Topographic axonal projection at single-cell precision supports local retinotopy in the mouse superior colliculus

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Abbreviated title: retinotopic RGC axon tiling in SC.

Key words: retinal ganglion cells; retinotopy; axonal imaging; superior colliculus; mouse.

Acronyms: 2D, two-dimensional; AAV, adeno-associated virus; CNMF, constrained non-negative matrix factorization; RF, receptive field; RGC, retinal ganglion cells; SC, superior colliculus; SD, standard deviation.

Abstract

Retinotopy, like all long-range projections, can arise from the axons themselves or their targets, but the underlying connectivity pattern remains unknown at the fine scale. To address this question, we functionally mapped the spatial organization of the input axons and target neurons in the mouse retinocollicular pathway at single-cell resolution using *in vivo* two-photon calcium imaging. We found a near-perfect retinotopic tiling of retinal ganglion cell axon terminals, with an average error below 30 μm or 2 degrees of visual angle. The precision of retinotopy was relatively lower for local neurons in the superior colliculus. Subsequent data-driven modelling ascribed it to a low input convergence, on average 5.5 retinal ganglion cell inputs to a postsynaptic cell in the superior colliculus. These results indicate that retinotopy arises largely from topographically precise input from presynaptic cells, rather than elaborating local circuitry to reconstruct the topography by postsynaptic cells.

Introduction

Topographic organization is central to brain function (Jbabdi et al., 2013; Patel et al., 2014). Connections between brain regions are often spatially arranged to have one-to-one mappings, and sensory processing relies on the transmission of topographically preserved information from receptor organs. In the visual system, for example, topographic visual representations are first formed in the retina and conveyed to the brain by retinal ganglion cells (RGCs) whose axons are bundled into an optic nerve (Masland, 2012). In general, neighboring RGCs project to neighboring regions in their targets (Frisén et al., 1998; McLaughlin et al., 2003; Cang and Feldheim, 2013; Arroyo and Feller, 2016). This forms a retinotopic map in the primary retinorecipient areas, such as the lateral geniculate nucleus (Malpeli and Baker, 1975) and the superior colliculus (SC; Dräger and Hubel, 1976; Cang et al., 2018), and retinotopy is likewise transferred throughout the entire visual pathways (Wandell et al., 2007; Cang and Feldheim, 2013).

How precisely is topographic information transmitted between brain regions? Only global-level relationship is known even for the best characterized cases, such as the retinal projection to SC (Frisén et al., 1998; McLaughlin et al., 2003; Cang and Feldheim, 2013; Arroyo and Feller, 2016). During development, RGC axons reach their target locations based on molecular guidance cues and activity-dependent refinement. Long-range projections of dense axonal fibers, however, preclude a precise anatomical characterization of the connectivity patterns at single-cell resolution (Hong et al., 2011). In the optic nerve, RGC axons are mixed and lose retinotopic organizations (Horton et al., 1979; Colello and Guillery, 1998). A fundamental question is then if RGC axons can nevertheless find precise targets even after they get "lost" in the long-range projection (Figure 1, Model 1), or if target neurons need to reconstruct the topography by elaborating local circuitry (Figure 1, Model 2). A recent study using a high-density electrode has implicated retinotopic RGC projections to the mouse SC along the probe shank (Sibille et al., 2022), favoring the former scenario. However, the precision of retinotopy at the level of individual axons and its relationship to that of target neurons remain elusive in any retinorecipient area.

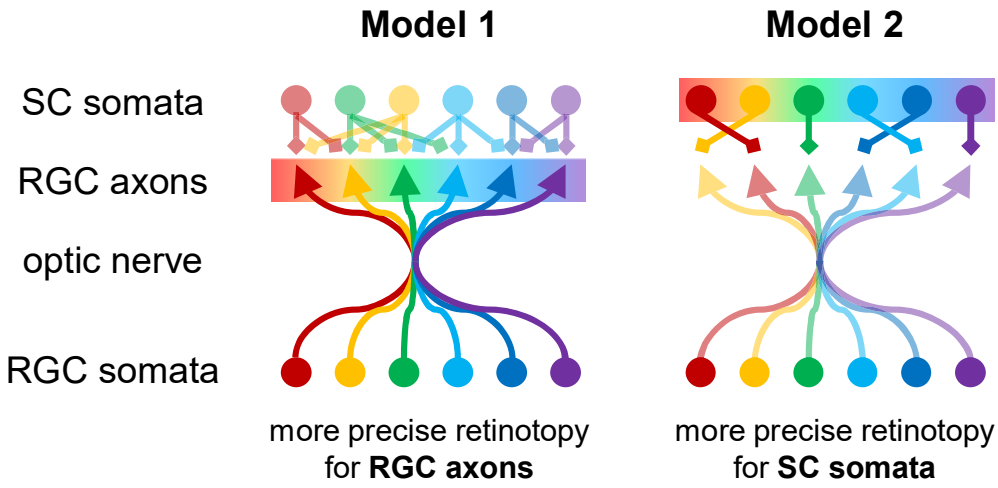


Figure 1: Possible retinocollicular projection models for topographic information transmission. Retinotopy in SC (color-coded) can arise from either precisely retinotopic projection of RGC axons, innervating exact target locations in SC (Model 1); or roughly retinotopic projection of RGC axons while SC neurons identify appropriate partners to recover the retinotopy (Model 2). In Model 1, retinotopy can be more precise for RGC axons than for SC somata if the synaptic connectivity is not topographically well organized. In Model 2, in contrast, SC somata should show more precise retinotopy than RGC axons. Note a loss of retinotopic organization in the optic nerve (Horton et al., 1979; Colello and Guillery, 1998).

To address this question, we performed functional mapping of RGC axon terminals and local neurons in SC at single-cell resolution in awake head-fixed mice using two-photon calcium imaging. Subsequent analyses on their spatial organizations allowed us to quantify and directly compare the precision of retinotopy between the pre- and post-synaptic sides. Moreover, these experimental data served as a basis of a computational modelling analysis for inferring key features on the topographic information transmission in the retinocollicular pathway. Specifically, by comparing the observed and simulated retinotopy patterns, here we addressed 1) to what extent RGC axon terminals are deviated from their retinotopically optimal target locations in SC; and 2) on average how many RGCs connect to individual SC neurons.

Results

For functional mapping of the mouse retinocollicular projections, we expressed axon-targeted calcium indicators (GCaMP6s; Broussard et al., 2018) in RGCs via intravitreal injection of recombinant adeno-associated viruses (AAVs) harboring the pan-neuronal human synapsin (hSyn) promoter, and monitored the visual responses of the labelled RGC axons in SC using *in vivo* two-photon microscopy (Figure 2A,B and Supplemental Movie 1). To segment axonal patches of individual RGCs and isolate their activity, we used constrained non-negative matrix factorization (CNMF) that allows us to extract morphological and temporal features from noisy time-lapse calcium activities based on their spatiotemporal correlations (Giovannucci et al., 2019). For a field of view of ~0.3 mm2 (0.57-by-0.57 mm), we detected 26 ± 9 axonal patches displaying independent activity patterns (median ± median absolute deviation here and thereafter unless otherwise noted; N=37 recording sites in total from 15 animals; e.g., Figure 2C,D). The size of the individual axonal patches (135 ± 25 μm; N=969; Supplemental Figure 1A) is consistent with the past anatomical measurement (Hong et al., 2011), with a substantial overlap between them over the SC surface (49 ± 11 %). This supports a successful signal extraction and a good coverage of RGC axonal labelling. These data allowed us to faithfully

reconstruct the local two-dimensional (2D) map of the individual RGC axon terminals in SC (e.g., Figure 2D).

