—Review—

Signal Processing in the Mammalian Retina

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Abstract

Visual information contains many different elements, such as contrast, color, brightness, and movement. Each element is extracted from visual information in a specialized neural circuit of the retina. Finally, the extracted signals are reconstructed into coded signals at the retinal ganglion cells and sent to the higher visual center in parallel for further processing. Each specialized neural circuit has both ON- and OFF-pathways, and the signal processing in the ON-pathway is a mirror image of that in the OFF-pathway. This review focuses on the dichotomy of neural circuits in the mammalian retina.
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Key words: retina, bipolar cell, amacrine cell

Introduction

In the mammalian retina, the processing of visual information signals starts at photoreceptors, where the light absorbed by the visual pigment is converted into electrical signals[1,2] (**Fig. 1**). Electrical signals generated in photoreceptors are transmitted to bipolar cells in the outer plexiform layer. Horizontal cells modulate signal transmission between photoreceptors and bipolar cells and play an important role in the formation of the concentric receptive field of bipolar cells (**Fig. 2**). Signals processed in bipolar cells are passed to retinal ganglion cells in the inner plexiform layer. Amacrine cells modulate the signal transmission between bipolar cells and retinal ganglion cells and help to extract visual elements, such as contrast, color, brightness, and movement. Finally, signals of individual elements are coded into trains of action potentials in retinal ganglion cells and sent to the higher visual center in parallel through the optic nerve, which is a bundle of axons of retinal ganglion cells.

In the retina, visual information is processed in parallel[1,3]. At the level of bipolar cells, light responses of photoreceptors are transmitted into ON- and OFF-pathways (**Fig. 1** and **Fig. 5**). The ON-pathway starts from rod bipolar cells or ON-cone bipolar cells, whereas the OFF-pathway starts from OFF-cone bipolar cells. Signals of the ON-pathway and the OFF-pathway are processed in parallel, and the signal processing of the ON-pathway is a mirror image of that of the OFF-pathway. To achieve the dichotomy of signal processing, the ON- and OFF-pathways have developed pathway-specific systems at multiple levels. This review describes the current level of understanding of such pathway-specific systems, that is, the center-surround organization of the receptive field of ON- and OFF-bipolar cells, the functional roles of AII amacrine cells in the daytime and at night, and the breakdown of symmetrical

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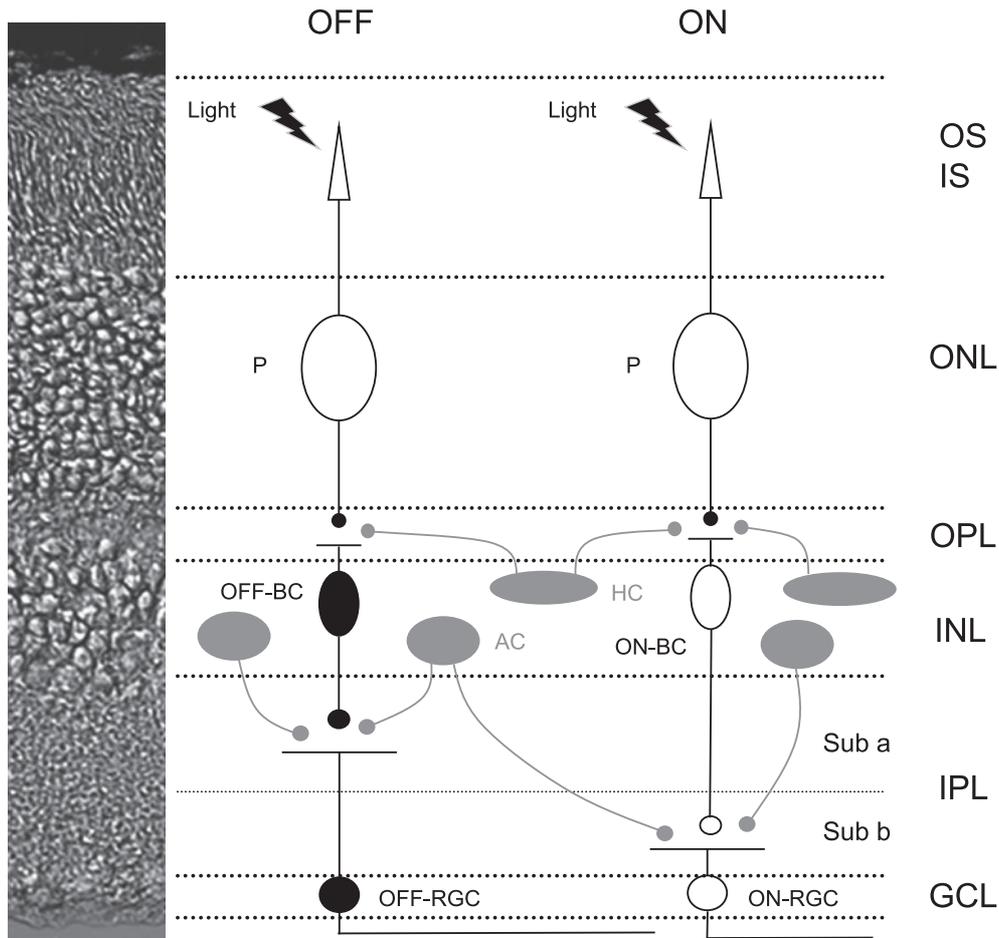Journal Website (http://www.nms.ac.jp/jnms/)

