

Supplementary Information for

Visual pathway origins: connectome of a human foveal retina

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Supplementary Data Tables 1 to 3

Supplementary Notes

26 **Supplementary Data Tables**

a	Summary		
	Type	Total (n)	Total (%)
	Photoreceptor	337	11.2 %
	Horizontal cell	265	8.8 %
	Bipolar cell	912	30.3 %
	Amacrine cell	293	9.7 %
	Ganglion cell	599	19.9 %
	Müller glia	555	18.5 %
	Astrocyte	32	1.1 %
	Microglia	15	0.5 %
	Total	3008	100%
b	Non-neuronal cells		
	Type	Total (n)	Total (%)
	Müller glia	555	92.2 %
	Astrocytes	32	5.3 %
	Microglia	15	2.5 %
	Total	602	100 %
c	Cone photoreceptors		
	Type	Total (n)	Total (%)
	LM cone	297	94.9 %
	S cone	16	5.1 %
	Total	313	100%
d	Rod and cone photoreceptors		
	Type	Total (n)	Total (%)
	Cone	313	92.9 %
	Rod	24	7.1 %
	Total	337	100%
e	Horizontal cells		
	Type	Total (n)	Total (%)
	H1	256	95.5 %
	H2	12	4.5 %
	Total	268	100%
f	Bipolar cells (BCs)		
	Type	Total (n)	Total (%)
OFF	FMB	302	33.1 %
	DB1	54	5.9 %
	DB2	92	10.1 %
	DB3	41	4.5 %
	Outer-x	15	1.6 %
	DBbroad	18	2.0 %
ON	IMB	264	28.9 %
	DB4	34	3.7 %
	DB5	45	4.9 %
	DB6	12	1.3 %
	BB	17	1.9 %
	RB	6	0.7 %
	Inner-x	5	0.5 %
	Unidentified	7	0.8 %
	Total	912	100%
g	Amacrine cells (ACs)		
	Type	Total (n)	Total (%)
	AC2 (AII, small)	75	25.6 %
	AC3 (small)	29	9.9 %
	AC6 (small)	24	8.2 %
	AC11 (small)	22	7.5 %
	AC5 (small)	20	6.8 %
	AC4 (small)	19	6.5 %
	AC7 (SAC, large)	17	5.8 %
	AC16 (large)	11	3.8 %
	AC9 (Interplexiform, large)	11	3.8 %
	AC10 (large)	10	3.4 %
	AC8 (large)	10	3.4 %
	AC19 (large)	8	2.7 %
	AC1 (A1?, large)	6	2.0 %
	AC12 (large)	6	2.0 %
	AC15 (large)	6	2.0 %
	AC17 (large)	6	2.0 %
	AC13 (large)	5	1.7 %
	AC18 (large)	5	1.7 %
	AC14 (large)	3	1.0 %
	Total	293	100 %
h	Ganglion cells (GCs)		
	Type	Total (n)	Total (%)
Major GCs	OFF midget	280	46.7 %
	OFF parasol	13	2.2 %
	ON midget	256	42.7 %
	ON parasol	13	2.2 %
	SBGC	12	2.0 %
Large Field GCs	LFGC1 (LBGC)	6	1.0 %
	LFGC2 (Large diffuse)	6	1.0 %
	LFGC3 (Recursive bistratified)	4	0.7 %
	LFGC4 (Inner/outer smooth monostratified)	5	0.8 %
	LFGC5 (Inner sparse)	4	0.7 %
	Total	599	100 %

a Cone 21 (LM cone 21)					d Cone 54 (S cone 6)				
	NO	GC types	Total synapse (#)	Total percent (%)		NO	GC types	Total synapse (#)	Total percent (%)
Major GCs	1	ON MG	63	13.8 %	Major GCs	1	ON MG	66	19.4 %
	2	OFF MG	105	23.0 %		2	OFF MG	129	37.9 %
	3	ON Parasol	51	11.4 %		3	ON Parasol	44	12.9 %
	4	OFF Parasol	213	46.1 %		4	OFF Parasol	64	18.8 %
	5	SBGC	1	0.2 %		5	SBGC	18	5.4 %
Large Field GCs	6	LFGC1 (LBGC)	9	2.0 %	Large Field GCs	6	LFGC1 (LBGC)	1	0.3 %
	7	LFGC2	3	0.7 %		7	LFGC2	14	4.1 %
	8	LFGC3	3	0.7 %		8	LFGC3	0	0.0 %
	9	LFGC4	5	1.1 %		9	LFGC4	3	0.9 %
	10	LFGC5	0	0.0 %		10	LFGC5	0	0.0 %
	11	Melanopsin?	4	0.9 %		11	Melanopsin	1	0.3 %
	12	Unidentified	1	0.2 %		12	Unidentified	0	0.0 %
Total			457	100%	Total			340	100%

