Retinal Amacrine Cells

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Introduction

The amacrine cells are the major interneurons in the proximal retina of vertebrates. They form the most diverse type of neuron in the retina, with 20–30 morphological subpopulations described in mammals, based on differences in somatic and dendritic architecture (Figure 1). The dendritic branches of these subtypes stratify within restricted levels of the inner plexiform layer (IPL), indicating selectivity in the synaptic connections made with bipolar cell axon terminals as well as the dendritic processes of neighboring amacrine and ganglion cells. It is believed that the structural diversity within the amacrine cell population reflects an equally large range of physiological properties and the corresponding functional roles played in formulating the retinal output signals expressed by the postsynaptic ganglion cells.

Amacrine Cell Morphology

The amacrine cells were named by Ramón y Cajal after a Greek derivation of ‘neurons lacking long fibers,’ based on the typically circumscribed space occupied by their dendritic arbors. The morphological classification of amacrine cells has been based on a number of structural parameters, including (1) size, position, and shape of the perikaryon; (2) size and symmetry of dendritic arbors, including the shape and caliber of branches and density of arborization; and (3) the stratification pattern of dendrites within the IPL. Beginning with the Golgi method, a wide variety of techniques have been employed to elucidate the different morphological subtypes of amacrine cell in the retina. It is now clear that 20–30 amacrine cell subtypes exist. A significant number of them are conserved across mammalian species and even show correspondence to amacrine cell subtypes in lower vertebrate retinas. Within a given retina, the amacrine cell subtypes form regular mosaics, but these show tremendous variability in intercellular spacing, indicating differences in their sampling of visuotopic space. It is interesting that any single subtype of amacrine cell forms only a small percentage of the total population. While the All subtype, which subserves the pathway that carries rod information, constitutes about 13% of all amacrine cells, no other subtype totals more than 5%. This division of amacrine cell subtypes suggests that each plays a separate, yet equally important, role in visual processing.

The somata of amacrine cells lie mainly within the proximal portion of the inner nuclear layer (INL), but those of many subtypes are displaced to the ganglion cell layer (GCL) or lie within the IPL. In some retinas, displaced amacrine cells even outnumber the ganglion cells in the GCL. On the basis of dendritic arbor size, amacrine cells can be divided generally into small-field cells, thought to play a role in local processing and higher acuity vision, and wide-field or long-range cells, involved in more-global retinal processing. Some of these long-range amacrine cells have very extensive dendritic arbors that span hundreds of micrometers laterally across the retina. Further, a number of amacrine cell subtypes, described recently in mammals, have very long, narrow, axonlike processes distinct from a conventional dendritic arbor (Figure 2). These fibers show the ultrastructure of typical axons in the central nervous system (CNS), including the expression of high-molecular-weight neurofilament proteins. In contrast to ganglion cells, these so-called polyaxonal amacrine cells bear multiple axonlike fibers that emerge from the soma or dendritic processes and course for several millimeters through the IPL but do not exit the retina via the optic nerve.

The synapses made by amacrine cells are confined to the IPL. Chemically mediated synapses are made with ganglion cell dendrites, bipolar cell axonal processes, or dendrites of other amacrine cells. The dendrites of amacrine cells can maintain both presynaptic and postsynaptic specializations. These synapses can occur juxtaposed along the same dendritic segment to form complex circuits. For example, amacrine cell processes that are postsynaptic to bipolar cells at ribbon synapses can make nearby reciprocal synapses back to the bipolar cell axon. This feedback synapse, which is inhibitory, is thought to modulate the release of neurotransmitter from the bipolar cell. A second type of complex amacrine cell circuit is called a serial synapse; in it, one amacrine cell makes contact with a second amacrine cell, which, in turn, synapses onto a third cell, all within a short distance.

