DSCAM-mediated control of dendritic and axonal arbor outgrowth enforces tiling and inhibits synaptic plasticity

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Mature mammalian neurons have a limited ability to extend neurites and make new synaptic connections, but the mechanisms that inhibit such plasticity remain poorly understood. Here, we report that OFF-type retinal bipolar cells in mice are an exception to this rule, as they form new anatomical connections within their tiled dendritic fields well after retinal maturity. The Down syndrome cell-adhesion molecule (Dscam) confines these anatomical rearrangements within the normal tiled fields, as conditional deletion of the gene permits extension of dendrite and axon arbors beyond these borders. Dscam deletion in the mature retina results in expanded dendritic fields and increased cone photoreceptor contacts, demonstrating that DSCAM actively inhibits circuit-level plasticity. Electrophysiological recordings from Dscam−/− OFF bipolar cells showed enlarged visual receptive fields, demonstrating that expanded dendritic territories comprise functional synapses. Our results identify cell-adhesion molecule-mediated inhibition as a regulator of circuit-level neuronal plasticity in the adult retina.

Significance

Adult neurons are not able to make new connections as easily as developing neurons can; however, future therapies aimed at regeneration and repair of neural circuits in the adult nervous system depend critically on the formation of such connections. Here, we studied a recently discovered cell population that has the unusual ability to make new connections into adulthood, but under normal conditions does not grow new axons or dendrites, so that no new cells are contacted. We manipulated this cell population to induce axon and dendrite outgrowth using transgenic methods and determined that this results in stable, functional connections with new cells.

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DSCAM Limits BC4 Plasticity by Enforcing Tiling of Dendritic Arbors.

We hypothesized that the ability of adult BC4s to expand dendritic arbors and contact previously not-contacted cones in WT mice is constrained by homotypic inhibition from neighboring BC4s. An important candidate for blocking circuit-level plasticity by preventing dendritic outgrowth is Dscam. Dscam is expressed in all OFF-type BCs, except BC1s (27, 37). To test the role of Dscam in establishing and maintaining BC4 tiling, we utilized genetic knockout mice and a floxed allele of Dscam in combination with HTR:Cre mice. HTR:Cre selectively targets Dscam deletion to BC4s with minimal disruption to retinal organization and no detectable change in BC4 cell number, common complications of Dscam deletion in other cell types (24) (Figs. S2 and S3). Dscams have been shown to regulate neurite laminar targeting through adhesion in Drosophila (38), chick (25), and at least one retinal cell type in mouse (26). Since altered laminar targeting may impact cell physiology (39), we first tested if selective deletion of Dscam in BC4 cells affects their laminar targeting. We found that this was not the case: conditional deletion of Dscam altered neither laminar targeting of BC4 axons (Fig. S2A–C and H) nor the distribution and density of upstream cone photoreceptors (Fig. S3A–F).

Next, we compared BC4 morphology in Dscam<sup>+/+</sup> and Dscam<sup>ΔF</sup> HTR:Cre retinas from P15 through 6 mo (Fig. 2A and B). Fluorescence coexpression and IHC validation showed that HTR:Cre deleted Dscam in many BC4s (Dscam<sup>ΔF</sup> BC4s) (Fig. 2A and B, red + green), but some nontargeted cells remained (~20%; Dscam<sup>ΔF</sup> BC4s) (Fig. 2A and B, green). This chimeric expression pattern allowed structural comparison of mutant and WT BC4s within the same retina (Fig. S2D). Since DSCAM in vertebrates and invertebrates acts as a homotypic cell-adhesion molecule (25, 40), we should expect a structural phenotype also in DSCAM-deficient BC4s.
With Dscam\(\text{+/+}\) BC4s (Fig. 2D) but the retinal area was not (Fig. 2E).

