as a function of time in the control condition, during application of MFA, and during washout of MFA. For both cells, $r_m$ and $R_m$ increased during application of MFA. Notice that for both cells, $R_m$ is smaller than $r_m$ in the control condition (see expanded graphs in insets) but becomes similar to $r_m$ during application of MFA and the 2 parameters become virtually identical when electrical coupling is blocked ($g_j = 0$). D: $I_{\text{hold}}$ for cells 1 and 2 as a function of time. E: average of $r_m$ and $R_m$ for all cells tested in the control condition ($\pm$ SE) and after block of electrical coupling ($g_j = 0$) with MFA (100 $\mu$M; $n$).
recorded cell to other coupled cells, cells were only kept for 5–10 min in the whole cell configuration after which the slices were fixed and processed to develop a visible reaction product. With such brief periods of whole cell recording, we never observed evidence for tracer coupling from the injected cell. Following morphological reconstruction, surface areas were calculated with Neurolucida Explorer. The morphological projections of six successfully reconstructed AII amacrine cells are illustrated in Fig. 6. The average surface area was $923 \pm 42 \mu m^2$ (range: 803–1,106 \mu m^2). If we assume the standard value of 0.01 pF/\mu m^2 for specific membrane capacitance (Gentet et al. 2000; Hille 2001; Major 2001), this corresponds to an average capacitance of $9.2 \pm 4.2 \mu F$ (range: 8.0–11.1 \mu F) for noncoupled AII amacrine cells.

With $r_m$ for isolated AII amacrine cells ranging between 1.5 and 6.5 G\Omega and the surface area ranging between 803 and 1,106 \mu m^2, the specific membrane resistance ($R_m$) can be estimated as 17–52 k\Omega cm^2 (assuming that surface area and $r_m$ are inversely correlated). From the average values of surface area and $r_m$, we obtain an average value for $R_m$ of 41 k\Omega cm^2. From these results, we can estimate a membrane time constant ($r_m = R_m \times C_m$) of 41 ms (range: 17–52 ms), again assuming a specific membrane capacitance of 0.01 pF/\mu m^2.

**DISCUSSION**

In this study we have presented estimates of the passive membrane properties of AII amacrine cells in the rat retina. Because these cells are electrically coupled to each other and to ON-cone bipolar cells, it is technically challenging to estimate their passive membrane properties. We used MFA to completely block the electrical synapses between AII amacrine cells and between AII amacrine and ON-cone bipolar cells (Pan et al. 2007; Veruki and Hartveit 2009) and used voltage-clamp responses measured after complete block of coupling to estimate the total resistance of the nongap junctional membrane of AII amacrine cells which ranged from 1.5 to 6.5 G\Omega. In a parallel set of experiments, we filled single AII amacrine cells with biocytin and, after developing the reaction product, reconstructed the morphology of AII amacrine cells. From the quantitative morphological data, we obtained estimates of total surface area which ranged from ~800 to ~1,100 \mu m^2 with an average of ~920 \mu m^2. Combining the estimates of membrane resistance and surface area allowed us to constrain the specific membrane resistance to the range 17–52 k\Omega cm^2 and the single-cell capacitance to 8–11 \mu F.

**Passive responses in the presence and absence of electrical coupling**

There is strong evidence from ultrastructural studies that AII amacrine cells are coupled via gap junctions to other cells of the same type and to ON-cone bipolar cells (Chun et al. 1993; Kolb 1979; Strettoi et al. 1992, 1994; Vardi and Smith 1996). Corresponding to this, injection of single AII amacrine cells with neurobiotin or biocytin can demonstrate tracer coupling to other AII amacrines and to ON-cone bipoars in whole-mount preparations.
preparations (Hampson et al. 1992; Mills and Massey 1995; Vaney 1991), and there is evidence that electrical coupling is present in in vitro slice preparations as well (Trexler et al. 2005; Veruki and Hartveit 2002a,b).

