

Figure S1. Light microscope images of an ON-cone bipolar cell (ON-cBC) (left) and a rod BC (right) in the rabbit retinal slice. Related to Figure 4.

Cells were filled with Alexa Fluor 488 and visualized with fluorescence. The two cells can be distinguished by the positions of their axon terminals in the inner plexiform layer and by the large bulbous morphology of the axon terminal of the rod BC. The ON-cBC was likely subtype CBb4 [S1]. Scale bar: 10 μm .

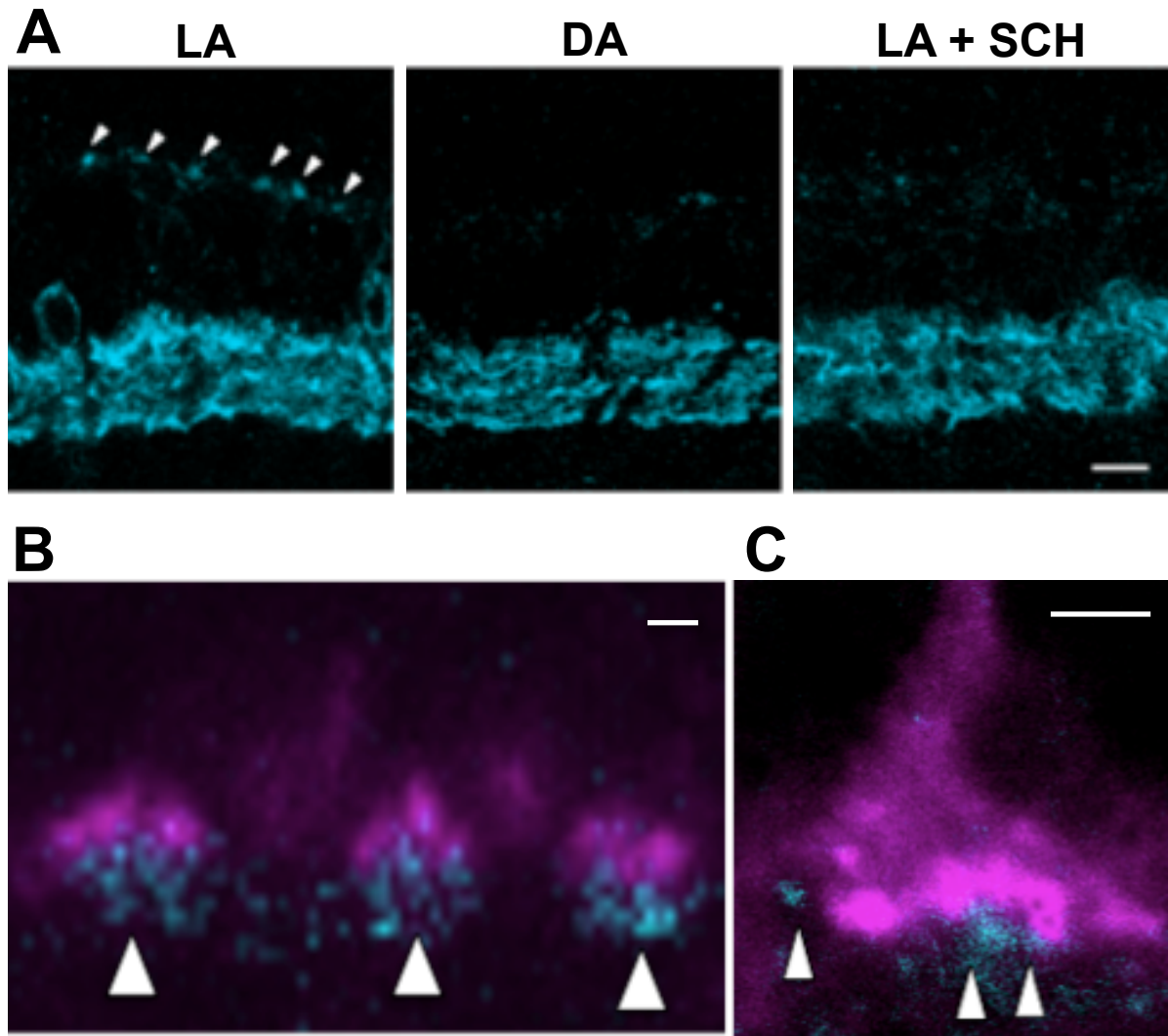


Figure S2. Maintained bright illumination increases GABA_A receptor (GABA_AR) expression just beneath cone pedicles by activating dopamine D₁ receptors (D₁Rs). Related to Figures 5 and 6.

(A) Single confocal scans of vertical retinal sections, which show single labeling of the $\beta 2/3$ subunit of GABA_ARs, revealed more immunoreactivity (IR) in the outer plexiform layer following maintained bright light adaptation (LA) than following maintained dark adaptation (DA) or following maintained bright illumination in the presence of the selective D₁R antagonist SCH23990 (SCH; 5 μ M). (B, C) Double immuno-staining of GABA_ARs and peanut agglutinin (PNA), a marker for cones in the rabbit retina [S2], revealed that following maintained bright illumination staining for the $\beta 2/3$ subunits of GABA_ARs consisted of clustered puncta located just beneath the cone pedicles. Image in C is from a different retina than in B and is at a higher magnification. GABA_ARs: cyan; cones (PNA): magenta. (A, B) Arrowheads denote GABA_AR-IR. (A-C) Scale bars: 20 μ m (A); 5 μ m (B); 1 μ m (C).