Increased dendritic field area without a change in retinal area implies increased dendritic field overlap concomitant with loss of dendritic tiling (41). To test this, we mapped the dendritic territories of neighboring BC4s in the Dscam\(\text{+/+}\) and Dscam\(\text{Δflox}\) retina (Fig. 2F). While dendritic field overlap between Dscam\(\text{+/+}\) BC4 pairs was negligible, as expected, overlap between neighboring Dscam\(\text{Δflox}\)-Dscam\(\text{Δflox}\) and Dscam\(\text{Δflox}\)-Dscam\(\text{Δflox}\) BC4 pairs (Fig. 2B) was substantial. Furthermore, Dscam\(\text{Δflox}\) retinas showed the predicted chimeric recombination pattern comprising WT-like tiling between Dscam\(\text{Δflox}\) BC4 pairs and loss of tiling between Dscam\(\text{Δflox}\) and Dscam\(\text{Δflox}\) BC4 pairs (Fig. 2B). Based on these data, we conclude that DSCAM acts between mouse BC4s in a cell-type–autonomous manner (i.e., between cells of same type). Using genetic knockout mice, we found a Dscam gene dosage-dependent change in BC4 tiling (WT < heterozygous < homozygous for the Dscam mutation), consistent with previous results (42, 43) (Fig. S4).

DSCAM Prevents BC4s from Contacting Cones Outside of Their Tiled Dendrite Territories. Next, we asked if loss of tiling is accompanied by an increase in the number of cone → BC4 anatomical connections. To answer this question, we counted the number of cones contacted by individual Dscam\(\text{+/+}\)/Dscam\(\text{Δflox}\) BC4s at ages between P15 and 6 mo (Fig. 3A–C). We found a significant increase in the number of cones contacted as a function of age in Dscam\(\text{Δflox}\) and Dscam\(\text{Δflox}\) BC4s, but not Dscam\(\text{+/+}\) BC4s (Fig. 3D).

We also found a significant increase in the total number of dendritic tips, number of dendritic tips per cone, and number of dendritic tips not at cones in Dscam\(\text{Δflox}\)/Dscam\(\text{Δflox}\) BC4s compared with Dscam\(\text{+/+}\) BC4s (Fig. 3E–G). To determine whether dendritic tips not ending at cones contacted rods, we stained retinas with dystroglycan, a marker specific to synaptic clefts and peanut agglutinin (PNA), which labels the cone axon terminal membrane (Fig. 3H). We found no difference in the number of rod contacts in Dscam\(\text{+/+}\) versus Dscam\(\text{Δflox}\) BC4s. However, the number of dendritic tips ending at either photoreceptor type was increased in Dscam\(\text{Δflox}\)/Dscam\(\text{Δflox}\) BC4s, compared with Dscam\(\text{+/+}\) BC4s (Fig. 3I). This phenotype, too, was dependent on Dscam gene dosage (WT < heterozygous < homozygous) (Fig. S5). Taken together, these data support a model in which Dscam-mediated recognition enforces BC4 dendritic tiling by preventing dendrite overlap.

In support of this model, we found that Dscam\(\text{Δflox}\) BC4 dendritic fields continue to grow, with significant increases in dendritic length and dendritic area between 3 and 6 mo of age (Fig. 4A–F). This contrasts with WT BC4s, in which length and area do not increase between 3 and 6 mo (Fig. 1I and J). Comparing increases in retinal area that occur as the retina ages to changes in BC4 dendritic area and overlap, we found that increases in dendritic area in Dscam\(\text{Δflox}\)/Dscam\(\text{Δflox}\) BC4s exceed retinal area growth and correlate with increases in BC4 overlap (Fig. 4G). Increases in WT BC4 dendritic area either match growth in retinal area or are not correlated with increases in dendrite overlap between adjacent BC4s (Fig. 4G). Increases in WT dendritic area between P35 and 3 mo occurred without similar changes in retina area or BC4 overlap. This suggested that BC4 tiling becomes tighter in the WT retina after P35. To test this, we measured unsampled space, defined as retinal area at the level of the OPL that was not sampled by the dendrites of any BC4 (Fig. 4H). We found a significant decrease in unsampled space in the WT retina between P35 and 6 mo of age (Fig. 4I).

