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Cross-talk between ON and OFF channels in the salamander retina: Indirect bipolar cell inputs to ON–OFF ganglion cells

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Abstract

It has been widely accepted that ON and OFF channels in the visual system are segregated with little cross-communication, except for the mammalian rod bipolar cell-AII amacrine cell-ganglion cell pathway. Here, we show that in the tiger salamander retina the light responses of a subpopulation of ON–OFF ganglion cells are mediated by crossing the ON and OFF bipolar cell pathways. Although the majority of ON–OFF ganglion cells (type I cells) receive direct excitatory inputs from depolarizing and hyperpolarizing bipolar cells (DBCs and HBCs), about 5% (type II cells) receive indirect excitatory inputs from DBCs and 20% (type III cells) receive indirect excitatory inputs from HBCs. These indirect bipolar cell inputs are likely to be mediated by a subpopulation of amacrine cells that exhibit transient hyperpolarizing light responses (AC_Hs) and make GABAergic/glycinergic synapses on DBC or HBC axon terminals. GABA and glycine receptor antagonists enhanced the ON and OFF excitatory cation current (ΔI_C) in type I ganglion cells, but completely suppressed the ON ΔI_C mediated by DBCs in type II cells and the OFF ΔI_C mediated by HBCs in types III cells. Dendrites of type I cells ramify in both sublamina A and B, type II cells exclusively in sublamina A, and type III cells exclusively in sublamina B of the inner plexiform layer. These results demonstrate that indirect, amacrine cell-mediated bipolar cell-ganglion cell synaptic pathways exist in a non-mammalian retina, and that bidirectional cross-talk between ON and OFF channels is present in the vertebrate retina.

Keywords: Depolarizing bipolar cells; Hyperpolarizing bipolar cells; ON–OFF amacrine cells; ON–OFF ganglion cells; GABA; Glycine

1. Introduction

The visual system processes light images through parallel information channels, and the ON and OFF channels are the two most important channels that carry "light on" and "light off" information from the retina to the brain (Dowling, 1987; Hubel & Wiesel, 1979). In the retina, the ON channel comprises the depolarizing bipolar cells (DBCs), the on-center ganglion cells and the ON responses of the ON–OFF ganglion cells, whereas the OFF channel consists of the hyperpolarizing bipolar cells (HBCs), the off-center ganglion cells and the OFF responses of the ON–OFF ganglion cells (Dowling, 1987). A widely accepted concept is that ON and OFF channels are segregated throughout the visual pathway, with little cross-communication between them. For example, anatomical studies have revealed that axons of DBCs make synaptic contacts with dendrites of ON ganglion cells in sublamina B of the inner plexiform layer (IPL) whereas axons of HBCs make synapses with dendrites of the OFF ganglion cells in sublamina A of the IPL (Kolb & Famiglietti, 1974; Strettoi, Dacheux, & Raviola, 1990). Additionally, it has been shown that application of L-AP4, an mGluR6 glutamate receptor agonist that suppresses DBC light responses (Slaughter & Miller, 1981), blocks light-evoked signals of ON ganglion cells, the ON responses of ON–OFF ganglion cells (Hensley, Yang, & Wu, 1993a), and ON responses of neurons in the visual cortex (Schiller, Sandell, & Maunsell, 1986).

A well known exception for ON and OFF channel segregation is the rod bipolar cell pathway in the mammalian

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retina. It has been shown that mammalian rod bipolar cells (which are ON bipolar cells, thus named DBC_Rs) do not make direct synaptic contacts with ganglion cells, but with AII amacrine cells (AIIACs) (Strettoi, Dacheux, & Raviola, 1994; Strettoi, Raviola, & Dacheux, 1992). AIIACs relay DBC_R signals to the ON cone bipolar cells (DBC_Cs) through electrical synapses and to OFF cone bipolar cells (HBC_Cs) through sign-inverting chemical synapses (Kolb & Famiglietti, 1974; Strettoi et al., 1994). DBC_Cs relay DBC_R-AIIAC signals to the ON ganglion cells, and HBC_Cs relay DBC_R-AIIAC signals to the OFF ganglion cells (Tsukamoto, Morigiwa, Ueda, & Sterling, 2001). Such AIIAC-mediated, unidirectional (ON → OFF) cross-talk between ON and OFF channels is believed to be mammalian-specific and only used for processing DBC_R signals. In this article, we present data which suggest that ON and OFF channel interaction occurs in a non-mammalian (tiger salamander) retina and the cross-talk is not limited to the DBC_R pathway, but through amacrine cell-mediated, bidirectional pathways that relay indirect DBC and HBC inputs to a subpopulation of ON–OFF ganglion cells.

In the tiger salamander retina, there are three types of ganglion cell light responses: the ON, OFF and ON–OFF, and the vast majority (about 80%) are ON–OFF cells (Hensley, Yang, & Wu, 1993b; Pang, Gao, & Wu, 2002) and they do not exhibit center-surround antagonism (Werblin, 1972; Wunk & Werblin, 1979). An earlier study has shown that dendrites of ganglion cells exhibiting ON excitatory postsynaptic current (ΔI_C) ramify in strata 6–10 (sublamina B), dendrites of cells with OFF ΔI_C ramify in strata 1–5 (sublamina A), and most ON–OFF ganglion cells have diffuse dendrites in both sublamina A and B (Pang et al., 2002). A subpopulation of ON–OFF ganglion cells, however, have dendrites that ramify exclusively in sublamina A or B. In this article, we present evidence indicating that the ON or OFF responses of this population of ON–OFF ganglion cells are not mediated by direct bipolar cell inputs, but indirectly by GABAergic/glycinergic amacrine cells. Our study demonstrates that indirect, amacrine cell-mediated cross-talk between ON and OFF channels is present in non-mammals, in addition to the well-known mammalian DBC_R-AIIAC synaptic pathway.

