LECTURE

Neurochemical organization of the first visual synapse

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Abstract. The retina employs two main synaptic relays in which information converges to higher order cells, and at the same time is modified by lateral inhibitory interneurons. At the first synaptic layer, rod and cone terminals contact second order neurons (horizontal and bipolar cells), and in turn, horizontal cells contact cones and bipolar cells. In this talk/review we describe the structures and the neurochemicals involved in transmitting the visual signal at this synaptic complex. (Keio J Med 51 (3): 154–164, September 2002)

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The retina employs two main synaptic relays, in which information passes forward via glutamatergic synapses. This information is simultaneously modified by lateral inhibitory connections at each layer (Fig. 1A). The main function of the forward and lateral processing is to form the center-surround receptive field structure of the light responses of several parallel circuits (Fig. 1B). In the first synaptic layer (termed ''outer plexiform layer'' or OPL), a rod terminal transmits information about single photons (scotopic range) to rod bipolar cells. A cone terminal transmits information about mesopic (twilight) and photopic (daylight) luminances to ten types of bipolar cell.[1–3] The bipolar cells are divided into two main classes: the OFF class, which depolarizes to glutamate, and the ON class, which hyperpolarizes to glutamate (Fig. 1B). Large cells, which collect information from many surrounding cones (horizontal cells), modify this transmission to provide surround information.[4–6] In the second synaptic layer (termed ''inner plexiform layer'' or IPL), bipolar cells provide information to ganglion cells, and this information is modified by about 21 types of amacrine cells. In this review, we shall discuss the neurochemical basis of the diverse responses in the rod- and cone synaptic complexes.

Results

Glutamate depolarizes OFF bipolar cells via ionotropic glutamate receptors

A single cone transmits information to about 400–1,000 second-order dendrites via two specialized structures: flat (or basal) contact and synaptic triad (Fig. 1C).[7] In primates, the basal contact consists of an electron-dense cone membrane apposing an electron-dense bipolar membrane; the cleft between them is filled with vertical striated material and the bipolar dendrite belongs to OFF bipolar cells. The synaptic triad consists of an electron dense synaptic ribbon, which is presynaptic to three processes: two ''lateral elements'' of horizontal cell processes, and a ''central element'' of an ON bipolar dendrite.

To determine which receptors are expressed by the OFF bipolar cells, we and others examined the localization of several ionotropic glutamate receptor subunits (iGluR) in the cat.[8–12] Others studied the localization of ionotropic subunits in primates.[13–15] It was found that OFF dendrites express most of the known iGluR subunits including the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) subunits

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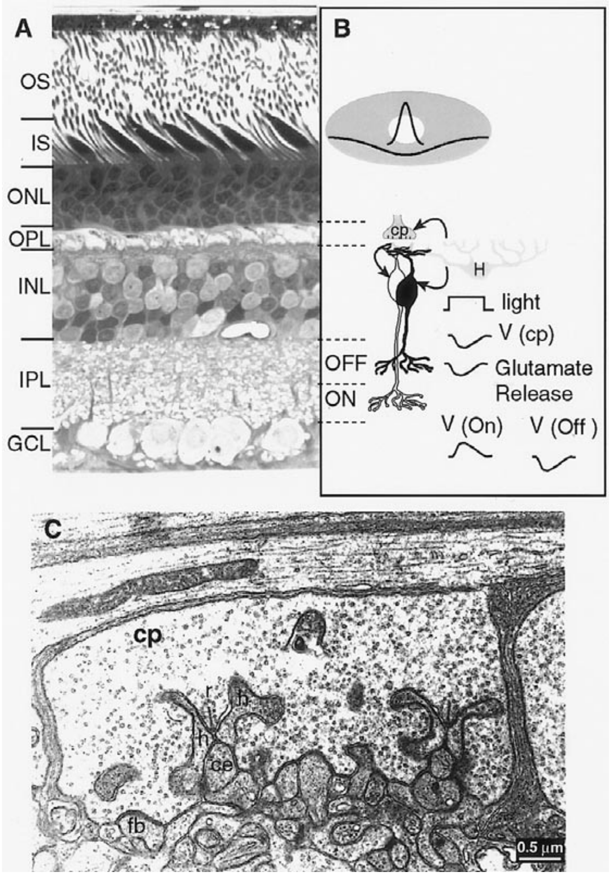

Fig. 1 The first visual synapse is a complex designed to transmit center surround information to parallel pathways. (A) A semi-thin (0.5 μm) epon radial section of a monkey retina stained with toluidine blue. Retinal layers for this and the rest of figures are: OS, outer segment; IS, inner segment; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer. Unless otherwise stated, all sections are radial. (B) A functional schematic of the cone circuits. The white circle represents the dimension of the bipolar receptive field center; within the center, light response decays according to a Gaussian distribution: strong in the very center, weaker at the outskirts. The gray circle and the corresponding Gaussian curve represent the receptive field surround. The surround is wider and shallower. A cone pedicle (cp) contacts horizontal cells (H) and ON (white) and OFF (black) bipolar cells. Voltage responses to light for ON (V(On)) and OFF V(Off)) cells are opposite. (C) An electron micrograph of a monkey cone pedicle (cp). The pedicle contains specialized ribbons (r), which are presynaptic to two lateral elements of horizontal cell processes (h) and a central element (ce) of an ON bipolar dendrite. In addition multiple OFF bipolar dendrites (fb) contact the pedicle at its base in a structure called ''flat contact''.

