Supplemental Information

Inventory of Supplemental Information

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Supplemental Figures and Legends

Figure S1

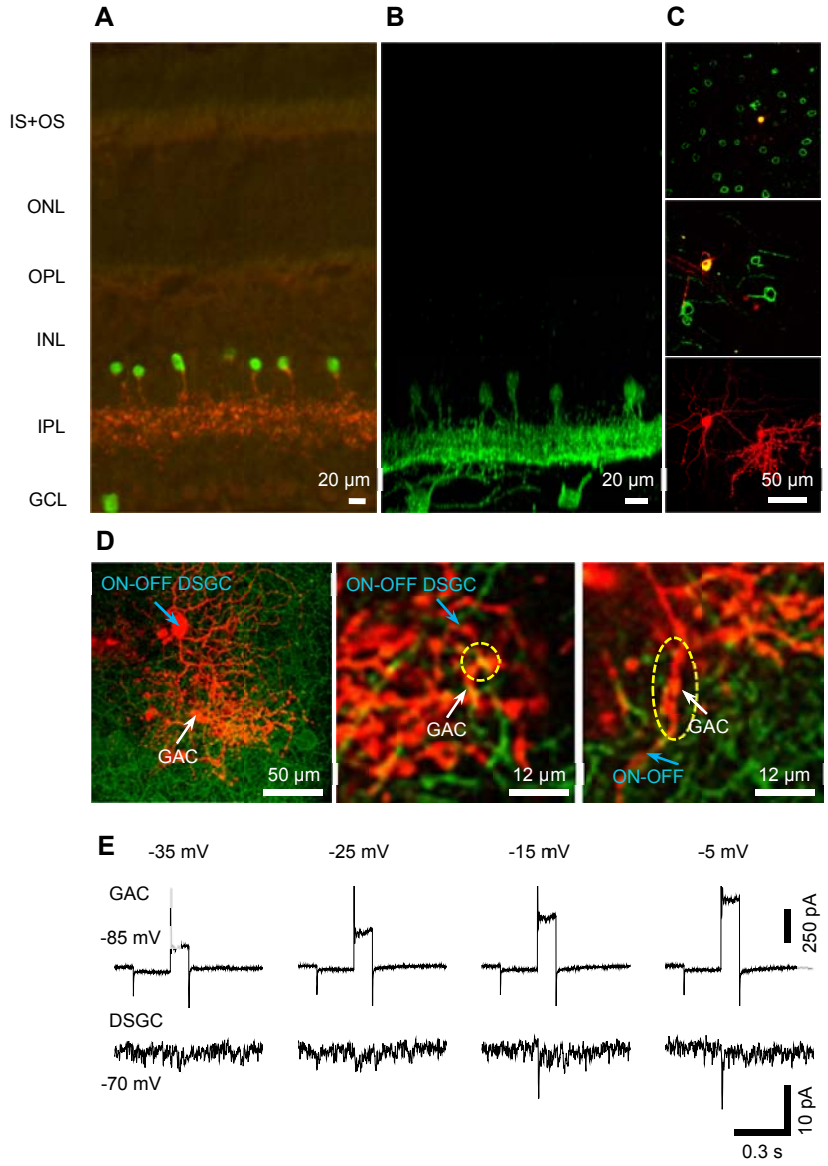

Figure S1 (related to Figure 1). (A-C) Anti-vGluT3 antibody and ChR2-YFP labeling of GACs. (A) Thin (25 µm) retinal section from a vGluT3-Cre/Rosa26YFP mouse, showing YFP expression (green) in GACs in the INL and a small number of neurons in the GCL. YFP-positive GACs are exclusively immunoreactive to a guinea pig anti-vGluT3 antibody (from R. Edwards, University of California at San Francisco, at