b Cone 22 (LM cone 22)					e Cone 103 (S cone 5)				
	NO	GC types	Total synapse (#)	Total percent (%)		NO	GC types	Total synapse (#)	Total percent (%)
Major GCs	1	ON MG	69	16.0 %	Major GCs	1	ON MG	22	7.2 %
	2	OFF MG	101	23.7 %		2	OFF MG	118	38.8 %
	3	ON Parasol	65	15.1 %		3	ON Parasol	19	6.3 %
	4	OFF Parasol	139	33.1 %		4	OFF Parasol	80	26.3 %
	5	SBGC	2	0.5 %		5	SBGC	43	14.1 %
Large Field GCs	6	LFGC1 (LBGC)	25	5.8 %	Large Field GCs	6	LFGC1 (LBGC)	1	0.3 %
	7	LFGC2	3	0.7 %		7	LFGC2	9	3.0 %
	8	LFGC3	3	0.7 %		8	LFGC3	2	0.7 %
	9	LFGC4	7	1.6 %		9	LFGC4	4	1.3 %
	10	LFGC5	2	0.5 %		10	LFGC5	0	0.0 %
	11	Melanopsin?	4	0.9 %		11	Melanopsin	0	0.0 %
	12	Unidentified	6	1.4 %		12	Unidentified	6	2.0 %
Total			431	100%	Total			304	100%

c Cone 58 (LM cone 58)				
	NO	GC types	Total synapse (#)	Total percent (%)
Major GCs	1	ON MG	39	13.8 %
	2	OFF MG	73	25.8 %
	3	ON Parasol	54	19.1 %
	4	OFF Parasol	98	34.6 %
	5	SBGC	0	0.0 %
Large Field GCs	6	LFGC1 (LBGC)	2	0.7 %
	7	LFGC2	11	3.9 %
	8	LFGC3	2	0.7 %
	9	LFGC4	4	1.4 %
	10	LFGC5	0	0.0 %
	11	Melanopsin	0	0.0 %
	12	Unidentified	0	0.0 %
Total			283	100%

Supplementary Data Table 2. Total excitatory synaptic output from a single cone.

Non-neuronal cells (related to Figure S1)	Microglia cells Astrocytes Müller Glia cells
Horizontal cells (related to Figures 2C, 2D)	H1 Horizontal cells H2 Horizontal cells
Photoreceptors (related to Figure 2A)	LM/S Cones + Rods
Bipolar cells (related to Figure 3)	IMB (ON midget bipolar cells) FMB (OFF midget bipolar cells) Blue cone bipolar cells (BB cells) ON diffuse bipolar cells (DB4, DB5, DB6) OFF diffuse bipolar cells (DB1, DB2, DB3) RB (rod bipolar) Outer-x Inner-x Giant DBbroad (diffuse bipolar type)
Amacrine cells (related to Figures 4, 5)	AC2 (All amacrine cells) AC7 (starburst amacrine cells) AC 8 AC 11
Ganglion cells (related to Figure 7)	ON and OFF midget ganglion cells and the midget circuit ON/OFF parasol ganglion cells Small bistratified ganglion cells LFGC1 (Large bistratified) LFGC2 (Large diffuse) LFGC3 (Recursive bistratified) LFGC4 (Smooth monostratified)

Supplementary Data Table 3. Links to cells in NeuroMaps. The second columns contain clickable links to view the various neurons that make up the HFseg1 volume.

38 **Supplementary Notes**

39 **Descriptions of Supplementary Notes on cell type identification**

40 **Rods and cones.** Rod and cone photoreceptors were distinguished by the well-established and
41 distinctive morphology of their axon terminals, the rod small spherules ($n = 24$) and the large cone
42 pedicles ($n = 313$). We also confirmed that the invaginating contact to the small number of rod
43 spherules in our volume derived from subsequently identified rod bipolar cells. Note also that rods could
44 also be distinguished from cones by the much smaller diameter of their axons within the Henle fiber
45 layer (HFL). These features were not quantified but are evident in the segmented 3D view of the cell's
46 morphology.