GluR1, GluR2, GluR2/3, and GluR4, and the kainate subunits GluR5, GluR6/7. Within the cell, receptor was concentrated in association with the OFF dendrite electron-dense membrane at the basal contact (Fig. 2A). These studies did not distinguish between different types of bipolar cells. However, a physiological study in the ground squirrel showed that different types of OFF bipolar cells express different receptors, some express the AMPA receptor, and some the kainate receptor.[16] Examination of the immunostainings also revealed that horizontal cells express both AMPA and kainate receptors, and that the receptor is concentrated at the electron-dense horizontal cell membrane just beneath the synaptic ribbon (Fig. 2B). ON bipolar cells generally do not express iGluR subunits, but in the cat, they did stain for the GluR2/3 and GluR4 subunits (Fig. 2C).[9] None of the second order neurons expressed the NMDA (N-methyl-D-aspartate) subunits.[17–19]

Glutamate hyperpolarizes ON bipolar cells via the metabotropic receptor mGluR6 coupled to the G-protein G_{o1}

In contrast to OFF bipolar cells, in which glutamate gates the receptor and opens cation channels, in ON bipolar cells, glutamate closes cation channels. About a decade ago, Nawy & Jahr[20] and Shiells & Falk[21] discovered that this glutamate response depends on a G-protein. Thus, a search to identify the receptor concentrated on the recently cloned metabotropic glutamate receptors. One such receptor, mGluR6, was found to be retina-specific, localized to dendritic tips of rod bipolar cells, and its deletion eliminated the dark-adapted electroretinogram (ERG) b-wave.[22,23] Since this wave originates from the ON responses of rod bipolar cells, its elimination proved that light response in ON bipolar cells requires mGluR6. Further studies showed mGluR6 to be expressed not only in rod bipolar cells, but also in ON cone bipolar cells.[8,24–26] To examine expression in higher mammals, we partially sequenced human mGluR6, and made an antibody that recognized this receptor in cat, rabbit and primates. In the monkey, mGluR6 was localized exclusively to dendrites of rod bipolar cells and ON cone bipolar cells (Fig. 3). Interestingly, the receptor was not concentrated under the synaptic ribbon close to the release site, but about 400 nm away at a region of the ON bipolar dendrite that was in contact with the electron-dense cone membrane (Fig. 3B, C).[26]

We next wished to identify the G-protein in the ON cells. Immunostainings for about 7 alpha subunits ($\alpha_{t1/2}, \alpha_q, \alpha_s, \alpha_i, \alpha_{olf}$, and α_o) revealed that only the α_o subunit was localized to dendritic tips of ON bipolar cells. Furthermore, this subunit was localized to dendrites of all types of ON bipolar cells; it was not localized to axon terminals of ON bipolar cells, or to OFF bipolar cells (Fig. 4).[27] Thus, G_o seemed a good candidate to mediate the ON response. Subsequent tests showed that the α_o subunit is capable of interacting with mGluR6 *in vitro*,[28] and that antibodies to this subunit reduce the light response.[29] The final proof that G_o

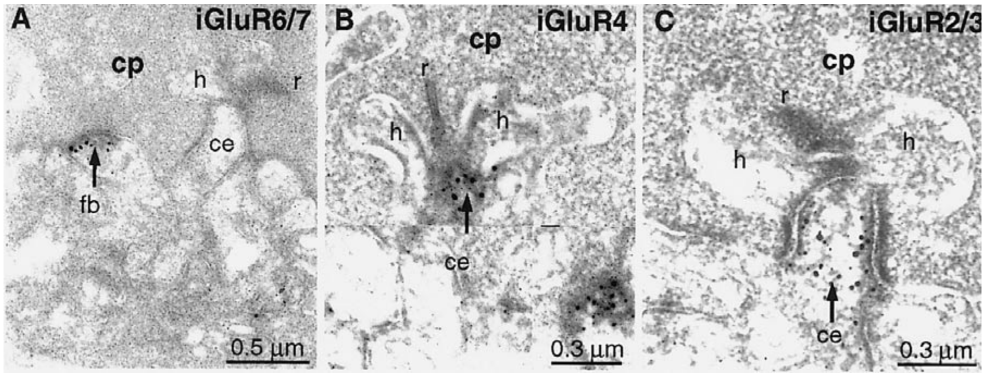

Fig. 2 Ionotropic glutamate receptor subunits (iGluR) are expressed by all types of second order neurons at their dendritic tips (electron micrographs, immunostain in cat). (A) iGluR6/7 is present at a flat contact formed by an OFF bipolar dendrite (fb). Note the exquisite localization to the membrane apposing the cone membrane. (B) iGluR4 is present in horizontal cell processes (h); it is highly localized to the electron-dense membrane just under the ribbon (r). (C) iGluR2/3 is present in a central element (ce), formed probably by an ON bipolar dendrite.

mediates the ON response came from testing the electroretinograms of mice deficient in both splice variants of the α_o subunit.[30] These mice lacked both the scotopic and the photopic b-waves (Fig. 5). The scotopic b-wave reflects the activity of rod bipolar cells, while the photopic b-wave reflects the activity of ON cone bipolar cells. The a-wave, which reflects the activity of photoreceptors, was normal. Thus, ON responses in all ON bipolar cells require G_o.

The next step was to determine which splice variant of $G\alpha_o$ is localized to these cells, and whether all ON bipolar cells express the same splice variant.[31] We first determined expression by RT-PCR (reverse transcription followed by polymerase chain reaction) and Western blotting using antibodies specific for the α_{o1} or the α_{o2} splice variants. Both splice variants were expressed. Immunostaining for the α_{o1} splice variant revealed a pattern similar to that obtained with an antibody that recognized both splice variants. Double staining for α_{o1} and protein kinase C (PKC) confirmed that rod bipolar cells stain for α_{o1} (Fig. 6A). Double staining for α_o (previously established to stain all types of ON bipolar cells) and α_{o1} showed colocalization in all bipolar cells (Fig. 6B). Thus, all types of ON bipolar cells express α_{o1}.

Localization of the α_{o2} splice variant required an indirect approach because the antibody for α_{o2} was not suitable for immunostaining. This was done using two antibodies that recognize both splice variants and applying them to mouse retina deficient in α_{o1}. Thus staining reflects expression of α_{o2}. Both antibodies gave similar staining: bipolar cell somas, dendrites, and a band in stratum 1 of the IPL. However, this stain was much weaker than the stain present in the wild type mouse. Double labeling for α_{o2} (with anti-α_o) and PKC showed that the two proteins colocalized, indicating that rod bipolar cells express the α_{o2} splice variant (Fig. 6C). In addition to rod bipolar cells, some bipolar cell somas negative for PKC were positive for the α_{o2} splice variant. This suggests that at least some cone bipolar cells also express α_{o2}.