It is interesting that the ratio of the number of synapses that ganglion cells make with amacrine cells and with bipolar cells varies across species. This ratio can also vary for different ganglion cell subtypes within the same retina. It has been suggested that ganglion cells with simple, concentric receptive fields receive synaptic inputs mainly from bipolar cells, whereas those with complex response properties,
such as direction or orientation selectivity, receive more amacrine cell synaptic input. This suggests that amacrine cell circuitry in the IPL underlies the processing of complex visual responses seen in some ganglion cells.

Electrical coupling via gap junctions occurs for almost all the subtypes of amacrine cell in mammalian retinas (Figure 3). This includes homologous coupling between adjacent amacrine cell neighbors or heterologous coupling between amacrine cells and ganglion cells or bipolar cells. These electrical synapses are thought to play a variety of roles in information processing. For example, AII amacrine cells receive inputs from rod bipolar cells and thereby process information during nighttime vision. The AII amacrine cells are extensively coupled to one another via gap junctions composed of connexin36 protein. The coupling between AII cells is believed to sum synchronous signals and reduce background noise, thus increasing the fidelity of the rod signals they carry. Further, the conductance of these gap junctions is affected by dopamine, which acts as a light-mediated neuromodulator. Thus, the size of the network formed by AII cells changes under different ambient, background light conditions. The result is that AII cell coupling is constantly changing to ensure the highest fidelity of responses to maintain the very sensitive rod signals in the proximal retina (see the section titled ‘All amacrine cells’). In support of this notion, the sensitivity of postsynaptic ganglion cells is reduced by about one log unit of illumination in the connexin36 knockout mouse retina, in which the AII amacrine cells are uncoupled.

**Amacrine Cell Neurotransmitters**

With one exception, all amacrine cells are inhibitory interneurons and typically use either \( \gamma \)-aminobutyric acid (GABA) or glycine as their neurotransmitter. The responses of virtually all ganglion cells are affected by exogenous application of these transmitters, either directly or through the complex circuitry of the IPL described above. The GABA receptors appear to be segregated, whereby \( \text{GABA}_A \) and \( \text{GABA}_B \) receptor subtypes subserve feedforward inhibition of amacrine cells onto other amacrine cells and ganglion cells, and \( \text{GABA}_C \) receptors mediate the feedback inhibition onto bipolar cell axon terminals. The combination of GABA effects via the different receptors is believed to influence the sustained or transient characteristics of amacrine and ganglion cell responses. In general, glycine is the transmitter used by small-field amacrine cells, whereas GABA is used by medium- and wide-field cells. Acetylcholine-containing amacrine cells, also called starburst amacrine cells, consist of mirror-image pairs with somata in the GCL and INL and dendrites monostratified in the inner and outer IPL, respectively. It is interesting that starburst amacrine cells are found in all vertebrate retinas, suggesting that they play an important role in visual processing (see the section titled ‘Starburst amacrine cells’). The acetylcholine release from starburst cells occurs by two methods: a small, tonic release that is independent of calcium and light and a second, larger acetylcholine release that is light dependent. It is interesting that starburst cells also release GABA, and so this neuron has mixed excitatory and inhibitory effects.

Dopaminergic amacrine cells form a distinctive subtype that is also found in all vertebrate retinas. Dopaminergic amacrine cells in the mammal have polyaxonal morphology, consisting of a relatively small dendritic arbor and numerous long axonal processes that extend more than 1 mm. Most processes stratify in the IPL, where they encircle the AII amacrine cells. As discussed below, dopaminergic amacrine cells control the electrical coupling between AII amacrine cells. The axons of some dopaminergic cells, particularly in nonprimate species, extend outward past the INL to terminate in the outer plexiform layer. The dendrites of...
dopaminergic amacrine cells receive most if not all the excitatory synaptic input from bipolar cells, whereas the release of dopamine occurs at varicosities within the axonal arbor. Thus, there is a polarity to the structure of dopaminergic amacrine cells in terms of their input and output synaptic circuits.

