Different Modes of Visual Integration in the Lateral Geniculate Nucleus Revealed by Single-Cell-Initiated Transsynaptic Tracing

Highlights

- Individual LGN cells integrate retinal inputs in one of three distinct modes
- Relay-mode cells integrate inputs from few retinal ganglion cells of mostly one type
- Combination- and binocular-mode cells combine inputs from many ganglion cell types
- The three integration modes exhibit different degrees of cell-type specialization

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In Brief

Rompani et al. employ single-cell-initiated transsynaptic tracing to decipher patterns of input integration in the thalamus. They show that individual cells in the lateral geniculate nucleus integrate retinal inputs in three distinct modes, each exhibiting different degrees of specialization.
Different Modes of Visual Integration in the Lateral Geniculate Nucleus Revealed by Single-Cell-Initiated Transsynaptic Tracing

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SUMMARY

The thalamus receives sensory input from different circuits in the periphery. How these sensory channels are integrated at the level of single thalamic cells is not well understood. We performed targeted single-cell-initiated transsynaptic tracing to label the retinal ganglion cells that provide input to individual principal cells in the mouse lateral geniculate nucleus (LGN). We identified three modes of sensory integration by single LGN cells. In the first, 1–5 ganglion cells of mostly the same type converged from one eye, indicating a relay mode. In the second, 6–36 ganglion cells of different types converged from one eye, revealing a combination mode. In the third, up to 91 ganglion cells converged from both eyes, revealing a binocular combination mode in which functionally specialized ipsilateral inputs joined broadly distributed contralateral inputs. Thus, the LGN employs at least three modes of visual input integration, each exhibiting different degrees of specialization.

RESULTS

Targeted, Single-Cell-Initiated Transsynaptic Tracing in the LGN

To address this question, we developed an in vivo experimental approach that revealed the dendritic stratification and morphology of ganglion cells projecting to a single principal cell in the LGN (Figure 1A). First, we injected V1 with an adeno-associated virus (AAV) that is capable of retrograde axonal transport and therefore resulted in the infection of principal cells, but not interneurons, in the LGN. The AAV expressed a Cre-GFP fusion protein: GFP allowed the targeting of fluorescent principal cells for single-cell electroporation using two-photon targeted shadow imaging (Kitamura et al., 2008), while Cre enforced nuclear localization of GFP. Nuclear GFP was necessary since cytoplasmic GFP also labeled the axon terminals of LGN-projecting cortical cells, which obscured GFP-expressing LGN cells.
Second, we performed targeted single-cell electroporation (Figure 1B) in a morphologically defined region of the LGN that corresponded to the same retinotopic location in each animal (Figure S1, available online). This is important since the diameter of the dendritic tree of some ganglion cell types changes significantly across the retina (Bleckert et al., 2014). To ensure single-cell specificity of the transsynaptic tracing (Marshel et al., 2010; Rancz et al., 2011; Wertz et al., 2015), in each animal we
electroporated a single GFP-expressing cell, up to 100 μm deep from the surface of the LGN, with a fluorescent dye, Alexa-594, and three plasmids: one expressing the avian TVA receptor in the presence of Cre recombinase, one expressing the rabies G glycoprotein, and one expressing the fluorescent protein tdTomato (Figure 1C). Third, we injected into the LGN a G-deleted, EnvA-coated rabies virus expressing mCherry. EnvA, a ligand of TVA, led to entry of the rabies virus into the TVA-expressing cell and the G glycoprotein led to the transsynaptic transfer of rabies virus to presynaptic partners of the targeted LGN cell (Figure 1D).

We implemented two safety mechanisms to limit transsynaptic spread of rabies to the presynaptic partners of only a single LGN principal cell. First, the expression of Cre recombinase in principal LGN cells, together with the use of Cre-dependent TVA, ensured the restriction of EnvA-coated rabies to principal cells only (Schwarz et al., 2015; Wall et al., 2010). Second, the sparse labeling of LGN cells with Cre, caused by the low efficacy of retrograde spread by the AAV, guaranteed that even in the unlikely case of additional electroporation of an untargeted nearby cell, the rabies could not enter the untargeted cell due to the absence of Cre recombinase and therefore TVA. However, we never observed dye filling, which is an indication of successful electroporation, in more than one cell.

This targeted, single-cell-initiated, monosynaptically restricted transsynaptic tracing led to the labeling of ganglion cells that were in close proximity to each other and had overlapping dendritic trees (Figure 1D). We refer to the ganglion cells within a single retina that provide monosynaptic inputs to a single LGN cell as a presynaptic ganglion cell cluster. Presynaptic ganglion cell clusters were positioned in similar retinotopic locations in each animal, around the ventral end of the vertical midline in the retina (40° ± 5°, mean ± SD, of visual angle from the center of the retina; Figure S1H). The size of the clusters varied, with a radius ranging from 1′ to 16′ of visual angle (5.9° ± 3.2°, mean ± SD) along the radial direction. Despite large variance in cluster size, the spread of the cluster along the radial direction and along a direction perpendicular to it was highly correlated (Pearson r = 0.87, p < 0.001) with an aspect ratio close to unity (Figure S1J). This suggests that LGN principal cells at a given retinotopic position receive visual input from a visual region that varies in size but is close to circular (Piscopo et al., 2013; Tang et al., 2016).

**Classification of Presynaptic Ganglion Cells**

To reveal the fine dendritic morphology of ganglion cells that provide input to a single LGN principal cell, we stained retinas post hoc with antibodies against mCherry and ChAT (which labels two retinal strata in the inner plexiform layer) and created concatenated confocal image stacks from a region of the retina that included the entire ganglion cell cluster. We then individually traced the dendritic trees of the ganglion cells in the cluster (Figure 1E) and quantified two morphological attributes of ganglion cells: the depth of stratification of their dendrites, using ChAT antibody as a ruler, and the diameter of their dendritic tree referring to a set of previously characterized ganglion cells (Farrow et al., 2013). Based on these parameters, we segregated the traced ganglion cells into 13 morphological types (Figures 2 and S2; Tables S1 and S2). Here we present data from 25 animals representing presynaptic ganglion cell clusters connected to 25 LGN principal cells.

