The Synaptic Organization of the Retina

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The basic architecture, signal flow and neurochemistry of signaling through the vertebrate retina is well-understood: photoreceptors, bipolar cells (BCs) and ganglion cells (GCs) are all thought to be glutamatergic neurons [1] and the fundamental synaptic chain that serves vision is photoreceptor → BC → GC [2]. But our understanding of detailed signaling is far from adequate and a complete description of synaptic interactions or signaling molecular mechanisms is lacking for any retinal cell network. The behavior of any one single GC remains to be characterized as a complete network model [3]. GCs express different mixtures of ionotropic glutamate receptors (iGluRs) and each receptor can be composed of many different subunits leading to a vast array of possible functional varieties [1]. The association of a defined cell class with any one functional iGluR mixture out of the many possible combinations remains to be established and current methods seem unable to make precise determinations. Network topologies are too numerous to resolve with physiological or pharmacologic data [4]. GCs contact many different amacrine cells (ACs), in addition to BCs, and the full description of all the inputs to any GC does not yet exist [5].

Physiology can only screen only a limited parameter space for any cell, thus limiting the rigor of any network models. Pharmacology is still an emergent field with many incomplete tools (despite our ability to describe the different major family of neurotransmitter receptors) and an immense diversity of neurotransmitter receptor subunit combinations, modulators and downstream effectors remains to be screened for any cell type. Molecular genetics, despite its power to provide complete or conditional inactivation of a single set of signaling elements, remains an ambiguous tool for deconstructing retinal networks. Morphology, augmented by immunohistochemistry and physiology, remain core tools in discovering new details of retinal organization. To a great extent, nothing has been as powerful as transmission electron microscopy for discovering retinal
networks. Mammalian night (scotopic) vision is a prime example. Its unique pathways were described by Helga Kolb and E.V. Famiglietti Jr. using electron microscopy [6]. Subsequent physiological analyses [7,8] provided clarification of how the network functions but would not have yielded the correct network architecture. Further complexities have been discovered by anatomical studies [9,10,11], including the fact that the network rewires in retinal degenerations [12]. But electron microscopy has not kept pace with the demands for high-throughput imaging until recently. We are now on the verge of a new era in imaging that will provide a deluge of new information about retinal circuitry [4]. Finally, the basics of retinal development and new findings in neuroplasticity are beyond the scope of this chapter[13,14], but the implications should be held in mind throughout: the connections we have long considered as static or hard-wired in retina display many of the same molecular attributes as plastic pathways in brain.

The basic signal flow in retina is overlaid on a well-studied cell architecture (Fig. 1). Retinal ON and OFF BC polarities are generated in the outer plexiform layer and mapped onto the inner plexiform layer into largely separated zones. The distal sublamina \( a \) receives inputs from OFF BCs and therein the dendrites of OFF GCs collect signals via BC synapses. The proximal sublamina \( b \) receives inputs from ON BCs and therein the dendrites of OFF GCs collect signals via BC synapses. ON-OFF GCs thus collect inputs from both sublayers.

1. Kinds of neurons

The retina is a thin, multilayered tissue sheet ... an image screen ... containing three developmentally distinct, interconnected cell groups that form signal processing networks:

Class 1 :: sensory neuroepithelium (SNE) :: photoreceptors and BCs

Class 2 :: multipolar neurons :: GCs, ACs, and axonal cells (AxCs)

Class 3 :: gliiform neurons :: horizontal cells (HCs)
Figure 1. A summary of major cell superclasses and synaptic connections in the mammalian retina. Photoreceptors include rods (cyan) and cones (green, blue) that hyperpolarize in response to light. All photoreceptors are glutamatergic and drive HC AMPA receptors on HCs, ON BC mGluR6 receptors, and OFF BC KA or AMPA receptors. All BCs are glutamatergic and drive either predominantly AMPA receptors on rod pathway interneurons or various mixtures of AMPA and NMDA receptors on cone pathway ACs and GCs. Homocellular gap junctions are formed between like pairs of cells (HCS, certain ACs) and heterocellular gap junctions are formed between different cells pairs (rods and green LWS cones; glycinergic rod ACs and ON cone BCs; some ACs and certain GCs). Two classes of feedback pathways exist. There is a putative HC → cone feedback path mediated by a pH sensitive process. AC → BC feedback is primarily GABAergic, as is AC → GC feedforward. Mammalian rod pathways are unique and not shared by any other vertebrate class. Rod BC signals are collected by a glycinergic rod AC that mediates a reentrant bifurcation into cone ON BC channels via gap junctions and cone OFF BC channels via glycinergic synapses. The outflow from the retina is largely split into ON GC channels that spike in response to light increments and OFF GC channels that spike in response to light decrements. The retina is precisely laminated into cellular and synaptic zones distal-to-proximal starting with the outer nuclear layer (ONL), the outer plexiform layer (OPL), the inner nuclear layer (INL), the inner plexiform layer (IPL), ganglion cell layer (GCL), and optic fiber layer (OFL). The INL is subdivided into the horizontal cell layer (HCL), bipolar cell layer (BCL), Müller cell layer (MCL) and amacrine cell layer (ACL). The IPL is subdivided into sublamina a that receives the output of OFF BCs and sublamina b that receives the output of ON BCs.
These three cell groups comprise over 60-70 distinct classes of cells in mammals [2,3,15,16,17] and well over 100-120 in most non-mammalian retinas [18].

The SNE phenotype

The SNE phenotype includes photoreceptors and BCs. These cells have polarized epithelial forms with apical ciliary-dendritic and basal axonal-exocytotic poles [19]. These two phenotypes form the first stage of synaptic gain in the glutamatergic photoreceptor → BC → GC → CNS vertical chain. This stage aggregates photoreceptor signals into BC receptive fields and highly amplifies photoreceptor signals. The basal ends of the BCs form the inner plexiform layer. As there are at least 12 kinds of BCs in mammals [16,20] and it has long been known that BCs delimit different functional zones therein, this suggests that the IPL contains strata with nearly 1 micron precision in lamination. Both photoreceptors and BCs use high fusion-rate synaptic ribbons as their output elements, fueled by hundreds to thousands of nearby vesicles. The retina is the only known tissue where SNE cells are arrayed in a serial chain.

As summarized in Figure 2, most mammals possess three classes of photoreceptors: rods expressing RH1 visual pigments, blue cones expressing SWS1 visual pigments and green cones expressing Long-Wave System green (LWS\(_G\)) visual pigments [21]. Conversely, the most visually advanced and diverse vertebrate classes (teleost fish, avians, reptiles) possess up to seven known classes of photoreceptors (RH1 rods, SWS1 UV/violet cones, SWS2 blue cones, LWS\(_R\) and RH2 green members of double cones, LWSR and RH2 green single cones) [22].
Similarly, the diversity of BCs in mammals is much lower (10-13) than that of non-mammalians.

Figure 2. Photoreceptor cohorts and connections in vertebrates. Non-mammalians display multiple pigment classes and cone types. There are five pigment classes and seven photoreceptor types for a freshwater turtle, including rods (comprising less than 10% of the photoreceptors) expressing class RH1 rhodopsins, three kinds of LWS cones (short members of double cones, long members of double cones with orange oil droplets, single cones with rod oil droplets), single cones expressing RH2 green cone pigments and a yellow oil droplet, single cones expressing SWS2 blue cone pigments and a UV-opaque clear oil droplet, and single cones expressing SWS1 UV cone pigments and a UV-transparent clear oil droplet. The connection patterns for non-mammalians are mixed rod-cone BCs and pure cone BCs, leading to mixed rod-cone GCs and pure cone GCs. Mammals display three pigment classes (one rod and two cone), two cone color types in non-primates and three color types in primates, including RH1 rods, SWS1 cones, and LWS cones. The LWS cone class forms one green type in most mammals, and red (LWSR) and green (LWSG) chromatypes in primates. The main connection rules for mammals are pure rod BCs and pure cone BCs, with only cone BCs driving GCs, with rod ACs (cyan) providing the re-entrant crossover.