In the present study, we observed a strong increase of apparent membrane resistance after application of a concentration of MFA (100 μM) that has been demonstrated to be sufficient to completely block electrical coupling between AII amacrine cells and between AII amacrine and on-cone bipolar cells (Veruki and Hartveit 2009). Because we recorded from pairs of electrically coupled AII amacrine cells, we could directly correlate the increase of apparent \( r_m \) with the block of electrical coupling by monitoring \( g_j \) between the recorded cells. If we make the reasonable assumption that MFA blocks not only the electrical synapses between the two coupled AII amacrine cells in the recorded pair, but all other electrical synapses in the slice potentially sensitive to MFA, including those between either recorded cell and the other nonrecorded cells to which they are directly coupled, one would indeed expect that the apparent membrane resistance of each cell would increase.

The DSEVC amplifiers used in these recordings do not provide explicit information about the capacitance of the recorded cells. However, if the apparent capacitance “seen” by a CSEVC amplifier in a conventional whole cell recording of an electrically coupled AII amacrine cell is measurable influenced by the electrical coupling, it is reasonable to expect that the apparent capacitance should be reduced after application of MFA. Although capacitative currents evoked by square-wave pulses in AII amacrine cells decay with a time course that cannot be satisfactorily described by a single-exponential function (Veruki and Hartveit, unpublished observations), the estimate of apparent capacitance obtained by the neutralization circuitry of a CSEVC amplifier should still reflect the effective surface area. In several previous studies with presumed normal electrical coupling, we have obtained whole cell capacitance measurements of AII amacrine cells with CSEVC amplifiers of \( \sim 14.5 \) pF [14.6 ± 0.4 pF; \( n = 89 \) cells (Veruki et al. 2003); 14.4 ± 0.4 pF; \( n = 62 \) cells (Veruki et al. 2008)]. With a specific membrane capacitance of 0.01 pF/μm², this corresponds to an average surface area of \( \sim 1,450 \) μm², a value that is \( \sim 160\% \) of the value obtained from quantitative, morphological reconstruction after filling cells with biocytin (average: \( \sim 920 \) μm²) and suggests that the measurements obtained in the control condition do indeed reflect the presence of electrical coupling. Because cells used for morphological reconstruction were kept in the whole cell configuration for a short time (5–10 min), it is likely that the light-microscopic morphology was relatively unaffected by diffusion of biocytin across electrical synapses to processes of neighboring coupled cells.

Specific resistance of nongap junctional membrane of AII amacrine cells

The results obtained in this study suggest that for AII amacrine cells, the nongap junctional conductance constitutes \( \sim 16\% \) of the total input conductance of a single cell. We obtained an approximate estimate for the specific membrane resistance of the nongap junctional membrane of AII amacrine cells by relating the total membrane resistance measured after blocking electrical coupling to the total surface area obtained after light-microscopic morphological reconstruction of single cells. The estimated range, 17–52 kΩ cm², is similar to estimates from rod bipolar cells (average: \( 25 \pm 19 \) kΩ cm²), obtained with considerably more accurate methods, involving compartmental modeling and directly fitting the current responses of individual models (based on morphological reconstruction) evoked by voltage pulses to the physiologically recorded responses from the same cells (Oltedal et al. 2009).

Experiments where we applied MFA to nucleated patches from AII amacrine cells suggested that MFA had no effect on the nongap junctional membrane, but there remains the possibility that MFA might have an effect on nonconnexon ion channels expressed in the dendrites of these cells (which would not be contained in nucleated patches). It will be technically challenging to investigate this experimentally as it does not seem possible to isolate AII amacrine cells intact from the retina.