The ganglion cell types found via single-cell-initiated transsynaptic tracing could reflect the overall distribution of ganglion cell types that provide input to the LGN. Alternatively, the targeted region of LGN may receive specific input. To distinguish between these scenarios, we compared the distribution of ganglion cell types between single-cell-initiated and bulk transsynaptic tracing. For bulk transsynaptic tracing, we coinjected V1 with a G-deleted rabies virus and a glycoprotein G-expressing AAV, which retrogradely infected principal LGN cells. Rabies virus then spread from infected LGN cells to presynaptic ganglion cells transsynaptically. The distributions of ganglion cell types were largely overlapping between single-cell-initiated and bulk transsynaptic tracing (Figure 3A), yet indicated a moderate, but significant, underrepresentation of type 2 in the ganglion cells projecting to the LGN region targeted for electroporation (Fisher’s exact test p = 0.001, but n.s. excluding type 2).

LGN cells could be divided into two groups depending on their retinal inputs. The majority of LGN cells (15/25) had presynaptic ganglion cells only in the contralateral eye and therefore were monocularly driven. A smaller fraction of LGN cells (10/25) had presynaptic ganglion cells in both eyes. The distribution of presynaptic ganglion cell counts was wide (ranging from 1 to 91; Figure S3A) and the number of cell types within a presynaptic ganglion cell cluster was also widely distributed (up to nine cell types; Figure 3B). The presynaptic ganglion cell count was similar in the studied time range 9–12 days post-infection (Spearman r = −0.15, p = 0.39), suggesting that by 9 days the number of labeled ganglion cells has saturated (Figure S3E) (Wertz et al., 2015). Our data did not indicate that the presynaptic ganglion cell count would depend on the age of the infected animal, which ranged from postnatal day (P) 22 to P40 at the time of rabies infection (Spearman r = 0.03, p = 0.88; Figure S3F); however, a larger sample would be needed to rule out this possibility. Furthermore, the diameter of the dendritic tree of ganglion cells was not correlated with animal age at the time of the removal of the retina (P32–P52; Figure S3G). Ganglion cell clusters contained 14.5 ± 18.4 cells per eye (mean ± SD, n = 35 clusters from 25 LGN cells).

**LGN Cells that Integrate Retinal Inputs in Relay Mode**

We first focused on the monocular LGN cells and asked whether the observed number of ganglion cell types per cluster could result from a random draw from the overall distribution of ganglion cell types as measured by bulk tracing, thereby just reflecting the ganglion cell count of each cluster. The number of cell types per cluster was significantly lower than expected by chance (p = 0.0001, Monte-Carlo simulation; Figures 3C and S4), and more than half of all clusters contained only one cell type (n = 3/15) or two cell types (n = 6/15; Figures 3D–3F and S5A). Moreover, in most clusters with two cell types (n = 5/6), all ganglion cells projected to shared strata of the inner plexiform layer, thereby suggesting functional similarity. Altogether, 7/15 monocular LGN cells displayed features of “relay” cells: their presynaptic ganglion cell cluster (Figures 3D, 3E, and S5A) consisted of either one ganglion cell type or one dominant ganglion cell type and a single outlier cell of a different but related type,
Figure 2. LGN-Projecting Ganglion Cell Types
Each row refers to a different type of ganglion cell observed in single-cell tracing.
(A) Dendritic arbors of three example ganglion cells (only one for type 13).
(B) Side projection of a representative ganglion cell (gray, antibody against ChAT; red dotted lines, ChAT strata). The boxes on the right represent the ten strata of the inner plexiform layer (black squares, dendrites of respective ganglion cell type; gray squares, ChAT strata). See Figure S2.
with both types extending their dendrites to shared strata of the inner plexiform layer (Figures 3E, 3F, and S5A). These presynaptic ganglion cell clusters contained one to five ganglion cells, and we refer to them as relay-mode clusters.

To characterize the ganglion cell types found in relay-mode clusters, we examined their depth of dendritic stratification in the inner plexiform layer of the retina. The inner plexiform layer is divided into two major regions: strata 6–10 are closer to the ganglion cell bodies and incorporate ganglion cell dendrites that signal increments of light intensity (ON region), while strata 1–4 are farthest from ganglion cell bodies and incorporate ganglion cell dendrites that signal decrements of light intensity (OFF region). Furthermore, the ChAT antibody-labeled strata divide the inner plexiform layer into three domains: a middle domain with more transient responses (strata 3–7) and inner and outer domains with more sustained responses (strata 1–2 and 8–9) (Baden et al., 2013; Borghuis et al., 2013; Roska and Werblin, 2001). Dendrites of ganglion cells in relay-mode clusters did not discriminate between ON and OFF regions, but they stratified almost exclusively (16/18 cells) in the inner and outer domains of the inner plexiform layer (Figures 3F, 3G, and S5A). Therefore, the relay-mode LGN cells integrate information mostly from the sustained ganglion cells.

Using bulk transsynaptic tracing, we occasionally found retinas containing only a few closely positioned cells in the entire retina, consistent with being presynaptic to a single LGN cell (n = 5 clusters in 30 retinas). Each of these clusters contained ganglion cells that almost exclusively stratified in the inner and outer domains of the inner plexiform layer (9/10 cells) and consisted of one to two ganglion cell types, indicative of relay-mode clusters (4/5 clusters) (Figure SSD). These clusters were located at random positions in the eye, suggesting that the relay mode is a general feature of LGN input processing, and not restricted to the retinotopic region targeted in single-cell-initiated transsynaptic tracings.

**LGN Cells that Integrate Retinal Inputs in Combination Mode**

In contrast to relay-mode clusters, the larger fraction of monocular LGN cells (8/15) had presynaptic ganglion cell clusters that were composed of different ganglion cell types (2–6 types, 6–36 cells total; Figures 3H–3J and SSB). Most of these “combination-mode clusters” contained as many ganglion cell types as expected from a random draw (5/8 clusters, Z scores between +0.8 and −1.1), but some of them contained fewer (3/8 clusters, Z scores between −2.3 and −2.8). Unlike the dendritic stratification of ganglion cells in the relay-mode clusters, the dendrites of ganglion cells in combination-mode clusters were not restricted to the inner and outer domains of the inner plexiform layer (5/8 stratified in strata 3–7) (Figures 3J, 3K, and SSB). Despite the presence of different ganglion cell types in combination-mode clusters, a strong bias toward a predominant ganglion cell type could indicate that these clusters mostly relay one information channel. We therefore measured ganglion cell type dominance, defined as the ratio of ganglion cells from the predominant ganglion cell type over the total ganglion cell count (Figure S6). Notably, in the majority of combination-mode clusters that contained a similar number of ganglion cell types as expected from a random draw, ganglion cell type dominance was close to chance level (p = 0.43, n = 5, Monte-Carlo simulation), ruling out that combination-mode clusters would have a strong bias toward one ganglion cell type. For the combination-mode clusters that contained a lower number of cell types than expected from a random draw, ganglion cell type dominance was correspondingly above chance level (p = 0.006, n = 3, Monte-Carlo simulation). However, ganglion cell type dominance in these clusters was similar to the expected value when we conditioned the probabilities on the respective number of ganglion cell types (p = 0.80, Monte-Carlo simulation conditional; Figure S6B), thereby ruling out that one of their two to three cell types largely dominated over the others. These results support the conclusion that all combination-mode clusters combine the information from distinct ganglion cell types.

