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TOPICAL REVIEW

The retinal hypercircuit: a repeating synaptic interactive motif underlying visual function

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Abstract The vertebrate retina generates a stack of about a dozen different movies that represent the visual world as dynamic neural images or movies. The stack is embodied as separate strata that span the inner plexiform layer (IPL). At each stratum, ganglion cell dendrites reach up to read out inhibitory interactions between three different amacrine cell classes that shape bipolar-to-ganglion cell transmission. The nexus of these five cell classes represents a functional module, a retinal 'hypercircuit', that is repeated across the surface of each of the dozen strata that span the depth of the IPL. Individual differences in the characteristics of each cell class at each stratum lead to the unique processing characteristics of each neural image throughout the stack. This review shows how the interactions between the morphological and physiological characteristics of each cell class generate many of the known retinal visual functions including motion detection, directional selectivity, local edge detection, looming detection, object motion and looming detection.

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Abbreviations CHAT, choline acetyltransferase; IPL, inner plexiform layer; LED, local edge detector.

Introduction

The retinal hypercircuit is formed by the interactions of three different morphologically defined classes of amacrine cells, the narrow, medium and broad field cell types, with the bipolar-to-ganglion cell pathway. These amacrine cell classes have been well documented in rabbit through the anatomical studies from the Masland lab (MacNeil & Masland, 1998; MacNeil *et al.* 1999, 2004; Masland, 2001*a*,*b*) creating a comprehensive morphological dictionary of mammalian retinal neurons. There exist more than 50 morphological retinal cell types as shown in Fig. 1, modified from the original papers. Interactions between a limited number of photoreceptor and horizontal cell types generate bipolar activity that drives the inner retina. The inner plexiform layer (IPL) is composed of about 12 distinct strata, visualized as discrete layering of bipolar cell axon terminals and corresponding ganglion cell dendrites. This stratification is laid down by an infrastructure that is prescribed early in development (Yamagata *et al.* 2002; Kim *et al.* 2010). Each of the strata carries a different representation of the visual world (Roska & Werblin, 2001; Werblin *et al.* 2001; Roska *et al.* 2006*a*). A similar stratification has also been described in non-mammalian vertebrates (Pang *et al.* 2004) suggesting that this stratification may be a general organizing principle of the vertebrate retina. Hypothetical dynamic patterns of activity across dendritic field of the different ganglion cell types are included in supplementary material.

Frank Werblin is Professor of Neurobiology at UC Berkeley. His initial study of retinal processing with John Dowling in 1969 was one of the first to define the physiological properties of retinal neurons from photoreceptors to bipolar cells to ganglion cells. He has continued to study the retinal circuitry that underlies neural processing. In retrospect, the initial studies missed the components of amacrine cell inhibition that we now know, from the work of the retinal research community, constitute the essential processing components that intersect the photoreceptor–bipolar–ganglion cell pathway to generate many sophisticated forms of neural behaviour. This review attempts to summarize what we now know about the inhibitory composition of the retina and show how different forms of inhibition contribute to visual function.

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The retinal hypercircuit is repeated throughout the volume of the inner plexiform layer

Bipolar cell stratification. For the most part, each stratum of the IPL receives synaptic input from a distinct population of bipolar cell axon terminals (Fig. 1*C*). Each of the separate populations of ON and OFF cone bipolar cells is functionally similar, but each derives its unique spatial response from the morphological relationship of its dendrites with the release sites at photoreceptor terminals, and its phase and kinetics from mGluR6 or AMPA/kainate receptors in different ratios (DeVries & Schwartz, 1999; DeVries, 2000; DeVries *et al.* 2006). Each bipolar synaptic terminal interacts with some combination of three main amacrine cell classes. These interactions are then 'read out' by ganglion cells generating a unique visual function at each stratum (Fig. 1*H*).

Correlation of morphology, pharmacology and response characteristics of amacrine cells with inhibitory currents in bipolar, amacrine and ganglion cells

Bipolar, amacrine and ganglion cells at each stratum receive inhibition from different combinations of the narrow (Fig. 1*D*), medium (Fig. 1*E* and *F*) and wide (Fig. 1*G*) field amacrine cells. The spatial, physiological response and pharmacological properties of each of the three amacrine cell types can be correlated with the three different forms of inhibition that have been measured in bipolar, amacrine and ganglion cells. These correlations are described below and summarized in Fig. 2.

Narrow field amacrine cells

Morphology, and pharmacology of narrow field amacrine cells. Populations of narrow field diffuse amacrine cells (Fig. 1*D*) span many sublaminae usually crossing the ON–OFF boundary of the IPL. The processes of these cells typically extend laterally by less than 100 μm. Many have been identified as glycinergic (Menger *et al.* 1998; Chen *et al.* 2010). An exception is the A2 amacrine cell, (not to be confused with the All amacrine cell) which has been shown to be GABAergic (Pourcho & Goebel, 1983). These cells generate a sustained or transient response at ON or OFF, but seldom at ON *and* OFF suggesting that they receive input exclusively from either the ON or OFF sublaminae. The response latency is typically about 160 ms (Chen *et al.* 2011).

Postsynaptic expression of narrow field amacrine cell inhibition. Bipolar, amacrine and ganglion cells all receive glycinergic inhibition with properties that correlate with the properties of the narrow field amacrine cells. Glycinergic inhibition is sustained, at either ON or OFF, but seldom ON *and* OFF, with latency around 160 ms. This glycinergic inhibition is typically elicited over a quite narrow spatial extent as measured in β and parasol and local edge detector ganglion cells (Chen *et al.* 2010; Chen & Werblin 2011; Russell & Werblin, 2010). In most cases, this glycinergic inhibition has been identified as