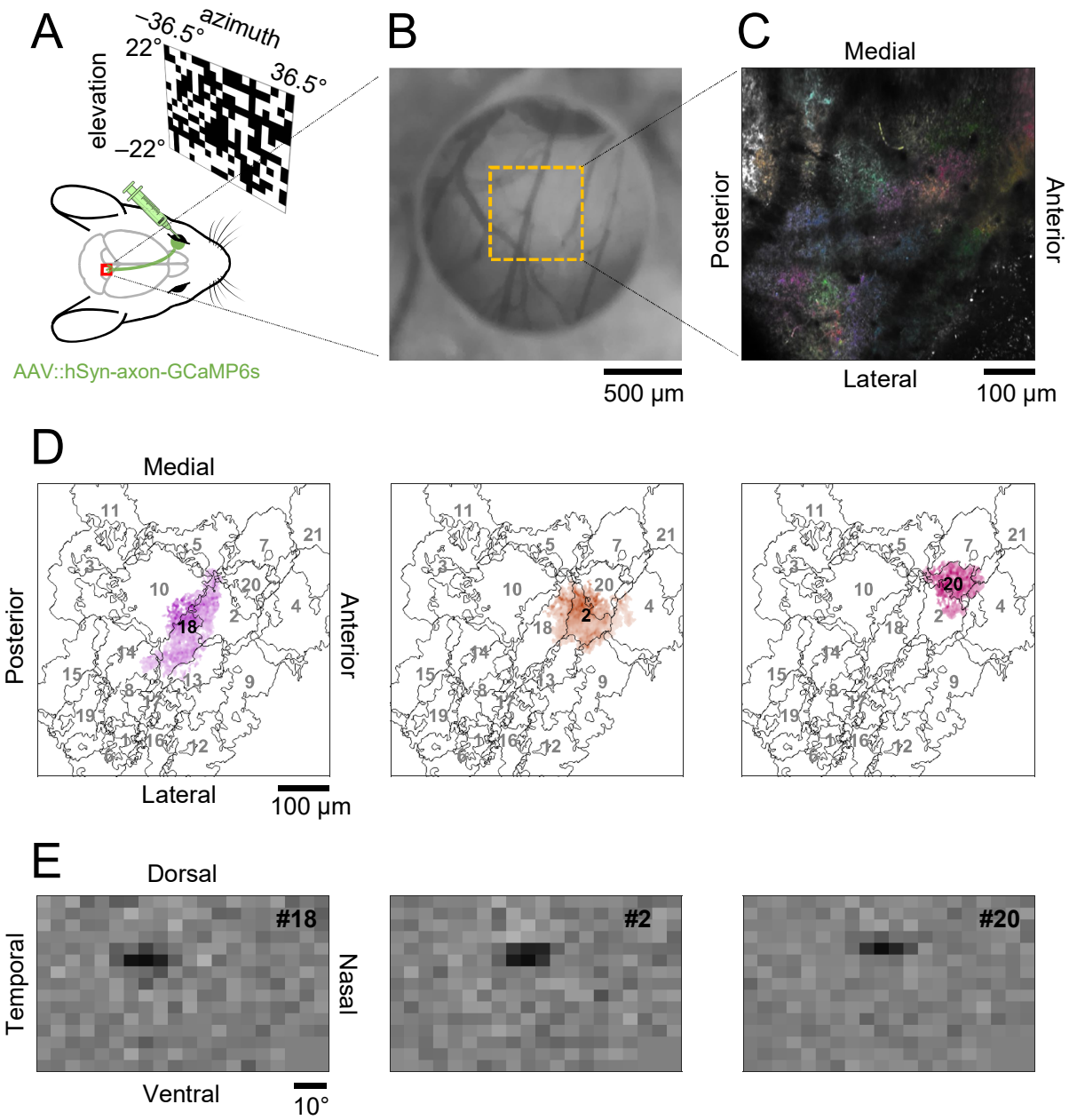
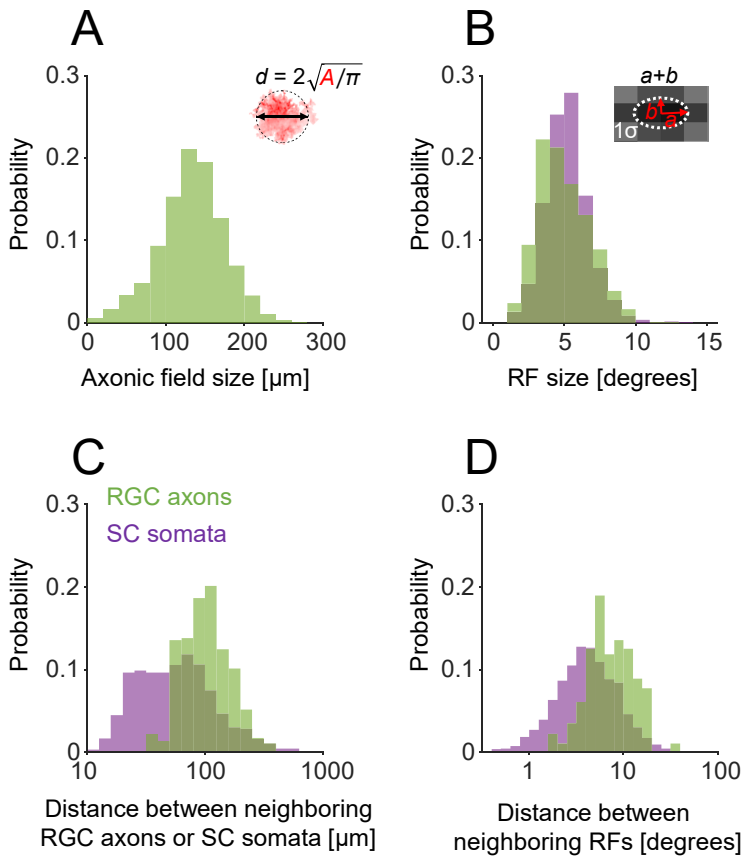
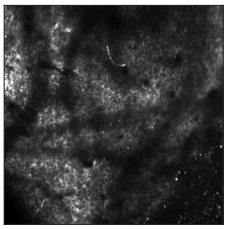


Figure 2: *In vivo* two-photon calcium imaging of retinal ganglion cell axon terminals in the mouse superior colliculus. *A*: Schematic diagram of the experimental set up for RGC axonal imaging in SC. *B*: Representative image of cranial window. Medial-posterior part of SC was clearly visible through a cylindrical silicone plug attached to a glass coverslip. *C*: Average intensity projection of representative axonal imaging data, overlaid with detected RGC axonal patches (N=21; color-coded). See also Supplemental Movie 1. *D*: Footprint of three representative RGC axonal patches (#18, 2, and 20 in distinct color; from left to right, respectively) overlaid with the profile of the rest patches (in grey). *E*: Corresponding RF of the three representative RGC axonal patches (from D), estimated by reverse-correlation analysis.



Supplemental Figure 1: Probability distributions of axonic and receptive field statistics. *A*: Size of the identified RGC axon terminals, d = 2(A/π)0.5, where A is the axonic field area from the CNMF analysis (135 ± 25 μm, median ± median absolute deviation; N=969). *B*: RF size of RGC axons (green; 4.8 ± 1.2 degrees; N=719) and SC somata (purple; 5.1 ± 0.9 degrees; N=1191), measured as the mean of long- and short-diameters (equivalently, the sum of long- and short-radii, a and b, respectively) of the 2D Gaussian profile at 1 σ fitted to the RF (e.g., Figure 2E). *C*: Distance between neighboring RGC axon centers (green; 100 ± 30 μm; N=761 pairs) or SC somata (purple; 56 ± 28 μm; N=2292 pairs). Neighboring pairs are identified by the Delaunay triangulation analysis (e.g., Figure 3C and Supplemental Figure 2F). *D*: Distance between neighboring RF centers of RGC axons (green; 7.2 ± 2.7 degrees; N=776 pairs) or SC somata (purple; 4.1 ± 1.9 degrees; N=2287 pairs). Neighboring pairs are identified by the Delaunay triangulation analysis (e.g., Figure 3D and Supplemental Figure 2G).



Supplemental Movie 1: *In vivo* two-photon calcium imaging of retinal ganglion cell axon terminals in the mouse superior colliculus. A snippet of motion-corrected movie (resampled at 30 Hz; original sampling rate, 15.4 Hz) showing the activity of RGC axons in the mouse SC in response to the random checkerboard stimuli (see Methods for details). See Figure 2 for the segmentation and Figure 3 for the tiling pattern analysis.

Presence of a well-defined receptive field (RF) is a characteristic feature of all RGC types (Masland, 2012; Baden et al., 2016). To map the RF of the identified RGC axons, we computed the response-weighted average of the presented random checkerboard stimuli (frame rate, 4 Hz; rectangular fields, 3.7° in width and 2.9° in height; e.g., Figure 2E) and fitted a 2D Gaussian at the peak latency to characterize the spatial RF profile. Most identified axonal patches had RFs within the stimulation screen (±22° in elevation and ±36.5° in azimuth from the mouse eye; Boissonnet et al., 2022). In accordance with *ex vivo* retinal physiology (Baden et al., 2016), the average RF size of the RGC axons was 4.8 ± 1.2 degrees (N=719; Supplemental Figure 1B), estimated as the mean of the long- and short-axis diameters of the 2D Gaussian profile at 1 standard deviation (SD). There was a weak but statistically significant correlation between the RF size and the RGC axonal patch size (Pearson's r = 0.19, p = 5e-7). The RFs locally tiled the visual field with 10 ± 5 % overlap at 1 SD Gaussian profiles, where the center of every RF occupied a unique location in the visual field. This ensures that these RFs belong to different RGCs because the RF center location of RGC axons should correspond well to the location of their somata in the retina. Hence, the RF tiling faithfully represents retinotopy.