To determine which splice variant is critical for the light responses, we examined the ERG response of mice lacking either α_{o1} or α_{o2}. Mice lacking the α_{o1} splice variant showed a reduced but significant a-wave, but completely lacked the b-wave. Mice lacking the α_{o2} splice variant had normal ERG a- and b-waves.[31]

Horizontal cells provide GABAergic signal to OFF and ON bipolar cells

About a decade ago, the neurotransmitter in mammalian horizontal cells was controversial because it was difficult to consistently show immunostaining for GABA (γ-aminobutyric acid) and GAD (glutamic acid decarboxylase) in these cells. Furthermore, initial staining for $GABA_A$ receptors was negative in the outer plexiform layer.[32] With the improvement of antibodies against GABA and optimizing fixation, we were able to show that all horizontal cells in cat and monkey contain GABA. We next investigated the localization of GAD. There are two isoform of GAD, one with a molecular weight of 65 kDa (GAD_{65}), and one with a molecular weight of 67 kDa (GAD_{67}).[33] Horizontal cells in cat and monkey stained for GAD; however, cat horizontal cells (both A- and B-types) stained for GAD_{67}, and monkey horizontal cells (both H1 and H2) stained for GAD_{65} (Fig. 7A).[34] Rabbit horizontal cells also stained for GAD, but the pattern was more complex: both types of horizontal cells stained for GAD_{67} in the tips of their processes, and type A also stained for GAD_{65}. This staining depended on eccentricity, at the visual streak somas and primary dendrites (but not their tips) stained for GAD_{65}; ventral to the visual streak,

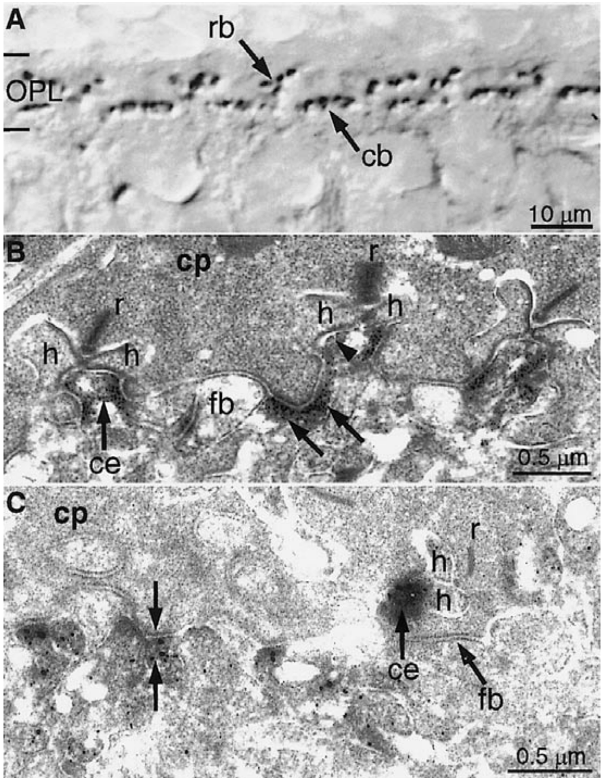

Fig. 3 mGluR6 is expressed by all ON bipolar dendritic tips in apposition to the electron-dense cone membrane. (A) Light micrograph, monkey, visualized with diaminobenzidine (DAB) reaction product. Punctate stain for mGluR6 is seen in two locations, in the upper part of the OPL, single puncta represent dendritic tips of rod bipolar cells (rb), and lower in the OPL puncta are organized in lines just under the cone pedicle; these represent cone bipolar dendrites (cb). (B) Electron micrograph, monkey. Three triads show that staining is restricted to the central elements (ce). No staining appears in flat (OFF) bipolar cells (fb). Within the central element, mGluR6 concentrates on the bipolar membrane region that apposes an electron-dense cone membrane (arrows). It is weak or undetectable just under the ribbon (arrowhead), where the dendrite apposes a horizontal cell membrane. (C) Electron micrograph, rat. mGluR6 is seen in one central element and in certain contacts resembling flat contacts (arrows). However, when these processes were traced over several sequential ultra-thin sections, it became clear that they were central elements.

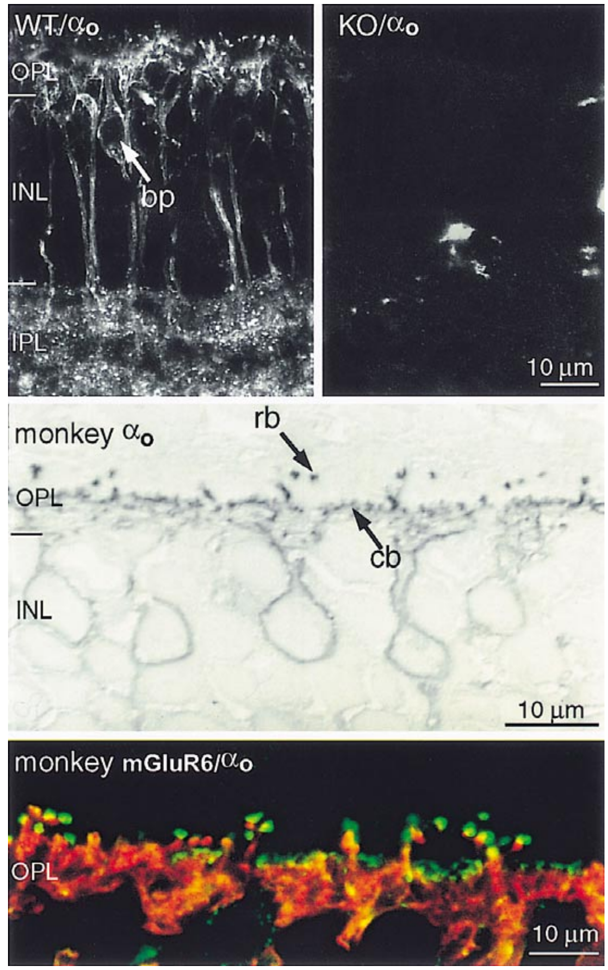

Fig. 4 Gα_o is expressed by all ON bipolar dendrites colocalized with mGluR6 in the dendritic tips. Top, wild type (WT) and Gα_o null (KO) mouse retinas immunostained for Gα_o. Immunostain is strong in bipolar somas and dendrites, and is also present in the IPL. Middle, semi-thin epon section of monkey retina visualized with DAB reaction product Stain for Gα_o is apparent in bipolar somas and their dendrites: both rod bipolar dendritic tips (rb) in the upper part of the OPL and cone bipolar dendritic tips (cb) located just below cone pedicles. Bottom, double staining for Gα_o and mGluR6. mGluR6 (green) is present at the tip of every dendrite stained for Gα_o (red).

