Electrical Synapses Between AII Amacrine Cells: Dynamic Range and Functional Consequences of Variation in Junctional Conductance

Margaret Lin Veruki, Leif Oltedal, and Espen Hartveit
Department of Biomedicine, University of Bergen, Bergen, Norway

Submitted 23 August 2008; accepted in final form 10 October 2008


All amacrine cells form a network of electrically coupled interneurons in the mammalian retina and tracer coupling studies suggest that the junctional conductance ($G_j$) can be modulated. However, the dynamic range of $G_j$ and the functional consequences of varying $G_j$ over the dynamic range are unknown. Here we use whole cell recordings from pairs of coupled AII amacrine cells in rat retinal slices to provide direct evidence for physiological modulation of $G_j$, appearing as a time-dependent increase from about 500 pS to a maximum of about 3,000 pS after 30–90 min of recording. The increase occurred in recordings with low- but not high-resistance pipettes, suggesting that it was related to intracellular washout and perturbation of a modulatory system. Computer simulations of a network of electrically coupled cells verified that our recordings were able to detect and quantify changes in $G_j$ over a large range. Dynamic-clamp electrophysiology, with insertion of electrical synapses between AII amacrine cells, allowed us to finely and reversibly control $G_j$ within the same range observed for physiologically coupled cells and to examine the quantitative relationship between $G_j$ and steady-state coupling coefficient, synchronization of subthreshold membrane potential fluctuations, synchronization and transmission of action potentials, and low-pass filter characteristics. The range of $G_j$ values over which signal transmission was modulated depended strongly on the specific functional parameter examined, with the largest range observed for action potential transmission and synchronization, suggesting that the full range of $G_j$ values observed during spontaneous run-up of coupling could represent a physiologically relevant dynamic range.

INTRODUCTION

Through their strategic position in the neural circuitry that constitutes the rod pathway of the mammalian retina, AII amacrine cells integrate and transmit scotopic visual signals (reviewed by Bloomfield and Dacheux 2001) and recent evidence suggests that they may also play a role in photopic (cone) vision (Manookin et al. 2008). Electron microscopic investigations have demonstrated that AII amacrine cells are connected via gap junctions to other AII amacrine cells (homologous connections) and to ON-cone bipolar cells (heterologous connections; Chun et al. 1993; Kolb 1979; Kolb and Famiglietti 1974; McGuire et al. 1984; Strettoi et al. 1992, 1994). There is evidence that both sets of gap junctions correspond functionally to electrical synapses (Trexler et al. 2005; Veruki and Hartveit 2002a,b). Although the electrical synapses between AII amacrine cells and cone bipolar cells are considered important for transmitting scotopic visual signals into the ON-pathway, the functional role of the electrical synapses between AII amacrine cells is less clear.

Understanding the functional role of electrical coupling between AII amacrine cells will require detailed, quantitative information about the strength of the electrical coupling and how it affects the biophysical and signal transmission characteristics of AII amacrine cells. For example, theoretical work has suggested that the electrical coupling could be important to remove noise from the visual signal (Smith and Vardi 1995; Vardi and Smith 1996; see also Lamb and Simon 1976) and that the performance of the noise-removal operation critically depends on the magnitude of the junctional conductance ($G_j$). Similarly, in recent theoretical and experimental studies of electrically coupled cells in other systems, the magnitude of $G_j$ was found to be important for the functional consequences of electrical coupling (e.g., Mancilla et al. 2007). There is also indirect evidence that the strength of electrical coupling between AII amacrine cells (measured as the spatial extent of tracer coupling) is influenced by the adaptational state of the retina (Bloomfield and Völgyi 2004; Bloomfield et al. 1997), possibly mediated by a concomitant change in the release of dopamine from specific amacrine cells (Hampson et al. 1992; Mills and Massey 1995; Xia and Mills 2004). Unfortunately, quantitative data concerning the conductance of the electrical coupling are difficult to obtain. It is even more difficult to obtain data concerning the quantitative relation between the strength of electrical coupling and the corresponding functional transmission characteristics. The estimates for $G_j$ between pairs of coupled AII amacrine cells in rat retina range from about 300 to about 1,500 pS (Veruki and Hartveit 2002a). These results raise a number of important questions. For example, does the range of observed $G_j$ values correspond to the total range over which $G_j$ can vary? To which extent does the range reflect variability in the strength of direct coupling and to which extent does it reflect variability in the number of additional, indirect pathways between the two recorded cells? Is it possible to directly demonstrate an up- or down-regulation of $G_j$ between electrically coupled AII amacrine cells? If so, how does this change the corresponding functional coupling characteristics?

Here, we report a spontaneous increase of $G_j$ during whole cell recordings of pairs of physiologically coupled AII amacrine cells. The increase occurred in recordings with low- but not high-resistance pipettes, suggesting that it was related to washout of the intracellular environment and perturbation of a physiological control system. We interpreted this increase as a
dynamic range within which $G_j$ may be physiologically modulated. To test the robustness of our measurements of $G_j$, we complemented the physiological recordings with theoretical simulations of simplified networks of electrically coupled cells. Although the spontaneous increase of $G_j$ suggested the presence of a physiological control mechanism, it also compromised our attempts to systematically correlate electrical coupling strength and signal transmission characteristics under stable and controlled conditions. Instead, we used dynamic-clamp electrophysiology (Robinson and Kawai 1993; Sharp et al. 1993; reviewed by Goyal and Marder 2006) to insert artificial electrical synapses between pairs of AII amacrine cells (not coupled to each other) and examined how the transmission properties varied when the conductance was systematically varied over its estimated dynamic range. Where possible, the results from dynamic-clamp experiments were compared with corresponding measurements from physiologically coupled cells.

METHODS

General aspects of the methods have previously been described in detail (Hartveit 1996; Veruki and Hartveit 2002a). Albino rats (4–7 wk postnatal) were deeply anesthetized with halothane or isoflurane in oxygen and killed by cervical dislocation (procedure approved under the surveillance of the Norwegian Animal Research Authority). Retinal slices were visualized with an infrared differential interference contrast videomicroscopy (Olympus BX51WI). 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). In some experiments, low extracellular Ca$^{2+}$ (0.15 mM) was used to reduce the frequency of spontaneous excitatory postsynaptic currents in AII amacrine cells (Veruki et al. 2003), in which case the concentration of Mg$^{2+}$ (3.35 mM) was increased correspondingly to 3.5 mM. The recording pipettes (5–7 MΩ for low-resistance pipettes; 24–30 MΩ for high-resistance pipettes) were pulled from borosilicate glass (GC150-11; Harvard Apparatus, Edenbridge, UK) and were filled with the following solution (in mM): 125 K-gluconate, 10 NaCl, 1 CaCl$_2$, 5 EGTA, 10 Heps, and 4 MgATP (pH was adjusted to 7.3 with KOH). Lucifer yellow was added at a concentration of 1 mg/ml. For perforated-patch recordings, we used low-resistance pipettes (5–7 MΩ) and added amphotericin B (Sigma; solubilized formulation) at a concentration of 0.5 mg/ml. Fresh aliquots were prepared at least every 3 h. The theoretical liquid junction potential was calculated with the computer program JPCalcW (Molecular Devices, Sunnyvale, CA) and membrane holding potentials were corrected for the liquid junction potential on-line or off-line.

For most recordings, a-amino-3-hydroxy-5-methyl-4-isoxazolopro-pionic acid (AMPA), γ-aminobutyric acid type A (GABA$_A$), and glycine receptors were blocked by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), bicuculline, and strychnine, respectively.

Drugs were added directly to the extracellular solution used to perfuse the slices. The concentrations of drugs were as follows (in μM; supplier Tocris Bioscience, Avonmouth, UK): 10 bicuculline methochloride, 10 CNQX disodium salt, 1 strychnine, 0.3 tetrodotoxin (TTX).

Electrophysiological recording and data acquisition

Whole cell, continuous single-electrode voltage- and current-clamp recordings with standard, low-resistance pipettes (5–7 MΩ) were performed with EPC9-dual or EPC10-triple patch-clamp amplifiers controlled by Pulse or PatchMaster software (HEKA Elektronik, Lambrecht/Pfalz, Germany). When targeting pairs of AII amacrine cells for recording, we established the cell-attached mode for both cells before breaking into either cell to reduce, as far as possible, differential washout of the intracellular environment in the two cells. When in voltage clamp, cells were held at a common membrane potential ($V_{m,\text{hold}}$) of −60 mV. For about 90% of the cells, the holding current at −60 mV was between −15 and +5 pA. If a cell developed a holding current > ±40 pA, subsequent data were not included in the analysis. Before sampling, signals were low-pass filtered (analog three- and four-pole Bessel filters in series) with a corner frequency (~3 dB) between one fifth and one third of the inverse of the sampling interval (10–100 μs). Currents caused by the recording pipette capacitance ($C_{pip}$) and the cell membrane capacitance ($C_{cell}$) were measured with the automatic capacitance neutralization circuitry of the amplifier. For each such measurement during dual voltage-clamp recording of electrically coupled cells, the test pulse stimuli were sent simultaneously to both recording amplifiers to eliminate junctional currents between the two cells. The average nominal capacitance for AII amacrine cells was 14.4 ± 0.4 (SE) PF ($n = 62$). Throughout these whole cell recordings, the series resistance ($R_s$) in both cells was regularly monitored by applying a series of 20-mV hyperpolarizing voltage pulses (16-ms duration). During such stimulation, the $C_{cell}$ neutralization circuitry of the amplifier was transiently disabled and the stimulus was simultaneously sent to both recording amplifiers. Capacitative transients were analyzed off-line by averaging consecutive responses (typically 20) and fitting the decay with triple-exponential functions to estimate the peak capacitative current and calculate $R_s$. The average $R_s$ was 21 ± 1 MΩ ($n = 62$).

