Supplementary Figure 1. Evidence for rod bipolar and All amacrine cells in the ground squirrel retina.

In the ground squirrel, an abundance of cone bipolar cells makes it relatively easy to study the contacts between rods and specific cone bipolar cell types. However, one might argue that the rod pathways in this cone dominant mammal are unrepresentative of pathways in the retinas of rod dominant mammals. In particular, aberrant connections between rods and b2 bipolar cells might result from the absence of a sensitive rod pathway including component rod bipolar and All amacrine cells. We present immunohistochemical evidence that the ground squirrel retina contains rod bipolar and All amacrine cells. (a) A putative rod bipolar cell (asterisk) was visualized following Neurobiotin injection (red). The slice was labeled by antibodies to calretinin (green), a marker for All amacrine cells in primate and rabbit retinas, and PKC-α (blue), a marker for rod bipolar cells. The injected bipolar cell (b) is positive for PKC-α labeling (c). The multi-stratified calretinin-labeled cell had one set of dendrites that co-fasciculated with rod bipolar cell axon terminals at the bottom of the inner plexiform layer (IPL) and another set (or sets) of dendrites suitably positioned in the middle of the IPL to contact both On and Off cone bipolar cells. Therefore, the calretinin positive cell has the anatomical characteristics of an All amacrine cell1. (d) Labeling with an antibody to PKC-α reveals axon terminals in two substrata of the IPL. A sparse substratum contains the terminals of rod bipolar cells (arrowhead), whereas a thicker layer contains the terminals of cone On bipolar cells. Antibodies to PKC-α exclusively label rod bipolar cells in rodent and rabbit retinas2. In ground squirrel and primate retinas, PKC-α antibodies additionally label On cone bipolar cell types3, 4.
Supplementary Figure 2. Rod- and cone-driven epscs have the same shape.

Representative b2 cell epscs obtained following a 1 ms rod or cone depolarization in the whole cell (WC, step from –70 to –30 mV) or loose seal (LS, depolarizing step that elicited a maximal epsc) mode. Responses are normalized. The similar epsc shapes obtained following rod and cone stimulation cannot be attributed to changes in intracellular calcium buffering capacity during whole cell recording since loose seal recording would not be expected to alter the intracellular milieu.
Supplementary Figure 3. Properties of the rod photoreceptor Ca\(^{2+}\) current.

(a) The membrane voltage of a rod was stepped from –70 mV to between –60 and 30 mV. Whole-cell currents were recorded before and during the application of the Ca\(^{2+}\) channel blocker Co\(^{2+}\) (1 mM). Subtracted currents are shown. The lowermost trace shows the pulse duration. Same cell as in Fig. 2c of main text. (b) Plot of steady current versus voltage from the traces in (a). (c) Average current-voltage plot (n = 5 rods). Currents from each cell were first normalized to the peak response from that cell. The voltage activation range of the rod Ca\(^{2+}\) current is similar to those of the Ca\(^{2+}\) currents in short (S)- and medium (M)- wavelength sensitive cones\(^5,6\).
Supplementary Figure 4. AMPA receptors mediate transmission at the rod to b2 cell synapse.

Response of a b2 cell to an 80 ms (lowermost trace) rod step depolarization before, during, and after exposure to GYKI 53655 (25 µM), an AMPA receptor selective antagonist. The rod was depolarized in the loose seal mode to a voltage that produced a maximal postsynaptic response. A similar degree of response suppression was observed at the cone to b2 cell synapse.7
Supplementary Figure 5. Paired-pulse responses recover with a similar time course at the rod and cone to b2 cell synapse irrespective of recording mode.

Plots of normalized epsc recovery as a function of interpulse interval were obtained following either rod or cone depolarization in either the loose seal (LS) or whole cell (WC) modes (n = 11 pairs; data re-plotted from main text Fig. 2d). The normalized response recovery was first calculated for each cell pair. Averages and standard deviations were then calculated for each response group (cone WC, n = 3; cone LS, n = 5; rod WC, n = 1; rod LS, n = 2). Inspection suggests that the recovery time courses of the rod- and cone-driven responses were similar irrespective of the recording mode.
Supplementary Figure 6. Current flows bidirectionally between rods and cones via electrical synapses. A rod step depolarization produces a graded depolarization in an adjacent cone.

