Visual Neuroscience (2011), **28**, 77–93.
Copyright © Cambridge University Press, 2010 0952-5238/10 $25.00
doi:10.1017/S0952523810000295

REVIEW ARTICLE

Bipolar cells in the zebrafish retina

V.P. CONNAUGHTON

Department of Biology, American University, Washington, District of Columbia

(Received June 1, 2010; Accepted September 1, 2010; First Published Online November 16, 2010)

Abstract

Zebrafish are an existing model for genetic and developmental studies due to their rapid external development and transparent embryos, which allow easy manipulation and observation of early developmental stages. The application of the zebrafish model to vision research has allowed for examination of retinal development and the characteristics of different retinal cell types, including bipolar cells. In particular, bipolar cell development, including differentiation, maturation, and gene expression, has been documented, as has physiological properties, such as voltage- and ligand-gated currents, and neurotransmitter receptor and ion channel expression. Mutant strains and transgenic lines have been used to document how bipolar cell connections and/or development may be altered, and toxicological studies examining how environmental factors may impact bipolar cell activity have been performed. The purpose of this paper was to review the existing literature on zebrafish bipolar cells, to provide a comprehensive overview of current information pertaining to this retinal cell type.

Keywords: Second-order neurons, Electrophysiology, Development, *Danio rerio*

Bipolar cell development and differential gene expression

Bipolar cells are the last neuronal cell type to differentiate in the zebrafish retina, forming at ~60 h postfertilization, hpf (~2.5 days postfertilization, dpf; Schmitt & Dowling, 1999). At this time, bipolar cells display typical morphology, with cell bodies in the inner nuclear layer (INL), axon terminals in the inner plexiform layer (IPL), and dendrites in the outer plexiform layer (OPL) (Schmitt & Dowling, 1999). By 74 hpf, mature ribbon synapses are observed in bipolar cell terminals, which are irregularly shaped and stratified within the IPL (Schmitt & Dowling, 1999; Biehlmaier et al., 2003). Recent work examining axon terminal development in ON-type bipolar cells labeled in transgenic *nyx::MYFP* zebrafish (Schroeter et al., 2006) showed that the irregular shape and stratification patterns observed ~3 dpf are due to the dynamic formation of axonal boutons (Fig. 1). Defined (adult-like) axon terminals are not present on bipolar cells at this age. Rather, filopodia extend from specific points along the proximal portion of the axon corresponding to the inner ON-sublamina in the IPL. The filopodia extend and retract laterally, a behavior similar to growth cone activity. As development continues, filopodia are lost and by 7–14 dpf, defined axon terminals are apparent (Schroeter et al., 2006).

Ultrastructural analysis of photoreceptor development suggests synaptic transmission between cones and second-order bipolar (and horizontal) cells is functional at 70–74 hpf (Schmitt & Dowling, 1999; Biehlmaier et al., 2003). Photoreceptor ribbons and their associated triad synapses formed from invaginating postsynaptic processes opposing the photoreceptor ribbons (i.e., Dowling & Werblin, 1969; Lasansky, 1973; Kolb & Jones, 1984) are first observed in the ventronasal patch at 65 hpf (Schmitt & Dowling, 1999), with subsequent development occurring throughout the retina by 74 hpf (Schmitt & Dowling, 1999; Biehlmaier et al., 2003). Probing cryostat sections with antibodies to synapse-associated proteins (SNAP-25, syntaxin 3, and SV2) identified immunopositive labeling beginning at ~2.5 dpf in ventral retina (Biehlmaier et al., 2003), consistent with the initial observations of synaptic ribbon formation. By 3–5 dpf, labeling for these synaptic proteins was observed across the entire retina (Biehlmaier et al., 2003). As noted above, bipolar cell development is also occurring at this time, and immunocytochemical data suggest differentiation of ON- and OFF-type bipolar cells may not occur simultaneously. Protein kinase C (PKC)-β_1 positive processes (characteristic of ON-type cells) are observed in the OPL at 2.5 dpf, with complete cell labeling observed at 4 dpf (Biehlmaier et al., 2003). In contrast, OFF-bipolar cell processes, identified by positive labeling for AMPA-type GluR4 glutamate receptors, are not observed until 3.5 dpf. These findings suggest that ON-type bipolar cells may develop first in zebrafish retina, though both ON and OFF cells are mature and evident by 7 dpf (Biehlmaier et al., 2003).

The differential expression of various genes, such as *chx10/vsx2* and *vsx1* (Passini et al., 1997; Clark et al., 2008; Vitorino et al., 2009), are important for bipolar cell differentiation, development, and maturation. Developmental expression patterns of *vsx1* and *vsx2* are complimentary (Passini et al., 1997). *In situ* hybridization identified strong initial expression of *vsx2*. By 31 hpf, *vsx1* transcripts are highly expressed in the presumptive INL region of the ventral patch, a pattern that coincides with downregulation of

Address correspondence and reprint requests to: V.P. Connaughton, Department of Biology, American University, 4400 Massachusetts Avenue, NW, Washington, DC 20016. E-mail: vconn@american.edu

https://doi.org/10.1017/S0952523810000295 Published online by Cambridge University Press