47 Unlike in macaque and marmoset retina, human S cone pedicles ($n = 16$) were not easily distinguished
48 by smaller overall size, reduced ribbon synapse number or lack of telodendritic contacts with
49 neighboring cone pedicles (Fig. 2a; Supplementary Table 1c). We therefore relied on the distinctive
50 dendritic and axonal morphology of the blue cone bipolar type (BB) that makes selective invaginating
51 contact with S cones and the concomitant lack of an ON-midget bipolar (IMB) connection. The L and M
52 cone pedicles have not been previously distinguished by morphology or connectivity, with one possible
53 exception. In the present dataset we have not yet attempted to determine if two groups of non-S cone
54 pedicles are present and have used LM to refer to the combined L and M cone pedicles ($n = 297$) (Fig.
55 2a; Supplementary Table 1c).

56 **Horizontal cells.** Morphological and connectional differences defining H1 and H2 horizontal cell types
57 in the primate retina are well established and largely consistent in the HFseg1 volume. However, H1
58 cells frequently contact S cones and greatly outnumbered H2 cells. The H1 cells ($n = 256$,
59 Supplementary Table 1e) were distinguished by small ($\sim 30\text{--}50\ \mu\text{m}$ diameter) and profusely branched
60 dendritic trees (Fig. 2c). Neighboring H1 cells overlapped extensively such that each cone pedicle was
61 contacted by several H1 cells. A characteristic single, long axon-like process arose from the soma and
62 extended to the edge of the volume within the OPL without branching. These long unbranched

63 processes are known to terminate in a profuse arbor that innervates rod spherules^{1, 2}. All H1 cell axons
64 in HFseg1 extended beyond the volume boundaries and did not contact the rod spherules within the
65 volume which were therefore innervated by axonal processes that originated from H1 cells outside the
66 volume. In contrast to H1 cells, the H2 cells (n = 12, Supplementary Table 1e) showed very large, and
67 loosely branched dendritic trees (>100 µm in diameter) that showed little dendritic overlap with
68 neighboring H2 cells³⁻⁵ (Fig. 2d). The long H2 cell dendrites tended to converge on and contact S cones
69 located within their fields, while only sparsely contacting LM cones (Extended Data Fig. 4). The axon of
70 the H2 cell is also distinguishes it from the H1 cell in that it takes a meandering course, branches
71 sparsely and gives rise to lateral elements in widely spaced cones; most of which are also S cones.

72 **Bipolar cells.** In foveate primates, flat (FMB, OFF type) and invaginating (IMB, ON Type) midget
73 bipolar cells show dominant connections to single cones and ganglion cells, whereas diffuse types
74 contact several cones and comprise 8 previously recognized types (DB1, 2, 3a, 3b, 4, 5, 6 and Giant)⁶
75 (Fig. 3). A single blue cone bipolar (BB) cell type shows selective targeting of S cones (Extended Data
76 Fig. 5), and a single rod bipolar type makes selective contact with rod spherules. It was possible to
77 recognize and annotate all of these types in HFseg1 with the exception of the DB3a-DB3b distinction
78 recently made in macaque and human retina⁷⁻¹² and the possible absence of the Giant type.

79 Of the cells with distinctive cone connectivity, FMB (n = 302, Supplementary Data Table 1f) and IMB
80 cells (264 cells, Supplementary Data Table 1f; Fig. 3a, 3b) accounted for 62% of all bipolar cells. Blue-
81 cone bipolar cells (BB cells; Fig. 3b, 3c; Extended Data Fig. 5) by contrast formed only 1.9% of all
82 bipolar cells (17 cells, Supplementary Data Table 1f). Of the presumed OFF diffuse bipolar types we
83 identified DB1¹³ (54 cells), DB2 (92 cells) and DB3 (41 cells, Supplementary Data Table 1f, Fig. 3a, 3c)
84 types. We could not further divide the outer DB3 cells into previously identified DB3a, DB3b types⁷⁻¹⁰.
85 Instead, we found only two bipolar populations that costratified in the IPL and tiled the IPL with their
86 axonal arbors that we therefore referred to as DB2 and DB3. The DB3 cells showed large axonal fields
87 and likely correspond to the calbindin-positive DB3a type in macaque, marmoset and human retina^{11, 12},

88 ^{14, 15}. If this association holds then DB3b cells are either absent or present at a very low density in the
 89 foveal DB population.