staining intensity decreased gradually and it was undetected at about 1.5 mm from the center of the streak.[35]

To further establish that horizontal cells are GABAergic it was necessary to show that GABA receptors are postsynaptic to horizontal cells. This was tested by examining the localization of the α_1 and $\beta_{2/3}$ subunits of the $GABA_A$ receptor. Both of these subunits localized to the OPL just beneath the cones (Fig. 7B).[36] Fine localization by electron microscopy showed that the strongest stain was present on dendrites in apposition to horizontal cell processes, both on OFF and ON dendrites (Fig. 7C, D).[37] To our surprise, we were unable to detect staining on cone or rod membranes.

Possible function of horizontal cell input to bipolar cells

Bipolar cells respond to light with a center-surround receptive field structure. The center is formed by convergence of several cones (in cat area centralis, 4–7)[38] onto a bipolar cell, and the response sign is determined by glutamate action: glutamate depolarizes OFF bipolar cells and hyperpolarizes ON bipolar cells. Because light reduces glutamate release, light on the OFF bipolar receptive field center hyperpolarizes, and on the ON receptive field center depolarizes. The surround is formed by input from horizontal cells; these cells collect input from a large number of cones (in cat, ~200)[39]

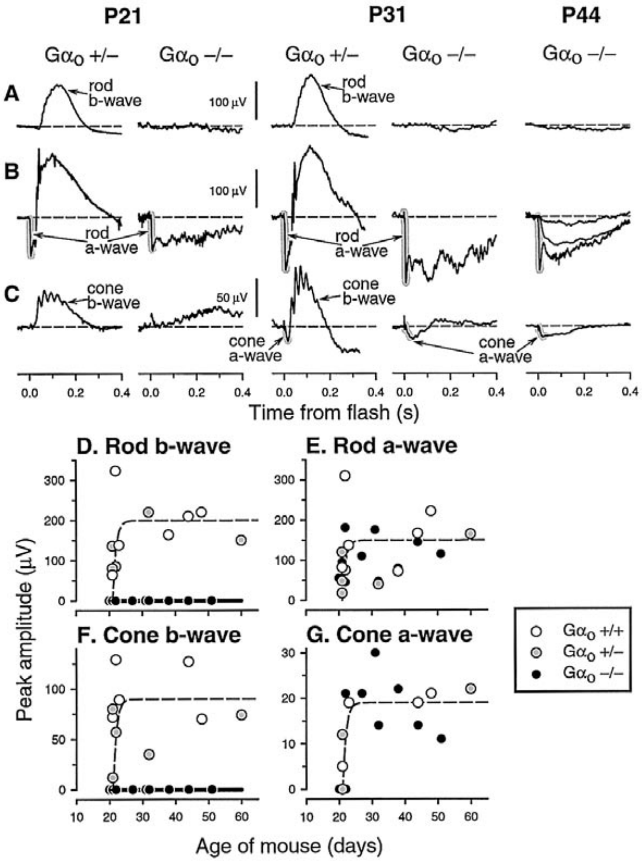

Fig. 5 Rod- and cone-driven b-waves are absent from the electroretinogram of the null mouse. (Row A) Animals dark-adapted for 2 hours were stimulated with dim flashes. Such flashes elicited a rod-driven, corneal-positive b-wave in the heterozygotes, but no positive-going responses in the null mice. The estimated flash intensities in photoisomerizations per rod (Φ) and the number of responses (n) averaged for each trace shown are as follows: for the P21 and P31 mice, $\Phi = 20$, $n = 11$; for the P44 mouse, $\Phi = 3$, $n = 40$. (Row B) Dark-adapted animals were stimulated with an intense flash (isomerizing ~1% of the rhodopsin). This elicited in the heterozygote a negative a-wave (shaded), followed by a positive-going b-wave. In the null mice the a-wave was normal, but the b-wave was absent. The flash intensities (Φ) and the number of responses (n) averaged were: for the P21 and P31 mice, $\Phi = 10^6$, $n = 2$–4. For the P44 mouse responses to three intensities are shown: $\Phi = 20$, $n = 20$; $\Phi = 500$, $n = 16$; $\Phi = 10^6$, $n = 2$. (Row C) Mice were adapted to a bright background (540 nm, 20,000 R* rod^{-1} s^{-1}) that completely suppressed the cGMP-activated current of the rods. They were then stimulated with an intense white flash that isomerizes about 1% of the M-cone pigment and 0.1% of the UV-cone pigment in adult mice. The cone driven a-wave was not visible in the P21 animals, but was pronounced in P31 and P44 animals (both $G\alpha_o$+/− and $G\alpha_o$−/−). A typical cone-driven b-wave (positive-going response with superimposed oscillations, peaking about 70–90 ms after the flash) was observed in the $G\alpha_o$+/− mice of all age groups, but was absent in the $G\alpha_o$−/− mice. For P21 and P31 $G\alpha_o$+/− mice, $n = 10$; for P21 $G\alpha_o$−/−, $n = 20$; for P31 and P44 $G\alpha_o$−/−, $n = 40$. The slow positive-going potential in P21 mouse is probably an artifact due to movement of the lightly anesthetized mouse. (D–G) ERG a- and b-waves' peak amplitude is variable due to variable contact with the electrode, and due to rapid growth between 21 and 30 day postnatal. However, at all ages, the rod- and cone-driven b-waves were missing from the $G\alpha_o$−/− mouse.

