or differences between active zones. However, the high degree to which SNARE protein function is conserved suggests that the phosphorylation state of SNAP-25 also regulates the transition between distinct vesicle pools in neurons, although the details of these pools may differ. For example, PKA-dependent SNAP-25 phosphorylation may regulate the rate of vesicle depriming from the neuronal RRP. It is worth noting that the rate of depriming is significantly slower in synaptic terminals of bipolar neurons than in chromaffin cells (Heidelberger et al., 2002). In light of the results of Nagy et al., one possibility is that the balance between phosphorylation and dephosphorylation favors the phosphorylated state in the bipolar neuron. Given the relationship between the neuronal RRP and synaptic efficacy, confirmation of the role of SNAP-25 and the identification of other factors that regulate pool size in neurons, such as the unidentified target of PKA activity, should be given high priority.

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Selected Reading

Heidelberger, R., Sterling, P., and Matthews, G. (2002). J. Neurophysiol. *88*, 98–106.

Mennerick, S., and Matthews, G. (1996). Neuron *17*, 1241–1249.

Nagy, G., Reim, K., Matti, U., Brose, N., Binz, T., Rettig, J., Neher, E., and Sørensen, J.B. (2004). Neuron *41*, this issue, 417–429.

Sakaba, T., and Neher, E. (2001). Neuron *32*, 1119–1131.

Sørensen, J.B., Nagy, G., Varoqueaux, F., Nehring, R.B., Brose, N., Wilson, M.C., and Neher, E. (2003). Cell *114*, 75–86.

Voets, T., Neher, E., and Moser, T. (1999). Neuron *23*, 607–615.

Design for a Binary Synapse

The mammalian rod transfers a binary signal, the capture of 0 or 1 photon. In this issue of *Neuron*, Sampath and Rieke show in mouse that the rod's tonic exocytosis in darkness completely saturates a G protein cascade to close nearly all postsynaptic channels. A full-sized photon event supresses exocytosis sufficiently to allow ~30 postsynaptic channels to open simultaneously. Thus, the synapse behaves like a digital gate, whose hallmark is reliability and resistance to noise.

Although we generally consider the human visual system as specialized for daylight, roughly 95% of our photoreceptors are rods, and in this respect we resemble the mouse. Rods dominate the photoreceptor sheet (outside the all-cone fovea), because from dusk till dawn, natural light provides less than one photon capture per rod over its integration time. The rod's transducer responds to a single photon capture by hyperpolarizing ~1 mV, a response that rises modestly above the noise (Baylor et al., 1984; Schneeweis and Schnapf, 1995; Field and Rieke, 2002). Such single-photon detectors must pack densely in order to maximize the photon catch and produce overall images whose quality is approximated in Figure 1A. But we would see such images only if most of the information embodied by the patterns of single photon capture actually reached the brain. That they do arrive is certain because a photon event reliably evokes several spikes in a ganglion cell (Barlow et al., 1971); however, there are serious obstacles—especially at the initial synapse onto the bipolar cell dendrite.

First, the rod's single-photon current in varies in amplitude (Figure 1B, upper trace). If it rises 5-fold above the noise, the event is easily spotted, but when it is much smaller, as occurs commonly in the mouse rod, the event can easily be taken for noise (Field and Rieke, 2002). Second, while this rod is producing a marginal photon signal, 19 other rods also contact the same bipolar cell and potentially contribute intrinsic noise (from their phototransduction cascades) and also synaptic noise (from their poisson vesicle release). Third, glutamate binding to a metabotropic receptor on the bipolar dendritic tip tonically activates a G protein cascade that closes cation channels in the dark. Fluctuations in this cascade could potentially cause fluctuations in the number of channels closed in the dark or open in light. This would contribute postsynaptic noise at each of 20 sites—that would accumulate at the bipolar soma and swamp the photon response from one rod.

A solution was proposed: let the initial synapse amplify nonlinearly to boost the larger voltages (likely to be photon events) more than smaller voltages (likely to be noise) (Baylor et al., 1984; van Rossum and Smith, 1998). This conjecture was proved by Field and Rieke (2002), who demonstrated in the bipolar cell that nonlinear amplification of single photon events strongly rejects noise and, along with it, the smaller single photon events. Sampath and Rieke (2004) now address the next big question: what causes this nonlinear amplification?