Another well-studied subtype is the indoleamine-accumulating or serotonergic amacrine cell. In the mammal, the serotonergic amacrine cells maintain very long and thin dendritic processes with numerous varicosities. Computational models suggest that varicosities act to electrically isolate individual dendritic segments, thereby allowing for independent processing of synaptic inputs. These cells receive input mainly from rod bipolar cells, but rather than transmit the signals forward to ganglion cells, the serotonergic amacrine cells make reciprocal synapses with the same rod bipolar axon terminals. These amacrine cells are thus thought to modulate the release of bipolar cell neurotransmitter under scotopic light conditions. Little is known about the effects of serotonin on ganglion cells, but application of serotonin antagonists appears to reduce the responses of on-center ganglion cells and to increase the responses of off-center cells.

In addition to conventional neurotransmitters, amacrine cells express a large number of neuropeptides, including substance P, enkephalin, somatostatin, neurotensin, glucagon, vasoactive intestinal peptide, neuropeptide Y, and cholecystokinin. The peptidergic amacrine cells have been best studied in the bird retina, in which they show differences in dendritic structure, particularly in the IPL strata they occupy. In the mammal, peptidergic amacrine cells are mainly subtypes of polyaxonal cells, suggesting that peptides are released over large areas of the retina and serve a neuromodulatory role. Many of these peptidergic amacrine cells accumulate or express more than one peptide and/or express the conventional inhibitory transmitters GABA and glycine. Individual amacrine cells may thereby have multiple roles in both local and global visual processing.

There have been relatively few studies of the effects of peptides on the responses of retinal neurons, but they appear to have general excitatory or inhibitory actions. For example, substance P excites retinal ganglion cells in the fish retina, whereas enkephalin appears to inhibit their activity in the amphibian. In mammals, ablation of neuropeptide Y amacrine cells results in a reduction of the receptive field size of certain ganglion cells that

Figure 2. Morphology of a subtype of polyaxonal amacrine cell in the rabbit retina. (a) Photomicrograph showing the extensive tracer coupling pattern following injection of Neurobiotin into a type I polyaxonal amacrine cell (asterisk). (b) Somata of tracer-coupled type I polyaxonal amacrine cells and the overlapping thick dendritic processes and thin axonal processes. Arrowheads indicate axonal processes emerging from proximal dendrites. (c) Camera lucida drawing providing a flat-mount view of a type 1 polyaxonal amacrine cell. The dendritic arbor is presented in black, and the axonal arbor and somata of tracer-coupled amacrine cells are shown in gray. Scale bars = 100 μm (a), 25 μm (b), 200 μm (c). From figures 1 and 2 in Völgyi B, Xin D, Amarillo Y, and Bloomfield SA (2001) Morphology and physiology of the polyaxonal amacrine cells in the rabbit retina. Journal of Comparative Neurology 440: 109–125.
respond preferentially to low acuity scenes. This peptide is thus thought to have a role in the circuitry underlying ganglion cell spatial tuning.

Amacrine Cell Physiology

Although there are 20–30 subtypes of amacrine cell in the mammalian retina, the physiology of fewer than ten subtypes has been studied in detail. Nevertheless, it is clear the amacrine cells show an extraordinary variety of response properties. In general, amacrine cells can be divided into transient and sustained categories on the basis of the temporal properties of their responses. In lower vertebrates, the transient cells usually show on–off responses, although transient ‘on cells’ and transient ‘off cells’ have been described in a number of species. Most sustained amacrine cells show the center-surround antagonistic receptive field organization displayed by bipolar cells and most ganglion cells. In classic experiments, amacrine cells were shown to be robustly excited by a windmill stimulus, indicating their sensitivity to moving objects.

In addition to the relatively simple center-surround receptive field of most amacrine cells, certain subtypes display complex physiology similar to that of some ganglion cells. These include amacrine cell subtypes with orientation selectivity derived from the spatial organization of excitatory and inhibitory inputs or an asymmetry in their dendritic field architecture (Figure 4). Amacrine cells with a response preference for the direction of stimulus motion have been reported. This includes the starburst amacrine cell, which shows a response preference for stimuli moving centrifugally away from the soma (see the section titled ‘Starburst amacrine cells’).