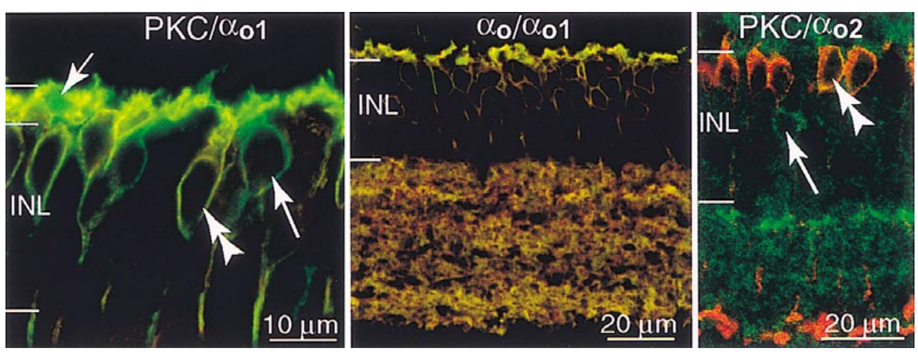

Fig. 6 ON bipolar dendrite express both splice variants of $G\alpha_o$ (mouse). (Left) double staining for $G\alpha_{o1}$ (green) and PKC (red, a marker for rod bipolar cells). All PKC positive cells also stain for $G\alpha_{o1}$ (double arrowhead), but certain somas negative for PKC are also positive (arrow). These are probably ON cone bipolar somas. Also, some processes, stained for $G\alpha_{o1}$, are unstained for PKC; these are cone bipolar dendrites (short arrow). (Middle) double staining for $G\alpha_{o1}$ (green) and $G\alpha_o$ (red, marks all ON bipolar somas). The two stains are 100% colocalized, indicating all ON bipolar cells express $G\alpha_{o1}$. (Right) double staining for PKC (red) and $G\alpha_o$ (green) on a $G\alpha_{o1}$-null mouse. ON this mouse, staining for $G\alpha_o$ labels cells expressing $G\alpha_{o2}$. $G\alpha_{o2}$ is expressed by rod bipolar cells (double arrowheads) and certain ON cone bipolar cells (arrow).

thus averaging light luminance over a wide area. A strong component of the surround is accomplished by feedback inhibition to cones. By feeding back to the cones, horizontal cells create surround already at the level of photoreceptors, and this is then transmitted to ON and OFF bipolar cells via glutamate. This feedback had been recognized more than 20 years ago in lower vertebrates,[4,40] and confirmed recently in mammals.[41,42] Surprisingly, however, the molecular mechanism of the feedback (i.e., which transmitter is released and which receptors responds to it) is still debated.[43]

Horizontal cells could also provide direct antagonistic surround to bipolar cells, via GABA receptors located on their dendritic tips.[37] However, this mode of

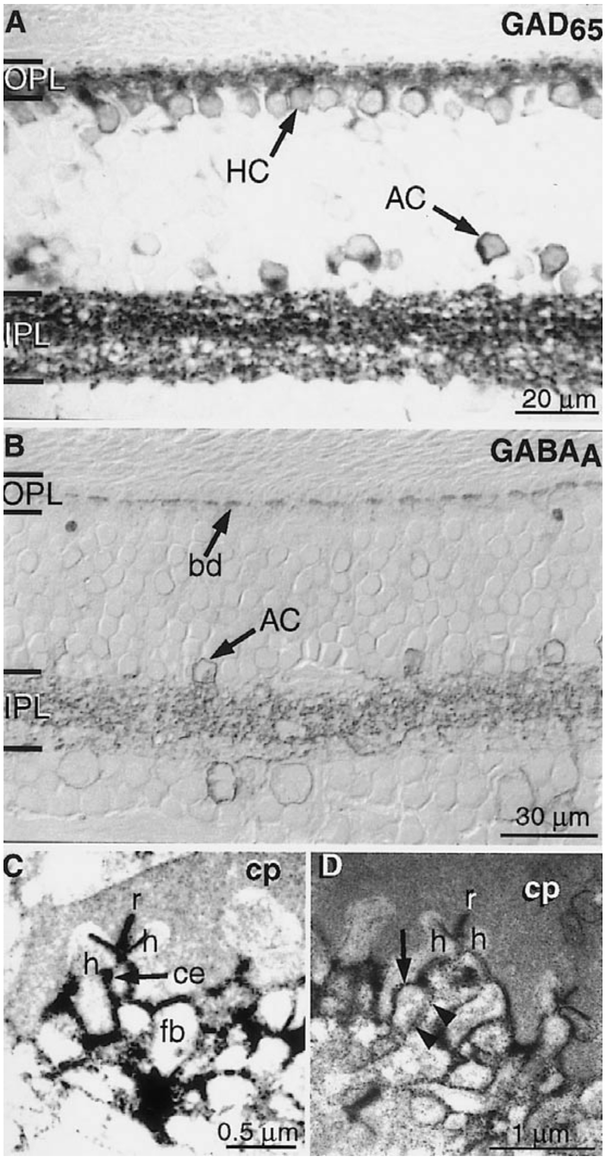

Fig. 7 Horizontal cells are GABAergic, probably releasing GABA onto ON and OFF bipolar dendritic tips expressing $GABA_A$ receptors (monkey). (A) A semi-thin section stained for GAD_{65}. Stain is present in both types of horizontal cells (HC) and their processes in the OPL, in amacrine cells (AC), and in the IPL. (B) A semi-thin section stained for the α_1 subunit of the $GABA_A$ receptor. Stain is present in the middle of OPL in bipolar dendrites (bd), and in amacrine cells and IPL. (C) Electron micrograph showing part of a cone pedicle (cp) and its postsynaptic processes. Staining for $GABA_A$ (α_1) is present in OFF bipolar dendrites forming flat contacts (fb), and in ON bipolar cells forming the central element of the triad (ce). (D) Electron micrograph. $GABA_A$ staining on a bipolar membrane (arrow) apposing a horizontal cell process (h) is much stronger than on the membrane facing another bipolar cell (arrowheads). *Stain is visualized with an un-intensified DAB reaction product.*

input requires separate treatment for the ON and OFF bipolar cells. In OFF bipolar cells, glutamate depolarizes and GABA could antagonize it by hyperpolarizing. In contrast, in ON bipolar cells, glutamate