Fig. 1 Photomicrograph of the vertical section of mouse retina (left) and schematic drawings of cone pathways of the mammalian retina.

Details of the rod circuit (rod and rod bipolar cells) are shown in Figure 5. Signals of cones (photoreceptors, P) are transmitted to ON-cone bipolar cells (ON-BC) and OFF-cone bipolar cells (OFF-BC) and sent in parallel to ON- and OFF-retinal ganglion cells (ON-RGC and OFF-RGC). Horizontal cells (HC) modulate synaptic transmission between cones (photoreceptors) and bipolar cells. Amacrine cells (AC) are important for the signal processing between bipolar cells and retinal ganglion cells. The location of synaptic contact between bipolar cells and retinal ganglion cells is pathway-specific. The OFF-pathway makes synaptic contacts in sublamina a (Sub a), whereas ON-pathway makes synaptic contacts in sublamina b (Sub b). OS, IS: outer and inner segments of photoreceptors; ONL: the outer nuclear layer; OPL: the outer plexiform layer; INL: the inner nuclear layer; IPL: the inner plexiform layer; GCL: the ganglion cell layer.

signal processing in cholinergic amacrine cells.

Formation of the Center-surround Response in the Outer Plexiform Layer

Bipolar cells have concentric receptive fields and are classified as ON- and OFF-type bipolar cells from the response of the receptive field center (**Fig. 2**)[1,2]. ON-type bipolar cells depolarize when their receptive field center is illuminated and hyperpolarize when their receptive field surround is illuminated. OFF-type bipolar cells hyperpolarize when their receptive field center is illuminated and depolarize when their receptive field surround is illuminated. ON-type bipolar cells include 5 subtypes of ON-cone bipolar cells and rod bipolar cells, whereas OFF-type bipolar cells have 4 subtypes of OFF-cone bipolar cells[4]. In this review, ON- and
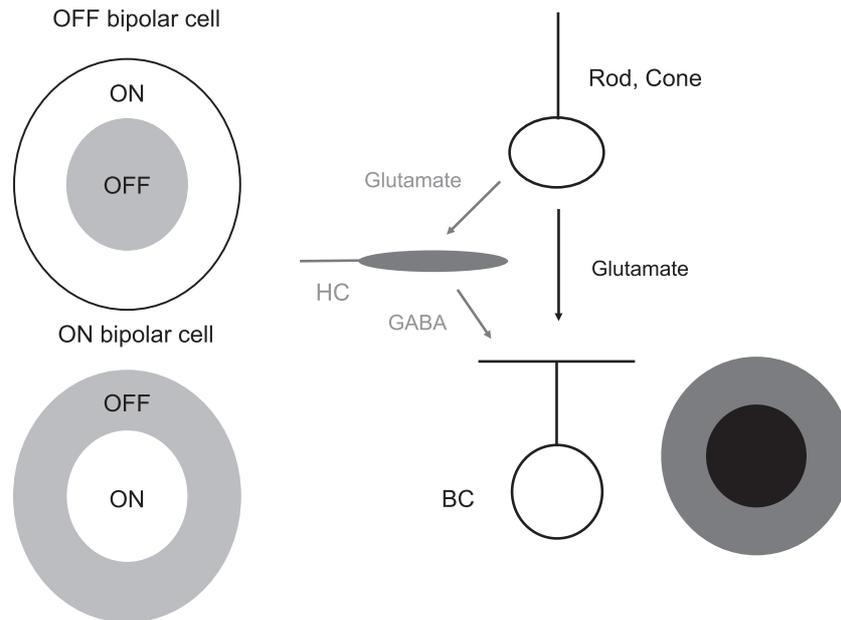
Fig. 2 Receptive field organization of bipolar cells.
Left: Schematic drawings of the concentric receptive fields of ON- and OFF-bipolar cells. Bipolar cells depolarize when the ON region (**white**) is illuminated, whereas bipolar cells hyperpolarize when the OFF region (**gray**) is illuminated. Right: Proposed mechanisms of center-surround antagonistic receptive field organization. The **black arrow** shows neural circuits responsible for center response, and the **dark gray arrows** are neural circuits responsible for surround responses. Glutamate released from photoreceptors (cones and rods) activates both bipolar cells (BC) and horizontal cells (HC) simultaneously. Glutamate acting on bipolar cells forms center responses (**black**), whereas GABA released from horizontal cells is responsible for the formation of surround responses (**dark gray**).