**Binocular LGN Cells**

Next, we investigated ganglion cell input integration in binocular LGN cells (Figures 4A and 4B). The percentage of binocular LGN cells (40%–50%) and their range of functional specialization were similar throughout age (Figure S7A). The ganglion cell count of presynaptic ganglion cell clusters was significantly higher in the binocular clusters than in the monocular clusters (p = 0.004 for the sum of ipsi- and contralateral cells, p = 0.018 for the largest cluster in either eye, n = 10 and 15, Mann-Whitney U test; Figures S3A and S3C) and the number of ganglion cell types per pair of binocular clusters was also significantly higher than in the monocular clusters (p = 0.004, Mann-Whitney U test; Figures 4B). Furthermore, 9/10 of binocular LGN cells received input from all three domains of the inner plexiform layer (Figures 4B and 4C). These results suggest that binocular LGN cells integrate, like combination-mode LGN cells, information from multiple different ganglion cell types.

We then compared the contralateral and ipsilateral clusters projecting to a single binocular LGN cell. Despite the diversity of retinal inputs to binocular LGN cells, the distribution of cells and cell types was highly non-random in several aspects. The number of ganglion cell types in ipsilateral clusters was lower than expected by chance (p = 0.00003, n = 9, Monte-Carlo simulation; Figures 3C, S4B, and S4C), indicating functional specialization. Moreover, ganglion cell type dominance was significantly above chance level in ipsilateral clusters (p = 0.00003, Monte-Carlo simulation), while ganglion cell type dominance in contralateral clusters was similar or even below chance level (Figures S7B and S7C). Accordingly, ganglion cell type dominance was significantly larger in ipsilateral than in contralateral clusters (p = 0.008, Wilcoxon signed-rank test; Figures 4D and S7B). While 8/10 contralateral clusters received inputs from all three domains of the inner plexiform layer, the number of ipsilateral clusters that received inputs exclusively from inner and outer domains (8/9) was highly significantly above chance level (p = 0.002, n = 9, Monte-Carlo simulation; Figures S7D and S7E). These results suggest that ipsilateral clusters receive input from fewer, selected types of ganglion cells and are therefore functionally specialized, while contralateral clusters combine ganglion cell types more broadly.

In addition to the functional specialization of the ipsilateral clusters, we found a second asymmetry between the ipsilateral...
Figure 3. Monocular Clusters of Presynaptic Ganglion Cells
(A) Distribution of ganglion cell types in single-cell (gray) or bulk tracing (white).
(B) Distribution of number of ganglion cell types per monocular cluster (gray) or per pair of binocular clusters (white, ipsi- and contralateral combined).
(C) The deviation of measured number of ganglion cell types from what is expected by a random draw (ipsilateral and contralateral binocular clusters shown separately). p values from Monte-Carlo simulations, **p < 0.01, ***p < 0.001; n.s., not significant.

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DISCUSSION

In summary, we used retrograde transsynaptic tracing from single LGN cells to determine how LGN principal cells of mice combine inputs from different ganglion cell types. Our analysis points to the co-existence of three integration modes by LGN principal cells.

In the relay mode (28% of the studied LGN cells), up to five ganglion cells of the same or mostly the same type (with one outlier cell) converged from the contralateral eye, with the single outlier cell being of a different, but related, cell type. Previous physiological recordings from cat LGN cells had suggested the existence of such outliers (Usrey et al., 1995). We found three clusters with a single retinal ganglion cell, which may indicate a private line from retina to cortex as shown for the cat LGN (Cleland et al., 1971; Hamos et al., 1987). A property of the relay-mode clusters was that ganglion cells stratified predominantly in the inner and outer domains of the inner plexiform layer, suggesting that this mode mainly transfers the information of sustained cells. In the combination mode (32% of the studied LGN cells), 6–36 ganglion cells of different types converged from one eye. In contrast to the relay-mode clusters, ganglion cell types found in combination-mode clusters were not restricted to specific retinal strata and were not dominated by any cell type. In the binocular mode (40% of the studied LGN cells), 4–91 ganglion cells of 2–9 different types converged from both eyes. Within the binocular mode, ipsilateral clusters contained more ganglion cell types and showed no dominance for any particular cell type. The inputs converging from both eyes were more similar than would be expected by chance, suggesting that the two eyes provide different information to each binocular LGN cell.

We found the number of ganglion cells projecting to single LGN cells to be higher than previously estimated by electric stimulation (Chen and Regher, 2000; Cleland et al., 1971; Mastroarande, 1992; Ziburkus and Guido, 2006). This higher convergence is consistent with ultrastructural studies in mice (Hammer et al., 2015; Morgan et al., 2016) and with physiological studies in cats, which considered it based on the low synchrony across Y-type LGN cells (Alonso et al., 1996; Yeh et al., 2009). The previous lower estimates of convergence could have resulted from limitations to detect weaker synapses or to stimulate every presynaptic ganglion cell axon individually.

There are also limitations to our study. First, we only studied the LGN of mice. In other animals with higher visual acuity and more pronounced laminar segregation, the proportion of the three modes could be different. Second, single-cell electroporation was done only in the binocular region of the LGN. The percentage of binocular clusters is expected to vary rostro-caudally in mouse LGN (Howarth et al., 2014) and to be layer dependent in animals with a more laminated LGN (Zeater et al., 2015).