In a second set of experiments, we used high-resistance pipettes (24–30 MΩ) with long, thin tips to obtain whole cell recordings of electrically coupled cells under conditions of reduced intracellular washout. A higher access resistance during recording will result in slowed diffusional exchange between the intracellular environment and the patch pipette (Pusch and Neher 1988; see also Horn and Korn 1992; Marty and Neher 1995). In these experiments, voltage clamp was achieved by using two discontinuous (switched) single-electrode voltage-clamp (DSEVC) amplifiers (SEC-05LX-BF; npi electronic, Tamm, Germany), each controlled by one of two instances of PatchMaster running on a single computer. These amplifiers switch between current injection and potential measurement at high frequency (e.g., Halfiwel 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 $R_s$ are reduced and potentially totally avoided. The switching frequency was set to about 30 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 four-pole Bessel filter) with a corner frequency (~3 dB) one fifth of the inverse of the sampling interval (100 μs). The voltage-clamp 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 LIH-1600 or ITC-18 laboratory interface (HEKA Elektronik). Synchronization of sampling by the two interfaces was controlled by hardware triggering via a digital line.

Conductance injection

We implemented artificial electrical synapses between pairs of recorded cells by conductance injection (dynamic-clamp) electrophys-
Electrical Synapses Between AII Amacrine Cells

The duration over which the spikes occurred, recordings from electrically coupled cells expressing Cx36, voltage dependence was implemented for the injected balance compensation (10- were performed with an EPC10-triple amplifier with 100% bridge- was determined from a fit by a Gaussian function and Yarom 1999). The time delay of the central peak of each spike dence level) divided by the corresponding integral limited by the correlogram peak over the synchronization width (at the 99% confi- Synchronization strength was calculated as the integral of the cross- width was defined as the width of the cross-correlogram peak at either is the total number of spikes in the reference cell. The synchronization coefficient was calculated from the membrane potentials of cell 1 and cell 2, respectively. The same value of $G_1$ was used for both directions of coupling ($G_{1,2}$ and $G_{2,1}$), given the evidence for symmetrical conductances between all amacrine cells (Veruki and Hartveit 2002a). No voltage dependence was implemented for the injected $G_1$, based on the evidence that the coupling between all amacrine cells is mediated by homomeric connexons containing connxin36 (C36; see discussion). In recordings from electrically coupled cells expressing C36, $G_1$ was almost unchanged over a voltage range of about ±80 mV (Srinivas et al. 1999; Teubner et al. 2000). For the SM-1 analog system, the rise time (10–90%) of a current command for a change in voltage is stated by the manufacturer as 290 ns. The SM-2 system was run with a sampling interval of 20 μs. All conductance injection experiments were performed with an EPC10-triple amplifier with 100% bridge-balance compensation (10-μs time constant).

General data analysis

Data were analyzed with FitMaster and PulseFit (HEKA Elektronik), IGOR Pro (WaveMetrics, Lake Oswego, OR), AxoGraph X (AxoGraph Scientific, Sydney, Australia), and DataView (Dr. W. J. Heitler, University of St. Andrews, UK).

Discrete cross-correlations were determined from trains of action potentials by calculating the temporal interval from the time of occurrence of each action potential in the reference cell to the time of occurrence of all action potentials in the nonreference cell. The intervals were binned (1-ms resolution) and used to generate a cross-correlation histogram where the value of each bin indicated the number of action potentials that occurred in the nonreference cell. For two neurons firing randomly, the expected level of correlation was calculated as

$$N_1 \times (1/T) \times b \times N_2$$

where $N_1$ is the total number of spikes in the nonreference cell, $T$ is the duration over which the spikes occurred, $b$ is the bin width, and $N_2$ is the total number of spikes in the reference cell. The synchronization width was defined as the width of the cross-correlogram peak at either the 99 or the 95% confidence level (Mann-Metzer and Yarom 1999). Synchronization strength was calculated as the integral of the cross-correlogram peak over the synchronization width (at the 99% confidence level) divided by the corresponding integral limited by the expected level of correlation (cf. Brivanlou et al. 1998; Mann-Metzer and Yarom 1999). The time delay of the central peak of each spike cross-correlogram was determined from a fit by a Gaussian function of the form (Brivanlou et al. 1998)

$$R = b + a \exp[-(t - d)^2/2w^2]$$

where $b$ is the baseline firing rate, $a$ is the amplitude of the peak, $w$ is the SD of the peak, and $d$ is the delay of the peak relative to zero time delay.

Normalized cross-correlograms of continuous membrane potential recordings were calculated as the correlation of the two records divided by the number of points and the SD of each voltage record. Accordingly, the cross-correlation amplitude can vary between +1 (100% correlated) and −1 (100% anticorrelated) and depends only on the degree of synchrony between the voltage records and not on their absolute amplitude. Sliding, color-coded two-dimensional (2D) cross-correlograms of pairs of voltage records of duration $T$ were calculated from $N$ consecutive pairs of data segments, each of duration $T/N$ s and shifted 100 ms forward in time relative to the previous segment. Each segment was mean-subtracted before calculating the cross-correlation, which is therefore mathematically equivalent to the cross-covariance. A matrix was then constructed where consecutive one-dimensional cross-correlation functions constitute consecutive columns with time running along the x-axis and the time lag of the correlation function running along the y-axis. The normalized correlation amplitude was coded by color. The time-averaged cross-correlogram was calculated as the average of each row of the 2D cross-correlogram.

For analysis of the low-pass filter transmission characteristics between pairs of all amacrine cells coupled via dynamic-clamp, we applied sinusoidal current stimuli of varying frequencies (1–1000 Hz) and calculated the coupling coefficient and phase lag for each frequency tested. The phase shift was calculated by cross-correlating the postsynaptic response with the presynaptic response and converting the temporal delay to phase by multiplying by 2π/$T$, where $T$ is the period of the stimulus. For each frequency, the coupling coefficient was normalized to the coupling coefficient for responses evoked at 1 Hz in the same cell pair.

Data were presented as means ± SE ($n =$ number of cells or cell pairs) and percentages are presented as percentage of control. Statistical analyses were performed using Student’s two-tailed r-tests and differences were considered statistically significant at the $P < 0.05$ level. For illustration purposes, most raw data records were low-pass filtered (digital nonlagging Gaussian filter; −3 dB at 0.5–2 kHz). Unless otherwise noted, the current traces in the figures represent individual traces.

Estimating steady-state coupling coefficient and junctional conductance

A pair of electrically coupled cells (cell 1 and cell 2) can be represented by the equivalent electrical circuit illustrated in Fig. IA (Bennett 1966, 1977; van Rijen et al. 1998). Each cell is represented by a simple resistor–capacitor (RC) circuit with a single lumped resistance ($r_1$ or $r_2$) and capacitance ($C_1$ or $C_2$). The resistance of the electrical synapse is represented by $r_o$, corresponding to a junctional conductance $G_1 (= 1/r_o)$. For the case where each cell is connected via electrical synapses to additional cells (see following text), this would change the effective values for $r_1$ and $r_2$, which would no longer correspond to the true membrane resistances of the cells and will henceforth be termed the nominal membrane resistance. When a current is injected on the presynaptic side, the electrical coupling coefficient is defined as the ratio of the voltage change in the postsynaptic cell to that in the presynaptic cell. The steady-state coupling coefficient in the direction from cell 1 to cell 2 ($k_{12}$) is given by the following equation (Bennett 1966, 1977)

$$k_{12} = \frac{r_2}{r_1 + r_2} \text{ or } k_{12} = \frac{r_2}{r_2 + (1/G_1)}$$

For the calculation of steady-state coupling coefficients between two recorded cells, only hyperpolarizing voltage responses were used. For a given cell pair, we estimated the coupling coefficient as the average of the coupling coefficients calculated for each direction of coupling. To estimate the steady-state $G_1$ between the two cells of a physiologically coupled pair, we used current responses obtained with dual
respectively.

sponding row and column indices, e.g., Cell(1, 1) and Cell(25, 26).

cells, arranged in a rectangular network of 25 rows (0, . . . , 24) and 26 columns, was used. For all simulations in this study, a larger network of 650 cells, arranged in 25 rows and 26 columns, was used.