(>20). This reduced diversity is a result of the Jurassic collapse of the mammalian visual system, where over half of the visual pigment genes, half of the neuronal classes and almost 2/3 of the photoreceptor classes were abandoned to exploit nocturnal niches. In addition, the
disproportionate proliferation of rods in the mammalian retina was accompanied by the loss of mixed rod-cone BCs in mammals and their replacement with pure rod BCs. How this occurred is unknown, but it cannot be due to an absolute selectivity of rod BCs for rods, as they will readily make contacts with cones when rods are lost in retinal degenerations. As we will see, the mammalian retina has exploited a re-entrant use of neuroepithelial ribbon synapses to enhance scotopic vision. The relationship between BCs and photoreceptors is still unclear, but there is both anatomical and molecular evidence that BCs were initially photoreceptors. For example, many non-mammalians possess BCs Landolt clubs, which are apical extensions extending from a BC primary cilium, extending past the outer plexiform layer into the outer nuclear layer, and containing packets of outer-segment-like membranes. Whether they are photosensitive has never been determined. Further, SWS1 blue cones and cone BCs share some SWS1 cis-regulatory sequences [23].

The multipolar neuron phenotype

The multipolar neuron phenotype [2] includes ACs, AxCs and GCs. Multipolar neurons can be further divided into axon-bearing (GCs, AxCs) and axonless cells (ACs). Mammals display roughly 30 kinds of ACs [15]. The roughly 15-20 kinds of mammalian GCs [3,17] are classical projection neurons, most of which have myelinated axons. GCs are completely postsynaptic at their dendrites and presynaptic at their axon terminals in CNS projections. So far, all are presumed to be glutamatergic. Conversely, ACs are local circuit neurons similar to periglomerular cells in the olfactory bulb. ACs lack classical axons and often have mixed pre- and postsynaptic contacts on their dendrites, though some ACs partition inputs and outputs into different parts of their dendritic arbors. Most ACs are GABAergic and the remainder are glycineric [24]. Several classes of ACs are dual transmitter cells, expressing both acetylcholine and GABA, serotonin and GABA (in non-mammalians) or peptides and GABA or glycine [1]. Differential mapping of these outputs has
never been achieved. In-between are the AxCs, also known as polyaxonal cells and intraretinal GCs, which have distinct axons that project within the retina [25,26,27]. One dramatic example of the AxC phenotype is the TH1 dopaminergic AxC [28]. This cell releases dopamine at unknown but probably axonal sites and likely glutamate at others [29], similar to nigrostriatal neurons [30]. While it has been postulated that TH1 cells were GABAergic, supportive evidence for that view has not emerged. But it is likely that some polyaxonal cells are GABAergic. There is no evidence for a glycinegic AxC. All multipolar neurons are characterized by numerous neurites branching in the plane of the retina, most of them collecting signals from patches of BCs. Multipolar neurons are among earliest to develop in the retina and quickly define the borders of the inner plexiform layer and its stratification. Their architecture clearly indicates that they sample patches of the signal space created by connected photoreceptors and BCs. Multipolar neurons all manifest somewhat classical “Gray”-like synapses, generally with small clusters of less than 200 vesicles.

The gliaform cell phenotype

This phenotype contains the horizontal cells (HCs), whose somas and processes are restricted to the outer plexiform layer [31]. Though HCs are multipolar, somewhat neuron-like, and may display axons, they do not spike. Further, they express many glial features such as intermediate filament expression and very slow voltage responses. Further, HCs produce high levels of glutathione and make direct contact with capillary endothelial cells in some species, suggesting they play homeostatic roles similar to glia. Even so, HCs clearly mediate a powerful network function, collecting large patches of photoreceptor input via AMPA receptors and providing a wide-field, slow signal antagonistic to the vertical channel. The mechanism of HC antagonism remains a matter of great uncertainty and debate. HCs do make conventional appearing synapses onto neuronal processes in the outer plexiform layer, and in fishes these synapses are made onto dendrites of glycinegic interplexiform cells, a form of AxC [32]. However, these are so sparse in
all species and contain so few vesicles that they cannot be the source of the large sustained opponent surrounds of retinal neurons that HC generate. HCs must use some other mechanism.

The phylogenetics of HCs has been thoroughly reviewed [33]. HCs in mammals are postsynaptic to cones at their somatic dendrites. One class of HCs common in mammals (foveal type I in primates, type A in rabbits & cats, and absent in rodents) contacts cones alone. A second class of HCs (extrafoveal type I in primates, type B in rabbit and cats, and the only known HC in rodents) displays axons several hundred microns long that branch profusely and form massive arborizations contacting hundreds to thousands of rods. A another class of primate HCs (type II) has axon terminals contacting cones and rods. Importantly, the axon of HCs appears to be electrically ineffective. Further, the axons of HCs are solely postsynaptic to photoreceptors, as far as is known and their function remains completely enigmatic. HCs also appear to be early-developing pioneer cells that define the outer plexiform layer. After the GCs and HCs define the layout of the inner and outer plexiform layers respectively, photoreceptors and BCs then mature and search for connections.

True glia and vasculature

The neurons of the retina are embedded in an array of vertical Müller glia that span the entire neural retina, forming 1/3 to 1/2 of the retinal mass and generating high-resistance seals at the distal and proximal limits of the retina. Most mammalian retinas are vascularized in three capillary beds: at the GC-inner plexiform layer border, the AC-inner plexiform layer border, and the outer plexiform layer. Squirrels (Sciurids) display two beds (at the GC-inner plexiform layer and AC-inner plexiform layer borders; and rabbits (Lagomorphs) have none at all, similar to all other non-mammalian vertebrates. The GC layer of many species also displays classical astrocytes, though their roles remains unclear. In brain, astrocytes carry out some of the operations attributed to
retinal Müller glia, including transport of spillover K\(^+\) and glutamate, and glucose supply via vascular > glial cell > neuron transcellular transport. Why and how most vertebrate retinas function without vasculature remains uncertain, but it is likely that Müller glia act as a surrogate vascular system with the added ability to accumulate large glycogen stores (like hepatocytes) as part of a glucose-skeleton homeostasis. The segregation of retinal astrocytes away from the inner plexiform layer remains a mystery.

Basic synaptic communication

With the discovery of the signaling mechanisms of the neuromuscular junction for decades ago [34], one might have thought that the archetypal synaptic format had been discovered. Yet it has become clear, especially in retina, that every kind of synapse is subtly different: diverse physics, topologies and molecular mechanisms lead to very different forms of synapses, most of which do not follow the single presynaptic “bouton” \(\rightarrow\) single postsynaptic target pattern of brain. Further, the arrangement of these systems into synaptic chains in retina is unlike any other known network, including olfactory bulb. In retina, the first stage of synaptic signaling is a direct SNE \(\rightarrow\) SNE synapse (Fig. 1): photoreceptor \(\rightarrow\) BC. No other instance of this topology has been discovered in any organism. There are at least six modes of presynaptic-postsynaptic pairing in retina.

1 Photoreceptor ribbon synapses: small-volume multi-target signaling

It is generally thought that all photoreceptor signaling is glutamatergic, but sporadic indications of cholinergic physiology and molecular markers have been found in many non-mammalians [1], so things may not be so simple. Glutamate release from photoreceptors is effected by high rates of vesicle fusion at active sites on either side of a large synaptic ribbon [35] positioned close to the pre-synaptic membrane. In general, the presynaptic zone is a protrusion or ridge with vesicle
fusion sites positioned on the slopes of the ridge (Fig. 4). The releasable vesicle pool is so large that photoreceptors and BCs are capable of maintaining continuous glutamate release in response to steady depolarizations. This, among other things distinguishes photoreceptors and BCs from ACs, which have very small presynaptic vesicle clusters.

