In the mammalian retina, processes of approximately 70 types of interneurons form specific synapses on roughly 30 types of retinal ganglion cells (RGCs) in a neuropil called the inner plexiform layer. Each RGC type extracts salient features from visual input, which are sent deeper into the brain for further processing. The layer. Each RGC type extracts salient features from visual input, which are sent deeper into the brain for further processing. The specificity and stereotypy of synapses formed in the inner plexiform layer account for the feature-detecting ability of RGCs. Here we analyse the development and function of synapses on one mouse RGC type, called the W3B-RGC. These cells have the remarkable property of responding when the timing of the movement of a small object differs from that of the background, but not when they coincide. Such cells, known as local edge detectors or object motion sensors, can distinguish moving objects from a visual scene that is also moving. We show that W3B-RGCs receive strong and selective input from an unusual excitatory amacrine cell type known as VG3-AC (vesicular glutamate transporter 3). Both W3B-RGCs and VG3-ACs express the immunoglobulin superfamily recognition molecule sidekick 2 (Sdk2), and both loss- and gain-of-function studies indicate that Sdk2-dependent homophilic interactions are necessary for the selectivity of the connection. The Sdk2-specific synapse is essential for visual responses of W3B-RGCs: whereas bipolar cells relay visual input directly to most RGCs, the W3B-RGCs receive much of their input indirectly, via the VG3-ACs. This non-canonical circuit introduces a delay into the pathway from photoreceptors in the centre of the receptive field to W3B-RGCs, which could improve their ability to judge the synchrony of local and global motion.

In situ hybridization revealed that both Sdk1 and Sdk2 were expressed in subsets of mouse retinal neurons (Fig. 1a–c). Sdk1- and Sdk2-positive cells were largely non-overlapping, as shown previously.
in chicks\textsuperscript{13,14}; however, a double-positive population was also present in mouse (Extended Data Fig. 1a). Expression was evident by embryonic day 17 and persisted into adulthood, spanning the periods of lamina formation and synaptogenesis (Extended Data Fig. 1b). Immunostaining showed that Sdk proteins were concentrated in the synapse-rich inner plexiform layer (IPL) (Fig. 1d), presumably owing to their carboxy-terminal synaptic localizing motif\textsuperscript{5,9}. Sdk proteins were concentrated in two of five strata within the IPL, S3 and S5. Sdk1- and Sdk2-positive puncta in S3 were non-overlapping, consistent with the complementary expression pattern of the genes (Fig. 1e). We generated mice in which a ligand-activated Cre recombinase (CreER) fused to distinct epitope tags was targeted to the first coding exon (Sdk1\textsuperscript{cre} and Sdk2\textsuperscript{cre}; Extended Data Fig. 2a–f). Interneurons and RGCs that expressed Sdk1 or Sdk2, identified using reporter lines or immunostaining, arborized in S3; neurons that expressed both Sdk1 and Sdk2 arborized in S5. Sdk1\textsuperscript{−}/Sdk2\textsuperscript{+} RGCs were W3B-RGCs, labelled by yellow fluorescent protein (YFP) in the TYW3 cell line, which we generated and characterized previously\textsuperscript{9,10}. Another set of morphologically similar RGCs, called W3D, which are dimly labelled in the TYW3 line, expressed neither Sdk1 nor Sdk2. Most Sdk1\textsuperscript{−}/Sdk2\textsuperscript{+} interneurons expressed the vesicular glutamate transporter 3 (VGlu3), encoded by Slc17a8 (refs 16–18); we refer to these cells as VG3-ACs. Both W3B-RGCs and VG3-ACs extend dendrites that arborized in S3 (Fig. 1f–k and Extended Data Fig. 2g–i). To determine whether VG3-ACs synapse on W3B-RGCs, we implemented an optogenetic strategy (Extended Data Fig. 3). We generated mice\textsuperscript{3,17} in which VG3-ACs expressed channelrhodopsin 2 (ChR2) fused to a red fluorescent protein, and W3B-RGCs were labelled with YFP. We targeted YFP-positive W3B-RGCs in explanted retinas with patch electrodes, and activated ChR2 in VG3-ACs using two-photon stimulation. Optogenetic stimulation of individual VG3-ACs evoked reliable postsynaptic currents in W3B-RGCs (Fig. 2a, top trace). Displacement of the laser (\(\sim 10\) µm) so that it was within the receptive field of the RGC but no longer illuminated a ChR2-expressing cell evoked no stimulus-locked current (Fig. 2a, bottom trace). Thus, responses were due to excitation of ChR2 rather than photoreceptors. Additional physiological and pharmacological studies demonstrated that VG3-ACs formed excitatory, glutamatergic connections on RGCs (Extended Data Fig. 4a–c), consistent with recent studies of VGlu3-containing neurons in retina\textsuperscript{9,20} and other brain areas\textsuperscript{51}.

We devised a test to determine whether the VG3-AC–W3B-RGC connection was monosynaptic. Although the light-activated ion channel ChR2 is highly calcium permeable, we found that it is insensitive to CdCl\(_2\), a blocker of endogenous voltage-activated calcium channels in nerve terminals (Extended Data Fig. 4d–h). Thus, in the presence of CdCl\(_2\), neurotransmitter can be released only from ChR2-positive terminals. Synaptic currents elicited by activating terminal arbors of ChR2-expressing VG3-ACs persisted in the presence of CdCl\(_2\) (Extended Data Fig. 4i–k). Gap junction blockers had no effect on these currents, ruling out the possibility that calcium entering through ChR2 in VG3-ACs permeated gap junctions to electrically coupled glutamatergic bipolar cells, which then synapsed on W3B-RGCs (Extended Data Fig. 4b). Together, these results demonstrate that VG3-ACs form synapses directly on W3B-RGCs.

To assess convergence of VG3-ACs onto W3B-RGCs, we recorded from W3B-RGCs while stimulating 60–200 VG3-ACs within 200 µm of their somata. All VG3-ACs and W3B-RGCs separated by \(\leq\)100 µm were connected, with the strength of the connection inversely proportional to the distance between them (Fig. 2b). Because the radii of VG3-ACs and W3B-RGCs dendritic arbors are \(\sim 25\) µm and \(\sim 60\) µm, respectively\textsuperscript{9,17,19,20}, we conclude that VG3-ACs are functionally connected to W3B-RGCs whenever their dendrites overlap (Fig. 2c).