1:5000 dilution). (B) z-Projection of a two-photon image stack taken from a wholemount vGluT3-Cre/ChR2-YFP mouse retina, showing ChR2-YFP expression in a regular population of GACs in the IPL and an irregular, heterogeneous population of neurons in the GCL. No YFP expression was detected in other neurons in INL, OPL, ONL, IS, or OS (A, B). (C) Two-photon images of the same retina as in (B), showing ChR2-YFP-expression in a regular, homogeneous population of GACs in INL (top) and irregular, inhomogeneous populations of neurons in GCL (middle). A pair of ChR2-YFP-expressing GAC (yellow, top panel) and ON DSGC (yellow, middle panel) were filled with Alexa 594 during dual whole-cell patch recording, revealing their dendritic morphology in a collapsed image from maximal-intensity projection of a two-photon image stack (bottom). No glutamatergic transmission was detected in this pair, presumably due to a lack of dendritic overlap. **(D-E) Glutamatergic transmission between GAC and ON-OFF DSGC under dual patch clamp.** (D) Left: whole-mount view of a maximally projected image of a pair of GAC and ON-OFF DSGC filled with Alexa 594 (red) during dual whole-cell recording in vGluT3-Cre/ChR2-YFP (green) retina, showing dendritic overlap between the two cells. Arrows: ON-OFF DSGC (blue) and ChR2-expressing GAC (white). Center and Right: whole-mount view of single two-photon optical sections, showing examples of dendritic crossing between the GAC (white arrow) and ON-OFF DSGC (blue arrow) in the ON sublamina (Center), and dendritic co-fasciculation in the OFF sublamina (Right). (E) Dual whole-cell recording from the same pair in (D) in the presence of L-AP4 (10 μM), ACET (10 μM), and HEX (300 μM), showing (in upper traces) voltage-gated currents in the GAC in response to depolarizing steps to -35, -25, -15, and -5 mV (100 ms in duration, preceded by a 200-

ms pre-step from -85 to -100 mV) and (in lower traces) inward postsynaptic currents in the ON-OFF DSGC at -70 mV. The presynaptic threshold for activating a detectable postsynaptic response (between -35 and -25 mV, n=3 pairs) is consistent with the activation voltage range of Ca^{2+} channels in GACs previously reported (Grimes et al., 2011), but this measured threshold voltage could have been affected by potential inadequacy in voltage clamp and/or presynaptic Ca^{2+} channel rundown under whole cell recording. No outward postsynaptic current (at holding potential 0 mV) was detected in these recordings.

Figure S2

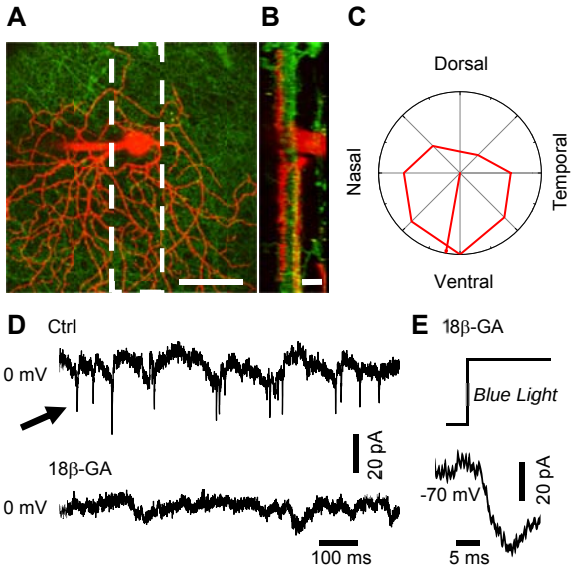

Figure S2 (related to Figure 2). Pharmacological effects of 18β-GA on gap junction-coupled spikelets in ventrally preferred ON-OFF DSGC. (A) Collapsed two-photon image (maximal projection from a z-stack) of an ON-OFF DSGC (filled with a red dye during patch clamp) in a vGluT3-Cre/ChR2-YFP (green) retina, showing a ventrally pointing, asymmetric dendritic field. Scale bar: 50 µm. (B) z-Projection of the

same image stack as in (A, boxed area selected). Scale bar: 20 µm. (C) Directional tuning curve from loose-patch recording of the cell in (A) in response to a white light bar (500x100 µm) moving (500 µm/s) in eight different directions, showing a ventrally preferred direction (superior coding visual field). (D) Gap junction-coupled spikelets (arrow, top trace) in the same ON-OFF DSGC shown in (A), evoked by voltage-clamping the same cell to 0 mV, which activated spikes in neighboring coupled superior coding ON-OFF DSGCs (Trenholm et al., 2013). Bath application of 18β-GA (25 µM, for 13 min) blocked the spikelets, indicating the effectiveness of the drug in blocking gap junction coupling between ON-OFF DSGCs (lower trace). (E) *Blue light*-evoked response remained intact after 14 minutes of 18β-GA (25 µM) application in the same ON-OFF DSGC.