**Passive membrane parameters of AII amacrine cells**

Cyttoplasmic resistivity \( (R_J) \), \( C_m \), and \( R_m \) are important parameters that influence the integrative properties and summation of synaptic inputs in single neurons. While there seems to be little variability in estimates of \( C_m \), which is often regarded as a biological constant (Hille 2001; Major 2001), there is considerably more variability in estimates of \( R_m \) and \( R_J \) (Major 2001; Spruston et al. 2008). With respect to \( R_J \), it has been difficult to design experiments that can estimate this parameter with high accuracy. In experiments with simultaneous somatic and dendritic whole cell recordings from layer 5 neurons in cerebral cortex (Stuart and Spruston 1998), CA1 pyramidal neurons (Golding et al., 2005), and cerebellar Purkinje neurons (Roth and Häusser 2001), computer modeling has indicated values for \( R_J \) between 70 and 200 Ω cm. The values obtained from these experiments have been considered to represent the most reliable estimates of \( R_J \) because the filtering of transient voltage changes in dendrites is very sensitive to the value of \( R_J \) (Spruston et al. 2008). Consistent with this, the modeling study of Oltedal et al. (2007) found that the decay of capacitative charging transients obtained in axon terminal recordings from retinal rod bipolar cells, as opposed to somatic recordings, was strongly influenced by changes of \( R_J \). In experiments with axon terminal recordings and morphological reconstruction followed by compartmental model fitting, our average estimate for \( R_J \) was \( \sim 130 \) Ω cm (Oltedal et al. 2009), very similar to the values obtained in the experiments with simultaneous somatic and dendritic recording from single cells.

It will be technically challenging to perform similar experiments to obtain more accurate estimates of \( R_m \), \( C_m \), and \( R_m \) for AII amacrine cells. Two obvious extensions of our experiments are unfortunately unlikely to provide the required answers. First, if one attempts to perform correlated physiological and morphological analysis on single cells, with recording of physiological responses and morphological reconstruction after filling the cells with biocytin, the long time required for MFA to block electrical coupling (Veruki and Hartveit 2009) means that biocytin would have time to diffuse to electrically coupled cells and ambiguate the cellular identity of filled processes during morphological reconstruction. Second, while blocking electrical coupling with MFA before establishing the recording could be a feasible strategy, we have been unable to obtain GΩ seals when MFA was already added to the extracellular solution (Veruki and Hartveit, unpublished observations). An alternative strategy for correlated physiological and morpholog-
ical analysis of single AII amacrine cells could be to obtain quantitative morphological analysis with high-resolution fluorescence microscopy based on multi-photon excitation microscopy (e.g., Schmidt-Hieber et al. 2007). If the fluorescent tracer used to obtain the cellular morphology does not permeate the electrical synapses between all amacrine cells, or between all amacrine and ON-cone bipolar cells, the long time required to block electrical coupling with MFA would be relatively unimportant.

Finally, it is possible that the use of genetically modified animals with deleted expression of Cx36 (e.g., Deans et al. 2002) could be helpful in estimating the passive membrane parameters of noncoupled all amacrine cells. While potentially very informative, such experiments will require detailed verification that the genetic modification has not altered other important functional or structural parameters (see e.g., De Zeeuw et al. 2003). It is also of concern that some of the heterologous gap junctions between all amacrine cells and ON-cone bipolar cells contain Cx45 in addition to Cx36 (Dedek et al. 2006; Han and Massey 2005; Lin et al. 2005; Maxeiner et al. 2005), suggesting that electrical coupling might not be completely abolished in Cx36 knockout animals.

Passive membrane parameters and synaptic integration in AII amacrine cells

All amacrine cells receive excitatory, glutamatergic input from rod bipolar cells at the arboreal dendrites and from some OFF-cone bipolar cells at the lobular appendages (Strettoi et al. 1992, 1994), and there is physiological evidence for the operation of both inputs (Singer and Diamond 2003; Veruki et al. 2003). All amacrine cells also receive inhibitory input from amacrine cells, presumably GABAergic and glycineric, at various cellular compartments, and there is functional evidence for glycineric, but not yet GABAergic, synaptic input (Gill et al. 2006; Weiss et al. 2008). Little is known, however, concerning the integration of the various types of chemical synaptic input to all amacrine cells. Accurate estimates of the passive membrane properties of all amacrine cells will be required for realistic modeling of signal integration and processing in neural networks involving these cells and their synaptic partners, including the extent and manner in which synaptic integration is influenced by the strength of electrical coupling. The importance of these questions is underscored by the extensive evidence for physiological regulation of the strength of electrical coupling between all amacrine cells (Bloomfield and Völgyi 2009; Hampson et al. 1992; Kothmann et al. 2009; Mills and Massey 1995; Urschel et al. 2006).

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