(a) An 80 ms rod step depolarization from −70 to −30 mV (timing trace below) produced both an initial transient and a delayed, secondary peak response (arrowhead) in a voltage-clamped b2 cell. The second component had a 20-80% rise time of 5.5 ± 1.0 ms (n = 7) and a time to peak of 15.3 ± 7.3 ms (n = 8). Second components were also observed when rods were step-depolarized in the loose seal mode (n = 3). We next addressed a potential mechanism for the second component—rod to cone electrical coupling. (b) Junctional currents from a rod-cone pair during a series of transjunctional voltage steps (lower traces). The junctional conductance was 170 pS (121 ± 80 pS in n = 9 rod-cone pairs; ground squirrel rod-cone and cone-cone junctional conductances are not significantly different (p = 0.03)). (c) Voltage responses of a cone during a series of depolarizing steps in a neighboring rod. The rod was stepped in voltage clamp from −70 mV to the values shown at right. (d) The start of the cone response is shown on an expanded time scale. The four smallest responses were fitted with a single exponential curve (τ = 5.8 ± 0.6 ms). A similar value was obtained from one additional rod-cone pair and five cone-cone pairs (τ = 6.5 ± 1.5 ms). For comparison, cones had a membrane time constant of 3.95 ± 0.63 ms when stepped from −70 to −80 mV (membrane resistance = 453 ± 71 MΩ, membrane capacitance = 8.96 ± 0.49 pF; n = 7). (e) A plot of peak cone membrane voltage change verses rod step voltage from c shows a roughly linear relationship. A typical rod step depolarization from −70 to −30 mV produced a 5 mV depolarization in adjacent cones. This depolarization should activate the cone L-type Ca^{2+} channels which mediate transmitter release.
Supplementary Figure 7. The second delayed response component can occur in the absence of a first transient component.

(a) The diagram shows two pathways over which signals in either a rod or a cone can reach a postsynaptic b2 cell. A direct pathway (cyan arrow) produces an initial, transient b2 epsc and involves contacts between a presynaptic rod (left panel) or cone (right panel) and the postsynaptic b2 cell. A second pathway (blue arrow) involves signal flow from a rod or cone across gap junction channels to an adjacent cone followed by synaptic transmission to the same b2 cell. Adjacent photoreceptors frequently contact the same bipolar cell. We hypothesize that this second pathway could contribute to the second, delayed, epsc response in b2 cells that can occur following either rod or cone step depolarization. Because of the numerical predominance of M-cones, it was easier to study the mechanism of the delayed epsc peak by depolarizing an M-cone and recording from a b2 cell. Similar results was obtained at one rod to b2 cell synapse. (b) Current responses of a b2 cell during a series of cone steps from –70 mV to between –60 and 95 mV (upper two panels). The bottommost panel shows the b2 responses to the same series of voltage steps after transmitter release in the voltage-clamped cone has run down. Pulse timing is shown by the lowermost trace. (c) The amplitudes of the first (red squares) and second (black squares, before, and blue squares, after, rundown) b2 response peaks from b. are plotted against presynaptic cone voltage.
In interpreting the results in (b) and (c), we assume that the initial transient epsc is caused by the ribbon-mediated exocytosis of docked vesicles at the terminal of the voltage-clamped cone. As the cone is stepped to progressively more depolarized potentials, the amplitudes of the initial epsc transient and cone Ca\textsuperscript{2+} current increase and then decrease in parallel\textsuperscript{16}. Ultimately, at very positive potentials (e.g., 95 mV), both Ca\textsuperscript{2+} influx and release from the clamped cone stop. However, it is precisely at these positive potentials that the second, delayed component is largest. It might be argued that there is a very small Ca\textsuperscript{2+} influx that remains during the most depolarized steps, and that this influx requires 5–10 ms to raise Ca\textsuperscript{2+} to the point where it can evoke release from the voltage-clamped cone. However, steps to hyperpolarized voltages (e.g., -50 mV) also produce a minimal Ca\textsuperscript{2+} influx, yet an early transient is evident. We conclude that the second component does not require Ca\textsuperscript{2+} influx into the voltage-clamped cone. After several minutes of whole cell recording, the initial synaptic transient usually disappears or “runs-down”. Rundown presumably results from a loss of essential intracellular release mediators as the size of the photoreceptor Ca\textsuperscript{2+} current is unchanged (unpublished observation). However, as shown in b., the second component persists, and continues to require extreme cone depolarizations. The results are most easily explained by assuming that large depolarizing voltage steps in the clamped cone produce slower depolarizations in neighboring unclamped cones via electrical synapses. The neighboring cones then release transmitter onto the voltage-clamped b2 cell. A similar second response component has been observed in salamander rods, where it is attributed to Ca\textsuperscript{2+}-dependent release from synaptic Ca\textsuperscript{2+} stores\textsuperscript{9}, and in goldfish bipolar cells\textsuperscript{10}, where it is thought to result from a Ca\textsuperscript{2+} dependence of ribbon vesicle turnover. Our observations and those in salamander rods are not entirely in conflict: Salamander rods are electrically coupled\textsuperscript{11}, and a contribution of coupling to the second component was not excluded; and, we have observed a small effect of store-mediated Ca\textsuperscript{2+} release on transmission in ground squirrel cones (unpublished result). Finally, according to our model, brief (1 ms) rod and cone depolarizations to between –40 and –20 mV evoke robust release from the voltage clamped cone, but are probably too brief and small to effectively depolarize neighboring unclamped cones, and thus rarely elicit secondary responses.
Supplementary Figure 8. Off bipolar cells respond more rapidly than On bipolar cells following a photoreceptor step depolarization.