In contrast to other retinal interneurons, many amacrine cells express voltage-gated sodium channels and display action potentials (Figure 5). Spiking has been reported in both transient and sustained amacrine cells and is often seen superimposed atop large excitatory potentials. In addition to large-amplitude somatic spikes, some amacrine cells display all-or-none sodium-mediated spikes similar to microspikes thought to be generated within the dendritic...
membrane (Figure 5(a)). In wide-field amacrine cells, including the polyaxonal cells, spikes are actively propagated centrifugally to terminal branches. In this scheme, somatic spikes are believed to rapidly invade the entire arbor to produce global signaling and release of neurotransmitter. In contrast, dendritic spikes provide only regional activation resulting in local signaling to postsynaptic cells. In this way, the multifocal impulse-generating capability of certain wide-field amacrine cells provides for a complexity in receptive field properties and integration of synaptic inputs.

The dopaminergic amacrine cells show a pacemaker activity of rhythmic, spontaneous bursts of action potentials (Figure 5(b)). This pacemaker activity is generated intrinsically by voltage-gated sodium channels that slowly depolarize the membrane to threshold levels. Since the polyaxonal dopaminergic amacrine cells act on distant cellular targets, the pacemaker activity likely ensures the tonic release of the neuromodulator. In addition to suprathreshold spiking, many amacrine cells show rhythmic subthreshold oscillations. This idea is supported by the finding that bipolar cell axon terminals display calcium-dependent spontaneous membrane oscillations, which may lead to pulsatile transmitter release and rhythmic activity of postsynaptic amacrine cells. In contrast, the oscillatory activity of some amacrine cell subtypes survives cell isolation in culture and thus must be generated intrinsically. The subthreshold oscillatory activity of bipolar and amacrine cells can produce periodic release of neurotransmitter that, in turn, will produce oscillatory activity in postsynaptic cells, particularly ganglion cells. As at other CNS loci,
subthreshold oscillations distributed among a network of cells can result in light-evoked synchronous activity as cells will tend to reach spike threshold together. The spontaneous oscillatory activity of amacrine cells may thereby serve to organize ensembles by coordinating the activity when activated by appropriate visual stimuli. This synchronous activity may serve to increase stimulus efficacy, encode additional information, and/or bind information about local visual features.

**Examples of Specific Amacrine Cell Functional Roles**

While it is clear that amacrine cells must play a wide variety of roles in visual processing, only a few of the many subtypes have been studied in detail. The physiology and morphology of two subtypes, the AII and starburst amacrine cells, have been extensively studied, and we now have a good understanding of their specific roles in retinal processing of visual signals. The results of these studies are summarized below.

**All Amacrine Cells**

In the mammalian retina, rod and cone photoreceptors synapse onto largely different bipolar cells, thereby segregating their signals into different vertical streams. Whereas up to 11 different morphological types of cone bipolar cells have been reported, showing both on- and off-center physiology, only a single type of rod bipolar cell exists. It is interesting that the axons of rod bipolar cells do not directly contact ganglion cells but instead contact mainly the small-field, bistratified AII amacrine cell. In turn, AII cells form sign-conserving electrical synapses with the axon terminals of on-center cone bipolar cells and sign-inverting glycinergic chemical synapses with the axon terminals of off-center cone bipolar cells. In this way, both on- and off-center scotopic signals utilize the cone pathways before reaching the ganglion cells and ultimately higher brain centers.

The gap junctions formed between AII cells and the on-center cone bipolar cells form nonrectifying electrical synapses across which the direction of signal flow changes with stimulus intensity. As mentioned above, rod signals generated under dim, scotopic light conditions move from the AII cells to the cone bipolar cells to be distributed to the ganglion cells. In contrast, under bright, photopic conditions, cone signals move in the opposite direction, from cone bipolar cells to the AII amacrine cells. The interconnecting gap junctions thus do double duty as conduits for both rod and cone signals.