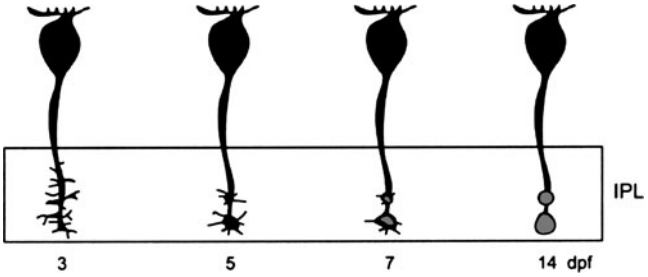


Fig. 1. Dynamic development of bipolar cell axon terminals. Summary diagram showing changes in bipolar cell axon terminals from 3 to 14 dpf. Bipolar terminals are initially observed in transgenic *nyx::MYFP* zebrafish as lateral extensions extending from the axon (3 dpf). As development proceeds, these extensions (filopodia) are replaced by defined axon terminals. By 14 dpf, adult-like axon terminals are apparent (Reprinted from Schroeter et al., 2006, *Visual Neuroscience* 23, 833–843).

vsx2. By 50 hpf, *vsx2* expression has been upregulated in the outer INL, where *vsx1* is also expressed (Passini et al., 1997). Using transgenic embryos, Vitorino et al. (2009) similarly observed *vsx2* expression across the entire retina at 24 hpf. With continued development, some cells upregulate *vsx2*, while others downregulate *vsx2* and express *vsx1*. By 96 hpf, however, all bipolar cells in double transgenic *vsx2:dsRed; vsx1:GFP* embryos express one of these genes, with most expressing *vsx1*. Consistent with strong initial expression of *vsx2* compared to *vsx1* (Passini et al., 1997), Vitorino et al. (2009) identified that bipolar cells expressing *vsx2* form from *vsx2*-positive precursor cells, while *vsx1*-positive cells develop from cells that initially expressed, but later downregulated, *vsx2*. Thus, bipolar cells in zebrafish originate from a single *vsx2*-expressing progenitor population with development of some subsequent cell types occurring only after downregulation of *vsx2* (Vitorino et al., 2009).

RIBEYE is a specific marker for synaptic ribbons (Schmitz et al., 2000). Two RIBEYE genes, *ribeye a* and *ribeye b*, each associated with a different RIBEYE protein, are expressed in zebrafish (Wan et al., 2005). Of these genes, *ribeye a* is localized to synaptic ribbons in both photoreceptor and bipolar cell terminals, while *ribeye b* expression is observed only in photoreceptor ribbons (Wan et al., 2005). Both genes are expressed beginning at 48 hpf (Wan et al., 2005). As *ribeye a* is the gene expressed in bipolar cells, Wan et al. (2005) used morpholinos to examine the specific function of this gene. They show that knockdown of *ribeye a* results in shorter photoreceptor ribbons, underdeveloped ribbons in bipolar cell terminals, a reduced number of PKC(+) bipolar cells, and a loss of optokinetic responses at 84 hpf (Wan et al., 2005). Since these changes in bipolar cells were observed after their initial differentiation, these researchers concluded that *ribeye a* is also important for later development and maturation of bipolar cells (Wan et al., 2005). Given that synaptic machinery is not apparent at the ultrastructural level until after bipolar cells have formed (Schmitt & Dowling, 1999) and that RIBEYE is involved in formation of synaptic ribbons (Magnupalli et al., 2008), it is logical that loss of RIBEYE would alter formation of synaptic structures but not alter initial bipolar cell differentiation. The fact that maturation of bipolar cells appears to be altered by knockdown of *ribeye a* suggests that synaptic activity may be important for final development of bipolar cells.

Expression and function of nyctalopin (nyx), a protein associated with congenital stationary night blindness (CSNB) in humans (Bech-Hansen et al., 2000), have been examined in mouse retina (i.e., Pesch et al., 2003). Mutant mice lacking *nyx* expression (= *no b-wave* or *nob* mice) have absent electroretinogram (ERG) b-waves (Pardue et al., 1998) and reduced visual sensitivity (Gregg et al., 2003), similar to humans with CSNB (Bech-Hansen et al., 2000; Khan et al., 2005). Nyctalopin expression is associated with mGluR6 glutamate receptors on the dendritic tips of ON-bipolar cells, and ON-bipolar cell glutamate responses are reduced in *nob* mice (Gregg et al., 2007). Taken together, these findings suggest that nyctalopin expression occurs postsynaptic to photoreceptor synapses and is required for proper functioning of ON-bipolar cells (Gregg et al., 2007). In zebrafish, nyctalopin is also expressed in a population of bipolar cells. Fluorescently labeled bipolar cells in the transgenic *nyx::MYFP* line are first observed in ventral retina at 2.5 dpf and across the entire retina at 4 dpf (Schroeter et al., 2006). At 5 dpf, nyx protein is identified in presumed ON-cone bipolar cell processes in both plexiform layers (Bahadori et al., 2006). Knockdown of *nyx* suppresses ERG b-wave activity in zebrafish (Bahadori et al., 2006), suggesting that nyctalopin plays a role in synaptic transmission between photoreceptors and ON-bipolar cells, similar to findings in mouse.

