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The Structure of Vertebrate Retinas

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Abstract: The vertebrate retina is formed from six distinct neuronal classes: (1) photoreceptors; (2) bipolar cells; (3) ganglion cells; (4) horizontal cells; (5) amacrine cells and (6) interplexiform cells. Most vertebrates possess a single type of rod photoreceptor and most non-mammalians have morphologically pleomorphic cone photoreceptors displaying different pigments and/or connectivities. Cartilaginous fishes and mammals possess monomorphic cones of similar forms regardless of pigment content. Bipolar cells range from ≈10 types in mammals to over 15 in cyprinid fishes. Many non-mammalians exhibit up to 4 types of cone-selective horizontal cells, plus a separate rod horizontal cell in fishes, while mammalian horizontal cells are usually of two types with the axon terminal of one contacting rods. Amacrine cells are diverse, with over 70 forms documented in cyprinid fish retinas and over 20 in mammals. Similar diversity characterizes ganglion cells, especially in cone-dominated non-mammalians. The distributions of interplexiform cells are poorly known, but many vertebrates appear to have one or more types containing GABA, glycine, or dopamine. Photoreceptors, bipolar cells and most ganglion cells contain molecular signatures characteristic of glutamatergic neurons, while all amacrine cells contain primary GABAergic or glycinergic signatures, regardless of whether a secondary transmitter is present (acetylcholine, serotonin, peptides).

Key words: photoreceptors, retinal neurons, neuronal patterning, neuronal stratification

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This chapter is designed as a brief key to the structural elements of vertebrate retinas, taking its form in part from traditional field guides, in part from Walls' *The Vertebrate Eye and Its Adaptive Radiation* [1], and in part from two decades of Taniguchi symposia. The citations are restricted to representative classic and exemplary recent sources that link to other important references. Other chapters in this book will elaborate upon the forms and actions of specific cell types. The chapter is built on six figures with detailed captions serving as the text.

Figure 1: The plan of the retina

The retinas of vertebrates (except for those with intracephalic lateral eyes, e.g. myxinoids) are composed of three operational layers: (1) a rod and cone photoreceptor "input" layer interdigitating with apical processes of the retinal pigment epithelium (RPE: a polygonal epithelium monolayer that seals the retina from the choroidal circulation); (2) an intermediate neuronal layer connecting the input and output layers; and (3) a ganglion cell (GC) "output" layer forming the innermost neuronal layer, sealed from the vitreous by the foot pieces of Müller cells (MCs: the radial glia of the retina). These layers include six distinct *histological* layers: the *photoreceptor layer*, the *outer plexiform layer* (OPL), the *inner nuclear layer* (INL), the *inner plexiform layer* (IPL), the *ganglion cell layer* (GCL) and the *optic fiber layer* (OFL). Layers more distant from the brain in the synaptic chain are "distal" and those closer are "proximal." The photoreceptor layer is split by the external limiting membrane (ELM: a high-resistance layer of tight junctions among MC distal processes and photoreceptors) into: (1) a proximal *outer nuclear layer* (ONL)

formed of photoreceptor inner limbs and a honeycomb of MC processes; and (2) a distal layer of photoreceptor outer limbs, MC microvilli extending past the ELM, and apical RPE processes. The neural retina is composed of interconnected neurons: bipolar cells (BCs), horizontal cells (HCs), amacrine cells (ACs), interplexiform cells (IPCs, not shown), ganglion cells (GCs) and rare biplexiform cells (not shown). Efferent fibers in many vertebrates enter the retina through the optic nerve and largely target ACs [2,3]. The OPL is the site of synaptic connectivity between photoreceptors and their targets: HCs, BCs and, sometimes, other photoreceptors. In most vertebrates the OPL is roughly laminated, cone synaptic pedicles forming a central layer and rod spherules positioned 1-5 μm distally. Proximal to the pedicles is a zone of mixed MC, BC, HC and IPC processes where some synaptic contacts take place. BC and HC preterminal dendrites arise there, coursing distally to contact photoreceptors. Rod and cone terminals are often partially insulated by MC processes. In avian retinas, the OPL is often bi- or tristratified, as there is insufficent room for all cone pedicles in one layer [4]. The INL in many vertebrates is divisible into overlapping distal → proximal HC, BC, MC and AC layers [5,6]. The HC layer of most vertebrates shows further stratification [7,8], as does the AC layer of vertebrates with large cone densities [6]. The IPL is heavily laminated, reflecting the distal → proximal layering of synaptic zones associated with construction of specific GC receptive field types [9]. The GCL in most vertebrates is a single layer of mixed neuronal types, predominantly containing GCs but also "displaced" ACs [10]. In animals with retinal areas of high cone density, the GCL can be packed six somas deep. The OFL includes GC axons, occasional astrocytes and is proximally delimited by the end feet of MCs. It is also a possible signal integration region: the "superficial" plexiform layer [11].

Glial cells (see Chapter by Puro): MCs are radial glia, comprising 30-50% of the retinal volume [5]. MC somas are located in the ACL or displaced towards the middle of the INL in thicker retinas. Proximal MC stalks enter the IPL and may divide into radial daughter stalks with lateral stratified extensions. MC end feet form the internal limiting membrane (ILM), in combination with astrocytes in some retinas: a permeability barrier of varied efficacy. Distal MC fibers wrap interstitial leaflets around BCs, branching heavily in the ONL in a honeycomb basket, and form the ELM. Microvillar extensions protrude past the ELM along the inner segments of photoreceptors. The ELM restricts molecular diffusion. Distinctive MC macromolecular signatures include glutamine synthetase, vimentin and glial fibrillary acidic protein (especially in traumatized MCs) . Micromolecular signatures include high intracellular taurine/glutamine levels and low glutamate/glycine/GABA levels [5,12]. Some MC functions include glutamate transport [13] and carbon chain recycling [14] (all species); GABA transport [15] (mammals, snakes, chondrichthyans, cyclostomes); K^+ buffering and siphoning [16]. Astrocytes are abundant near the optic nerve head in many species, and are sparsely distributed among the endfeet of MCs across the retinas of several species [17]. They participate in forming the ILM in many vertebrates.

Retinal pigment epithelium (see Chapter by Tamai): The RPE is a monolayer of polarized epithelial cells coupled by gap junctions, forming the distal blood-retinal barrier. The basal surface apposes Bruch's membrane and the basolateral surface is sealed from the apical RPE processes by tight junctions. RPE functions include [18]: transport of all-trans retinol from the basal and apical extracellular spaces; storage, isomerization, and oxidation of retinol; partitioning retinal to the subretinal space; recognition and phagocytosis of photoreceptor outer segments; transport of oxygen and metabolites into the retina; dehydration and ionic regulation of the subretinal space. RPE cells in pigmented animals contain prolate ellipsoidal melanosomes (melanin granules ≈ 1 μm long), whose function seems to be absorption of image-degrading stray photons. In many non-mammalians, especially fishes and anuran amphibians, RPE apical processes extend nearly to the ELM, ensheathing light-adapted cones [19]. In these species, melanosomes show vectorial movements, concentrating into RPE somas in dark-adaptation and dispersing into apical processes in light adaptation. Many marine fishes possess additional pigmented organelles associated with optical isolation of outer segments. Teleost RPE apical processes in dorsal retina contain immobile reflective plates (often guanine crystals) that further optically isolate cones. Melanosome concentration in the dark-adapted retina exposes the plates for "second-chance" capture of reflected photons by rods.