Figure S3

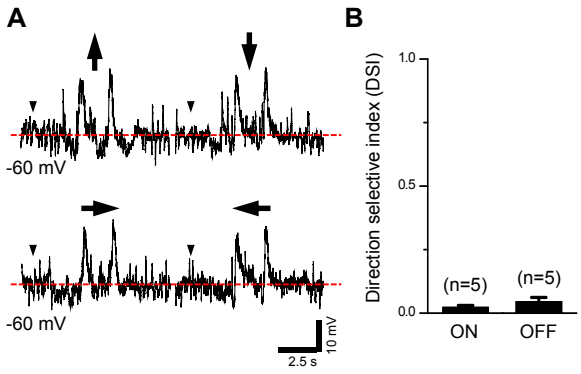

Figure S3 (related to Figure 3). Somatic voltage responses of GACs to a moving light bar. (A) Example traces of current-clamp recording from a GAC in response to a white light bar (600x200 µm) moving (300 µm/s) in four orthogonal directions (arrows), showing ON and OFF depolarizing responses to the leading and the trailing edge of the

moving bar. A small hyperpolarization is discernable preceding the depolarization, presumably due to surround inhibition. No obvious directional preference was detected in somatic voltage responses. Broken red line: resting membrane potential (-60 mV); triangle: onset of moving white bar stimulation, with the leading edge emerging 900 μm away from soma of GAC. (B) Summary of direction selective index (DSI, defined below) from five GACs, showing no direction selectivity.

$$\mathrm{DSI} = \frac{\sqrt{(\mathrm{R}_0 - \mathrm{R}_{180})^2 + (\mathrm{R}_{90} - \mathrm{R}_{270})^2}}{\mathrm{R}_0 + \mathrm{R}_{90} + \mathrm{R}_{180} + \mathrm{R}_{270}}$$

where R_0, R_{90}, R_{180}, and R_{270} are amplitudes of peak depolarization to a light bar moving in the four orthogonal directions, respectively.

Figure S4

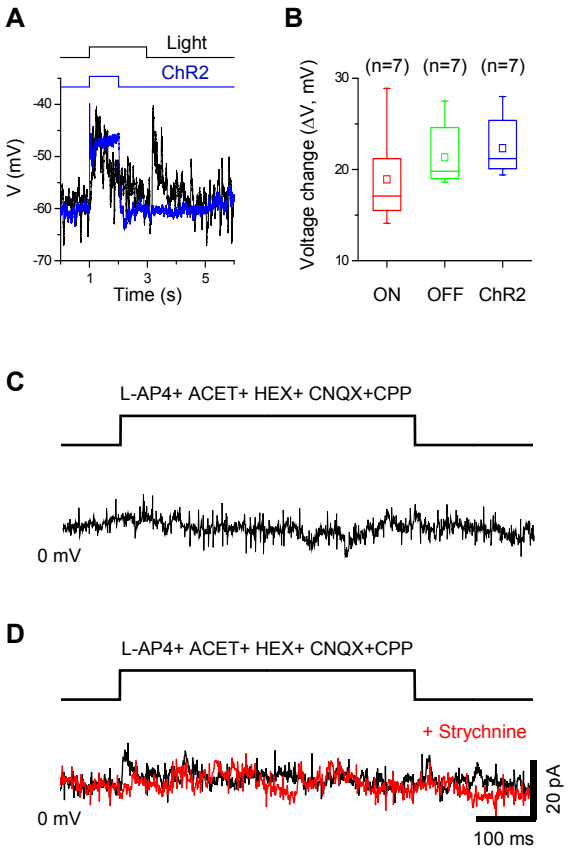

Figure S4 (related to Figure 4). (A, B) Comparison of somatic voltage responses of GACs to visual input and blue light activation of ChR2. (A) Voltage responses of a ChR2-expressing GAC to 2s-long flashes of a center light spot (black trace, 60 μm in radius) and to 1s-long *blue light* stimulation of ChR2 (blue trace). ChR2 stimulation was conducted in the presence of light-response blocking antagonist cocktail, L-AP4 (20 μM), ACET (20 μM) and HEX (300 μM). (B) Statistical summary of the range (ΔV) of membrane depolarization from resting membrane potentials, showing overlaps in response amplitudes to center light spot and ChR2 stimulations. Resting membrane potentials were not significantly changed after application of the light-response blocking cocktail (slightly hyperpolarized from -58.9 ± 1.9 to -59.7 ± 3.3 mV; mean ± s.d., p=0.67, paired Student's *t*-test). (Bottom and top of the box: 1st and 3rd quartile, respectively; horizontal line in the box: median (2nd quartile); square in the box: mean; whiskers at bottom and top: min and max, respectively). **(C, D) Little or no glycinergic synaptic transmission detected from ON-OFF DSGCs to GACs.** (C) An example of voltage-clamp recording (at 0 mV) from an ON-OFF DSGC, showing no detectable outward postsynaptic current in response to *blue light* stimulation in the presence of L-AP4 (20 μM), ACET (10 μM), HEX (300 μM), CNQX (40 μM) and CPP (20 μM). Similar results were obtained in 9 out of 11 ON-OFF DSGCs tested. (D) Responses of an ON-OFF DSGC to *blue light* stimulation (black trace) at 0 mV in the presence of L-AP4 (20 μM), ACET (10 μM), HEX (300 μM), CNQX (40 μM) and CPP (20 μM), showing small (~10 pA) outward postsynaptic currents. This small current was blocked by strychnine (1 μM). Only 2 out of 11 ON-OFF DSGCs tested showed small strychnine-sensitive outward currents similar to shown here.