The strong connectivity of VG3-ACs to W3B-RGCs could be a simple consequence of the overlap of their arbors\textsuperscript{22–24}; as predicted by Peters’ rule, which posits that connectivity is proportional to the proximity of pre- and postsynaptic arbors\textsuperscript{22–24}. To test this idea, we measured the connectivity of VG3-ACs to four Sdk2-negative RGC types for which we had marker lines: W3D-RGCs, W7-RGCs and two types of ON–OFF direction-selective RGCs (ooDSGCs); W3D-RGC and W7-RGC dendrites intermingle with those of W3B-RGCs, and ooDSGC dendrites straddle those of W3B-RGCs. Optogenetic stimulation of VG3-ACs elicited excitatory postsynaptic currents from W3D-RGCs, W7-RGCs and ooDSGCs that were qualitatively similar to, but only \(\sim 10\%\) as strong as those in W3B-RGCs (Fig. 2e, f and Extended Data Fig. 5). The weakness resulted from a twofold decrease in the fraction of pairs that were detectably connected, and a fivefold decrease in the peak synaptic currents for connected pairs (Fig. 2g, h).
We also assayed input to W3B-RGCs from six other types of interneurons that arborize in or at the border of S3. In all cases, connectivity was many-fold lower than that between VG3-ACs and W3B-RGCs (Fig. 2d–h). Together, these data demonstrate that Peters’ rule is insufficient to explain patterns of connectivity in the neuropil of the retina.

To address whether Sdk2 is involved in the establishment of the strong VG3-AC to W3B-RGC connection, we used the Sdk2<sup>-</sup> line, in which insertion of CreER generates a Sdk2-null allele (Extended Data Fig. 2). Sdk2<sup>–</sup> mice are viable, fertile and exhibit no external deficits. We detected no alterations in retinal structure or in the numbers or positions of any cell types examined (Extended Data Fig. 6). However, physiological analysis revealed a 20-fold reduction in the strength of synaptic connections between VG3-ACs and W3B-RGCs (Fig. 3a–d). Thus, Sdk2 is required for the selective connectivity of VG3-ACs to W3B-RGCs.

We sought morphological correlates of the synaptic disruption observed in Sdk2 mutants. To this end, we imaged single VG3-ACs and W3B-RGCS labelled in a transgenic line or by dye injection. The size, shape and laminar restriction of VG3-AC and W3B-RGC arbors were generally normal in Sdk2<sup>+/c</sup> mice (Fig. 3e, f). However, branch number and length were modestly reduced in mutant W3B-RGCS and modestly increased in VG3-ACs in both cell types, some branches extended beyond the normal termination zone (Fig. 3g–l and Extended Data Figs 6m–o and 7). Defects in laminar restriction were similar to, although less striking than, those observed in chick retina and W3B-RGCs labelled in a transgenic line or by dye injection. The synaptic connections between VG3-ACs and W3B-RGCs (Fig. 3a–d).

Physiological analysis revealed a 20-fold reduction in the strength of synaptic connections between VG3-ACs and W3B-RGCs (Fig. 3a–d). However, physiological analysis revealed a 20-fold reduction in the strength of synaptic connections between VG3-ACs and W3B-RGCs (Fig. 3a–d).

Defects should be observed only at synapses in which both part-partner connections are Sdk2-positive. Deletion of Sdk2 had no effect on the strength of coupling in any of four such cases: connections of Sdk2-negative bipolar and amacrine cells to W3B-RGCS and of VG3-ACs to Sdk2-negative RGCS (Fig. 3b–d and Extended Data Fig. 8a–r). These results also provide evidence that loss of Sdk2 does not affect the overall properties of VG3-ACs or W3B-RGCS. In addition, we generated mice in which Sdk2 could be expressed as a Cre-dependent manner in any cell (Extended Data Fig. 8s). In combination with the Sdk2<sup>+</sup> line, this allowed us to restore Sdk2 in VG3-ACs selectively. In this case, connectivity was as low as in the Sdk2<sup>–</sup> mutants (Extended Data Fig. 8t).

Together, these results support the idea that Sdk2 promotes connectivity by a homophilic mechanism.

We next asked what role the Sdk2-specified VG3-AC-to-W3B-RGC connection has in the function of W3B-RGCS. To this end, we recorded responses of W3B-RGCS to visual rather than optogenetic stimulation. As reported previously<sup>9,10,11</sup>, a bright spot flashed over the dendritic arbor (the receptive field centre) of control W3B-RGCS elicited a burst of action potentials at both the onset and the offset of the light, with the OFF response substantially larger than the ON response. In Sdk2<sup>–</sup> mice, the ON response persisted but the OFF response was nearly abolished (Fig. 4a and Extended Data Fig. 9a). This result was unexpected, because input from amacrine cells generally modulates RGC responses but does not generate them<sup>5</sup>. To address whether this phenotype resulted from decreased excitation or enhanced inhibition, we recorded synaptic currents in response to the same stimulus. Consistent with results from the voltage recording, a flashing spot elicited excitatory currents at both light onset and offset, with OFF currents larger than ON currents in both Sdk2 mutants, the OFF current was nearly abolished while the ON current was less affected (Fig. 4c, d). By contrast, inhibitory currents evoked in W3B-RGCS by full-field stimulation, presumably derived from conventional amacrine cells, were unaffected in Sdk2 mutants (Fig. 4e and Extended Data Fig. 9b). The effect was specific in that light-evoked inward currents in W3B-RGCS in Sdk2<sup>–</sup> and Sdk2<sup>+</sup> retinas. Data from 14 W3B-RGCS in 8 Sdk2<sup>–</sup> mice and 11 W3B-RGCS in 9 Sdk2<sup>+</sup> mice. Data in b–d and g–i indicate mean ± s.e.m.

The effect we observed could have resulted from defects in other Sdk2-expressing retinal cells (Fig. 1k), or compensatory alterations during development. To test these possibilities, we selectively ablated mature VG3-ACs. We expressed the diphtheria toxin receptor (DTR) in VG3-ACs, injected diphtheria toxin in adult animals, recorded from W3B-RGCS ~10 days after injection, and verified loss of VG3-ACs after recording (Extended Data Fig. 9d, e). Ablation of VG3-ACs in adulthood led to an even greater loss of light-evoked excitatory OFF responses than observed after global deletion of Sdk2; inhibitory responses in W3B-RGCS and excitatory responses in nearby RGCs were unaffected (Fig. 4e, g and Extended Data Fig. 9f–h; see also ref. 20). Together, these results lead to the conclusion that visual input are
delivered to W3-RGCs in an unusual way: while bipolar cells synapse directly on most RGCs, W3-RGCs receive OFF input indirectly via VG3-ACs (Fig. 4b). The small, statistically insignificant decrease in the ON response may reflect the presence of other ON inputs that compensate for loss of that normally supplied by VG3-ACs. Nevertheless, VG3-ACs provide the main excitatory drive to the W3B-RGCs in response to a full field flash (n = 7 W3B-RGCs in 5 controls and 5 W3B-RGCs in 4 Sdk2ce/ce mice). I. Peak excitatory currents elicited from W3B-RGCs in response to a spot flash (n = 5 W3B-RGCs in 3 control mice and 11 W3B-RGCs in 5 Vglut3-cre;DTR mice; **p < 0.01, Student’s t-test). J. Sdk2 mutants are localized at synapses and required in both partners, we speculate that it acts homophilically to promote appropriate connections. In its absence, functional connections fail to form or are not maintained, leading to markedly decreased synaptic strength.

Taken together with previous studies, our data support a multi-step model for synaptic specificity in the IPL. First, one set of recognition molecules, including cadherins and plexins, direct arbors to appropriate sublaminae. Within sublaminae, proximate partners connect at low levels, consistent with Peters’ rule. Finally, recognition molecules such as Sdk proteins, and perhaps other immunoglobulin superfamily adhesion molecules, act to bias connectivity in favour of specific pairings.