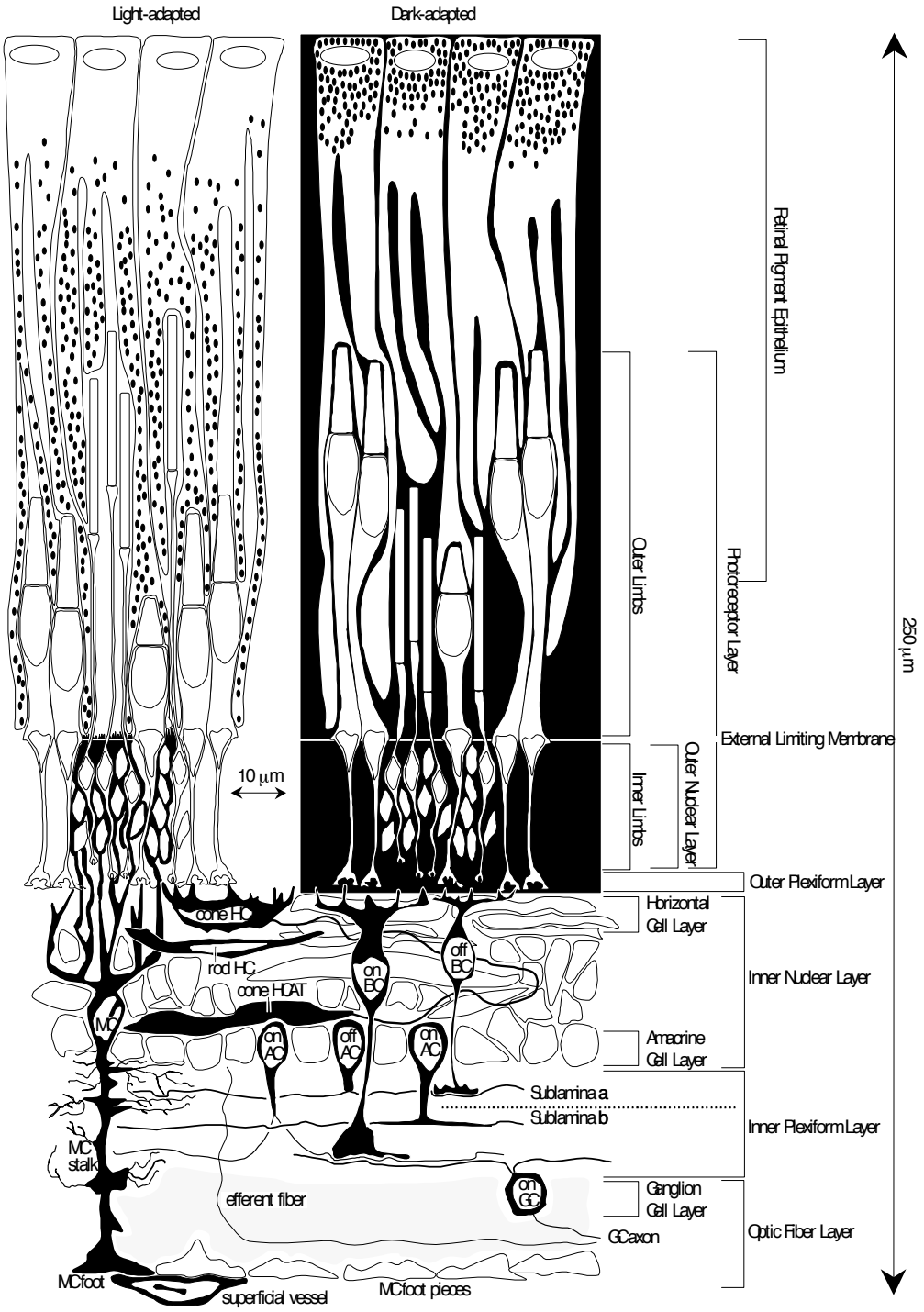
Light-adapted
Dark-adapted
Retinal Pigment Epithelium
Photoreceptor Layer
Outer Limbs
Inner Limbs
Outer Nuclear Layer
External Limiting Membrane
250 µm
10 µm
Outer Plexiform Layer
Horizontal Cell Layer
Inner Nuclear Layer
Amacrine Cell Layer
Inner Plexiform Layer
Ganglion Cell Layer
Optic Fiber Layer
cone HC
rod HC
cone HCAT
MC
on BC
off BC
on AC
off AC
on AC
Sublamina a
Sublamina b
MC stalk
efferent fiber
on GC
GCaxon
MCfoot
superficial vessel
MCfoot pieces

Figure 2: Basic photoreceptor forms. Far left: a *light-adapted* goldfish long single cone. Far right: a *dark-adapted* goldfish rod. Center top: a horizontal section though the basal outer segment of the cone. Center bottom: synaptic terminals of a cone and rod.

Most retinas are “duplex”, containing rods and cones. Pure rod retinas are rare (e.g. ratfishes). Cones and rods are specialized neuroepithelial cells with multiple compartments. The *outer segment* (OS) contains hundreds to thousands of free disks or contiguous membrane formed of flattened plasma membrane folds in which various opsins (the protein auxochrome of the visual pigment) are inserted (see Chapters by Shichida, Yau and Kawamura). The OS connects to the inner segment (IS) via a cytoplasmic neck through which a 9+0 cilium extends. The *ellipsoid*, the most distal part of the IS, contains a dense packet of mitochondria. The *myoid* contains diffuse structures including the endoplasmic reticulum and golgi apparatus. In teleosts and anurans the myoid is motile, contracting cones and extending rods in the photopic state and the reverse in the scotopic state, regulated in part by diffusible signals from the neural retina [20]. The ELM is the physical border between the outer and inner *limbs* of photoreceptors and defines the optical entrance aperture of the outer limb. In most vertebrates cone nuclei are positioned at the distal border of the ONL and often protrude past the ELM. In rod-rich species, rod nuclei form an irregular, stacked proximal sublayer. Rods and cones have an axon fiber, ≈ 0.5-2 μm in diameter in many species, terminating as a synaptic ending at the distal margin of the OPL.

A cone outer segment (COS) often literally resembles a truncated cone. The plasma membrane forms tightly stacked free lamellae on most of the COS circumference but fuses with the plasma membrane on the ciliar side [19]. In most bony fishes an accessory outer segment (AOS) of unknown function and large volume connects to the COS by a thin isthmus extending up the ciliar side of the OS. The base of the OS in many non-mammalian cones and rods is ringed by a palisade of actin-stiffened cytoplasmic fingers that may extend over half the length of a COS in some species. Each COS is surrounded by apical RPE processes, although mammals and avians show a large space around each COS composed of a complex extracellular matrix. The myoid of teleost cones is an active motor complex, extended (microtubule mediated) in dark-adapted and contracted (actin-mediated) in light-adapted retinas [21]. Non-mammalian cone myoids are often striped by longitudinal fins that interdigitate with MV microvilli [19,22,23]. Proximal to the ELM the cones become smooth, with sparse cytoplasm around the nuclear bulge, narrowing proximally to an axon fiber that expands into a synaptic pedicle.

The cone pedicle (see Chapter by Copenhagen) is a cupola-shaped chamber filled with synaptic vesicles and a few cisterns, vacuoles and coated vesicles (endocytic compartments). Teleost cones possess ≈ 12 presynaptic specializations (up to 40 in primates) each shaped as a linear *synaptic ridge* beneath which the arciform density forms a groove into which one edge of the synaptic ribbon (a pentalaminar plate) is inserted. The ribbon "striping" is the cross-sectioned plate whose two cytoplasmic surfaces to serve as vesicle tethering sites, feeding two rows of docking and fusion sites along each slope of the ridge for vesicular glutamate release [24]. Most pedicles are contacted by large horizontal cell (HC) processes lateral to the ribbon, with dendrites of certain ON-center bipolar cells (BCs) usually occupying the center lacunae at various distances from the release site [25]. In many non-mammalians telodendrial processes of other cones may occupy a central postsynaptic position near the ribbon, presumably for synaptic cone-cone signaling [26]. OFF-center BCs are usually positioned away from the ribbon at specialized adhesion points with cones [27]. These sites may contain postsynaptic receptors but apparently do not indicate cone release sites. Diffusion from the synaptic ridge appears to suffice. Cones and BCs also possess potent glutamate transporters whose detailed spatial localization is unknown [28].

Rod outer segments (ROSs) differ from those of cones. After a few lamella are formed at the base of the ROS, the rims of facing extracellular membrane surfaces fuse and then separate from the plasma membrane, creating free disks within a plasma membrane case [29]. Rod disks often possess deep incisures where the plasma membrane sharply indents the disk [30]. In non-mammalians, calyceal processes are essential in shaping the growing ROS, but the lamellar zone and the processes are short. Most ROSs have no identifiable AOS. As in cones, the ellipsoidal mitochondrial pack is positioned at the distal limit of the IS. Fish rod myoids are motile and contract in dark-adapted and extend in light-adapted states; both processes involve actin binding [21]. Rod synaptic terminals are often smaller than those of cones and in most fishes and mammals, containing a single ribbon. Fish rod spherules enclose lateral rod HC ribbon contacts, with mixed rod-cone ON-center BC dendrites crowding into a central position and mixed rod-cone OFF-center BCs positioned away from the ribbon [31].