2. Methods

Larval (1- to 2-year-old) tiger salamanders (*Ambystoma tigrinum*) purchased from Charles D. Sullivan, Co. (Nashville, TN) and KON's Scientific Co. Inc. (Germantown, WI) were used in this study. All animals were handled in accordance with the policies on treatment of laboratory animals of Baylor College of medicine and the National Institutes of Health. Before each experiment, salamanders were anesthetized in MS222 until the animal gave no visible response to touch or water vibration. The animals were then quickly decapitated and the eyes were enucleated. The procedures of dissection, retinal slicing and recording were described in previous publications (Werblin, 1978; Wu, 1987). Dissection and recording were done under infrared illumination with a dual-unit Nitemare infrared scope. Oxygenated Ringer's solution was introduced continuously to the superfusion chamber by gravity, and the control Ringer's contained 108 mM NaCl, 2.5 mM KCl, 1.2 mM $MgCl_2$, 2 mM $CaCl_2$, and 5 mM Hepes (adjusted at pH 7.7). All chemicals (obtained from Research Biochemical International (Natick, MA) or Sigma (St. Louis, MO)) were dissolved in control Ringer's solution. Picrotoxin, I4AA and strychnine solutions were freshly made each time, and they were introduced and washed via the bath superfusion system. It took about 2–3 min superfusion time for picrotoxin, I4AA and strychnine to exert action on most cells and 5–10 min to wash away the drug action. A photostimulator was used to deliver light spots (of diameter 600–1200 μm) to the retina via the epi-illuminator of the microscope. The intensity of unattenuated ($\log I = 0$) 500 nm light was 2.05×10^7 photons $\mu m^{-2} s^{-1}$.

Voltage-clamp recordings were made with an Axopatch 200B amplifier connected to a DigiData 1200 interface and pClamp 6.1 software. Patch electrodes of 5 MΩ tip resistance (when filled with an internal solution containing 118 mM Cs methanesulfonate, 12 mM CsCl, 5 mM EGTA, 0.5 mM $CaCl_2$, 4 mM ATP, 0.3 mM GTP, 10 mM Tris, 0.8 mM Lucifer yellow, and when adjusted to pH 7.2 with CsOH) were made with Narishige or Sutter patch electrode pullers. The chloride equilibrium potential, E_{Cl}, with this internal solution was about −60 mV. The equilibrium potential of cation current was determined by the reversal potential of glutamate-induced current in morphologically identified bipolar cells in Ringer's containing 2 mM Co^{2+} (Wu & Maple, 1998). Light-elicited photoreceptor and amacrine cell inputs to bipolar cells were studied by recording the light-evoked cation and chloride currents, ΔI_C and ΔI_{Cl}, at holding potentials E_{Cl} and E_C, respectively. Estimates of the liquid junction potential at the tip of the patch electrode prior to seal formation varied from −9.2 to −9.6 mV. For simplicity, we corrected all holding potentials by 10 mV.

Intracellular recordings were made from amacrine cells in flat-mounted whole retinas with micropipettes drawn with a modified Livingston puller with Omega Dot tubing (1.0-mm o.d. and 0.5-mm i.d.). The micropipettes were filled with 2 M potassium acetate and had tip resistances measured in Ringer's solution of 100–600 MΩ.

Three-dimensional cell morphology was visualized in living retinal slices through the use of Lucifer yellow fluorescence with a confocal microscope (Zeiss 510). Images were acquired by using a 40× water immersion objective (n.a. = 0.75), the 458 nm excitation line of an argon laser, and a long pass 505 nm emission filter. Consecutive optical sections were superimposed to form a single image using the Zeiss LSM-PC software, and these compressed image stacks were further processed in Adobe Photoshop 6.0 to improve the contrast. Background images of the retinal slices were also acquired using transmitted light. The level at which dendritic processes stratified in the IPL was characterized by the distance from the processes to the distal margin of the IPL. We selected cells in the ganglion cell and amacrine cell layers with somas situated beneath the surface of the slice and they usually had relatively intact processes (assessed by rotation of the stacked images).

3. Results

3.1. Bipolar cell inputs to the majority of ON–OFF ganglion cells (type I) persist in the presence of amacrine cell neurotransmitter blockers

In about 75% (54/72) of the ON–OFF GCs, direct bipolar cell inputs, represented by the light-evoked cation current ΔI_C (recorded at E_{Cl}), persisted in the presence of amacrine cell neurotransmitter blockers, and we named these cells type I cells. Fig. 1A show the stacked confocal fluorescent image of a type I cell in the retinal slice, and it exhibited typical ON–OFF GC morphology with an axon and dendrites diffusely distributed in both sublamina A and B of the inner plexiform layer (IPL). The light-evoked current responses of this cell to a 2.5-s light step (500 nm, −2) at six holding potentials in normal Ringer's, in the presence of 100 μM picrotoxin + 1 μM strychnine + 10 μM I4AA