Our results also reveal a role of the VG3-AC–W3B-RGC synapse in visual function. In canonical retinal circuits, bipolar cells relay visual input from photoreceptors to RGCs, whereas amacrine cells, which have been presumed to be inhibitory, modulate this input. By contrast, VG3-ACs provide the main excitatory drive to the W3B-RGCs. Thus, the VG3-AC–W3B-RGC synapse is a component of a non-canonical retinal circuit in which some of the visual input is relayed to W3B-RGCs through VG3-ACs rather than arriving directly through bipolar cells. This seemingly cumbersome arrangement could improve the sensitivity of W3B-RGCs to the visual features that best excite them—the motion of small objects whose movements are asynchronous with those of the background.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

Received 11 October 2014; accepted 22 June 2015.

Published online 19 August 2015.

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Figure 4 | Excitatory input reaches W3B-RGCs via VG3-ACs. a, Average firing rate recorded from W3B-RGCs in Sdk2ce/ce (blue, n = 21) and Sdk2ce/ce (red, n = 10) retina in response to a small spot flashed for 1 s. Dark lines, average; shadowing denotes s.e.m. Bin width, 50 ms. b, Canonical and non-canonical pathways for delivering OFF input to RGCs. c, Excitatory currents recorded from W3B-RGCs in response to a full field flash (Vth = −5 mV). d, Average peak current (Ipeak) from experiments such as shown in c (n = 7 W3B-RGCs in 4 Sdk2ce/ce mice and 8 W3B-RGCs in 4 Sdk2ce/ce mice; *p < 0.01, Student’s t-test). e, Ipeak of inhibitory currents recorded from W3B-RGCs in response to a full field flash (Vth = −5 mV) (n = 7 W3B-RGCs in 5 controls and 5 W3B-RGCs in 4 Sdk2ce/ce mice). f, Ipeak of excitatory currents recorded from W3B-RGCs in response to a spot flash (n = 5 W3B-RGCs in 3 control mice and 11 W3B-RGCs in 5 Vglut3-cre;DTR mice; **p < 0.01, Student’s t-test). g, Ipeak of inhibitory currents recorded from W3B-RGCs in response to a full field flash (n = 5 W3B-RGCs in 5 control mice) and 11 W3B-RGCs in 5 Vglut3-cre;DTR mice. h, Object motion stimulus-evoked responses in W3B-RGCs. W3B-RGCs spike vigorously to a grating passed over their receptive field centre (local). They are silenced when the moving grating extends to their surround (global) but not when the centre grating leads or lags that in the surround (differential), as quantified in i, l. Responses measured in W3B in response to a stimulus where the centre grating began its movement at different times relative to that of the surround. Negative delays are when the centre led the surround. Responses were normalized (Norm) to those evoked by a centre grating alone (n = 7 W3B-RGCs). j, Responses measured in W3B-RGCs to global (G) and differential (D) motion stimuli normalized to that elicited in control W3B-RGCs with local motion stimuli (n = 10 W3B-RGCs in 5 control mice, 6 W3B-RGCs in 4 Sdk2ce/ce mice and 9 W3B-RGCs in 3 diphtheria-toxin-treated Vglut3-cre;DTR/TYW3 mice). OMS, object motion sensitivity. Data in e–g, i and j are mean ± s.e.m.
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Acknowledgements We thank E. Soucy and J. Greenwood for assistance with constructing the two-photon microscope, the Genome Modification Facility at Harvard for generating mouse lines, and E. Feinberg for insight into the ion selectivity of ChR2. This work was supported by grants from the NIH (NS029169 and EY022073) to J.R.S., NSERC (Canada) and Banting Postdoctoral Fellowships to A.K., a HHMI-Life Sciences Research Foundation Postdoctoral Fellowship to X.D., and an NIH fellowship (F31 NS055488) to Y.K.H.

Author Contributions A.K., M.Y. and J.R.S. planned experiments, analysed data and wrote the paper. A.K. performed electrophysiological and histological experiments, M.Y. performed genetic and histological experiments, X.D. developed methods and generated reagents and Y.K.H. generated reagents and performed in situ hybridization. The authors declare no competing interest.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to J.R.S. (sanesj@mcb.harvard.edu).
METHODS

Animals. We modified a lambda-phage-mediated recombineering method to generate Sdk1 and Sdk2 targeting vectors in which the first coding exons of the Sdk1 and Sdk2 genes were replaced by an epitope-tagged CreER-T2 recombinase complementary DNA. Tags were three tandem copies of the haemagglutinin (HA) tag (amino acid sequence YPYDVPDYA) for Sdk1, and six tandem copies of the Myc tag (amino acid sequence EQKLISEEDL) for Sdk2. Loci were modified by homologous combination in V6.5 embryonic stem cells, and chimaeras were produced by the Harvard University Genome Modification Facility. High percentage chimaeras transmitting the knock-in alleles were bred to animals expressing FLP recombinase from the β-actin promoter to remove the SV40-NEO cassette.

Thyl-STOP-YFP, TYW3, TYW7, TYW9, Kog4-cre and Neto1-cre mice were generated in our laboratory. Thyl-STOP-YFP expresses YFP after excision of a stop cassette by Cre recombinase. TYW3 mice express YFP in subsets of RGCs. TYW7 mice express YFP in two types of RGCs, which are present at similar densities; one has its dendrites in the distal half of S3; the other has its dendrites in S1. TYW9 mice express YFP in osoDSGCs that prefer nasal motion. Neto1-cre and Kog4-cre mice express Cre recombinase in type 2 and type 5 bipolar cells, respectively, as well as in subsets of RGCs. Vglut3-cre mice were obtained from R. Seals and R. Edwards. In these mice, Cre was inserted into exon 1 of a bacterial artificial chromosome that contained Vglut3, and this vector was used to generate a transgenic line. Nex-cre mice, in which Cre is targeted to the endogenous Neurod6 locus, were obtained from K. Nave via L. Reichardt; this line expresses Cre in non-GABAergic non-glycinergic (nGnG) and glycinergic SGC amacrine cells. DAT-cre mice, in which Cre is targeted to the endogenous DAT (also known as Sletα3) locus, were obtained from X. Zhaung via V. Murthy; this line expresses Cre in type II catecholaminergic amacrine cells. ChAT-cre mice, in which the Cre recombinase gene was targeted to the endogenous ChAT gene, were obtained from Jackson Laboratories; this line expresses Cre in starburst amacrine cells (SAG). Hb9-cre transgenic mice, which express green fluorescent protein (GFP) in osoDSGCs that prefer nasal motion, were obtained from K. Eggan. Rosa-LOX-STOP-LOX-Chr2(H134R)-tdTomato mice (Ai27), which express channelrhodopsin after excision of a stop cassette by Cre recombinase, were provided by H. Zeng. Rosa-CAG-LOX-STOP-LOX-DTR mice, which express DTR following excision of a stop cassette by Cre recombinase, were obtained from Jackson Laboratories. The Rosa-CAG-LOX-CHERRY-LOX-GFP line was from S. Dynecki. Six3-cre mice were provided by W. Klein.