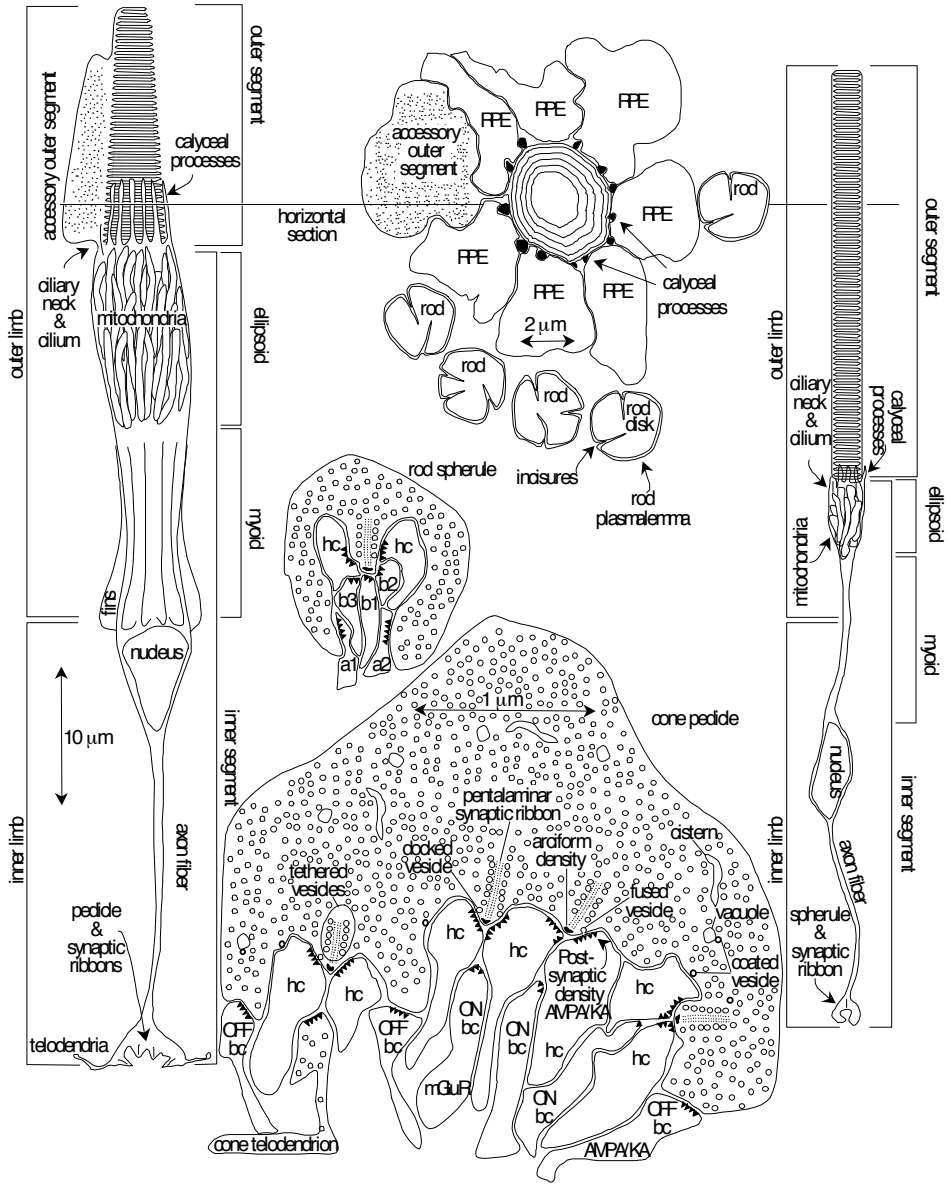
outer segment
accessory outer segment
calyceal processes
ciliary neck & cilium
mitochondria
ellipsoid
myoid
fins
nucleus
inner segment
10 μm
inner limb
outer limb
axon fiber
pedicle & synaptic ribbons
telodendria
horizontal section
PPE
accessory outer segment
rod
2 μm
rod disk
incisures
rod plasmalemma
rod spherule
hc
b2
b3
b1
a1
a2
1 μm
cone pedicle
pentalaminar synaptic ribbon
arciform density
cistern
docked vesicle
tethered vesicles
fused vesicle
vacuole
coated vesicle
Post-synaptic density AMPA/KA
ON bc
OFF bc
mGluR
cone telodendrion
AMPA/KA
spherule & synaptic ribbon

Figure 3: Diverse forms of vertebrate photoreceptor cohorts.

Different vertebrate systems have been exploited for structural, physiological, biochemical, developmental, genetic and psychophysical analyses of retinal function. Most non-mammalians have pleomorphic cones. The photoreceptor types of six popular models are presented here. The goldfish, *Carassius auratus* displays one form of teleost photoreceptor cohort containing double cones (DCs) of unequal members patterned with single cones (SCs), and rods filling remaining space [32,33]. Retinaldehyde (retinal) or dehydroretinal chromophores predominate in marine and fresh-water species/phases, respectively, though mixtures are common. The long goldfish DC (LD) contains a dehydroretinal-based pigment absorbing maximally at 625 nm: P625. The short (SD) cone contains P535. A variable number of long SCs contain P625 (LSR) or P535 (LSG) and short SCs (SS) contain P435. In young animals, miniature SS (MSS) cones with oblique axon fibers contain P360 [31]. Both rod and cone outer limbs can move tens of microns in response to adaptive signals. Other fishes have simpler cone sets and often display long-wave pigments in both members of either unequal or equal DCs. In most fishes rods outnumber cones 5-10:1.

The fresh water turtle *Psuedemys scripta elegans* typifies known chelonian retinas [35]. Unequal DCs are formed by a long principal (PC) and short accessory (AC) cone, both containing P620, with a yellow-orange carotenoid oil/wax droplet (o) in the PC. The AC lacks a droplet (as in all tetrapods) and has a distinctive paraboloid (p). The paraboloid is a glycogen storage body placed between the mitochondrial pack (m) and a supranuclear sac (s). Two SC types contain P620 and either red (r) or pale green (pg) droplets. P540 SCs contain a yellow orange (y) droplet and P460 SCs contain a clear droplet. P460 SCs have oblique axons. Rods (P520) lack oil droplets but have larger outer segments than cones, multiple synaptic ribbons and are sparser than in teleosts. Marine turtles have similar cell types but both droplets and pigments (retinal-based) are blue-shifted [50].

Avian retinas, represented here by the rock dove (*Columba livia*), have slender versions of the cones of reptiles [37]. PCs and ACs contain retinal-based P562 with a yellow-orange droplet in PCs and none in ACs (though yellowish granules often appear). P562 SCs have red droplets, P506 SCs have yellow droplets, P450 SCs have visibly clear droplets and P400 cones have UV-transparent droplets (t). Rods contain P506.

Amphibian retinas are diverse in photoreceptor form and switch from juvenile dehydroretinal to retinal-dominated adult pigments. Anurans such as *Rana pipiens*, possess two kinds of rods, named according to their appearance in a fresh, unbleached receptor mosaic. "Red" rods in adults contain P502 and smaller "green" rods contain P432. Not all frog cones have been classified but PCs contain P580 with a clear oil droplet and ACs contain P502 and no oil droplet. SCs with clear oil droplet contain P580, but blue and UV-sensitive cones are present in anurans. Certain urodeles, here represented by juvenile phase tiger salamanders (*Ambystoma tigrinum*), are polyploid organisms with huge cells: red (P520) and green rods (P432), PCs (P618), ACs (P520), and SCs (P618, P432, P380) [38-41].

Mammalians have cones of only two or three spectral types. Rods contain P500, red cones (long-wave sensitive, LWS) cones P556, green cones (mid-wave sensitive, MWS) P534, and blue cones (short-wave sensitive) P431 [42]. While superficially monomorphic, subtle shape differences exist between blue and non-blue cones, the former being slightly longer and more tapering [43]. Depending on whether the plane of section is distal (D) or proximal (P) relative to the ellipsoid of the blue cones, they may appear larger or smaller than surrounding cones in horizontal sections.

