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Supplemental Information

Target-Specific Glycinergic Transmission from VGluT3-Expressing Amacrine Cells Shapes Suppressive Contrast Responses in the Retina

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Figure S1

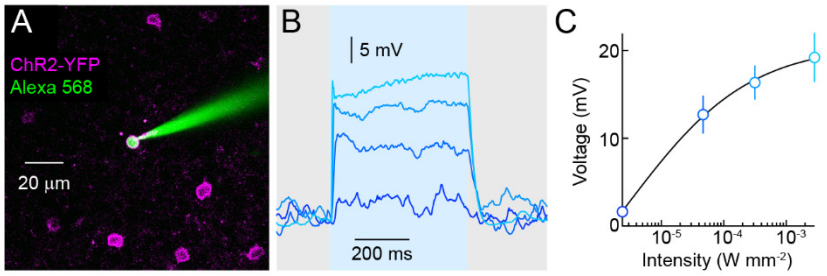

Figure S1 Optogenetic activation of VG3-ACs (related to Figure 1)
(A) Representative 2-photon image of a VG3-AC targeted for patch clamp recording (Alexa 568 in *green*) in a *VG3-ChR2* (YFP fluorescence in *magenta*) retina. (B) Representative voltage responses of a VG3-AC stimulated with steps of blue light (426 – 446 nm, *shaded area*) of increasing intensity. (C) Summary data (mean ± SEM) of the intensity response function of optogenetic stimulation of VG3-ACs (n = 4).

Figure S2

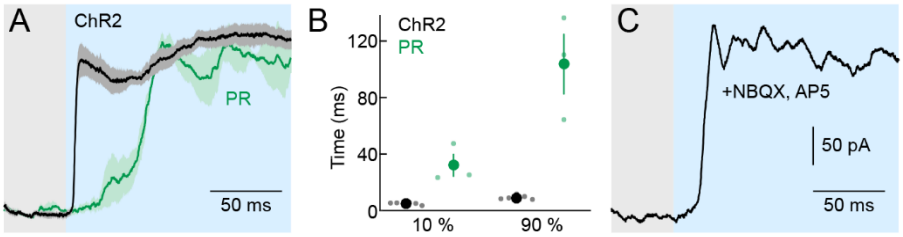

Figure S2 Kinetics and glutamate-blocker-resistance of optogenetic responses in SbC-RGCs (related to Figure 2)

(A) Channelrhodpsin-2 (ChR2)-mediated (*black*) and photoreceptor-mediated (*green*) IPSCs in SbC-RGCs elicited by steps of blue light (3.15 $*10^{-4}$ W mm^{-2}, 426 – 446 nm, *shaded area*). *Lines* (*shaded areas*) indicate normalized mean (± SEM) responses, facilitating comparisons of response timing. (B) Summary data of the time after stimulus onset before 10 % and 90 % of the peak amplitudes are reached (ChR2-mediated, *black*; photoreceptor-mediated, *green*). *Dots* show data from individual cells (ChR2-mediated, n = 6; photoreceptor-mediated, n = 3) and *circles* (*errorbars*) indicate mean (± SEM) of the respective population ($p < 0.002$ and $p < 0.001$ for ChR2-mediated vs. photoreceptor-mediated responses to 10 % and 90 %, respectively). (C) Representative IPSC in and SbC-RGC elicited by optogenetic stimulation of VG3-ACs in the presence of NMDA (30 µM D-AP5) and AMPA (40 µM NBQX) receptor blockers. These blockers were added to inhibitors of metabotropic glutamate (20 µM L-AP4) and kainate receptors (10 µM ACET), which were used in all optogenetic experiments to block transmission of photoreceptor signals to bipolar cells.

Figure S3

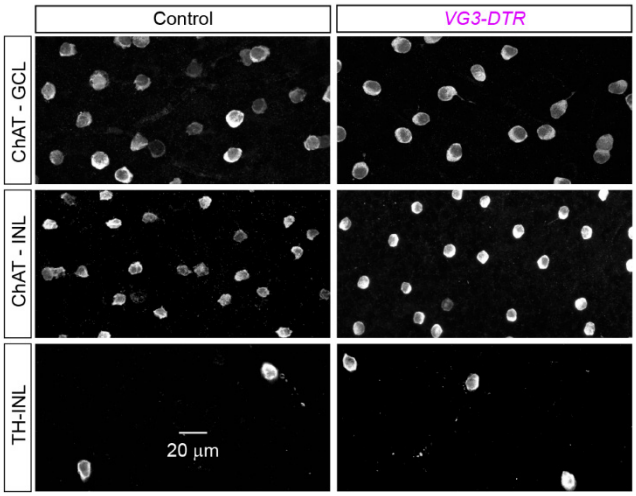

Figure S3 Selectivity of the genetic VG3-AC removal (related to Figure 3)
Representative z-axis projections of confocal image stacks of control (*left column*) and *VG3-DTR* (*right column*) retinas stained for choline acetyltransferase (ChAT, *top* and *middle row*) and tyrosine hydroxylase (TH, *bottom row*). Projections were either restricted to the inner nuclear layer (INL, *middle* and *bottom row*) or the ganglion cell layer (GCL, *top row*). Images were taken one week after diphtheria toxin injections and show that the density of the respective amacrine cell types is unaffected by removal of VG3-ACs.

Figure S4

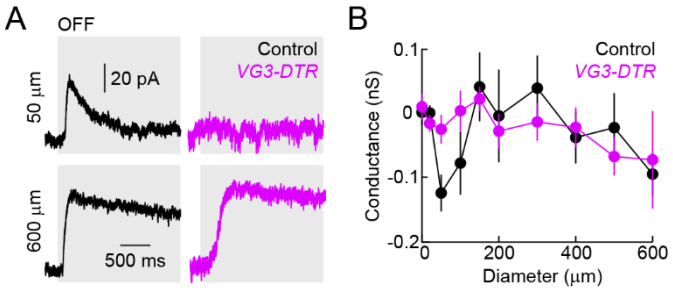

Figure S4 Genetic removal of VG3-ACs alters modulation of tonic excitatory input to SbC-RGCs by OFF stimuli in a size-selective manner (related to Figure 4)

(A) Representative excitatory postsynaptic current traces of SbC-RGCs during presentation of light decrements (OFF) in small (50 µm diameter, *top*) and large (600 µm diameter, *bottom*) circles recorded in control (*left*, *black*) and *VG3-DTR* (*right*, *purple*) retinas. (B) Summary plots (mean ± SEM) of excitatory conductances of SbC-RGCs of control (n = 7, black) and *VG3-DTR* (n = 7, *purple*) retinas elicited by OFF stimuli of different sizes. In *VG3-DTR* mice, suppression of tonic excitation of SbC-RGCs by small OFF stimuli is reduced ($p < 0.02$ for control vs. *VG3-DTR* at 50 µm).