Their assay was technically difficult, requiring them to slice a mouse retina in the dark (using infrared goggles) and then record the photocurrent from a tiny bipolar cell body without disturbing its delicate synaptic input from the rods. Nonlinearity was assessed by the Hill exponent, which expresses the relation between stimulus intensity and response. The relation is linear when the exponent is 1. Under these conditions, and using flash strengths somewhat greater than 1 photoisomerization (Rh*) per rod, the Hill coefficient was typically ~1.5, indicating "supralinearity," i.e., bigger responses are amplified more (Field and Rieke, 2002). Sampath and Rieke first tested whether feedback from interneurons, such as a horizontal cell that integrates input from about 1000 rods (Figure 1C; Nelson et al., 1975), causes the nonlinearity. They blocked all potential feedback by applying antagonists of AMPA and NMDA glutamate receptors that excite these interneurons. The Hill coefficient was unaffected, showing that supralinearity at these intensities is not caused by feedback and must be intrinsic to the synapse between rod and bipolar dendrite.
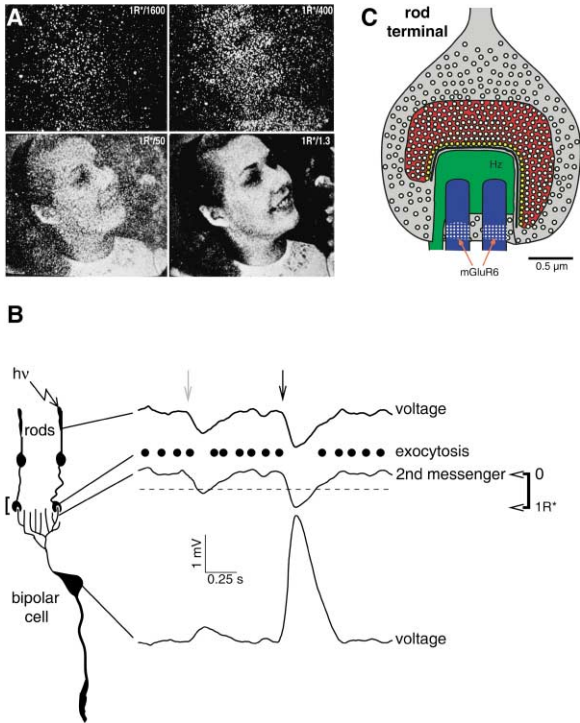
Figure 1. Functional Architecture of a Binary Synapse

(A) Four images of the same face created by light flashes of different intensity, all equivalent to less than 1 Rh*/rod. Reprinted from Rose (1973) with permission.

(B) Rod bipolar dendrite resembles a candelabrum, and each of its 20 tips connects to a single rod synaptic terminal, shown at higher magnification in (C). Upper trace shows noisy dark voltage in rod outer segment. Light arrow indicates a small hyperpolarization that might be a photon event (Rh*) but is hard to distinguish from noise; dark arrow indicates a larger hyperpolarization that is more likely to represent an Rh*. Dots in next trace represent the temporal pattern of vesicle release: high rate in dark with poisson variability, but a longer pause in release caused by the larger hyperpolarization. Second messenger trace also fluctuates somewhat in darkness (0 photon condition), but because some stage before or at the channel level is saturated, these fluctuations do not open channels until the level reaches the dashed line. Then most channels open simultaneously to strongly amplify the bipolar voltage.

(C) The rod synaptic terminal contains a large, crescentic ribbon (red) that tethers several hundred vesicles near to the curved active zone that docks 100 vesicles for immediate release. The mGluR6 receptors are expressed, not at the dendritic tips, but lower down, more than 500 nm from the release sites (see text). Hz marks one of the two horizontal cell spines that invaginates the rod to collect signals and provide feedback. Reprinted from Rao-Mirotznik et al. (1995) with permission.

They next asked whether exocytosis, which is maximal in the dark, is sufficient to cause saturation of the transduction cascade in the bipolar dendrite. If so, the bipolar cell would be relatively insensitive to small decreases in glutamate, which might arise from poisson fluctuations in exocytosis (Figure 1B). To test this, they measured the bipolar current in darkness, when the channels should be maximally closed by glutamate, and added a high concentration of APB, a known agonist to mGluR6. The dark current was hardly affected, indicating that the bipolar cascade is maximally active in the dark. But would this be caused by glutamate saturation of the mGluR6 receptors, plausible from previous mea-

surements and calculations (de la Villa et al., 1995; Rao-Mirotznik et al., 1998), or downstream, by saturation of the G protein cascade?