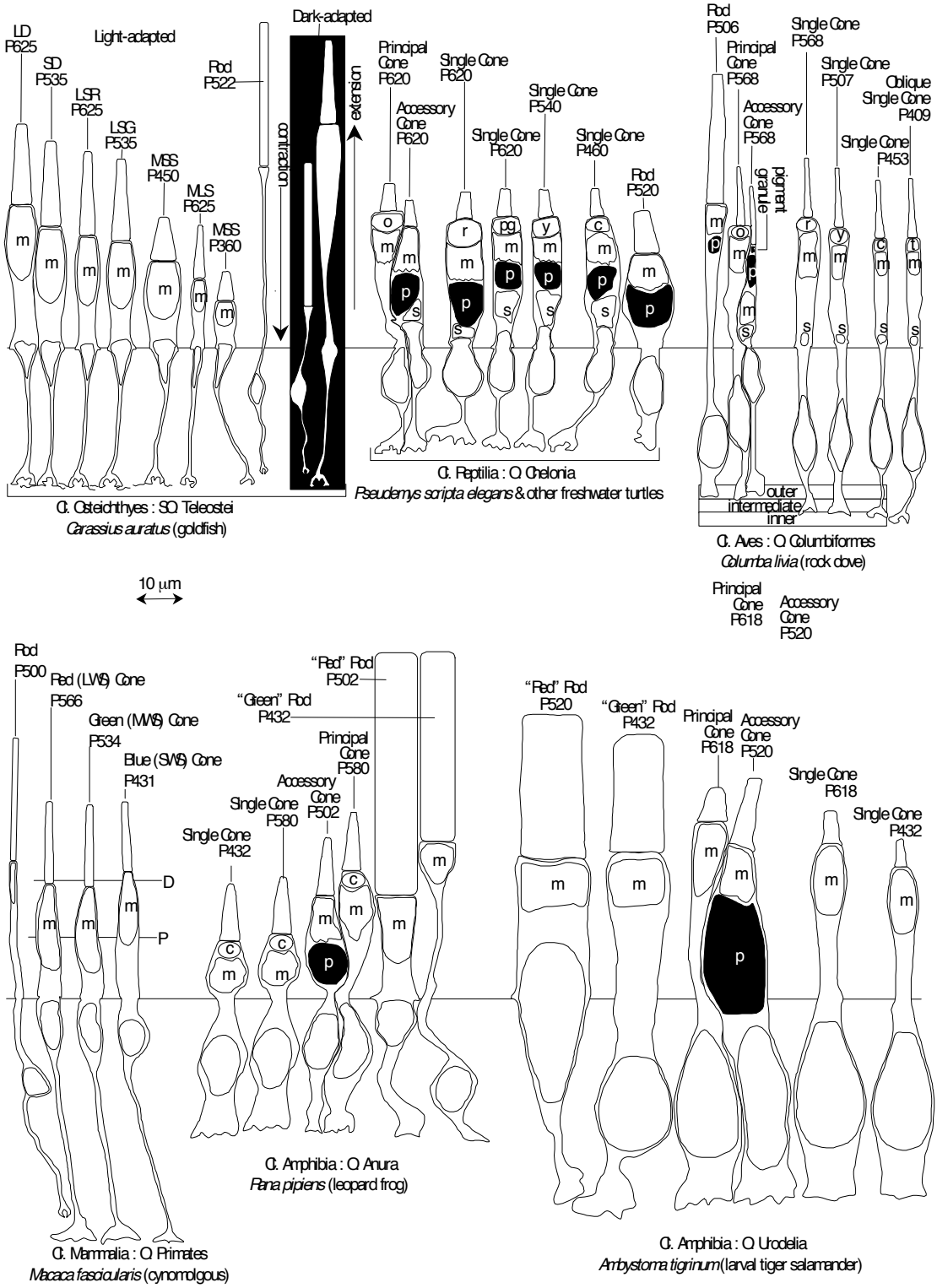
LD P625
SD P535
LSR P625
LSG P535
MSS P450
MLS P625
MSS P360
Light-adapted
Dark-adapted
Rod P522
contraction
extension
Cl. Osteichthyes : SO. Teleostei
Carassius auratus (goldfish)
Principal Cone P620
Accessory Cone P620
Single Cone P620
Single Cone P540
Single Cone P460
Rod P520
Cl. Reptilia : O. Chelonia
Pseudemys scripta elegans & other freshwater turtles
Rod P506
Principal Cone P568
Accessory Cone P568
Single Cone P568
Single Cone P507
Oblique Single Cone P409
Single Cone P453
pigment granule
outer
intermediate
inner
Cl. Aves : O. Columbiformes
Columba livia (rock dove)
10 μm
Principal Cone P618
Accessory Cone P520
Rod P500
Red (LWS) Cone P566
Green (MWS) Cone P534
Blue (SWS) Cone P431
D
P
"Red" Rod P502
"Green" Rod P432
Principal Cone P580
Accessory Cone P502
Single Cone P580
Single Cone P432
Cl. Amphibia : O. Anura
Rana pipiens (leopard frog)
"Red" Rod P520
"Green" Rod P432
Principal Cone P618
Accessory Cone P520
Single Cone P618
Single Cone P432
Cl. Amphibia : O. Urodelia
Ambystoma tigrinum (larval tiger salamander)
Cl. Mammalia : O. Primates
Macaca fascicularis (cynomolgous)

Figure 4: Vertebrate neuronal patterns:

The visual image is cast on the screen of the retina and the planar distribution of photoreceptors and neurons impacts the quality of the neural representation. Most vertebrates have no easily discernable patterning for cones containing middle or long-wave pigments but almost all have patterned short-wave or blue cones. In some cases, the cones are not locally patterned but are distributed differentially. In mice, green and blue cones are largely segregated to the dorsal and ventral parts of the retina, respectively [44]. In the primate retina there is a distinct differential depletion of blue cones in the fovea [45,46]. Bony fishes display the most orderly patterns, with true mosaics of DCs and SCs. In many fishes (e.g. perch and goldfish), the rhomboid is a common motif [32,46,47]. DCs form the sides of rhombs and SCs are positioned in the centers. When UV cones are present, they occupy some of the rhomb corners. Perch DCs are equal cones, while goldfish DCs are unequal, with LD and SD members usually taking alternating positions. Amphibian photoreceptor mosaics have not been thoroughly described, but green rods are randomly interspersed among the more numerous red rods. Mosaics are difficult to discern in reptiles or avians, but patterning is subtly present. The PCs and one type of SC in pigeon retinas fluoresce under 365 nm illumination and can be seen to form two distinct orderly arrays [48]. In primates, only blue cones have a non-random pattern [45,49]. Patterns are less obvious but still pervasive among the complex matrix of higher-order retinal neurons. When unique types of cells are isolated from contaminating cohorts, most retinal neurons are highly patterned, and random placements are rarities [50]. Even in retinas with many neuronal types it is possible to observed fine-scale, ordered neuronal placements: in the marine fish, the dragonet, three different kinds of BC terminals form distinct arrays [51]. On a scale two orders of magnitude greater, outer alpha GCs of a cichlid fish are independently patterned, with many different GC types interspersed [52].