To label LGN principal cells, we injected AAV into the cortex broadly. Therefore, rabies tracing was initiated from LGN cells projecting to all cortical layers (we expect predominantly to layer 4), yielding an overall estimate for the distribution of ganglion cell

(D) A representative relay-mode cluster.
(E) Reconstruction of the ganglion cells in (D).
(F) Dendritic stratification of the ganglion cells in (D). Each column refers to one presynaptic ganglion cell. Number on black boxes refers to the corresponding ganglion cell type.
(G) The distribution of ganglion cells in relay-mode clusters based on dendritic stratification. Strata 1 and 2, OFF-sustained; 3 and 4, OFF-transient; 6 and 7, ON-transient; 8 and 9, ON-sustained responses.
(H) A representative combination-mode cluster.
(I) Reconstruction of the ganglion cells in (G).
(J) Dendritic stratification of the ganglion cells in (H).
(K) The distribution of ganglion cells in combination-mode clusters based on dendritic stratification. Cells were counted in all layers in which they stratify (G and K). See Figures S5–S6.
Figure 4. Binocular Clusters of Presynaptic Ganglion Cells
(A) Representative binocular clusters of an LGN cell.
(B) Dendritic stratification patterns of the ganglion cells in (A).
(C) The distribution of ganglion cells in binocular clusters based on dendritic stratification; gray, ipsilateral; white, contralateral clusters. Cells were counted in all layers in which they stratify.
(D) Pairwise comparison of ganglion cell type dominance between pairs of ipsilateral and contralateral clusters.
(E) Comparison between the expected (black bars) and observed (red line) distribution of the mean Z score of absolute differences in ganglion cell count (ipsilateral – contralateral). Expected distribution based on binomial model.

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inputs. For specialized types of LGN cells, specific projection rules may apply; for instance, LGN cells projecting to layers 1 and 2 have been reported to display relay-mode integration from direction-selective retinal ganglion cells (Cruz-Martín et al., 2014). The projection rules from retinal ganglion cells of different types to LGN principal cells at different depths and, respectively, the projections from these cells to distinct cortical layers are under intense investigation (Cruz-Martín et al., 2014; Hagihara et al., 2015; Sun et al., 2016).

In olfaction, relay-like circuits mediate innate olfactory behavior, while circuits that combine olfactory features are the substrates for learned odor behavior (Sosulski et al., 2011; Stettler and Axel, 2009). Relay- and combination-mode LGN cells could potentially also be components of neuronal circuits with different behavioral relevance. The convergence of different ganglion cell types we found in the monocular and binocular combination modes suggests that the visual thalamus not only relays but also combines different retinal channels to generate new visual channels. Since some retinogeniculate inputs could be more potent drivers of LGN activity than others (Cleland et al., 1971; Hamos et al., 1987; Hong et al., 2014; Mastronarde, 1992; Morgan et al., 2016; Usrey et al., 1999), the inputs from different ganglion cell types might be weighted differently to elicit activity of an individual LGN cell. Furthermore, this weighting could be influenced by long-range inputs from a variety of brain regions that project to the LGN. Why and how individual LGN cells functionally integrate converging visual channels for each mode, and how integration may change in different behavioral states, remain open and intriguing questions.

**STAR METHODS**

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**SUPPLEMENTAL INFORMATION**

Supplemental Information includes eight figures and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2017.01.028.

**AUTHOR CONTRIBUTIONS**

S.B.R. developed and performed transsynaptic tracing, analyzed data, and wrote the text. F.E.M. conceived and performed probabilistic modeling, analyzed data, and wrote the text. A.W. helped with tracing. C.Z. and C.N.R. assisted with experiments. K.Y. made two plasmid constructs. B.R. analyzed data and wrote the text.

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**REFERENCES**


(F and G) Pairwise comparison of (F) number of ganglion cell types, and (G) Z scores of the number of ganglion cell types between pairs of ipsilateral and contralateral clusters. Red, c > i, more cells in the contralateral eye; black, i > c, more cells in ipsilateral eye. Blue lines in (G), mean Z scores. Lines connect corresponding cluster pairs (D, F, and G).

(H) Pairwise comparison of the measured and expected number of ganglion cell types shared between an ipsilateral and corresponding contralateral cluster. Expected values are based on the random draw hypothesis with conditional probabilities to correct for reduced numbers of ganglion cell types ipsilaterally (Figure S8D).

*p values from Wilcoxon signed-rank test in (D) and (F) and Monte-Carlo simulations in (E) and (G) (two-sided) and (H) (left-tailed), *p < 0.05, **p < 0.01, ***p < 0.001; n.s., not significant. See Figures S7 and S8.


# STAR METHODS

## KEY RESOURCES TABLE

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<td>Cat# 67527</td>
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<tr>
<td>Plasmid: pAAV-EF1a-double floxed-hChR2(H134R)-EYFP-WPRE-hGHpA</td>
<td>Laboratory of Karl Deisseroth</td>
<td>N/A</td>
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<td><strong>Sequence-Based Reagents</strong></td>
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<td>CVS-11 glycoprotein mRNA sequence</td>
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<td>Forward primer for AAV titer quantification: GGCTGTGGGGCAGCAGACAA</td>
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<td>Reverse primer for AAV titer quantification: CCAAGGAAAGGACGATGATTTC</td>
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(Continued on next page)
CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Botond Roska, upon signing an MTA (botond.roska@fmi.ch).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Animals

Animals were used in accordance to standard ethical guidelines as stated in the European Communities Guidelines on the Care and Use of Laboratory Animals, 86/609/EEC. Experiments were approved by the Veterinary Department of the Canton of Basel-Stadt. Animals were C57BL/6J wild-type, 3-6 weeks old female mice (Charles River), maintained on a 12 hr light/dark cycle, and fed with irradiated food (KLIBA NAFAG irradiated rodent breeding diet 3302.PM.V20, Provimi Kliba AG) and autoclaved chlorinated tap water. Mice were kept in Individually Ventilated Cages (Blue Line IVCs cages, Tecniplast) with bedding (Lignocel BK8-15, Rettenmaier & Söhne GmbH & Co KG) and nesting material (Plexx). Health monitoring was done according to FELASA Guideline 2014.

METHOD DETAILS

Plasmids

We used the following two plasmids to produce AAVs. pAAV-CMV-hGHintron-GFP-Cre-WPRE-SV40 was used to express Cre recombinase fused to GFP and was purchased from Penn Vector Core. To obtain pAAV-EF1a-DIO-CVS11-G-WPRE-hGhpA, we linearized pAAV-EF1a-double floxed-hChR2(H134R)-EYFP-WPRE-hGhpA (kindly provided by K. Deisseroth, Stanford University) using Nhel/AcsI sites. A CVS11G insert with Nhel/AcsI overhang sites was synthesized (GenScript) based on rabies virus strain CVS-11 glycoprotein mRNA sequence (GenBank EU126641.1) and cut using Nhel/AcsI. The linearized vector and the insert were ligated to yield pAAV-EF1a-double floxed-CVS11G-WPRE-hGhpA.

