Supplementary Figure 1

Light responses of rod photoreceptors in mouse whole mount retina.

a, First column shows the mean and standard error of rod depolarization evoked by a 800 μm spot at 1090 R/s intensity. In the second and third column rod depolarization to 800 μm spot are compared in two different experimental conditions. Second column: rod depolarization was recorded immediately after getting electrical access to rods. Third column: rod depolarization was recorded at the end of the experimental series shown in Fig. 1a. Significance was tested by Mann-Whitney U test, p = 0.37. b, Normalized response magnitudes in whole mount rods at five background intensities and different spot sizes. c, Rod depolarization at high background light levels increased with increasing positive contrast. d, Rods responded with hyperpolarization to negative contrast, and the hyperpolarization increased with increasing negative contrast.
Supplementary Figure 2

Rod responses in Gnat2<sup>cpfl3</sup> mouse.

a, Rod responses at two different background intensities in Gnat2<sup>cpfl3</sup> mice. The stimulus was a spot of 800 μm. b, Quantification, responses were normalized to the maximum hyperpolarization evoked by the 0.26 R<sup>*</sup>/s stimulus.
Supplementary Figure 3

Channelrhodopsin expression in horizontal cells.

We delivered a bi-stable channelrhodopsin (bi-ChR2) to horizontal cells using conditional adeno-associated viruses. A single subretinal injection led to the labeling of horizontal cells across the entire retina.
Supplementary Figure 4

Sequence of light stimulation in the experiments using reversible inactivation of horizontal cells.

First, the retina was stimulated with a test flash, a white spot of 800 µm in diameter shown for 2s. The test flash was not bright enough to activate bi-ChR2 (Fig. 4j). Second, a switch flash was presented. The switch flash was a blue full-field light step that lasted 50 ms. The switch flash was brighter than the test flash and it activated bi-ChR2 (Fig. 4j). Third, a test flash was presented again 10s after the switch flash. After waiting for 5 min we started a new “test flash-switch flash-test flash” cycle. The cycle was then repeated.
Supplementary Figure 5

The influence of picrotoxin and strychnine on the depolarizing rod response.

The effect of the GABA receptor blocker picrotoxin and the glycine receptor blocker strychnine on the depolarizing rod response evoked by a 800 µm diameter spot at 1090 R°/s intensity.
Supplementary Figure 6

The 'seesaw' model at daylight intensities.

Light stimulus leads to cone and, subsequently, horizontal cell hyperpolarization. Horizontal cell hyperpolarization, in turn, depolarizes rods via a sign-inverting synapse.
Supplementary Figure 7

Layout of the upright two-photon microscope.
Supplementary Figure 8

Layout of the optical path.

A two-photon laser source provided a laser beam, which was attenuated by polarization optics and was scanned using mirrors mounted on an upright microscope. The fluorescent signal from labeled cells was split and detected by two photomultipliers. An infrared camera was used to visualize the patch electrode and retinal cells. Infrared light, light for photoreceptor stimulation and light for bi-ChR2 stimulation were provided by a DLP projector. The light provided by the DLP projector was gated by a fast shutter and was modified by neutral density and band pass filters.