To enable expression of Sdk2 under Cre-dependent control, we generated a line using a previously described strategy. A cassette encoding Venus and Sdk2, separated by tripleF2A (three tandem repeats of foot-and-mouth disease virus 2A peptide sequence) was cloned into a Rosa26-STOP-CAG-targeting vector to generate Rosa-CAG-LOX-STOP-LOX-Venus-3F2A-Sidekick2-WPRE-FRT-neo-FRT. Homologous recombinants were selected in the V6.5 embryonic stem cell line and chimaeras were generated. Germ-line chimaeras were crossed to a Fip mouse to obtain germ-line transmissions and to remove the FRT-neo-FRT sequence.

Animals were used in accordance with NIH guidelines and protocols approved by Institutional Animal Use and Care Committee at Harvard University. Mice were maintained in a C57Bl/6J background. Both male and female mice were used in this study. Animals were 40 to 100 days old at the time of euthanasia unless otherwise stated in the text or figure legend. Genotypes of mice were known to investigators at the time of the experiment, and there was no randomization in assignment of animals for specific experiments.

RT–PCR. RNA was prepared from the brains of wild-type or knockout mice using EZNA Total RNA kit I (Omega bio-tek), reverse-transcribed by SuperScript III (Life Technologies), and amplified using Taq DNA polymerase (EconoTaq PLUS, 710) confocal microscope using a 63x water immersion objective. Images were acquired on a LSM 710 confocal microscope using a 63x water immersion objective. Images were acquired at a resolution of 1,024 × 1,024 pixels with a step size of 0.2–0.5 μm. ImageJ was used to generate maximum intensity projections of singly labelled neurons and skeletonized dendrites in the x–y and x–z planes.

Histology. Mice were euthanized by intraperitoneal injection of pentobarbital and either encotted immediately or transcardially perfused with Ringer’s solution followed by 4% (w/v) paraformaldehyde (PFA) in PBS. Eyes were removed and fixed in 4% (w/v) PFA in PBS on ice overnight, sunk in 30% (w/v) sucrose/PBS, and mounted in the OCT compound. Immunostaining of cryosections was carried out as described previously. For double immunostaining with two different mouse antibodies, we used the Zenos Horseradish Peroxidase Mouse IgG1 Labelling Kit (Life Technologies) to label one of them, and detected reaction product with TSA-Plus kits (Perkin-Elmer Life Sciences). For immunodetection of proteins maximally differ in amino acid sequence. cDNAs encoding these fragments were fused to a poly-histidine tags were inserted into a PET vector (Novagen). Fusion proteins were produced in BL21 bacteria and purified using a His-column (Life Technologies). Animals were immunized and antisera produced by Covance Research. Antibodies were affinity purified using the antigen fusion proteins as bait. We also generated mouse polyclonal antibodies to mouse Sdk1 and Sdk2 by immunizing Sdk1- and Sdk2-knockout mice with L cells (ATCC) that had been transfected with full-length mouse Sdk1 or Sdk2 cDNA as described previously. Antibodies were tested on Sdk1- and Sdk2-expressing HEK cells (HEK-293T, ATCC) and on retinal tissue from Sdk1(z/m) and Sdk2(z/m) mice to verify specificity.

Antibodies used in this study were: rabbit monoclonal antibody to oestrogen receptor alpha (Clone SP1, from Epitomics or Abcam); goat anti-Myc (NB600-335, from Novus); rat anti-αH (3F10, from Roche Diagnostics); anti-Bn3a (clone, S3A.2), rabbit anti-synapsin I (AB1543P), mouse anti-Caleptin (clone, 6b8.2), goat anti-ChAT antibodies, and sheep anti-tyrosine hydroxylase from Millipore; AP2 (clone, 3B5), SV2, anti-synaptotagmin 2 (clone, ZNP1) from Developmental Studies Hybridoma Bank; mouse anti-VGlut1 (clone, D28/299), mouse anti-pan-MAGUK (clone, D28/86), mouse anti-HCN4 (clone, N141.10), and mouse anti-Vesicular acetylcholine transporter (clone, N6/30) from NeuroMab; mouse anti-SATB2 (clone, 4B19) from Abcam; rabbit anti-fluorescin (Life Technologies); and rabbit anti-protein kinase C alpha (P4334) from Sigma. Rabbit antibody to Dab1 was a gift from B. Howell. Rabbit anti-lucifer yellow and anti-fluorescein were from Invitrogen. Chicken anti-GFP and rabbit anti-mCherry were generated as described previously. Nuclei were labelled with NeuroTrace Nissl 435 (Life Technologies). Secondary antibodies were conjugated to DyLight 488, DyLight 594, or Alexa 467 (Jackson ImmunoResearch).

For in situ hybridization, rhinops were synthesized from Sdk1 or Sdk2 cDNAs using digoxigenin- or fluorescein-labelled UTP and hydroxylised to around 500 base pairs as described previously. Probes were detected using anti-digoxigenin or anti-fluorescin conjugated to alkaline phosphatase, followed by reaction with nitroblue tetrazolium (NTB) and/or nitroblue tetrazolium substrate for 24–36 h; or using anti-digoxigenin and anti-fluorescein antibodies conjugated to horseradish peroxidase, followed by amplification with tyramide conjugates (TSA-Plus system; Perkin-Elmer Life Sciences).

Imaging. Images of immunostained retinal wholemounts were acquired on a LSM 710 confocal microscope using a 63× water immersion objective. Images were acquired at a resolution of 1,024 × 1,024 pixels with a step size of 0.2–0.5 μm. ImageJ was used to generate maximum intensity projections of singly labelled neurons and skeletonized dendrites in the x–y and x–z planes. Channelrhodopsin excitation. ChR2-tdTomato-positive interneurons were first identified with channelrhodopsin excitation. Examples of double immunostaining with two different mouse antibodies, we used the Zenos Horseradish Peroxidase Mouse IgG1 Labelling Kit (Life Technologies) to label one of them, and detected reaction product with TSA-Plus kits (Perkin-Elmer Life Sciences). For immunodetection of proteins. IHC was performed as described previously. For double immunostaining with two different mouse antibodies, we used the Zenos Horseradish Peroxidase Mouse IgG1 Labelling Kit (Life Technologies) to label one of them, and detected reaction product with TSA-Plus kits (Perkin-Elmer Life Sciences). For immunodetection of proteins.
raster or spiral scan trajectories (25–30 mW sample plane power at 920 nm).
Dwell times in these scan patterns were 0.02–0.05 ms pixel−1, which was less than the rise time of the current produced by a stationary spot (Extended Data Fig. 3c, g–j). Pixel size was 0.6 μm2 and scan patterns typically contained 100 pixels with their total time synchronized to the laser shutter, which opened for 2 ms.