Isoneuronal Organization Is Disrupted at the Cone Synapse in Dscam Mutant BC4s. Mouse Dscam prevents excessive adhesion between cells of a given cell type, while Drosophila DscamI helps to provide isoneuronal cues—those intrinsic to individual neurons—that prevent excessive overlap of a given cell’s own dendrites (44–47). Isoneuronal recognition is provided in retinal starburst

![Image](https://example.com/image.png)
amacrine cells by γ-protocadherins, but how isoneuronal avoidance is mediated in most retinal cell types remains unknown (48). We next measured if isoneuronal BC4 dendrite organization was disrupted in the Dscam mutant retina. Compared with Dscam+/− BC4s, Dscam-null BC4 innervation of cones was highly disorganized and the dendrites of a single BC4 appeared to clump together (Fig. 5A). Indeed, Dscamfl disc BC4 dendrites covered significantly more cone field in the DscamFF HTBCr retina was increased (Fig. 5C). Reconstructions of individual BC4 cone innervations showed a significant increase in the proportion of cones with crossing dendrites in Dscamfl disc BC4s compared with Dscam+/− BC4s (Fig. 5D and E). Conceivably, this could be caused by clumping of multiple BC4 dendrite tips constraining dendrite avoidance, but we found increased overlap in BC4 dendrites even in cases where a cone was innervated by a single Dscam mutant BC4 (Fig. 5B).

**Loss of DSCAM Causes Enlarged BC4s Visual Receptive Fields.** Our anatomical data show that BC4s lacking Dscam have enlarged dendritic arbors. To test whether these cells make functional synaptic connections with cone photoreceptors throughout their extended dendritic field, we performed whole-cell recordings of fluorescein-labeled BC4s in the whole-mount retina in vitro (Fig. 6A and B), and measured their spatial receptive fields using binary white noise stimulation (Fig. 6C). Dscamfl disc BC4s, identified by HTR-GFP and Ai9 coexpression in DscamFF HTBCr mice, showed similar time course and strength of excitatory and inhibitory synaptic conductance compared with Dscam+/− BC4s, identified by HTR-GFP expression in Dscam+/− mice, including both ON and OFF inhibition and excitation (Fig. 6A–C). These data indicate that presynaptic neurons and basic response properties of DscamΔfl disc BC4 cells were unchanged. However, DscamΔfl disc BC4s had significantly larger receptive fields compared with Dscam+/− BC4s (Fig. 6E and F), demonstrating that extended dendrites make functional synaptic connections with cone photoreceptors over a larger retinal area compared with WT BC4s.

**DSCAM Enforces Tiling of BC4 Axonal Arrows.** BCs are strongly polarized neurons characterized by axonal and dendritic arbors extending in opposite directions from the soma. DSCAM expression in BC4s is localized to the distal dendrites (27) (Fig. 5F and G). In RGCs, the function of DSCAM differs depending on cellular compartment. DSCAM prevents clumping in dendrites
adjacent to other Dscamflx BC3bs, and Dscamflx BC3b phenotypes were not significantly different from WT BC3bs (Fig. 8–E). Based on these data, we conclude that DSCAM plays a similar role in controlling dendritic field area in BC3b and BC4.

**DSCAM Actively Inhibits BC4 Plasticity After Development.** The results presented thus far show that constitutive loss of Dscam expression leads to expanded BC3b and BC4 dendritic fields. Conceivably, this phenotype is caused by the absence of DSCAM during development. Alternatively, it may reflect an active role for DSCAM in maintaining BC dendritic territory after development is complete (>1 mo). To distinguish between these two scenarios, we deleted Dscam in BCs in adult mice using two independent approaches. In the first approach, late deletion of Dscam was mediated by Pou4f2-Cre. Pou4f2-Cre targets the conditional allele of Dscam in BCs after 1 mo of age (51). Using this approach, we found no differences in dendrite area, dendrite overlap, or cones contacted in Dscamff vs. Dscamff Pou4f2Cre BC4s before recombination (up to 1 mo of age) (Fig. 9A, B, and E–G). However, at this time point a phenotype is clearly observed in the Dscamff Htr::Cre mice compared with WT (Fig. 2F). After recombination, at ages 3 and 6 mo, we found significant increases in each of these measurements (Fig. 9C–G). We found no change in retinal area at any of the time points (Fig. 9H).

