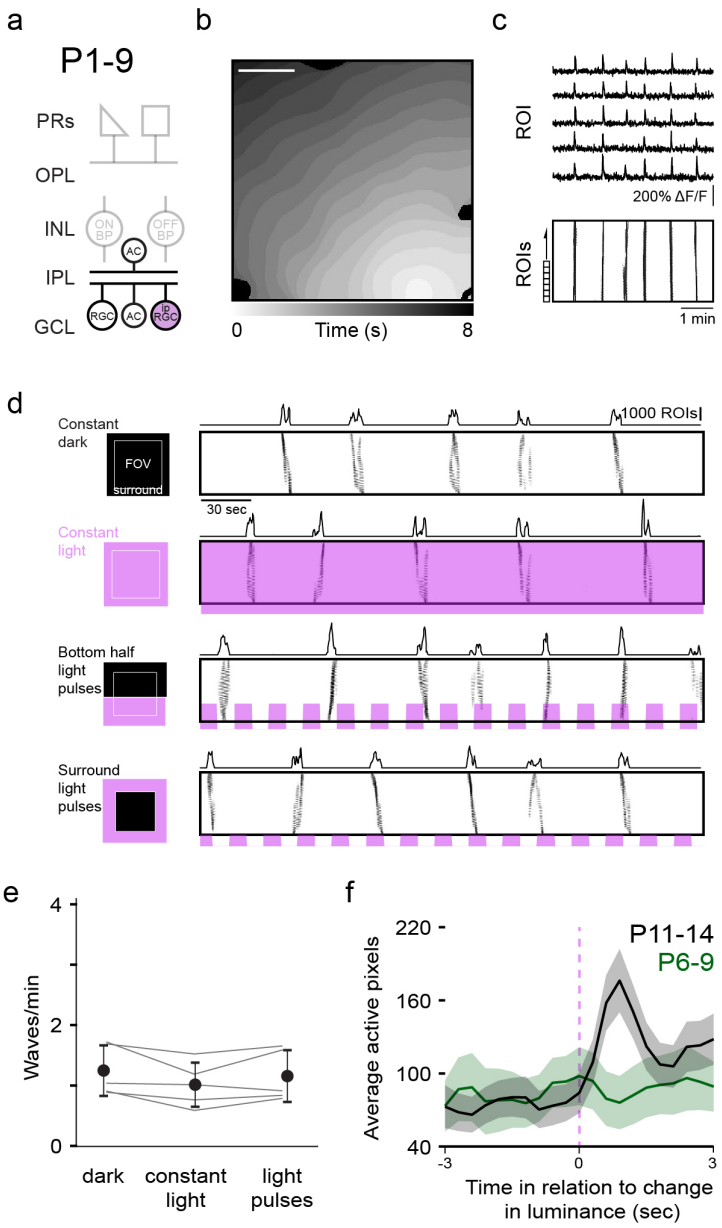
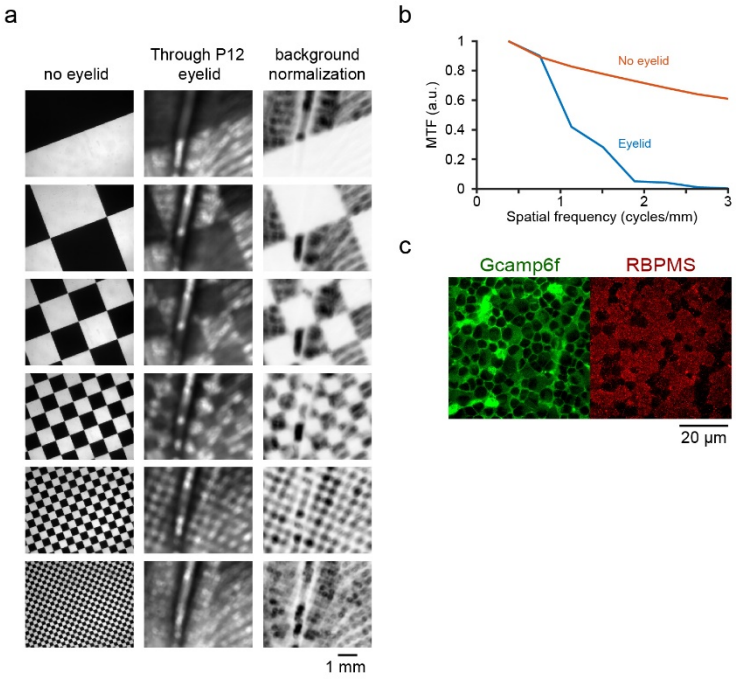


Supplementary Figure 1, Related to Figure 1. Light does not modulate the frequency of cholinergic waves.