We arrived at these parameters by measuring the kinetics of ChR2 responses on HEK cells and retinal neurons that expressed ChR2 (H134R) (Extended Data Fig. 3). Our goal was to scan interneuron somas (typically 10 × 10 pixels in size) and activate them with a staircase of powers that would emulate the square current pulses used when stimulating interneurons via a sharp electrode. Responses in ChR2-expressing HEK cells elicited by a stationary spot of infrared laser light at a range of wavelengths between 800 and 960 nm (840 nm, 860 nm and 920 nm are shown in Extended Data Fig. 3b, c) led us to conclude that 920 nm would be a good compromise between response size, rise time and sample plane power. Currents typically had <2 ms rise times (10–90%) with peak amplitudes of 150–175 pA at 25 mW sample plane power (Extended Data Fig. 3a). Increasing stimulus duration beyond 2–3 ms produced no increase in peak amplitude and instead the response showed signs of desensitization19–21 (Extended Data Fig. 3a).

Responses in ChR2-expressing HEK cells elicited by a stationary spot of infrared laser light at a range of wavelengths between 800 and 960 nm (840 nm, 860 nm and 920 nm are shown in Extended Data Fig. 3a) are not contaminated by light responses. Nevertheless, we avoided exposure times exceeding 2 ms to prevent any photoreceptor-cell damage on cultured cells as well as retinal tissue at sample plane powers exceeding 25 mW sample plane power (Extended Data Fig. 3a). Increasing stimulus duration beyond 2–3 ms produced no increase in peak amplitude and instead the response showed signs of desensitization48–51 (Extended Data Fig. 3a). Taken together, these results would suggest that responses evoked by 920 nm light would saturate at dwell times greater than 2 ms pixel−1.

Next, we measured the spatial dimension of two-photon laser stimulation of ChR2. To do this, we recorded from ChR2-expressing HEK cells while stimulating the HEK cell at different positions away from the edge of the cell membrane (Extended Data Fig. 3d), or while stimulating the HEK cell at different heights beginning at the cell membrane (Extended Data Fig. 3f). Taken together, these results indicate that two-photon laser stimulation of ChR2 has maximal x–y–z dimensions of approximately 3 × 3 × 3 μm. Next, we recorded from ChR2-positive RGCs or SACs, highlighted their somas with ROIs and steered the beam through these ROIs in raster or spiral scan trajectories at different dwell times to produce currents of 250–350 pA with <2 ms rise times (Extended Data Fig. 3g–j). Empirically, this dwell time tended to be 0.02–0.05 ms pixel−1. Currents were similar at both dwell times. Finally, we determined the relationship between inward currents on W3B to stimulation of VG3-ACs at different sample plane powers. This experiment revealed a classical sigmoid relationship between the size of the postsynaptic response and the strength of the stimulus (power at the sample plane) delivered to VG3-ACs consistent with calcium dependence of transmitter release29 (Extended Data Fig. 3f). Sample plane powers used in our analysis of connectivity (25–30 mW) sit on the plateau of this curve and are 4–6 times the power levels that evoke a minimal response. We observed signs of infrared-light induced damage on cultured cells as well as retinal tissue at sample plane powers greater than 40 mW.

Under the conditions we used, photoconverter-evoked light responses on RGCs were rarely seen. To confirm this independence, we activated ChR2 and photoconverters independently and observed distinctly different kinetics (Extended Data Fig. 4g). These data establish that our functional connectivity measurements are not contaminated by light responses. Nevertheless, we avoided exposure times of >100 ms owing to a well described relationship between exposure time and infrared-evoked light responses29. In these cases, however, ChR2-positive interneurons were cleaved from their photoreceptors to produce currents of 250–350 pA with <2 ms rise times (Extended Data Fig. 3g–j). Empirically, this dwell time tended to be 0.02–0.05 ms pixel−1. Currents were similar at both dwell times. Finally, we determined the relationship between inward currents on W3B to stimulation of VG3-ACs at different sample plane powers. This experiment revealed a classical sigmoid relationship between the size of the postsynaptic response and the strength of the stimulus (power at the sample plane) delivered to VG3-ACs consistent with calcium dependence of transmitter release29 (Extended Data Fig. 3f). Sample plane powers used in our analysis of connectivity (25–30 mW) sit on the plateau of this curve and are 4–6 times the power levels that evoke a minimal response. We observed signs of infrared-light induced damage on cultured cells as well as retinal tissue at sample plane powers greater than 40 mW.

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Visual stimuli. Visual stimuli were delivered via a projector as described26. In brief, all visual stimuli were written in Matlab using the psychophysics toolbox and displayed on the projector with a background intensity set to 1 × 104 R°/s−1. In which R° denotes activated rhodopsin.

The ON receptive field is larger than the OFF receptive field in W3B-RGCs and slightly off-centre spots can bias the relative sizes of ON and OFF inward currents. To ensure that this innate feature of W3B-RGCs did not influence our measurements of ON and OFF current sizes, we delivered white noise stimuli to W3B-RGCs while recording inward currents. Next, we reverse correlated these currents to the stimuli and created an average that described the receptive field centre. These receptive field was used as stimulus to measure the sizes of ON and OFF inward currents. Currents in wild-type animals had a canonical shape and showed that the peak OFF current is typically 4–8 times larger than ON current (Fig. 4). For our differential motion stimulus, we first mapped the W3B-RGCs receptive field using a grid of flashing spots and measured spikes in 1 = 0 mode. Next, we centred a circular object region (100–150 μm) over the receptive field, bounded it with a grey annulus (50–70 μm) and created a background region that extended from the annulus to edge of our projected image. Differential motion stimuli were constructed as previously reported22 and consisted of bars of 40–72 μm in width that alternated in intensity about our adapting grey level and moved at a speed of 100 μm s−1, for a single bar width. For local motion, bars were passed within the object region and exited the background region before or after bars moved in the background: 333.33 ms, 166.67 ms, 100 ms, 83.33 ms, 66.67 ms, 50ms, 33.33 ms, 16.67 ms, 0 ms (Fig. 4i). These correspond to a difference of 20, 10, 5, 4, 3, 2, 1 and 0 frames of our 60-Hz projector. Responses at these different timings were collected and normalized to the response obtained under local motion only. W3B-RGCs are strongly silenced by activation of their surround, and as a result do not fire when bar movement in the two regions were synchronous. The OMS index (Fig. 4j) was computed by normalizing the average firing rate in response to differential and global motion to that measured in response to local motion. Average firing rates used for this procedure are shown in Extended Data Fig. 10.

**Diphtheria toxin injection.** Diphtheria toxin (Sigma) was first dissolved in PBS at 1 mg mL−1 and then aliquoted at ~80°C. Freshly thawed diphtheria toxin aliquot was diluted in PBS and delivered as intraperitoneal injection at 1 μg per 50 g body weight11 to ~7–10-week-old Vglut3-cre; Rosa-LOX-STOP-LOX-DTR;TWY3 mice. The dose was repeated four times at 2-day interval. As controls, we injected diphtheria toxin in control animals and saline in Vglut3-cre; Rosa-LOX-STOP-LOX-DTR;TWY3 animals.