The AII amacrine cells also form gap junctions between one another, forming an extensive electrical syncytium. Computational models suggest that this coupling increases the signal-to-noise ratio of AII cell responses by summing synchronous responses and decreasing asynchronous noise. It is interesting that the conductance of these homologous AII cell–AII cell junctions is affected by light via modulation of dopamine release by changes in dark-light adaptation (Figures 6(a)–6(c)). Under dim, rod-mediated light conditions, the relationship between coupling and ambient light intensity has two phases: AII cells are relatively uncoupled in the dark-adapted retina but...
show a dramatic increase in coupling when dim background stimuli are presented (Figure 6(d)).

What is the function of the light-induced changes in AII cell–AII cell coupling? One idea is that these changes reflect the need for AII cells, as vital elements in the rod pathway, to remain responsive throughout the scotopic-mesopic range. In this scheme, dark adaptation is analogous to starlight conditions, under which rods will only sporadically absorb photons of light. The need, then, is for AII cells to preserve these isolated signals above the background noise. Accordingly, the AII cells are relatively uncoupled in that there are few correlated signals to sum, and so extensive coupling would serve to dissipate and thereby attenuate the few isolated responses rather than enhance them. As dawn approaches, more photons become available. In turn, AII amacrine cells show an increase in coupling, which provides for summation of synchronous activity over a wider area, thus preserving the fidelity of these rod-driven, correlated signals at the expense of spatial acuity. This transition in coupling between dark-adapted retinas and those illuminated with dim background light suggests two basic operating states for AII cells under scotopic/mesopic light conditions: (1) responding to single photon events and (2) summing signals over a relatively large area to sum synchronized events above the background noise. Overall, the AII cell coupling ensures that the most sensitive rod signals are maintained at the ganglion cell level and transmitted to higher brain centers.

Starburst Amacrine Cells

A unique subtype of ganglion cell found in the retinas of many species is the direction-selective (DS) unit. DS cells respond vigorously to stimulus movement in the preferred direction yet show little or no activity following movement in the opposite or null direction. The ON–OFF variety of DS ganglion cell can be divided into four subtypes, each preferring a direction of movement roughly corresponding to the attachment points of the extraocular muscles. The mechanism underlying the computation of direction of motion has been the subject of investigation for more than 40 years. Pharmacologic studies indicate that acetylcholine and GABA can modify the selectivity of DS ganglion cells. In particular, application of GABA antagonists abolishes the direction selectivity of DS ganglion cells, suggesting that asymmetric inhibition resulting from null direction stimulus
movement plays a crucial role. Taken together, these data suggest a role for the starburst amacrine cells, which release both acetylcholine and GABA. As mentioned earlier, starburst amacrine cells are of two mirror-image subtypes. The starburst-\(a\) cell has a perikaryon in the INL and monostratifies within sublamina-\(a\) of the distal IPL, whereas the starburst-\(b\) cell is displaced to the GCL and monostratifies in sublamina-\(b\). It is now clear that the dendrites of both the starburst-\(a\) and -\(b\) subtypes costratify and are presynaptic to the bistratified processes of ON–OFF DS ganglion cells.

Recent studies have provided compelling evidence that starburst cells indeed play a key role in the generation of direction selectivity. Specific ablation of starburst cells, either through pharmacological manipulation or genetic targeting, abolishes the selectivity of DS cells. Electrophysiological studies have shown that starburst cells provide direct inhibition to DS ganglion cells, suggesting that they provide the GABAergic null inhibition crucial for the direction selectivity of DS cells. Furthermore, the null inhibition from starburst cells is itself direction selective, being stronger for stimulus movement in the null direction.

Taken together, these data suggest that starburst amacrine cells manufacture the direction-selective responses in the retina. In particular, two intrinsic properties of starburst amacrine cells are believed crucial to the generation of DS ganglion cell responses. First, computational and calcium-imaging studies have indicated that the dendritic branches of starburst cells are electrically isolated from one another. This means that single starburst cell dendrites can perform computations independently and simultaneously. Second, light stimulation in the centrifugal direction (away from the soma) produces a greater voltage response than movement in the centripetal direction. It is unclear what establishes these two properties of starburst cells, but potassium channels, calcium channels, chloride ion transporters, and membrane cable properties have all been implicated.