OFF-type bipolar cells are referred to as 1) ON- and OFF-bipolar cells or 2) rod bipolar cells, ON-cone bipolar cells, and OFF-cone bipolar cells.

The center response is mediated by glutamate released from photoreceptors, whereas the surround response is thought to involve GABAergic feedback to bipolar cells mediated by horizontal cells (**Fig. 2**). When photoreceptors are illuminated, they exhibit decreased glutamate release onto both ON- and OFF-bipolar cells, as well as horizontal cells. This reduction of glutamatergic inputs to horizontal cells also acts to reduce GABA release onto both ON- and OFF-bipolar cells from horizontal cells. Thus, photoreceptor responses to light stimulation reduce both glutamatergic and GABAergic inputs for both ON- and OFF-bipolar cells. Therefore, ON- and OFF-bipolar cells should have different mechanisms to produce opposite responses to glutamatergic or GABAergic inputs.

Center responses are mediated by the glutamate receptors on the dendrites of bipolar cells (**Fig. 2**). Because photoreceptors release glutamate in the dark, glutamate receptors of both ON- and OFF-bipolar cells receive glutamatergic inputs in the dark. To generate 2 types of light response to glutamatergic inputs, ON- and OFF-bipolar cells have developed different types of glutamate receptor on their dendrites (**Fig. 3**). OFF-bipolar cells use ionotropic glutamate receptors (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid [AMPA]/kainic acid [KA] type)[5], whereas ON-bipolar cells use metabotropic glutamate receptors (mGluR6)[6–8]. When OFF-bipolar cells receive glutamatergic inputs in the dark, AMPA/KA-type glutamate receptor-coupled cation channels are active. Therefore, OFF-bipolar cells are kept in a depolarized state in the dark. When glutamate release from photoreceptors is halted by light stimulation, OFF-bipolar cells
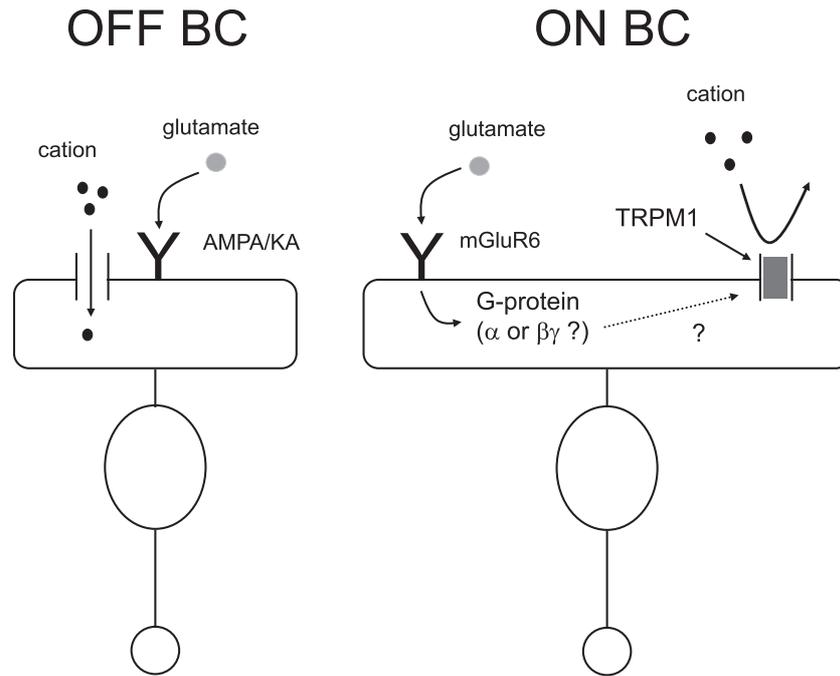
OFF BC　　　　　　　ON BC