time step of 1 µs, the results were very similar. For the simulations, we constructed an idealized network consisting of 650 cells containing the injected cell, the steady-state voltage response fell globally to 3 nS. When the responses were measured along the row of cells that were judged to have spatially overlapping, potentially electrical synapses. When AII amacrine cells were filled with biocytin and morphologically reconstructed, the average surface area was 800 ± 48 µm² (n = 3). Assuming a specific membrane capacitance of 0.01 pF/µm² (Hille 2001), the capacitance of a single cell would be about 8 pF. In our NEURON model, each cell was therefore represented by a cylinder with surface area of about 800 µm² (diameter and length set arbitrarily to 15 and 17 µm, respectively). The cytoplasmic resistivity (Rc) was set to 125 Ω·cm (e.g., Hallermann et al. 2003; Oltedal et al., unpublished observations) and the specific membrane resistance (Rm) was set to 26 kΩ·cm². The value for Rm was determined by iterative adjustment in repeated simulations of a rectangular network where the junctional coupling (Gj) was fixed at 700 pS [the average value reported by Veruki and Hartveit (2002a)] until the input resistance of a single cell was about 470 MΩ (the average input resistance recorded at a single AII amacrine cell in the current-clamp configuration; n = 24). Although the value for Rm is reasonable, it does not explicitly take into account the contribution of coupling to on-cone bipolar cells. The reversal potential (Erev) of the leak current (e_pas), was set to −60 mV (the holding potential used during acquisition of the experimental traces and a value close to the resting potential of AII amacrine cells). For simulations of responses in paired recordings of two neighboring coupled cells, we sampled the activity of Cell(12, 12) and Cell(12, 13) in the middle of the network (unless otherwise noted). For dual voltage-clamp recordings, an idealized single-electrode voltage clamp (SEClamp; taken from the standard repertoire of point processes in NEURON) was used to inject current into one of the cells. Before each simulation run, the model was initialized to steady state (Carnevale and Hines 2006). The simulation results were analyzed as the physiological recordings with respect to estimating Gj (from voltage-clamp recordings) and steady-state coupling coefficient (from current-clamp recordings). To investigate whether the network was large enough to avoid boundary effects, we injected a 100-pA current pulse in Cell(12, 12) with Gj set globally to 3 nS. When the responses were measured along the row of cells containing the injected cell, the steady-state voltage response fell to ≤5% of that in the injected cell in Cell(12, 18) and to about 0.6% in the cell at the edge of the network [Cell(12, 25)].

Computer simulations were performed with NEURON (version 6.0.3) running under Mac OS X (10.4) (Carnevale and Hines 2006). All simulations were run with a variable time step (absolute error tolerance set to 0.0001). When we repeated some simulations with a fixed time step of 1 µs, the results were very similar. For the simulations, we constructed an idealized network consisting of 650 cells, arranged in a rectangular network of 25 rows (0, . . . , 24) and 26 columns (0, . . . , 25), where each cell is referred to by its corresponding row and column indices, e.g., Cell(i, j) for the cell located in row i and column j. In this network, each interior cell is connected to its four nearest neighbors (see Fig. 1B for a smaller network of the same type). This is likely to be an underestimation of the average number of AII amacrine cells and on-cone bipolar cells electrically coupled to a single AII amacrine cell. To our knowledge, there are no published reports that have examined this experimentally. We attempted to estimate the number of AII amacrine cells from the distribution of rat AII amacrine cells illustrated by Wässle et al. (1993; their Fig. 6A) by calculating the corresponding Dirichlet domains (e.g., Wässle et al. 1981). For each domain, we counted an average of 5.8 neighboring domains (range 4–8; n = 110 domains). The electrical coupling between neighboring cells was modeled by a linear conductance Gi (NEURON code adapted from software available in the ModelDB database at http://senselab.med.yale.edu/ modeldb/showmodel.asp?model=43039&file=gap-modeldb/gap.hoc; Migliore et al. 2005). In whole cell recordings of AII amacrine cells in the slice preparation, the average capacitance (see earlier text) is likely to overestimate the true capacitance of a single cell because of electrical coupling. When all amacrine cells were filled with biocytin and morphologically reconstructed, the average surface area was 800 ± 48 µm² (n = 3). Assuming a specific membrane capacitance of 0.01 pF/µm² (Hille 2001), the capacitance of a single cell would be about 8 pF. In our NEURON model, each cell was therefore represented by a cylinder with surface area of about 800 µm² (diameter and length set arbitrarily to 15 and 17 µm, respectively). The cytoplasmic resistivity (Rc) was set to 125 Ω·cm (e.g., Hallermann et al. 2003; Oltedal et al., unpublished observations) and the specific membrane resistance (Rm) was set to 26 kΩ·cm². The value for Rm was determined by iterative adjustment in repeated simulations of a rectangular network where the junctional coupling (Gj) was fixed at 700 pS [the average value reported by Veruki and Hartveit (2002a)] until the input resistance of a single cell was about 470 MΩ (the average input resistance recorded at a single AII amacrine cell in the current-clamp configuration; n = 24). Although the value for Rm is reasonable, it does not explicitly take into account the contribution of coupling to on-cone bipolar cells. The reversal potential (Erev) of the leak current (e_pas), was set to −60 mV (the holding potential used during acquisition of the experimental traces and a value close to the resting potential of AII amacrine cells). For simulations of responses in paired recordings of two neighboring coupled cells, we sampled the activity of Cell(12, 12) and Cell(12, 13) in the middle of the network (unless otherwise noted). For dual voltage-clamp recordings, an idealized single-electrode voltage clamp (SEClamp; taken from the standard repertoire of point processes in NEURON) was used to inject current into one of the cells. Before each simulation run, the model was initialized to steady state (Carnevale and Hines 2006). The simulation results were analyzed as the physiological recordings with respect to estimating Gj (from voltage-clamp recordings) and steady-state coupling coefficient (from current-clamp recordings). To investigate whether the network was large enough to avoid boundary effects, we injected a 100-pA current pulse in Cell(12, 12) with Gj set globally to 3 nS. When the responses were measured along the row of cells containing the injected cell, the steady-state voltage response fell to ≤5% of that in the injected cell in Cell(12, 18) and to about 0.6% in the cell at the edge of the network [Cell(12, 25)].

RESULTS

Identification of AII amacrine cells in retinal slices

All amacrine cells were targeted for recording according to the size and location of the cell body in the inner nuclear layer and the thick primary dendrite descending into the inner plexiform layer (Fig. 2A). For recording cell pairs with a high probability of physiological coupling, we targeted neighboring cells that were judged to have spatially overlapping, potentially contacting, arboreal dendrites in the proximal part of the inner
plexiform layer \((n = 19\) cell pairs\). For recording cell pairs with no physiological coupling, we targeted cells with somata that were \(\geq 50 \mu m\) apart \((n = 17\) cell pairs\). After filling the cells with Lucifer yellow, fluorescence microscopy allowed visualization of both cells’ complete morphology (Fig. 2B).

EXCEPT WHERE NOTED, RECORDINGS WERE PERFORMED WITH LOW-RESISTANCE PIPETTES AND CONTINUOUS (CONVENTIONAL) PATCH-CLAMP AMPLIFIERS. (SEE METHODS).

In the following, we first present an analysis of physiological coupling between pairs of neighboring AII amacrine cells and the time-dependent increase of \(G_j\) and steady-state coupling coefficient observed in whole cell recordings with low-resistance pipettes. The physiological experiments are complemented by computer simulations that support the ability of our experiments to estimate \(G_j\) between neighboring cells in a 2D syncytium. Next, we use dynamic-clamp electrophysiology to insert artificial electrical synapses between nonneighboring AII amacrine cells. This technique made it possible to finely and reversibly control \(G_j\) over the same range observed for physiologically coupled cells and allowed us to investigate the quantitative relationship between \(G_j\) and a series of functional coupling characteristics that are likely to be important for signal transmission between AII amacrine cells under physiological conditions.

**Physiological coupling between neighboring AII amacrine cells**

With both cells of a pair in the whole cell voltage-clamp configuration, we tested for electrical coupling by applying voltage steps to one cell (“presynaptic”) and recording the current responses in both the stimulated and the nonstepped (“postsynaptic”) cells (Veruki and Hartveit 2002a). In the presynaptic cell, hyperpolarizing and depolarizing voltage steps evoked inward and outward currents, respectively (Fig. 3A).

When cells were physiologically coupled, the hyperpolarizing and depolarizing voltage steps applied to the presynaptic cell evoked outward and inward currents, respectively, in the postsynaptic cell (Fig. 3A). With both cells of a coupled pair in the current-clamp configuration, depolarizing current pulses applied to the presynaptic cell depolarized both cells and hyperpolarizing current pulses hyperpolarized both cells (Fig. 3B). For electrically coupled cells, we always observed physical overlap between the cells’ dendritic processes in the inner plexiform layer (as visualized with fluorescence microscopy).

For physiologically coupled cells, we estimated \(G_j\) with both cells in voltage clamp by applying a series of small depolarizing and hyperpolarizing voltage pulses to the presynaptic cell and recording the resulting currents in both cells. From plots of the junction current \((I_j)\) versus the junction voltage \((V_j)\), we calculated \(G_j\) as the slope of a straight line fitted to the \(I_j–V_j\) relationship. The conductance for a cell pair was calculated as the average of the conductance values measured in each direction. Similar to previous observations, the conductance was very similar for both directions of measurement (Veruki and Hartveit 2002a).