As schematized in Figure 7, centrifugal movement of light along one dendritic branch of a starburst amacrine cell will result in a robust depolarization and the release of GABA onto postsynaptic DS ganglion cells. This provides the null inhibition to the DS cells. Stimulus movement in the opposite, or centripetal, direction will not produce a large response in the starburst cell, and thus no GABAergic inhibition of the postsynaptic DS ganglion cells will be evoked. Thus, the preferred and null directions for a DS ganglion cell can be divided into four parts that are electrically isolated and thus function autonomously. Stimulus movement in the centripetal direction evokes a depolarization of the starburst cell dendritic branch and a release of \(\gamma\)-aminobutyric acid.

This produces the null inhibition of the postsynaptic DS ganglion cell. The preferred direction of the DS cell is opposite to the null or centrifugal in the starburst cell dendritic branch. Each starburst cell dendritic branch synapses with a different subtype of ON–OFF DS ganglion cell and thereby produces its preferred direction responses of upward, downward, leftward, and rightward.

**Figure 7** Schematic showing how a starburst amacrine cell generates the direction selectivity of the four ON–OFF direction-selective (DS) ganglion cell subtypes that show different preferred directions: temporal, nasal, superior, and inferior. The dendritic arbor of the starburst amacrine cell (center) can be divided into four parts that are electrically isolated and thus function autonomously. Stimulus movement in the centripetal direction evokes a depolarization of the starburst cell dendritic branch and a release of \(\gamma\)-aminobutyric acid. This produces the null inhibition of the postsynaptic DS ganglion cell. The preferred direction of the DS cell is opposite to the null or centrifugal in the starburst cell dendritic branch. Each starburst cell dendritic branch synapses with a different subtype of ON–OFF DS ganglion cell and thereby produces its preferred direction responses of upward, downward, leftward, and rightward.
ganglion cell are established by the direction of stimulus movement along a starburst cell dendritic branch. Since the dendritic branches of the starburst cell are electrically isolated, each can provide a different directional preference expressed by the four subtypes of ON–OFF DS ganglion cells. In this scheme, each subtype of DS ganglion cell must be postsynaptic to different dendritic branches of a given starburst amacrine cell. Indeed, recordings from DS ganglion cells indicate that while starburst cells lying on the null side of a DS cell provide inhibition, those on the preferred side do not. Likewise, morphological data indicate that DS ganglion cells selectively synapse only with particular starburst amacrine cell processes within their dendritic field.

Conclusions

Amacrine cells form an extensive, heterogeneous group of retinal interneurons that are positioned to modify the output signals carried by the postsynaptic ganglion cells. Despite the large diversity of amacrine cell morphology found in the retina, no one subtype dominates, suggesting each plays a distinct important role in visual processing. This idea is supported by the extraordinary variety of physiological response properties found for amacrine cells, despite the fact that only a few subtypes have been studied so far. These properties include complex receptive fields, including orientation and direction selectivity, somatic and dendritic spiking, pacemaker activity, rhythmic subthreshold oscillations, and electrical isolation of dendritic segments. Further, amacrine cells partake in a variety of complex circuitry that includes both chemical and electrical synapses. In fact, nearly every neurotransmitter, neuromodulator, and neuropeptide found in the CNS has been identified in specific subtypes of retinal amacrine cells. Taken together, these data indicate that the amacrine cells play diverse yet specific roles in the integration of visual signals passing through the retina.

See also: Activity in Visual Development; Fovea: Primate; Retina: An Overview; Retinal Development: An Overview; Vision: Light and Dark Adaptation.

Further Reading


Encyclopedia of Neuroscience (2009), vol. 8, pp. 171-179