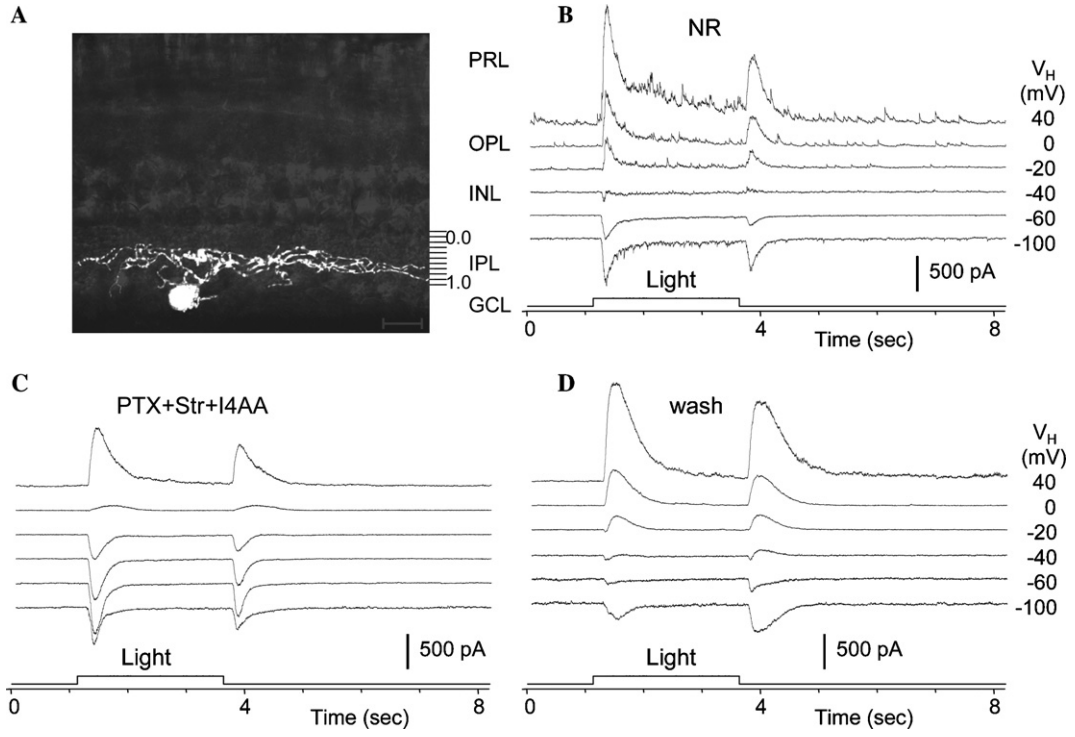

Fig. 1. (A) Stacked confocal fluorescent image of a type I ON–OFF ganglion cell in the retinal slice. PRL, photoreceptor layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer (0–1:0–100% of IPL depth); GCL, ganglion cell layer. Calibration bar, 20 μm. (B) The light-evoked current responses of this cell to a 2.5 s light step (500 nm, −2) at six holding potentials in normal Ringer's, (C) in the presence of 100 μM picrotoxin + 1 μM strychnine + 10 μM I4AA (P + S + I), and (D) after wash.

(P + S + I), and after wash are shown in Fig. 1B–D, respectively. We used these three compounds because they have been shown to block light-evoked inhibitory inputs from amacrine cells to bipolar cell axon terminals and ganglion cells in the tiger salamander retina (Gao, Maple, & Wu, 2000; Pang et al., 2002). In addition to the light-evoked current responses at light onset and offset, there were also discrete spontaneous postsynaptic currents (sPSCs), mediated by single or multiples of glutamatergic, GABAergic and glycinergic synaptic vesicle release (Arkin & Miller, 1988a; Gao & Wu, 1998, 1999; Taylor, Chen, & Copenhagen, 1995). The current traces in P + S + I were smoother, because GABAergic and glycinergic spontaneous inhibitory postsynaptic currents (sIPSCs) were blocked (Gao & Wu, 1998). The light-evoked currents in P + S + I reversed near –5 mV, consistent with the idea that P + S + I suppresses the light-evoked inhibitory chloride current (ΔI_{Cl}) mediated by GABAergic and glycinergic amacrine cells, and that the residual ON and OFF ΔI_C are mediated by DBCs and HBCs through a glutamate-gated cation conductance (with a reversal potential ranging from −10 to +10 mV) (Diamond & Copenhagen, 1993; Gao & Wu, 1999; Mittman, Taylor, & Copenhagen, 1990). By comparing the ΔI_C at $E_{Cl} = -60$ mV with and without P + S + I, it is evident that the excitatory light-evoked inputs from bipolar cells are larger and more prolonged when amacrine cell inputs are blocked (quantitative measurements were provided in a previous study (Pang et al., 2002)). This is consistent with the idea that GABAergic and glycinergic amacrine cells not only directly activate chloride conductances in ganglion cells, but also decrease and shorten the output signals of the bipolar cells through the feedback synapses made on bipolar cell axon terminals (Dong & Werblin, 1998; Lukasiewicz, Maple, & Werblin, 1994a). Similar results were obtained in 54 (out of a total of 72) ON–OFF GCs, indicating that the majority of ON–OFF GCs in the salamander retina receive direct DBC and HBC inputs, as blockade of amacrine cell inputs does not suppress ON and OFF ΔI_C in these cells.