GTP-γ-S, applied via the recording pipette, gave a clear answer. This analog of GTP is poorly hydrolyzed and thus greatly extends G protein activity. Consequently, if darkness saturated the external receptors but not the internal cascade, GTP-γ-S should increase the dark current. It did not, proving that darkness fully activates the internal cascade. Conceivably, this would contribute to the supralinear response because smaller events would not relieve the saturation. To test whether full activation of the cascade requires glutamate to saturate the mGluR6 receptors, Sampath and Rieke reduced the number available for the light response with low concentrations of the high-affinity APB. If mGluR6 saturation caused the nonlinearity, APB would not affect the Hill exponent, but the exponent actually increased, suggesting that the key saturation is due to factors downstream of the receptor. This conclusion was confirmed with a high-affinity antagonist which also suggested that in darkness the receptors are not strongly saturated.

Perhaps most importantly, Sampath and Rieke determined by noise analysis the single channel current (∼0.27 pA), which allowed them to calculate the number of channels open in dark and light. They found in darkness that at each synapse only 0.5–2.0 channels are open—supporting the idea of essentially complete saturation. A large photon event in the rod (Figure 1A, heavy arrow) suppresses glutamate release for long enough to relieve the second messenger saturation and open most of the channels at that synapse, about 30. This causes approximately 6-fold amplification in the rod bipolar cell (Figure 1B), enough to evoke a burst of exocytosis at the bipolar synaptic terminal (Singer and Diamond, 2003).

To create a nonlinear synapse with the sharp thresholding of a digital gate requires that many other aspects of the synapse cooperate to keep the G protein cascade saturated in the dark. For example, the high tonic rate of exocytosis (Rao-Mirotznik et al., 1998; van Rossum and Smith, 1998) demands a huge reservoir of readily releasable vesicles—which corresponds to the rod's huge synaptic ribbon and active zone (Figure 1C). To minimize fluctuations of glutamate at the mGluR6 receptors, all vesicles should be released at roughly the same distance from the receptors. This is achieved by bending the active zone into a semicircle and placing the receptors at its center (Figure 1C; Rao-Mirotznik et al., 1995; Vardi et al., 2000). Finally, despite the lack of evidence for feedback under the conditions of the present experiments, the rod almost certainly receives horizontal cell feedback (Figure 1C). Whether these lateral connections regulate sensitivity during the single photon response or at higher backgrounds remains to be determined because the present experiments were performed somewhat above the single photon regime. Nevertheless, the present report delivers a generous increment in our understanding of a binary synapse and the molecular basis for night vision.

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Selected Reading

Barlow, H.B., Levick, W.R., and Yoon, M. (1971). Vision Res. *S3*, 87–101.

Baylor, D.A., Nunn, B.J., and Schnapf, J.L. (1984). J. Physiol. *357*, 575–607.

de la Villa, P., Kurahashi, T., and Kaneko, A. (1995). J. Neurosci. *15*, 3571–3582.

Field, G.D., and Rieke, F. (2002). Neuron *34*, 773–785.

Nelson, R., Lützow, A.V., Kolb, H., and Gouras, P. (1975). Science *189*, 137–139.

Rao-Mirotznik, R., Harkins, A., Buchsbaum, G., and Sterling, P. (1995). Neuron *14*, 561–569.

Rao-Mirotznik, R., Buchsbaum, G., and Sterling, P. (1998). J. Neurophysiol. *80*, 3163–3172.

Rose, A. (1973). Vision: Human and Electronic (New York: Plenum Press).

Sampath, A.P., and Rieke, F. (2004). Neuron *41*, this issue, 431–443.

Schneeweis, D.M., and Schnapf, J.L. (1995). Science *268*, 1053–1056.

Singer, J.H., and Diamond, J.S. (2003). J. Neurosci. *23*, 10923–10933.

van Rossum, M.C.W., and Smith, R.G. (1998). Vis. Neurosci. *15*, 809–821.

Vardi, N., Duvoisin, R.M., Wu, G., and Sterling, P. (2000). J. Comp. Neurol. *423*, 402–412.