HHS Public Access

Author manuscript

Nat Neurosci. Author manuscript; available in PMC 2010 October 01.

Published in final edited form as:

Nat Neurosci. 2010 April ; 13(4): 414–416. doi:10.1038/nn.2507.

A fast rod photoreceptor signaling pathway in the mammalian retina

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Abstract

Rod photoreceptors were recently shown to contact Off cone bipolar cells, providing a novel pathway for rod signal flow in the mammalian retina. By recording from pairs of rods and Off cone bipolar cells in the ground squirrel, we measured the synaptic responses of mammalian rods unfiltered by the slow kinetics of the rod bipolar cell response. We show that vesicle fusion and turnover in mammalian rods is fast, and that this new pathway can mediate rapid signaling.

Rods signal over a ~10^5-fold range of light intensities and use two pathways to communicate with postsynaptic neurons. At the dimmest intensities, small graded signals flow to rod bipolar cells where a metabotropic glutamate receptor-linked cascade provides low-pass temporal and threshold filtering1. At brighter intensities, larger signals flow to cone photoreceptors over a second pathway mediated by rod-cone gap junctions2. Anatomical evidence for a third rod pathway was initially obtained in the ground squirrel3 and subsequently in other mammals4, 5. In this pathway, rod terminals, called spherules, directly contact a subset of Off cone bipolar cells. The function of the third pathway is unclear. Only a 5–20% of the rods contact Off cone bipolar cells, and contacts are on the external surface of the spherule away from vesicle release sites within invaginations4, 5. If the third pathway is functional, then the rapidly-responding AMPA/kainate receptors on Off cone bipolar cells can be used to measure the properties of rod transmitter release. Measurements at an amphibian rod to Off bipolar cell synapse suggest that release is dominated by a component with slow kinetics that is matched to the slow time course of the rod photoresponse6, 7.

We first identified the cone bipolar cell types that contacted rods. Cone bipolar cells were labeled by injecting a fluorescent tracer. Photoreceptor terminals were localized either by tracer injection or by labeling with antibodies to the GluR4 and GluR5 subunits of

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Author Contributions

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postsynaptic AMPA and kainate receptors8, respectively. Small receptor clusters that contained both GluR4 and GluR5 labeled puncta corresponded to the locations of rod spherules (Fig. 1a,b;Supplementary Fig. 1). One bipolar cell type, the “Off” b2, contacted the rod spherules within its dendritic field (Fig. 1c,d). At rod terminals, the tips of b2 cell dendrites were colocalized with GluR4-labeled puncta (Fig. 1e,f). In ground squirrel cones, GluR4-labeled puncta mark the sites of invaginating synapses8. Similar rod contacts were observed in 16 of 19 injected b2 and 1 of 4 On b5 cells. Contacts were absent in other Off (5 b3 and 5 b7) and On (6 unidentified) bipolar cell types. Experiments with two fluorescent tracers confirmed that b2 cells directly contacted rod terminals (Fig. 1g,h). The results suggest that rods signal directly to b2 bipolar cells, and thus we measured synaptic transmission by simultaneously recording from a rod and a nearby b2 cell9.

Ribbon-mediated release has both transient and sustained components. The shape of the transient component is related both to the size of a membrane-docked pool of vesicles and the rate of vesicle fusion10. Transient excitatory postsynaptic currents (epscs) were measured in a b2 cell following a brief rod or cone depolarization. Rod depolarization triggered a b2 cell epsc with a peak amplitude of −94 ± 44 pA (mean ± s.d.). Subsequent loose seal (*i.e.*, on-cell) depolarization9 of a cone that contacted the same b2 cell produced a peak response of −273 ± 205 pA (Fig. 2a; n = 9). The ~3-fold difference in response amplitude corresponded to a ~3-fold difference in the number of anatomical contacts at rod (2.3 ± 0.7, n = 7) versus cone (7.0 ± 2.5, n = 14) to b2 cell synapses. Synaptic responses initiated by rods and cones had similar 20–80% rise times (rod = 0.59 ± 0.33 ms versus cone = 0.45 ± 0.22 ms, n = 9) and decay time constants (τ = 2.64 ± 1.32 ms versus 2.18 ± 1.04 ms, n = 9; Fig. 2a, **inset**). The rapid time course of the rod-initiated epsc did not result from the inclusion of a fast Ca^{2+} buffer (10 mM BAPTA; Supplementary Methods) in the whole cell recording pipette solution, since transient epscs were obtained when rods were also depolarized in the loose seal mode (n = 4 of the 9 experiments; Supplementary Fig. 2.). Consistent with the fast epsc onset, rod Ca^{2+} currents rapidly activated during a depolarizing voltage step (τ_{onset} < 0.5 ms; Fig. 2b; Supplementary Fig. 3). The AMPA receptor antagonist GYKI 53655 (25 μM) reduced epsc amplitude by 85–95% at rod (n = 2; Supplementary Fig. 4) and cone9 to b2 cell synapses, suggesting that both synapses contain the same receptor type. The rapid epsc time course and resistance to fast Ca^{2+} buffers are consistent with a close association between Ca^{2+} channels and vesicle docking sites in both rods and cones8.