3.2. ON bipolar cell inputs to about 5% of ON–OFF ganglion cells (type II) can be suppressed by amacrine cell neurotransmitter blockers

In 4 of the 72 ON–OFF GCs (about 5%), P + S + I blocks ΔI_{Cl} and ON ΔI_C. These cells had dendrites that ramified in sublamina A (strata 1–5) of the IPL and exhibited transient ON and OFF responses, and we named them type II cells. Fig. 2A shows the stacked confocal fluorescent image of a type II cell in the retinal slice, with dendrites stratified in strata 2 and 4 of the IPL and an axon. The light-evoked current responses of this cell to a 2.5-s light step (500 nm, −2) at six holding potentials in normal Ringer's, in the presence of 100 μM picrotoxin + 1 μM strychnine + 10 μM I4AA (P + S + I), and after wash are shown in Fig. 2B–D, respectively. Similar to the type I cells, these cells also exhibit discrete spontaneous sPSCs. The light-evoked currents in P + S + I also reversed near −5 mV, consistent with the idea

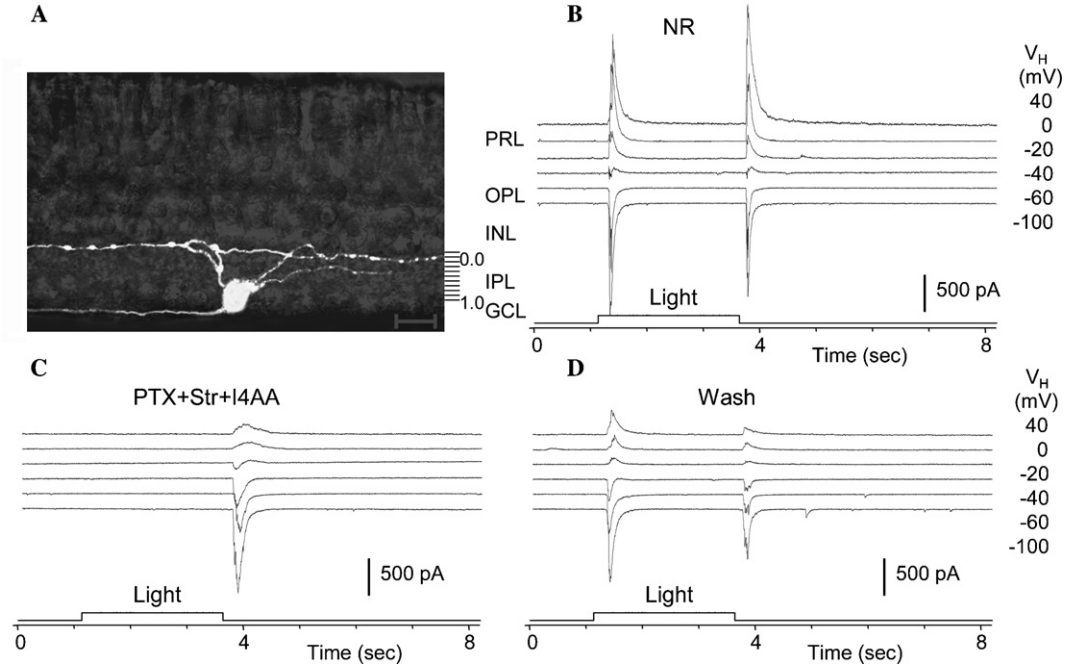

Fig. 2. (A) Stacked confocal fluorescent image of a type II ON–OFF ganglion cell in the retinal slice. PRL, photoreceptor layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer (0–1:0–100% of IPL depth); GCL, ganglion cell layer. Calibration bar, 20 μm. (B) The light-evoked current responses of this cell to a 2.5 s light step (500 nm, −2) at six holding potentials in normal Ringer's, (C) in the presence of 100 μM picrotoxin + 1 μM strychnine + 10 μM I4AA (P + S + I), and (D) after wash.