AAV-mediated gene transfer. Viral-mediated gene transfer was performed as described26. For initial connectivity measurements, adeno-associated virus (AAV) (2.5 mg) was injected into the eye of the mouse (H134R)-YFP-WPRE, AV-9-20297P, Penn Vector Core) was at a titre of ~1 × 1013 genome copies per ml, as described previously26. All of these viral infection experiments were repeated using the Ai27 line. Similar results were obtained with both methods, and results from both methods were pooled.
Statistical methods. No statistical method was used to predetermine sample size. Data sets were tested for normality and statistical differences were examined using the Student’s t-test (Igor Pro). Variance in the estimate of the mean is shown as s.e.m.

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Extended Data Figure 1 | Expression of Sdk1 and Sdk2 in developing retina.

a, Double-label in situ hybridization for Sdk1 and Sdk2 at P10. Arrow indicates a retinal ganglion cell that expresses both Sdk genes. Label in INL includes amacrine and bipolar cells, as summarized in Fig. 1k. Other images (not shown) reveal that Sdk2 is also expressed by horizontal cells. b, In situ hybridization for Sdk1 and Sdk2 RNA at indicated postnatal ages. Scale bars, 10 µm.
Extended Data Figure 2 | Generation and characterization of Sdk1 and Sdk2 knock-in mice. 

(a) Targeting vectors used to generate Sdk1<sup>lox/lox</sup> (a) and Sdk2<sup>lox/lox</sup> (b) mice. HEK293 cells transfected with expression vectors encoding Sdk1 or Sdk2, followed by staining with mouse antibodies to Sdk1 and Sdk2. 

(c) HEK293 cells transfected with expression vectors encoding Sdk1 or Sdk2, followed by immunoblotting with rabbit polyclonal antibodies to Sdk1 and a mouse monoclonal antibody to Sdk2 (CS22). 

(d) HEK293 cells transfected with expression vectors encoding Sdk1 or Sdk2, followed by immunoblotting with rabbit polyclonal antibodies to Sdk1 and a mouse monoclonal antibody to Sdk2 (CS22). 

(e) Retinal sections from P30 Sdk1<sup>lox/lox</sup> and Sdk2<sup>lox/lox</sup> mice stained with mouse antibodies to Sdk1 and Sdk2. Signal on blood vessels is nonspecific. 

(f) Reverse transcription PCR (RT–PCR) from Sdk1<sup>lox/lox</sup>, Sdk1<sup>lox/lox</sup>, Sdk2<sup>lox/lox</sup> and wild-type (WT) mice. Total RNA was prepared from brain. G3PDH, glyceraldehyde-3-phosphate dehydrogenase. 

(g) VG3-ACs and W3B-RGCs are Sdk1-negative. CreER expressed in P30 Sdk1<sup>lox/lox</sup> mouse was stained with antibodies to the oestrogen receptor and vesicular glutamate transporter 3 (VG3). Bottom row shows CreER and YFP double staining in a Sdk1<sup>lox/lox</sup>; T Y W 3 mouse. 

(h) Sdk2-expressing W3B cells express the RGC marker Brn3a, but not the amacrine cell marker AP2. Calretinin is expressed in all the SACs, a subpopulation of type II catecholaminergic cells (CAII) amacrine cells and some RGCs. 