Various vertebrate rods and cones differ greatly in the number of ribbons and postsynaptic targets arrayed within the presynaptic terminals. For example, most mammalian and teleost fish rods have very small presynaptic spherules shaped like a grape $\approx 3 \, \mu m$ in diameter with a small entrance aperture leading to an enclosed extracellular invagination or vestibule in which thin postsynaptic dendrites are contained (Fig. 3). Importantly, glial processes are excluded from the interior of the spherule and any glutamate release must diffuse out of the spherule to reach the Müller glia. However, mammalian rods express the EAAT5 glutamate transporter [1] and likely regulate their own intrasynaptic glutamate levels. Each spherule contains one or two synaptic ribbons and a few postsynaptic targets [36]. In fishes, the postsynaptic targets are the dendrites of roughly five kinds of mixed rod-cone bipolar cells [37] and one kind of rod horizontal cell [33]. Thus each ribbon serves no less than six different types of postsynaptic targets. In mammals, only two targets are common: the dendrites of one kind of rod BC and the axon terminals of HCs. There are some instances of sparse OFF BC contact in mammals, but this seems to vary with species and may be an evolutionary relict with variable expression rather than a major signaling pathway [38,39,40].

In sum, rod spherules form a sparse-ribbon $\rightarrow$ small volume, sparse-target architecture.

Cones and rod terminals in some non-mammalians (e.g. urodele amphibians) adopt a different topology, with the presynaptic ending expanding to form a foot-piece or pedicle some 3-5 $\mu m$ wide shaped either like a cupola (fishes) whose broadly concave interior admits some 50-100 or more fine dendrites served by roughly 12 synaptic ribbon sites [41]; or like a true pediment (e.g.
primate cones) whose shallow concavity is studded with up to 50 ribbon sites [42] (Fig. 3). Cone pedicles in primates target at least ten different kinds of BCs and at least two kinds of HCs. Mouse cone pedicles are smaller but still target eleven kinds of BCs [20] and one kind of HC. In sum, 

cone pedicles form a multi-ribbon \(\rightarrow\) small volume, multi-target architecture.

Figure 3. Basic organization of mammalian photoreceptor synaptic terminals. Primate cone terminals contain many ribbons, mitochondria clustered at the head of the pedicle and thousands of synaptic vesicles (white dots), some of which form organized zones near the plasma membrane opposite an array of cone-specific postsynaptic processes including type H1 and H2 HCs as lateral invaginating elements (primate H1 cells tend to avoid SWS1 cones, while H2 cells contact all cones). Cone ON BCs tend to center their dendrites between the lateral HC processes at varying distances from the synaptic ribbon, forming so-called invaginating and semi-invaginating contacts. Most cone OFF BCs position their dendrites outside the HC processes at so-called flat contacts. It is thought that most of these express KA receptors. Some occasional OFF BCs processes invaginate, and they may express AMPA receptors.
Figure 4. A detailed schematic of synaptic organization at cone (left) and BC (right) ribbon synapses. Each synaptic ribbon is a pentalaminar structure in cross-section, in reality a disc or lozenge-shaped solid with its two broad faces serving as attachment sites for tethered vesicles and its small paramembrane face attached to a dense structure known as the arciform density (composition unknown). Ribbons serve as the major site for the “readily releasable” pools of synaptic vesicles for continuous glutamate transmission, and facilitate high-speed formation of docked vesicles. Upon depolarization of the presynaptic membrane, voltage-gated calcium channels (VGCCs, black barrels) open, allowing docked vesicles to fuse and release glutamate into the synaptic cleft. At some distance from the ribbon, endocytosis mediates vesicle recovery. BC ribbons tend to be shorter than photoreceptor ribbons. Cytoplasmic glutamate (orange) is formed glutamine via mitochondrial phosphate-activated glutaminase and loaded into vesicles via vGlut vesicular transporters. Glutamate release by vesicle fusion diffuses away from the release site (shaded orange) and is cleared by both presynaptic and distant Müller glia glutamate transporters (white barrels). Müller glia synthesis glutamine from glutamate via glutamine synthetase (GS) and exports glutamine via transporters (grey barrels). Similarly, neurons import glutamine via transporters. Vertebrate photoreceptors also express presynaptic cystine-glutamate (Xc-) exchangers (orange barrels) whose function is unknown. Glutamate released by cones activates ON BCs via mGluR6 receptors (light blue barrels), HCs via AMPA receptors (dark blue barrels) and OFF BCs via either AMPA or KA receptors (bright blue barrels). HCs are positioned at the highest glutamate concentration zone and OFF BCs at the lowest. Glutamate released by BCs activates ACs and GCs via AMPA receptors (dark blue barrels) and NMDA receptors (gold barrels). Feedback at photoreceptors appears to be mediated by either focal connexin (yellow barrel) or a pH modulator (magenta barrel) close to the photoreceptor VGCC. Feedforward from HCs to BCs may be GABAergic in some species and mediated by GABAC receptors (dark red barrels). Feedback at BCs is mediated by vesicular GABA (red shading) release from ACs targeting largely GABAC receptors. Feedforward from ACs to BCs is mediated by largely by GABAA receptors (bright red barrels). ACs and mammalian Müller glia also have GABA transporters that clear the synaptic space. GABA is converted via the GABA-transaminase (GABA-T) complex to glutamate in glia. Cone synaptic terminals also have a number of other proteins including Na-Ca exchangers (NCKX), plasma membrane Ca transporters (PMCA), transient receptor potential channels (TRPC), metabotropic glutamate receptors (mGluR) and possibly histamine receptors (H2). BC terminals may share some of these.
2 BC ribbon synapses: semi-precise target signaling.

Like photoreceptors, BC signaling is generally considered glutamatergic [1]. But once again, sporadic evidence of exceptions exist. In mammals (especially primates) and amphibians, some BCs contain molecular markers of GABAergic function [24] (or at least GABA-related metabolism), though no robust physiology supports this. In contrast to photoreceptors, BC synaptic endings are topological spheroids, usually multiple (depending on BC type), with dozens to hundreds of ribbons abutting the surface. Further, BCs form no invaginations, so there is no restricted volume into which glutamate is injected by vesicle fusion. In most cases each ribbon is directly apposed to a pair of postsynaptic targets, usually ACs. This is termed a dyad and, while monads, triads and tetrads do occur, dyads dominate by far. This means that large BC terminals such as those found in teleost fishes can drive up to one to two hundred distinct processes. Mammalian BCs driven many fewer targets and most BCs have elaborate, branched terminals with connecting neurites often as small as 100 nm. In contrast to photoreceptors, the targets of BCs are focal. BC terminals largely fully encapsulated by neuronal processes at their release sites to which they are presynaptic or postsynaptic, with rarely direct contact between the terminal and Müller glia near the synaptic release zone. This means that any glutamate that escapes from the synaptic cleft may travel some distance before glial glutamate transporters can clear it. Thus the potential for glutamate overflow at BC synapses is substantial. This may be particularly important for the activation of NMDA receptors, as they are suspected to be displaced from the primary AMPA receptors used in the inner plexiform layer. Thus, BCs form multi-ribbon → semi-precise target architectures.

3 AC and AxC conventional fast synapses: precise presynaptic → postsynaptic signaling.
ACs and AxCs are the only retinal cells that make synaptic contacts resembling CNS “Gray”-like, non-ribbon conventional synapses. ACs target BCs, GCs or other ACs. The targets of most AxCs are not well known but appear likely to be ACs and GCs. Though each AC may make many hundreds of synapses, each synapse contacts only and only one postsynaptic target, similar to classical multipolar neurons in CNS and spinal cord [43]. The dominant fast transmitters of AC systems are GABA and glycine, with GABAergic neurons making up half to two-thirds of the AC population depending on species [1]. Additional transmitters such as acetylcholine, peptides, or serotonin (in non-mammalians) are also associated with GABAergic (in most cases) or glycinergic systems [1] [44]. Acetylcholine (ACh) is a fast excitatory transmitter and is found in paramorphic starburst ACs in mammals and also uses conventional synapses [1]. However, we don’t yet know of any differences in architecture between GABA and ACh utilizing synapses in retina. This poses a bit of a problem in interpreting ultrastructure without complete neurochemical information or serial section reconstruction. In the CNS, many excitatory glutamate neurons tend to make perforated Gray type I synapses: synapses with annular or otherwise discontinuous “rivers” of synaptic release sites and asymmetric density (very dark postsynaptic densities). Most inhibitory neurons tend to make non-perforated, spot-like Gray type II synapses: synapses with a solid disc of presynaptic vesicles and roughly equal presynaptic and postsynaptic densities. What, exactly, cholinergic or non-ribbon glutamate synapses should look like in retina remains unknown.