Other genes known to be important in eye and/or retinal development have also been identified in zebrafish bipolar cells. For example, *Zrx3* is a paired-class homeobox gene expressed in the outer INL at 41 hpf and throughout the adult INL (Chuang et al., 1999). Differential and overlapping expression of three *Lin7* genes (*Lin7a*, *Lin7b*, and *Lin7c*) is observed in the INL during development, and Lin7 immunoreactivity (IR) was observed in most/all bipolar cells in adult retina (Wei et al., 2006). *Pax6* expression has also been observed in presumed bipolar cells at 3–4 dpf (Lakowski et al., 2007).

Bipolar cell types, their physiological properties, and receptor expression patterns

Morphological types

In other cyprinid fish, such as goldfish (Sherry & Yazulla, 1993) and rudd (Scholes, 1975), numerous morphological types of bipolar cells have been identified based on overall morphology, including somal size and location in the INL, dendritic arborization, and axon terminal ramification patterns. Subsequent work using the gene gun DiOlistic technique (Gan et al., 2000) in zebrafish retinal slices (Connaughton et al., 2004) identified 17 morphological types of bipolar cells (Fig. 2). These cells were separated into presumed OFF types (denoted as B_{off} types; $n = 7$) with axon terminal(s) ramifying exclusively in sublamina a of the IPL, presumed ON types (B_{on} cells; $n = 6$) with terminal(s) ramifying exclusively in sublamina b, and multistratified cells (B_{off}–B_{on} cells; $n = 4$) with terminals in both sublaminae (Connaughton et al. 2004). A variety of bipolar cell types have also been identified in zebrafish based on neurochemical signature (Marc & Cameron, 2001).

In addition to morphological diversity, some types of bipolar cells can be selectively labeled with antibodies to various intracellular molecules. For example, labeling retinal cryostat sections with an antibody to either PKC-α (Caminos et al., 2000) or PKC-α/β (Yazulla & Studholme, 2001) selectively labels ON-bipolar cells with large axon terminals ramifying deep in the IPL. These are B_{on}-s6L cells in zebrafish (Connaughton et al., 2004), which are morphologically similar to goldfish Mb-type bipolar cells. PKC(+) bipolar cells are also observed in chicken, rat, and tench (another teleost) (Caminos et al., 2000). Interestingly, though PKC (+) cells with large terminals, such as Mb-type cells, are found in all three fish species (goldfish, tench, and zebrafish), the somata and

https://doi.org/10.1017/S0952523810000295 Published online by Cambridge University Press