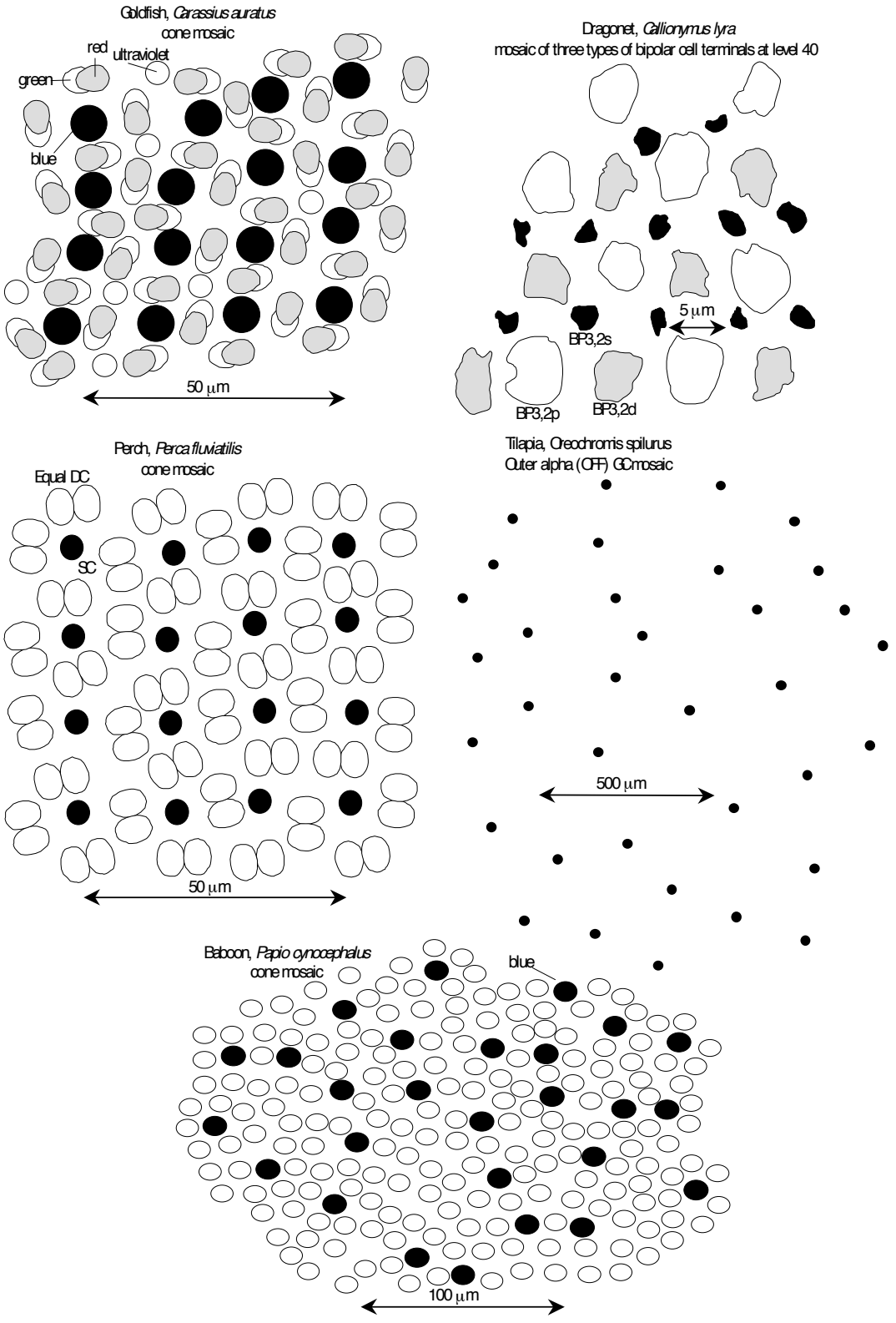
Goldfish, *Carassius auratus*
cone mosaic
red
ultraviolet
green
blue
50 μm
Dragonet, *Callionymus lyra*
mosaic of three types of bipolar cell terminals at level 40
5 μm
BP3,2s
BP3,2p
BP3,2d
Perch, *Perca fluviatilis*
cone mosaic
Equal DC
SC
50 μm
Tilapia, Oreochromis spilurus
Outer alpha (OFF) GC mosaic
500 μm
Baboon, *Papio cynocephalus*
cone mosaic
blue
100 μm

Figure 5. Signal flow in the retina (see Chapters by Kaneko, Wässle, Kaneko, Tachibana and Dacey). Vertical channels are glutamatergic and lateral channels are predominantly GABAergic/glycinergic.

The detailed forms of BCs, HCs, ACs and GCs have been described in many other sources and will be treated here only schematically [53-56]. The photoreceptor and BC neurotransmitter is glutamate [57]. In non-mammalians, rod signals are collected by special subsets of BCs but, even so, one of those is clearly a homologue of the mammalian rod BC. ON-center cells bear type III metabotropic glutamate receptors (mGluR6) [58]. OFF-center BCs are driven through ionotropic AMPA/KA receptors. Multiple mixed rod-cone BCs are known in goldfish, differing in form, rod weightings and cone selectivities [59]. Pure cone BCs are diverse in fishes and the connectivities of most remain unknown, though pure green, pure blue and mixed cone BCs have been established [60,61]. The mammalian retinal BC cohort is simpler: one rod BC, ≈ 7-9 types of diffuse cone BCs contacting all cone types [9]. Primates and ground squirrel retinas possess, in addition, both ON- and OFF-center midget BCs that contact but a single cone in central retina and a only few cones in the periphery. The restriction to a single cone means that foveal/central midget cells are potentially color-biased. Primates also have a blue cone BC [62].

BC axon terminal positions reveal the rich laminar organization of the IPL. OFF-center BCs terminate in the distal third to half of the IPL (sublamina **a**) and ON-center BCs in the proximal two-thirds to half (sublamina **b**) [63, 64]. AC and GC dendritic arbors laminate according their BC sources: OFF-center ACs/GCs in sublamina **a**, ON-center ACs/GCs in sublamina **b** and ON-OFF cells in both. GC populations in vertebrates are diverse and several known morphologies correlate with stimulus-response patterns [9](see Chapters by Rodeick and Sterling).

Lateral processing in the retina shapes attributes of vertical channels . HCs (see Chapters by Miyachi, Kaneko and Toyoda) are feedback and possibly feedforward inhibitory interneurons, many varieties of which display GABAergic markers [15]. The GABAergic nature of HC transmission remains controversial. Most HCs lack *bona fide* presynaptic specializations, although clear HC→IPC vesicular contacts are known [65,66]. GABAergic HC feedback transmission is thought to be mediated by reverse transport (see Chapter by Schwartz). In fishes, GABAergic markers have been associated only with non-color coded cone HCs; all other HC types lack GABAergic markers [56]. All HCs seem to bear pharmacologically similar sets of AMPA/KA receptors. Pure rod HCs do not exist in tetrapods and rod contacts are made by axon terminal fields arising from cells whose somas contact cones [55]. While variations exist, most mammalian HCs resemble rabbit HCs. Type A HCs are strongly coupled, lack axons, have large processes and contact cones [55, 67]. Type B HC somas contact cones and have an extensive axonal arbor contacting rods [67]. The mechanism of mammalian HC feedback remains mysterious. It could be GABAergic, but most mammalian HCs cells apparently lack markers characteristic of CNS GABA neurons. Subcellular localizations of GABAergic markers have now been found in central rabbit HCs [68].

ACs (see Chapter by Masland) are the key lateral interneurons of the retina and are extremely diverse [53,54,56]. They directly shape BC responses by feedback/feedforward inhibition and GC responses by feedforward. Mammalian ACs contain GABA, glycine or both [5]. All non-mammalian ACs or IPCs contain GABA/glycine/both, except the dopaminergic interplexiform cell of teleost fishes [69]. ACs are highly stratified, reflecting BC organization. Every BC type receives extensive GABAergic AC input and anatomical evidence indicates that each BC likely has a unique cohort of AC inputs, though some ACs may service many BCs [15].

IPCs (see Chapter by Hashimoto) may exist in more forms than have been clearly delineated but the two best studied forms are the dopaminergic (DA) and glycinergic (gly) IPCs of teleost retinas [69]. DA IPCs primarily receive GABAergic input in the IPL and target cells in the OPL, typified by HCs. Gly IPCs are more complex and receive input from HCs, bypassing the BC filter altogether, and targeting cells in the inner retina.