4 AC, AxC and efferent slow transmitter synapses: large volume signaling.

Dopamine (and possibly norepinephrine / epinephrin) as well as peptides in retina appear to be released by a non-focal, Ca^{2+} dependent vesicular system [45], but without any clear postsynaptic associations. Dopamine (and likely the other slow transmitters) acts via volume conduction [46] and modulates a range of cellular responses largely via G-protein coupled receptors (GPCRs). In non-mammalians, efferent systems from CNS target ACs with fast neurotransmitter synapses,
especially GABA [5]. In mammals, all known efferents appear to release either histamine or serotonin, likely as volume signaling systems [47].

5. HC non-canonical signaling

HCs generate potent, large-field, slow surround signals in retinal GCs, BCs, and even in non-mammalian cone photoreceptors [48,49,50]. There is evidence for both feedforward signaling via the cone → HC → BC path [51] and feedback signaling via the cone → HC → cone → BC path [52,53]. The efficacy and sustained nature of the feedback signal is such that no known vesicular mechanism could maintain it other than a ribbon-style synapse. This poses a significant problem as vesicular HC synapses are very rare and small. Several models of non-canonical signaling have been proposed including synaptic pH regulation [54], hemi-junction mediated ephaptic signaling [55], and even transporter-mediated signaling. Some of these will be discussed in detail below, but this very unusual functionality is further evidence that HCs are not classical neurons.

6. Coupling types and Coupling patterns

While gap junctional coupling was first discovered between HCs, only in the past decade has it become clear how powerful and pervasive intercellular coupling is in retina [56,57]. There are two simple classes of coupling: homocellular and heterocellular (between like and different classes of cells respectively). The participant connexins in each case are respectively likely to be homo- or heterotypic (similar or dissimilar connexin types) [37]. The strength of coupling is partly associated with the size of the junctions, as they represent summed parallel conductances, but also with functional modulation by various signaling pathways such as dopamine D1 receptors, that decrease conductances between coupled HCs [45,58] and coupled ACs [59], and dopamine D2 receptors that modulate rod-cone coupling [60]. The significance of coupling is clear in certain cases, such as the ability of HCs to spatially integrate signals over large fields (> 1mm diameter) or
the crossover of rod signals into cones via heterocellular rod-cone and rod AC-cone BC coupling. However, such knowledge does not readily extrapolate, and other coupling patterns are poorly understood, such as heterocellular AC-GC coupling and even HC-BC coupling.

Fast, Focal Neurochemistry, Synaptic Currents and Amplification

One of the most powerful discoveries of the last two decades has been the diversity of the primary fast neurotransmitter receptors of the vertebrate nervous system. Again, the primary signaling channel of retina is the vertical glutamatergic chain from photoreceptors to brain [1]. Rods, cones and BCs encode their voltage responses as time-varying glutamate release. The targets of photoreceptors and BCs, in turn, decode time-varying extracellular glutamate levels as time-varying currents with glutamate receptors. There are two major classes of glutamate receptors: ionotropic and metabotropic (iGluRs and mGluRs, respectively). The iGluRs are separable into two distinct families: the AMPA/KA receptors and NMDA receptors. AMPA and KA receptors are related but pharmacologically and compositionally distinct. Four basic classes of glutamate receptor subunits (GluR1, 2, 3, 4) can be recruited to form a tetrameric AMPA receptor. Similarly, five basic classes of KA receptors (GluR5, 6, 7 and KA1, 2) can be assembled into tetrameric KA receptors. With some exceptions, these receptor assemblies can have nearly any stoichiometry. NMDA receptors are a distinct group of iGluRs in several ways. First, they have an obligate NR1 and NR2 tetrameric subunit composition. Second, they are coincidence detectors that require dual binding site activations: one by glutamate and another by a glycine-like endogenous agonist. There is substantial evidence that this co-ligand may be D-serine released from Müller glia [61]. Finally, the mGluRs represent a complex collection of GPCRs whose functions are far from clear.

Different classes of neurons express different types or different combinations of receptors and, in the end, the glutamate receptor profile of a cell is diagnostic for its class. Mammalian BCs are
unique in expressing either mGluR6, KA or AMPA receptors as their glutamate decoding system. BCs seem functionally monolithic in having their signals dominated by one of these three receptor systems. But immunochemical and mRNA expression analysis suggest that these associations are not so precise, and iGluR subunit expression occurs in nominally mGluR6-driven cells [62]. HCs predominantly express AMPA receptors, but show no NMDA mediated responses. Finally, ACs and GCs resemble CNS neurons in expressing AMPA receptors augmented by varying amounts of NMDA receptors.

The key glutamate receptor systems of retina operate on the principle of cation permeation [1,63]. When activated by glutamate binding, iGluRs generate increased channel conductances and carry inward currents carried by cations: mostly Na$^+$ and Ca$^{2+}$. Thus the canonical iGluR AMPA, KA and NMDA families of receptors are nominally sign-conserving (>) depolarizing systems that “copy” the polarity of the time-varying presynaptic source voltage input in the postsynaptic target. The facts that many inputs converge on one postsynaptic cell; that small presynaptic voltages can modulate the release of many vesicles (in SNE cells); and that glutamate gates large postsynaptic conductance changes to cations with a positive reversal potential means that such synapses have high gain. Signals from photoreceptor to brain are successively amplified by a chain of glutamate synapses.

The group III mGluR6 system is unique and, in retina, is expressed by ON BCs. No known multipolar neuron in the CNS uses this receptor as its primary signal modality. As a classical GPCR, with Go$\alpha$ as its cognate G-protein [64], the binding of glutamate triggers a cascade of signals that ultimately leads to the closure of cation channels on BC dendrites, thus moving the BC membrane potential closer to the K$^+$ equilibrium potential. Thus mGluR6 receptors are nominally sign-inverting (>i) hyperpolarizing systems that “invert” the polarity of the time-varying presynaptic
source voltage input in the postsynaptic target. The modulation of a strong cation current renders the mGluR6 mechanism high-gain in spite of its inverted polarity.

The differential expression of iGluRs and mGluR6 in BCs creates the two fundamental signal processing channels of the retina: OFF and ON BCs respectively [1]. Unknown mechanisms regulate the expression of glutamate receptors in BCs. In general, BCs that express iGluRs such as KA or AMPA receptors do not express functional mGluR6 receptor display, and vice versa. However, there is evidence that BCs expressing mGluR6 also express low levels of iGluR protein, but there is yet no evidence that such iGluR subunits contribute to an electrically detectable signaling event [62].

There are additional mGluRs expressed in retina, including both the group I mGluR1 and mGluR5 and group III mGluR 4, 7 and 8, all largely expressed in varying patterns in the inner plexiform layer [65]. Their roles are thought to be associated with presynaptic glutamate feedback.