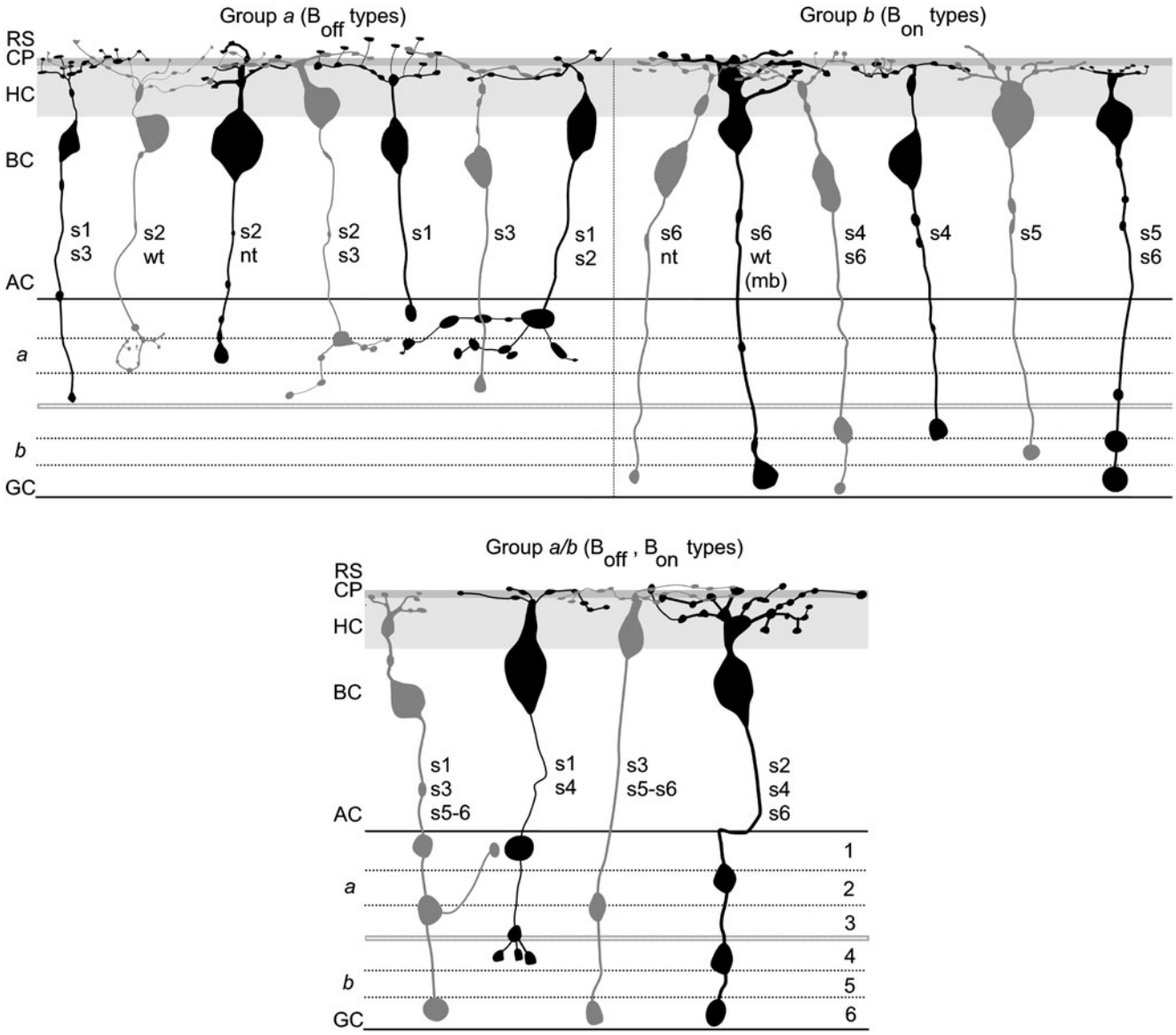


Fig. 2. Seventeen morphological types of bipolar cells have been identified in adult zebrafish retina. Bipolar cells, labeled using the DiOlistic technique in zebrafish retinal slices, were classified primarily by axon terminal ramification patterns in the IPL. Cells with the morphology of OFF bipolars (Group a, B_{off} cells) had axon terminals restricted to sublamina a of the IPL. Presumed ON-type (Group b; B_{on} cells) cells had axon terminals restricted to sublamina b of the IPL. Multistratified (Group a/b; B_{off}–B_{on} cells) cells had axon terminals in both sublaminae. In each panel, the IPL is separated into six sublayers (1–6), shown at the right. RS, rod spherule layer; CP, cone pedicle layer; HC, horizontal cell layer; BC, bipolar cell layer; AC, amacrine cell layer; GC, ganglion cell layer (Reprinted from Connaughton et al., 2004, *The Journal of Comparative Neurology* 477, 371–385).

terminals of these cells are smallest in zebrafish (Caminos et al., 2000). The large size of goldfish Mb terminals has allowed them to be directly examined physiologically (i.e., Heidelberger & Matthews, 1991, 1992; Matthews et al., 1994; Burrone & Lagnado, 1997; Matthews, 1999; Hull & Von Gersdorff, 2004; Vigh & von Gersdorff, 2005; Hull et al., 2006; Palmer, 2006; Jones & Palmer, 2009); such experiments have not yet been performed in zebrafish. Smaller ON-type bipolar cells were also labeled with the PKC-α/β antibody (Yazulla & Studholme, 2001). More recently, two transgenic zebrafish lines, *xfz3* and *xfz43*, that express eGFP (enhanced GFP) in some bipolar cell types have been generated (Zhao et al., 2009). Bipolar cells labeled in the *xfz3* line were identified as multistratified B_{off}-s1/s3/s5 cells and B_{on}-s3/s5 cells, whereas the eGFP-expressing cells in the *xfz43* line were B_{off}-s2/s3 and B_{on}-s4 bipolars (Zhao et al., 2009). The ability to develop transgenic zebrafish lines expressing fluorescent markers in populations of bipolar cells, such as *xfz43* and *zfx3* (Zhao et al., 2009), *nyx::MYFP* (Schroeter et al., 2006), and *vsx2:dsRed; vsx1:GFP* (Vitorino et al., 2009) described above, provides a powerful tool to examine function, morphology, and response of these cells in normal conditions as well as during perturbations, development, and/or disease.

Glutamate

In all vertebrate retinas examined to date, immunocytochemical studies have identified glutamate-positive photoreceptor, bipolar,

https://doi.org/10.1017/S0952523810000295 Published online by Cambridge University Press

and ganglion cells, suggesting that these cells release glutamate as their neurotransmitter. This labeling pattern has been confirmed in zebrafish (Connaughton et al., 1999; Marc & Cameron, 2001), and studies have examined glutamate-evoked responses and glutamate receptor expression on bipolar cell processes (Table 1).

