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 $\rightarrow$ BC $\rightarrow$ GC [2]. But, our understanding of detailed signaling is far from adequate and a complete description of synaptic interactions or signaling mechanisms is lacking for any retinal network [3]. For example, 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]. At a larger scale, network topologies are too numerous to resolve with current physiological or pharmacologic data [4]. Each GC contacts many different amacrine cells (ACs) and a full description of the inputs to any given GC does not yet exist [5]. Physiology can only screen only a limited parameter space for any cell. Pharmacology is still an emergent field with many incomplete tools 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 modulate signaling elements, remains an ambiguous tool for analyzing retinal networks. Morphology, augmented by immunochemistry and physiology, remains the core tool in discovering new details of retinal organization. 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 :: gliaform neurons :: horizontal cells (HCs)

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 includes photoreceptors and BCs. These cells are polarized neuroepithelia with apical ciliary-dendritic and basal axonal-exocytotic poles [19]. They form the first stage of synaptic gain in the glutamatergic photoreceptor → BC → GC → CNS vertical chain. This aggregates photoreceptor signals into BC receptive fields and amplifies their signals. The basal ends of the BCs form the inner plexiform layer. There are at least 12 kinds of BCs in mammals [16,20] and BCs delimit different functional zones in the IPL, suggesting 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\textsubscript{G}) visual pigments \cite{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\textsubscript{R} and RH2 green members of double cones, LWSR and RH2 green single cones) \cite{22}.

Similarly, the diversity of BCs in mammalians is lower (10-13) than non-mammalians (>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 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 \cite{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 \( \approx 30 \) kinds of ACs [15]. The 15-20 kinds of mammalian GCs [3,17] are classical projection neurons. GCs are postsynaptic at their dendrites and presynaptic at their axon terminals in CNS projections. So far, all are presumed to be glutamatergic. 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 glycinergic [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]. 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 phenotypo 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]. Some polyaxonal cells are GABAergic. There is no evidence for a glycinergic AxC. Multipolar neurons are characterized by numerous neurites branching in the plane of the retina, most collecting signals from BCs. Multipolar neurons are among earliest to develop in the retina and quickly define the borders of the IPL and its stratifications. 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, 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 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 glycinergic 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. Another class of primate HC (type II) has axon terminals contacting cones and rods. Importantly, the axon of HCs appears to be electrically inactive and these somatic and terminal regions are believed to act independently. 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 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, with diverse physics, topologies and molecular mechanisms leading to very different forms of synapses, most of which do not follow the single presynaptic “bouton” → 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 → SNE synapse (Fig. 1): photoreceptor → 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 thought that all photoreceptor signaling is glutamatergic, but sporadic indications of cholinergic physiology and molecular markers have been found in many non-mammalians [1]. 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. 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
small grape-like presynaptic spherules $\approx 3 \mu m$ 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
μm 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 → small volume, multi-target architecture.

2 BC ribbon synapses: semi-precise target signaling.

Like photoreceptors, BC signaling is generally considered glutamatergic [1]. Sporadic evidence of exceptions exist. In mammals (especially primates) and amphibians, some BCs contain biomarkers of GABA-related metabolism [24]. 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. 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. Large BC terminals such as those found in teleost fishes can drive up to two hundred distinct processes. Mammalian BCs drive 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 primary AMPA receptors. 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]. Acetylcholine (ACh) is a fast excitatory transmitter and is found in paramorphic starburst ACs in mammals and also uses conventional synapses [1]. However, we know of no distinguishing anatomical differences between GABA- and ACh-utilizing synapses in retina.

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 the other slow transmitters likely act via volume conduction [46] and modulate 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] and, now, the rod → HC → rod [54]. 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). Vesicular HC synapses are very rare and small. Several models of non-canonical signaling have been proposed including synaptic pH regulation [55], hemi-junction mediated ephaptic signaling [56], and even transporter-mediated signaling. Some of these will be discussed in detail below, but this 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 [57,58]. 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 associated with the size of the junctions, as they represent summed parallel conductances, and with functional modulation by various signaling pathways. Activated dopamine D1 receptors decrease conductances between coupled HCs [45,59] and coupled ACs [60], and dopamine D2 receptors modulate rod-cone coupling [61]. 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 tetrameric subunit compositions. Second, they are coincidence detectors that require dual activations by glutamate and by a glycine-like endogenous agonist. There is substantial evidence that this co-ligand may be D-serine released from Müller glia [62]. Finally, the mGluRs represent a complex collection of GPCRs whose functions are far from clear.

Different classes of neurons expresses 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 [63]. 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 [1,64]. When activated, iGluRs generate increased channel conductances and carry inward currents carried mostly by 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 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 signaling modality. As a classical GPCR, with G$\alpha$ as its cognate G-protein [65], 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 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 [63].