(i) SACs (ChAT-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type II catecholaminergic cells (CAII) amacrine cells and some RGCs. i, SACs (ChAT-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type II catecholaminergic cells (CAII) amacrine cells and some RGCs. i, SACs (ChAT-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type II catecholaminergic cells (CAII) amacrine cells and some RGCs. i, SACs (ChAT-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type II catecholaminergic cells (CAII) amacrine cells and some RGCs. i, SACs (ChAT-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type II catecholaminergic cells (CAII) amacrine cells and some RGCs. i, SACs (ChAT-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type II catecholaminergic cells (CAII) amacrine cells and some RGCs. i, SACs (ChAT-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type II catecholaminergic cells (CAII) amacrine cells and some RGCs. i, SACs (ChAT-positive), type I catecholaminergic (tyrosine hydroxylase-positive), type II catecholaminergic cells (CAII) amacrine cells and some RGCs. i, SACs (ChAT-positive), type II catecholaminergic cells (CAII) amacrine cells and some RGCs. i, SACs (ChAT-positive), type II catecholaminergic cells (CAII) amacrine cells and some RGCs. i, SACs (ChAT-positive). Scale bars, 10 µm.
Extended Data Figure 3 | Optimization of optogenetic methods. a, Sample current recorded from a ChR2–YFP-expressing HEK cell in response to a stationary PSF-sized spot of 920-nm laser stimulation for 7 ms. b, Plot of average peak current measured on ChR2–YFP-expressing HEK cells in response to a stationary point spread function (PSF)-sized spot of laser stimulation at 840 nm, 860 nm and 920 nm for a range of different powers. The 920 nm light produced the largest currents for the least power. c, Plot of the rise time (10–90%) of currents to a stationary, PSF-sized spot of laser stimulation at 840 nm, 860 nm and 920 nm for a range of different powers. The 920 nm light produced the shortest rise time for the least power. d, Cartoon of a HEK cell with four adjacent PSF-sized spots that start on the edge of the cell and extend off (left). Sample currents evoked by stimulation of these regions. Currents decrease in size as the PSF moves away from the cell. e, Quantification of the experiment illustrated in d for 5 cells. The curve was obtained in only one direction and reflected about the y-axis to give a measure of the x-y spread of ChR2 excitation. f, Cartoon of a HEK cell stimulated by a stationary, PSF-sized spot at 10 adjacent 1-μm planes that extend from the cell surface to ~10 μm. Sample currents evoked by this procedure at the indicated z-positions. Average peak current measured on four cells for this procedure (right) show the z-extent of ChR2 excitation. g, Sample current evoked in a ChR2-expressing RGC in response to stimulating an ROI (10 × 15) with a dwell time of 0.03 ms, taking a total time of 5 ms. h, Sample current evoked in a ChR2-expressing SAC in response to stimulating an ROI (10 × 15) with a dwell time of 0.03 ms, taking a total time of 5 ms. i, Average peak current evoked in RGCs and SACs in response to the stimuli shown in g and h (n = 6 cells). j, Average rise time (10–90%) of currents evoked on RGCs and SACs in response to the stimuli shown in g and h (n = 6 cells). k, Plot of average peak current measured in a W3B-RGC in response to stimulation of VG3-ACs with two-photon stimulation (920 nm) for a range of sample plane powers. Average current size shows a sigmoid relationship. Responses plateau at 25 mW sample plane power (n = 6 W3B-VG3 pairs in two animals). l, Ipeak versus power relationship from b has been re-plotted for comparison. Ipeak versus sample plane power measured on W3B-RGCs to VG3-AC replotted from k. Sample currents beside the curve were evoked by the powers indicated. Responses require a threshold amount of excitation in VG3-ACs; probably owing to calcium-dependent vesicle release mechanisms (n = 6 W3B-VG3 pairs in two animals).
Extended Data Figure 4 | VG3-ACs form direct excitatory synapses on W3B-RGCs. **a,** Average current ($I$)–voltage ($V$) plot of VG3-AC evoked currents on W3B-RGCs. Currents were normalized to the maximum inward current per cell ($n = 10$ VG3-W3B pairs). **b,** Currents are abolished by inhibitors of AMPA-type glutamate receptors (DNQX, 20 µM), but are unaffected by inhibitors of GABA (picrotoxin (PTX), 100 µM), glycine (strychnine (STR), 3 µM) receptors and gap junctions (18β-glycyrrhetinic acid (18β-GA), 25 µM) ($n = 8–10$ per condition). **c,** Latency of VG3-AC-evoked currents on W3B ($n = 623$ VG3s and 14 RGCs). **d,** Inward currents measured in HEK cells transiently transfected with constructs containing ChR2–GFP. The first, twenty-fifth and fiftieth responses to a train (1 Hz) of 60 stimuli are shown for HEK cells recorded in control and CdCl₂-containing solution. **e,** Peak currents from the experiment shown in **a,** plotted as a percentage of the initial peak size. Currents in CdCl₂ appear to decrease slightly over time. **f,** Quantification of peak currents measured in CdCl₂-containing solution expressed as a percentage of those found in control solution. ChR2 activity is largely unaffected by the presence of CdCl₂ ($n = 6$ cells, in control solution, 10 cells in 500 µM and 8 cells in 1 mM CdCl₂ solution). **g,** Responses of a ChR2-positive RGC to stimulus train that alternated between two-photon excitation of ChR2 on the RGC soma and one-photon activation of photoreceptors above the RGC. The first, fifteenth and thirtieth responses to a train are shown. ChR2 responses are insensitive to the calcium channel blocker but light responses are not. **h,** Peak current measured on the final pulse of the train expressed as a percent of the first for the experiments performed like that in **e,** $I_{\text{ChR2}}$ is largely immune to the calcium channel blocker CdCl₂ ($n = 4$ ChR2-positive RGCs). **i,** Heat map of VG3-AC responses measured in a W3B-RGC in control solution (left) and the same heat map measured in the presence of 500 µM CdCl₂. VG3-AC responses initiated by soma stimulation require functional voltage gated calcium channels in the nerve terminals. **j,** Heat map of VG3-AC responses measured in the same W3B-RGC in CdCl₂ solution shown in **f** in response to a 10 × 10 stimulus grid (grid square = 5 × 5 pixels) in the inner plexiform layer. Activating ChR2 on VG3-AC nerve terminals produces responses in W3B-RGCs in spite of silencing voltage-activated calcium channels globally. Scale bars, 65 ms and 34 µm. **k,** Currents recorded from W3B-RGCs (−60 mV) in response to stimulation of VG3-ACs at their somas (INL) or terminals (IPL) in control and CdCl₂ (200 µM) containing solution (left). Average peak current evoked by either soma (INL) or nerve terminal (IPL) stimulation in CdCl₂ solution expressed as a percentage of that found in control (right, $n = 276$ VG3-ACs and 5 W3B-RGCs). Currents evoked by IPL stimulation in CdCl₂-containing solution result from Ca²⁺ influx via ChR2 on VG3-AC terminals. VG3-ACs synapse directly with W3B-RGCs. **l,** Sample currents evoked in W3B-RGCs, W3D-RGCs and ooDSGCs by blue light (one-photon) stimulation of ChR2 positive VG3-ACs. Experiments were done in the presence of a cocktail of blockers: 10 µM ACET, to block the OFF pathway; 20 µM L-AQP, to block the ON pathway, and 300 µM hexamethonium, to block cholinergic nicotinic receptors19,51. **m,** Average peak currents from experiments like those in **I** ($n = 15$ W3B-RGCs, $n = 7$ W3D-RGCs and $n = 13$ ooDSGCs). These results from one-photon stimulation of a population of VG3-ACs confirm the conclusion from two-photon stimulation of single VG3-ACs (Fig. 2): these amacrine cells innervate W3B-RGCs far more strongly than W3D-RGCs or ooDSGCs. By one-photon stimulation, the currents evoked in ooDSGCs are stronger than those in W3D-RGCs, whereas they are similar in the two-photon data. This difference probably arises from their larger dendritic size. On the basis of the dendritic diameter of VG3-ACs (~50 µm; Figs 2c and 3r), W3B-RGCs (~115 µm; Figs 2c and 3l), W3D-RGCs (~125 µm; data not shown) and ooDSGCs (200 µm; ref. 37), we can estimate that dendrites of W3B-RGCs, W3D-RGCs and ooDSGCs overlap dendrites of ~24, ~29 and ~100 VG3-ACs, respectively. Given the percentage connectivity in shown in Fig. 2, we estimate that each W3D-RGC is innervated by 10–15 VG3-ACs, whereas each ooDSGC is innervated by ~35 VG3-ACs.
Extended Data Figure 5 | Synaptic connectivity of VG3-ACs and W3B-RGCs. a, b, Strength of connections as a function of distance from six interneuron types to W3B-RGCs (a) and VG3-ACs to four RGC types (b). Number of synaptic partners assayed shown above and sample currents shown below each graph. The W7 population contained six nearly-disconnected and ten connected pairs, presumably corresponding to the S1-laminating and S3-laminating W7 subsets. Normalized peak currents \( I_{\text{peak}} \) from each pair were normalized to the average maximum response from VG3-AC–W3B-RGCs.
Extended Data Figure 6 | Normal retinal architecture in Sdk2 mutants. Sections of Sdk2<sup>+/−</sup> and Sdk2<sup>−/−</sup> retinas (P30) were stained with antibodies to cell-type-specific markers, synaptic components or fluorescent proteins. No differences between mutants and controls were detected in cells other than VG3-ACs and W3B-RGCs. a, Brn3a labels most RGCs. b, AP2 labels all amacrine cells. c, d, Synapsin 1 and SV2 are associated with synaptic vesicles. e, VGlut1 is concentrated in photoreceptor and bipolar terminals. f, PSD-95 family members, stained with anti-MAGUK, are associated with synaptic sites, usually excitatory postsynaptic densities. g, Synaptotagmin 2 is concentrated in bipolar cells types 2 and 6. h, Anti-HCN4 labels type 3a bipolar cells. i, Anti-disabled-1 (Dab1) labels AII amacrines. j, Anti-VACHT labels dendrites of SACs. k, Anti-calretinin labels subsets of RGCs and amacrine cells, including SACs. l, Anti-protein kinase C-α (PKCα) labels rod bipolar cells and a subset of amacrine cells. m, Anti-VGlut3 labels VG3-ACs. Sprouting is evident in the mutant. n, Single-cell reconstructions of VG3-ACs labelled sparsely with GFP in retinal cross-sections. o, Quantification of sprouting in mutant VG3-ACs. Scale bars, 10 μm.
Extended Data Figure 7 | Morphological analysis of VG3-ACs and W3B-RGCs in Sdk2 mutants.  