We electroporated three plasmids into single cells. pAAV-EF1a-CVS11-G-WPRE-hGhpA expressed the rabies glycoprotein G and was described previously (Wertz et al., 2015). pAAV-EF1a-tdTomato-WPRE-hGhpA expressed the red fluorescent marker tdTomato.
and was described previously (Wertz et al., 2015). pAAV-EF1a-DIO-TVA-WPRE-hGHpA expressed the avian TVA receptor in the presence of Cre recombinase. To obtain the plasmid pAAV-EF1a-DIO-TVA-WPRE-hGHpA, we linearized pAAV-EF1a-double flexed-hChR2(H134R)-EYFP-WPRE-hGHpA (kindly provided by K. Deisseroth, Stanford University) using NheI/AseI sites. A TVA insert was PCR amplified using pCMMP-TVA800 (kindly provided by E. Callaway, Salk Institute; Addgene plasmid # 15778) as a template, and recombined into the linearized vector using the In-Fusion 2.0 PCR Cloning Kit (TAKaRA).

**AAV production and titration**

HEK293T cells were transfected with an AAV inser plasmid, an AAV-helper plasmid encoding the AAV proteins Rep2 and Cap for serotype (serotype 7), and another helper plasmid harboring adeno viral genes (kindly provided by C. Cepko, Harvard Medical School, Boston, MA, USA) using branched polyethylenimine (PEI, no.23966, Polysciences). For transfection, 10 cell culture dishes 15 cm in diameter were transfected at 80% confluence of HEK293T cells. A stock solution was made for each transfection that included 200 µg of the AAV-helper plasmid harboring adeno viral genes, 70 µm of the AAV-helper plasmid encoding Rep2 and Cap, and 70 µg of the AAV inser plasmid, 48 mL of DMEM and 1360 µL of polyethylenimine, with the stock solution incubated at room temperature for 15 min before 5 mL being added to each dish. 60 hr after transfection, cells were released from the plate by vigorous pipetting and re-suspended in lysis buffer (150 mM NaCl, 20 mM tris pH 8.0). The AAV stock was then freeze-thawed three times between dry ice/ethanol bath and a 37°C water bath and MgCl2 was added to a final concentration of 1mM. Nuclease (Nucleoburclease, no. 50310, BPS Bioscience) was then added to a final concentration of 250 U/ml and incubated at 37°C for 10 min. Cell debris was removed by centrifugation at 4,000 rpm for 20 min. Viral stocks were then purified using a discontinuous iodixanol gradient (Opti Prep, D1556, Sigma). This gradient was then added to a final concentration of 4 layers: the first layer is composed of 50 mL 10 × PBS, 0.5 mL 1 M MgCl2, 0.5 mL 2.5 M KCl, 100 mL 5 M NaCl, 125 mL Optiprep (iodixanol) and H2O to 500 ml, the second layer is composed of 50 mL 10 × PBS, 0.5 mL 1 M MgCl2, 0.5 mL 2.5 M KCl, 200 mL Optiprep, 1 mL of 0.5% (wt/vol) phenol red and H2O to 500 ml, the third layer is composed of 50 mL 10 × PBS, 0.5 mL 1 M MgCl2, 0.5 mL 2.5 M KCl, 333 mL Optiprep and H2O to 500 ml, and the forth layer is composed of 500 mL Optiprep, 0.25 mL 0.5% phenol red, 0.5 mL 1 M MgCl2 and 0.5 mL 2.5 M KCl. The viral fraction was extracted from the third layer after ultracentrifugation for 90 min at 242,000 g. AAVs were then purified three times in Millipore Amicon 100K columns (Amicon, UFC910008, Millipore) and resuspended in a final volume of 200 µL PBS and frozen at –80°C in aliquots of 20 µL. Encapsidated viral DNA was quantified by TaqMan RT-PCR (forward primer: GCCGCTTGGGGACTGCACA; reverse primer: CCAAAGAAAAGGACGATGATTTC; probe: TCCGTGGTGTTGTCG) following denaturation of the AAV particles by Proteinase K, and titers were calculated as genome copies (GC) per ml. These procedures were used to produce two different AAVs: AAV-CMV-hGHintron-GFP-Cre-WPRE-SV40 (titer: 1.28E+12 GC/mL, serotype 7) made from the plasmid pAAV-CMV-hGHintron-GFP-Cre-WPRE-SV40, AAV-EF1a-DIO-CVS11-G-WPRE-hGHpA (titer: 1.70E+14 GC/mL, serotype 7) made from the plasmid pAAV-EF1a-DIO-CVS11-G-WPRE-hGHpA.

**Rabies virus production**

Rabies production was similar to previous methods (Osakada and Callaway, 2013; Wickersham et al., 2010) and identical to a recent method (Wertz et al., 2015). The G-deleted, G-coated SADΔG-mCherry rabies virus was amplified in B7GG cells, which express the rabies glycoprotein G of the B19 strain of rabies. This amplification was done by infecting 5 10-cm plates of 80% confluent B7GG cells with 3 multiplicity of infection (MOI) of G-deleted, G-coated SADΔG-mCherry rabies virus and incubated for 6 hr. After infection, cells were trypsinized for 30 min and transferred to 25 10-cm plates. Every 3 days after splitting, supernatant was collected, filtered and stored at 4°C for 10 min. Cell debris was removed by centrifugation at 4,000 rpm for 20 min. Viral stocks were then purified using a discontinuous iodixanol gradient (Opti Prep, D1556, Sigma). This gradient was then added to a final concentration of 4 layers: the first layer is composed of 50 mL 10 × PBS, 0.5 mL 1 M MgCl2, 0.5 mL 2.5 M KCl, 100 mL 5 M NaCl, 125 mL Optiprep (iodixanol) and H2O to 500 ml, the second layer is composed of 50 mL 10 × PBS, 0.5 mL 1 M MgCl2, 0.5 mL 2.5 M KCl, 200 mL Optiprep, 1 mL of 0.5% (wt/vol) phenol red and H2O to 500 ml, the third layer is composed of 50 mL 10 × PBS, 0.5 mL 1 M MgCl2, 0.5 mL 2.5 M KCl, 333 mL Optiprep and H2O to 500 ml, and the forth layer is composed of 500 mL Optiprep, 0.25 mL 0.5% phenol red, 0.5 mL 1 M MgCl2 and 0.5 mL 2.5 M KCl. The viral fraction was extracted from the third layer after ultracentrifugation for 90 min at 242,000 g. AAVs were then purified three times in Millipore Amicon 100K columns (Amicon, UFC910008, Millipore) and resuspended in a final volume of 200 µL PBS and frozen at –80°C in aliquots of 20 µL. Encapsidated viral DNA was quantified by TaqMan RT-PCR (forward primer: GCCGCTTGGGGACTGCACA; reverse primer: CCAAAGAAAAGGACGATGATTTC; probe: TCCGTGGTGTTGTCG) following denaturation of the AAV particles by Proteinase K, and titers were calculated as genome copies (GC) per ml. These procedures were used to produce two different AAVs: AAV-CMV-hGHintron-GFP-Cre-WPRE-SV40 (titer: 1.28E+12 GC/mL, serotype 7) made from the plasmid pAAV-CMV-hGHintron-GFP-Cre-WPRE-SV40, AAV-EF1a-DIO-CVS11-G-WPRE-hGHpA (titer: 1.70E+14 GC/mL, serotype 7) made from the plasmid pAAV-EF1a-DIO-CVS11-G-WPRE-hGHpA.