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 [66]. 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 dopamine [45]. In mammalian retinas, dopaminergic neurons are largely AxCs that arborize primarily in the distal-most layer of the IPL, appear to receive direct inputs from BCs, and have predominantly ON-type responses [67]. 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 [68], the uncoupling of rod-driven glycinergic ACs [60], enhanced spike speeds [69] (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 [70]. 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 [60], 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 [71].

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 neurotensin, 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 by Müller glia likely represents the last line of defense [1]. 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. 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. 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 (>), 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 photoreceptors, this decreases the rate of synaptic glutamate release and (in conjunction with 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. Thus photoreceptor → HC and OFF BC signaling is 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 [72] and blocks rod ON BCs responses in fishes. However, upon light adaptation, fish ON BCs change their behaviors. In response to photopic lights that activate cones, the “ON” 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 [73,74]. Thus, in photopic “dark”, glutamate 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 [3,64,75]. Thus all BC output synapses are sign-conserving (Fig. 1). The bulk of AC → BC, AC or GC signaling is either GABAergic or glycineergic via increased anion conductances [76]. Thus these synapses are characteristically sign-inverting (>i). GABAergic and glycineergic 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 takes a significant amount of inhibition to control glutamate synapses. Some inhibitory mechanisms involve the metabotropic GABA\(\beta\) receptor, which is a GPCR that can lead to a tremendous increase in potassium currents, but can also show paradoxical excitation [73]. Since potassium currents are usually outward (positive current flowing outward), GABA\(\beta\) can produce a strong and long-lasting inhibition near threshold. Different GABA receptors tend to be expressed at different sites (ionotropic GABA\(\alpha\) on ACs and BCs, ionotropic GABA\(\beta\) on BCs), but the distribution of GABA\(\beta\) 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, currents 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 [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 of them. 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 [5]. While there are no network models that use such chains, the minimum architecture for such a chain is BC → AC → AC → BC or GC. If we the AC outputs are sign-inverting, the net transfers of the chain is > >i >i > (sign-conserving). Importantly, the low gain of GABAergic and glycinergic synapses prevents such chains from being runaway excitations [5,77]. What roles might such networks play? In non-mammalian, 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. [78] argued that in rabbit, the only synaptic crossover networks are glycine → GABA.

Feedback, feedforward and nested feedback / feedforward

Designing analog operational amplifier networks is very similar to evolving a retina: every stage of forward amplification needs feedback control [79]. In retina, sign-inverting GABAergic mechanisms are used as feedback and feedforward control systems. Feedback is the most powerful way to set synaptic gain, improve signal-to-noise ratios, and improve synaptic bandpass. We will skip the mathematic demonstration of this, but note that it has been widely discussed [76,80].
the other hand, feedforward is an 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 it is 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 is the chloride reversal potential. We 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 and depolarize the
cell. 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. 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 [81]. 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 organization. Where does the surround come from? Anatomically, the vast number of AC synapses at the BC synaptic terminal, as well as evidence of GABA\textsubscript{c} 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 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 [55]. Conversely, GABAergic drugs have no effect on these large surrounds (in mammals). So what about ACs and all those synapses? Why

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.
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 smaller. Second, many ACs themselves show antagonistic center-surround organization, likely due to AC >i AC chains [82,83]. 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).

As we have described earlier, the synaptic chains that drive GCs in all retinas are grouped into ON and OFF pathways. In non-mammalians, 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 spike train: rods >m ON BCs, rods > OFF BCs and BCs > 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 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 >m ON rod BCs > gly rod ACs :: ON cone BCs > 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[84] > 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 [84].

The rod circuit is all the more complex for the involvement of GABAergic ACs, also known as S1 and S2 classes [11]. These \( \gamma \) 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 [85]. This feedback likely further speeds the initially sluggish rod threshold response.

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 [86,87,88]. 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 starburst GABAergic/cholinergic ACs and other GABAergic ACs [89,90]. 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\textsubscript{A} receptor antagonists block this strong inhibition and convert DS GCs into non-directional cells [86]. 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’etre 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.

The Synaptology of Color - HC surrounds again?