How well does the tiling pattern of RGC axons in SC agree with that of their RFs? As expected from the global retinotopy in SC (Dräger and Hubel, 1976; Cang et al., 2018; Sibille et al., 2022), relative positions of the RGC axonal patches (e.g., Figure 3A) agreed well with those of the corresponding RFs (e.g., Figure 3B) regardless of their cell types. For quantification, we first computed the Delaunay triangulation using the geometric centers of the individual axonal patches or the RF center locations as landmark points in each space (e.g., Figure 3C,D, respectively). This triangulation features the adjacency relationship regardless of their absolute positions, where all the adjacent pairs of the landmark points in a given space are connected as a dual graph of the Voronoi tessellation that separates the space into territories close to each landmark point. The distances between the centers of neighboring RGC axons and those between their RFs were identified to be 100 ± 30 μm (N=761 pairs;

Supplemental Figure 1C) and 7.2 ± 2.7 degrees (N=776 pairs; Supplemental Figure 1D), respectively. As a measure of the agreement between the two tiling patterns, we then calculated the fraction of the common edges between the two Delaunay triangulations (blue edges; 88.2% for the example in Figure 3C,D). Throughout our datasets, we found a near-perfect match between the tiling patterns of RGC axons in SC and their RFs (84 ± 5%; N=36 recordings from 12 animals; Figure 4A) regardless of the recording depth within the superficial SC layer (120-220 µm deep from the surface; Figure 4B). This observation is consistent with the precise axonal projection model (Model 1 in Figure 1) whereby RGC axon terminals retinotopically tile the SC surface at single-cell precision.

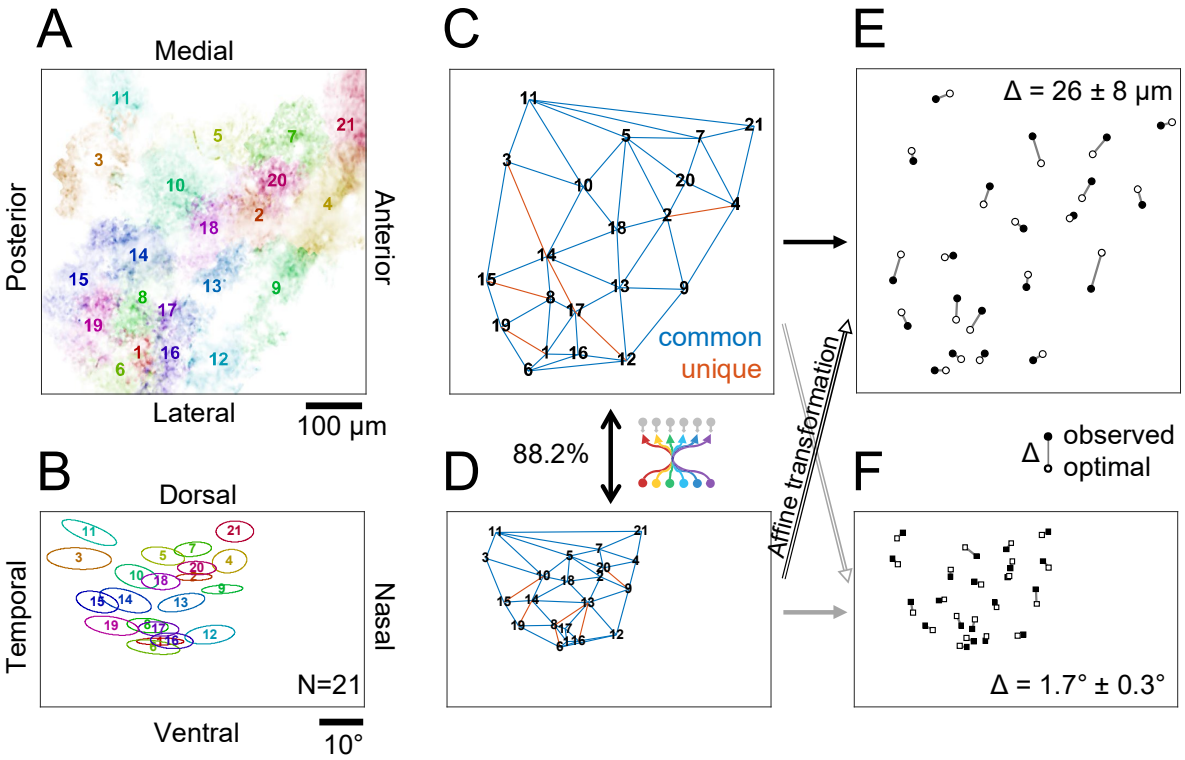
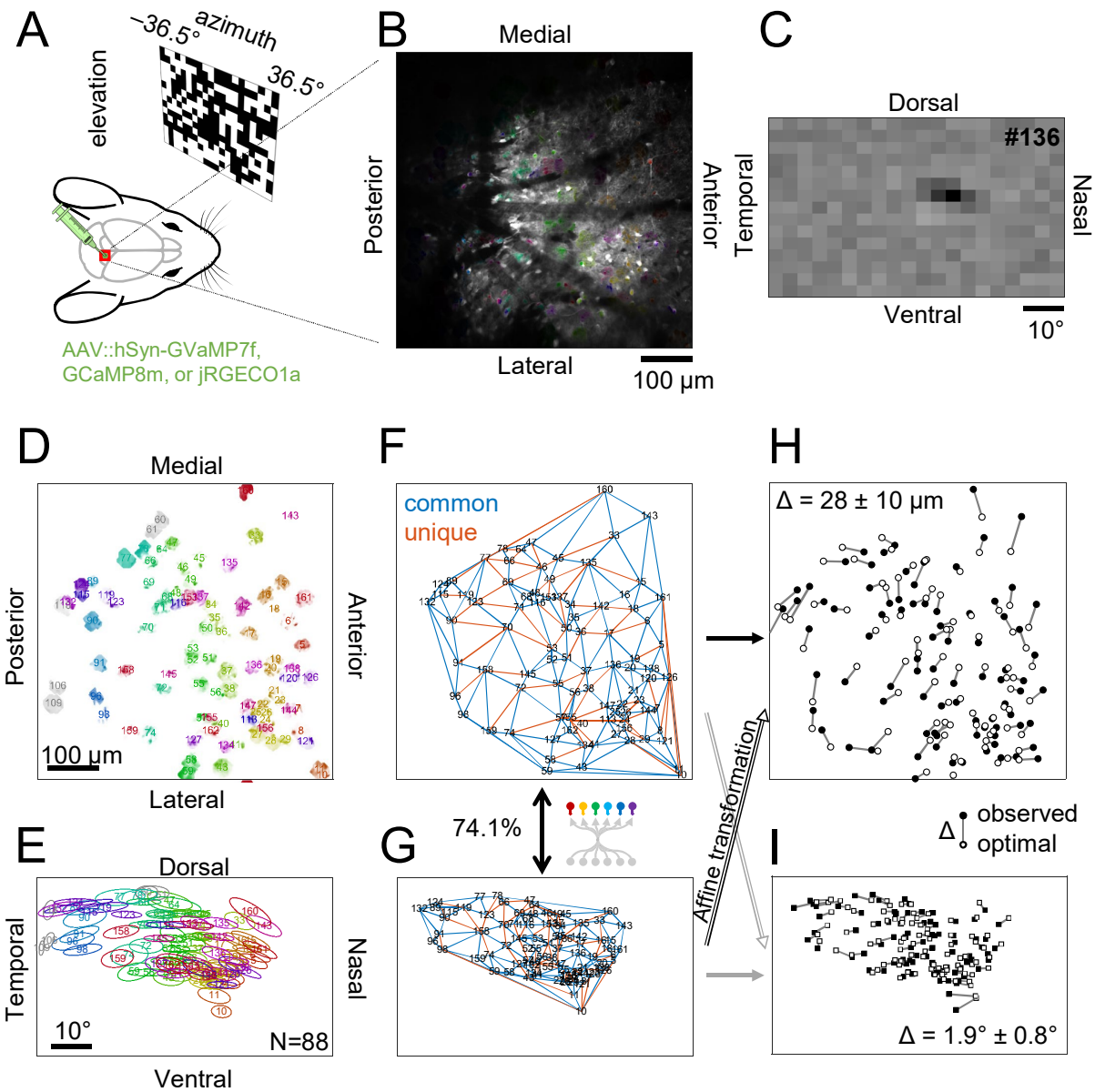