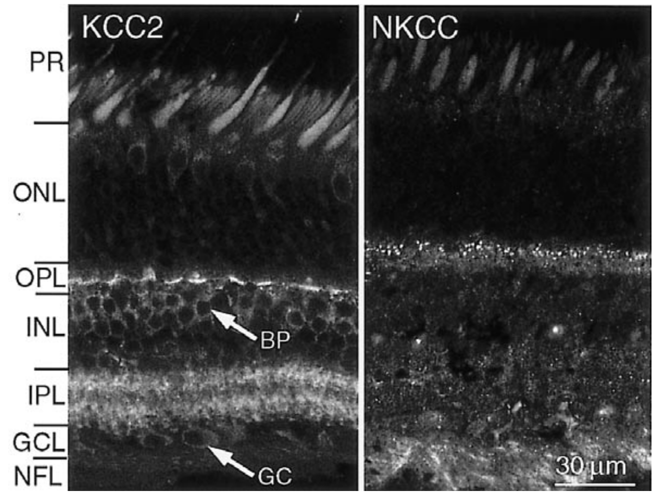

Fig. 8 Retina expresses two types of chloride cotransporters: KCC2 (a chloride extruder), and NKCC (a chloride accumulator) (monkey). Staining for KCC2 is present in OPL, bipolar somas (BP) in INL, in IPL, and in ganglion cell somas (GC). Staining for NKCC is strong and punctate in the upper part of the OPL, and somewhat diffused lower in the OPL. Stain is very weak in the IPL, and strong in the nerve fiber layer (NFL). PR, photoreceptors.

hyperpolarizes, so for GABA to antagonize it, it should depolarize. GABA could depolarize a cell if the chloride equilibrium potential (E_{Cl}) is maintained higher than the resting potential. Early reports measuring $[Cl^-]_i$ in mudpuppy, indeed found that $[Cl^-]_i$ in ON bipolar cells was higher than in OFF bipolar cells.[44] We tested this conjecture in mammalian retinas by examining the expression pattern of cation-coupled chloride cotransporters.

Retina expresses two types of chloride cotransporters: KCC2 and NKCC

Three families of cation-coupled chloride cotransporters are known: the K-Cl cotransporter (KCC) uses K^+ gradient to extrude Cl^-, and the Na-K-Cl (NKCC) and Na-Cl cotransporters (NCC) use Na^+ gradient to accumulate Cl^-. Neuronal cotransporters are mainly KCC2 and NKCC1.[45,46] In retina, immunostaining for KCC2 gave strong staining both in OPL and in IPL (Fig. 8). In OPL, the pattern resembled ‘‘dashed lines’’, in IPL it was punctate throughout the layer. Immunostaining for NKCC gave strong staining only in the OPL with puncta high in the OPL and somewhat diffused stain lower in the OPL (Fig. 8).

Distribution of NKCC and KCC2 in adult retina correlates with known E_{Cl}

Horizontal cells express NKCC, the chloride accumulator (Fig. 9, left).[47] This fits robust evidence that

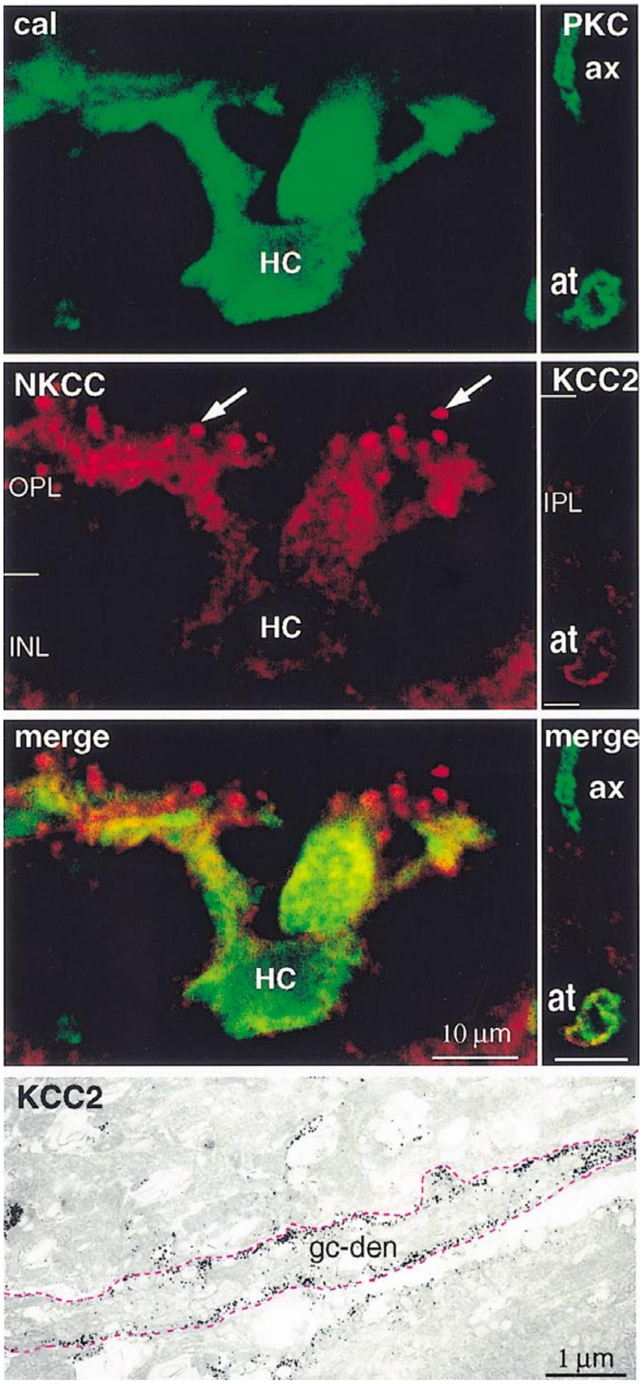

Fig. 9 Staining for the chloride cotransporters agrees with known E_{Cl}. (Left three) double labeling for NKCC and calbindin (marker for horizontal cells), rabbit. Stainings are colocalized in horizontal cell somas (HC) and their primary processes. Strong punctate stain for NKCC in the upper OPL is not clocalized with horizontal cell processes (see figure 10). (Right three) double labeling for KCC2 and PKC (marker for rod bipolar cells), rabbit. Stained with PKC is an axon of a rod bipolar cell (ax) and its terminal in stratum 5 of the IPL (at). KCC2 was localized in the axon terminal but not in the axon itself. Scale bar, 5 µm. (Bottom) electron micrograph, monkey. Staining for KCC2 is associated with the plasma membrane of ganglion cell dendrites (highlighted in pink, gc-den).