Using whole-cell patch-clamp techniques in zebrafish retinal slices, glutamate-gated currents were recorded from bipolar cells following focal application of L-glutamate (1 mM) onto the dendritic arbor (Connaughton & Nelson, 2000). Results from this work identified four glutamate-evoked responses in zebrafish bipolar cells. OFF-type bipolar cells with axon terminals ramifying exclusively in IPL sublamina a responded to glutamate application with a conductance increase and a reversal potential (E_{rev}) ~0 mV (Fig. 3). A similar response was elicited following the application of the ionotropic glutamate agonist kainate, consistent with OFF-bipolar cell responses reported in other species. Glutamate- and kainate-gated currents were also recorded from multistratified bipolar cells in zebrafish with terminals in both sublaminae a and b (Connaughton & Nelson, 2000) suggesting that OFF-bipolar cell axon terminals are not restricted to the distal IPL. ON-bipolar cells with terminals ramifying exclusively in sublamina b responded to glutamate application with one of three possible conductance mechanisms: (a) a conductance decreases with an E_{rev} ~0 mV (Figs. 3 and 4) that could also be elicited using the ON-bipolar–selective metabotropic glutamate receptor agonist APB (L-(+)-2-amino-4-phosphonobutyric acid or LAP4); (b) a conductance increases with an $E_{rev} \sim E_{Cl}$ (Fig. 4) characterized as I_{glu}, the glutamate receptor with transporter-like pharmacology associated with a chloride conductance (i.e., Grant & Dowling, 1995, 1996); or (c) a current that was outward going at all holding potentials tested (−60 to +20 mV) with no apparent reversal potential resulting from the activation of both the APB-sensitive current and the I_{glu} (Connaughton & Nelson, 2000). These findings for zebrafish ON-type bipolar cells are similar to responses recorded in white bass (Grant & Dowling, 1995, 1996). Of these three mechanisms, the majority (~60%) of recorded zebrafish bipolar cells expressed the glutamate-gated chloride conductance; APB responses were present in only 4% of recorded ON-type cells (Connaughton & Nelson, 2000). APB-elicited currents were identified in bipolar cells with axon terminals ramifying deep in the IPL [i.e., B_{on}-s6L (Mb-type) cells], while I_{glu} was identified in bipolar cells with smaller, or multiple, synaptic boutons. Multistratified (Group a/b) ON-type bipolars in zebrafish expressed I_{glu} exclusively and have axon terminals located proximally in both sublaminae (Connaughton & Nelson, 2000). Expression of different glutamate conductance mechanisms on teleost ON-bipolar cells is suggested to underlie different photoreceptor inputs to these cells (Grant & Dowling, 1995, 1996; Wong, Cohen, et al., 2005; Wong & Dowling, 2005, and see below).

Light responses have not been recorded from zebrafish bipolar cells. However, light responses from bipolar cells in the closely related giant danio (Wong, Cohen, et al., 2005; Wong & Dowling, 2005) show results that are similar to the above described glutamate responses as multiple glutamate conductance mechanisms were found to underlie ON-bipolar light responses in this species (Wong, Cohen, et al., 2005; Wong & Dowling, 2005). Whole-cell patch-clamp recordings of light- and D-aspartate–evoked responses in giant danio ON-cone (Cb) bipolar cells were mediated by chloride currents through the activation of TBOA-sensitive glutamate transporters (EAAT or excitatory amino acid transporters). In contrast, mixed (Mb-type) ON-type bipolar cells were found to coexpress EAAT and LAP4-sensitive metabotropic receptors (mGluR6) (Wong, Cohen, et al., 2005). Light-evoked responses recorded from these Mb cells confirmed that both conductance mechanisms are present and, as noted above, that each mechanism underlies different photoreceptor inputs: mGluR6 mediates rod-driven responses, while both EAAT and mGluR6 mediate cone-driven responses, with most cone inputs activating EAATs (Wong, Cohen, et al., 2005). Giant danio multistratified (Cab) bipolar cells have axon terminals in both IPL sublaminae (i.e., Group a/b cells of Connaughton & Nelson, 2000) and coexpress OFF-type AMPA/kainate receptors and ON-type EAAT receptors on their dendrites (Wong & Dowling, 2005). In these cells, the polarity of response depends on the ratio/number of each receptor type expressed. ON–OFF responses, resulting from differential inputs to these receptors from different cone types, were also observed in some Cab cells (Wong & Dowling, 2005). Similarly, multistratified salamander bipolar cells, with terminals in both sublaminae, are able to generate both ON- and OFF-light responses (Wu et al., 2000) due to the expression of both metabotropic and ionotropic (AMPA/kainate) receptors on the dendrites (Pang et al., 2004). Coactivation of both the receptor types in giant danio also results in color-opponent responses in Cab cells (Wong & Dowling, 2005).

The above glutamate- (zebrafish) and light-evoked (giant danio) responses were recorded in adult animals. Similar mechanisms are also present in larval retinas. Patch-clamp recordings of bipolar cells in retinal slices from 8 dpf zebrafish larvae (Fig. 3) have identified kainate- and APB-evoked glutamate conductance mechanisms, as well as adult-like depolarization-elicited potassium currents (Connaughton, 2001). Recent molecular work has identified the expression of *eaat7* in zebrafish bipolar cells using *in situ* hybridization (Kastenhuber et al., 2010) and mGluR6 is found in the larval OPL (Huag et al., 2010). These findings indicate that glutamatergic synaptic transmission present in adult retinas is also apparent and functional in zebrafish larvae.