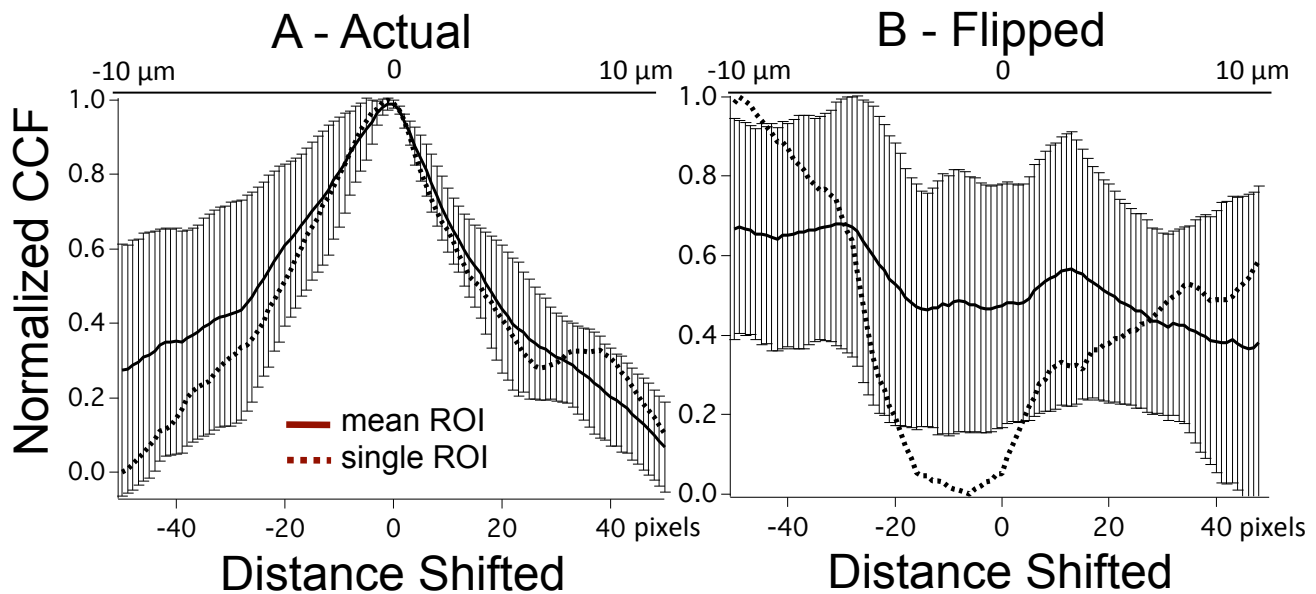


Figure S3. Colocalization analysis using JACoP plugin for NIH ImageJ. Related to Figure 5.

(A) Normalized cross correlation factor (CCF) for regions of interest (ROIs) from Goa labeled ON-BC dendrites (green channel) and GABA_AR-positive puncta (red channel) following maintained bright background illumination. As described previously [S3], CCF analysis was performed by shifting the red channel (GABA_ARs) in the x-direction pixel by pixel (lower x-axis) relative to the green channel (Goa-positive ON-BCs) and calculating the CCF. The peak of the CCF occurred when the red channel was not shifted (= 0 pixel shift), indicating co-localization of the red and green channels. (B) To exclude the possibility that GABA_AR-IR was randomly distributed, the red channel (GABA_ARs) was flipped horizontally and the normalized CCF re-calculated. In this case, the peak was not close to a shift of 0 pixels, that is, the CCF required a strong shift to reach its maximum. This result indicates that the colocalization observed in A was not a consequence of the random distribution of GABA_AR-IR, but rather that the ON-cBC dendrites and GABA_ARs were truly co-localized. (A, B) These results are consistent for an averaged number of ROIs (mean, solid line, n = 6) and for a single representative ROI (dotted line). Lower x-axis denotes the distance shifted in pixels; the upper x-axis denotes the distance shifted in microns. The standard deviation (SD) of the CCF at 0 pixels: actual was 0.01; flipped was 0.31. The size of the ROIs was 500 x 100 pixels (50 μm x 10 μm). Error bars denote SD for each data point.

SUPPLEMENTAL REFERENCES

- S1. MacNeil, M.A., Heussy, J.E., Dacheux, R.F., Raviola, E., and Masland, R.H. (2004). The population of bipolar cells in the rabbit retina. *J. Comp. Neurol.* 472, 73-86.
- S2. Hack, I., and Peichl, L. (1999). Horizontal cells of the rabbit retina are non-selectively connected to the cones. *Eur. J. Neurosci.* 11, 2261-2274.
- S3. Hilgen, G., von Maltzahn, J., Willecke, K., Weiler, R., and Dedek, K. (2011). Subcellular distribution of connexin45 in OFF bipolar cells of the mouse retina. *J. Comp. Neurol.* 519, 433-450.