a, b, Dye-injected W3B-RGCs were imaged and skeletonized as described in Methods. Projections on rotated stacks counterstained with anti-VAChT antibodies are shown in h, c, d. Similar to a, b, except for VG3-ACs labelled sparsely with tdTomato and counterstained with anti-ChAT to label the somas of SACs. Scale bars, 25 μm. e, f, Mean intensity (± s.e.m.) of dye-labelled W3B-RGC dendrites (e) and VG3-AC dendrites (f) across the IPL from images such as those shown in a–d.  
g, h, En face or laminar projections of skeletonized dendritic arbors from VG3-ACs labelled sparsely with tdTomato in Sdk2<sup>+/−</sup> and Sdk2<sup>−/−</sup> retinae. i, j, En face or laminar projections of skeletonized dendritic arbors from dye-filled W3B-RGCs in Sdk2<sup>+/−</sup> and Sdk2<sup>−/−</sup> retinae.
Extended Data Figure 8 | Electrical and synaptic properties of Sdk2 mutant VG3-ACs and W3B-RGCs. 

a–l, Strength of connections as a function of distance from three interneuron types (SACs, type 5 bipolar cells (BC5) and type II catecholaminergic cells (CAII)) to W3B-RGCs and from VG3-ACs to W3D-RGCs in Sdk2ce/ce (black) and Sdk2ce/ce (red) retinae. 

e–h, Normalized peak current. 

i–l, Average current amplitudes in a 30-ms window after the stimulus pulse. 

m–o, Latencies of currents detectable above noise. Number of pre- and postsynaptic partners assayed shown below each column. 

p, Responses evoked in a W3B-RGC (green dot) in a Sdk2 mutant after stimulation of 66 VG3-ACs (genotype: TYW3; Sdk2ce/ce; Vglut3-cre; LOX-STOP-LOX-ChR2-tdTomato). 

q, Average latencies for currents detectable above noise in control. 

r, Sodium currents in W3B-RGCs Sdk2ce/ce and Sdk2ce/ce retinæ. Sample currents evoked by a step from −60 mV to −5 mV (left), and average peak sodium current amplitude measured on W3B-RGCs in Sdk2ce/ce and Sdk2ce/ce retinae. 

s, Retinal cross-sections from wild-type mice and those that overexpress Sdk2 broadly using the Six3-cre driver and the Sdk2 swap transgene. Sdk2 is expressed strongly from the swap transgene, which is shown as a schematic below the micrographs. 

t, Strength of connections from VG3-ACs to W3B-RGCs in Sdk2ce/ce mutants in which Sdk2 expression was rescued in VG3-ACs. Data were derived from 121 VG3-ACs and 3 W3B-RGCs. Fit to the control data (Fig. 3a) re-plotted in blue.
Extended Data Figure 9 | Effective deletion of VG3-ACs by diphtheria toxin. a, Spike responses of W3B-RGCs in Sdk2$^{+/+}$ and Sdk2$^{-/-}$ retinae in response to a $\sim$100-µm flashing spot centred on the receptive field. OFF responses are strongly reduced in the absence of Sdk2. b, Inhibitory currents recorded ($V_h = -5$ mV) from W3B-RGCs in Sdk2$^{+/+}$ and Sdk2$^{-/-}$ retinae in response to a full field flash for 1 s. c, $I_{\text{peak}}$ measured from excitatory currents in non-W3B-RGCs ($n = 8$ in 4 Sdk2$^{+/+}$ mice and 8 in 4 Sdk2$^{-/-}$ mice).

d, Sample images of tdTomato-positive VG3-ACs in retinae from diphtheria-toxin-treated control (d) and Vglut3-cre; Rosa-CAG-LOX-STOP-LOX-DTR mice (e). Scale bar, 40 µm. 
f, Average firing rate recorded from W3B-RGCs in control (blue, $n = 21$) and diphtheria-toxin-treated Vglut3-cre:DTR/TYW3 (red, $n = 12$) retinae in response to small spots flashed for 1 s. Dark lines, average; shadowing denotes s.e.m. Bin width, 50 ms. 
g, Excitatory currents recorded ($V_h = -65$ mV) from W3B-RGCs in control and diphtheria-toxin-treated Vglut3-cre:DTR/TYW3 retinae in response to small spots flashed for 1 s.
h, Inhibitory currents recorded ($V_h = -5$ mV) from W3B-RGCs in control and diphtheria-toxin-treated Vglut3-cre:DTR/TYW3 retinae in response to small spots flashed for 1 s.
Extended Data Figure 10 | A delay line in the differential motion response.

a, Average latency to peak firing rate in W3B-RGCs and non-W3B-RGCs in response to a spot flashed over their receptive field centre (n = 21 non-W3B-RGCs and 18 W3B-RGCs). Spike responses on W3B-RGCs are delayed (**P < 0.01, Student’s t-test). b, Possible mechanisms for the delay. In non-W3B-RGCs excitatory currents produced by bipolar cells drive the neuron to fire. In W3B-RGCs, spikes could be delayed because the interposition of VG3-ACs delays the onset of the excitatory postsynaptic current (EPSC) (W3B e delay) or because of a transient inhibitory postsynaptic current that arrives at the same time as the EPSC and delays the cell from reaching threshold (W3B i delay). c, d, Excitatory (c) and inhibitory (d) currents measured from non-W3B-RGCs and W3B-RGCs in response to a flashing spot centred on the receptive field or a full field flash. Dotted lines denote the stimulus onset, non-W3B-RGC EPSC onset and W3B-RGC EPSC onset (red). The onset of EPSCs in W3B-RGC lags behind those found on non-W3B-RGCs by ~30 ms. No significant transient inhibition was observed in the receptive field centre (d). e, f, Histogram of latency to the onset of the ON (e) and OFF (f) excitatory current measured on W3B-RGCs (light blue, light red) and non-W3B-RGCs (blue, red). W3B-RGCs lag non-W3B-RGCs by ~32–40 ms and also have higher variance about the mean (n = 29 non-W3B-RGCs and 27 W3B-RGCs). g, Average peak of inhibitory currents measured on W3B-RGCs in response to a ~100-μm diameter flashing spot and a full field flash (n = 6 wild-type W3B-RGCs; *P < 0.05, Student’s t-test). h, Slope of the light-evoked excitatory current between 10% and 90% of the peak. Slopes of currents in W3B-RGCs are significantly lower than those of non-W3B-RGCs (n = 29 non-W3B-RGCs and 27 W3B-RGCs; **P < 0.001). The non-W3B-RGCs included W3D-RGCs, ooDSGCs, α-RGCs and some unidentified RGCs. i, Average maximal firing rates measured in W3B-RGCs to local, global and differential motion stimuli (n = 10 W3B-RGCs in 5 control mice, 6 W3B-RGCs in 4 Sdk2cre mice and 9 W3B-RGCs in 3 diphtheria-toxin-treated Vglut3-cre;DTR/TYW3 mice; **P < 0.01, Student’s t-test). Firing rates in global and differential motion were normalized to that elicited in control W3B-RGCs with local motion stimuli for Fig. 4j.