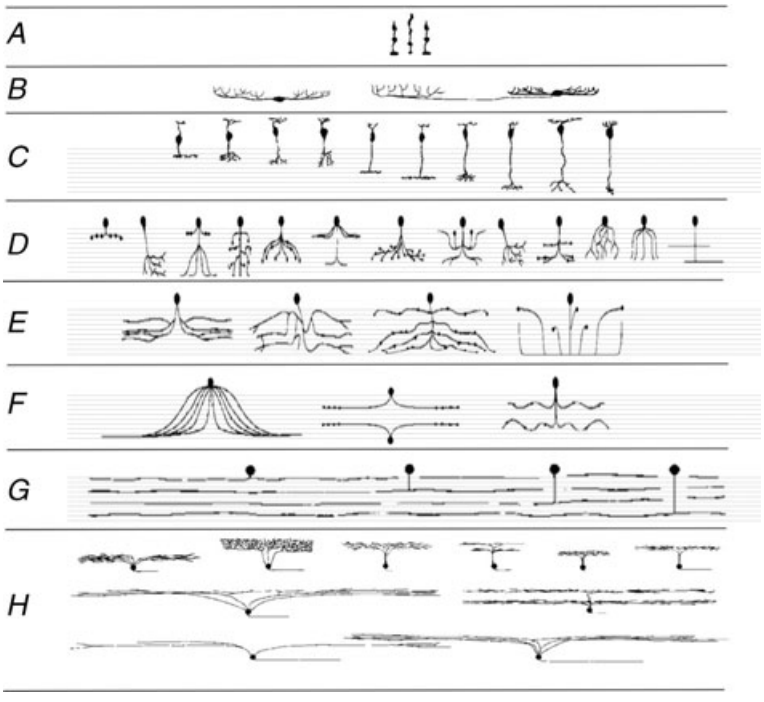


Figure 1. A dictionary of morphological cell types in the mammalian retina modified from Masland (2001*a*)
This figure shows more than 50 different morphological cell types. *A*, retinal rods and cones; *B*, horizontal cells; *C*, bipolar cells, with axon terminals in each stratum; *D*, narrow field diffuse amacrine cells; *E*, medium field laterally stratified amacrine cells that have NOT been well characterized; *F*, A17, starburst and DAPI 3 amacrine cells and wide field laterally stratified amacrine cells; *G*, wide field amacrine cells (the lateral scale is compressed here; *H*, ganglion cells.

'crossover inhibition' (Roska *et al.* 2006*a*,*b*; Molnar & Werblin, 2007*b*; Hsueh *et al.* 2008; Molnar *et al.* 2009; Werblin, 2010). Crossover inhibition is characterized as OFF cells receiving ON inhibition and ON cells receiving OFF inhibition.

Medium field amacrine cells

Morphology and pharmacology of medium field amacrine cells. The processes of most medium field amacrine cells (Fig. 1*E*) extend across the ON–OFF boundary. Some have processes that avoid specific sublaminae (MacNeil & Masland, 1998). Many have been identified as GABAergic (Chen *et al.* 2010). They extend laterally by about 200 μm and respond with sustained or transient activity with latency of about 200 ms. An exception is the DAPI 3 cell that is identified as glycinergic (Wright *et al.* 1997).

Postsynaptic expression of medium field amacrine cell inhibition. A local GABAergic inhibition extending about 200 μm beyond the receptive field centre has been measured in bipolar, other amacrine, and ganglion cells (Cook *et al.* 2000; Lukasiewicz *et al.* 2004; Ichinose & Lukasiewicz, 2005; Eggers & Lukasiewicz, 2006*b*, 2010); Eggers *et al.* 2007; Hsueh *et al.* 2008; Molnar *et al.* 2009; Chen *et al.* 2010). This GABA inhibition extends laterally by about 200 um. GABA inhibition has never been measured as crossover, i.e. ON GABA inhibition never affects OFF cells, and OFF GABA inhibition never affects ON cells. This is surprising because most of the medium field amacrine cells, particularly those in Fig. 1*E* and *F*, span the ON–OFF boundary. The DAPI 3 amacrine cell extends laterally by about 200 μm, and provides local glycinergic inhibition to starburst amacrine cells (Euler *et al.* 2002). A correlation between medium field amacrine cells and GABA content has been identified (Chen *et al.* 2010).

Wide field amacrine cells

Morphology and pharmacology of wide field amacrine cells. Separate populations of wide field (polyaxonal) amacrine cells are shown in Fig. 1*G*. They usually ramify at a single stratum and are found at every stratum of the IPL (MacNeil & Masland, 1998; Volgyi *et al.* 2001). The response is spike-like and quite transient, at either ON, OFF or ON–OFF, with latency less than 100 ms. Wide field cells propagate action potentials along widely ramifying narrow axonal processes that can extend over distances greater than 1 mm. The receptive fields of the polyaxonal cells extend beyond the central processes that have been described as dendritic due to electrical coupling (Volgyi *et al.* 2001). Other classes of wide field amacrine cells with