We next compared sustained transmitter release at rod and cone synapses. We measured the replenishment rate of a release-ready pool of vesicles by first depleting the pool with a brief depolarization and then applying a second depolarization, after a variable interval, to measure pool recovery9. Recovery time courses were comparable for rod- and cone-driven responses (Fig. 2c,d). The comparable recovery time courses are unlikely to result from alterations in intracellular Ca^{2+} buffering during whole cell recording since most of the recordings were obtained in the loose seal mode (Supplementary Fig. 5). The size of the steady synaptic current during a prolonged cone depolarization provides another measure of vesicle turnover. The ratio of steady to peak currents at rod and cone to b2 cell synapses

were similar (rod = 3.6 ± 0.4%, n = 5; cone = 4.0 ± 1.5%, n = 6). The results suggest that ground squirrel rods and cones replenish their releasable pool of vesicles at similar rates.

The dynamic regulation of free Ca^{2+} within the salamander rod terminal appears to greatly prolong the time course of transmission6, 7. A similar prolonged release component occurs in goldfish bipolar cell terminals11. We occasionally observed small prolonged components at both rod and cone to b2 cell synapses (Supplementary Fig. 6). However, these secondary responses may not depend entirely on intracellular Ca^{2+} stores, as we found that current spread from a stimulated photoreceptor to electrically coupled neighbors can produce a delayed response in a common postsynaptic bipolar cell (Supplementary Figs. 6,7).

Rod light responses are slower than cone light responses, and it is thought that rapid signaling is less important for rod vision. Against this view, rods can mediate vision during 30 Hz flicker12; rod photoresponses to bright flashes activate and decay in under 100 and 200 ms13, respectively; and, ganglion cell spike trains are precise to within 2–10 ms under rod-driven conditions14. When light hyperpolarizes photoreceptors, release stops and vesicles accumulate at membrane docking sites. When photoreceptors depolarize at light-off, Ca^{2+} influx causes a burst of vesicle fusion which produces a transient excitatory response in Off bipolar cells15. We show (Fig. 2e) that a slow photoreceptor depolarization, designed to mimic the recovery time course of a rod after a bright flash, triggers a transient response in a postsynaptic b2 cell (20–80% rise = 3.4 ms; time to peak = 7.3 ± 2.6 ms; n = 4). The rod depolarization at light-off is also directly signaled to rod bipolar cells and, indirectly, through rod-cone gap junctions and cones, to On and Off cone bipolar cells. We compared the speed of transmission following a step depolarization at the rod to Off b2 cell synapse, the rod-cone electrical synapse, and at a photoreceptor to On bipolar cell synapse (Supplementary Figs. 6,8). Signals in the rod to b2 cell pathway rose and reached their peak 5–10 times faster than the signals in the other pathways. We conclude that the kinetics of transmission at rod and cone to b2 cell synapses are similar, and that the rod to b2 cell synapse can mediate rapid signaling at light-off in a mammalian retina.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by National Institutes of Health grant (R01EY12141) to S.H.D., (F32EY017257) to W.L., and the National Eye Institute Intramural Research Program to W.L and S.C.