Most physiological data of zebrafish retinal activity has been recorded using ERGs. ERGs have been documented using intact larvae and adults (Grant & Dowling, 1996; Hughes et al., 1998; Bilotta et al., 2001; Patterson et al., 2002; Saszik et al., 2002; Seeliger et al., 2002; Ren & Li, 2004; Vitorino et al., 2009), isolated larval eyes (Wong et al., 2004), and adult eyecup preparations (Wong, Adolph, et al., 2005; Nelson & Singla, 2009). ERGs reflect whole-retina responses to light, and each component of the ERG is attributed to the response of a specific retinal cell type(s). For example, zebrafish OFF-bipolar cell responses mediated by AMPA/kainate-type glutamate receptors are represented by the ERG d-wave (Wong et al., 2004; Wong, Adolph, et al., 2005), as also reported in frog (Xu & Karwoski, 1995), though a component of the a-wave is also attributed to OFF-bipolar cell signals (Nelson & Singla, 2009). In contrast, the b-wave, which reflects the overall activity of ON-bipolar cells (i.e., Stockton & Slaughter, 1989), is more complex in zebrafish as it involves multiple conductance mechanisms, consistent with data from patch-clamp recordings. In particular, the glutamate transporter antagonist TBOA blocks most of the b-wave response in larval zebrafish (and giant danio) ERGs, while LAP4 application results in only a slight reduction in amplitude (Wong et al., 2004; Wong, Adolph, et al., 2005), suggesting that glutamate transporters mediate the majority of the b-wave response.

ERGs have also been used to identify different photoreceptor inputs to bipolar cells, and both rod and cone contributions to the b-wave have been identified (Ren & Li, 2004; Wong, Adolph, et al., 2005). As discussed above, cone contributions are mediated by two glutamate receptor mechanisms, while rod inputs to the

https://doi.org/10.1017/S0952523810000295 Published online by Cambridge University Press

https://doi.org/10.1017/S0952523810000295 Published online by Cambridge University Press

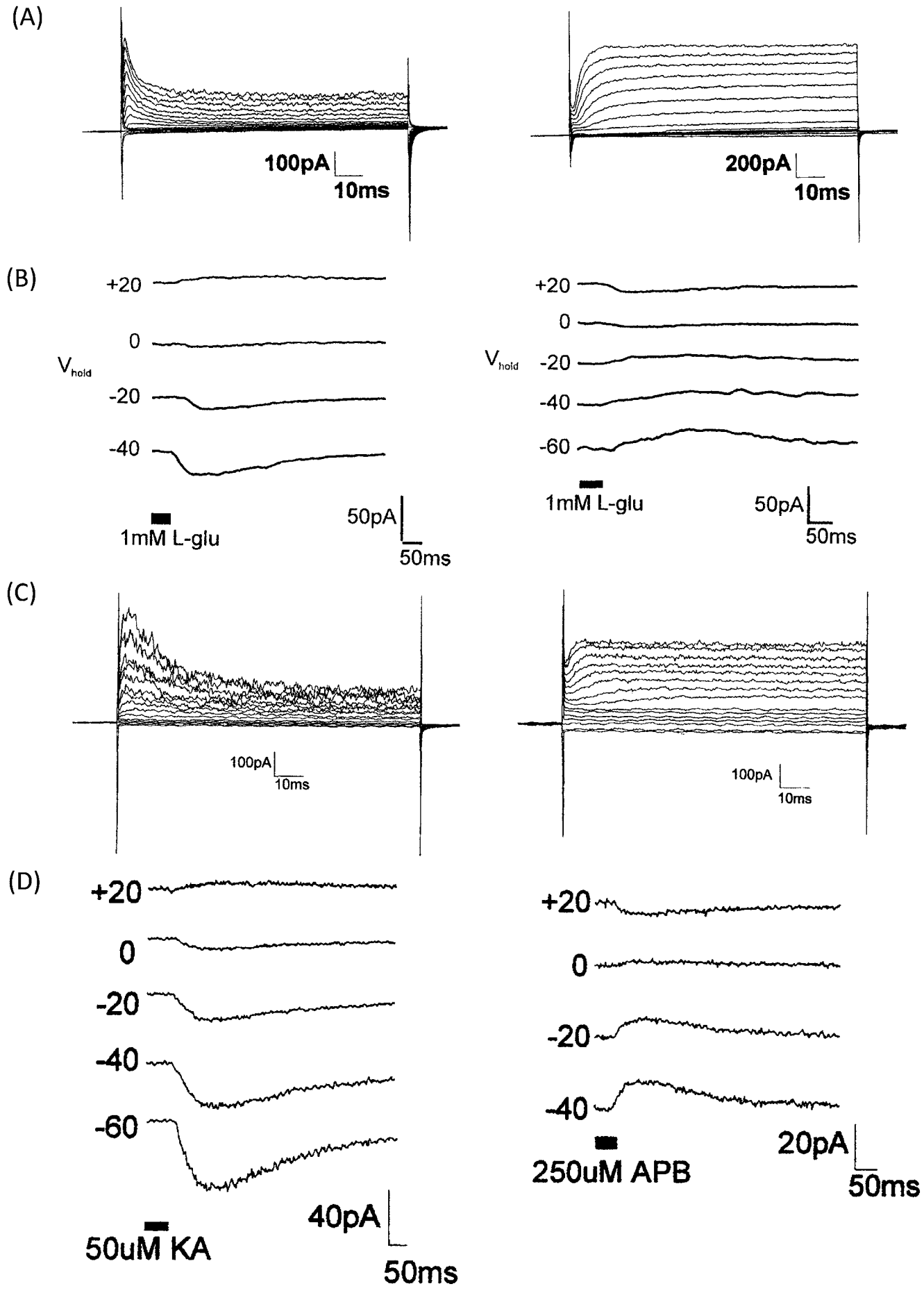
Table 1. *Physiological and immunocytochemical characteristics of zebrafish bipolar cells*