**Time-dependent increase of junctional conductance**

When physiologically coupled cell pairs were held in the whole cell recording configuration for longer periods of time \((30–90 \text{ min})\), we observed a marked, time-dependent increase of \(G_j\). This is illustrated by the example voltage-clamp traces for a cell pair obtained 5, 35, and 55 min after establishing the whole cell recording configuration (Fig. 4A). After 35 and 55...
min of recording, the responses in the postsynaptic cell were markedly larger than those after 5 min of recording. A similar increase was observed for responses obtained when cell 2 was the presynaptic cell (not shown). For the corresponding $I_j$ versus $V_j$ curves (Fig. 4B), the slope of the curves increased as a function of time, corresponding to an increase in $G_j$, and $G_j$ increased steadily throughout the period of recording (Fig. 4C, arrow). The increase of $G_j$ over time was observed for all cell pairs recorded for $>30$ min ($n = 14$ cell pairs). The average early $G_j$ was $500 \pm 91$ pS (range $140–1,200$ pS; $\sim 3–5$ min after establishing the whole cell configuration) and after $30–90$ min of whole cell recording, the average $G_j$ had increased to $1,800 \pm 290$ pS (range $420–3,500$ pS; $P = 9.1 \times 10^{-5}$; paired t-test). The average increase for the 14 cell pairs was $300 \pm 53\%$ (range $120–670\%$). In Fig. 4C (open circles), the increase of $G_j$ over time is plotted for six of these cell pairs (where we had more than five conductance measurements), illustrating that for some cell pairs, the increase of $G_j$ was almost linear as a function of time. Although the values for nominal membrane resistance and $R_b$ typically varied over the duration of the longer-lasting recordings, the calculation of $G_j$ takes these parameters into account (see METHODS). The increase of $G_j$ was specific to the cell pair recorded from, as opposed to being a global phenomenon that affected the whole population of cells in the preparation. This was revealed by the low magnitude of junctional conductances initially observed for new cell pairs in the same batch of slices. We never observed a consistent reduction of $G_j$ over time.

These recordings of physiologically coupled AII amacrine cells suggested that $G_j$ is subject to run-up during whole cell recording, commencing as soon as the whole cell recording configuration has been established. A likely explanation for the increase is that it is caused by washout of essential intracellular constituents and perturbation of a physiological modulatory system that controls $G_j$ between coupled cells (see DISCUSSION). If this interpretation is correct, it should be possible to reduce the run-up of $G_j$ by decreasing intracellular washout. We first attempted to reduce washout by recording pairs of electrically coupled AII amacrine cells in the perforated-patch configuration. Unfortunately, the success rate of maintaining both cells of a coupled pair in the intact perforated-patch configuration for extended periods of time was too low. Instead, we performed whole cell voltage-clamp recordings from pairs of coupled AII amacrine cells with high-resistance pipettes ($24–30$ MΩ) and DSEVC amplifiers. In this configuration, an increase of $G_j$ over time was not observed for five of five cell pairs, with stable recordings lasting $>100$ min (Fig. 4C, filled circles). For the five cell pairs, the average early $G_j$ was $280 \pm 30$ pS (range $190–360$; averaged over $5$ min). After about $90$ min of recording, the average $G_j$ was $320 \pm 20$ pS (range $270–380$; averaged over $5$ min), not significantly different from the early value ($P = 0.2$; paired t-test). We consider the most likely reason for the absence of time-dependent increase of $G_j$ in these recordings to be reduced intracellular washout following the use of high-resistance pipettes.

If intracellular washout in a paired recording of electrically coupled cells leads to an increase of $G_j$, this raises the question whether there is a comcomitant increase of $G_j$ between either recorded cell and the other nonrecorded cells to which they are directly coupled. To investigate this, we calculated the nominal membrane resistance of both cells in cell pairs recorded for $>30$ min (see earlier text). For the cells recorded with low-resistance pipettes ($n = 14$ cell pairs), the early resistance ($760 \pm 67$ MΩ) was significantly higher than the late resistance ($440 \pm 46$ MΩ; $n = 28$ cells; $P < 0.0001$; paired t-test), consistent with an increase in $G_j$ between each cell and its nonrecorded, coupled neighbors. For the cells recorded with high-resistance pipettes, there was no difference between the early ($850 \pm 80$ MΩ) and late ($800 \pm 99$) nominal membrane resistance ($n = 10$ cells; $P = 0.44$; paired t-test).
Estimating junctional conductance of electrically coupled cells in two-cell circuits versus 2D syncitia

Whereas the experimental measurements obtained from neighboring pairs of electrically coupled AII amacrine cells suggested that \( G_j \) increased over time in whole cell recordings with low-resistance pipettes, there are some methodological problems that are likely to influence our estimates. First, it is unclear to what extent the correction procedures for estimating \( G_j \) in paired recordings work as expected. Second, the two-cell circuit (Fig. 1A) is an obvious simplification compared with a 2D network (Fig. 1B), both because each cell is likely to be connected to additional cells and because there will be indirect pathways for current flow between the two cells in addition to the direct pathway. This potentially challenged our ability to obtain correct measurements of \( G_j \) between coupled pairs of cells, irrespective of whether \( G_j \) was constant or subject to time-dependent increase. The condition with run-up is complicated by possible differences between the local \( G_j \) between the two cells of a coupled pair and the global \( G_j \) in the rest of the network. The degree of such spatial heterogeneity in networks of coupled AII amacrine cells is unknown.

To investigate the influence of these factors on our measurements, we simulated voltage-clamp recordings from a pair of neighboring cells centrally located in an idealized network of rectangularly arranged unbranched cells (see Fig. 1B). Each cell was configured to roughly mimic the membrane resistance and capacitance of a single AII amacrine cell (see METHODS), but could in principle also represent an ON-cone bipolar cell. In the simulations, we varied \( G_j \) between the two cells (\( G_j, \text{local} \)) for a series of conditions that differed with respect to the \( G_j \) of all other electrical synapses in the network (\( G_j, \text{global} \)). As in the physiological recordings, the presynaptic cell was stepped from its holding potential and the resulting currents were measured in this cell and in the postsynaptic cell. For each combination of \( G_j, \text{local} \) and \( G_j, \text{global} \), we estimated \( G_j, \text{local} \) both with and without correction for finite nominal membrane resistance and nonzero series resistance. Figure 5, A–E shows the results for five different conditions (\( G_j, \text{global} = 0, 200, 700, 1,500, \) and \( 3,000 \, \text{pS} \)). For each condition, \( G_j, \text{local} \) was varied in steps of \( 200 \, \text{pS} \) between \( 0 \) and \( 3,000 \, \text{pS} \). For \( G_j, \text{global} = 0 \, \text{pS} \), corresponding to the simple two-cell network (Fig. 1A), it can be seen that without correction (Fig. 5A, open circles), \( G_j, \text{local} \) was consistently underestimated except for values \( \leq 1,000 \, \text{pS} \) and that the errors were eliminated by the correction procedure (Fig. 5A, filled circles). Very similar results were obtained for \( G_j, \text{global} = 200 \, \text{pS} \) (Fig. 5B) and \( 700 \, \text{pS} \) (Fig. 5C); at \( 700 \, \text{pS} \), the results obtained with correction slightly overestimated \( G_j, \text{local} \), indicating a contribution of the indirect pathways for current flow. For \( G_j, \text{global} = 1,500 \) and \( 3,000 \, \text{pS} \), the contribution of the indirect pathways increased correspondingly. This resulted in an overestimation of \( G_j, \text{local} \) with the absolute error depending on the value of \( G_j, \text{global} \) but not on the value of \( G_j, \text{local} \) (Fig. 5, D and E). At these higher values for \( G_j, \text{global} \) the calculated values for \( G_j, \text{local} \) obtained without correction were relatively close to the theoretical values when \( G_j, \text{local} \approx G_j, \text{global} \), overestimated when \( G_j, \text{local} < G_j, \text{global} \) and slightly underestimated when \( G_j, \text{global} < G_j, \text{local} \) (Fig. 5, D and E). This had the nonintuitive consequence that for the condition when \( G_j, \text{local} = G_j, \text{global} \), the estimates of \( G_j, \text{local} \) without correction were closer to the theoretical values, compared with estimates with correction when the conductance values were \( \approx 1 \, \text{nS} \) (Fig. 5F). With correction, \( G_j, \text{local} \) was increasingly overestimated for increasing theoretical values of \( G_j \) (Fig. 5F).

Because the cells recorded in a slice preparation are located closer to the surface, and not centrally in a 2D network, we repeated the simulations in Fig. 5C (\( G_j, \text{global} = 700 \, \text{pS} \)) and Fig. 5F (\( G_j, \text{global} = G_j, \text{local} \)) for two neighboring cells located at or close to the edge of the network [Cell(0 . . 2, 12) and Cell(0 . . 13)]. In all cases, the results were qualitatively almost identical to those obtained for the centrally located cells, with only minor differences in the estimated values of \( G_j, \text{local} \) for a given value of \( G_j, \text{global} \) (data not shown).