Figure 3: Retinal ganglion cell axons retinotopically tile the mouse superior colliculus at single-cell precision. *A,B*: RGC axon patches in SC from a representative recording session (A; N=21, color-coded; from Figure 2C) and their corresponding RFs (B; 1 SD Gaussian profile). *C,D*: Delaunay triangulation of the RGC axonal patch locations (C; from A) and the RF centers (D; from B). The two triangulation patterns are nearly identical (88.2%; blue, common edges in both patterns; red, unique edges only in either pattern). *E*: Comparison between the observed tiling pattern of RGC axons (filled circles; from C) and the retinotopically ideal pattern (open circle) obtained by applying an optimal Affine transformation to the corresponding RF locations (in D) that minimizes the discrepancy between the two patterns (Δ = 26 ± 8 µm). *F*: Corresponding comparison between the observed (filled squares; from D) and ideal (Affine-transformed pattern in C) RF tiling patterns of RGC axons (Δ = 1.7 ± 0.3 degrees).



Supplemental Figure 2: Tiling pattern analysis of local neurons in the mouse superior colliculus. Figure panels are shown in the same format as in Figures 2 and 3. *A*: Schematic diagram of the experimental set up for SC somatic imaging. *B*:Average intensity projection of representative imaging data, overlaid with detected SC somata (N=88; color-coded). *C*: Representative RF of a local SC neuron (#136) from reverse-correlation analysis. *D,E*: Tiling pattern of SC somata (D) from a representative recording session (B) and their corresponding RF tiling pattern (E; 1 SD Gaussian profiles with the same color-code as in B). *F,G*: Delaunay triangulation of the SC somatic locations (F; from D) and the RF centers (G; from E), showing a good agreement between them (74.1%; blue, common edges in both patterns; red, unique edges only in either pattern). *H,I*: Comparison between the observed and retinotopically ideal tiling patterns of SC somata (H; Δ = 28 ± 10 µm) or their RFs (I; Δ = 1.9 ± 0.8 degrees).

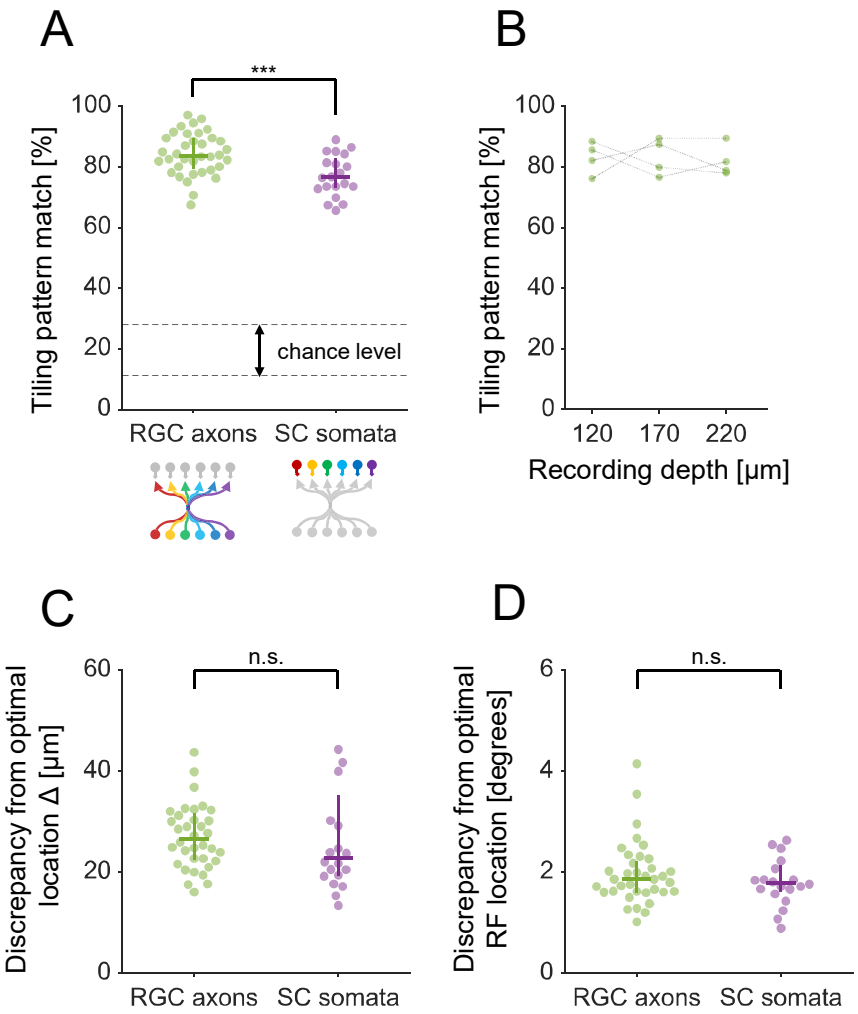


Figure 4: Tiling patterns of retinal ganglion cell axons are retinotopically more precise than those of local neurons in the mouse superior colliculus. *A*: RGC axons (84 ± 5%; N=36 recordings from 12 animals; e.g., Figure 3) showed a significantly higher precision of retinotopy than SC somata (77 ± 5%; N=20 recordings from 20 animals; e.g., Supplemental Figure 2); p = 0.001, rank sum test. The error bars show the median and interquartile range; and the dotted lines represent the chance level at p = 0.05 (11 - 28%; bootstrap with 10,000 repetitions). *B*: The precision of the retinotopic tiling of RGC axons was not dependent on the projection depth from the SC surface (N=4 animals; 83 ± 5, 83 ± 6, and 82 ± 5% at 120, 170, and 220 µm deep, respectively; mean ± SD; p = 0.9, one-way analysis-of-variance). *C*: Deviation between the observed and retinotopically ideal locations of the RGC axons (27 ± 4 µm; e.g., Figure 3E) or SC somata (23 ± 5 µm; e.g., Supplemental Figure 2H); p = 0.27, rank sum test. *D*: Deviation between the observed and retinotopically ideal RF locations of the RGC axons (1.9 ± 0.3 degrees; e.g., Figure 3F) or SC somata (1.8 ± 0.3 degrees; e.g., Supplemental Figure 2I); p = 0.66, rank sum test.

To further quantify the precision of the RGC axonal projection, we compared the observed position with the ideal one that forms perfect retinotopy (e.g., Figure 3E). Specifically, we used a linear method to estimate this retinotopically optimal tiling pattern of RGC axons:

185 i.e., an Affine transformation that best mapped the observed RF tiling pattern onto the
186 corresponding observed axonal tiling pattern (see Methods for details). We found that the
187 average discrepancy between the observed and retinotopically optimal RGC axonal locations
188 in SC was 27 ± 4 μm (Figure 4C). This is much shorter than the distance between neighboring
189 RGC axon centers (100 ± 30 μm, N=761 pairs; Supplemental Figure 1C) or the axonal patch
190 size (135 ± 25 μm, N=969; Supplemental Figure 1A), and thus will not have a measurable
191 impact on the retinotopy. Likewise, the extent to which the observed RF tiling pattern deviated
192 from the linear optimal one (1.9 ± 0.3 degrees; Figure 4D; see Figure 3F for example) was
193 much smaller than the RF size of RGC axons (4.8 ± 1.2 degrees, N=719; Supplemental Figure
194 1B) or the spacing between the RFs (7.2 ± 2.7 degrees, N=776 pairs; Supplemental Figure
195 1D). These data support that RGCs can precisely innervate their axons to their target locations
196 and faithfully transmit the information about retinotopy despite a loss of topographic
197 organization along the optic nerve (Horton et al., 1979; Colello and Guillery, 1998).