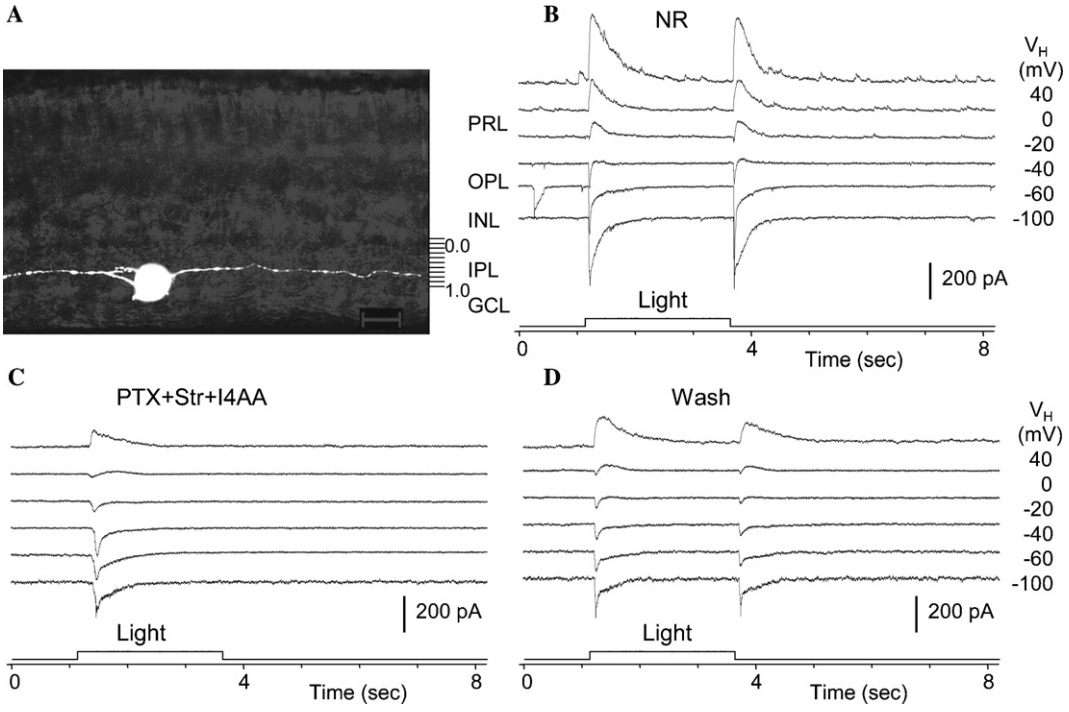

Fig. 3. (A) Stacked confocal fluorescent image of a type III ON–OFF ganglion cell in the retinal slice. PRL, photoreceptor layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer (0–1:0–100% of IPL depth); GCL, ganglion cell layer. Calibration bar, 20μm. (B) The light-evoked current responses of this cell to a 2.5 s light step (500 nm, −2) at six holding potentials in normal Ringer's, (C) in the presence of 100 μM picrotoxin + 1 μM strychnine + 10 μM I4AA (P + S + I), and (D) after wash.

that P + S + I suppresses the light-evoked inhibitory chloride current (ΔI_{Cl}) mediated by GABAergic and glycinergic amacrine cells. In contrast to type I cells, P + S + I completely blocked ON ΔI_C and reduced and prolonged OFF ΔI_C in all four type II cells. This suggests that the ON ΔI_C in these cells is not mediated directly by DBCs, but through the GABAergic/glycinergic amacrine cells, perhaps by a DBC→AC→HBC synaptic pathway (see Fig. 5 below).

3.3. OFF bipolar cell inputs to about 20% of ON–OFF ganglion cells (type III) can be suppressed by amacrine cell neurotransmitter blockers

In 14 of the 72 ON–OFF GCs (about 20%), P + S + I not only blocks ΔI_{Cl}, but also the OFF ΔI_C. These cells had dendrites that ramify in sublamina B (strata 6–10) of the IPL and exhibited transient ON and OFF responses, and we named them type III cells. Fig. 3A show the stacked confocal fluorescent image of a type III cell in the retinal slice, with dendrites stratified in stratum 7 of the IPL and an axon. The light-evoked current responses of this cell to a 2.5-s light step (500 nm, −2) at six holding potentials in normal Ringer's, in the presence of 100 μM picrotoxin + 1 μM strychnine +10 μM I4AA (P + S + I), and after wash are shown in Fig. 3B–D, respectively. Similar to the type I and type II cells, these cells also exhibit discrete sPSCs. The light-evoked currents in P + S + I reversed near 5 mV, consistent with the idea that P + S + I suppresses the light-evoked inhibitory chloride current (ΔI_{Cl}) mediated by GABAergic and glycinergic amacrine cells. In contrast to type I cells, P + S + I blocked OFF ΔI_C and slightly reduced ON ΔI_C in all 14 type III cells. This suggests that the OFF ΔI_C in these cells is not mediated directly by HBCs, but through the GABAergic/glycinergic amacrine cells, perhaps by a HBC → AC → DBC synaptic pathway (see Fig. 5 below).

3.4. Depolarizing and hyperpolarizing amacrine cells in the tiger salamander retina

Results described above indicate that the HBC inputs to about 20% of ON–OFF GCs and DBC inputs to about 5% of ON–OFF GCs in the salamander retina are not direct,