In the second approach, we used the tamoxifen-dependent CreER system (52, 53), which allowed greater flexibility in the timing of Dscam deletion. Dscamff and Dscamff CreER mice were injected with tamoxifen at 1 mo and retinas harvested and analyzed at 3 mo. Comparing CreER-targeted vs. non-CreER–targeted BC4s (Fig. 9I and J), we found significant increases in dendrite area, dendrite overlap, and number of cones contacted (Fig. 9K–M). As before, we found no change in retina area (Fig. 9N). These data demonstrate that Dscam actively enforces dendritic tiling throughout the lifespan. By blocking dendritic outgrowth, DSCAM prevents BCs from contacting additional cones over time, thus inhibiting circuit-level BC plasticity in the mature retina.

**Discussion**

We provide insight into the regulation of neuronal plasticity. We report that OFF-type retinal BCs and BC4s retain the ability to extend and refine components of their dendritic arbors within a defined tiled territory long after the retina is considered developmentally mature. We identify Dscam as a gene that enforces BC axonal and dendritic tiling and inhibits BC plasticity through interactions between homotypic cells. We demonstrate that abolishing Dscam expression in BC3bs and BC4s promotes dendritic outgrowth, and show that BC4 dendritic extensions make functional synaptic connections with more cone photoreceptors than in WT controls, resulting in enlarged visual receptive fields. Finally, we show that deleting Dscam in the adult retina is sufficient for triggering BC dendrite outgrowth. This demonstrates that Dscam is required for maintaining dendritic territories throughout life. This is a demonstration of cell-adhesion molecule-mediated inhibition of axon and dendrite outgrowth as a regulator of circuit-level plasticity. Because adult plasticity is a key objective for clinical approaches to retinal regeneration and repair, interfering with Dscam or its downstream signaling pathways may serve as a potential strategy for promoting neurite outgrowth and synaptogenesis in the adult CNS.

Several factors limit neuronal plasticity in the mature CNS. In the spinal cord, myelin and extracellular matrix proteins provide inhibitory cues that prevent sprouting and outgrowth of neurons following damage (54–58). Within the retina, restriction of axon outgrowth in mature RGCs is mediated, in part, by the downregulation of factors that promote cytoskeletal dynamics (59). Throughout the CNS, the bioavailability of growth factors critical for promoting plasticity during development and for learning and memory is reduced with age (17). Here, we expand our understanding of the
Loss of DSCAM-Dependent Axon and Dendrite Tiling Results in Formation of New Cone Contacts. While most retinal neurons have axonal and dendritic fields that overlap extensively, ranging from about 3-fold in nonfoveal ganglion cells to about 30-fold for starburst amacrine cells (67–69), BCs have tiled dendritic and axonal arbors (20). In this study, we identify Dscam as the regulator of tiling in two Dscam-expressing OFF BC types (BC3b, BC4) (27). The tiled organization of dendritic and axonal arbors of these BC types suggest that a similar mechanism acts to prevent overlap in other BC types, but the genes that mediate this, as well as tiling in other vertebrate neuron populations, remain to be identified. Four types of OFF BCs express Dscam and all have dendrites that converge onto the cone synapse (27) or axonal arbor that overlap, and yet DSCAM protein appears to function by preventing dendrite and axonal overlap independently in these cell types. This is consistent with the extensive overlap of the dendritic arbors of different Dscam-expressing cell types in the IPL and a model wherein Dscam does not provide cell-type-specific recognition cues, but rather acts to prevent excessive adhesion or preserve tiling by acting in the context of a larger cell-type identity code (24). This contrasts with Dscam function in chick where DSCAM protein is localized to a narrow band of the IPL and plays a role in laminar targeting, a function that may be mediated in mouse by semaphorins and plexins (70, 71), contactins (72), and cadherins (39). The localization of DSCAM protein in the mouse IPL at the base of synaptic invaginations, possibly leaving their dendrite tips free to explore and expand (62, 63). An additional difference between ON cone BCs and mouse OFF BC3a, BC3b, and BC4s is that the latter make direct synaptic contacts with rods in addition to cones, analogous to the primate DB3 BCs (33, 50, 64). The dendritic field of these OFF BC types reportedly contains an excess of potential presynaptic rod contacts, with only about one in five rods directly contacted (62, 63). We did not observe an increase in the number of rods contacted, even as dendritic field size increased. Thus, synaptic input to BCs appears to be controlled independent of dendritic arbor size, with reported feedback from axonal contacts as a potential mechanism (66).