Supplemental Experimental Procedures

Patch-clamp recording, visual stimulation, two-photon imaging and optical activation of ChR2 in the whole-mount mouse retina

Mouse retinas were dissected under dim red or dim white light illumination. Patch-clamp recordings were made from GCs and SACs in the whole-mount retina under visual control with IR-DIC optics of an Olympus BX51WI upright microscope (with a 60×, 1.0 NA objective, LUMPlanFL/IR, Olympus USA, New York) and an IR video camera (Hamamatsu, Model C2400, Artian Technology Group, Champaign, IL). GACs were identified under brief epi-fluorescence illumination and recorded under dim trans-illumination from the same microscope (Olympus BX51WI). Stable light responses were obtained from GACs after 1-3 min of dark adaptation following the establishment of a patch clamp configuration. Electrophysiological data were recorded with a Multiclamp 700B patch-clamp amplifier (Molecular Devices, CA), stored on Power Lab (AD Instruments, Colorado Springs, CO), and analyzed with pClamp10 (Molecular Devices) and Origin 9 software (Origin Lab Corp., Northampton, MA). Liquid junction potential was calculated using pClamp 10 software and corrected. Pharmacological agents were applied to the retina *via* bath perfusion.

Visual stimuli were generated with the software VisionWorks (Vision Research Graphics, Inc, Durham, NH) on a miniature black-and-white transmissive LCD (diagonal dimension: 18 mm; resolution: 800 × 600 pixel; refresh rate: 60 Hz; contrast ratio: 100:1; Model: SGA4LCD, CRL Opto Ltd., Dunfirmline, Scotland), which was illuminated by a 100-W halogen bulb from the microscope trans-illumination port. The stimulus image on the LCD screen was projected through the microscope condenser lens onto

photoreceptor outer segments of the retina. The visual stimulus intensity at the retina was 0.006 nW μm^{-2} (~2×10^5 photons μm^{-2} s^{-1}) with an intensity-weighted mean wavelength of 573 nm.

Z-series stacks of two-photon images of ChR2-YFP-expressing cells and whole-cell patch-clamped cells (filled with Alexa Fluor 594) in the whole-mount retina were taken with a two-photon imaging system (Ultima, Prairie Technologies, Middleton, WI), which was configured on the same Olympus BX51WI microscope with a Ti:Sapphire pulsed laser (MaiTai, Newport, CA) tuned to 910-920 nm. Images were processed with Prairie View (Prairie Technologies) and Image J (http://rsb.info.nih.gov/ij).

ChR2 was activated by intense *blue light* from either a high power LED (λ_{peak}, 470 nm) focused on the retina through the condenser lens of an Olympus BX51WI upright microscope, or from an epi-fluorescence light source (100W Hg bulb, band-pass filtered at 465 ± 15 nm, focused on the retina through the 60×, NA/0.9 objective lens, and controlled by a Uniblitz shutter, Vincent Associates, Rochester, New York). The intensity of the *blue light* measured at the retina was 22 nW μm^{-2} (5.5 $x10^{10}$ photons μm^{-2} s^{-1}) and 8 nW μm^{-2} ($2x10^{10}$ photons μm^{-2} s^{-1}) for the blue LED and the Hg bulb, respectively.

Histology

Immunostaining of retinal sections was done as described (Grimes et al., 2011). Eyes were postfixed for 2 h and then rinsed in 0.1 mM phosphate buffer (PB). Cornea was cut away, lens removed, and eyecup cryoprotected overnight in 30% sucrose. Sections (16 µm thick) were cut in eyecups on a cryostat and were blocked in PBS plus 5% normal

donkey serum and 0.25% Triton X-100. The sections were then incubated with a guinea pig anti-VGLUT3 antibody at 1:5000 (R. Edwards Lab) in blocking buffer for 1–3 days at 4°C. Sections were washed in the same buffer and then incubated with secondary antibodies at room temperature for 2 h.