a. Left: schematic of retinal circuitry in P1-9 mice when cholinergic waves occur. Purple indicates light-sensitive cells. OPL: outer plexiform layer; INL: inner nuclear layer; IPL: inner plexiform layer; GCL: ganglion cell layer; RGC: retinal ganglion cell; ipRGC: intrinsically photosensitive RGC; AC: amacrine cell. Scale bar = 200 μm.
b. Heat map representing the spread of an example cholinergic wave observed with two-photon calcium imaging in retina expressing GCaMP6f in synapsin 1 positive cells.
c. (Top) Time course of the fractional change in fluorescence (ΔF/F) for five example ROIs acquired at a frame rate of 3 Hz. (Bottom). Raster plots of Ca^{2+} transients > 15% ΔF/F for all ROIs. The total imaging duration is 5 minutes.
d. Raster plots depicting retinal waves under four light conditions: dark, constant light, surrounding light pulses, and light pulses across the bottom of the FOV. The sum of active ROIs

is displayed above each raster plots.
e. Summary data depicting mean ± SD wave frequency for dark, light, and flickering light conditions. ns, n = 5 retinas per group.
f. Waveform average depicting sum of ROIs ± SEM triggered around changes in luminance at P6-9 (cholinergic waves, green) and at P11-14 (glutamatergic waves, black; same data from Figure 1f).

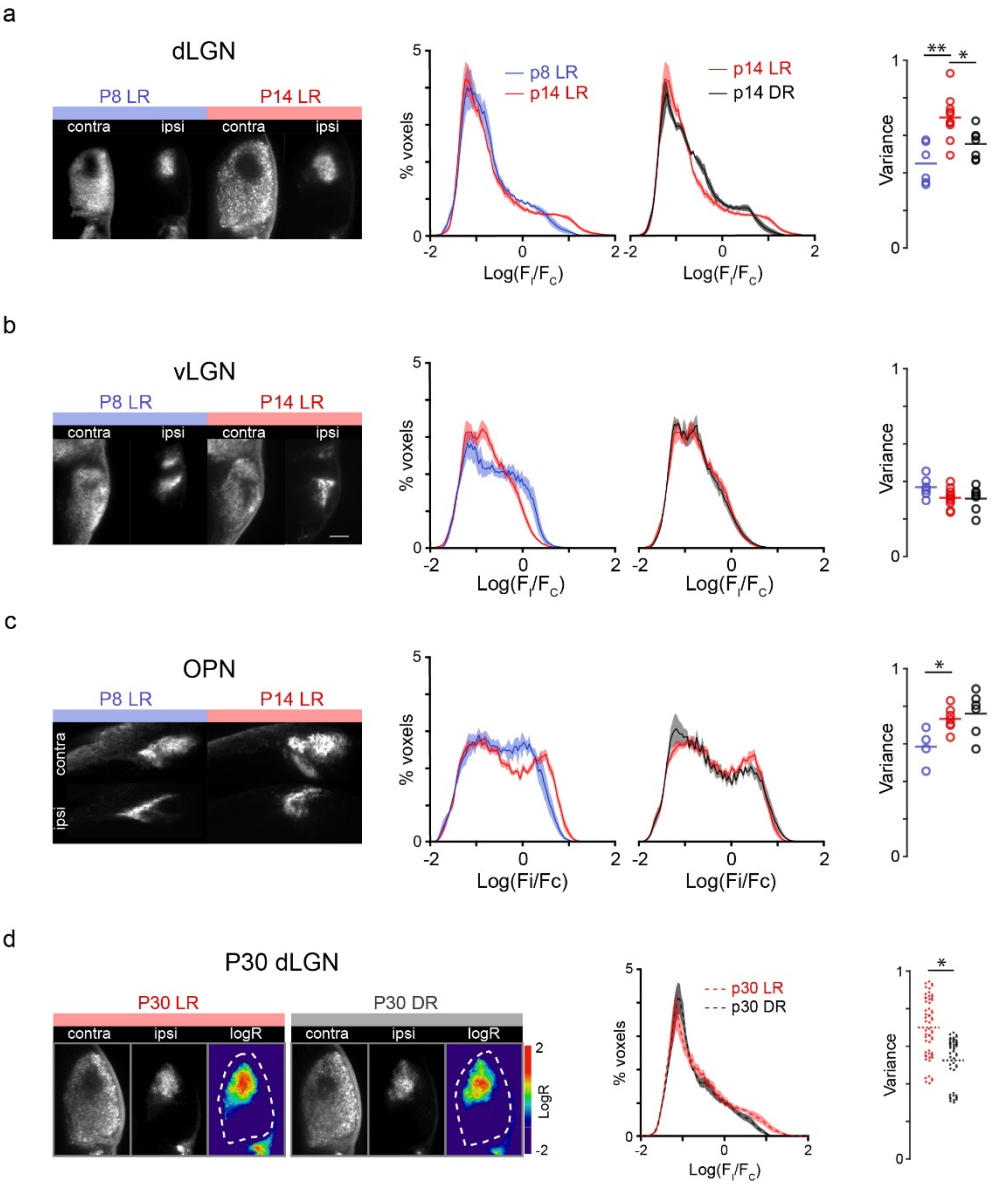


Supplementary Figure 2, Related to Figure 1. Spatial patterns are transmitted through the closed eyelid of P12 mice.

a. Left: Checkerboard patterns of increasing spatial frequency were generated using a DMD and an LED with a peak emission at 375nm, projected onto the sample plane with a 5x/0.15NA objective, and were imaged in the transillumination path with a 2.5x/0.075NA objective. A slant edge was also transmitted to allow for the measurement of the modulation transfer function (MTF). Center: The eyelid from a P12 mouse was mounted on a slide and placed near the image plane of the illumination pattern, and the resulting transmitted image was collected. The relative transmission efficiency of the eyelid was 20%. Right: To better observe the resulting transmitted image independent of additional spatial patterning due to the eyelid fusion line and hairs on the eyelid, the images were normalized by dividing the patterned images by a background image containing the eyelid with full field illumination.