E_{Cl} in horizontal cells is positive to E_{rest}: (1) GABA depolarizes horizontal cells in lower vertebrates and in mammals[44,48,49]; (2) $[Cl^-]_i$ measured with a chloride-sensitive electrode predicts E_{Cl} at about −17 mV, i.e., ~10 mV positive to dark E_{rest}.[44,48]

Bipolar axon terminals (of rod bipolar and OFF cone bipolar cells) express KCC2, the chloride extruder (Fig. 9, right). This fits evidence that their GABA feedback from amacrine cells is inhibitory, i.e., hyperpolarizing, which implies E_{Cl} negative to E_{rest},[50,51] (reviewed by Freed).[52]

Ganglion cells express KCC2. Somas (Fig. 8) and dendrites, identified by EM (Fig. 9, bottom), were stained in the plasma membrane.[47] Also, co-staining rat retina with anti-KCC2 and anti-thy1 (a marker for ganglion cell dendrites) showed strong colocalization.[53] Direct measurement of $[Cl^-]_i$ or E_{Cl} under conditions that do not disturb $[Cl^-]_i$ are rare because most recordings are performed whole cell, where Cl^- is free to diffuse from the electrode into the cell. Nevertheless it is clear that GABA hyperpolarizes ganglion cells: (1) $[Cl^-]_i$ measured by a chloride-sensitive electrode, estimated E_{Cl} at −49 mV, about 20 mV negative to dark E_{rest}[44]; (2) Blocking $GABA_A$ and glycine receptors increases excitation.[54–57] Whether ganglion cells also express NKCC is unclear.

OFF bipolar dendrites express KCC2 and ON bipolar dendrites express NKCC

Staining in OFF bipolar dendrites was examined by electron microscopy. When stained for KCC2, OFF bipolar cell dendrites stained at their tips (Fig. 10A), but these remained unstained for NKCC (Fig. 10C). Staining for ON bipolar cells was examined both by colocalization with mGluR6 (Fig. 10B) and by electron microscopy (Fig. 10C, D). Dendritic tips of rod bipolar cells and ON cone bipolar cells stained for NKCC, but not for KCC2.

Discussion

The neurochemical organization of the first visual synapse is unique in its complexity (summarized in Fig. 11), and it is designed to transmit center-surround antagonistic signal to about 10 parallel pathways.

Parallel pathways

There are about 10 parallel pathways in photopic vision, about 5 in the OFF channel and 5 in the ON channel. Within each channel, the parallel pathways are thought to transmit different temporal frequen-

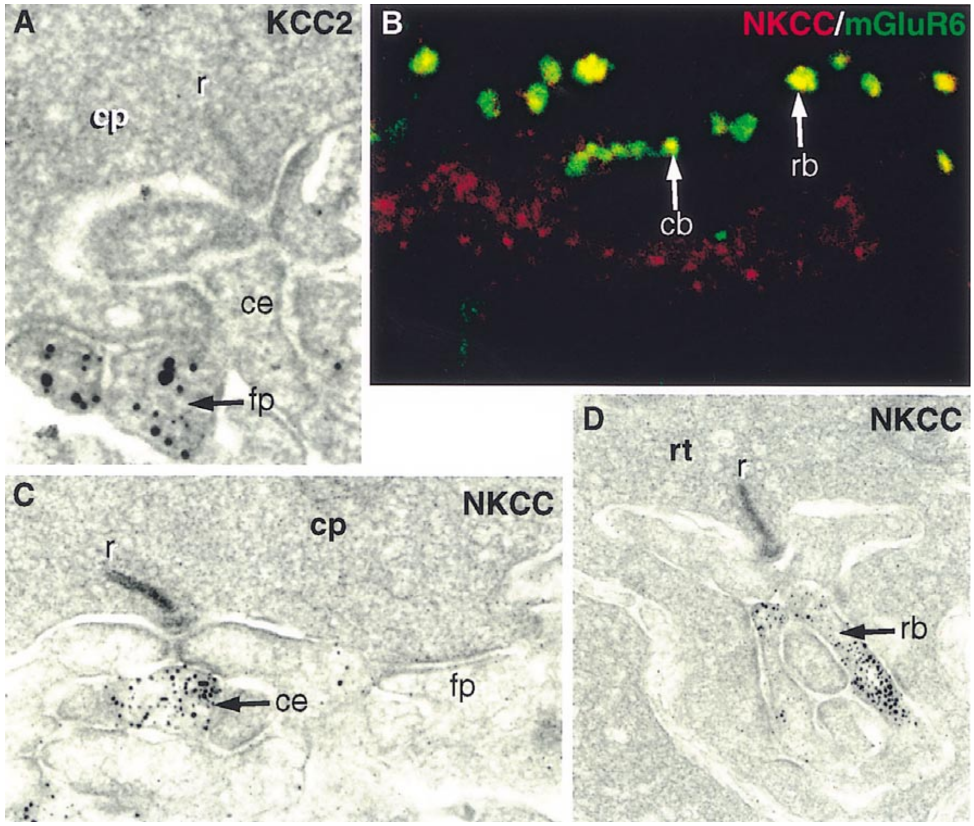

Fig. 10 Dendritic tips of OFF bipolar cells express KCC2 and those of ON bipolar cells express NKCC (monkey). (A, C, D) Electron micrographs. (A) KCC2 is present in flat contacts (fp) and not in central elements (ce); cp, cone pedicle; r, ribbon. (B) Double staining for NKCC and mGluR6. Staining is colocalized in large puncta representing rod bipolar dendritic tips (rb) and in smaller puncta representing dendritic tips of ON cone bipolar cells (cb). Diffuse stain for NKCC below the puncta represents horizontal cells' primary dendrites. (C) NKCC is expressed by the central element and is absent from flat bipolar dendritic tips. (D) NKCC is expressed by rod bipolar dendritic tips (rb); rt, rod terminal.

cies.[3,58–61] The fundamental differences between the OFF and the ON channels are now understood. The OFF bipolar cells express ionotropic glutamate receptors; when bound to glutamate, these receptors open and permit an influx of cations. In contrast, the ON pathways express mGluR6. When bound to glutamate, this receptor activates the G-protein G_{o1}, which closes a non-specific cation channel. In the absence of glutamate the channel is maintained at an open state, which depends on intracellular [cGMP]. However, the identity of the channel, and how G_{o1} causes its closure are still unknown.