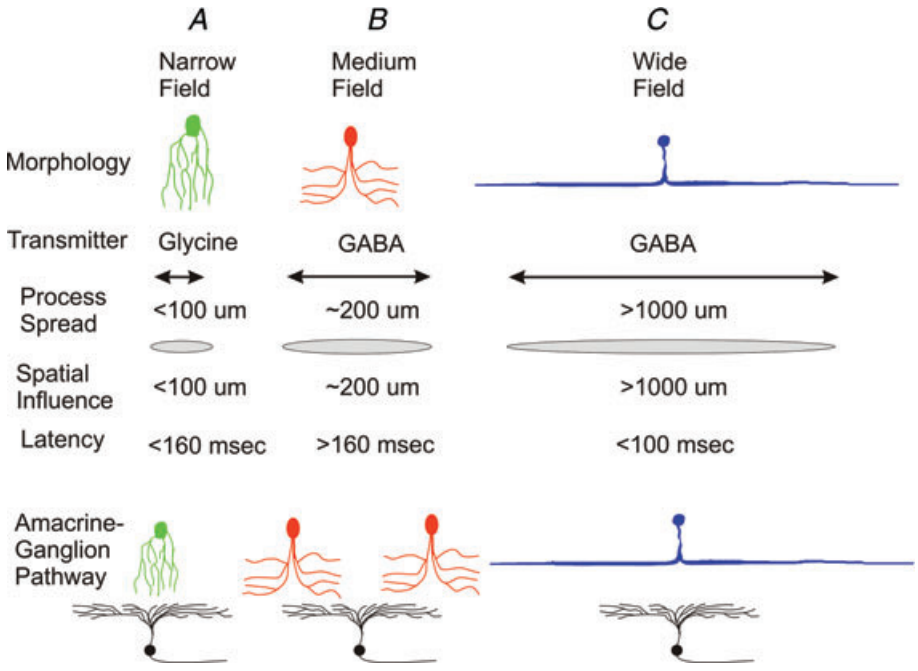


Figure 2. Correlating postsynaptic response profiles of local glycinergic, local GABAergic and broad GABAergic inhibition with narrow field, medium field and wide field amacrine cells
A, local glycine inhibition is quite narrow, less than 100 μm, and usually confined within the dendritic field of the postsynaptic cell. *B*, local GABA inhibition extends about 200 μm beyond the dendrites of the postsynaptic cell. *C*, broad GABA inhibition has the shortest latency, and integrates activity over broad regions extending beyond 1 mm. These three forms of postsynaptic activity closely resemble the physiology, known pharmacology and timing of the narrow, medium and wide field amacrine cell types.

similar morphology, but likely serving other functions, have been identified such as the serotonin accumulating amacrine cell at the most proximal margin of the IPL (Vaney, 1986; Borghuis *et al.* 2011). The processes of the A18 or dopaminergic amacrine cell lie along the inner margin of the IPL in cat (Kolb *et al.* 1981).

Postsynaptic expression of wide field amacrine cell inhibition. Both bipolar and ganglion cells, but not amacrine cells, receive a transient inhibitory input at ON, OFF or ON–OFF (Roska & Werblin, 2001; Werblin *et al.* 2001; Roska *et al.* 2006*a*; Hsueh *et al.* 2008; Chen *et al.* 2010). This input is driven by annuli of diameter up to 1 mm (Chen *et al.* 2010) or by rapid changes in broad field stimuli, increasing over areas greater than 1 mm. The response latency is short, usually less than 100 ms. Cells affected by this short latency transient inhibition are found in the strata between the choline acetyltransferase (CHAT) bands of the IPL (Roska & Werblin, 2003).

Summarizing these correlations: narrow field amacrine cells generate local glycinergic inhibition falling within the dendritic field of postsynaptic cells, medium field amacrine cells generate local GABAergic inhibition that extends between 200 and 300 μm beyond the dendrites of the postsynaptic cells, and wide field (polyaxonal) amacrine cells generate broad transient GABAergic inhibition that can extend to more than 1 mm. These correlations are shown in Fig. 2.

The lateral and vertical repeating motif at the synaptic nexus of amacrine, bipolar and ganglion cells

Interactions between the three amacrine cell classes and bipolar cell terminals form a repeating hypercircuit within each stratum as shown in Fig. 3. The hypercircuit is also repeated about a dozen times at different strata distributed throughout the depth of the IPL. A representation of the stacked layers of hypercircuits is shown in Fig. 6.

Inhibitory roles of the amacrine cells: narrow cells mediate crossover inhibition, medium cells mediate lateral inhibition and wide amacrine cells mediate saccadic suppression

Narrow amacrine cells carry crossover inhibition to correct for synaptic rectification. Most cones and bipolar cells generate a transient response of one polarity at ON and a transient response of opposite polarity at OFF, operating around a neutral, ambient potential level as shown in voltage responses in Fig. 4 (Molnar & Werblin, 2007*b*). Synaptic release depends upon calcium entry at the synaptic terminal, and calcium activation increases exponentially with depolarization. As a consequence of the non-linear calcium activation curve, transmitter release is greater at the transient depolarization phase than at the transient hyperpolarization phase. This asymmetry leads to the non-linear postsynaptic currents shown in Fig. 4. The crossover pathway corrects for this non-linear distortion of the signal. Through crossover, non-linear ON activity is inverted and 'crossed over' to the OFF pathway via the narrow field glycinergic amacrine cells, then added to excitation to reconstruct the original linear signal in the OFF ganglion cell. Glycinergic crossover inhibition of this form has been measured in bipolar, amacrine and ganglion cells (Molnar & Werblin, 2007*b*; Hsueh *et al.* 2008; Molnar *et al.* 2009; Werblin, 2010).

Medium-size up to 200 μm laterally oriented amacrine cells mediate lateral inhibition, edge enhancement and gain control

Many of the morphological amacrine cell types in Fig. 1*E* and *F* extend laterally for up to 200–300 μm. These cells have been shown to contain, and probably release, GABA (Chen *et al.* 2010) making them likely candidates for local lateral GABAergic inhibition. Many studies have described a GABAergic inhibitory influence

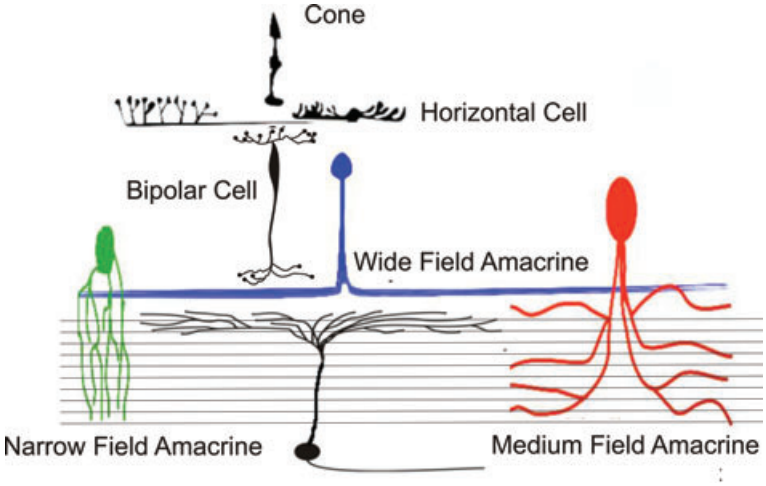


Figure 3. One stratum representation of the retinal hypercircuit distilled from the different morphological cell types shown in the Masland dictionary in Fig. 1
This includes a cone, a horizontal cell, a bipolar cell, a narrow diffuse vertically oriented amacrine cell, a medium field laterally oriented amacrine cell, a wide monostratified amacrine cell, probably a polyaxonal amacrine cell and a ganglion cell. The cell types are represented as icons, not meant to resemble any specific cell type within a class.