Fig. 3　Ionic mechanisms of response of receptive field center to light in bipolar cells.
OFF-bipolar cells have ionotropic AMPA/KA-type glutamate receptors on their dendrites, whereas mGluR6 constitutes the dendritic glutamate receptors of ON-bipolar cells. OFF BC: OFF-bipolar cells; ON BC: ON-bipolar cells.

hyperpolarize owing to the reduction of glutamatergic inputs from photoreceptors. On the other hand, when ON-bipolar cells receive glutamatergic inputs in the dark, mGluR6 activates an intracellular signal cascade, which works to close mGluR6-coupled cation channels. Therefore, ON-bipolar cells are hyperpolarized in the dark. When the receptive field center is illuminated, mGluR6 is inactivated owing to the reduction of glutamatergic inputs from photoreceptors. Therefore, the mGluR6-coupled intracellular signal cascade becomes inactive, and ON-bipolar cells are depolarized. Until 2009, cyclic guanosine monophosphate-mediated cation channels were the most plausible candidate for mGluR6-coupled cation channels, and G-protein-coupled machinery was proposed as the intracellular signal cascade[9–11]. Studies in transient receptor potential cation channel subfamily M member 1 (TRPM1)-knockout mouse showed that TRPM1 is the cation channel of ON-bipolar cells[12,13]. Although the proposed mGluR6-coupled cation channel has changed, G-protein-coupled machinery remains the most plausible candidate for the intracellular signal

cascade[14,15]. However, whether the pivotal subunit of G-protein for the signal transduction is $G\alpha$[14] or $G\beta\gamma$[15] is unclear.

The mechanisms of the surround response of bipolar cells are much more complex and remain somewhat unclear. The GABAergic feedback from horizontal cells should be excitatory on ON-bipolar cells but must be inhibitory on OFF-bipolar cells (**Fig. 2**). Bipolar cells have $GABA_A$ and $GABA_C$ receptors[16], but both receptors are ionotropic and coupled with Cl^- channels, although their contributions to total GABA responses differ among bipolar cell subtypes[17]. Therefore, the opposite GABA responses cannot be explained by a difference in ionic mechanisms between GABA receptor subtypes. One attractive hypothesis is that OFF-bipolar cells and ON-bipolar cells have different types of Cl^- transporter on their dendrites[18] (**Fig. 4**). Namely, Cl^- transporters on the dendrites of OFF-bipolar cells are K-Cl cotransporters (KCC2), which export the intradendritic Cl^- to the extracellular space. On the other hand, Cl^- transporters on the dendrites of ON-bipolar cells are Na-K-Cl