**Cortical AAV infection**

Injections to V1 were performed in mice anesthetized with FMM (fentanyl 0.05 mg/kg, medetomidine 0.5 mg/kg, midazolam 5.0 mg/kg). Coliquifilm (S01XA20, Allergan) was applied to the eyes to prevent dehydration. Three 0.4 mm diameter holes were drilled above V1 at the following coordinates: first hole 3 mm lateral and 4.2 mm posterior from bregma, second hole 2 mm lateral and 4.2 mm posterior from bregma, third hole 2.5 mm lateral and 3.2 mm posterior from bregma. 1 µL of AAV-CMV-hGHintron-GFP-Cre-WPRE-SV40 was front-loaded into a glass needle (Warner Instruments, Model G100-4, resistance 1-3 MΩ) and one third of the volume was injected into each of the holes via an oil injector (IM-9B, Narashige).

**Targeting the same retinotopic location in the LGN in each mouse**

Mice were injected with 1 µL of the anterograde tracer CTB-Alexa 555 (C-34776, Invitrogen, dissolved in PBS to a final concentration of 1 µg/ µL) into the contralateral (right) eye and 1 µL of CTB-Alexa 488 (C-34775, Invitrogen, 1 µg/ µL in PBS) into the ipsilateral (left) eye. This injection resulted in a large region of the left LGN labeled with axons of the contralateral ganglion cells and a smaller region with axons of the ipsilateral ganglion cells (Figures S1C–S1E). In top view, the overlap between the ipsi- and contralateral axons...
defined a specific area of the LGN. A defined point in this area could be targeted for electroporation even without the guidance of CTB injection since it could be consistently determined in each animal by anatomical landmarks (Figure S1F).

Targeted single cell electroporation and rabies virus injection

Mice were kept under anesthesia as described above. A craniotomy was made on the left hemisphere of the mouse skull. The cortex and hippocampus above the LGN were aspirated to produce an opening of 2.5 mm in diameter centered on the LGN. This opening allowed imaging and access for the electroporation pipette. The opening above the LGN was filled with Ringer’s solution (150 mM NaCl, 2.5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 10 mM HEPES in ddH₂O, 0.2 µm sterile filtered). The animal was then placed under a two-photon microscope, which was equipped with two detection channels, one for GFP and one for Alexa-594 (Farrow et al., 2013) as well as with a red LED light source (630 nm Red LED Array Light Source, LiU630A, Thorlabs). The surface of the LGN was centered under a 4x objective (MPlan N 5x/.1NA, Olympus) using the red LED light and a defined retinotopic location was identified for electroporation using local landmarks as described (Figure S1F). Electroporation solution containing 40 µL intracellular solution (130 mM K-methanesulphonate, 10 mM HEPES, 7 mM KCl, 2 mM Na₂-ATP, 2 mM Mg-ATP, 0.05 mM EGTA in ddH₂O, 710 mOsm, pH 7.2), 1.5 µL pAAV-ef1a-DIO-TVA-WPRE-hGHPA (4 µg/µl, dissolved in ddH₂O), 1.5 µL pAAV-ef1a-CVS11-G-WPRE-hGHPA (2.6 µg/µl dissolved in ddH₂O), 1.5 µL pAAV-ef1a-ttdTomato-WPRE-hGHPA (4.5 µg/µl dissolved in ddH₂O), and 2.5 µL Alexa-594 (1 mM in intracellular solution, A-10438, Thermo Fisher) was made and filtered (0.2 µm Ultrafree-MC GV Centrifugal Filter, UFC30GVOS, Millipore). The electroporation solution was loaded into a glass needle (Standard Wall Borosilicate Tubing with Filament, BF100-50-10, Sutter Instruments, resistance 12-30 MΩ) and placed onto a needle holder connected to the head stage of an electroporation device (Axoporator, 800A, Molecular Devices) mounted on a manipulator (MPC 200, Sutter Instruments). With 100 mbar positive pressure, the pipette was lowered to the surface of the LGN using visual control under the red LED illumination. Once at the surface of the LGN, the 4x objective was switched to a 40x objective (LUMPlanFl 40x/0.8NA Water immersion, Olympus) and the tip of the pipette containing Alexa-594 dye was located using epifluorescence light. Next, epifluorescence imaging was switched to two-photon imaging of GFP and Alexa-594. A GFP-positive cell near the needle was selected and electroporated using the following settings: voltage = −10 V, DC offset = 0, train = 1 s, frequency = 100 Hz, pulse width 50 µs. Then the electroporation pipette was withdrawn. A glass needle (Premium Standard Wall Borosilicate, Model G100-4, Warner Instruments, resistance 1-3 MΩ) was cut at the tip and front-loaded with 1 µL of Envelope-A-coated SADΔG-mCherry rabies virus. In order to minimize the dilution of rabies virus Ringer’s media from the LGN surface was removed with a pipette. The rabies virus was then injected into the LGN within 100 µm from the electroporated cell. After 80% of the virus was injected, we waited 10 min to allow virus diffusion within the tissue before retracting the pipette. Finally, the surgical window was filled with KWIK-CAST (World Precision Instruments) solution, allowed to solidify, then closed with super glue gel and dental cement. The mouse was placed in a heated cage to recover. 9-12 days later, the mouse was sacrificed to remove its retinas.