A rod step depolarization in the loose seal mode elicited a b2 cell epsc that emerged from baseline with a latency of 1.10 ± 0.47 ms, had a rise time of 0.59 ± 0.34 ms, and reached a peak at 3.6 ± 1.7 ms (n = 9; see black trace for an example). The blue trace shows a representative On bipolar (b5) cell response to a similar cone depolarization. On bipolar cell responses had slower rise times (13.5 ± 6.1 ms; n = 12 pairs), and longer times to peak (25.9 ± 8.9 ms). The On bipolar cell epsc was likely to result from a direct synaptic input, since a plot of peak response amplitude versus On bipolar cell membrane voltage had a negative slope that intercepted the x-axis at 2.3 ± 5.8 mV (n = 4; results not shown). We used a cone to On bipolar cell synapse in this experiment because the paucity of rod bipolar cells precluded recording from a rod to rod bipolar cell synapse. An assumption is that the signal rise at the cone to On bipolar cell synapse is either equal to or faster than that at the rod to rod bipolar cell synapse. The results suggest that On bipolar cell responses are ~10-fold slower than Off bipolar cell responses following transient photoreceptor exocytosis. A similar delay in synaptic signaling was observed in turtle On and Off bipolar cells\textsuperscript{12, 13}. For comparison, the time constant for signal rise at the rod to cone electrical synapse was 5–7 ms (from Supplementary Figure 6).
Supplementary Methods

Preparation and electrophysiology. The procedures for making ground squirrel (Spermophilus tridecemlineatus) slices have been described\(^5\),\(^7\). During recording, the tissue was superfused with a solution that contained (in mM): NaCl, 115; KCl, 3.1; MgSO\(_4\), 2.28; CaCl\(_2\), 2.0; glucose 6; succinate, 1; lactate, 1; malate, 1; pyruvate, 1; NaHCO\(_3\), 25; 0.01 strychnine; 0.05 picrotoxin (pH 7.4 with 5% CO\(_2\)). The pipette solution contained (in mM): KCl, 80; CsCl, 30; MgSO\(_4\), 2; HEPES 20; tetrapotassium BAPTA (Molecular Probes), 10 or EGTA, 5; ATP, 5; GTP 0.5; and either Neurobiotin (Vector Laboratories), 0.1 or Alexa Fluor 568 (Molecular Probes), 0.1. pH was adjusted to 7.4 with KOH. Slices were mounted on a Zeiss Axioscope 2 microscope and superfused continuously at 31–33ºC. Recordings were obtained with Axopatch 200B amplifiers (Molecular Devices), and currents low-pass filtered at 1 or 5 kHz using the 4 pole Bessel filter on the amplifier. Data was digitized at 10 kHz using an ITC-18 interface (Instrutech) controlled by a G4 PowerMac or Dell computer running IgorPro 6.0 (WaveMetrics). Data was analyzed with custom-made software (IgorPro 6.0). Values are expressed as mean ± s.d. Cone and bipolar cell membrane voltages were maintained at −70 mV unless otherwise indicated. Rod and cone photoreceptors were sometimes depolarized in the on-cell configuration by forming a loose seal with a patch pipette the tip of which was “dirtied” by prior contact with cell membranes. In this configuration, maximal bipolar cell epsc responses were obtained by stepping the photoreceptor pipette from −70 mV to a voltage between −30 and +100 mV. We were concerned that large voltage steps might produce extracellular currents that could polarize neighboring photoreceptors, but recordings from pairs of neighboring S- and M-cones, which are not electrically coupled, showed no crosstalk between a polarized cone and its voltage-clamped neighbor\(^8\). Also, the observed selective signaling between rods and S-cones\(^6\), on the one hand, and b2 Off bipolar cells (rather than b3 or b7 Off bipolar cells), on the other, militates against a more widespread cone activation. Rod photoreceptors were stimulated with a 470 nm LED which delivered the equivalent of 4400 photons-µm\(^2\) at a wavelength of 505 nm during a 10 ms flash. Unless otherwise mentioned, chemicals were from Sigma-Aldrich.

Tracer injection and immunocytochemistry. For tracer injections, a sharp electrode was tip filled with 1–2% Neurobiotin (Vector Laboratories) and back-filled with 3M KCl. Impaled bipolar cells were injected with 0.5–1.0 nA current for 1-2 minutes using an intracellular electrometer (IE-210, Warner Instruments). After recording or injection, slices were fixed and processed for immunocytochemistry as previously described\(^6\),\(^8\). The following antibodies were used: GluR4 (1:100; Chemicon), GluR5 (1:50; Santa Cruz Biotechnology), and rhodopsin (1:500, Chemicon). Slices were usually mounted and imaged in the whole- or flat-mount orientation. Images were acquired with a LSM-510 META or a Leica SP4 confocal microscope (Zeiss) and edited with LSM image software and Photoshop 7.0 (Adobe Systems).
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