Global Neurochemistry and Modulation

There are a number of alternative neurochemical mechanisms that appear to operate on a larger spatial scale than conventional synapses. The most thoroughly described, though incompletely understood, is the catechol dopamine [45]. In mammalian retinas, dopaminergic neurons are largely AxCs that arborize primarily (but not exclusively) in the distal-most layer of the IPL, appear to receive direct inputs from BCs, and have predominantly ON-type responses [66]. The exact sites of dopamine release are not known, although these cells clearly have small accumulations of synaptic vesicles distributed sparsely in their processes. Data from many species suggest that dopamine acts largely via volume conduction [46]: i.e., dopamine diffuses throughout the retina and targets high-affinity type D1 and D2 receptors distributed on almost every class of cell,
including Müller cells. Actions triggered by D1 receptors include the uncoupling of HCs [67], the uncoupling of rod-driven glycinergic ACs [59], enhanced spike speeds [68] (faster waveforms). All of these are consistent with the process of converting the retina from a scotopic to a photopic state. Dopaminergic neurons are also thought to contain a fast transmitter as well. Previous evidence in the mouse suggested that they were GABAergic, but other studies support the possibility that they are glutamatergic.

A similar role is posited for the rapidly diffusing neuroactive gas NO. A variety of retinal cells are posited to produce NO via the neuronal NO synthase (nNOS) pathway, activated by binding of Ca-activated calmodulin [69]. Thus synaptic currents with high Ca-permeability (e.g. those mediated by NMDA receptors) can turn on nNOS, which catalyzes the oxidation of one of the guanido nitrogens of arginine to NO. NO appears to have the capacity to diffuse through transcellular regimes (how far is in debate) and activate soluble guanyl cyclases to produce cGMP. Acting via cGMP-dependent protein kinase G or directly on cyclic-nucleotide gated cation channels, cGMP can effect a number of modulatory actions. One site of action is thought to be the heterocellular coupling of ON cone BCs and glycinergic rod ACs [59], and increased cGMP appears to uncouple this network, again a light-adaptive action. While many cells appear to be able to produce NO, the best known architecture for NOS-containing neurons is a wide-field GABAergic AC [70].

Many retinas contain a number of neuropeptides as cotransmitters, largely in wide-field GABAergic ACs with cone BC inputs. These peptides include somatostatin, substance P and neotensin, with many more in non-mammalian, such as enkephalins [44]. This probably with the least understood neurochemical aspect of the retina. In general, it is thought that peptides act via specific peptide receptor GPCRs to modulate various ion channels. However, very little research on retinal peptides has been carried out in recent years.
Modulation by transporters

An important part of every signaling process is termination. Given the ability of glutamate to activate excitotoxic processes, the expression of high-affinity sodium-glutamate transporters (symporters) by Müller glia likely represents the last line of defense [1]. In terms of signaling, a molecule of glutamate must have already left the synaptic cleft by diffusion to encounter glial glutamate transporters. However, both photoreceptors and BCs express glutamate transporters, presumably near the sites of synaptic release [1] and these likely play a major role in determining the temporal dynamics of extracellular glutamate levels, though this has been difficult to quantify. Even so, every fast transmitter has corresponding presynaptic transporter mechanism including GABA, glycine and even acetylcholine (ACh). Though acetlycholinesterase terminates the action of ACh, the cleavage product choline can activate ACh receptors. In retrospect, it is not surprising that cholinergic neurons express choline transporters. Though numerous studies support the roles of transporters in signal termination, the precise localization of transporters has not been achieved.

Signal processing

The roles of synaptic networks are to convert graded sensory photoreceptor potentials into patterns of action potentials for long-range transmission to the CNS, and perform spatial, temporal, and spectral signal processing on the input signals of the photoreceptors. This latter action converts the retinal image into the parallel signaling behaviors of 15-20 different classes of retinal GCs in mammals, more in non-mammalian. As we shall see in the next section, collections of retinal neurons form networks in which these operations are effected. The concept of signal processing, as derived from electrical engineering, is particularly relevant [49]. Each kind of synapse, each kind of cell, and each topology of network is invoked in various ways to generate the kinds of “filters” through which the visual scene must be encoded. The physiological analysis of retina in
the 1970s (especially as carried out by Naka) represented a sea-change in thinking; a move away from Sherringtonian concepts of spinal excitation, inhibition, and circuits (loops) and towards engineering notions of polarity, inversion, networks and filters.

Sign-conserving (>) and sign-inverting (>i, >m) transfers.

The behavior of a photoreceptor is neither excitatory nor inhibitory. Photoreceptors encode time-varying changes in light intensity with fairly faithful (though nonlinear) time-varying changes in voltage. As glutamatergic neurons, one would normally think of them as “excitatory” in brain, but a more robust concept is derived by looking at the behaviors of target neurons. HCs (driven by AMPA receptors) and OFF BCs (driven by AMPA or KA receptors) merely copy the polarity of presynaptic photoreceptors (Fig. 1). When light hyperpolarizes the presynaptic voltage of photoreceptors, this decreases the rate of synaptic glutamate release and (in conjunction with ongoing glutamate transport) leads to a decrease in synaptic glutamate levels. Since AMPA and KA receptors are iGluRs, decreased synaptic glutamate means that AMPA and KA receptor-gated currents will decrease and the HCs and OFF BCs will hyperpolarize. Conversely, when a fly navigates across the visual field, local darkening will depolarize some photoreceptors and the HCs and OFF BCs will follow suit. Thus photoreceptor → HC and OFF BC signaling is formally termed sign-conserving (>). In addition, iGluRs typically mediate high-gain responses (i.e. strong amplification) and, over modest voltage ranges this amplification is symmetric and polarity invariant. ON BCs behave in a totally different manner. In mammals, all ON BCs express functional mGluR6 receptors that activate a cation channel when unbound and close it upon binding of glutamate. Thus, when photoreceptors decrease their glutamate release, this leads to decreased mGluR6 receptor binding and the opening of cation channels and depolarization of ON BCs (Fig. 1). This is an explicit, high-gain, metabotropic sign-inverting (>m) synaptic transfer.
Non-mammalians display a twist on this mechanism that perhaps reveals the evolutionary history of mammalian ON BCs. The apparent homologue of the ancestral mammalian rod BC exists in the retinas of modern fishes as the mixed rod-cone BC. This cell has an unusual behavior in that it has different reversal potentials and conductance changes for different stimuli. Scotopic lights that activate rods generate ON responses that display a positive reversal potential (like a cation) and an increase in conductance (like a channel opening). This is very like mammalian rod and cone ON BCs, and indeed it appears to have the same pharmacology: 2-amino-4-phosphonobutyrate (AP4) is an agonist at mGluR6 receptors [71] and blocks rod ON BCs responses in fishes. However, upon light adaptation, fish ON BCs change their behaviors. While remaining “ON” in response to photopic lights that activate cones, the reversal potential moves to very negative values (like an anion) and the cells display a decrease in conductance (like a channel closure). In fact, the cone-driven ON responses of fish BCs are mediated by an anion channel coupled to a glutamate transporter [72,73]. Thus, in photopic “dark”, glutamate cone release activates the transporter and its coupled chloride current, leading to hyperpolarization of the BC. Thus the fish cone → ON BC synapse is sign-inverting, but not metabotropic. The degree of its amplification is also unknown.

In the inner plexiform layer, BC → AC and GC signaling is all mediated by AMPA or AMPA +NMDA receptors [3,63,74]. Thus all BC output synapses are sign-conserving (Fig. 1). The bulk of AC → BC, AC or GC signaling is either GABAergic or glycinergic via increased anion conductances [75]. Thus these synapses are characteristically sign-inverting (>i). GABAergic and glycinergic transmission is also usually very low gain, often because the reversal potential is very close to the membrane potential and/or the total conductance change experienced by a target cell leads to a tremendous decrease in total cell input resistance, thus decreasing signal efficacy. In any case, it take a significant amount of inhibition to control glutamate synapses. Some inhibitory
mechanisms involve the metabotropic GABA\textsubscript{B} receptor, which is a GPCR that can lead to a tremendous increase in potassium currents, but can also show paradoxical excitation \cite{73}. Since potassium currents are usually outward (positive current flowing outward), GABA\textsubscript{B} can produce a strong and long-lasting inhibition near threshold. Different GABA receptors tend to be expressed at different sites (ionotropic GABA\textsubscript{A} on ACs and BCs, ionotropic GABA\textsubscript{C} on BCs), but the distribution of GABA\textsubscript{B} receptors is less well understood.