	Glutamate		GABA		Glycine		Dopamine		Voltage-gated currents	
	-IR patterns	Membrane response	-IR patterns	Membrane response	-IR pattern	Membrane response	-IR patterns	Membrane response	-IR patterns	Membrane response
ON cells	Glutamate-IR observed in all cells[a,b]; NR2B-(+) dendrites in subtype of cone contacting ON-bipolar cell[c]; mGluR1α(+) (Mb) cell body, dendrites[c,d]	3 responses[e]: Increase Cl^- conductance (I_{glu}); decreased conductance (APB-type receptor); outward current (coactivation of I_{glu} and APB receptors)	Two classes of bipolar cells (BC1 and BC6) contain GABA[b];	Increased Cl^- conductance[f]; $GABA_A$ = bicuculline sensitive, picrotoxin sensitive[f]; $GABA_C$ = TPMPA insensitive, Zn^{+2} sensitive, some picrotoxin sensitivity[f];	—	Glycine application increases membrane conductance in ~30% of all cells[f]	D1-type receptors (D1R) on large (Mb) terminals[d]	Decrease amplitude of delayed rectifying K^+ current (I_K) and change activation time constant; involved D1-type receptors[h]	Some ON-bipolar cells display PanNa-IR[d]; Kv1.1 on presumed ON-cells[d]	In retinal slices, some cells express I_A/I_{sust}, others express I_K/I_h; All cells express I_{Ca}; in most ON-cells I_{Ca} is sustained (L-type) $I_{K(Ca)}$ is present[i]; in isolated cells, both I_A and I_K were recorded[h]
OFF cells	GluR4(+) dendrites contacting rods and cones[c,d]; GluR4 colocalizes with PSD95 on OFF-bipolar dendrites[d]; Glutamate-IR observed in all cells[a,b]	Increase conductance (AMPA/KA)[e]	$GABA_{A\alpha3}R$-IR (Mb cells, OFF cells)[d]	5% (OFF) cells depolarized by GABA[g]	—		—	—	All OFF-bipolar cells are PanNa(+)[d]	In retinal slices, some cells express I_A/I_{sust}, others express I_K/I_h[i]; all cells express I_{Ca}; $I_{K(Ca)}$ also observed[i]

Membrane responses to glutamate, GABA, glycine, and dopamine recorded from zebrafish bipolar cells are summarized, as are -IR patterns associated with these ligands. Similar data are also presented for voltage-gated current responses. Most published information pertains to ON or OFF cells in general, though information related to a specific subtype of bipolar cell is indicated, if known.—indicates no data has been published.

References cited within the table are noted by superscripts.

[a]Connaughton et al. (1999).
[b]Marc and Cameron (2001).
[c]Klooster et al. (2009).
[d]Yazulla and Studholme (2001).
[e]Connaughton and Nelson (2000).
[f]Connaughton et al. (2008).
[g]unpublished observations.
[h]Yu and Li (2005).
[i]Connaughton and Maguire (1998).



https://doi.org/10.1017/S0952523810000295 Published online by Cambridge University Press

b-wave are only mediated by metabotropic receptors (Wong, Adolph, et al., 2005). More specifically, ERGs have been used to identify the contributions of different cone types (i.e., red, green, blue, UV) to ON-bipolar cell responses (i.e., Hughes et al., 1998; Saszik et al., 2002; Nelson & Singla, 2009). For example, in their recent analysis of the photopic ERG in adult zebrafish, Nelson and Singla (2009) identified that cone inputs to the two subelements of b-wave (PII) vary: the PIIm component generated by metabotropic (APB-sensitive) responses receives inputs predominantly from blue and UV cones, while cone inputs to the nonmetabotropic (presumed transporter) PIInm component are spectrally broad, consistent with more uniform inputs from different cone types. Similarly, Saszik et al. (2002) reported that the b-wave in adult zebrafish was differentially sensitive to APB, with APB application reducing responses to blue and UV, but not longer, stimulating wavelengths. Taken together, these results indicate that zebrafish ON-bipolar cells receive inputs from spectrally different cones and that these inputs are mediated by the activation of different types and/or combinations of glutamate receptors. Furthermore, spectral sensitivity functions, determined from ERG recordings (Hughes et al., 1998; Patterson et al., 2002; Saszik et al., 2002), identify color-opponent and nonopponent mechanisms that contribute to the b-wave response. The short wavelength portion of the spectral sensitivity function was attributed to nonopponent UV and blue cone inputs. In contrast, spectral sensitivity functions at longer (middle to long) wavelengths result from opponent/inhibitory mechanisms between red (L) and green (M) or between green (M) and blue (S) cones (Hughes et al., 1998). Clearly, circuitry within the distal zebrafish retina is complex and includes both distinct pathways for each cone type as well as interaction/integration among different inputs to bipolar cells (i.e., Nelson & Singla, 2009).