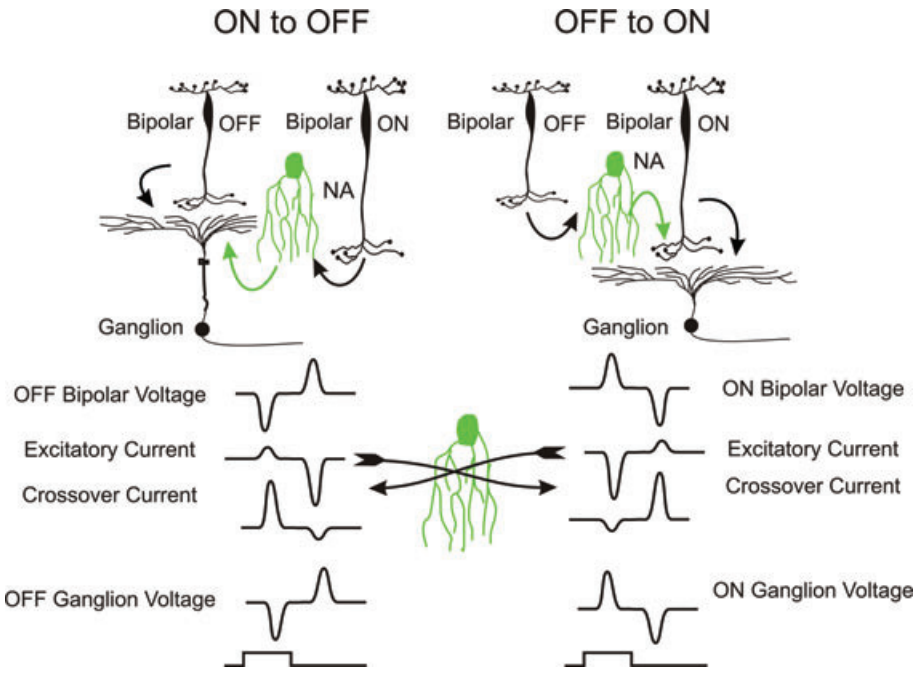


Figure 4. Circuitry underlying crossover inhibition
Upper left, the narrow field amacrine cell (green) is driven by the ON bipolar cell and serves to inhibit activity in the OFF bipolar, amacrine and ganglion cells. Upper right, the narrow field amacrine cell is driven by the OFF bipolar and inhibits the ON neurons. Lower left, OFF bipolar voltage leads to excitatory current. This current combines with the crossover current from the ON-driven amacrine cell to generate a replica of the OFF bipolar voltage in the OFF ganglion cell. NA: narrow field amacrine cell.

from a local surround in bipolar and ganglion cells, spanning the range from 200 to 300 μm. Lateral inhibitory GABAergic lateral inhibition has been measured in both salamander (Wu, 1986; Lukasiewicz & Werblin, 1990; Lukasiewicz *et al.* 2004; Eggers & Lukasiewicz, 2006*b*; Chen *et al.* 2010) and mammalian retinas (Eggers & Lukasiewicz, 2006*a*, 2010; Eggers *et al.* 2007; Chen *et al.* 2010; Russell & Werblin, 2010). The circuitry for lateral inhibition from medium field amacrine cells is sketched in Fig. 5.

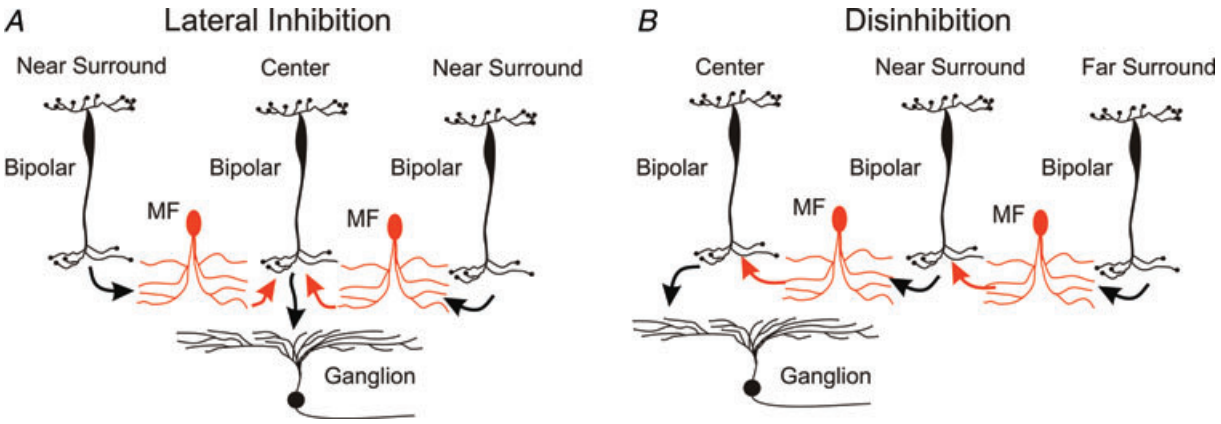


Figure 5. Circuitry for lateral GABAergic inhibition and possible pathway for inhibition of inhibition without direct amacrine-to-amacrine synaptic contact
A, circuitry for lateral GABAergic inhibition. Many studies have shown a local GABA inhibition extending about 200 μm beyond the ganglion cell dendritic field. The narrow amacrine cell is too narrow; the wide amacrine cell is too broad and transient. This suggests that the inhibitory interneuron is likely to be the medium amacrine cell. Right, possible pathway for inhibition of inhibition without direct amacrine-to-amacrine synaptic contact. The right-most medium interneuron inhibits the bipolar cell that provides excitation to the left medium amacrine cell thereby reducing the activity of the left amacrine cell. This pathway allows for inhibition of inhibition without a direct inhibitory synapse between medium amacrine cells. MF: medium field amacrine cell.