198 Thus far we have focused on the local retinotopy at the presynaptic input level, and
199 demonstrated a precise topographic organization of the RGC axons in the mouse SC (Figures
200 2-4). What about the postsynaptic side? Taking a similar approach, we next examined the
201 retinotopy of SC somata at single-cell resolution (Supplemental Figure 2). Specifically, using
202 *in vivo* two-photon calcium imaging, we mapped the RFs of local neurons in the superficial SC
203 layer (58 ± 27 cells/recording from 20 animals; RF size, 5.1 ± 0.9 degrees; RF overlap, 37 ±
204 11 %; N=1191 cells in total; Supplemental Figure 1B), and performed the same tiling pattern
205 analysis using the Delaunay triangulation (neighboring somata distance, 56 ± 28 μm, N=2292
206 pairs, Supplemental Figure 1C; neighboring RF distance, 4.1 ± 1.9 degrees, N=2287 pairs,
207 Supplemental Figure 1D). As expected, we found that the tiling patterns of SC somata and
208 their corresponding RFs agreed well in general (77 ± 5%, Figure 4A). However, the agreement
209 was significantly lower for SC somata than for RGC axons ($p = 0.001$, rank sum test; Figure
210 4A), indicating that local cellular-level retinotopy is less precise for the postsynaptic neurons
211 than for the input axons. Moreover, the average discrepancies between the observed and

retinotopically optimal locations of SC somata (23 ± 5 μm; Figure 4C) or their RFs (1.8 ± 0.3 degrees; Figure 4D) were comparable to those for RGC axons (p = 0.27 and 0.66, respectively; rank sum test), suggesting that the connectivity between RGC axons and SC neurons is not necessarily made to improve the precision of local retinotopy. Thus, our data disagree with the selective connectivity model whereby SC neurons selectively integrate inputs from appropriate presynaptic partners to reconstruct the topography at single-cell resolution (Model 2 in Figure 1). Instead, we suggest that retinotopy in SC arises primarily from precise RGC axonal projections (Model 1 in Figure 1), without much need to elaborate the postsynaptic connectivity.

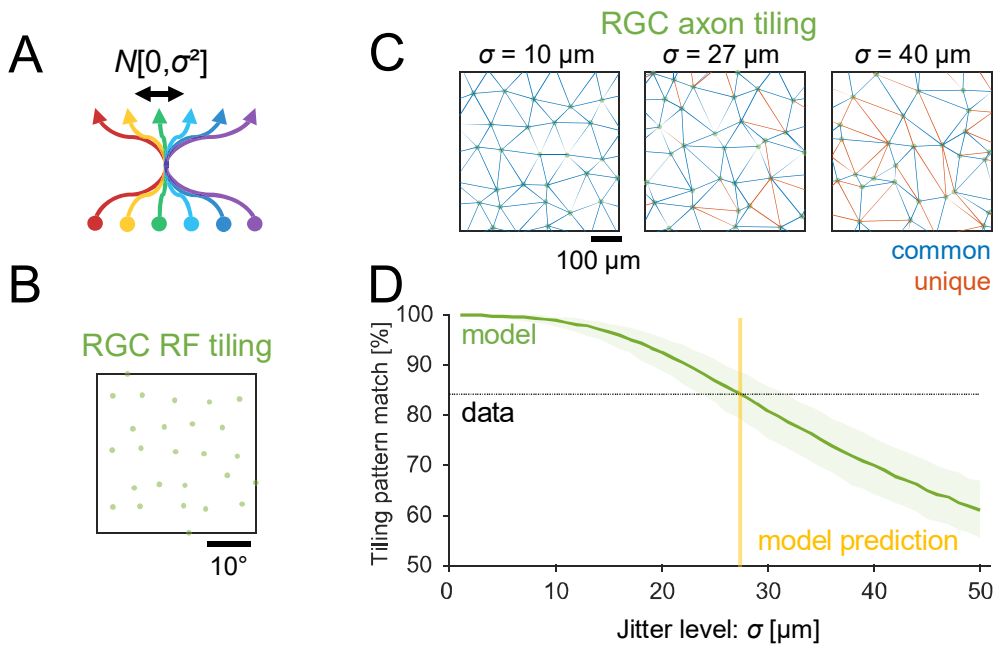


Figure 5: Data-driven model prediction on the precision of retinal ganglion cell axonal projection to the mouse superior colliculus. *A*: Schematic of a retinocollicular projection model. We assumed that 1) a jitter of RGC axonal projection follows a Gaussian distribution $N[0,\sigma^2]$; and 2) the tiling pattern of RGC RF centers corresponds to that of the cell locations in the retina. See Methods for details. *B,C*: Representative tiling patterns of simulated RGC RF centers (B; on a 10%-jittered hexagonal lattice) and the corresponding axon centers at different jitter levels (C; σ = 10, 27 and 40 μm from left to right panels, respectively). Common (blue) and unique (red) triangulation edges are also shown in each panel of C, when compared to the RF tiling pattern in B. The hexagonal lattice spacing was set to be 7.2 degrees and 100 μm for RGC RFs and axons, respectively, from the experimental data (Supplemental Figure 1). *D*: Correspondence of the triangulation edges between simulated RGC RF and axon tiling patterns at different projection jitter levels (median with 95% confidence interval; 1,000 repetitions). The intersection with the experimentally identified value (horizontal dotted line, 84% from Figure 4A) gives a model prediction on the precision of RGC axonal projection (vertical yellow line; σ = 27 ± 4 μm, with 95% confidence interval).

237 Our tiling pattern analysis showed a near-perfect retinotopy already at the level of the
238 axonal inputs to the mouse SC and no further improvement in the precision of retinotopy for
239 local SC neurons (Figures 3 and 4). What are the conditions to achieve such topographic
240 organizations in the retinocollicular pathway? To address this question, we next performed a
241 computational modelling analysis (see Methods for details). Specifically, by comparing the
242 observed and simulated tiling patterns on the pre- and post-synaptic sides, here we quantified
243 the following two parameters: 1) the precision of RGC axonal projection to a target location in
244 SC (Figure 5); and 2) the number of connecting RGCs to individual SC neurons (Figure 6).

245 We first modelled the tiling patterns of RGC axons at different jitter levels to identify
246 how small the projection error needs to be to recapitulate the observed precision of retinotopy
247 (Figure 5A). The tiling pattern of RGC somata – or equivalently, that of RGC RFs – was
248 simulated as a 2D hexagonal lattice with a small additive Gaussian noise, where the standard
249 deviation of the jitter followed 10% of the lattice spacing to replicate the dense packing of the
250 cell bodies in the retina (e.g., Figure 5B). The tiling pattern of RGC axons in SC was then
251 simulated by introducing additional Gaussian noise to the simulated RGC RF tiling pattern,
252 where the standard deviation σ of this additional noise determines the jitter level of the axonal
253 projection (e.g., Figure 5C). Here we set the axonal lattice spacing to be 100 μm based on our
254 experimental data (Supplemental Figure 1C), and ran the tiling pattern analysis as we did on
255 our experimental data to quantify the precision of retinotopy in the model. As expected, the
256 larger the jitter was, the less precise the retinotopy was (Figure 5D). This allowed us to
257 determine the jitter size that agreed with the observed precision level of retinotopy (84%;
258 Figure 4A): i.e., $\sigma = 27 \pm 4$ μm (with 95% confidence interval). This is consistent with the
259 average discrepancy between the observed and retinotopically optimal tiling patterns ($\Delta = 27$
260 μm; Figure 4C), hence validating our modelling framework and further supporting our estimate
261 on the precision of the RGC axonal projection.