Bulk rabies tracing

Mice were injected with 1 µL of a 1:1 mixture of two AAV’s: AAV-CMV-hGHintron-GFP-Cre-WPRE-SV40 and AAV-ef1a-DIO-CVS11-G-WPRE-pGHPA into V1, as described above. 10 days later, the V1 of the same mice were injected with 1 µL G-coated SADΔG-mCherry rabies virus. After 10 days rabies infection, mouse retinas were removed.

Immunohistochemistry

After euthanasia, eyes were removed and dissected in PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na₂HPO₄, 1.47 mM KH₂PO₄, pH 7.4). The retinas were fixed in 4% PFA for 30 min to 2 hr. The retinas were transferred into wells of a 24-well plate filled with PBS and washed 3 times by moving the retinas from one well to the next. After washing, retinas were transferred to wells containing 30% sucrose in PBS (w/v) and allowed to sink for 30 min to overnight. Retinas were frozen in fresh 30% sucrose in PBS in 1.5 mL eppendorf tubes by placing the tubes in dry ice. The tubes were thawed in a 37°C heat block. The freeze-thaw cycle was repeated two additional times. Retinas were washed 3 more times in PBS then incubated with heavy blocking solution (10% NDS, 1% BSA, 0.1% triton, 0.02% Sodium Azide in PBS) for 2 hr to overnight before being incubated with primary antibodies for 7 days shaking at room temperature. Primary antibodies used were rabbit anti-RFP (600-401-379, Rockland, 1:1000 v/v) and goat anti-ChAT (ab144P, Millipore, 1:200 v/v) with antibody being diluted in light block solution (heavy block solution described above diluted 1:2 in PBS). Retinas were washed three additional times in PBS before being transferred into the secondary antibody solution for 2 hr at room temperature. Secondary antibodies were Alexa 568 donkey anti-rabbit (A10042, Life Technologies, 1:500 v/v) and Alexa 633 donkey anti-goat (A-21082, Life Technologies, 1:200 v/v) in light block solution. Nuclei were stained with Hoechst 33342 (H1399, Thermo Fisher, 1:1000 v/v) together with the secondary antibody. Slices were washed three more times in PBS before being mounted on slides with ProLong Gold (P36934, Thermo Fisher), a zero thickness coverslip placed on the samples, kept from compressing the retinas with slivers of Paraflim (52858-000, VWR) being placed next to the retinas, which were allowed to cure overnight at room temperature in the dark before being stored at 4°C in the dark. Using the finding that the mouse retina has a dorso-ventral gradient of ultraviolet (UV) cone opsin expression, the ventral retina was identified by staining with rabbit anti-UV cone opsin primary antibody (NG1919862, Millipore, 1:200 v/v) and Alexa 488 donkey anti-rabbit secondary antibody (R37118, Thermo Fisher, 1:500 v/v) (Wikler et al., 1996).
Confocal microscopy

Confocal microscopy was performed on a Zeiss LSM 720. Overview images of the retina and brain were obtained with a 10x (Plan-APoCHROMAT 10x/0.45 NA, Zeiss) or 20x air objective (Plan-APoCHROMAT 20x/0.8 NA, Zeiss). Confocal images for manual tracing and direct inspection were taken with either a 40x (Plan-APoCHROMAT 40x/1.3 NA oil immersion, Zeiss) or 63x (Plan-APoCHROMAT 63x/1.4 NA oil immersion, Zeiss) objective.

Experimental design

Inclusion and exclusion criteria of any data or subjects: we did not exclude data.

QUANTIFICATION AND STATISTICAL ANALYSIS

Tracing the morphology of individual ganglion cells in a cluster

The morphology of each neuron in a cluster was traced using an approach originally developed for tracing neurons in 3D electron microscopy. Briefly, professional image annotators performed skeleton tracing in confocal image stacks using the tracing software PyKNOSSOS (Ariadne-service). First, confocal image stacks containing all ganglion cells in a given cluster were divided into cubes for dynamic data loading. Second, ganglion cell bodies were manually labeled as starting points for skeleton tracing. Third, image annotators traced skeletons from each starting point. Fourth, based on three independent tracings, a consolidated reconstruction was generated for each neuron. Fifth, to generate a final reconstruction, each mismatch point was retraced locally by additional annotators. Sixth, the final reconstruction was loaded into Imaris (Bitplane) and overlaid onto the original ChAT and Hoechst staining using the MatlabXL plugin and the Filament Tracer module. To analyze the morphology and dendritic stratification of single ganglion cells of a cluster, reconstructions of single ganglion cells were loaded as individual channels.

Classification of ganglion cell type by morphology

All presynaptic ganglion cell clusters together consisted of 507 ganglion cells. For sparse clusters consisting of ≤ 20 cells, 90% of cells could be classified, for dense clusters with 20–88 cells, 45% of cells could be classified. The morphological type of individually traced ganglion cells was determined based on two parameters (with the exception of type 5): dendritic stratification and dendrite diameter, which were quantified in high-resolution confocal stacks of individually traced cells (see above) with the “Slice and Sur-pass” modules of Imaris (Bitplane). First, ganglion cells were grouped based on dendritic stratification. Second, for each group we determined the distribution of dendritic diameter. Some of the groups had dendritic size distributions with a larger spread compared to the spread of the dendritic size distributions of previously defined, morphologically similar ganglion cell types in the same stratum (PV1-PV8 ganglion cell types, which were classified based on their genetic, morphological and physiological properties; Figure S1 of Farrow et al., 2013). For these groups (strata 4, strata 8,9 and strata 1,8,9) we used prior knowledge about the dendritic diameter of PV cells as starting point for our classification. We classified cells having dendrites in a given stratum as two cell types if the nonparametric Siegel–Tukey test rejected the hypothesis that their dendritic size has the same variability as that of the PV ganglion cell type that projects its dendrites to the same stratum (in addition, we confirmed the inequality of the distributions using the Kolmogorov-Smirnov test). If the Siegel–Tukey test rejected the null hypothesis, we split the cells into two types based on the procedure as follows. We fitted the distribution with two Gaussian distributions. According to the optimal fit, the group was divided into two subgroups. Specifically, first, we took the rabies-traced ganglion cells that have dendrites in stratum 4 (Figure S2A). This is the same stratum where PV5 cells project their dendrites. The null hypothesis that the variability of the dendritic sizes of the rabies traced cells is the same as the variability of the dendritic sizes of PV5 cells was rejected by the Siegel–Tukey test (p = 0.002, also note Kolmogorov-Smirnov test: p = 0.0003). Therefore, we split the rabies-traced cells in stratum 4 to Types 3 and 4. Second, we took the rabies-traced ganglion cells that have dendrites in strata 8,9 (Figure S2B). This is the same stratum where PV1 cells project their dendrites. The null hypothesis that the variability of the dendritic sizes of the rabies traced cells is the same as the variability of the dendritic sizes of PV1 cells was rejected by the Siegel–Tukey test (p = 0.00009, also note Kolmogorov-Smirnov test: p = 0.0003). Therefore we split the rabies-traced cells in strata 8,9 to Types 7 and 8. Third, since in the previous study (Farrow et al., 2013) we did not characterize any ganglion cell types with dendrites in strata 1,8,9, we tested the variance of the dendritic size of these cells against PV1 cells which stratify in layer 8,9 and also against PV6 cells which stratify in layer 1 (Figure S2C). The Siegel–Tukey test (p = 0.01 against PV6, p = 0.004 against PV1, also note Kolmogorov-Smirnov test: p = 0.01 against PV6, p = 0.05 against PV1) rejected the null hypothesis and therefore we split the rabies-traced cells in strata 1,8,9 to Types 10 and 11. One exception to this identification scheme based on dendritic diameter is ganglion cell type 5, which contained unique dendritic varicosities that were absent in the other two ganglion cell types that project to the same stratum, and therefore was classified as its own morphological cell type (Figures S2G and S2H). The JAM-B cell type was observed in the bulk rabies tracing in the dorsal retina, where it has a characteristic asymmetric dendritic field (Kim et al., 2008). In the ventral retina however, where single-cell initiated clusters were exclusively located, JAM-B cells have a symmetric dendritic field (Kim et al., 2008) and we could not distinguish them from other ganglion cells projecting to layers 1 and 2.