There remains some controversy as to whether GABAergic amacrine cells mediate lateral inhibition in all mammalian retinas. McMahon *et al.* (2004) recording from primate ganglion cells, found no evidence for GABAergic lateral inhibition. But others (Zaghloul *et al.* 2007; Russell & Werblin, 2010) used a high spatial frequency luminance-neutral inverting grating designed to be invisible to horizontal cells, and elicited a clear GABA surround response.

GABAergic interactions at the inner retina have also been interpreted as mediating gain control (Beaudoin *et al.* 2007, 2008; Demb, 2008; VanLeeuwen *et al.* 2009) or forming an antagonistic surround (Lukasiewicz *et al.* 2004; Ichinose & Lukasiewicz, 2005; Chen *et al.* 2010; Eggers & Lukasiewicz, 2010).

Disinhibition. Chen *et al.* (2010) found no local GABAergic amacrine cell inhibition measured in other medium field amacrine cells, precluding GABAergic amacrine to amacrine inhibition. However, other studies suggest that GABAergic amacrine cells can inhibit other GABAergic amacrine cells (Lukasiewicz *et al.* 2004; Eggers & Lukasiewicz, 2006*a*, 2010; Eggers *et al.* 2007). In some studies the effects of GABA inhibition directly to bipolar cells has been reported, refining the spatial properties of GABA feedback to bipolar cells (Kaneko & Tachibana, 1987, 1988; Tatsukawa *et al.* 2005; Eggers & Lukasiewicz, 2006*a*, 2010) and affecting the time course of bipolar responses (Molnar & Werblin, 2007*a*). It is possible that some of the GABA inhibition may be fed back to bipolar cell terminals as shown in Fig. 5 right. This pathway allows for disinhibition but does not require any serial GABAergic amacrine cell synapses.

Wide field monostratified GABAergic amacrine cells mediate saccadic suppression and object motion

The widely ramifying amacrine cells, represented the cells in Fig. 1*G*, resemble the polyaxonal amacrine cells that have been identified and characterized pharmacologically as GABAergic (Volgyi *et al.* 2001; Wright & Vaney, 2004). There are at least six subtypes (Volgyi *et al.* 2001), three that respond at ON and OFF and three that respond only at ON. (There seem to be no OFF responding polyaxonal amacrine cells.) Their processes extend over broad distances across the IPL up to 1.5 mm, and the cells, in most cases, appear to be strongly electrically coupled (MacNeil & Masland, 1998; Volgyi *et al.* 2001). The soma of the Type 1 cell lies within the IPL itself (Wright & Vaney, 2004).

Polyaxonal amacrine cells that lie between the CHAT bands have been implicated functionally in mediating saccadic suppression (Roska & Werblin, 2003). Polyaxonal cells have also been invoked for mediating the detection of object motion (Olveczky *et al.* 2003, 2007; Baccus *et al.* 2008). Because these cells incorporate voltage-dependent sodium channels, depolarizing responses are accelerated and peak very early. Consequently, ganglion cells are transiently inhibited by wide field amacrine cell input before the more slowly rising bipolar cell excitatory input peaks. Full inhibition is expressed before excitation, blocking spiking in ganglion cells (Cook & Werblin, 1994; Volgyi *et al.* 2001). These wide field amacrine cells receive glycinergic, but no GABAergic inhibition (Chen & Werblin, 2011). Serotonin-containing (Vaney, 1986) amacrine cells are found along the proximal border of the IPL. Dopaminergic amacrine cells are located at the outer margin of the IPL (Dowling & Ehinger, 1978; Dacey, 1988, 1990) and the response can be either sustained or transient (Zhang *et al.* 2007). A sketch of the circuitry associated with different strata of the wide field amacrine cells is shown in Fig. 6.

Hypercircuit interactions that generate *visual* functions

Each *visual* function is formed by a specific combination of hypercircuit elements. In some cases synaptic rectification is required; in others crossover inhibition linearizes transmission. Responses involving the local edge detector, movement detection and directional selectivity require rectification. With rectification, changes in release at ON are not balanced with the decrement in release at OFF. Similarly, the leading and trailing edges of a stimulus are asymmetrical, leading to motion detection. With rectification compensated by crossover the change in bipolar transmitter release signalling the *arrival* of activity is balanced by the change in release signalling the *departure* of activity, precluding motion detection. Visual functions like looming detection and edge detection require balanced linear activity. The signals generated to flashes and movement with and without rectification are illustrated in Fig. 7.

Visual functions requiring rectification

Local edge detector. The local edge detector (LED) receives rectified input from bipolar cells responding to luminance-neutral textured input. Activity can be elicited by inverting gratings with period near 50 μm matching the span of the bipolar cell dendrites (Zaghloul *et al.* 2007; Russell & Werblin, 2010). The LED appears to receive both ON and OFF bipolar input but is dominated by OFF activity. A luminance-neutral inverting grating with similar resolution can activate a GABAergic inhibitory surround, in these LEDs. This GABAergic inhibition extends about 200 μm beyond the dendritic field suggesting that it is carried by one of the classes of medium amacrine cells shown in Fig. 1*E*. The circuitry underlying the LED behaviour is shown in Fig. 8.