OFF BC

KCC2　　　　　● GABA

K　Cl　　　　Cl

Low Cl

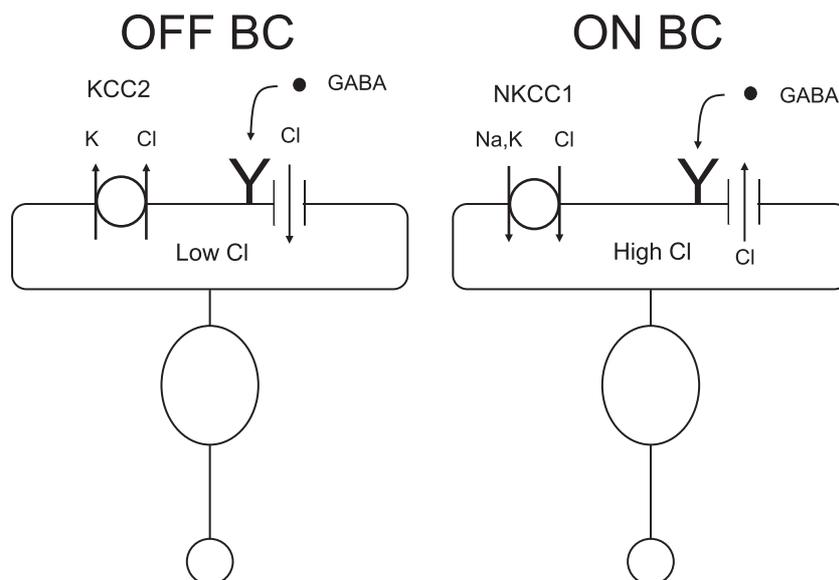## ON BC

NKCC1　　　　● GABA

Na,K　Cl

High Cl　　Cl

Fig. 4　Hypothetical ionic mechanisms of response of receptive field surround to light in bipolar cells.
OFF-bipolar cells have K-Cl cotransporter (KCC2), whereas ON-bipolar cells have Na-K-Cl cotransporter (NKCC1). In OFF-bipolar cells, KCC2 lowers the intradendritic Cl^- concentration. In ON-bipolar cells, NKCC1 elevates the intradendritic Cl^- concentration. OFF BC: OFF-bipolar cells; ON BC: ON-bipolar cells.

cotransporters (NKCC1), which pump extracellular Cl^- into the intradendritic space. The difference in Cl^- transporters might allow Cl^- transport in opposite directions and produce a difference in intradendritic Cl^- concentration. Therefore, when the extracellular Cl^- concentration is constant, the equilibrium potential of Cl^- (E_{Cl}) differs between ON- and OFF-bipolar cells. Because the reversal potentials of $GABA_A$ and $GABA_C$ receptors follow the E_{Cl}, a shift in the E_{Cl} would change the polarity of GABA responses. When the intradendritic Cl^- concentration is low, the E_{Cl} is negative compared with the resting potential, and GABA acts as an inhibitory neurotransmitter. This is the case for OFF-bipolar cells. If the intradendritic Cl^- concentration is elevated and the E_{Cl} becomes positive compared with the resting potential in ON-bipolar cells, GABA might act as an excitatory neurotransmitter. This hypothesis is supported by the fact that the intracellular Cl^- concentration of ON-bipolar cells is higher than that of OFF-bipolar cells[19] and by the fact that the intracellular Cl^- concentration of dendrites is higher than that of axon terminals in ON-bipolar cells[20]. Although the presence of an intracellular Cl^- concentration gradient in ON-bipolar cells is also supported by intracellular Cl^- imaging[21], the hypothesis cannot fully explain how opposite GABA responses are generated. First, the reported elevation of intracellular Cl^- concentration in ON-bipolar cells is not sufficient to reverse the inhibitory response to an excitatory response[19]. This insufficient elevation of intracellular Cl^- concentration might reflect the fact that the measurement of the intracellular Cl^- concentration at somas underestimates the intracellular Cl^- concentration at dendrites. Second, the observed gradient of the intracellular Cl^- concentration is insufficient to reverse the polarity of GABA responses[20]. Third, some ON-bipolar cells show a change in the intracellular Cl^- concentration (increase or decrease), but others show no change in intracellular Cl^- concentration, as monitored with Cl^--sensitive dye[21]. Therefore, the question of how the surround response of bipolar cells is formed remains unresolved.
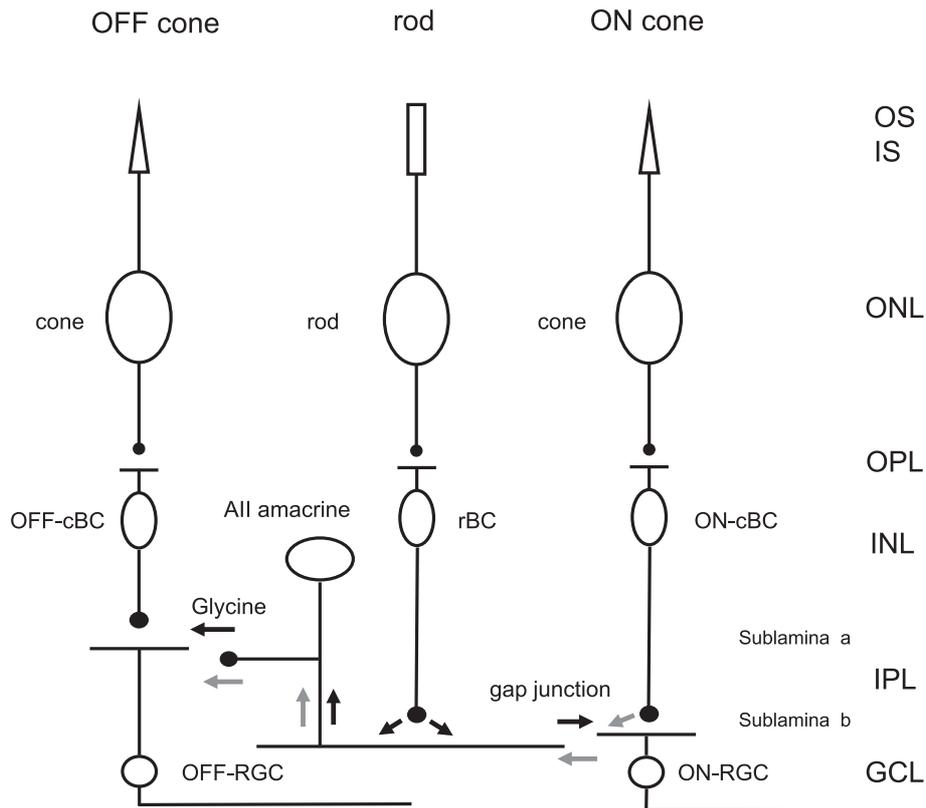
Fig. 5 Signal flow of rod-AII amacrine cell pathway in daytime and at night in the mammalian retina.
Black arrows show signal flow at night. **Gray arrows** show signal flow in the daytime. OFF-cBC: OFF-cone bipolar cells; ON-cBC: ON-cone bipolar cells; rBC: rod bipolar cells; OFF-RGC: OFF-retinal ganglion cells; ON-RGC: ON-retinal ganglion cells; OS, IS: outer and inner segments of photoreceptors; ONL: the outer nuclear layer; OPL: the outer plexiform layer; INL: the inner nuclear layer; IPL: the inner plexiform layer; GCL: the ganglion cell layer.