Classification of integration modes

LGN cells were classified as “relay mode” if they received monocular input and if the presynaptic ganglion cell cluster contained cells of a single morphological cell type, or cells of one cell type plus a single outlier cell that shared dendritic strata with the predominant
cell type. LGN cells were classified as “combination mode” if they received monocular input and did not fulfill the criterion for relay mode. LGN cells were classified as “binocular” if they received inputs from both eyes. Although we cannot exclude the possibility that our transsynaptic labeling might have missed a subset of presynaptic ganglion cells (in which case 28% relay-mode and 60% monocular clusters would be upper estimates), our results clearly show that most LGN cells receive inputs from diverse retinal channels. It is important to note that monocular clusters showed a significantly increased number of exclusively inner- and outermost stratifying clusters (n = 8/15) compared to a random draw from the contralateral cell-types found in binocular clusters (p = 0.003, Monte-Carlo simulation), confirming that monocular clusters are not a random subsample of binocular clusters, but display distinct modes of integration.

Unclassified cells
In especially dense clusters, not all presynaptic ganglion cells could be classified. When comparing the data to the random expectation, this was automatically corrected for by simulating cell-type numbers based on classified cells only. When comparing number of ganglion cell types between eyes (Figures 4F, S3B, and S3D), the number of ganglion cell types was corrected for unclassified cells by multiplying the measured number of ganglion cell types (Gmeasured) with the expected number of ganglion cell types (Exp[G]), given the total count of classified and unclassified cells (Exp[G | Ntotal]), divided by the expected number of ganglion cell types given the number of classified cells (Exp[G | Nclassified]). The number of ganglion cell types corrected for unclassified cells was therefore: 

$$G_{corrected} = G_{measured} \cdot \frac{\text{Exp}[G | N_{total}]}{\text{Exp}[G | N_{classified}]} \cdot \frac{\text{Exp}[G | N_{total}]}{\text{Exp}[G | N_{classified}]}.$$ 

Expected values were calculated based on the random-draw hypothesis. Similar results were obtained if unclassified cells were not accounted for.

Probabilistic modeling
The data were analyzed with respect to the null hypothesis that presynaptic ganglion cells were randomly drawn from a pool of ganglion cells with a given distribution of cell types. The number of cell types expected from a random draw increases monotonously with the total number of cells (Figure S4A). Distributions were either calculated exactly, or estimated by Monte-Carlo simulations with sufficient sampling size (as verified by repetitions or comparison to exact calculations). Assuming a large pool of potential presynaptic partners, we modeled the random draw as a multinomial distribution (draw with replacement; black, magenta, blue in Figure S4A), which provides a lower-bound for the respective hypergeometric distributions (draw without replacement; gray, green in Figure S4A for a pool size of 100). Ganglion cell types that were not found during bulk tracing were assigned 1 or 0.001 (black or magenta in Figure S4A). All Monte-Carlo simulations reported in the paper are based on the most conservative distribution (black in Figure S4A), i.e., a multinomial model with cell-type distribution as determined by bulk tracing, and missing cell-types accounted for as 0.001 cells. In addition, we verified that under the alternative model assumptions (magenta, gray, blue in Figure S4A) similar results were obtained for all analyses.

To test against the null-hypothesis that presynaptic ganglion cells in binocular clusters were randomly distributed across the contra- and ipsilateral eye, a binomial model was applied with p = 0.73, matching the median fraction of contralateral cells over total cell count in binocular clusters. An asymmetric test-statistic (|ipsilateral – contralateral |, the absolute difference between contra- and ipsilateral cell counts) was chosen in order to reveal a potential asymmetry in the distribution of cells across the two eyes. To normalize differences prior to averaging, z-scores were calculated based on the expected mean and s.d. of |ipsilateral – contralateral |, given the binomial model.

Deviations from random expectation are reported as differences of measured minus expected values or, respectively, as z-scores, defined as the measured minus expected value, divided by the expected standard deviation. Please note that data points for which the expected standard deviation becomes zero (e.g., for the distribution of cell type dominance, conditioned on one cell-type), z-scores become NaN (“Not A Number”) even though measured and expected value are equal.

Analyses and simulations were performed with MATLAB (MathWorks).

Statistics
Unless noted otherwise, p values were derived from symmetric confidence intervals of the Monte-Carlo simulated distributions of the given test-statistics (e.g., the mean z-score). Non-parametric tests (Mann-Whitney U, Wilcoxon signed-rank) were applied for all group comparisons. Two-sided p values are provided, with the exception of Figure 4H where we test against the null hypothesis that ipsi and contralateral eyes share more cell types than expected by chance. Pearson correlation coefficients are provided for linear relationships as noted, Spearman’s rank correlation coefficients are provided otherwise. Unless noted “n” refers to the number of ganglion cell clusters.