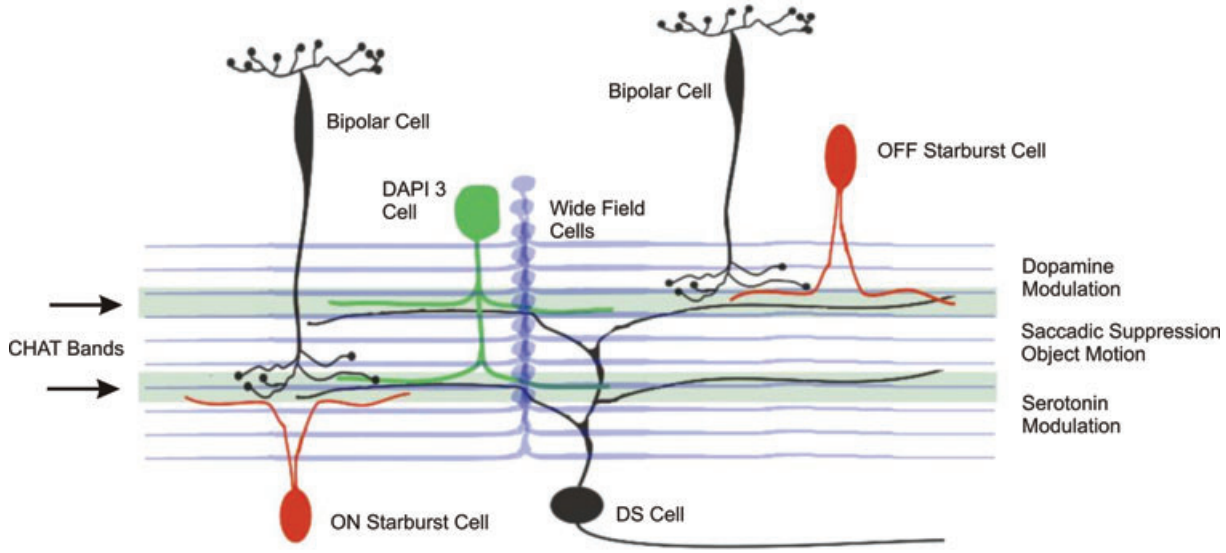


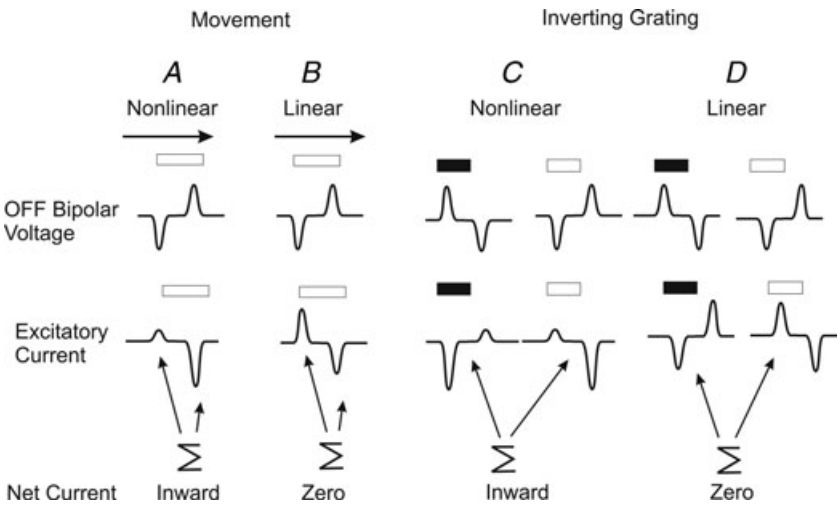
Figure 6. Wide field cells occupy each stratum
Wide field amacrine cells (light blue) in the central strata of the IPL between the levels of the DS dendrites or the CHAT bands are activated by change over large regions of the retina, and feed both forward and back to provide transient inhibition during a saccade (saccadic suppression). Wide field cells outside the CHAT bands serve neuromodulatory functions. DS circuitry is shown here to establish landmarks within the IPL for the different functional levels of wide field amacrine cell activity.

Directional selectivity. DS cells also receive rectified input from the population of bipolar cells within their dendritic field, thereby yielding a motion-sensitive response. The DS system incorporates the very special properties of the starburst amacrine cells in a complex circuitry (O'Malley & Masland, 1993; Yang & Masland, 1994, Peters & Masland, 1996, Chiao & Masland, 2002; Fried *et al.* 2002, 2005; Jeon *et al.* 2002; Lee & Zhou, 2006). The population of starburst cells provides more inhibition to the DS cell from the null side than from the preferred side. This inhibition also feeds back to the bipolar cells. The starburst cells are GABAergic and are mutually inhibitory, consistent with other medium field amacrine cells. In summary, GABA inhibition from the null side reduces release from bipolar cells, and inhibits ganglion cells and starburst cells on the preferred side, as shown by the three leftward-directed orange arrows *b*, *c* and *d* in Fig. 9.

Visual functions requiring linearity

Looming detector. The looming detector is a wide dendritic field OFF cell that responds to the increasing dimension of a dark shadow expanding across its dendritic field (Munch *et al.* 2009). This cell type is insensitive to lateral movement across its dendritic field. Insensitivity to lateral movement requires that the excitatory response

Figure 7. Rectified signals generate response to movement and texture
Left panel, responses to movement. Bipolar responses are balanced at the leading and trailing edges of the moving bar. *A*, with non-linear release the increase in excitatory current at the leading edge of a moving bar is larger than the decrease at the trailing edge, generating a net inward current during movement. *B*, increases in excitation and inhibition are balanced generating zero excitation to movement. Right panel, responses to inverting grating. *C*, for non-linear input a net inward current is generated at the initiation of a dark bar (left) and at the termination of a light bar (right) leading to inward current at each inversion of the grating. *D*, for linear input no net current is generated at each inversion because ON and OFF responses are balanced.