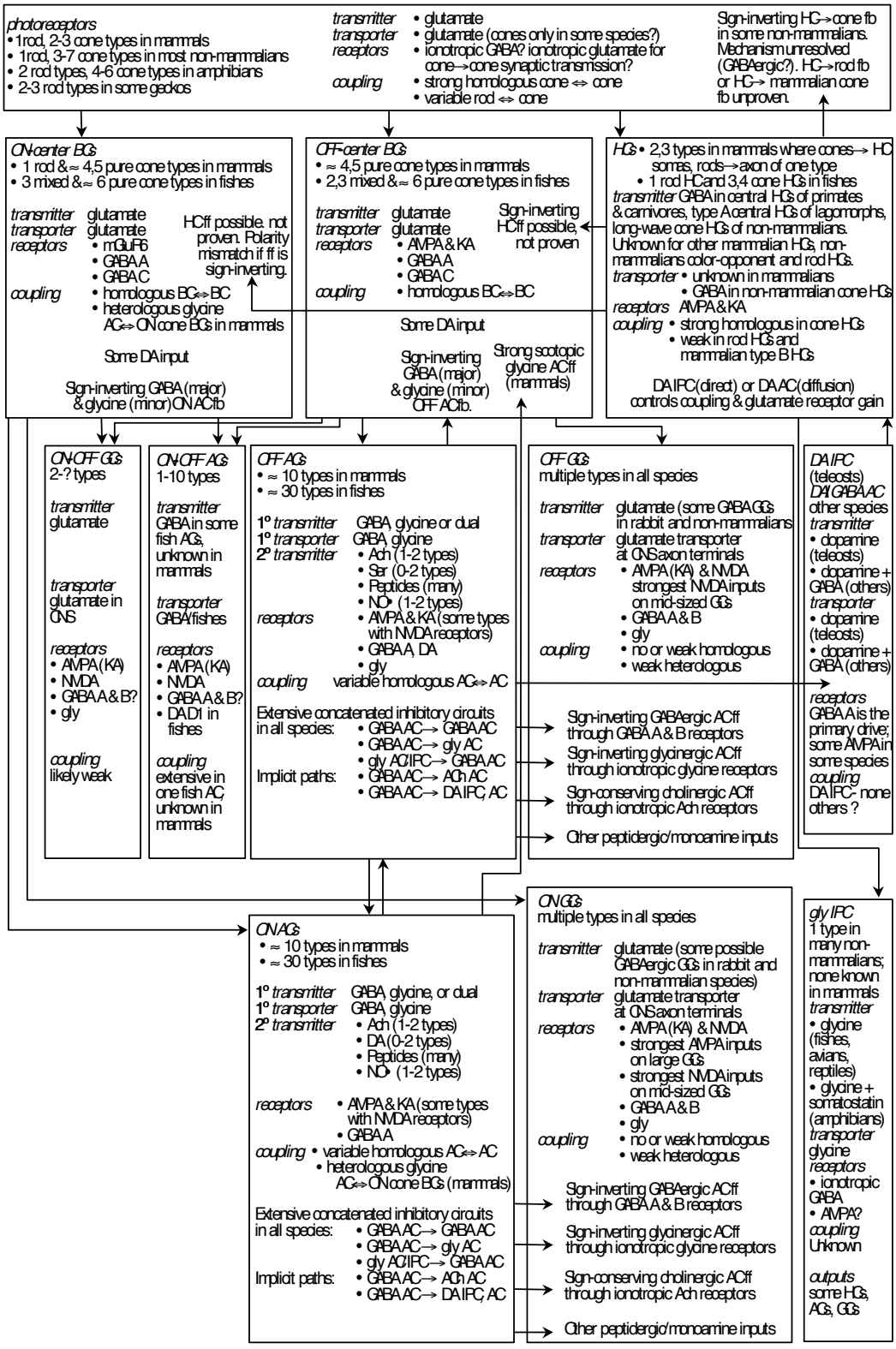
photoreceptors
• 1 rod, 2-3 cone types in mammals
• 1 rod, 3-7 cone types in most non-mammalians
• 2 rod types, 4-6 cone types in amphibians
• 2-3 rod types in some geckos
transmitter • glutamate
transporter • glutamate (cones only in some species?)
receptors • ionotropic GABA? ionotropic glutamate for cone→cone synaptic transmission?
coupling • strong homologous cone ⇔ cone
• variable rod ⇔ cone
Sign-inverting HC→cone fb in some non-mammalians. Mechanism unresolved (GABAergic?). HC→rod fb or HC→ mammalian cone fb unproven.
ON-center BCs:
• 1 rod & ≈ 4,5 pure cone types in mammals
• 3 mixed & ≈ 6 pure cone types in fishes
transmitter glutamate
transporter glutamate
receptors • mGluR6
• GABA A
• GABA C
coupling • homologous BC⇔BC
• heterologous glycine AC⇔ON cone BCs in mammals
HCff possible, not proven. Polarity mismatch if ff is sign-inverting.
Some DA input
Sign-inverting GABA (major) & glycine (minor) ON ACfb
OFF-center BCs:
• ≈ 4,5 pure cone types in mammals
• 2,3 mixed & ≈ 6 pure cone types in fishes
transmitter glutamate
transporter glutamate
receptors • AMPA & KA
• GABA A
• GABA C
coupling • homologous BC⇔BC
Sign-inverting HCff possible, not proven
Some DA input
Sign-inverting GABA (major) & glycine (minor) OFF ACfb.
Strong scotopic glycine ACff (mammals)
HCs • 2,3 types in mammals where cones→ HC somas, rods→axon of one type
• 1 rod HC and 3,4 cone HCs in fishes
transmitter GABA in central HCs of primates & carnivores, type A central HCs of lagomorphs, long-wave cone HCs of non-mammalians. Unknown for other mammalian HCs, non-mammalians color-opponent and rod HCs.
transporter • unknown in mammalians
• GABA in non-mammalian cone HCs
receptors AMPA & KA
coupling • strong homologous in cone HCs
• weak in rod HCs and mammalian type B HCs
DA IPC (direct) or DA AC (diffusion) controls coupling & glutamate receptor gain
ON-OFF GCs
2-? types
transmitter glutamate
transporter glutamate in ONS
receptors
• AMPA (KA)
• NMDA
• GABA A & B?
• gly
coupling likely weak
ON-OFF ACs
1-10 types
transmitter GABA in some fish ACs, unknown in mammals
transporter GABA/fishes
receptors
• AMPA (KA)
• NMDA
• GABA A & B?
• DA D1 in fishes
coupling extensive in one fish AC, unknown in mammals
OFF ACs
• ≈ 10 types in mammals
• ≈ 30 types in fishes
1º transmitter GABA, glycine or dual
1º transporter GABA, glycine
2º transmitter
• Ach (1-2 types)
• Ser (0-2 types)
• Peptides (many)
• NO• (1-2 types)
receptors
• AMPA & KA (some types with NMDA receptors)
• GABA A, DA
• gly
coupling variable homologous AC⇔AC
Extensive concatenated inhibitory circuits in all species:
• GABA AC→ GABA AC
• GABA AC→ gly AC
• gly AC/IPC→ GABA AC
Implicit paths:
• GABA AC→ ACh AC
• GABA AC→ DA IPC, AC
OFF GCs
multiple types in all species
transmitter glutamate (some GABA GCs in rabbit and non-mammalians)
transporter glutamate transporter at ONS axon terminals
receptors
• AMPA (KA) & NMDA
• strongest NMDA inputs on mid-sized GCs
• GABA A & B
• gly
coupling
• no or weak homologous
• weak heterologous
Sign-inverting GABAergic ACff through GABA A & B receptors
Sign-inverting glycinergic ACff through ionotropic glycine receptors
Sign-conserving cholinergic ACff through ionotropic Ach receptors
Other peptidergic/monoamine inputs
DA IPC (teleosts)
DA GABA AC other species
transmitter
• dopamine (teleosts)
• dopamine + GABA (others)
transporter
• dopamine (teleosts)
• dopamine + GABA (others)
receptors
GABA A is the primary drive; some AMPA in some species
coupling
DA IPC - none others ?
ON ACs
• ≈ 10 types in mammals
• ≈ 30 types in fishes
1º transmitter GABA, glycine, or dual
1º transporter GABA, glycine
2º transmitter
• Ach (1-2 types)
• DA (0-2 types)
• Peptides (many)
• NO• (1-2 types)
receptors
• AMPA & KA (some types with NMDA receptors)
• GABA A
coupling
• variable homologous AC⇔AC
• heterologous glycine AC⇔ON cone BCs (mammals)
Extensive concatenated inhibitory circuits in all species:
• GABA AC→ GABA AC
• GABA AC→ gly AC
• gly AC/IPC→ GABA AC
Implicit paths:
• GABA AC→ ACh AC
• GABA AC→ DA IPC, AC
ON GCs
multiple types in all species
transmitter glutamate (some possible GABAergic GCs in rabbit and non-mammalian species)
transporter glutamate transporter at ONS axon terminals
receptors
• AMPA (KA) & NMDA
• strongest AMPA inputs on large GCs
• strongest NMDA inputs on mid-sized GCs
• GABA A & B
• gly
coupling
• no or weak homologous
• weak heterologous
Sign-inverting GABAergic ACff through GABA A & B receptors
Sign-inverting glycinergic ACff through ionotropic glycine receptors
Sign-conserving cholinergic ACff through ionotropic Ach receptors
Other peptidergic/monoamine inputs
gly IPC
1 type in many non-mammalians; none known in mammals
transmitter
• glycine (fishes, avians, reptiles)
• glycine + somatostatin (amphibians)
transporter
glycine
receptors
• ionotropic GABA
• AMPA?
coupling
Unknown
outputs
some HCs, ACs, GCs