Intrinsic Plasticity of OFF BCs. Our mechanistic understanding of BC dendrite and axon development is based primarily on studies in ON BCs (23, 36, 60, 61). Here, we demonstrate that OFF BC dendritic arbor and synaptic development is qualitatively different from ON BCs. Whereas ON BCs have the same number of anatomical connections in the adult retina as they do around the time of eye-opening (18, 60), we show that OFF BCs continue to make and refine anatomical connections within their dendritic territories until at least 6 mo of age. The difference in dendritic plasticity between ON and OFF BCs may be related to the different innervation patterns of ON and OFF BCs in the mammalian retina. While ON cells make invaginating contacts at synaptic ribbon-containing sites, OFF cells make flat contacts at the base of synaptic invaginations, possibly leaving their dendrite tips free to explore and expand (62, 63). Addition difference between ON cone BCs and mouse OFF BC3a, BC3b, and BC4s is that the latter make direct synaptic contacts with rods in addition to cones, analogous to the primate DB3 BCs (33, 50, 64). The dendritic field of these OFF BC types reportedly contains an excess of potential presynaptic rod contacts, with only about one in five rods directly contacted (62, 63). We did not observe an increase in the number of rods contacted, even as dendritic field size increased. Thus, synaptic input to BCs appears to be controlled independent of dendritic arbor size, with reported feedback from axonal contacts as a potential mechanism (66).
microtubule disruption (73). Conservation of such a system in mouse would be consistent with the findings of this study that *Dscam* maintains tiling and inhibits dendrite and axonal outgrowth by acting in conjunction with additional factors for cell-type specificity.

BC tiling facilitates efficient transmission of spatiotemporal information from photoreceptors to RGCs (35). Interestingly, we report that the physiological tiling of OFF BCs is less strict than the anatomical tiling, with the anatomical BC visual receptive field diameter roughly twofold larger than the dendritic field diameter. This is likely due to cone–cone gap junction coupling (74). By developing a model system in which we can selectively and inducibly increase the receptive field of identified BC types, we can now test directly how loss of tiling impacts information encoding at the level of the retinal ganglion cells. Since *Dscam* is widely expressed throughout the CNS (75–77), similar perturbations of receptive and projective fields in the brain should be of potential interest for exploring links between mutations in *Dscam* and autism in humans (78, 79).

**DSCAM Deletion in Mature Neurons Triggers Dendrite Outgrowth and Synaptic Contact.** During development, Dscams promote axon growth and guidance (49, 80–84), laminar targeting (25, 38), and avoidance (22, 24, 44, 45, 85, 86). In this study, we report that DSCAM acts in mature neurons to actively suppress dendrite growth, a novel mechanism to inhibit plasticity. Widespread ablation of photoreceptors has been shown to result in rod BC dendrite sprouting and innervation of rods located outside of the ablated region, helping to restore visual function in the ablated region (87). Surviving cone BCs, on the other hand, failed to sprout and innervate cones outside of the lesion (87). Our study predicts that targeting *Dscam* in this context will activate BC...
dendrite outgrowth and facilitate restoration of cone-driven visual function in BCs proximal to the lesion site.

Other cell-adhesion molecules that regulate neuron self-organization through homotypic binding include the MEG-1023 and the γ-proteohoein complex (48, 88), while semaphorins and plexins restrict axon and dendrite arbors across the vertical plane of the retina (i.e., photoreceptors to RGCS) (70, 71, 89, 90). Whether deletion of factors like MEGF and semaphorins in the adult retina is also sufficient to induce neurite outgrowth is not known but worth exploring.

The identification of signaling pathways downstream of Dscam and the availability of pharmacological agents to up- and down-regulate these pathways (91, 92) suggest that targeted intervention could be performed to promote or inhibit neurite outgrowth in cases where DSCAM function may be lacking or over-exuberant, including in autism (78, 79, 93), fragile-X syndrome, and Down syndrome (94, 95). Because of its sensitivity to graded changes in Dscam expression (e.g., homozygous vs. heterozygous for Dscam mutations), the model system presented here provides a powerful research platform for understanding how manipulation of neurite outgrowth impact neural circuit function.