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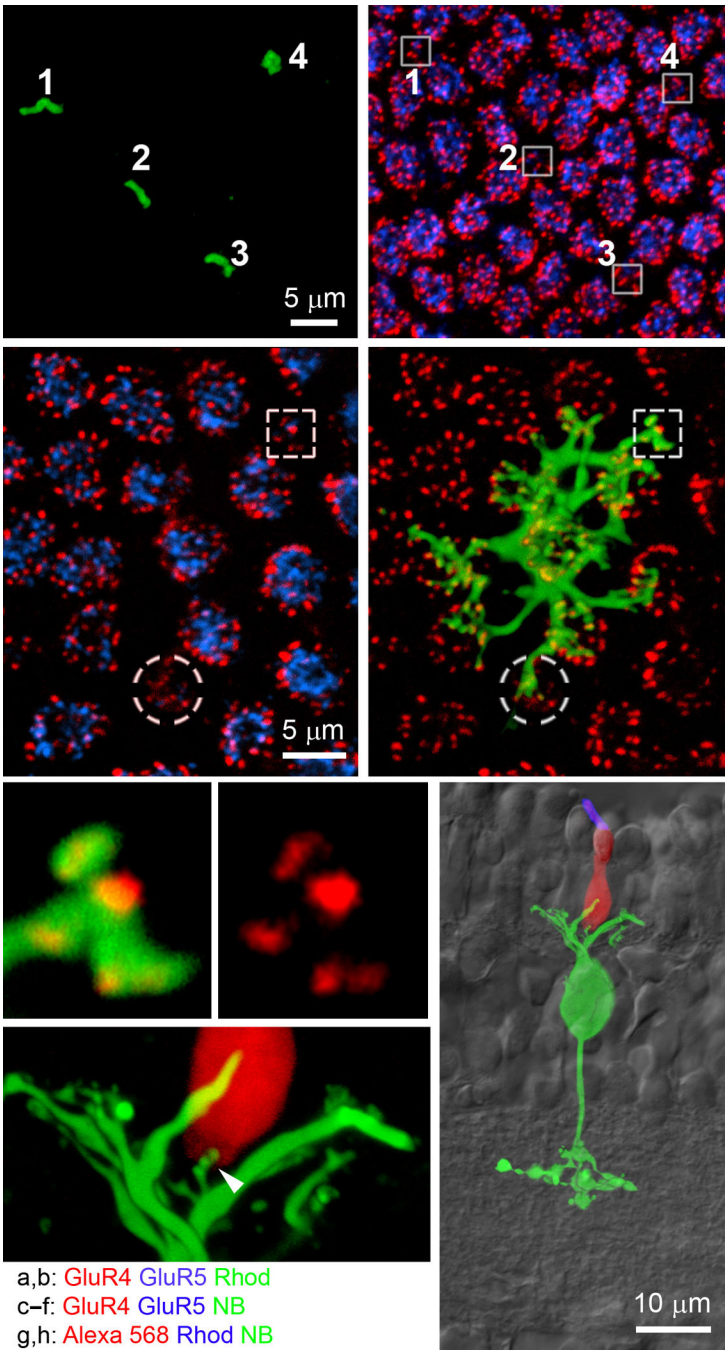

Figure 1.

Anatomical contacts between rods and b2 Off cone bipolar cells. **(a)** Rod outer segments (numbered) were labeled with an antibody to rhodopsin in a flat-mounted retina. **(b)** A different image plane shows the corresponding small clusters (squares) of GluR4 and GluR5 labeled puncta. **(c,d)** A b2 Off cone bipolar cell was labeled with Neurobiotin (NB). The b2 cell contacted all the terminals within its dendritic field including those of a rod (square) and an S-cone (circle). **(e,f)** The dendritic endings at the rod terminal colocalize with GluR4 puncta. **(g)** A tracer-injected rod (Alexa Fluor 568) and b2 cell in a retinal slice (n = 3). The

rod outer segment was labeled with an antibody to rhodopsin (Rhod). The b2 cell was identified by its level of axon termination. (**h**) Magnified image of the rod terminal showing a contact (arrowhead) with a b2 cell dendrite. Experimental use of animals was approved by the Institutional Animal Care and Use Committee at Northwestern University.