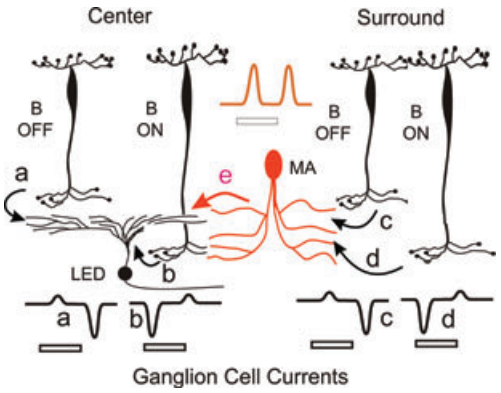


Figure 8. Circuitry for local edge detector
The centre LED receives non-linear synaptic input at centre from both OFF (*a*) and ON (*b*) bipolar cells. The LED also receives a GABA inhibitory input (e) from medium field amacrine cells in the surround that are driven by OFF (*c*) and ON (*d*) non-linear bipolar input. In addition, these cells receive local ON and OFF early transient very narrow glycinergic input, likely to be from the narrow field amacrine cells that serves to delay the LED response (not shown here).
(e) texture sensitive GABAergic inhibitory input from LED surround.
MA: medium field amacrine cell.

at the leading edge of the laterally moving shadow be countered by a decrease of excitation (or increase in inhibition) at the trailing edge of the moving shadow. This is accomplished by a crossover inhibition like that shown in Fig. 3 where the onset and offset responses are made equal and opposite. Linearization is achieved by crossover inhibition mediated by glycinergic amacrine cells which in this case are the AII amacrine cells. It is also necessary that the timing of the increase and decrease in excitation be synchronized. This is accomplished by the electrical synapse from ON cone bipolar cells to AII amacrine cells that then make glycinergic inhibitory contact with the OFF ganglion cell. That synapse linearizes the cell's response and suppresses responses to moving targets but permits response to expanding targets (Munch *et al.* 2009). The circuitry for the looming detector is shown in Fig. 10.

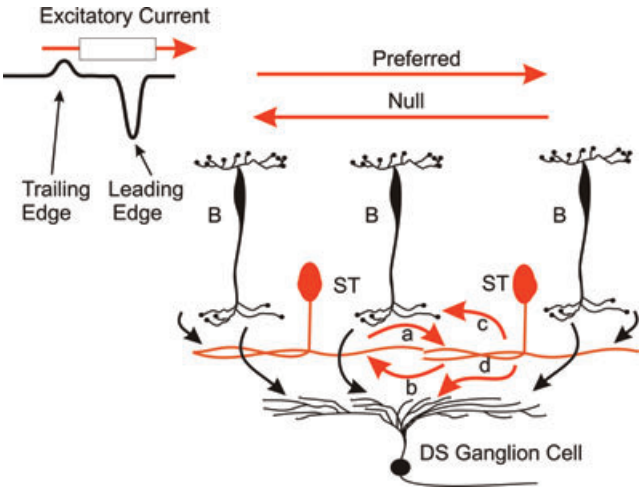


Figure 9. Circuitry for the directionally selective ganglion cell
The DS cell receives nonlinear, motion-sensitive input from populations of ON and OFF bipolar cells. Only the ON components of the DS and starburst (ST) cells are shown here. Starburst amacrine cells, respond symmetrically to centrifugal movement from centre to surround. They mutually inhibit each other (*a* and *b*) symmetrically. But inhibition from starburst to bipolar (*c*) and ganglion cells (*d*) is stronger from the 'null' than the 'preferred' side. This null side inhibition provides the asymmetry that leads to directional selectivity in the DS cell. *B*: bipolar cell, ST: starburst cell. Although only 2 starburst cells are shown here, there may be as many as 70 starburst cells within the dendritic field of the DS cell. The glycinergic DAPI 3 cells likely play a role, but that has not yet been defined.

Figure 11 summarizes the known roles of the three different classes of amacrine cells. Narrow field amacrine cells carry glycinergic inhibition vertically through the IPL while medium and wide field amacrine cells carry GABAergic inhibition laterally across the IPL. It is likely that different combinations of these inhibitory interneurons will be involved in mediating other yet-to-be-discovered visual functions. If these general rules continue to apply, we could expect the glycinergic releasing, ACh receiving DAPI 3 cells that ramify in the CHAT bands to be involved in directionally selective cross-over inhibition.

Some remaining mysteries about the amacrine cells

The likely role of the different morphological amacrine cell types is summarized in Fig. 11. But there remain many unanswered questions about amacrine cell function. Some of these mysteries are addressed below.

Why are polyaxonal amacrine cells monostratified? A subset of monostratified wide field amacrine cells are polyaxonal and responsible for saccadic suppression. The polyaxonal amacrine cells, thought to be responsible for saccadic suppression (Roska & Werblin, 2003) and object motion (Olveczky *et al.* 2003) are rigorously monostratified between the CHAT bands (MacNeil & Masland, 1998). The bipolar input at each stratum has a specific time course (DeVries, 2000; DeVries *et al.* 2006), so these amacrine cells' responses must accurately interact with the timing of bipolar to ganglion cell activity. It must block activity during the saccade, but release the pathways from inhibition immediately following the saccade. Having a specific polyaxonal amacrine cell population at each stratum synchronized with the timing of bipolar activity at that stratum may be critical for its function in either saccadic suppression or object motion. But if general motion during a saccade elicits suppression, why are we able to follow a bird in flight through the forest where the background is moving rapidly across the visual field? If retinal drift during object motion activates