90 In the inner half of the IPL, DB4 (n = 34), DB5 (n = 45) and DB6 cells (n = 12, Supplementary Data
 91 Table 1f; Fig. 3) were distinguished by axonal morphology, non-overlapping spatial arrangement and/or
 92 stratification depth in the IPL. A small number of cells that remain to be studied in detail could include
 93 the Giant type (n = 7, Supplementary Data Table 1f). Lastly, rod bipolar cells (RB, 6 cells, 0.7% of total
 94 BCs, Supplementary Data Table 1f) made invaginating contacts^{16, 17}, with rod spherules and showed
 95 small unbranched axonal arbors that contained far fewer ribbon synapses (15.8 ± 2.2 ribbons, n = 5)
 96 compared to diffuse or midget bipolar cells (see the detailed number of synapses in Extended Data Fig.
 97 9)^{18, 19}. As presented in the Results x-type bipolar cells (n = 20, Supplementary Data Table 1f) were
 98 further distinguished by a lack of an outward extending dendrite but the presence of abundant ribbon
 99 synapses in the axonal arbor (see also Extended Data Fig. 6). DBbroad cells (n = 18, Supplementary
 100 Data Table 1f)) were distinguished by an axonal arbor that extended variably into both the outer-OFF
 101 and inner-ON IPL and was presynaptic to both ON and OFF ganglion cell types. In the OPL, the
 102 DBbroad cell dendrites remain to be completely proofread, but thus far we found only basal contacts
 103 with cone pedicles and have placed the DBbroad cells provisionally in the OFF bipolar category, though
 104 the lack of invaginating cone contacts does not preclude an ON-type response from these cells²⁰.

105 **Amacrine cells.** Amacrine cell types show a great morphologically diversity and have been divided into
 106 many more types than other retinal cell classes. The result is that with a few well studied exceptions
 107 there is little consensus on the number of amacrine cell types and how they compare across species.
 108 To annotate HFseg1 amacrine cells we started with the high density, small field AII amacrine (AC2, n = 75
 109 cells, Supplementary Data Table 1g) that surprisingly accounted for over 25% of the amacrine cells with cell
 110 bodies in the volume. These cells showed the characteristic lobular appendages making synaptic
 111 output to cone bipolar cell axon terminals and long arboreal dendrites stratified near the IPL-GCL
 112 border, despite the lack of rod bipolar axon terminals at this foveal location (Figs. 4a and 5). We

divided all small field ($< 100 \mu\text{m}$ diam) amacrine cells, including AC2, into six provisional types (AC2, 3, 4, 5, 6 and 11) based on dendritic morphology, spatial tiling and IPL stratification depth, pending a more detailed analysis of their synaptic connectivity. Typical of the small field types, AC11 illustrated in Figure 4 ($n = 22$, Supplementary Data Table 1g) showed fine, densely branched, dendrites that defines a group of small field cells conventionally referred to as “knotty” amacrine²¹⁻²³. The broadly stratified AC11 may correspond to the strongly parvalbumin positive cells described in macaque monkey²⁴. Small field cells together accounted for ~65% of the amacrine cells in HFseg1.

Cells with larger ($> 100 \mu\text{m}$) dendritic fields were divided into 13 provisional types based mainly on morphology (including nuclear staining pattern, see Extended Data Fig. 3) and stratification depth in the IPL. Some of these types were easily classified by well documented morphology (e.g., the starburst amacrine cells illustrated in Fig. 4 or the interplexiform cells illustrated in extended data Fig. 8). Almost all starbursts were found in the ganglion cell layer (GCL, see Fig. 4d), where they were the most numerous AC type (14/40 cells, 35%), consistent with previous estimates^{25, 26}. Other large field types like AC12 ($n = 6$; Extended data Fig. 8; Supplementary Data Table 1g) or AC8 (10 cells, Fig. 4g, 4h; Supplementary Data Table 1g) showed a stereotyped morphology and stratification pattern that made them relatively easy to classify. Several large field types showed sparsely branching dendrites and varied stratification patterns; for these groups and analysis of synaptic connectivity will be important for confirming or extending the current amacrine cell classification.