Cone and Rod Pathways in the Mammalian Retina

The neural circuit in the mammalian retina has 2 cone pathways (ON and OFF) and 1 rod pathway (**Fig. 5**)[1]. The rod pathway mediates signal transmission in scotopic (night) conditions or mesopic conditions (dawn and dusk), whereas the cone pathways mediate signal transmission in photopic conditions (daytime). Cone signals are sent in parallel to ON-cone bipolar cells and OFF-cone bipolar cells. The signals processed in ON- and OFF-cone bipolar cells are sent in parallel to ON- and OFF-ganglion cells. The rod pathway mediates signal transmission from rod to rod bipolar cells. All rod bipolar cells, however, are ON-type bipolar cells and have no direct synaptic contact with ganglion cells. To send

signals of rod bipolar cells to ON- and OFF-ganglion cells, the mammalian retina has developed AII amacrine cells. AII amacrine cells receive excitatory synaptic inputs from rod bipolar cells and send signals to both ON- and OFF-cone bipolar cells. AII amacrine cells have electrical synaptic contact (gap junctions) with ON-cone bipolar cells[22–27] and form glycinergic synapses with OFF-cone bipolar cells[28,29]. Therefore, AII amacrine cells, when excited by synaptic inputs from rod bipolar cells, send excitatory signals to the axon terminals of ON-cone bipolar cells via gap junctions in a sign-conserving manner and send inhibitory signals to the axon terminals of OFF-cone bipolar cells through glycinergic synapses in a sign-inverting manner. Because they receive synaptic inputs from the rod pathway, AII amacrine cells are thought to function
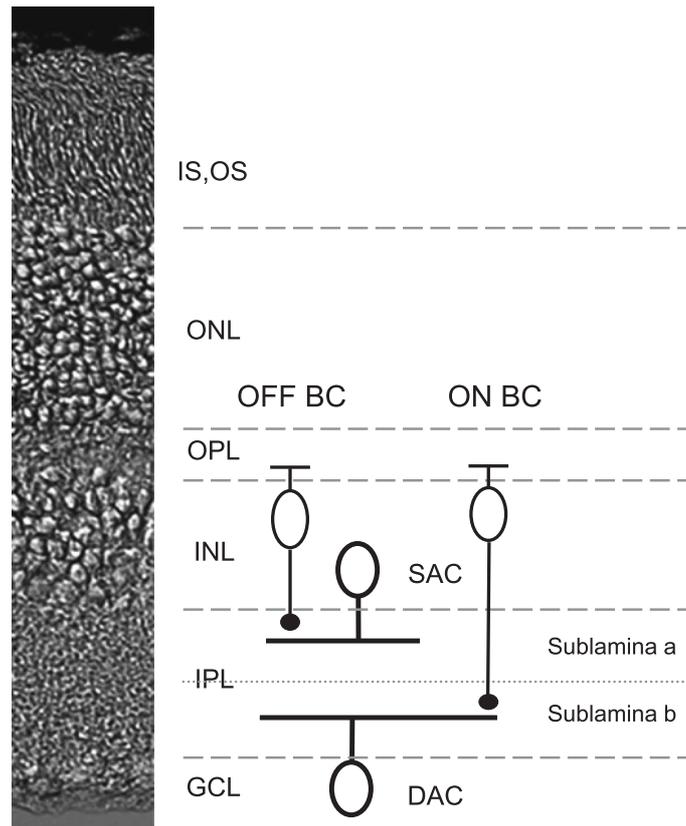
Fig. 6 Schematic drawings of neural circuit of cholinergic amacrine cells.
SAC: starburst amacrine cells (OFF-type); DAC: displaced amacrine cells (ON-type); OFF BC: OFF-bipolar cells; ON BC: ON-bipolar cells; OPL: the outer plexiform layer; INL: the inner nuclear layer; IPL: the inner plexiform layer; GCL: the ganglion cell layer.