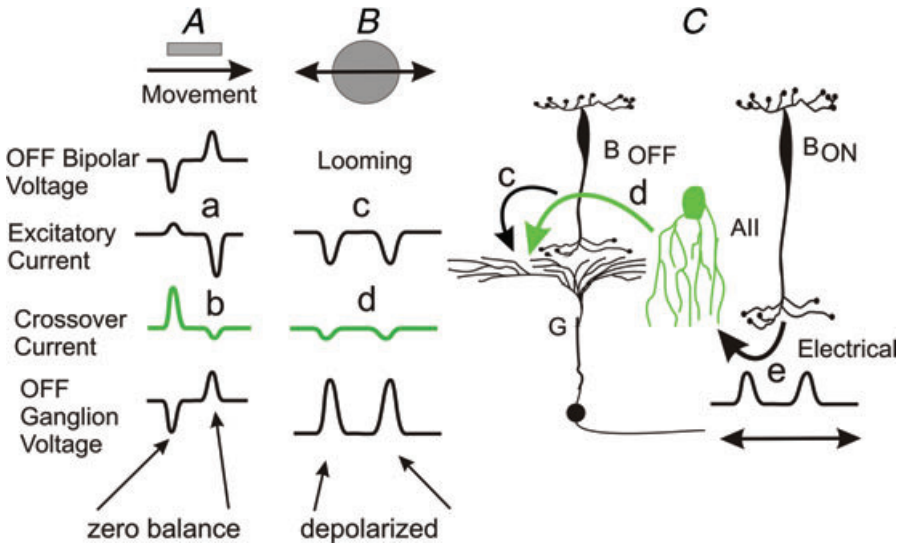


Figure 10. Circuitry and signals underlying the looming detector
To ensure that this cell does not respond to translational movement, the synaptic inputs to this cell are 'linearized' by crossover inhibition, mediated by AII glycinergic amacrine cells (AII). *A*, the cell is unresponsive to lateral movement because excitation (*a*) and inhibition (*b*) add, so the leading and trailing edges of the stimulus generate equal and opposite activity resulting in zero balance. *B*, the cell is responsive to looming because the OFF excitation at both edges (*c*) adds to ON inhibition at both edges (*d*). The expanding shadow only has a leading dark edge (no trailing edge) generating only inward currents to depolarize the looming detector. *C*, looming circuitry: the AII amacrine cell receives outward currents (*e*) from the ON bipolar cell and delivers inward currents (*d*) that add to the excitatory currents (*c*) to the looming detector (*G*). B OFF: OFF bipolar cell, B ON: ON bipolar cell, G: ganglion cell.

similar suppressive amacrine cells, we should be subject to numerous scotomas in time making it unlikely that we would ever see anything at all in the presence of ongoing retinal drift.

Why are medium field GABAergic lateral inhibitory amacrine cells vertically diffuse? The contacts between bipolar cell terminals and ganglion cell dendrites are stratum-by-stratum specific as shown in Fig. 1*C* and *H*. But the medium field GABAergic amacrine cells that provide local lateral inhibition to these bipolar and ganglion cells are characteristically *multistratified* although the strata at which these amacrine cell processes ramify seem to be strata-specific (MacNeil & Masland, 1998). Although these amacrine cells span the ON–OFF sublaminae there is no clear measurement of 'cross-over inhibition' mediated by GABA: ON centre activity is inhibited by ON local surrounds, and OFF centre activity is inhibited by OFF local surrounds. This suggests that signals in individual processes may be confined to specific strata and that activity in the processes of these cells is asynchronous and not measurable from patch recording at the cell body. The A17 amacrine cell and the starburst amacrine cell are examples of amacrine cells that display independent asynchronous activity in individual processes (Euler *et al.* 2002; Grimes *et al.* 2010; Borghuis *et al.* 2011).

Why are vertically oriented amacrine cells diffuse? The vertically oriented glycinergic amacrine cells serve to reconstruct the rectified signals transmitted by each retinal cell type by crossing ON to OFF or OFF to ON activity

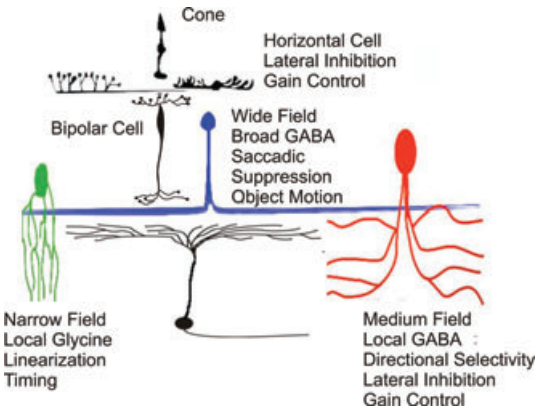


Figure 11. Some of the functional roles of the three main amacrine cell types. Wide field cells mediate both saccadic suppression and object motion. Narrow field amacrine cells mediate crossover inhibition that linearizes activity after synaptic rectification. The LED response is delayed by an initial rapid glycinergic inhibition, likely mediated by narrow field amacrine cells. A peripheral component of contrast gain control is initiated in the cell periphery and feeds back to bipolar and forward to ganglion cells (Zaghloul *et al*. 2007). This is likely mediated by medium field GABAergic amacrine cells. The specialized GABAergic amacrine cell, the starburst cell is a main component of directional selectivity.

(Molnar & Werblin, 2007*b*; Hsueh *et al.* 2008; Molnar *et al.* 2009). But which ON stratum or strata does a diffuse amacrine cell receive input from, and to which OFF stratum or strata does it deliver its ON signal? Timing may not be as critical in these narrow field glycinergic amacrine cells as it is for the wide field cells (although the rapid timing of the glycinergic input delays the LED response). While the wide field inhibition is quite transient and must be properly synchronized with bipolar activity, the narrow field inhibition is more sustained and slower, reducing the need for timing precision.

What is the value of layer-by-layer crossover reconstruction that does not completely linearize the signal? Most retinal neurons receive crossover inhibition that serves to reconstruct the signals that have been distorted by rectification at synaptic transmission (Molnar & Werblin, 2007*a*,*b*; Hsueh *et al.* 2008; Molnar *et al.* 2009; Werblin, 2010). This reconstruction must occur at each processing level: if the signal is filtered before it is reconstructed, it can never be completely reconstructed by further crossover inhibition (Molnar *et al.* 2009; Werblin, 2010). Yet the reconstruction never completely balances the non-linearity introduced by the synaptic rectification. What could be the value of this partial reconstruction if the visual signal becomes more non-linear as the signal proceeds through the retina? One possibility is that the retina makes good use of varying degrees of non-linearity in generating the appropriate visual signals.

Future studies. This review summarizes much of our understanding of the retinal circuitry that underlies visual function. Recent successful efforts to unravel circuitry have relied upon imaging of the processes of either starburst or A17 amacrine cells. These studies were successful because individual processes of amacrine cells behave in significantly functional ways that are not accessible to patch electrode recording. It is likely that many of the future significant advances in our understanding of visual function will move beyond patch recording and require probing at the processes of individual neurons with studies similar to those of Borghuis *et al.* (2011).

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