Figure 6: Functional lamination of the IPL

One of the most striking features of the retina is the lamination of the IPL, which is far richer than suggested by mere divisions into ON and OFF, or rod-ON /cone-ON / cone –OFF (see Chapter by Wässle). It has been traditional to divide the IPL into five arbitrary, equal sublayers, but biological lamination is clearly more complex. For example, the levels of the IPL at which various types of goldfish BCs terminate are known in detail, even if all the connections are not, and demonstrate that specific ganglion cells must send dendrites to different levels to acquire signals from those cells. OFF-center Ma BCs differentially stratify over levels 10-30 while ON-center Mb BCs stratify in levels 75-95. Pure cone BCs are very diverse and include cells with double (C2a, C2b) and triple (C3a, C3b) stratifications within sublayers a and b as well as across the entire IPL (C2ab – likely green cone BCs; C3ab – likely blue cone BCs; and C4ab – of unknown connectivity). Markers for molecules such as calbindin indicate that functional sublamination can be ever more precise and that individual neuronal strata can be less than 2 μm in width [70]. In many retinas, simple structural observations imply the existence of a minimum of 15 sublayers, some of which are very thick and likely to be subdivided further [50]. The GABAergic AC stratification pattern of the pigeon retina reveals a minimum of 15 sublayers [71]. Even the thin goldfish IPL (25-30 μm) is highly stratified. By accounting for the laminar positions of BCs, kainate (KA) and NMDA sensitivity, cholinergic processes (ACh), and various known types of ACs, a minimum of 15 functional sublayers emerges there also. If any GC differs from another type only slightly in its level of arborization in the IPL, the differential composition of that layer will confer upon unique stimulus selectivities on that GC. Indeed, the key characteristic of most of the 20-30 amacrine cell types of the rabbit retina is the stratification pattern of each cell's dendrites [71].

In summary, the diverse photoreceptor/neuronal types are arrayed in patterns, many of which have yet to be discovered, and their synaptic connections are partially revealed by the fine laminar organization of the inner plexiform layer. Differential distributions of neurotransmitters and receptor subtypes confer upon each cell distinct input/output characteristics. The molecular, structural and biophysical attributes of retinal processing will be discussed in the following chapters.

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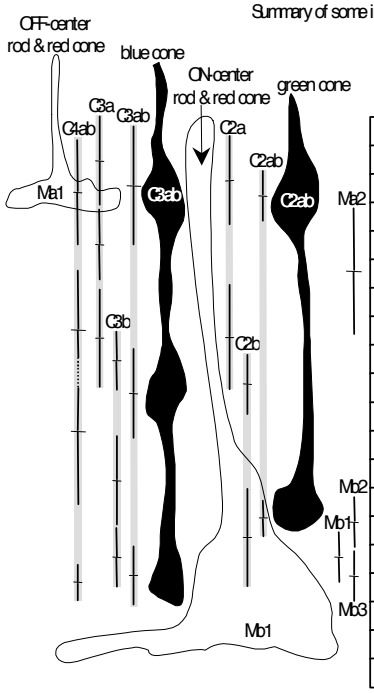
Robert E. Marc: The Structure of Vertebrate Retinas // CODE: Toyoda // Figure 6

Summary of some innervation patterns at each of 20 levels of the goldfish IPL



	ACL	Apparent biological strata
5	KA-insensitive GABA ACs, efferents, peptide ACs, glycine gap	1
10	S1 Serotonin/GABA ACs, Ma1 BCs, NMDA sensitive neurons (peak 1), glycine gap	2
15	GABA ACs, OFF Ma1 BCs, glycine ACs	3
20	GABA ACs, OFF Ma1 BCs, glycine ACs	
25	OFF Starburst ACh/GABA ACs, OFF Ma2 BCs	4
30	OFF Starburst ACh/GABA ACs, OFF Ma2 BCs, glycine ACs (peak 1)	
35	GABA ACs, ACh gap, glycine ACs (peak 1)	5
40	OFF Radiate ACh/GABA ACs, glycine ACs (peak 1)	6
45	GABA ACs, NMDA sensitive neurons (peak 2)	7
50	GABA ACs, peptide ACs, Glycine ACs (peak 2) BC terminal minimum	8
55	GABA ACs, peptide ACs, Glycine ACs (peak 2), BC terminal minimum	
60	NMDA sensitive neurons (peak 3), S2 Serotonin-accumulating GABA ACs	9
65	ON Radiate ACh/GABA ACs	10
70	GABA ACs, ACh Gap	11
75	ON Starburst ACh/GABA ACs	12
80	ON Mb BCs, glycine AC (peak 3)	13
85	ON Mb BCs, glycine AC (peak 3)	
90	ON Mb BCs, A*b* pyriform GABA ACs	14
95	ON Mb BCs, A*b* pyriform GABA ACs	
100	S1 Serotonin/GABA ACs, peptide ACs, A*b* pyriform GABA ACs	15

GCL

References

1. Walls GL. The Vertebrate Eye and Its Adaptive Radiation. Cranbrook Institute of Science, Bulletin 19,1942;785 pp.
2. Stell WK, Walker SE, Chohan KS, Ball AK. The goldfish nervus terminalis: A luteinizing-hormone releasing hormone and molluscan cardioexcitatory peptide immunoreactive olfactoretinal pathway. Proc Natl Acad Sci USA 1984;81:940-944.
3. Zucker CL, Dowling JE. Centrifugal fibers synapse on dopaminergic interplexiform cells in the teleost retina. Nature 1987;300:166-168.
4. Mariani AP, Leure-duPree AE. Photoreceptors and oil droplet colors in the red area of the pigeon retina. J Comp Neurol 1978;182:821-838.
5. Kalloniatis M, Marc RE, Murry RF. Amino acid signatures in the primate retina. J Neurosci 1996;16:6807-6829.
6. Kalloniatis M, Fletcher E. Immunocytochemical localization of amino acid neurotransmitters in the chicken retina. J Comp Neurol 1993;*336*:174-193.
7. Mitarai G, Asano T, Miyake Y. Identification of five types of S-potential and their corresponding generating sites in the horizontal cells of the carp retina. Jap J Ophthalmol 1974;18:161-176.
8. Stell WK, Lightfoot DO. Color-specific interconnections of cones and horizontal cells in the retina of the goldfish. J Comp Neurol 1975;*159*:473-502.
9. Wässle H, Boycott BB. Functional architecture of the mammalian retina. Physiological Reviews 1991;*71*:447-480.
10. Vaney D. The mosaic of amacrine cells in the mammalian retina. Prog Retinal Res 1990;*9*:49-100.
11. Wieniawa-Nariewicz E, Hughes A. The superficial plexiform layer: A third retinal association area. J Comp Neurol 1992;324:463-484.
12. Marc RE, Murry R, Fisher SK, Linberg K, Lewis G, Kalloniatis M. Amino acid signatures in the normal cat retina. Invest Ophthalmol Vis Sci 1998;39:1685-1693.
13. Brew H, Attwell D. Electrogenic glutamate uptake is a major current carrier in the membrane of axolotl retinal glial cells. *Nature* 1987;327:707-709.
14. Poitry-Yamate CL, Poitry S, Tsacopoulos M. Lactate released by Müller glial cells is metabolized by photoreceptors from mammalian retina. J Neurosci 1995;15:5179-5191.
15. Marc RE. The structure of GABAergic circuits in ectotherm retinas. In: Mize R, Marc, RE, Sillito, A (eds.), GABA in the Retina and Central Visual System, Elsevier, Amsterdam, 1992;61-92.
16. Newman E, Reichenbach A. The Müller cell: A functional element of the retina. Trends in Neuroscience 1996;19:307-311.
17. Schnitzer J. Astrocytes in mammalian retina. In: Osborne N, Chader G (eds.) Progress in Retinal Research Vol 7. Oxford: Pergamon Press;1988:209-232.
18. Bok D. The retinal pigment epithelium: a versatile partner in vision. J Cell Sci 106, Suppl 17;1993:189-195.
19. Fineran BA, Nicol JAC. Studies on the eyes of New-Zealand parrot-fishes (Labridae). Proc Roy Soc Lond B 1974;186:217-247.
20. Iuvone PM. Cell biology and metabolic activity of photoreceptor cells: light-evoked and circadian regulation. In: Djamgoz MBA, Archer SN, Vallerga S (eds), Neurobiology and Clinical Aspects of the Outer Retina. Chapman & Hall, London, 1995;25-55.
21. Burnside B, Dearry A. Cell motility in the retina. In: Adler R, Farber D (eds.) The Retina: A Model for Cell Biology Studies Part I. Academic Press, Orlando, 1986;151-206.
22. Schaeffer SF, Raviola E. Membrane recycling in the cone cell endings of the turtle retina. J Cell Biol 1978;802-825.
23. Kolb H, Jones J. Light and electron microscopy of the photoreceptors in the retina of the red-eared slider, *Pseudemys scripta elegans*. J Comp Neurol 1982;20:331-338.
24. Sterling P. Retina. In: Shepherd GM (ed.) The Synaptic Organization of the Brain, 4th edition, Oxford University Press, NY, 1998;205-253.
25. Raviola E, Gilula NB. Intramembrane organization of specialized contacts in the outer plexiform layer of the retina. J Cell Biol 1975;65:192-222.
26. Lasansky A. Synaptic organization of cone cells in the turtle retina. Phil Trans Roy Soc Lond B 1971;262:365-387.
27. Stell WK, Ishida AT, Lightfoot DO. Structural basis for on- and off-center responses in retinal bipolar cells. Science 1977;198:1269-1271.