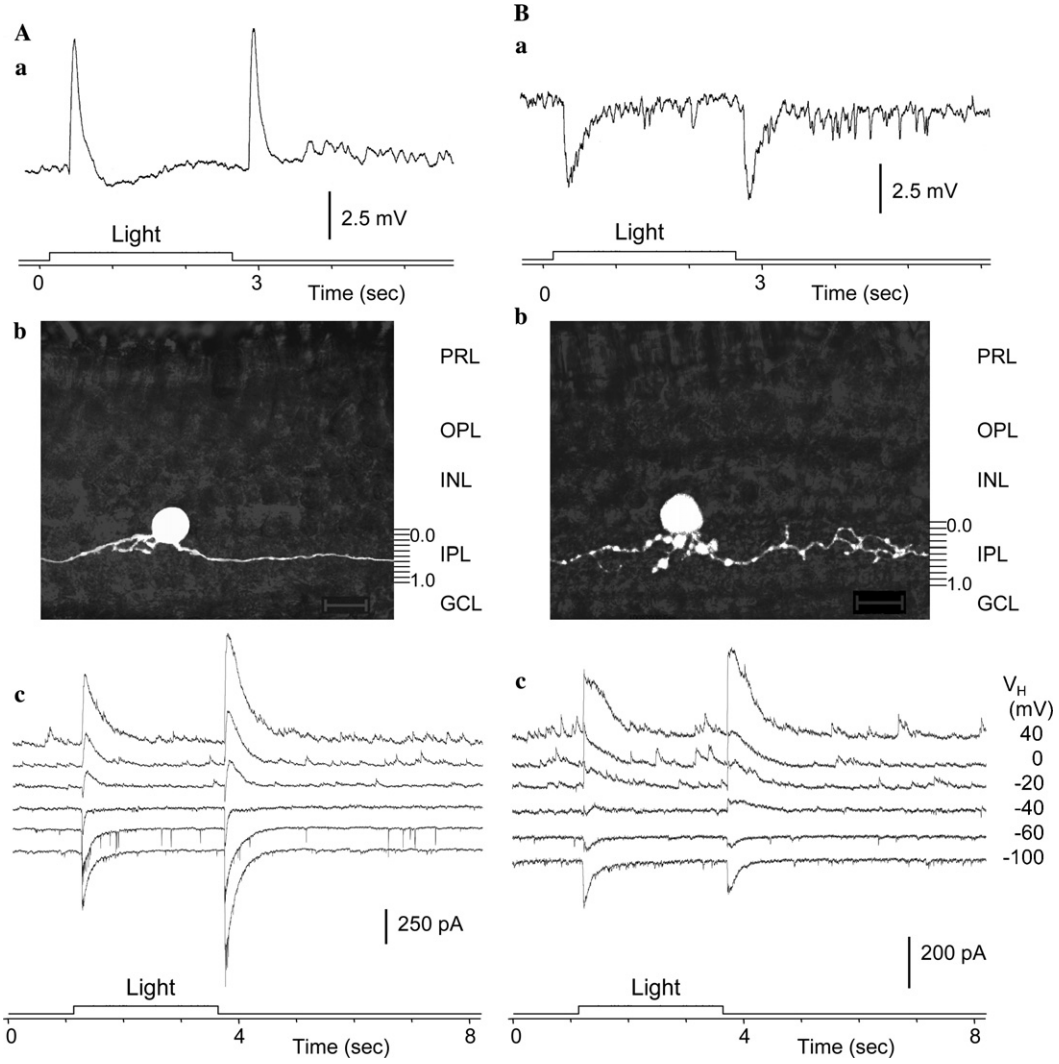

Fig. 4. (A) Depolarizing transient ON–OFF AC (AC_D). (a) Voltage response to a 2.5-s light step (500 nm, −2), dark membrane potential, −77 mV. (b) Stacked confocal fluorescent image in the retinal slice. PRL, photoreceptor layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer (0–1:0–100% of IPL depth); GCL, ganglion cell layer. Calibration bar, 20 μm. (c) The light-evoked current responses of this cell to a 2.5-s light step (500 nm, −2) at six holding potentials in normal Ringer's. (B) Hyperpolarizing transient ON–OFF AC (AC_H). (a) Voltage response to a 2.5-s light step (500 nm, −2), dark membrane potential, −41 mV. (b) Stacked confocal fluorescent image in the retinal slice. (c) The light-evoked current responses of this cell to a 2.5-s light step (500 nm, −2) at six holding potentials in normal Ringer's.

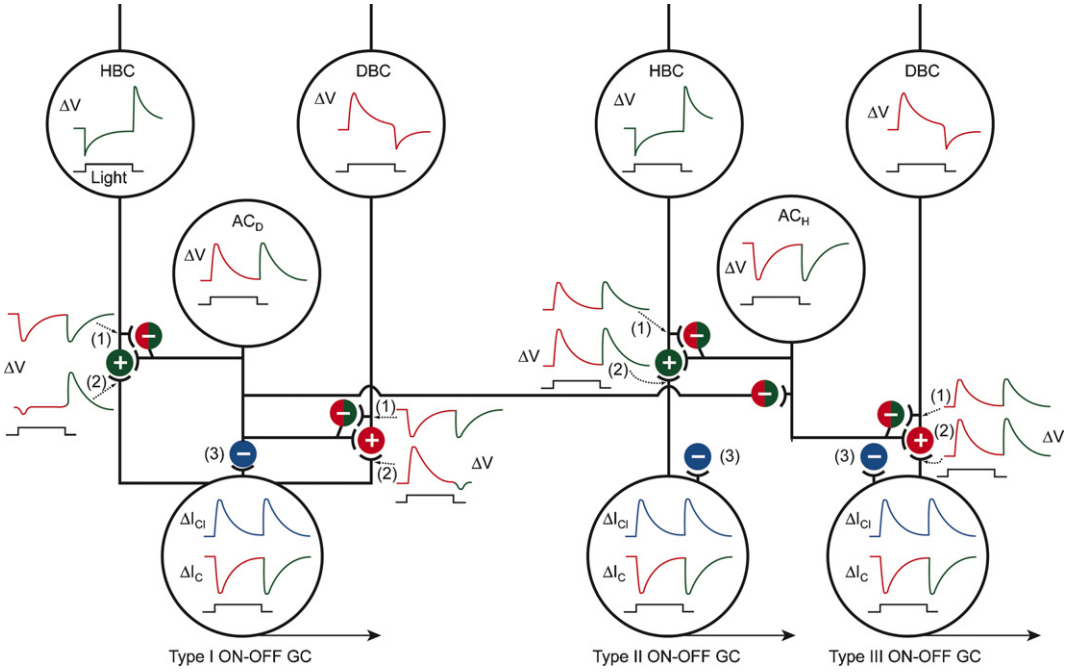

Fig. 5. Schematic diagram of synaptic pathways mediating light responses of type I (left portion), type II, and type III (right portion) ON–OFF ganglion cells. DBC, depolarizing bipolar cell; HBC, hyperpolarizing bipolar cell; AC_D, depolarizing transient amacrine cell; AC_H, hyperpolarizing transient amacrine cell; +, sign-preserving synapse; −, sign-inverting synapse. ΔV, light-evoked voltage responses in bipolar cells and amacrine cells. At each bipolar cell axon terminal region the postsynaptic voltage signal (2) is the sum of the bipolar cell soma signal and the amacrine → bipolar cell feedback signal (1). ΔI_C, light-evoked cation current (under voltage clamp) resulting from the postsynaptic signal (2) (or sum of postsynaptic signals (2) of HBC and DBC in type I cell); ΔI_{Cl}, light-evoked chloride current resulting from amacrine cells through GABAergic/glycinergic inhibitory synapses (3). Red, DBC (ON) pathway; green, HBC (OFF) pathway; blue, inhibitory synapses from amacrine cells to ganglion cells.