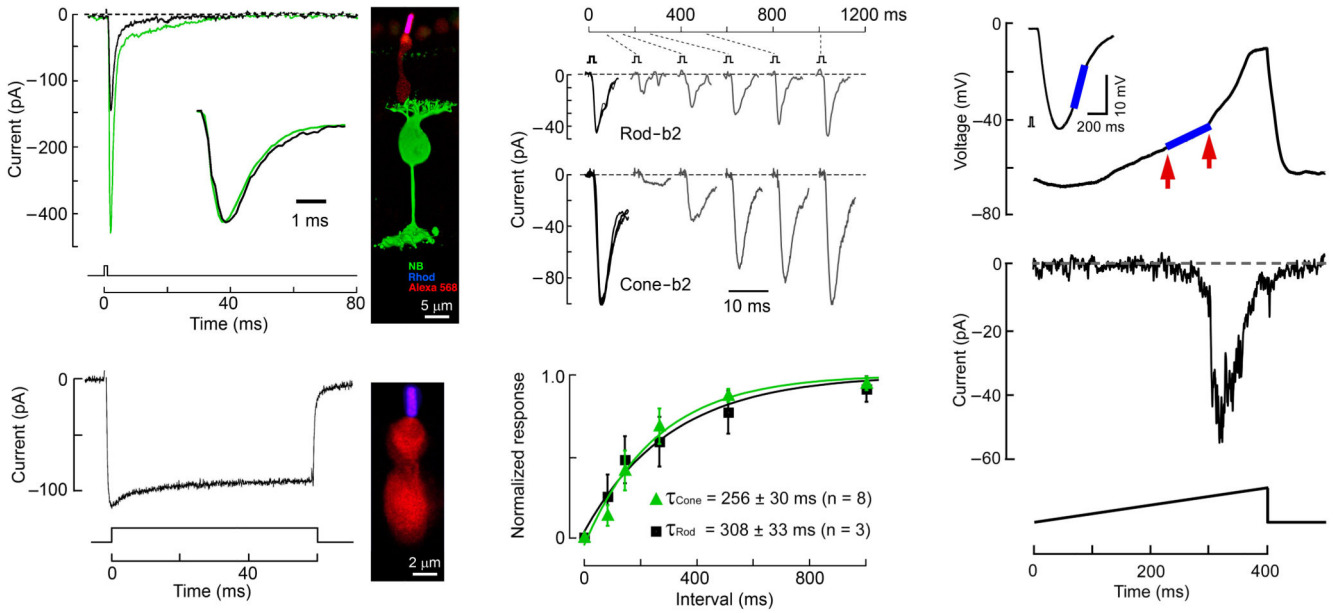

Figure 2.

Synaptic transmission between rods and b2 bipolar cells. **(a)** Left, current response of a b2 cell to a 1 ms depolarization from −70 to −30 mV in a rod (black trace) and subsequently in a nearby M-cone (green trace). The cone was depolarized in the "loose seal" configuration to elicit a maximal response. Inset, response transients normalized and superimposed. Right, morphology of the recorded rod (Alexa Fluor 568) and b2 cell (NB), and rhodopsin (Rhod) antibody labeling. **(b)** Ca^{2+} current (left, Co^{2+}-subtracted) during a rod step from −70 to −20 mV. Recorded rod (left) filled with Alexa Fluor 568 (red) and labeled with an antibody to rhodopsin (blue). **(c)** Two 15 ms depolarizations were applied to either a rod (upper) or a cone (lower) with increasing inter-pulse intervals. The responses in a b2 cell to the first pulses are shown in black and to the second pulses are shown in gray. **(d)** Normalized rod-initiated (black) and cone-initiated (green) responses were plotted against inter-pulse interval (mean ± s.d.). **(e)** A current injection "ramp" (lowermost trace) in a presynaptic cone produced a steady voltage change (blue line) and a transient current in a postsynaptic b2 cell. The line between the two arrows (from −50 to −40 mV) was superimposed on the corresponding voltage range of a rod light response measured in current clamp (inset). Flash (10 ms) intensity equaled 4,400 photons-µm^2 at an equivalent wavelength of 505 nm. Rod membrane potential in darkness = −30.7 mV. A cone-triggered response was used for illustration because it is larger than the rod-triggered response. The kinetics of rod- and cone-triggered responses should be the same.