Synaptic chains and polarity.

The effect of cascading synapses through various pathways can be estimated in terms of polarity and gain. For example, though the mechanism of HC action is very poorly understood, its efficacy is not in doubt. As first established in fish retinas, current injected into HCs have stereotyped actions on different GCs. The net pathway from HCs to ON GCs is sign-conserving. The net pathway from HCs to ON GCs is sign-conserving \cite{48,49}. From a signal processing perspective, this means that the polysynaptic chain from a given HC, somehow reaching a BC and thence to an ON GC must contain either no sign-inverting elements or an even number. The path to an OFF GC must contain an odd number of sign-inverting elements. This poses fundamental constraints on where and how signals flow in the retina and can be used as a model for network investigations. Similarly, we know that there are chains of two and three serial ACs in the inner plexiform layer of most retinas \cite{5}. While there are no network models that explicitly invoke such chains, the minimum architectures for such chains are BC → AC → AC → BC or GC and BC → AC → AC → AC → BC or GC respectively. If we presume that the AC output are sign-inverting, then the net transfers of the two chains are > >i >i > (sign-conserving) and > >i >i >i > (sign-inverting). Importantly, the low gain of GABAergic and glycinergic synapses prevents such chains from being runaway excitations in the former case \cite{5,76}. In brain and spinal cord, GABAergic synapses tend
to have gains of \(<1\). Thus a chain three sign-inverting synapse would have an amplification of around 0.125 and inhibitory mechanisms are thus self-attenuating. What roles might such networks play? In non-mammalians, the bulk of GABAergic ACs also receive some form of GABAergic input as evidenced by their pharmacology. It was presumed that similar networks existed in mammals, but recently Hsueh et al. [77] argued that in rabbit, the only synaptic crossover networks are glycine → GABA. While this does not square with the anatomy of the rabbit retina, it suggests that this net-sign-conserving crossover network may be particularly potent.

Feedback, feedforward and nested feedback / feedforward

Designing analog operational amplifier networks is very similar to evolving a retina or any control system: every stage of forward amplification needs some form of feedback control [78]. In retina, sign-inverting GABAergic mechanisms are used as both feedback and feedforward control systems. In a formal sense, feedback is the most powerful way to set the net synaptic gain, improve the signal-to-noise ratio, and improve synaptic bandpass. We will skip the mathematic demonstration of this, but note that it has been widely discussed [75,79]. On the other hand, feedforward is a very effective way to generate strong antagonistic mechanisms in target cells. These architectures are clearly at play in retina but we have only a hazy idea of their importance. For example, blocking GABAergic inhibition converts directionally selective GCs into non-selective cells, but has little effect on the center-surround organization of other GCs, despite the abundance of GABAergic synapses in the inner plexiform layer. Thus is it hard to generalize function from anatomy. Conversely, it is impossible to understand function without anatomy.

Caveats
Three major problems have emerged in understanding how GABAergic (or any classical inhibitory transmitter) works in retina. The first problem is the chloride reversal potential. We actually have a very poor idea of which way GABA receptor-gated anion currents will flow: inward or outward. Small retinal cells may have the ability to adjust intracellular chloride levels with various ion transport systems. The KCC2 system tends to export Cl while the NKCC system tends to import it [51]. If intracellular Cl is locally high, opening an anion channel may evoke an outward negative current (a net positive current) and depolarize the cell. Thus GABA may act in a sign-conserving way at some synapses. However, most studies of GABAC receptors at the synaptic terminals of retinal BCs support the view that it is inhibitory [1]. A second problem is temporal delay. Imagine a cell responding to a light input with a sinusoidal voltage. Then imagine a surrounding cell giving a similar response and providing feedback with a sign-inverting polarity. That’s all well and good. But if the feedback was delayed so that the phase is shifted by 180 degrees, the “inhibitory” local surround would sum with the center response. There is much evidence to suggest that simple AC → BC inhibitory feedback cannot explain all BC responses [80]. Finally, it has been long assumed that BCs were effectively isopotential, and that simple lumped-parameter calculations would suffice to model their network functions. But reconstructions of BCs in mammals [4] suggest that the isopotentiality assumption is not correct and that complex local information processing can be effected at the synaptic terminals without any evidence of that filtering appearing at the BC soma.

5. Networks.

The Synaptology of Center-Surround Organization (Fig. 5)
The long-standing view of any BC or GC receptive field is that it has antagonistic center-surround
Figure 5. The synaptic flow that forms GC receptive fields. Cone signals (C) converge on BCs (B) which then converge on GCs (G), creating the canonical receptive field center, represented by a peak in the signal strength form the GC. The coupled HC layer (H:H) forms the large, slow antagonistic surround, while narrower ACs (A) form fast, small surrounds with damp oscillatory wings. The HCs dominate sustained signaling, so typical receptive field maps of the light required to excite cells represent BC+HC contributions. For ON cells, a spot of bright light will excite, while flanking regions of darkness will excite. For OFF cells, a spot of darkness will excite, while flanking regions of light excite. The red zones indicate regions outside the field where neither excites.
organization. The question has long been: where does the surround come from? Anatomically, the vast number of AC synapses at the BC synaptic terminal, as well as evidence of GABA<sub>c</sub> receptor function, suggested that ACs should have a powerful surround effect [5]. Conversely, direct current injection into non-mammalian HCs clearly shows an effective, low-frequency dominated, sustained path from HCs to GCs. Reconciling the two mechanism has been problematic but is likely simple. HCs and ACs function on very different time and space scales. HCs are slow, sustained (beyond the capacity of any normal neuron) and have immense receptive fields due to strong coupling by gap junctions. Thus the presence of very large antagonistic surrounds in GCs is likely driven through HCs. Experiments using fast pH buffers such as HEPES block these surrounds [54]. Conversely, GABAergic drugs have no effect on these large surrounds (in mammals). Evidence for GABAergic function in non-mammalian HCs is abundant, but clearly suffers from some of the same problems as conventional synaptic models. Thus it is likely that HCs in fishes serve multiple roles with multiple mechanisms, including pH or connexin mediated cone modulation, GABA transporter modulation of GABA<sub>c</sub> receptors in the outer plexiform layer (on various targets), direct synaptic control of some BC dendrites and glycinergic interplexiform cells, and even direct sign-conserving control of some BCs vie heterocellular coupling at the dendritic level. But the dominant surround mechanism remains the HC >i cone >m ON BC and HC >i cone > OFF BC chains.

So what about ACs and all those synapses? Why don’t they create the large surrounds of GCs? First, ACs have much smaller receptive fields than HCs and their range of action will thus be spatially much smaller. Second, many ACs themselves show antagonistic center-surround organization, likely due to AC >i AC chains [81,82]. Third, ACs are very fast and their actions at the BC terminal likely have more to do with feedback stabilization of synaptic gain than creating large, slow antagonistic surrounds. ACs work in a highly time-and-space restricted domain.
The Synaptology of Mammalian rod pathways - evolution of a new amplification scheme (Fig. 6).

Figure 6. The synaptology of the mammalian scotopic network (symbols as in Fig. 1). Rod signals predominantly reach GCs by three pathways. The main dark-adapted pathway flows from rods $\rightarrow$ rod BCs $\rightarrow$ glycinergic rod ACs which then redistributes the signal back into the cone BC channels via gap junctions (ON BCs) or glycinergic synapses (OFF BCs), and thence to GCs. The second, less sensitive path is via rod:cone gap junctions. A rarer path, not found in all mammals, is the occasional sampling of rod signals by OFF BCs.