but mediated by amacine cells. The vast majority, about 80% of over 100 ACs we recorded from the tiger salamander retina are transient depolarizing ON–OFF cells with dark membrane potentials ranging from −70 mV to −87 mV (Yang, Gao, & Wu, 2002). Only about 10% of ACs give rise to sustained responses. The remaining 10% of the ACs have much more positive dark membrane potentials (near −40 mV) and these cells give transient *hyperpolarizing* responses at light onset and offset. Fig. 4Aa and Ba shows voltage responses of a depolarizing transient ON–OFF AC (AC_D) and a hyperpolarizing transient ON–OFF AC (AC_H) to a 2.5-s light step (500 nm −2), recorded with intracellular microelectrodes. The dark-membrane potentials of the two cells were −77 and −41 mV, respectively.

The stacked confocal fluorescent images of these two types of transient ACs in retinal slices are shown in Fig. 4Ab and Bb, and their current responses at six holding potentials recorded with whole-cell patch electrodes under voltage clamp conditions are shown in Fig. 4Ac and Bc. There are two major differences between AC_Ds and AC_Hs under voltage clamp. First, the light-evoked currents in the AC_D reversed between −30 and −10 mV whereas those in the AC_H reversed near −50 to −55 mV. This is because the light-induced cation current (ΔI_C recorded at $E_{Cl} = -60$ mV) in the AC_Hs is weaker than that in AC_Ds, and thus the reversal potential of the light responses in AC_Hs were closer to E_{Cl}. Second, the zero-current potentials of the AC_Ds were near −70 mV and those of the AC_Hs were near −40 mV, similar to the dark membrane potentials of the AC_D and AC_H recorded with intracellular microelectrodes shown in Fig. 4Aa and Ba.

3.5. Synaptic circuitry mediating type I, II, and III ON–OFF GCs

Results in Fig. 1 suggest that AC feedback signals to DBC and HBC axon terminals reduce and shorten ΔI_C in type I ON–OFF GCs (since P + S + I enhances and broadens ΔI_C). We therefore propose that ACs that feedback to type I ON–OFF GCs exhibit depolarizing light responses (similar to the cell in Fig. 4A). Light increases the release of GABA/glycine from these ACs, opens chloride channels in bipolar cell axon terminals, and causes a delayed hyperpolarization (dark membrane potentials of salamander bipolar cells are near −40 mV (Yang & Wu, 1997) and E_{Cl} is near −60 mV). The schematic diagram of bipolar cell and amacrine cell synaptic circuitry mediating type I GC responses is given on the left portion of Fig. 5.

On the other hand, we propose that ACs which make sign-inverting synapses on DBC and HBC axon terminals presynaptic to type II and type III ON–OFF GCs have hyperpolarizing light responses (similar to the AC_H in Fig. 4B). Light decreases the release of GABA/glycine from these ACs, closes chloride channels in bipolar cell axon terminals, and causes a depolarization that adds to the depolarizing ON (from DBCs) and OFF (from HBCs) signals.

The schematic diagram illustrating bipolar cell and amacrine cell synaptic inputs to type II and III ON–OFF GCs is given on the right portion of Fig. 5.

4. Discussion

Results presented in this article suggest that ON–OFF ganglion cells with dendrites ramifying in both sublamina A and B (type I cells) receive excitatory inputs (ΔI_C) directly from both HBCs and DBCs, those with dendrites ramifying in sublamina A (type II cells) receive excitatory inputs only from HBCs, and those with dendrites ramifying in sublamina B (type III cells) receive excitatory inputs only from DBCs. Type II ON–OFF GCs receive indirect DBC inputs via AC_Hs that make GABAergic/glycinergic synapses on HBC axon terminals, and type III ON–OFF GCs receive indirect HBC inputs through AC_Hs that make GABAergic/glycinergic synapses on DBC axon terminals (Lukasiewicz, Maple, & Werblin, 1994c; Maple & Wu, 1998) (Fig. 5). These synaptic arrangements were derived based on the following results. First, GABA and glycine receptor antagonists completely suppressed ON ΔI_C of type II ON–OFF GCs and OFF ΔI_C of type III ON–OFF GCs, suggesting that DBC inputs (ON ΔI_C) to type II cells and HBC inputs (OFF ΔI_C) to type III cells cannot be direct, as these compounds do not affect bipolar cell light responses (Hare & Owen, 1996), and bipolar cell output synapses to GCs are largely glutamatergic (although a small population of GABAergic bipolar cells has been identified in the salamander retina (Yang, Zhang, & Yazulla, 2003), they cannot gate cation current (ΔI_C)). Secondly, it has been shown that GABAergic/glycinergic ACs make sign-inverting chemical synapses on bipolar cell axon terminals (Lukasiewicz, Maple, & Werblin, 1994b), and P + S + I suppresses AC inputs to bipolar cells (Gao et al., 2000; Pang et al., 2002). Therefore it is reasonable to propose that AC_Hs (with hyperpolarizing light responses) generate the sign-inverting postsynaptic signals to HBC axon terminals presynaptic to type II ON–OFF GCs and in DBC axon terminals presynaptic to type III ON–OFF GCs. Since AC_H responses are ON–OFF, they add to the HBC OFF response and are solely responsible for the ON response in the HBC axon terminals presynaptic to the type II ON–OFF GCs. Hence P + S + I *reduces* the OFF ΔI_C and completely abolishes the ON ΔI_C in type II GCs. Similarly, AC_Hs add to the DBC ON response and are solely responsible for the OFF response in the DBC axon terminals presynaptic to the type III ON–OFF GCs, and thus P + S + I *reduces* the ON ΔI_C and completely abolishes the OFF ΔI_C in type III ON–OFF GCs. Thirdly, AC_Ds (with depolarizing light responses) result in the sign-inverting postsynaptic signals to DBC and HBC axon terminals presynaptic to type I ON–OFF GCs, and thus P + S + I *enhances* the ON and OFF ΔI_C in type I GCs. Moreover, AC_Ds contribute to the light-evoked chloride current (ΔI_{Cl}) in AC_Hs and GCs through sign-inverting GABAergic/glycinergic synapses. Finally, our data (Fig. 4) suggest that AC_Hs have several distinct properties: (1) their light responses are predominantly mediated by ΔI_{Cl} (presumably mediated by AC_Ds) while ΔI_C(from DBCs and HBCs) are weak, thus their light responses reverse between −50 and −55 mV; (2) their dark membrane potentials are nearly −40 mV instead of near −70 mV in AC_Ds, and thus they give transient ON–OFF *hyperpolarizing* responses; and (3) their dendrites ramify diffusely in strata 2–7 of the IPL, and thus they can make synaptic contacts with both type II and type III ON–OFF GCs.