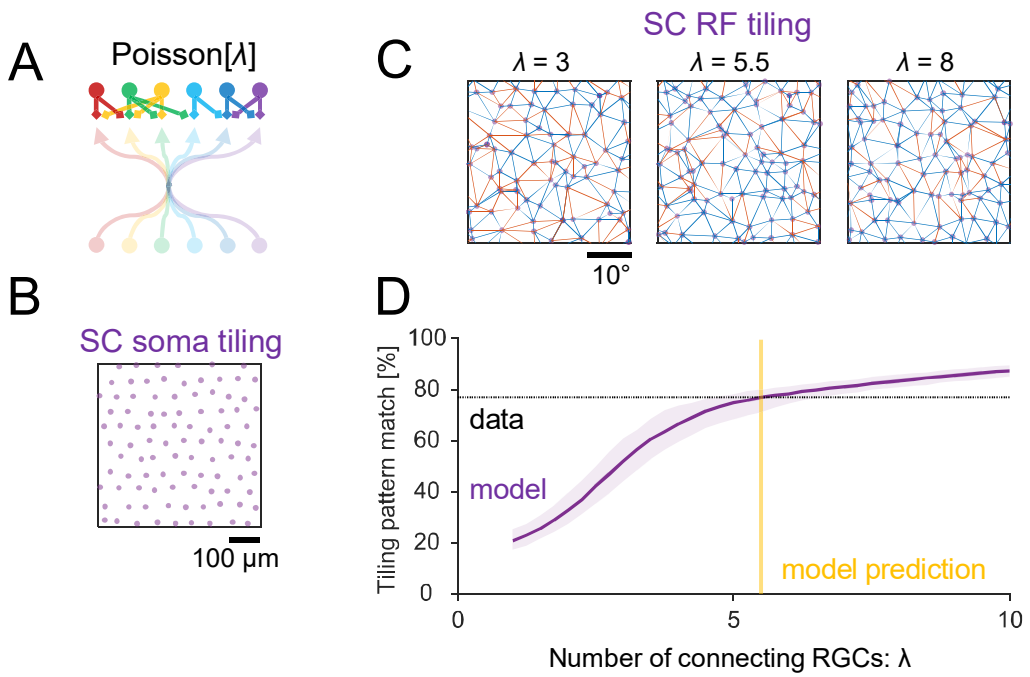


Figure 6: Data-driven model prediction on the number of connecting retinal ganglion cells to individual neurons in the superior colliculus. *A*: Schematic of a retinocollicular mapping model. We assumed that 1) each SC neuron integrates inputs from λ nearest neighbor RGC axons, where λ follows a Poisson distribution; and 2) the connectivity weights depend on the amount of overlap between SC dendritic field (radius, 200 µm; Gale and Murphy, 2014) and RGC axonic field (135 µm; Supplemental Figure 1A). RGC axonal tiling was simulated with σ = 27 µm (Figure 5). See Methods for details. *B,C*: Representative tiling patterns of simulated SC soma centers (B; on a 10%-jittered hexagonal lattice with 56 µm spacing; Supplemental Figure 1C) and the corresponding RF centers at different integration levels (G; λ = 3, 5.5, and 8, from left to right panels, respectively), overlaid with common (blue) and unique (red) triangulation edges. *D*: Correspondence of the triangulation edges between simulated SC soma and RF tiling patterns at different input convergence levels (median with 95% confidence interval; 1,000 repetitions). The intersection with the experimentally identified value (horizontal dotted line, 77% from Figure 4A) gives a predicted number of connecting RGCs to individual SC neurons (vertical yellow line; λ = 5.5 ± 1.0, with 95% confidence interval).

We next modelled the retinotopy of SC somata on top of the optimal RGC projection model described above (jitter size, σ = 27 µm), using the average number of RGC inputs to SC neurons, λ, as a key model parameter (Figure 6A). The simulated tiling pattern of SC somata (e.g., Figure 6B) was generated in a similar way to that of RGC somata, but with a lattice spacing of 56 µm based on our experimental data (Supplemental Figure 1C). For each simulated SC cell, the RF center location was then determined as a weighted average of the RF centers of neighboring RGC axons (Figure 6C), where we assumed that the number of

286 connecting RGCs followed a Poisson distribution (mean, λ), and that the connectivity strength
287 was proportional to the amount of overlap between the SC cell's dendritic field (radius, 200
288 μm; Gale and Murphy, 2014) and the RGC's axonic field (135 μm; Supplemental Figure 1D).
289 Here we introduced a rather simple connectivity rule as implicated by our experimental data
290 solely from the retinotopy viewpoint (Figures 3 and 4), while details on the cell-type specific
291 connectivity are beyond the scope of our modelling framework. The tiling pattern analysis on
292 the simulated SC cells then showed that the larger the number of connecting RGCs was, the
293 more precise the retinotopy was (Figure 6D). This suggests that the observed relatively less
294 precise retinotopy for SC somata (77% as opposed to 84% for RGC axons; Figure 4A) results
295 from a low input convergence. Indeed, by comparing the model outcome with the experimental
296 data, here we derived that on average SC neurons receive inputs from $\lambda = 5.5 \pm 1.0$ RGCs
297 (with 95% confidence interval). This is consistent with the observation in the previous
298 electrophysiological studies (Chandrasekaran et al., 2007; Sibille et al., 2022).

299 Discussion

300 Using *in vivo* two-photon axonal imaging, we conducted functional mapping of the mouse
301 retinocollicular projection, and demonstrated a precise retinotopic tiling of RGC axon terminals
302 in SC at single-cell resolution (Figures 2 and 3). Here we calculated the projection error size
303 in two different ways: 1) based on the deviation from a linearly-estimated retinotopically-ideal
304 target location (Figures 3 and 4), and 2) by data-driven computational modelling (Figure 5).
305 Both methods consistently found that the projection jitter was below 30 μm, much smaller than
306 the observed RGC axonic field size (135 μm; Supplemental Figure 1). These axons can thus
307 be innervated to their exact target locations to faithfully transmit topographic information from
308 the retina, even after a loss of topographic organization through the long-range projection via
309 the optic nerve (Horton et al., 1979; Colello and Guillery, 1998).

310 In contrast, we found that the local retinotopy of SC somata was no better than that of
311 RGC axons (Figure 4 and Supplemental Figure 2). The connectivity between RGCs and SC

312 cells is thus not necessarily made to retain or improve the topography. Instead, assuming no
313 selectivity in the connectivity patterns, our modelling analysis indicates that a reduced
314 precision of local retinotopy on the postsynaptic side can be a direct consequence of a low
315 input convergence level (Figure 6). Based on our experimental data, we derived from our
316 model that on average SC neurons receive inputs from ~5.5 RGCs (Figure 6). This is
317 consistent with the past electrophysiological measurements (Chandrasekaran et al., 2007;
318 Sibille et al., 2022), justifying our model framework and conclusion.

319 Taken together, we suggest that retinotopy in the mouse SC arises largely from
320 topographically precise projection of RGC axons, rather than local circuit computation by SC
321 neurons. Nevertheless, the precision of axonal projection was not perfect. Postsynaptic circuit
322 mechanisms should then be indispensable as well in retaining topography, especially for
323 higher-order processing because otherwise retinotopy will no longer be recognizable after a
324 cascade of signal transmission along the visual hierarchy (e.g., below chance level after six
325 ~80% precision transmissions). It is a future challenge to investigate the cellular-level
326 topographic organization in other brain areas, including the retinotopy in the downstream
327 visual pathways (Wandell et al., 2007), and clarify the contribution of pre- and post-synaptic
328 circuit mechanisms in each area.

329 Having a precise retinotopy at single-cell resolution facilitates spatial information
330 processing not only at a global level, but also at local circuit levels. For example, looming
331 detection has been suggested to arise de novo in the superficial SC layer (Lee et al., 2020).
332 In principle, this can be achieved even in the absence of retinotopy by elaborating the wiring
333 among local neurons. It is, however, much more efficient to exploit precise topographic
334 information conveyed from the retina because the connectivity length and its complexity can
335 be minimized to locally process spatial information at any point in the visual field. This will also
336 help align different topographic maps in the same brain area to function coherently, such as
337 the retinotopy, orientation, and ocular dominance maps in SC (Feinberg and Meister, 2015;

de Malmazet et al., 2018). The observed precise spatial organization we demonstrate here suggests that the wiring efficiency indeed matters for local circuit computation.

How can then such a precise retinotopic projection be formed? Retinotopic map formation in SC occurs during the first postnatal week in mice, involving both genetic and activity-dependent factors (Cang and Feldheim, 2013; Arroyo and Feller, 2016). These factors also play a key role in the development of a fine-scale organization in other sensory systems, such as the tonotopic map in the cochlear nucleus (Krasewicz and Yu, 2023), and the chemotopic map in the olfactory bulb where olfactory sensory neurons expressing the same olfactory receptor type project exclusively to the same single glomerulus (Imai et al., 2010). While overall sensory map formation is genetically predetermined by molecular cues (e.g., ephrin-Eph signaling; Frisén et al., 1998; Krasewicz and Yu, 2023), a precise topography is established only after refinement that involves spontaneous activity, such as retinal waves during development (Arroyo and Feller, 2016), and eventually experience-driven alignment (McLaughlin et al., 2003). In particular, here we suggest that this refinement process of the retinocollicular projection should be extremely precise, to the extent that retinotopy arises at a single-cell resolution regardless of the RGC types in adult animals (Figures 3 and 4). It is then possible that neuronal circuits are in general wired more precisely than previously thought to exploit topographic information for their function, including long-range projections to other retinal targets (Liang, et al., 2018) as well as those in other systems, such as the callosal projections and entorhinal-hippocampal networks (Jbabdi et al., 2013; Patel et al., 2014).