As we have described earlier, the synaptic chains that drive GCs in all retinas are grouped into ON and OFF pathways. In non-mammals, rod and cone pathways both use this direct chain to target the CNS. Thus rods signals undergo two-stage amplification before being encoded as a GC spike train: rods $\rightarrow$ ON BCs, rods $\rightarrow$ OFF BCs and BCs $\rightarrow$ GCs. In mammals, a new amplification scheme evolved using cone BCs as the output stages, with rod BCs and glycinergic (gly) rod ACs as interneurons. Mammalian rod BCs are homologous to non-mammalian mixed rod-cone BCs, but have lost both cone inputs and, nominally, the ability to target GC dendrites. Nevertheless, six possible rod networks arising from three primary pathways (Fig. 6) exist in mammals, here grouped by amplification.

3-stage amplification

(1) rods $\rightarrow$ ON rod BCs $\rightarrow$ gly rod ACs $\rightarrow$ ON cone BCs $\rightarrow$ ON GCs
(2) rods >m ON rod BCs > gly rod ACs >i OFF cone BCs > OFF GCs

2-stage amplification

(3) rods :: cones >m ON cone BCs > ON GCs

(4) rods :: cones > OFF cone BCs > OFF GCs

(5) rods > OFF cone BCs > OFF GCs (sparse and species variable)

(6) rods >m ON cone BCs[83] > ON GCs (sparse)

Thus, rod vision is parsed into ranges served by different networks: (i) the gly rod AC network with two arms of three-stage amplification for threshold scotopic vision and (ii) the rod :: cone → cone BC → GC two-stage amplification for high brightness (moonlit) scotopic vision. Additional rod > cone BC contacts have been shown in some mammals [38,39], but whether these additional pathways are structural errors in evolution or functional is not certain, as their incidences vary across mammalian species [83].

The rod circuit is all the more complex for the involvement of GABAergic ACs, also known as S1 and S2 classes [11]. These γ rod ACs have dendritic arbors 1 mm in diameter and contact over 1,000 rod BCs with reciprocal feedback synapses, with S2 cells providing twice the number of feedback synapses as S1 [84]. This feedback likely further speeds the initially sluggish rod threshold response. One very interesting feature of this circuit is the problem of switching from scotopic to photopic states. If rods saturate in photopic regimes, the mGluR6 mechanism should leave rod BCs and gly rod ACs in a persistent depolarized state. If so, the gly rod AC should pass maximal, constant ON signals into ON cone BCs and OFF signals into OFF cone BCs, corrupting the true cone-driven BC pathways. Clearly there is a mechanism to counter this, as light-adapted
gly rod ACs actually show cone-driven OFF behavior. There are likely two mechanisms involved. First, NO-activated increases in cone BC cGMP may uncouple the gly rod AC :: ON cone BC network [59]. Second, gly rod ACs also receive a significant GABAergic synaptic input from unknown sources: likely cone-driven ACs [4]. This may actively antagonize any persistent depolarization from light-adapted rod BCs. Clearly there is more to learn about the rod-driven network in mammals.

The Synaptology of Motion - AC surrounds from afar (Fig. 7)

While the roles of ACs in forming the center-surround features of sustained GCs are cryptic, their primacy in encoding motion is established. Directionally-selective (DS) GCs respond to targets moving in a preferred direction, but remaining silent when targets move in the opposite, “null” direction [85,86,87]. DS GCs come in two classes: ON-OFF and ON GCs. These networks engage BCs and perhaps several different AC inputs, including the ON and OFF subtypes of [87] starburst GABAergic/cholinergic ACs and other GABAergic ACs [88,89]. OFF starburst ACs hyperpolarize to light and are driven by OFF cone BCs. ON starburst cells have somas displaced to the GCL, depolarize to light and are driven by ON cone BC inputs. Each class stratifies with and synapses on the dendrites of DS GCs. The precise classes of other γ ACs in DS GC networks are not known, but the functional roles of GABAergic inhibition are emerging. At least one GABAergic AC inhibits the starburst ACs, and others inhibit DS GCs. Thus, as stimuli come from the preferred side, a combination of excitatory glutamatergic BC and cholinergic starburst AC signals converge on the GC in advance of GABAergic inhibition. The excitatory gain is likely enhanced by the the BC > starburst AC > GC chain, which should have greater gain than a direct BC > GC transfer. In the null direction, a strong GABA signal reaches the DS GC in advance of the excitatory input and prevents it from reaching spike threshold. GABA\(\lambda\) receptor antagonists block this strong inhibition and
convert DS GCs into non-directional cells [85]. The inhibition seems so strong (almost like veto synapses in cerebellum) that the BC > starburst AC > GC circuit can’t break through. In fact, that may be the *raison d’être* for starburst ACs: to break through any residual inhibition in the preferred direction as GABA inhibition is strong *even in the preferred direction*. This is likely an archetype for all AC circuits, where spatial properties, timing and convergence of multiple cell classes select for fine grain features such as edges, texture or flicker.

Figure 7. One possible synaptology for the mammalian ON-center DS GC network (symbols as in Fig. 1). ON DS GCs collect glutamatergic excitatory signals from ON cone BCs and cholinergic excitatory signals from ON starburst ACs. This amplifies the center response. ON starburst ACs also provide GABAergic feedback onto BCs. However, additional GABAergic inputs exist input in the DS strata of the IPL. Such cells may receive inputs in the surround on one side and send axons (dotted) to target distant DS GC dendrites. Thus stimuli approaching from the left will excite and those from the right will inhibit.
The Synaptology of Color - HC surrounds again?

Humans and old-world primates have cone mosaics with sparse blue (B, SWS1) cone arrays [90] surrounded by randomly distributed red cones and green cones (R, LWSr; G, LWSg). Most mammals possess dichromatic vision via B and G cone opponencies. Complete trichromatic vision has two opponent processes [91]: (1) Blue/yellow (B/Y) opponency (where the Y signal is the sum of R and G cones signals) [92,93]; and red/green (R/G) opponency. Both pose conceptual problems. The R and G pigment genes are tandem head-to-tail LSW arrays on the X chromosome [21,91]. LSW cones can express only one pigment, either LWSr or LWSg, creating either R or G cones [94,95] and this may be the only gene product that discriminates R and G cones. No molecular marker is known that BCs can use to select between them and, similarly, no downstream marker is known discriminate between BCs connected to R or G cones. This suggests that the connectivity of R and G systems is probabilistic. But, R/G opponency is robust in trichromatic primates.

R/G opponency. In the foveola, each midget BC contacts only one cone and each midget GC contacts only one midget BC. Thus four types of center/surround R/G color opponency emerge: R+/G-, R-/G+, G+/R- and G-/R+ [96]. If the behavior of a single foveal R cone is not confounded by R::G coupling, a midget BC > midget GC chain should manifest a pure R or G center. Thus all midget GCs will be color opponent (Fig. 8) since their surrounds, whether derived from HCs or ACs, should be “yellower” than both: always greener than a R cone or redder than a G cone. HCs do not show any spectral selectivity for R or G cones and sum their inputs [97]. While it was once posited that even the surrounds were pure opponent (pure R versus pure G) via selective contact of opponent BCs by ACs, electron microscopy shows this is not likely [98]; that the AC driven surrounds of midget GCs encode mixtures of R and G cones. Even so, some midget GCs show
Midget pathways arise from midget BCs that contact only one cone and midget GCs that contact only one BC. This creates pure red (R) or green (G) center GCs in both ON and OFF channels. However the surround information from HCs and ACs collect from both R and G channels, generated “yellow” (Y) surrounds. GCs that are R/Y will generate net R/G percepts (shown in parenthesis), with similar outcomes for the three other possible R and G channels. OFF midget blue channels are likely antagonists by “yellow” H1 HCs and either Y or “white” (W) ACs that collect from all classes of cone BCs. GCs that are B/W will generate net B/Y percepts (shown in parenthesis), with similar outcomes for the three other possible R and G channels. It is also possible that diffuse BCs that contact all LWS cones (summarized as Y cones) may have surrounds from “white” H2 HCs (either by feedback as shown or feedforward) that generate Y/W+ fields leading to Y/B+ percepts. Finally, the best known B pathway involves selective for SW51 B cones. The GCs that collect these signals are small bistratified cells with ON B BC inputs in sublamina b and Y selective OFF BC inputs in sublamina a. There are at least three possible -Y channels: H1 HC feedforward to B cones, direct OFF BC inputs from LWS-selective BCs, and W opponent inputs from non-selective ACs. The inability of GABA antagonists to block Y opponency in these cells suggest that the HC network dominates.
nearly pure opponent surrounds [99,100,101], perhaps because of the patchiness of R and G cone distributions [102] and the small size of midget GC surrounds. Thus there is much more to be understood about midget GC networks. Why don’t broad yellow-sensitive (Y) HC surrounds dominate midget BCs, as they do for B/Y opponent GCs? Perhaps the patchiness of cones is partially reflected in a spectrally inhomogeneous HC layer if the coupling of foveolar HCs is weaker than in the periphery.