Several previous studies have suggested cross-talk between DBC and HBC pathways in sustained ganglion cells (Arkin & Miller, 1988a, 1988b; Belgum, Dvorak, & McReynolds, 1982). Since sustained ganglion cells exhibited antagonistic surround responses, whereas ON–OFF ganglion cells do not (Werblin, 1972; Wunk & Werblin, 1979), it is likely that the cross-talks in the two classes of GCs are mediated by different mechanisms. In sustained GCs, GABA/glycine receptor antagonists only affect the inhibitory input but not the bipolar cell (center) input (Belgum, Dvorak, & McReynolds, 1984) whereas in the type II and III ON–OFF GCs they completely block not only ΔI_{Cl}, but also ΔI_C (ON response in Fig. 2C and OFF response in 3C), suggesting that the DBC inputs to type II and HBC input to type III ON–OFF GCs are indirect.

The synaptic arrangements for type II and III GCs (Fig. 5, right portion) illustrate AC_H-mediated indirect DBC and HBC inputs to ON–OFF ganglion cells in the tiger salamander retina. This suggests that amacine cell-mediated indirect bipolar-ganglion cell pathways exist in vertebrate retinas, besides the mammalian rod bipolar cell—AII amacrine cell-cone bipolar cell-ganglion cell synaptic circuitry. There are several differences between the two pathways. First, the salamander AC_Hs relay indirect inputs between ON and OFF (DBC and HBC) pathways whereas the mammalian AII ACs relay the rod pathway to the cone pathway. The second difference is that AII ACs exhibit depolarizing light responses and they send sign-preserving signals to cone DBCs via gap junctions and sign-inverting signals to cone HBCs via chemical synapses (Kolb, 1994), while the salamander AC_Hs exhibit hyperpolarizing responses that make sign-inverting GABergic/glycinergic chemical synapses on both DBCs and HBCs. Thirdly, the mammalian DBC_R-AIIAC pathway is unidirectional ($DBC_R \rightarrow DBC_C/HBC_C$) whereas the salamander AC_H pathway is bidirectional (DBC → HBC → OFF GC response in type II cells and HBC → DBC → ON GC response in type III cells). Despite these differences, the general principle of indirect bipolar cell input to ganglion cells through amacrine-bipolar cell-ganglion cell pathways hold in both salamander and mammalian retinas. It is also worth noting that a recent study has shown that ON responses are present in some ganglion cells in the mGluR6 (glutamate receptors mediating DBC light responses) knockout mice (David Copenhagen, personal communication). Additionally, ON → OFF cross inhibition has been observed in the guinea pig retina (Zaghloul, Boahen, & Demb, 2003). It is possible similar cross-talk between ON and OFF channels also exist in mammalian retinas.

Indirect bipolar cell-ganglion cell synaptic pathways in vertebrate retina allow more flexibility in signal transmission and modulation. If all bipolar cell inputs to GCs were direct, such as the type I ON–OFF GCs (Fig. 5, left portion), then all information channels would have similar properties and be modulated by similar regulators, as all bipolar cell (with the possible exception of small population of GABAergic bipolar cells (Yang et al., 2003)) use glutamate as their neurotransmitter (Marc, Liu, Kalloniatis, Raiguel, & Van Haesendonck, 1990; Wu & Maple, 1998). With indirect, amacrine cell-mediated bipolar cell-ganglion cell pathways, mammalian rod signals are selectively amplified by the AIIAC coupled network (Pang, Gao, & Wu, 2004), and the salamander ON or OFF channels in the type II and type III GCs can be selectively modulated by amacrine cells which use GABA/glycine as neurotransmitters and have much wider receptive fields that permit lateral signal regulations in the inner retina.

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