Materials and Methods

Mouse Strains. All animal procedures were performed in accordance with protocols approved by the Animal Use and Care Committees at the University of Idaho and the University of Louisville, and were in compliance with National Institutes of Health guidelines. We used two different transgenic mouse lines manipulating Dscam: (i) Dscam<sup>Δflox</sup> and (ii) Dscam<sup>Δflox</sup>; one Cre-dependent reporter mouse: Gt(Rosa26)Sor5tm1(CAG-tdTomato)Hze (referred to as Ai9; The Jackson Laboratory, stock no: 007909) (96); three Cre recombinase-expressing transgenic mouse lines: (i) Tg(HttTa-cre)KM07Gas (referred to as Htt<sup>Cre</sup>; Transgenic Mouse Resource Center, stock no: 035395) (31), (ii) Tg(HuR-cre) (courtesy of Vann Ben-Nett, Duke University, Durham, NC) (40), and (iii) Tg(GRF+D]325tm1([CreERT2])y1 (referred to as CreER<sub>T2</sub>; The Jackson Laboratory, stock no: J008463) (52); and one GFP transgenic mouse: Htr2a-EGFP (referred to as HTR-GFP; MMRRC, stock no: D0118) (32). Mice were maintained on a mixed genetic background containing C57BL/6J, C3H, and FVB. Mutant alleles of Pde6b were crossed out of the colony. Mice were fed ad libitum and kept on a 12-h light-dark cycle. A minimum of three retinas from three mice were used in each measurement, except for Pou422-Cre at 6 mo of age, in which case four retinas from two mice were used.

Genotyping. Mice were genotyped following standard procedures using previously reported primer sequences (97). Morphological analyses were performed blind to genotype. All mice taken for study were given a unique alphabetical code that was dissociated from the genotypes to those performed experiments until data collection was complete. Tail biopsies from each mouse were saved for post hoc verification of genotype, if needed.

Microscopy. Micrographs were captured using either a Nikon Spinning Disk confocal microscope or an Olympus Scanning Laser confocal microscope (FV1000). Image processing was performed using Fiji (National Institutes of Health) or Adobe Photoshop (Adobe Systems) and was limited to adjustments of brightness or contrast applied uniformly across the image.

Immunohistochemistry. IHC was performed as previously described (27). In all images containing HTR-GFP, the GFP was amplified using IHC; the tdTomato RFP from the Ai9 reporter was not amplified. See SI Materials and Methods for details about antibodies and stains.

Statistical Analysis. Statistical analyses were performed in Microsoft Excel or Matlab. A summary of the statistical tests performed and P values for each measurement can be found in Table S1; resources can be found in Table S2. To test for statistical differences between two groups with numerical data, we used the Student’s t test; for three or more groups with numerical data we used a one-way ANOVA with a Tukey post hoc test; for categ-orical data we used a hypothesis test comparing two independent proportions; for IPL lamination data we used a Kruskal–Wallis test with Dunn’s post hoc test. A P value < 0.05 was considered statistically different and is denoted by an asterisk in the graphs in the figures. Horizontal lines above columns in figures represent comparisons between the column under the left end of the line and all columns to the right. An asterisk above a point on the line represents a significant difference between the left-most column and the column under the asterisk. Error bars show mean ± SEM.

Cell Counts, Cell Spacing, and Retinal Thickness. For total cell counts, eight images (four central, four peripheral), equally sampled from the dorsal,
ventral, nasal, and temporal regions, were captured from whole-mount retinas stained with cell type specific markers at 1-μm increments along the z axis. A 60x magnification (160,000-μm² field size) was used to capture dopaminergic amacrine cells (DACs). Image stacks were imported into Fiji and cell bodies were manually marked using the multipoint tool. The total number of cells per retina was calculated by extrapolating the number of cells in the eight sample areas to the total retinal area. Retinal area was determined by montage imaging of the entire whole-mount retina and using the polygon tool in Fiji. To determine cell spacing, x- and y-coordinates of each annotated cell soma were exported from FIJI and imported into WinDRP to compute the nearest-neighbor regularity index (NNRI) and the packing factor (98). Procedures for quantifying morphological characteristics of identified BC types are described in SI Materials and Methods.