28. Rauen T, Rothstein JD, Wässle H. Differential expression of three glutamate transporter subtypes in the rat retina. Cell Tiss Res 1996;286:325-336.
29. Steinberg RH, Fisher SK, Anderson DH. Disc morphogenesis in vertebrate photoreceptors. J Comp Neurol 1980;190:501-518.
30. Cohen AI (1965) New details of the ultrastructure of the outer segments and ciliary connectives in the rods of human and macaque retinas. Anat Rec 1965;152:63-80.
31. Stell WK. Functional polarization of horizontal cell dendrites in the goldfish retina. Invest Ophthalmol 1976;15:895-908.
32. Marc RE, Sperling HG The color receptor identities of goldfish cones. Science 1976;191: 487-489.
33. Stell WK, Hárosi FI. Cone structure and visual pigment content in the retina of the goldfish. Vision Res 1976;16:647-657.
34. Bowmaker JK, Thorpe A, Douglas RH. Ultraviolet-sensitive cones in the goldfish. Vision Res 1991;31:349-352.
35. Ohtsuka T. Relation of spectral types of oil droplets in cones of turtle retina. Science 1985; 229: 874-877.
36. Liebman PA, Granda AM. Microspectrophotometric measurements of visual pigments in two species of turtle, *Pseudemys scripta* and *Chelonia mydas*. Vision Res 1971;11:105-114.
37. Bowmaker JK, Heath LA, Wilkie SE, Hunt DM. Visual pigments and oil droplets from six classes of photoreceptor in the retinas of birds. Vision Res 1997;37:2183-2194.
38. Hárosi FI. Absorption spectra and linear dichroism of some amphibian photoreceptors. J Gen Physiol 1975;66:357-382.
39. Makino CL, Taylor WR, Baylor DA. Rapid charge movements and photosensitivity of visual pigments in salamander rods and cones. J Physiol 1991;442:761-780.
40. Makino CL, Dodd RL. Multiple visual pigments in a photoreceptor of the salamander retina. J Gen Physiol 1996;108: 27-34.
41. Mariani AP. Photoreceptors of the larval tiger salamander retina. Proc Roy Soc Lond B 1986;227:483-492.
42. Merbs SL, Nathans J. Absorption spectra of human cone pigments. Nature 1992;356:433-435.
43. Ahnelt PK, Kolb H, Pflug R. Identification of a subtype of cone photoreceptor, likely to be blue sensitive, in the human retina. J Comp Neurol 1987;255:18-34.
44. Szél A, Rölich P, Caffé AR, Julisson B, Aguirre G, Van Veen T. Unique topographic separation of two spectral classes of cones in the mouse retina. J Comp Neurol 1992;325:327-342.
45. Curcio CA, Allen KA, Sloan KR, Lerea CL, Hurley JB, Klock IB, Milam AH. Distribution and morphology of human cone photoreceptors stained with anti-blue opsin. J Comp Neurol 19991;312:610-624.
46. Marc RE, Sperling HG. The chromatic organization of the goldfish cone mosaic. Vision Res 1976;16: 1211-1224.
47. Engstrom K. Cone types and cone arrangements in teleost retinae. Acta Zool 1963;44:179-243.
48. Marc RE. Chromatic organization of the retina, In: McDevitt D (ed.) Cellular Aspects of the Eye, Academic Press, NY, 1982 pp. 435-473.
49. Marc RE, Sperling HG. The chromatic organization of primate cones. Science 1977;196:454-456.
50. Wässle H, Reimann HJ. The mosaic of nerve cells in the mammalian retina. Proc. Roy. Soc. Lond. B. 1978;200:441-461.
51. Van Haesendonck E, Missotten L. Stratification and square pattern arrangements in the dorsal inner plexiform layer in the retina of *Callionymus lyra L.* J Ultrastruct Res 1983;83:296-302.
52. Cook JE, Becker DL. Regular mosaics of large displaced and non-displaced ganglion cells in the retina of a cichlid fish. J Comp Neurol 1991;306:668-684.
53. Ramón y Cajal S. La retiné des vertébrés. La Cellule 1892, Vol. 9.
54. Kolb H, Nelson R, Mariani A. Amacrine cells, bipolar cells and ganglion cells of the cat retina. Vision Res 1981;21:1081-1114.
55. Djamgoz MBA, Wagner HJ, Witkovsky P. Photoreceptor-horizontal cell connectivity, synaptic transmission and neuromodulation. In: Djamgoz MBA, Archer SN, Vallerga S (eds.), Neurobiology and Clinical Aspects of the Outer Retina. Chapman & Hall, London, 1995;155-194.
56. Wagner HJ, Wagner E. Amacrine cells in the retina of a teleost fish, the roach (*Rutilus rutilus*): A Golgi study on differentiation and layering. Phil Trans Roy Soc B 1988;321:263-324.
57. Marc RE, Liu W-LS, Kalloniatis M, Raiguel S, Van Haesendonck E. Patterns of glutamate immunoreactivity in the goldfish retina. J Neurosci 1990;10:4006-4034.

58. Nakajima Y, Iwakabe H, Akazawa C, Nawa H, Shigemoto R, Mizuno N, Nakanishi S. Molecular characterization of a novel retinal metabotropic glutamate receptor mGluR6 with a high agonist selectivity for L-2-amino-4-phosphonobutrate. J Biological Chem 1993;268:11868-11873.
59. Ishida AT, Stell WK, Lightfoot DO. Rod and cone inputs to bipolar cells in goldfish retina. J Comp Neurol 1980;191: 315-335.
60. Scholes JH. Colour receptors and their synaptic connexions in the retina of a cyprinid fish. Phil. Trans. R. Soc. Lond.1975;270B:61-118.
61. Sherry DM, Yazulla S. Goldfish bipolar cells and axon terminal patterns: A golgi study. J Comp Neurol 1993;329:188-200.
62. Kouyama M, Marshak DW. Bipolar cells specific for blue cones in the macaque retina. J Neurosci 1992;12:1233-1252.
63. Famiglietti EV Jr, Kolb H. Structural basis for ON- and OFF-center responses in retinal ganglion cells. Science 1976;194:193-195.
64. Famiglietti EV Jr, Tachibana M, Kaneko A. Neuronal architecture of ON and OFF pathways to ganglion cells in the carp retina. Science 1977;198:1267-1268.
65. Marc RE, Liu W-LS. Horizontal cell synapses onto glycine-accumulating interplexiform cells. Nature 1984;311:266-269.
66. Marshak DW, Dowling JE. Synapses of the cone horizontal cell axons of the goldfish retina. J Comp Neurol 256:430-443.
67. Dacheux RF, Raviola E. Horizontal cells in the retina of the rabbit. J Neurosci 1982;2:1486-1493
68. Johnson MA, Vardi N. Regional differences in GABA and GAD immunoreactivity in rabbit horizontal cells. Vis Neurosci 1998;15:743-753.
69. Marc RE. Interplexiform cell connectivity in the outer retina. In: Djamgoz MBA, Archer SN, Vallerga S (eds.), Neurobiology and Clinical Aspects of the Outer Retina. Chapman & Hall, London, 1995;pp 369-393.
70. Pochet R, Pasteels B, Seto-Ohshima A, Bastianelli E, Kitajima S, Van Eldik LJ. Calmodulin and calbindin localization in retina from six vertebrate species. J Comp Neurol 1991;314:750-762.
71. Marc RE. Neurochemical stratification in the inner plexiform layer of the vertebrate retina. Vision Res 1986;26:223-238.
72. MacNeil MA, Masland RH. Extreme diversity among amacrine cells: Implications for function. Neuron 1998;20:971-982.