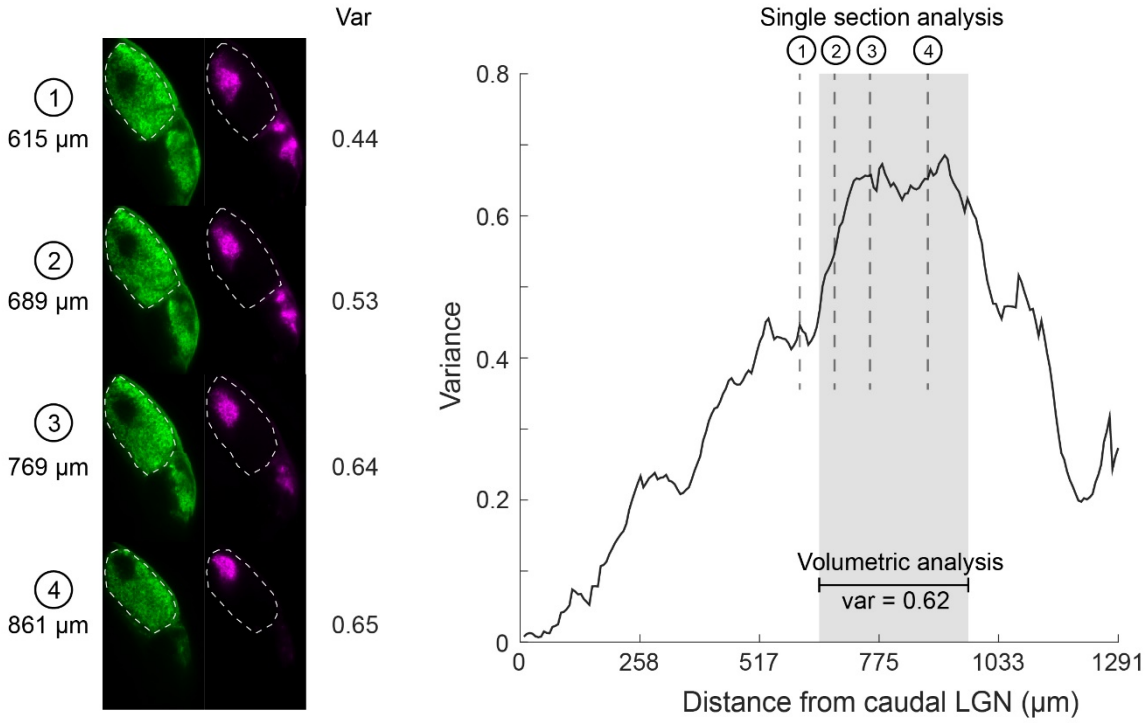
b. The MTF of the eyelid was measured using the slant edge image in (a). To ensure only the MTF of the slant edge was measured, the images were first normalized to suppress spatial modulation independent of the projected image, and then a direction dependent Fourier transform filter was used to preserve edges only in the direction of the slant.

c. Gcamp6f expression in retinal neurons in the retinal ganglion cell layer. Green: anti GFP to stain for Gcamp6f; Red: anti RBPMS to stain for retinal ganglion cells.



Supplementary Figure 3, Related to Figure 4. Eye-specific segregation in retinorecipient brain regions across development.

a. Left: fluorescence images of retinal projections to the dLGN from the contra- and ipsilateral eyes for a light-reared (LR) P8 (blue) and P14 (red) wild-type mouse. Scale bar = 200 µm. Middle: Mean ± SEM of the distributions of log R for each condition. Right: Summary data comparing variance of log R distributions across conditions. *: $p < 0.05$ ANOVA followed by Tukey-Kramer post-hoc test.
b. Same as **a** for the vLGN.
c. Same as **a** for the olivary pretectal nucleus (OPN).
d. Same as **a** for LR and DR P30 mice (DR mice were dark-reared from birth to P14).



Supplementary Figure 4, Related to Figure 4. Variance of LogR distributions across 2-dimensional slices changes rapidly.

Left: Four sets of fluorescence images from optical coronal sections. The four sets shown represent the locus where eye-specific segregation is typically studied in the dLGN.
Right: Variance across every optical section in the dLGN. The four sets of coronal slices shown at left are represented by the dotted lines. Note that taking variance across a volume of voxels is more resistant to sudden changes than taking variance across pixels in a single or few 2-dimensional slices.