Ganglion cells. The morphology of the major, relatively high density, ganglion cell types, the midget, parasol and small bistratified cells is well established, and we therefore were able to annotate these clearly recognized types unequivocally without the need for detailed proofreading. As reviewed in the Results these cells accounted for ~96% of the total ganglion cells with cell bodies within the HFseg1 volume, leaving only 25 ganglion cells that remained to be characterized.

We divided these remaining large field cells into 5 provisional types. Large field type GC1 (LFGC1, 6 cells, Supplementary Data Table 1h; Fig. 7d, 7h) was postsynaptic to blue-cone bipolar cells and had a

138 dendritic tree that co-stratified with the small bistratified cells. These cells likely correspond to the large
 139 bistratified GC observed in the human fovea²⁷ and marmoset and macaque retinal periphery^{28, 29}.
 140 Consistent with input from blue-cone bipolar cells these cells showed an ON-response to S cone
 141 modulation in macaque retina²⁸. Large field GC2 (LFGC2, 6 cells, Supplementary Data Table 1h; Fig. 7i,
 142 7l) showed broadly stratified and densely branched dendritic trees; a counterpart in monkey retina
 143 remains unclear³⁰ but similar morphology has been observed in human peripheral retina³¹. A third cell
 144 group (LFGC3, 4 cells, Supplementary Data Table 1h; Fig. 7j, 7m) stratifies across the center of IPL
 145 (Fig. 7j, 7m) and appears to correspond to previously described recursive bistratified cells, identified as
 146 ON-OFF direction selective cells in the macaque monkey retina³². A fourth cell group (LFGC4, 5 cells,
 147 Supplementary Data Table 1h; Fig. 7k, 7n) shows large cell bodies and radiate dendritic branching near
 148 the center of the IPL co-stratified with the inner-ON and outer-OFF parasol cells (3 inner and 2 outer
 149 stratified) and could correspond to the ON and OFF smooth mono-stratified types identified in macaque
 150 and marmoset retina^{29, 32, 33}. A fifth group of cells shows very sparsely branching dendrites stratified in
 151 the inner IPL (LFGC5, 4 cells, Supplementary Data Table 1h) that could correspond to inner melanopsin
 152 cells³⁴ (or an inner large sparse type recognized in macaque and marmoset^{29, 30}). Lastly, a few very
 153 sparsely branching GC processes (not linked to cell bodies within the volume) stratified along the outer
 154 border of the IPL were also present, likely corresponding to outer melanopsin ganglion cells³⁴. If
 155 included in our current total this would give 7 large field types and a total of 12 ganglion cell types in
 156 HFseg1.

157 **Glial cells.** The common radial glia of the retina, the Müller cells (MC, n = 555, Fig. 1; Supplementary
 158 Data Table 1b), were unequivocally recognized as a clear population of cell bodies in the approximate
 159 middle of the INL with large, irregularly shaped and euchromatic nuclei and a relatively filamentous,
 160 dark cytoplasm. A sparsely distributed population of glial cells was also present in the GCL (Extended
 161 Data Figs. 1b and 3b) and provisionally identified as astrocytes³⁵ (n = 32, Supplementary Data Table
 162 1b). Astrocyte cell bodies and cytoplasm were similar in appearance to Müller cells. However, astrocyte

163 cell bodies apposed blood vessels in the GCL (Extended Data Fig. 3b) and gave rise to multiple
164 dendrites that extended radially into the GCL and IPL (Extended Data Fig. 1b) and tended to fasciculate
165 along blood vessels. Dendrites of these astrocytes were also restricted to the GCL and IPL and thus did
166 not show the thick extension to the outer retina characteristic of Müller cells (Extended Data Fig. 1b).
167 Our identification of small population microglial cells (n = 15, Supplementary Data Table 1b) was based
168 on a characteristic dendritic morphology, nuclear morphology distinct from both Müller cells and
169 astrocytes (Extended Data Fig. 3a-3c) and very lightly stained cytoplasm containing apparent cellular
170 debris. The density of microglial cells was low, consistent with previous measurements in the macaque
171 monkey foveal retina^{36, 37}. In the current volume we have not yet attempted an analysis of blood vessel
172 and associated pericytes, though these cellular elements are also segmented and are available to
173 annotate and further characterize.

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176 **Supplementary References**

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