Methods

No statistical method was used to predetermine the sample size. All experiments involving animals were performed under the license 233/2017-PR from the Italian Ministry of Health. The data analyses were done in Python and Matlab (Mathworks). The statistical significance level was set to be 0.05. All summary statistics were described as median ± median absolute deviation unless otherwise noted.

364 # Animals

365 Female C57BL/6J mice (15 for axonal imaging; 20 for somatic imaging) were used at around
366 6-10 weeks of age at the time of the first surgery. Mice were kept on a 12-h light / 12-h dark
367 cycle and given water and food *ad libitum*. After surgery, the animals were kept in groups
368 operated on the same day. Mice were between 12-24 weeks of age at the time of imaging
369 experiments.

370 # Intravitreal viral injections

371 Intravitreal injection of recombinant adeno-associated virus (AAV) 2, pseudotyped with a
372 hybrid of AAV1 and AAV2 capsids, was used to deliver hSyn-axon-GCaMP6s expression
373 cassette to the mouse retinal ganglion cells (RGCs; Broussard et al., 2018). For the viral
374 injection, mice were anaesthetized (induction, 4% isoflurane in oxygen; maintenance, 1.8-
375 2.0%) and kept on a heated plate (Supertech Physiological Temperature Controller) to avoid
376 hypothermia. Both eyes were protected by saline drop or viscous eye ointment (VitA-POS,
377 Ursapharm). The scleral surface on the left eye was exposed and a small piercing was made
378 with a sterile 28-30G needle in between the sclera and the cornea. An injection pipette (~50
379 μm tip diameter with 30-40° bevel) prefilled with a virus solution (~1.5×10^{14} vg/mL in
380 phosphate-buffered saline with 0.001% Pluronic F68 and 0.001% FastGreen) was then
381 inserted into the vitreous chamber approximately 1 mm deep. The injection pipette was made
382 from a borosilicate glass capillary (1B120F-3, WPI) with a pipette puller (DMZ, Zeitz) and a
383 microgrinder (EG-45, Narishige). After a good sealing of the pipette was formed, 1.2 μL of the
384 virus solution was injected at a rate of 10 nL/s using a microinjection pump (either Neurostar
385 NanoW or WPI NanoLiter 2010) with mineral oil (Sigma, M5904) filled in the displacement
386 space by a stainless steel plunger. The pipette was slowly withdrawn at least 5 minutes after
387 the completion of the injection, and the treated eye was covered with the eye ointment. The
388 animal was then allowed to recover from anesthesia in a warmed-up chamber and brought
389 back to its home cage.

390 # Intracranial viral injections

391 Pseudotyped AAV, composed of AAV2 rep and AAV9 cap genes or a hybrid of AAV1 and
392 AAV2 cap genes, was locally injected to the mouse superior colliculus (SC) for the expression
393 of genetically-encoded calcium indicators (jGCaMP7f, jGCaMP8m or jRGECO1a) under pan-
394 neuronal human synapsin (hSyn) promoter. The intracranial viral injection was made at the
395 same time as the cranial implantation as described below. After making a craniotomy over the
396 right SC, an injection pipette (~30 μm tip diameter; WPI 1B120F-3 borosilicate glass capillary
397 pulled with Zeitz DMZ puller) prefilled with a virus solution (~5×10^{12} to ~4×10^{14} vg/mL in
398 phosphate-buffered saline) was inserted across the dura at coordinates from Bregma around
399 -4 mm AP, 0.5-0.7 mm ML, and then slowly advanced until ~1.25 mm deep. The virus solution
400 (0.4-0.6 μL) was injected at a rate of 2 nL/s with a microinjection pump (either Neurostar
401 NanoW or WPI NanoLiter 2010). The pipette was slowly withdrawn at least 10 minutes after
402 the completion of the injection, followed by the cranial window implantation procedure.

403 # Cranial implantations

404 We adapted methods described in Feinberg and Meister (2015) for the cranial window
405 implantation over the mouse SC. A cranial window assembly was made in advance, where
406 the surface of a circular glass coverslip (5 mm diameter, 0.13-0.15 mm thickness; Assistent
407 Karl Hecht) was activated by a laboratory corona treater (BD-20ACV Electro-Technic
408 Products) and fused to a cylindrical silicone plug (1.5 mm diameter, 0.75 - 1.00 mm height;
409 Kwik-Sil, WPI) by baking it for 24 hours at 70-80°C.

410 For the implantation, animals were anaesthetized (induction, 4% isoflurane in oxygen;
411 maintenance, 1.5-2.0%) and placed inside a stereotaxic apparatus (Stoelting 51625).
412 Throughout the surgery, temperature was maintained at 37°C using a heated plate (Supertech
413 Physiological Temperature Controller) to avoid hypothermia, and the eyes were protected with
414 eye ointment (VitA-POS, Ursapharm). After disinfecting and removing the scalp (Betadine
415 10%, Meda Pharma), the skull surface was scratched and cleaned to ensure good cement

416 adhesion. A craniotomy of a size about 3.0 mm (anterior-posterior; AP) by 2.5 mm (medial-
417 lateral; ML) was made over the right SC using a high-speed surgical drill (OmniDrill35, WPI)
418 with a 0.4 mm ball-tip carbide bur (Meisinger). To prevent bleeding, the craniotomy was treated
419 by hemostatic sponges (Cutanplast, Mascia Brunelli) soaked with sterile cortex buffer (NaCl
420 125 mM, KCl 5 mM, Glucose 10 mM, HEPES 10 mM, $CaCl_2$ 2 mM, $MgSO_4$ 2 mM, pH 7.4).
421 For SC somata imaging, viral injections were made as described above. The implant was then
422 placed in a way to push the transversal sinus and posterior cortex ~0.5 mm forward and
423 position the silicone plug over the medial-caudal region of the right SC. Tissue adhesive
424 (Vetbond, 3M) was used to fix and seal the implant. A custom-made titanium headplate (0.8
425 mm thick) was then cemented to the skull using acrylic cement powder (Paladur, Kulzer) pre-
426 mixed with cyanoacrylate adhesive (Loctite 401, Henkel).

427 After the surgery, the animal was recovered from anesthesia in a warmed-up chamber
428 and returned to its home cage. For postoperative care, animals were given intraperitoneally 5
429 mg/kg Rimadyl (Zoetis) and 5 mg/kg Baytril (Bayer) daily for 3-5 days. We waited for another
430 10-15 days until the cranial window completely recovered before starting *in vivo* two-photon
431 imaging sessions (e.g., Figure 2B).

432 Visual stimulation

433 Visual stimuli were presented to the subject animals as described previously (Boissonnet et
434 al., 2022). In short, a custom gamma-corrected digital light processing device was used to
435 project images (1280-by-720 pixels; frame rate, 60 Hz) to a spherical screen (radius, 20 cm)
436 placed ~20 cm to the contralateral side of an animal's eye, stimulating the visual field ±22° in
437 elevation and ±36.5° in azimuth (Figure 2A and Supplemental Figure 2A). We presented 1)
438 random water-wave stimuli (2-10 min) for generating binary masks for signal source extraction
439 in calcium image analysis (see below); and 2) randomly flickering black-and-white
440 checkerboard stimuli (10 min) for receptive field mapping, with rectangular fields 3.7° in width
441 and 2.9° in height, each modulated independently by white noise at 4 Hz.

442 ## *In vivo* two-photon imaging

443 Prior to *in vivo* imaging sessions, animals were habituated to stay head-fixed on a custom-
444 made treadmill disc (8-10 habituation sessions in total over a week, each for 2 hours). For the
445 imaging session, animals were kept on the treadmill with their head fixed for no longer than 2
446 hours (2-5 sessions/animal). Two-photon calcium imaging was done on a galvo-resonant
447 multiphoton microscope (Scientifica HyperScope with SciScan image acquisition software)
448 equipped with a mode-locked tunable laser (InSight DS+, Spectra-Physics) and a plan fluorite
449 objective (CFI75 LWD 16X W, Nikon). In each imaging session, we performed single-plane
450 time-lapse recordings (field of view, approximately 0.65-by-0.65 mm) at a depth of 120-220
451 μm from the SC surface. The fluorescent signal (excitation wavelength, 920 nm for axon-
452 GCaMP6, GCaMP7f, and GCaMP8m; 1040 nm for jRGECO1a; average laser power under
453 the objective, 40-80 mW) was bandpass-filtered (BP 527/70 or BP 650/100 after beam-splitter
454 FF580-FDi01, Semrock) and detected with a non-descanned gallium arsenide phosphide
455 photomultiplier tube (Hamamatsu GaAsP PMT). Each frame was acquired with 1024-by-1024
456 pixels (16-bit depth) at 15.4 Hz for RGC axonal imaging, and 512-by-512 pixels (16-bit depth)
457 at 30.9 Hz for SC somata imaging.