Also unsolved is what molecular mechanisms underlie the differences between cell types. In the ON pathways, we have shown that all types of ON bipolar cells express mGluR6 and G_{o1}, thus the differential responses have to arise either from their different distance from the release site,[16] or by differential modulations of the cascade. In the OFF pathways, different bipolar types may express different combinations of the four iGluR subunits that form a receptor, but this has not been resolved yet.

Bipolar cell center-surround receptive field

It is clear from multiple recordings that feedback from horizontal cells to cones contributes to bipolar cells surround by shifting the activation curve for Ca^{2+}, but how horizontal cells manage to shift this curve is not clear. Some investigators believe that the inhibition is GABAergic, but others attribute the feedback to an ephatic effect, in which the shift is created by a current through hemichannels located on the two appossing horizontal cell membranes under the ribbon.[43] To add to the puzzle, we should note that even the morphology and the location of the feedback synapse have not been identified because the synaptic complex contains no conventional structures that might disclose the location of the feedback synapse.

In addition to feedback, horizontal cells affect bipolar cell responses by directly synapsing onto them. It is now accepted that the horizontal to bipolar synapse is GABAergic, and that bipolar cells respond via $GABA_A$ and $GABA_C$ receptors. We have shown that OFF bipolar cells express KCC2 and we predict that

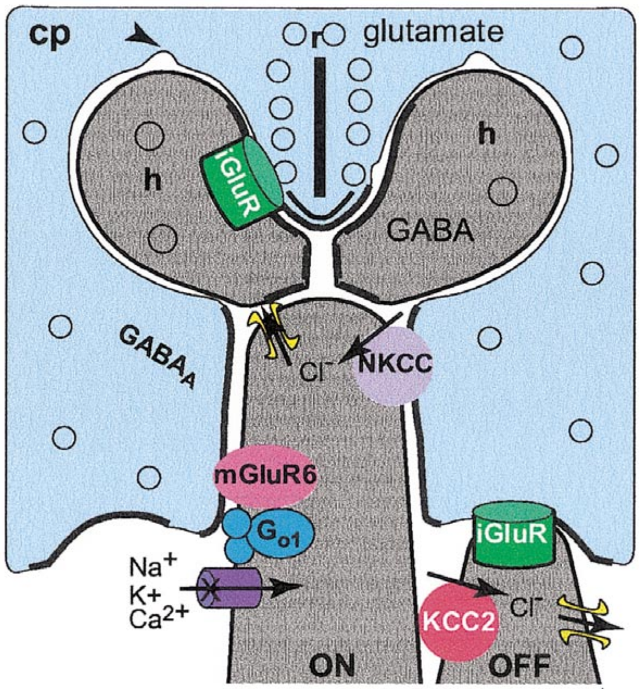

Fig. 11 The first visual cone synaptic complex: structure and neurochemistry. The cone pedicle (cp) releases glutamate onto horizontal cell processes (h) and ON and OFF bipolar cell dendrites by an exocytotic process that occurs at the release site located just under the synaptic ribbon (r) (to which synaptic vesicles are tethered). Endocytosis occurs at regions flanking the release site (arrowhead). The cone contacts the second order neurons at three specialized regions, at these cotacts, the cone membrane is electron-dense (thick lines). The contact between cone and horizontal cell is at the invagination, just beneath the ribbon; the horizontal cell membrane at this contact is electron-dense; and it expresses ionotropic glutamate receptors (iGluRs). The contact between cone and OFF bipolar dendrite (flat contact) is at the cone base; the bipolar membrane is electron-dense and it expresses iGluRs. The contact between cone and ON bipolar dendrites is at the mouth of the invagination, the bipolar membrane is only slightly dense; it expresses mGluR6, G_{o1}, and an unidentified non-selective cation channel. Horizontal cell processes contact ON bipolar cells at the triad, but they probably contact both ON and OFF bipolar dendrites as they ascend to invaginate the cone. These contacts are largely unspecialized, but they are revealed by $GABA_A$ receptor that concentrates on bipolar membranes apposing horizontal cells. Along with the $GABA_A$ receptor, ON bipolar dendrites express NKCC and OFF bipolar dendrites express KCC2.

they maintain an E_{Cl} negative to rest. Under these conditions, GABA would antagonize responses to glutamate and will contribute to surround response. ON bipolar dendrites express NKCC, so they are predicted to maintain an E_{Cl} positive to rest. If so, GABA could contribute to surround response by depolarizing the cells. Recent measurement of E_{Cl} in bipolar cells confirmed that E_{Cl} in rod bipolar cells was higher than that in ON cone bipolar cells, which was higher than in OFF cone bipolar cells.[62] The difference between rod bipolar cells and OFF cells was significant, but the difference between ON and OFF cone bipolar cells was not.

Does the ON rod bipolar cell maintain a chloride gradient?

Theoretical considerations require that the rod bipolar's dendrite maintains different E_{Cl} than its axon terminal. As explained above, dendrites should depolarize to GABA, but axon terminals should hyperpolarize. This is because GABAergic amacrine cells that feedback onto the rod bipolar axon terminal depolarize to light.[63] Thus when a light stimulus depolarizes rod bipolar cells, it also depolarizes the A17 amacrine cell. GABA would then be released with a delay and hyperpolarize the rod bipolar cell. This prediction is supported by the expression pattern of the chloride cotransporters (NKCC in dendritic terminal, KCC2 in axon terminal). E_{Cl} measurements by physiological methods are controversial, one study measured the same E_{Cl} for GABA applied to dendrites and to axons,[62] and another study measured a difference.[64] Whether or not it is theoretically possible to maintain a gradient within such a compact cell is not clear because it depends on the level of activity of the chloride transporters and on the diffusion parameters within the cell. It should be mentioned that chloride gradients within larger cells (hippocampal neurons) have been reported.[65]

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