DSCAM Localization. All DSCAM localization was performed in cryo-sections of Dscam<sup>+</sup> retinas by staining them with antibodies against HTR:GFP, calceinilin, and DSCAM. Confocal images were then taken using a scanning-laser confocal microscope at 1,200x magnification, sampling at 0.5-μm increments about the z axis. Image stacks were then taken into Fiji where they were analyzed by the following metrics.

DSCAM localization on BC4 dendrites. Dendrite tips and cell bodies of BC4s and DSCAM colocalized to BC4 dendrites were identified and marked using the multipoint tool. This distance from DSCAM puncta to the nearest dendrite tip was measured by tracing from the puncta, along the dendrite, to the terminal. The length of each dendrite was measured in the same way, but from the dendrite tip back to the cell body.

DSCAM colocalization with BC4 axons. Using image stacks, volumes surrounding isolated BC4 axons were identified and all of the DSCAM puncta within that area were marked with the BC4 axon channel hidden. Then the channel was made visible and the puncta colocalized with the axon were recorded. As a control, the BC4 axon channel was flipped about the horizontal axis and the measurement was performed again.

Electrophysiological Recordings. Whole-cell electrophysiological recordings from fluorescence-labeled bipolar cells were performed as described previously (99). Each retina was mounted photoreceptor-side down on nitrocellulose filter paper, centered on a square pattern of four 1.3-mm-diameter apertures to permit visual stimulation from below and electrode access from above. A fifth aperture, offset ventrally, was used to focus the stimulus onto the photoreceptors. Recordings were performed on a custom-built two-photon fluorescence microscope (Olympus BX-51) controlled by Sciamimage software (http://www.sciamimage.org). We used concentric and parfocal IR bright-field and two-photon imaging capability. BC4 cells were selectively recorded by targeting HTR:GFP-expressing, or HTR:GFP and Ai9 coexpressing cells in inner nuclear layer of Dscam<sup>+</sup> and Dscam<sup>-/-</sup> HTR:GFP mice, respectively (for details, see ref. 100). Typical access resistance was 25-40 MΩ; typical input resistance was 350-700 MΩ. Pipette internal solution contained: 120 mM Cs-methanesulfonate, 5 mM TEA-Cl, 10 mM Hepes, 10 mM BAPTA, 3 mM NaCl, 2 mM CaCl<sub>2</sub>, 4 mM Mg-ATP, 0.4 mM Na3GTP, and 10 mM phosphocreatine-Tris (pH 7.3, 280 mM). A red fluorescent dye (Sulforhodamine 101; thermo Fisher) Excitatory currents were recorded at a holding potential near the reversal potential for chloride, E<sub>G</sub> (−67 mV), corrected for the liquid junction potential (−9 mV); inhibitory currents were recorded at the reversal potential for cations, E<sub>H</sub> (0 mV).

Visual Stimulation. Visual stimuli were generated with an iMac computer using Matlab and Psychophysics Toolbox software (Mathworks; www.psychtoolbox.org). Stimuli were displayed using a DLP video projector (60 fps refresh rate; HP AX325AA; Hewlett-Packard), with the image projected onto the photoreceptor layer using a 20x objective (Olympus) in place of the microscope condenser. The stimulus was focused under visual guidance and focus accuracy was verified post hoc for each recorded cell. Pixel size at level of the photoreceptors was 3.25 × 3.25 μm. Stimuli comprised binary white noise checkerboard stimuli with 6 checks, checking frequency of 6 checks per second (100% Michelson contrast); stimulus frames per second (to measure spatial receptive fields and contrast-modulated spots (100% Michelson contrast; 100-μm diameter; 1 Hz) to measure excitatory and inhibitory conductances.

Tamoxifen Dosing. Tamoxifen (TS648; Sigma Aldrich) was dissolved at 20 mg/ml in sesame seed oil by incubation at 37 °C until dissolved (about 4 h). Next, 100 μl of tamoxifen solution was injected subcutaneously in the mouse tail (1 mg/10 g body weight) daily for 5 d starting at postnatal day 30. Mice were maintained for an additional 3 mo before being taken for study.

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