In summary, these simulations suggested that the correction procedure works adequately for pairs of cells embedded in larger networks and that the voltage-clamp measurements are able to reliably detect changes in \( G_j, \text{local} \) over a large range. If \( G_j, \text{global} \) increases at the same rate as \( G_j, \text{local} \), estimates without correction are actually very close to the theoretical values. However, when \( G_j, \text{global} \) is \( < 1,000 \, \text{pS} \) (Fig. 5, A–C), the network has little influence on the estimates of \( G_j, \text{local} \) between pairs of coupled cells.
Time-dependent increase of junctional conductance is accompanied by an increase of the steady-state coupling coefficient

The time-dependent increase of $G_j$ raises the question whether, and to what extent, it is accompanied by concomitant changes in functional coupling characteristics, as observed by recording the cells in the current-clamp configuration. We examined this by analyzing the steady-state coupling coefficient as a function of time. Figure 6 shows representative changes observed for the same cell pair illustrated in Fig. 4. At about 5 min, $G_j$ was about 150 pS. At about the same time, the coupling coefficient was about 0.11 (Fig. 6A). In parallel with the increase of $G_j$, the coupling coefficient increased such that after 35 min it was about 0.18 and after 55 min it was about 0.33 (Fig. 6A). When we plotted all measurements of coupling coefficient for this cell pair as a function of time, we observed a steady increase of coupling coefficient throughout the period of recording (Fig. 6B). Parallel measurements of $G_j$ and coupling coefficient at early and late recording time points were made for 10 cell pairs, all of which displayed an increase in $G_j$. The coupling coefficient increased from 0.23 ± 0.04 after about 5–10 min to 0.37 ± 0.04 after ≥30 min of recording ($P = 0.002$; paired t-test). For a few cell pairs, we obtained relatively long-lasting recordings with frequently repeated measurements of both $G_j$ and coupling coefficient at approximately identical time points. For four of these cell pairs, the measurements spanned a sufficiently large range of $G_j$ values that the relationship between $G_j$ and coupling coefficient could be adequately examined (Fig. 6C; same cell pair as illustrated in Figs. 4 and 6A and B). The data points were moderately well fitted with $Eq. \; 5$, giving a value for the nominal membrane resistance ($r_m$) of 400 MΩ (580 ± 120 MΩ; $n = 4$ cell pairs). The relationship between the data points and the fitted curve described by $Eq. \; 5$ suggested that the increase of $G_j$ between the two cells recorded from was accompanied by a decrease in the nominal membrane resistance of the postsynaptic cell, corresponding to an increase in $G_j$ between this cell and its nonrecorded, coupled neighbors (see earlier text). These results suggested that even when two cells are coupled to other cells in a network, their functional connection can still be adequately described by the two-cell circuit (Fig. 1A). To further explore this, we used computer simulations to analyze coupling coefficients in more extensive networks.

For a two-cell circuit (Fig. 1A), the relationship between $G_j$, postsynaptic resistance, and steady-state coupling coefficient is given by $Eq. \; 5$. If we extend the network by coupling each of the two cells to other cells, but with no indirect pathways for current flow between the two original cells, the relationship should still be described by $Eq. \; 5$ because the additional coupling reduces the values only for the parameters $r_1$ and $r_2$ without changing $r_c$ ($G_r$). For a 2D rectangular network of electrically coupled cells (as in Fig. 1B), the relationship between $G_j$ and steady-state coupling coefficient for two neighboring cells will be more complicated because of the additional indirect pathways of current flow. To investigate the relationship between $G_j$ and steady-state coupling coefficient under varying conditions of $G_r$ and effective network connectivity, we simulated current-clamp recordings from a pair of neighboring cells centrally located in our previously used network of rectangularly arranged cells.

In the simulations, we varied $G_j$ between the two central target cells ($G_{j, local}$; Fig. 1B) for a series of conditions that differed with respect to the $G_r$ of all other electrical synapses in the network ($G_{j, global}$). As in the physiological recordings, a current pulse was injected into the presynaptic cell and the resulting voltage responses were measured in this cell and in the postsynaptic cell. For each combination of $G_{j, local}$ and $G_{j, global}$, we calculated the steady-state coupling coefficient. Figure 7 shows the results for five different conditions of $G_{j, global}$ (0, 200, 700, 1,500, and 3,000 pS), as well as the condition where $G_{j, local} = G_{j, global}$. For each condition, the steady-state coupling coefficient increased monotonically when $G_{j, local}$ increased from 0 to 3,000 pS, but the slope of the relationship was largest for $G_j$ values ≤1,000 pS (Fig. 7). The effect of $G_{j, global}$ on the steady-state coupling coefficient depended on the value of $G_{j, local}$. For $G_{j, local} ≥700$ pS, the steady-state coupling coefficient decreased when the value of $G_{j, global}$ increased (Fig. 7). The degree of reduction was most pronounced for values of $G_{j, global} ≤700$ pS and diminished with increasing values of $G_{j, global}$ (Fig. 7). For $G_{j, local} <500–700$ pS, the steady-state coupling coefficient increased when the value of $G_{j, global}$ increased ≥700 pS (Fig. 7). This change in the relationship between junctional conductance and steady-state coupling coefficient for electrically coupled cells in two-cell circuits versus 2D syncytia

![Image](http://jn.physiology.org/)
between $G_j$ (local) and steady-state coupling coefficient reflects that the indirect pathways for current flow became more important than the direct pathway. For the condition when $G_j$ (local) = $G_j$ (global), the shape of the relationship between $G_j$ (0–3,000 pS) and steady-state coupling coefficient was almost identical to that for $G_j$ (global) = 0 pS (after normalizing the curves to their peak coupling coefficient; not shown), with little increase in steady-state coupling coefficient for values of $G_j$ > 1,000 pS (Fig. 7). Like the curve for $G_j$ (global) = 0 pS (corresponding to the two-cell circuit; Fig. 1A), the curves for $G_j$ (global) = 200 and 700 pS were well fit by Eq. 5, but for larger values of $G_j$ (global) and for $G_j$ (global) = $G_j$ (local), the fit became increasingly inadequate (not shown), reflecting an increasing contribution of the indirect pathways for current flow.

Inserting artificial electrical synapses between AII amacrine cells by dynamic-clamp electrophysiology

The time-dependent increase of $G_j$ in whole cell recordings could be measured directly in voltage clamp and was reflected as an increase in the steady-state coupling coefficient in current clamp. With computer simulations, we explored the adequacy of our procedures to detect and quantify changes in $G_j$ and investigated the relationship between $G_j$ and steady-state coupling coefficient in an idealized network of electrically coupled cells. An increase in $G_j$ should also influence other properties of signal transmission mediated by electrical synapses, such as low-pass filter characteristics (including spike transmission characteristics) and synchronization of subthreshold membrane potential fluctuations (Veruki and Hartveit 2002a). To investigate how the magnitude of $G_j$ influences these signal transmission characteristics ideally requires precise and reversible control of the $G_j$ such that it can be manipulated as the independent variable. This was not possible in recordings with low-resistance electrodes where $G_j$ increased continuously, albeit with variable rates. In addition, although whole cell recordings with high-resistance pipettes avoided the time-dependent increase of $G_j$, the observed values of $G_j$ did not cover a large enough range of conductance values. An alternative strategy would be to use computer simulations of network models. Currently, there is not sufficient quantitative information to generate models of AII amacrine cells with a high level of morphological and physiological realism, such as models that incorporate voltage-gated conductances involved in spike generation. Therefore to complement our physiological recordings and computer simulations, we used dynamic-clamp (conductance injection) electrophysiology to insert artificial electrical synapses with user-specified conductance between pairs of AII amacrine cells (for related applications, see Bem et al. 2005; Mancilla et al. 2007; Merriam et al. 2005; Perez Velazquez et al. 2001; Sharp et al. 1992). This approach can be considered a simulation where the parameters to be examined are under tight experimental control and the remaining parameters are by definition correct because they are “computed” by the real cells (Goaillard and Marder 2006).

For conductance injection experiments, we targeted pairs of AII amacrine cells without direct physiological coupling. For the majority of recordings, the two cells of a pair were located in the same slice, with a minimum soma-to-soma distance of about 50 μm (Fig. 2B). For some recordings, the two cells were located in different slices. In voltage-clamp recordings (without conductance injection), no postsynaptic response was evoked by presynaptic voltage steps up to ±30 mV (from a holding potential of −60 mV; data not shown). When we used conductance injection to insert a symmetrical artificial electrical synapse ($G_{j,12} = G_{j,21}$) between the two cells, pulses of current injected into the presynaptic cell changed the membrane potential of both the presynaptic and postsynaptic cells (Fig. 8, A and B). As expected, when the input resistances measured at the two cells were approximately equal, inserting an electrical synapse with symmetrical $G_j$ yielded approximately equal responses in both directions (Fig. 8, A and B). The steady-state coupling coefficient increased monotonically with increasing $G_j$ (0–3,000 pS; Fig. 8C), but the largest increase in steady-state coupling coefficient for a given increase in $G_j$ was observed for the lowest conductances (Fig. 8D), similar to observations with physiological coupling (Fig. 6C). With $G_j$ = 3,000 pS, the steady-state coupling coefficient reached approximately 0.6 (Fig. 8D). The average data were well fitted by Eq. 5 (Fig. 8D), with a best-fit value for $r$ of 460 MΩ. For this analysis, the equivalent circuit in Fig. 1A can be considered to represent two networks, instead of two single cells, connected via the junctional resistance $r_c$.