in scotopic or mesopic conditions. However, a recent study has found that AII amacrine cells also work in photopic conditions[30]. When ON-cone bipolar cells are activated in photopic conditions, they send excitatory signals to AII amacrine cells via gap junctions. Then, excited AII amacrine cells finally inhibit OFF-ganglion cells, presumably by glycinergic inhibition of the OFF-pathway. Thus, signal flows from AII amacrine cells to ON-cone bipolar cells at night but in the opposite direction in the daytime. Because the gap junction is an electrical synapse whose signal flow is usually bidirectional, how signal transmission between AII amacrine cells and ON-cone bipolar cells is regulated in the daytime and at night is an interesting question.

Cholinergic Amacrine Cells

Amacrine cells are a group of axonless neurons classified into more than 20 subtypes[31]. Cholinergic amacrine cells are one of the best-characterized types of amacrine cells[32,33] and probably function to form a specific receptive field of direction-selective retinal ganglion cells[34–37]. Cholinergic amacrine cells are further classified into ON-type and OFF-type. Somas of OFF-cholinergic amacrine cells are located in the inner nuclear layer, and their dendrites arborize in the scleral side (sublamina a) of the inner plexiform layer (**Fig. 6**). Somas of ON-cholinergic amacrine cells are located in the ganglion cell layer, and their dendrites arborize in the vitreal side (sublamina b) of the inner plexiform layer. OFF-cholinergic amacrine cells receive synaptic inputs

from OFF-bipolar cells in sublamina a, whereas ON-cholinergic amacrine cells receive synaptic inputs from ON-bipolar cells in sublamina b. Because they resemble a "starburst" from above, cholinergic amacrine cells are called "starburst amacrine cells"[33]. In addition, ON-cholinergic amacrine cells are sometimes called "displaced amacrine cells". ("Displaced amacrine cells" are also a population of different subtypes of amacrine cells whose somas are located in the ganglion cell layer.) ON- and OFF-cholinergic amacrine cells are thought to function as mirror images of each other. However, recent studies have shown that ON- and OFF-cholinergic amacrine cells use P2X-purinoceptors, ionotropic purinergic receptors, in different ways[38,39]. $P2X_2$-purinoceptors are densely distributed in OFF-cholinergic amacrine cells but sparsely distributed in ON-cholinergic amacrine cells[39], and P2X-purinoceptors modulate the firing pattern of OFF-type retinal ganglion cells but not that of ON-type retinal ganglion cells[38]. At present, the functional roles of modulation specific for the P2X-purinoceptor-mediated pathway remain somewhat unclear. Since mouse lines showing selective green fluorescent protein labeling of cholinergic amacrine cells have been established, these cells have become "approachable". Breakdown of symmetry of P2X-purinoceptors in cholinergic amacrine cells means that studies of currently "unapproachable" amacrine cell subtypes might provide further pathway-specific signal-processing systems.

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