458 ## Calcium image analysis

459 For preprocessing of RGC axon data, the original 1024-by-1024 pixel images were first
460 downsampled to 512-by-512 pixels (2-by-2 pixels averaging) to reduce noise. To correct
461 motion artefacts, we performed two iterations of Fourier-based rigid image registration in
462 ImageJ, followed by cropping the image border by 16 pixels; and then ten iterations of non-
463 rigid motion correction (NoRMCorre) in CaImAn (Giovannucci et al., 2019), followed by a 12-
464 pixel border crop. The resulting images (456-by-456 pixels; e.g., Supplemental Movie 1)
465 represent a field of view of around 0.57-by-0.57 mm (1.3 μm/pixel).

466 From the preprocessed images, we identified the axonal patches of individual RGCs
467 and extracted their signals in CaImAn (e.g., Figure 2C,D). Specifically, using a part of the

468 recordings (3,000-5,000 frames representing the random water-wave stimulus presentation
469 period), we first ran two iterations of constrained non-negative matrix factorization (CNMF) in
470 CaImAn, where we set the number of expected components (*params.K*) to be 60 as an
471 initialization parameter. From the identified components, we then manually selected those with
472 a uniformly-filled oval-like shape that had a size of around 50-150 μm as biologically relevant
473 ones (Hong et al. 2011), and converted them into binary spatial masks to run two iterations of
474 masked CNMF for processing the entire time-lapse recordings. The resulting set of spatial
475 components (*estimates.A*) and deconvolved neural activities (*estimates.S*) was used for the
476 subsequent analyses.

477 The area of the individual RGC axonal patches P_i was estimated from the identified
478 spatial components in CaImAn (1.3 μm/pixel), from which the radius was estimated as
479 $(P_i/\pi)^{0.5}$ under the assumption of a circular patch shape (Supplemental Figure 1A). The
480 fraction of the overlap between identified axonal patches was calculated as the ratio of the
481 areas between the intersection of any two patches $\cup_{i \neq j}(P_i \cap P_j)$ and the union of all patches
482 $\cup_i P_i$.

483 For SC soma data, we first ran a sequence of the rigid and non-rigid motion corrections
484 in CaImAn, followed by image cropping from 512-by-512 pixels into 480-by-480 pixels in
485 ImageJ (1.3 μm/pixel). Using a part of the recordings (3,000 frames from the random water-
486 wave stimulus presentation period), we then ran two iterations of CNMF in CaImAn, where the
487 images were divided into 6-by-6 (36 in total) patches and the expected number (*params.K*)
488 and size (*params.gSig*) of neurons were set to be 5 per patch and 5-by-5 pixels in half size,
489 respectively, as initialization parameters. From the identified putative cells, we manually
490 selected those with a uniformly-filled round shape of around 10-20 μm in size as biologically
491 relevant ones, and converted them into binary spatial masks to run two iterations of masked
492 CNMF in CaImAn on the entire time-lapse recordings. The resulting set of spatial components
493 (*estimates.A*) and deconvolved neural activities (*estimates.S*) was used for the subsequent
494 analyses.

495 # Receptive field analysis

496 The receptive fields (RFs) of the identified RGC axon patches or SC somata were estimated
497 by reverse-correlation methods using the random checkerboard stimuli (Chichilnisky, 2001).
498 Specifically, we calculated the response-weighted average of the stimulus waveform (0.5 s
499 window; 1/60 s bin width), and characterized its spatial profile by the two-dimensional (2D)
500 Gaussian curve fit at the peak latency (e.g., Figure 2E and Supplemental Figure 2B). The RF
501 center was assigned to the center of that 2D Gaussian profile, and the RF size was estimated
502 as twice the mean standard deviation (SD) of the long and short axes (Supplemental Figure
503 1B). The fraction of the overlap between the RFs (1 SD Gaussian profiles) was computed
504 similarly as for the axonal patches. The Pearson correlation coefficient between the RF size
505 and the RGC axonal patch size was calculated with the 95% interval of the data to eliminate
506 the outliers. Those cells that had the RF center on the border or outside the stimulus screen
507 were eliminated from the tiling pattern analysis described below. Those recordings that had
508 less than 10 cells with RF centers on the stimulus screen were also excluded from the tiling
509 pattern analysis.

510 # Tiling data analysis

511 To compare the tiling patterns between RGC axon patches / SC somata and their RFs (e.g.,
512 Figure 3 and Supplemental Figure 2), we first computed the Delaunay triangulation of their
513 centroid locations using the Euclidean distance in each space (e.g., Figure 3C,D and
514 Supplemental Figure 2C,D). As a measure of similarity between the two tiling patterns, we
515 then calculated the number of common edges, divided by the mean of the total number of
516 edges in each triangulation. The chance level was calculated by a bootstrap method (10,000
517 repetitions; Figure 4A).

518 We used the least squares method to identify an optimal Affine transformation for
519 mapping a given RF tiling pattern onto the corresponding tiling pattern of RGC axons (e.g.,
520 Figure 3E), or vice versa (e.g., Figure 3F). The Euclidean distance of the cell or RF locations

521 between the observed and affine-transformed tiling patterns was then used as a measure of
522 the precision of local retinotopy (Figure 4C,D).

523 Modelling of retinocollicular mapping

524 We modelled the retinocollicular mapping in four steps (Figures 5 and 6).

525 1. The tiling pattern of RGC somata was simulated as a 2D hexagonal lattice with a
526 Gaussian jitter $N[0, \sigma^2]$. The standard deviation σ was set to be 10% of the lattice
527 spacing L to recapitulate the dense packing of the cell bodies in the retina. We
528 assumed that the tiling pattern of RGC RFs was equivalent to the corresponding
529 somatic tiling pattern (e.g., Figure 5B; L = 7.2 degrees from Supplemental Figure 1D).
530 2. The tiling pattern of RGC axons in SC (i.e., retinocollicular projection) was then
531 simulated by introducing additional Gaussian noise $N[0, \sigma^2]$ to the RGC RF tiling
532 pattern from step 1 (e.g., Figure 5C) but with L = 100 μm (Supplemental Figure 1C).
533 When σ = 0 μm, the tiling pattern of RGC axons is identical to the somatic tiling pattern,
534 showing perfect retinotopy (i.e., 100% tiling pattern match).
535 3. The tiling pattern of SC somata was simulated as a 2D hexagonal lattice (L = 56 μm;
536 Supplemental Figure 1C) with a Gaussian jitter (σ = 0.1L) as in step 1 (e.g., Figure 6B).
537 4. The RF of each SC neuron was calculated by integrating inputs from λ nearest
538 neighbor RGC axons, where λ follows a Poisson distribution. Specifically, assuming
539 that the connectivity strength depends on the amount of overlap between SC dendritic
540 field (radius, 200 μm; Gale and Murphy, 2014) and RGC axonic field (135 μm;
541 Supplemental Figure 1A), we defined the SC RF center location as the weighted
542 average of the RF centers of the connecting RGCs (e.g., Figure 6C).

543 To identify the precision of RGC axonal projection to SC (Figure 5), we ran the steps 1 and 2
544 at different jitter levels σ (from 0 to 50 μm in steps of 1 μm; 1,000 repetitions each) and
545 calculated the similarity between the simulated tiling patterns of RGC axons and their RFs
546 using the triangulation method as described above (Figure 5D). We then determined the jitter

level σ where the simulated tiling pattern similarity agreed with the experimental data (84%; Figure 4A).

To estimate the average number of connecting RGCs to individual SC neurons (Figure 6), we ran the steps 1-4 at different mean values λ (from 1 to 10 RGCs in steps of 0.25; 1,000 repetitions; σ = 27 μm for step 2 from Figure 5D) and calculated the similarity between the simulated SC somatic and RF tiling patterns using the triangulation method as described above (Figure 6D). We then determined the input convergence level λ where the simulated tiling pattern similarity agreed with the experimental data (77%; Figure 4A).

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