Because dynamic clamp using single-electrode current clamp with high-resistance electrodes is problematic (see Brette et al. 2008 for an extensive discussion), we used low-resistance electrodes for these experiments. With longer-lasting recordings (>30 min), we observed a time-dependent decrease in the nominal membrane resistance (from $770 ± 160$ MΩ early in the recording to $420 ± 42$ MΩ late in the recording; $P = 0.02$; paired t-test; $n = 18$ cells), similar to the decrease seen in paired recordings of cells with direct electrical coupling and consistent with an increase in $G_j$ between each cell recorded and its directly coupled neighbors. We therefore limited the analysis of recordings with dynamic-clamp electrophysiology to the minimum period (typically ~10 min) necessary to test the required range of conductances.
Relation between junctional conductance and synchronization of subthreshold membrane potential fluctuations

Physiologically coupled pairs of AII amacrine cells display synchronization of subthreshold membrane potential fluctuations (Veruki and Hartveit 2002a). To quantitatively explore the relationship between $G_j$ and the degree of synchronization of subthreshold membrane potential fluctuations, we recorded the spontaneous activity of pairs of AII amacrine cells while systematically varying the conductance of an artificial electrical synapse ($0–3,000$ pS). Spiking was blocked by adding $300$ nM TTX to the bath. For each value of $G_j$, the degree of synchronization was examined by calculating sliding-window, 2D cross-correlograms during epochs of $15$ s. Figure 9, A–D shows 2D cross-correlograms for the same cell pair for $G_j$ values of $0$, $400$, $1,000$, and $3,000$ pS (top panels) and average cross-correlograms over the epoch of each 2D cross-correlogram (bottom panels). With increasing $G_j$, there was a monotonic increase in the strength of synchronization of the subthreshold membrane potential fluctuations between the two cells. The slope of the relationship between the average peak amplitude of the individual cross-correlograms and $G_j$ was largest for conductances between 0 and 1,000 pS and the average peak amplitude of the cross-correlograms reached about 0.75 at $G_j = 3,000$ pS (Fig. 9E, filled circles). The relationship obtained for cells coupled by artificial electrical synapses corresponded reasonably well with values obtained for physiologically coupled cells (Fig. 9E, open circles).

Relation between junctional conductance and spike transmission characteristics

The relationships between $G_j$ and steady-state coupling coefficient and between $G_j$ and strength of synchronization of subthreshold membrane potential fluctuations displayed the highest slope for $G_j$ values $\leq 1$ nS and a progressively diminishing influence of $G_j$ at higher values. This raises the question whether the physiologically relevant dynamic range of $G_j$ really is $>0$ to 1 nS? It is possible that $G_j$ values $\geq 1$ nS could have a strong influence on the transmission of voltage waveforms with higher-frequency components (e.g., action potentials). AII amacrine action potentials are slow with peak amplitudes between 10 and 30 mV and the rate of spontaneous spiking can vary considerably between cells (Boos et al. 1993; Tamalu and Watanabe 2007; Veruki and Hartveit 2002a). In physiologically coupled cells, presynaptic action potentials evoke discrete, low-amplitude, postsynaptic depolarizations (electrical postsynaptic potentials [electrical PSPs]) (Veruki and Hartveit 2002a). Figure 10A shows an example of spontaneous spiking in an AII amacrine cell (cell 1). With an artificial electrical synapse ($G_j = 200$ pS) between this cell and another AII amacrine cell (cell 2), a spike in the presynaptic cell evoked an electrical PSP in the postsynaptic cell (Fig. 10A). An increase of $G_j$ increased the amplitude of the electrical PSPs (Fig. 10A). For each value of $G_j$, the waveforms of the evoked electrical PSPs were averaged after aligning them by a spike in the presynaptic cell (364–401 electrical PSPs for each condition). The results for one cell pair, with averaged presynaptic spikes and electrical PSPs, are illustrated in Fig. 10, B and C. With increasing $G_j$, the amplitude of the electrical

![Image](http://jn.physiology.org/)

**Fig. 8.** Inserting an artificial electrical synapse between a pair of nonneighboring AII amacrine cells by dynamic-clamp (conductance injection) electrophysiology, relation between $G_j$ and steady-state coupling coefficient. A: with a pair of nonneighboring AII amacrine cells (as in Fig. 2B) in current clamp, 500-ms current pulses ($I$) of $–50$, 0, and $+50$ pA are applied to cell 1, whereas voltage responses are recorded from both cells ($V_1$ and $V_2$). An artificial electrical synapse between the 2 cells implemented by conductance injection with $G_j = 600$ pS (symmetrical). Diagram at left indicates recording configuration; variable resistor symbolizes the artificial electrical synapse between the 2 cells (A, B). Injection of negative current results in hyperpolarization of both cells and injection of positive current results in depolarization of both cells. B: same as in A, but current pulses are applied to cell 2. C: with a pair of AII amacrine cells in current clamp, 500-ms current pulses ($I$) of $–50$, 0, and $+50$ pA are applied to cell 1, whereas voltage responses are recorded from both cells ($V_1$ and $V_2$). Artificial electrical synapse between the 2 cells implemented by conductance injection with $G_j$ (symmetrical) as indicated (200, 1,000, and 3,000 pS). Injection of negative current results in hyperpolarization of both cells and injection of positive current results in depolarization of both cells. Notice increasing response in cell 2 with increasing $G_j$. D: relationship between $G_j$ (0–3,000 pS) and steady-state coupling coefficient (as in C, 3–11 cell pairs for each data point). Data points plotted as means ± SE and fitted by Eq. 5.
PSPs increased gradually, with a peak amplitude of about 2 mV at a junctional conductance of 3,000 pS, corresponding to a coupling coefficient at the peak of about 30% (Fig. 10D; n = 3 cell pairs). The 10–90% rise time was quite variable at the lowest values of \( G_j \) (Fig. 10E). With increasing \( G_j \), the 10–90% rise time was reduced to values around 2 ms and displayed only a moderate reduction with further increases of \( G_j \leq 3,000 \) pS (Fig. 10E). Increasing \( G_j \) also accelerated the voltage decay during the repolarization phase of the action potential in the presynaptic cell (Fig. 10B).

Relation between junctional conductance and synchronization of action potentials

An important function of electrical coupling between neurons is synchronization of action potentials (Connors and Long 2004). Physiologically coupled pairs of AII amacrine cells display temporally precise synchronization of spiking (Veruki and Hartveit 2002a). The influence of \( G_j \) on spike transmission suggests that spike synchronization will be influenced as well. To explore the relationship between \( G_j \) and the degree of spike synchronization quantitatively, we recorded spontaneous action potentials from pairs of cells while systematically varying \( G_j \) of the artificial electrical synapse. For six cell pairs, we observed spontaneous spiking in both cells when the membrane potential was close to \(-60 \) mV. For each value of \( G_j \), we repeatedly sampled the activity for 10-s epochs, with 5-s periods before and after each repetition when the cells were uncoupled (no injected conductance). In this way, any spontaneously occurring fluctuations in the isolated spiking activity of each cell could be observed and taken into account. Figure 11, A–D (left and middle panels) shows segments of voltage records for a cell pair with \( G_j \) set to 0, 400, 1,000, and 3,000 pS, leading to enhanced spike synchronization. To analyze the synchronization quantitatively, we calculated spike cross-correlation histograms of simultaneously recorded spike trains for each conductance value. With increasing \( G_j \), the spike cross-correlation histograms displayed a sharp peak near zero time delay and the amplitude of the peak increased with increasing conductance (Fig. 11, A–D, right panels). As judged by eye, a peak was already present at conductance values of 100 and 200 pS, although the amplitude was not large enough to reach the upper 99% confidence limit of the cross-correlation histogram (not shown). From each cross-correlation histogram, we estimated the synchronization strength, the synchronization width, and the time delay of the peak from zero (see METHODS). Synchronization strength increased monotonically, and almost linearly, with increasing \( G_j \) (Fig. 11E; \( n = 4 \) cell pairs). At a conductance of 700 pS, the average conductance previously reported for physiologically coupled pairs of AII amacrine cells relatively early during whole cell recording (Veruki and Hartveit 2002a), the average synchronization strength for artificial coupling was 10.3 (Fig. 11E). This is very similar to the average synchronization strength for physiologically coupled cells (11.4; Veruki and Hartveit 2002a). When we fitted the relationship between \( G_j \) and synchronization strength (Fig. 11E) with a straight line (not shown), we obtained a slope of 8.4 nS.

Consistent with previous observations for physiologically coupled cells, there could be one or two peaks in the spike cross-correlation histograms, one on each side of zero time delay (Veruki and Hartveit 2002a). We fitted each histogram with a Gaussian function to obtain an estimate for the time delay (the delay of the major peak from zero). The time delay decreased with increasing \( G_j \) (Fig. 11F). At lower conductance levels (≤1,000 pS), the average time delay ranged between 4 and 8 ms. For conductance levels >1,000 pS, there was an approximately linear relationship with a slope of 0.87 ms/nS (linear fit; not shown), with a time delay of about 2 ms for a maximum conductance of 3,000 pS.

The synchronization width (the width of the cross-correlation peak at the 99% confidence level) decreased slightly with
increasing $G_j$ (Fig. 11G, filled symbols) and ranged between 3 and 4 ms for conductance values between 1,000 and 3,000 pS. If we instead measured the synchronization width as the width of the peak at the 95% confidence level, a decrease of width with increasing $G_j$ became obvious (Fig. 11G, open symbols). At a conductance of 700 pS, the average synchronization width at the 99% confidence level was about 4 ms. This is in the lower range of values previously measured for pairs of physiologically coupled cells (3–13 ms; average 7.2 ms; see Veruki and Hartveit 2002a). When we compared the average relationships obtained for cells coupled by artificial electrical synapses with individual data points obtained for physiologically coupled cells, the correspondence was reasonably good for synchronization strength (Fig. 11E, open circles), time delay (Fig. 11F, open circles), and synchronization width (data not shown).