B/Y opponency: GCs that convey blue signals are thought to be of two varieties: large and small bistratified B+/Y- GCs [92,96] receive B cone ON BCs synapses in sublamina b and Y inputs from diffuse OFF BCs in sublamina a (Fig. 30). Though it was thought that B/Y centers and surrounds overlapped tremendously, but recent data suggest that the Y surround from HCs via HC >i cone feedback is much larger [53]. Recent anatomical evidence suggests the existence of a midget B cone OFF BC pathway in monkey [103] and a diffuse B cone OFF BC in rabbit [104]. Further, melanopsin GCs are putative B-/Y+ GCs with large receptive fields [105]. Which cells carry “the” blue signal remains uncertain as human patients lacking the mGluR6 receptor (and thus lacking ON BC signaling) apparently have quite excellent photopic sensitivity and apparently no color deficits.

Revising the retinal synaptic networks with disease

It was once thought that retinal networks were laid down once and for all in development by a process independent of sensory experience, but that is now clearly incorrect. No less correct is the idea that, during the process of photoreceptor deconstruction and after photoreceptor death, the neural retina remains largely normal. These and many other studies show that retina behaves much like CNS in response to challenges such as oxidative stress, denervation and trauma by remodeling its synaptic connectivity and reprogramming neural signaling rules. For example, the loss of
photoreceptors in retinitis pigmentosa leads to the retraction of BC dendrites and the evolution of new axon-like structures; the generation of abundant new processes from retinal neurons of all kinds; the formation of new synaptic zones in the form of microneuromas; the switch from mGluR6 to iGluR expression in former ON rod BCs; and the ultimate death of many neurons [12,106,107]. These changes challenge many strategies to restore vision by genetic, molecular, cellular and bionic schemes. But beyond that, they demonstrate two very important concepts. First, synaptic communication is likely never static and that signaling mechanisms are stabilized by active mechanisms. Second, the rules used by any neuron to decide which glutamate (or other) receptors to express are not known. We do not understand which transcriptional regulators make the decision to choose mGluR6 initially, much less choose AMPA or KA receptors in response to reprogramming.

Box 1

The mGluR6 receptor and vision.

Ionotropic glutamate receptors comprise the primary excitatory signaling pathways of the retina and CNS but the retina is specialized to express mGluR6 receptors (the human GRM6 gene) in both rod and cone ON BCs. GRM6 is expressed predominantly in retina, with minor transcript detection in brain. This high-gain, sign-inverting receptor converts light-activated hyperpolarizations of rods and cones into depolarizing signals in BCs. While there have been several theories advanced for the evolution of two polarity channels for visual processing (ON and OFF), no compelling data have emerged to support them. Recently, certain human night-blind patients were found to have GRM6 mutations [108]. Importantly, there appeared to be no significant photopic visual deficit in these patients, despite the absence of a photopic b-wave. For some time, the importance of mGluR6 in driving blue ON center cone BCs and GCs has been
discussed in the literature. Using functional magnetic resonance imaging (fMRI) in rodents, Mauck et al [109] have shown that the intraocular administration of the ON BC blocker 2-amino-4-phosphonobutyrate attenuates blue-driven fMRI blood-oxygen level dependent (BOLD) signals in the LGN. Remarkably, cortical blue-driven BOLD signals were enhanced, suggesting that blue percepts are effectively or even primarily carried by OFF channels. This suggests that plasticity need not be invoked to explain involved human photopic vision in patients with GRM6 defects. But the question remains: what does the world look like when viewed solely through OFF channels?

Box 2

Glutamate excitotoxicity.

Glutamate excitotoxicity has often been invoked as a mechanism in many retinal diseases, but the evidence is controversial. Reported elevations of vitreal glutamate once claimed to be associated with high-tension glaucoma [110] have not been validated, but the tidal wave of interest in excitotoxicity has nevertheless lasted for over a decade, despite the fact that glaucoma-mediated loss of GCs clearly did not match glutamate excitotoxic patterns in retina. Starburst ACs are the most glutamate sensitive neurons in the inner retina [63], yet there are no known AC losses in glaucoma. The problem has been exacerbated by the apparent neuroprotective effect of NMDA receptor antagonists in animal models of glaucoma [111]. But this may be a red herring. Whatever truly causes slow cell death in retinal GCs in glaucoma likely invokes Ca$^{2+}$-mediated apoptosis, and NMDA antagonists (like many other drugs) decrease constitutive Ca$^{2+}$ loads in neurons. Use of weak NMDA antagonists as neuroprotectants may have no lasting role in glaucoma therapeutics. Conversely, glutamate is likely a major player in retinal damage in diabetes and ischemic insults, but neuroprotection is difficult to achieve in those cases. It is likely that the major mechanism of
excitoxicity in hypoxic retina is initiated by reverse transport of glutamate by BCs, the repository of most of the glutamate in the inner retina. In such cases, competitive non-translocated transporter ligands may be safer neuroprotectants than ionotropic glutamate receptor antagonists.

Box 3

Retinal Remodeling in Retinal Degenerations.

Mammalian retinal degenerations initiated by gene defects in rods, cones or the retinal pigmented epithelium (RPE) often trigger loss of the sensory “photoreceptor” retina, effectively leaving the neural “inner” retina deafferented. The neural retina responds to this challenge by remodeling, first by subtle changes in neuronal structure and gene expression and later by large-scale reorganization. Retinal degenerations in the mammalian retina generally progress through three phases. Phase 1 initiates with expression of a primary insult, followed by phase 2 photoreceptor death that ablates the sensory retina via initial photoreceptor stress, phenotype deconstruction, irreversible stress and cell death, including bystander effects or loss of trophic support. During this period, survivor cones often host the dendrites of rod BCs that have lost their inputs, but it appears that these BCs undergo reprogramming, downregulating mGluR6 expression and upregulating iGluR expression. This is corruptive of visual processing. The loss of cones heralds phase 3: a protracted period of global remodeling of the remnant neural retina.

Remodeling resembles the responses of many CNS assemblies to deafferentation or trauma, and includes neuronal cell death, neuronal and glial migration, elaboration of new neurites and synapses, rewiring of retinal circuits, glial hypertrophy and the evolution of a fibrotic glial seal that isolates the remnant neural retina from the surviving RPE and choroid. New data indicate that remodeling in degenerative retinal diseases may be even more severe than initially believed: in advanced human RP, in the LD rat model, and perhaps even AMD, both glia and neurons break
through into the choroid and emigrate from the retina, further depleting the remnant retina of functioning neurons. Retinal remodeling is not plasticity, but represents the pathologic invocation of mechanisms resembling developmental and CNS plasticities. Together, neuronal remodeling and the formation of the glial seal may abrogate many cellular and bionic rescue strategies. However, survivor neurons appear to be stable, healthy, active cells and given the evidence of their reactivity to deafferentation, it may be possible to influence their emergent rewiring and migration habits.
Bibliography