Humans and old-world primates have cone mosaics with sparse blue (B, SWS1) cone arrays [91] surrounded by randomly distributed red cones and green cones (R, LWS\textsubscript{R}; G, LWS\textsubscript{G}). Most mammals possess dichromatic vision via B and G cone opponencies. Complete trichromatic vision has two opponent processes [92]: (1) Blue/yellow (B/Y) opponency (where the Y signal is the sum of R and G cones signals) [93,94]; 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 [21,92]. LSW cones can express only one pigment, either LWS\textsubscript{R} or LWS\textsubscript{G}, creating either R or G cones [95,96] and this may be the only gene product that discriminates R and G cones. This
suggests that the connectivity of R and G systems is probabilistic. Even so, 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 (Fig. 8) [97]. 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 R cones or redder than G cones. HCs do not show any spectral selectivity for R or G cones and sum their inputs [98]. It was once posited that the GC surrounds were pure (pure R versus pure G) via selective contact of opponent BCs by ACs, but electron microscopy shows this is not so [99]; that the AC driven surrounds of midget GCs encode mixtures
of R and G cones. Even so, some midget GCs show nearly pure opponent surrounds [100,101,102], perhaps because of the patchiness of R and G cone distributions [103] and the small size of midget GC surrounds. Thus there is much more to be understood about midget GC networks. For example, why don’t broad yellow-sensitive (Y) HC surrounds dominate midget BCs, as they do for B/Y opponent GCs?

B/Y opponency: GCs that convey blue signals are thought to be of two varieties: large and small bistratified B+/Y- GCs [93,97] 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, recent data suggest that the Y surround comes from HCs via HC >i cone feedback and is much larger [53]. Recent anatomical evidence suggests the existence of a midget B cone OFF BC pathway in monkey [104] and a diffuse B cone OFF BC in rabbit [105]. Further, melanopsin GCs are putative B-/Y+ GCs with large receptive fields [106]. 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 clearly incorrect. No less correct is the idea that, during the process of photoreceptor deconstruction and death, the neural retina remains normal. Many 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 [12,107,108]. 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**

- The retina expresses mGluR6 receptors in both rod and cone ON BCs
- The human mGluR6 gene GRM6 is expressed predominantly in retina
- The mGluR6 receptor converts rod / cone hyperpolarizations into depolarizing BC signals
- The mGluR6 receptor dominates scotopic, but may not be essential for photopic vision
- Some human night-blind patients have GRM6 mutations [109], with no photopic deficit
- The mGluR6 drives blue ON center cone BCs and GCs
- fMRI in rodents [110] shows that blockade of mGluR6 receptors attenuates blue-driven signals in the LGN
- Simultaneously, blue-driven cortical signals are enhanced, suggesting that blue percepts are effectively or even primarily carried by OFF channels.
- OFF center BCs can support photopic vision
- The perceptual world under pure OFF vision is yet unknown

**Box 2 Glutamate excitotoxicity**

- Glutamate excitotoxicity has often been invoked as a mechanism in retinal diseases
- The evidence is mixed and controversial. On balance:
  - Elevations of vitreal glutamate in glaucoma [111] have not been validated
  - Glaucoma-mediated loss of GCs does not match known excitotoxic patterns in retina
  - Starburst ACs the most glutamate sensitive cells in the retina [64]
  - There are no established AC losses in primate glaucoma
• NMDA receptor antagonists appear neuroprotective in models of glaucoma [112]
• The mechanism of NMDA neuroprotection may be indirect
  • Retinal GC death in glaucoma likely involves Ca\(^{+2}\)-mediated apoptosis
  • NMDA antagonists (like many drugs) will decrease Ca\(^{+2}\) loads in neurons
  • Weak NMDA antagonists likely have no lasting role in glaucoma therapeutics
• Glutamate is likely a major player in retinal damage in diabetes and ischemic insults
• Neuroprotection is difficult to achieve in those cases as it involves AMPA receptors.
• Excitotoxicity in hypoxic retina is likely initiated by reverse transport of glutamate by BCs
• Competitive, non-translocated transporter ligands may be safer ocular neuroprotectants

Box 3 Retinal Remodeling in Retinal Degenerations
• Primary photoreceptor or RPE degenerations leave the neural “inner” retina deafferented
• The neural retina responds by remodeling in phases, first by subtle changes in neuronal structure and gene expression and later by large-scale reorganization.
• In Phase 1, expression of a primary insult activates photoreceptor and glial stress signals
• In Phase 2, ablation of the sensory retina via complete photoreceptor loss or cone-sparing rod loss triggers revision in downstream neurons.
• Total photoreceptor loss triggers wholesale bipolar cell remodeling
• Cone-sparing degenerations trigger BCs reprogramming, down-regulating mGluR6 expression and up-regulating iGluR expression.
• Loss of cone triggers Phase 3: a protracted period of global remodelin, including
  • 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
• In advanced disease, glia and neurons may enter the choroid and emigrate from the retina
• Retinal remodeling represents the pathologic invocation of plasticity mechanisms
• Remodeling likely abrogate or attenuate many cellular and bionic rescue strategies
• However, survivor neurons are stable, healthy, active cells
• It may be possible to influence their emergent rewiring and migration habits.

Bibliography