For pairs of isolated sinoatrial node cells, dynamic-clamp electrophysiology has been used to systematically study the relationship between $G_j$ of the artificial coupling and the functional characteristics of spontaneous spiking (Verheijck et al. 1998). Interestingly, it was observed that entrainment of spike frequency can occur already at relatively low conductance values (<500 pS). To investigate similar phenomena for coupled AII amacrine cells, we recorded from pairs of spontaneously spiking cells while varying $G_j$ between 0 and 3,000 pS. For each cell pair, we plotted the average spiking frequency of each cell, both in the uncoupled condition and during electrical coupling, as a function of $G_j$. For the example illustrated in Fig. 11H, the two cells displayed different spiking frequencies in the uncoupled condition (open symbols) and the frequencies were relatively stable over time. When the cells were coupled by an artificial electrical synapse (filled symbols), there was clear frequency entrainment, in that the spiking frequencies of the two cells approached each other (Fig. 11H). Frequency entrainment could be observed for conductances of about ≈500 pS. At the highest conductances (>2,000 pS), the firing frequencies of the two cells were almost identical. Similar results were seen for four additional cell pairs where the spontaneous spiking frequencies were sufficiently different in the uncoupled condition that entrainment could be observed during electrical coupling.

Relation between junctional conductance and low-pass filter characteristics

The results obtained for steady-state coupling coefficient, synchronization of subthreshold membrane potential fluctuations and transmission of action potentials suggested that the influence of $G_j$ on a specific functional parameter depends on the frequency components of the presynaptic voltage waveform. In general, transmission of signals mediated by electrical synapses have the functional characteristics of a low-pass filter (Bennett 1966, 1977; Galarreta and Hestrin 1999; Gibson et al. 1999, 2005; Nolan et al. 1999; Veruki and Hartveit 2002a,b; Zhang and Wu 2005). Specifically, an increase in stimulus frequency leads to a decrease in coupling coefficient and an increase in phase lag, but the influence of $G_j$ has not been measured. We examined the quantitative relationships for pairs of AII amacrine cells by applying sinusoidal current stimuli to one cell (frequency 1–1,000 Hz) and inserting an artificial electrical synapse with conductance ranging from 0 to 3,000
pS. Action potentials were blocked by adding TTX. Both the stimulated and the nonstimulated cells responded with a sinusoidal voltage response, but the response in the noninjected cell had a lower amplitude and lagged behind the response in the injected cell. An example of this is illustrated in Fig. 12A for $G_j$ values of 200, 1,000, and 3,000 pS and a stimulus frequency of 40 Hz. With increasing $G_j$, there was an increase in the coupling coefficient and a decrease in the phase lag (Fig. 12A).

Figure 12B illustrates quantitatively how an increase of stimulus frequency resulted in decreasing coupling coefficient (increasing signal attenuation). An increase of $G_j$ shifted the coupling coefficient versus $G_j$ relationship to the right (Fig. 9B, left). For each curve, corresponding to a specific value of $G_j$, we calculated the corner frequency as the frequency at which the normalized coupling coefficient falls to $1/\sqrt{2} (\sim 0.707)$. The corner frequency versus $G_j$ relationship was approximately...
linear (cf. Zhang and Wu 2005), ranging from approximately 10 Hz at 200 pS to approximately 40 Hz at 3,000 pS (Fig. 12B, right). Fitting the data points with a straight line yielded a slope of 11.2 Hz/nS.

We also used the conductance injection experiments to generate similar curves for the phase lag versus $G_j$ relationship (Fig. 12C). At the lowest value of $G_j$, the phase lag increased from 0° to between 100 and 150° when the frequency of the sinusoidal current was increased from 1 to 1,000 Hz (Fig. 12C). With increasing $G_j$ ($\leq 3,000$ pS), there was a marked reduction in the phase lag (Fig. 12C). To the extent that the transmission of specific presynaptic voltage waveforms (subthreshold, suprathreshold) is linear, the curves for coupling coefficient and phase lag will predict the postsynaptic coupling to any arbitrary presynaptic waveform for specific values of $G_j$ (cf. Gibson et al. 2005).

**DISCUSSION**

We have observed a spontaneous, time-dependent increase of $G_j$ between electrically coupled pairs of neighboring AII amacrine cells during longer-lasting whole cell recordings. The increase was observed with low- but not high-resistance pipettes and suggested that the electrical coupling strength is controlled by a physiological modulatory system that can be perturbed by intracellular washout. In this study, our primary focus was the investigation of the dynamic range of $G_j$ and the consequences for the functional transmission characteristics. The intracellular transduction mechanism involved in the control of coupling strength was not examined, but remains an important topic for future studies. By taking advantage of the spontaneous increase, we were able to estimate a dynamic range of $G_j$. It is possible that this range reflects a minimum dynamic range that is under physiological control under in vivo conditions. Using dynamic-clamp electrophysiology, we inserted artificial electrical synapses between pairs of AII amacrine cells, allowing us to vary and finely control the conductance of the artificial electrical synapse within the same range that we observed during recording of physiologically coupled cells. This approach was necessary because we could not otherwise control the physiological $G_j$ in a direct and reversible manner over the required range. Thus we were able to systematically study the quantitative relationship between $G_j$ and a series of specific functional transmission characteristics that are likely to be important for the integrative function of the network of AII amacrine cells.

**Gap junctions and electrical synapses between AII amacrine cells**

Consistent with electron microscopic observations of gap junctions between the arboreal dendrites of AII amacrine cells in the inner plexiform layer (Kolb and Famiglietti 1974), intracellular injection of Neurobiotin into an AII amacrine cell is followed by diffusion of the tracer to a variable number of neighboring AII amacrine cells (Bloomfield et al. 1997; Hampson et al. 1992; Mills and Massey 1995; Vaney 1991; Xia and Mills 2004). Simultaneous dual recording of pairs of cells demonstrates strong electrical coupling with transmission characteristics expected for electrical synapses (Veruki and Hartveit 2002a). In a study of rabbit retina, Strettoi et al. (1992) reported that an AII amacrine cell examined in...
The single-channel conductance for Cx36 connexons has been estimated to vary between 50 and 150 pS (see references in Vardi and Smith 1996). Based on these values, one can estimate a range of conductance values from about 800 pS to 19 nS. However, because electrical coupling between AII amacrine cells is mediated by homomeric connexons containing Cx36 (Deans et al. 2002; Feigenspan et al. 2001; Mills et al. 2001), the estimate of single-channel conductance must be revised. In expression systems, the connexon open probability (P_{open}), and the connexon single-channel conductance. When parameter values for the gap junctions between AII amacrine cells were unknown, Vardi and Smith (1996) used published values from other systems. Their values for connexon density ranged between 2,000 and 8,000 per μm² (Baldridge et al. 1989; Perachia 1973; Witkovsky et al. 1983). P_{open} was taken as 1–2% (Lin and Faber 1988; see also Zhang and Wu 2005), and the single-channel conductance was assumed to vary between 50 and 150 pS (see references in Vardi and Smith 1996). Based on these values, one can estimate a range of conductance values from about 800 pS to 19 nS. However, because electrical coupling between AII amacrine cells is mediated by homomeric connexons containing Cx36, the conductance pipes, we observed a run-up of G_j with maximum values ranging from about 400 to about 3,500 pS. If we assume that the run-up is caused by washout of intracellular constituents related to neuromodulatory control of G_j (see following text), it suggests that the overall range corresponds to a range within which G_j is under physiological control. In their study of thalamic reticular neurons, Landsman and Connors (2005) found that G_j was reduced by about 20% following synaptic or pharmacological activation of metabotropic glutamate receptors. It is difficult, however, to directly compare these results with those obtained in our study because it is not known where the control values for G_j between reticular thalamic neurons were located within their dynamic range.

### Relationship between junctional conductance and functional coupling characteristics

Electrical coupling can be a mechanism for synchronization of both spiking and subthreshold membrane potential fluctuations. After having estimated the minimum dynamic range of G_j between pairs of physiologically coupled AII amacrine cells, we used dynamic-clamp electrophysiology to quantitatively investigate the relationship between G_j and a series of functional coupling characteristics. For spiking and subthreshold membrane potential fluctuations, we observed a monotonic relationship between the conductance of the artificial electrical synapse and the strength and temporal precision of synchronization. For some functional properties, the relationship was approximately linear over the dynamic range of G_j (e.g., strength of spike synchronization) or displayed only a small reduction in the slope of the relationship for the highest values of G_j (e.g., the peak amplitude of spike-evoked electrical PSPs). For other functional properties, however, the slope was larger for junctional conductances between 0 and about 1 nS (e.g., for the time delay of the central peak in spike cross-correlograms and for the peak cross-correlation of subthreshold membrane potential fluctuations). In these cases, it might be speculated that a modulatory control system would exert its greatest influence when operating in the corresponding range of junctional conductances. For functional properties such as synchronization of spiking, spike frequency entrainment and subthreshold membrane potential fluctuations, the effect of coupling is seen already at low values of G_j (100–500 pS). Further work will be required to determine whether the full range of G_j values observed here is indeed used in the physiological regulation of electrical coupling between AII amacrine cells. Some studies have suggested that under certain conditions, electrical coupling between neurons can lead to antiphase spike synchronization (e.g., Pfuetzner et al. 2003; Sherman and Rinzel 1992). We have never observed this phenomenon for electrically coupled AII amacrine cells, irrespective of whether the cells were coupled physiologically or with conductance injection.

When the transmission between electrically coupled cells was analyzed as a linear filter by using sine-wave stimuli, the extent of low-pass filtering (quantified as attenuation and phase lag of transmission) was strongly reduced by increasing the injected conductance from 200 pS to 3 nS. The reduced attenuation corresponded to an approximately linear change in cutoff frequency from about 10 Hz (at 200 pS) to about 40 Hz (at 3 nS), whereas the largest changes in phase lag occurred for conductances ≤1 nS.

### Modulatory control of junctional conductance of electrically coupled AII amacrine cells

Although it has been demonstrated directly in only a few cases, it is generally considered that phosphorylation of connexins can influence the open probability of the corresponding connexons (e.g., McMahon et al. 1989; reviewed by Moreno and Lau 2007). In the case of electrical coupling between AII amacrine cells, there are currently two opposing theories: one proposing that increased phosphorylation of Cx36 leads to reduced open probability (Urshel et al. 2006) and the other proposing that increased phosphorylation leads to increased open probability (Kothmann et al. 2008). In the first case, phosphorylation is hypothesized to be mediated by protein kinase A (PKA), following activation of dopamine D1-type receptors on AII amacrine cells and increased intracellular concentration of cAMP (Hampson et al. 1992; Urshel et al. 2006). In the second case, phosphorylation is hypothesized to be driven by an unknown mechanism, perhaps activated by Ca^{2+} influx, whereas dephosphorylation is suggested to be driven by activation of D1 receptors on AII amacrine cells and activation of a protein phosphatase via phosphorylation by PKA (Kothmann et al. 2008). Importantly, both theories can...
account for the reduced extent of tracer coupling between AII amacrine cells following pharmacological activation of D1-type receptors (Hampson et al. 1992; Urschel et al. 2006). Our finding of increased \( G_i \) following whole cell recording of AII amacrine cells with low-resistance pipettes does not support one theory over the other because intracellular washout could lead to either reduced phosphorylation or reduced dephosphorylation. To date, there is no direct electrophysiological evidence that activation of D1-type receptors can modulate \( G_i \) between coupled AII amacrine cells. Furthermore, there is no evidence for the presence of D1 receptors on AII amacrine cells (cf. Veruki and Wässle 1996). If enhanced \( G_i \) is mediated by an increase in open probability, as opposed to an increase in connexon density and/or single-channel conductance (see following text), the dynamic range of \( G_i \) observed during time-dependent run-up in our study corresponds approximately to a 10-fold increase in open probability.

In addition to modulatory control of the junctional conductance of electrical synapses between AII amacrine cells, there is also the possibility for an indirect enhancement of the conductance between AII amacrine cells by increasing the conductance between AII amacrine cells and ON-cone bipolar cells, thought to be regulated by the NO–cGMP-signaling system (Mills and Massey 1995). Whereas the electrical synapses between AII amacrine cells contain Cx36, there is evidence that the synapses between AII amacrine and ON-cone bipolar cells contain Cx36 on the AII side and either Cx36 or Cx45 on the bipolar side (Dedek et al. 2006; Han and Massey 2005; Lin et al. 2005; Maxeiner et al. 2005). In contrast to the increase of \( G_i \) seen in paired recordings of AII amacrine cells, we have not observed a time-dependent increase of \( G_i \) during dual recordings of AII amacrine cells and ON-cone bipolar cells (Veruki and Hartveit, unpublished observations) and we consider it unlikely that an up-regulation contributed to the measurements reported here.

Artificial electrical coupling via dynamic-clamp electrophysiology versus physiological electrical coupling

In our experiments, we implemented a single artificial electrical synapse between a pair of simultaneously recorded AII amacrine cells via whole cell recording electrodes located at the soma of each cell. It is important to consider whether, and to what extent, this configuration is able to mimic the effects of electrical synapses mediated by natural gap-junction coupling between neighboring cells. First, dynamic-clamp electrophysiology is of course only able to mimic the electrical effects of gap-junction coupling. Any functions the gap junctions between AII amacrine cells might have with respect to metabolic coupling will not be mimicked. We consider it unlikely that such mechanisms would be important for the results reported here. Second, it might be argued that, although the artificial electrical synapses were implemented between the cell bodies of AII cells, the physiological electrical synapses correspond to gap junctions between the arboreal dendrites of AII amacrine cells (Chun et al. 1993; Kolb and Famiglietti 1974; Strettoi et al. 1992). Unfortunately, the arboreal dendrites of AII amacrine cells are not accessible to electrophysiological recording. Although it has been suggested that AII amacrine cells are electrototically compact (Vardi and Smith 1996), the degree of electrotonic filtering between the cell body and the arboreal dendrites is currently unknown. It is interesting to note, however, that at least in cat retina, there is evidence for gap junctions between AII amacrine cells not only at their arboreal dendrites, but also between their cell bodies (Vardi and Smith 1996). If similar electrical synapses also exist in rat retina, artificial electrical synapses implemented via whole cell soma recordings are not as unrealistic as they might seem at first. Finally, although dynamic-clamp electrophysiology allowed us to control \( G_i \) between two AII amacrine cells, it did not allow us to control \( G_i \) globally in the network of coupled AII amacrine cells. Although it is likely that \( G_i \) of all electrical synapses in the network of coupled AII amacrine cells is modulated in the same direction by a physiologically adequate stimulus (increase vs. decrease), the degree of spatial heterogeneity is unknown. In addition, computer simulations of a simplified network of coupled cells suggested that the errors introduced by our inability to control the global \( G_i \), in parallel with the local \( G_i \) corresponding to the artificial electrical synapse, are less important for global conductance levels <1,000 pS when the transmission is dominated by direct electrical coupling. The most important shortcoming of the dynamic-clamp experiments might be that we were unable to examine the influence of global changes in \( G_i \) on transmission and synchronization of action potentials. For the network simulations, there was little influence on the steady-state coupling coefficient of changes in global \( G_i \) beyond about 1 nS. Unfortunately, there is currently insufficient information available to attempt reasonably realistic network simulations of spike transmission and synchronization between AII amacrine cells.

In the case of AII amacrine cells, additional current pathways mediated by other cells coupled to both cells recorded from (cf. Gibson et al. 2005), can be mediated via both AII amacrine cells and ON-cone bipolar cells (Veruki and Hartveit 2002a,b). With a pair of AII amacrine cells coupled by an artificial electrical synapse, it cannot be excluded that at the start of recording there were indirect electrical connections between the cells, but that the effective conductance was too small to be measured. During recording, intracellular washout could enhance the conductance and add this to the magnitude of the injected conductance. This would lead to a distorted relationship between the nominal \( G_i \) imposed by the dynamic-clamp and the functional coupling characteristics. Except in the recordings where we connected pairs of AII amacrine cells located in different slices, we cannot eliminate the possibility that such a mechanism could, in principle, exist. We do doubt, however, any quantitative contribution because measurements with the conductance of the artificial electrical synapse set to zero confirmed a lack of coupling throughout the period of recording.

Dynamic range of junctional conductance and physiological relevance

Based on recordings of physiologically coupled AII amacrine cells and time-dependent run-up of \( G_i \), we estimate that the dynamic range of conductance values between two coupled cells can be from 0 to about 3 nS. In our experiments with artificial electrical synapses between pairs of AII amacrine cells, we injected values of conductance from 0 to 3 nS and explored the quantitative relation between the magnitude of \( G_i \)
and a series of functional coupling characteristics. This raises the question whether the observed dynamic range for $G_i$ is physiologically exploited. Whereas the most likely explanation for the time-dependent run-up of $G_i$ is increased open probability following intracellular washout and altered phosphorylation of Cx36 (see preceding text), we cannot exclude the possibility that longer-lasting whole cell recordings also could be accompanied by an increase in the total number of channels in the gap-junction contacts between coupled AII cells. Assuming a continuous turnover of connexons, an increase in the total number of channels could be caused by an increase in the number of inserted channels, a decrease in the number of sequestered channels, or both (for review, see Laird 2005). At the moment, we do not have evidence either to support or to refute this hypothesis. Although it is likely that short-term modulatory control of $G_i$ between electrically coupled AII amacrine cells would be preferentially mediated by changing the open probability of Cx36 connexons, there could be an additional, presumably slower, modulatory control of the total number of connexons. It will be interesting to see whether dual recordings from pairs of electrically coupled AII amacrine cells under conditions of minimal intracellular washout (e.g., by using high-resistance pipettes) can be used to investigate the transduction mechanisms responsible for the presumed physiological regulation of the strength of electrical coupling.

ACKNOWLEDGMENTS

We thank Dr. Robert G. Smith for helpful discussions about electrical coupling in neuronal networks.

GRANTS

This study was supported by Research Council of Norway Grants NFR 161217, 165328, and 178105 and the Faculty of Medicine at the University of Bergen.

REFERENCES