In agreement with the above physiological data, a recent morphological study (Li & Dowling, 2010) identified different spectral classes of bipolar cells by comparing their dendritic spread and morphology to the photoreceptor mosaic in transgenic SWS1-GFP fish. These authors report that some bipolar cells are connected to several different cone types (i.e., red, green, blue or green, blue, and UV), while others have more selective contacts, such as green and UV cone connections. Their results also indicate that some zebrafish bipolar cells are connected to both rods and cones, while others exclusively contact cones (Li & Dowling, 2010).

Immunocytochemical studies have localized glutamate receptor subtypes on bipolar dendrites. GluR4-IR is found on zebrafish OFF-bipolar cell processes, and mGluR1α-IR is localized to the dendrites of large ON-type bipolar cells (Yazulla & Studholme, 2001; Klooster et al., 2009), findings that are consistent with glutamate-elicited current responses generated in these cells (Connaughton & Nelson, 2000). These findings are also consistent with IR patterns found in goldfish, as some ON-type bipolar cells, including Mb cells, display mGluR1α-IR (Yazulla et al., 2001; Joselevitch et al., 2007), and GluR4-IR was identified on presumed OFF-bipolar processes in the OPL (Vandenbranden et al., 2000). Catfish bipolar cells are also labeled with antibodies to metabotropic glutamate receptors (Gafka et al., 1999). Interestingly, immunoreactive puncta labeling the NR2B subunit of NMDA receptors (but not NR1; Yazulla & Studholme, 2001) were also identified on ON-bipolar dendrites in zebrafish (Klooster et al., 2009), though NMDA-evoked responses were not recorded from these cells (Connaughton & Nelson, 2000).

GABA and glycine

Inhibitory GABAergic and glycinergic processes are found in both plexiform layers in the zebrafish retina (Connaughton et al., 1999; Marc & Cameron, 2001; Yazulla & Studholme, 2001). These processes are associated with horizontal (GABA), amacrine (GABA, glycine), and/or interplexiform (glycine) cells, as reported in other species (Marc & Lam, 1981; Kalloniatis & Marc, 1990; Yazulla & Studholme, 1990; Sherry et al., 1993). Within both plexiform layers, GABA labeling is more prominent. GABA-IR is found throughout the IPL, except for an immunonegative band in mid-IPL that labels positively with an antibody to glycine (Connaughton et al., 1999). Furthermore, $GABA_A$ α-3 receptor-IR is observed on PKC(+) ON-type and all OFF-type bipolar cells (Yazulla & Studholme, 2001), suggesting that these cells receive GABAergic inputs. Immunoreactivity for the $GABA_C$ receptor ρ-subunit was observed in the outer INL and as puncta throughout the IPL. Glycine receptor-IR was more prominent in the zebrafish IPL than the OPL (Yazulla & Studholme, 2001), consistent with IR patterns in goldfish where glycine receptors were identified on both Ma and Mb cell terminals (Yazulla & Studholme, 1991).

GABA- and glycine-gated currents (Fig. 5) have been identified using voltage-probe responses in isolated cells and whole-cell patch-clamp techniques in zebrafish retinal slices (Connaughton et al., 2008). Interestingly, focal application of GABA onto bipolar cell dendrites in retinal slices did not evoke a response, though isolated axotomized cells responded robustly to GABA application, a discrepancy presumably due to the loss of dendritic processes during retinal slice preparation (Connaughton et al., 2008). Consequently, retinal slices were used to characterize GABA-evoked responses from bipolar axon terminals, while dendritic responses were better examined in isolated cells. GABA-gated currents have also been reported in bipolar cells in a variety of other species, including salamander (Lukasiewicz & Shields, 1998), skate (Qian et al., 1997, 2001), hybrid bass (Qian & Dowling, 1995), rat (Euler & Wassle, 1998), carp (Han et al., 1997), and goldfish (Tachibana & Kaneko, 1987). Overall, GABA-evoked currents in zebrafish (Table 1) have

Fig. 3. Voltage- and glutamate-gated currents are present in bipolar cells in adult and larval retina. Whole-cell voltage clamp recordings of adult (**A** and **B**) and larval (**C** and **D**) bipolar cells. (**A**) Depolarizing voltage steps elicited two different outward potassium currents: a rapidly activating and inactivating I_A current (left) and a delayed rectifying I_K current (right). Expression of these depolarization-elicited outward currents allowed adult bipolar cells to be separated into two populations (Reprinted from Connaughton & Maguire, 1998, *The European Journal of Neuroscience* 10, 1350–1362). These currents were also identified in bipolar cells recorded from 8 dpf retinal slices. (**C**) ON- and OFF-type bipolar cells were identified by their glutamate-evoked responses. In adult bipolar cells (**B**), OFF-bipolar cells responded to glutamate application with a conductance increase and a reversal potential ~0 mV (left). This response was also elicited by the agonist kainate (not shown). Some ON-bipolar cells expressed an APB-sensitive metabotropic response to glutamate application characterized by a conductance decrease (right) and a reversal potential ~0 mV (Reprinted from Connaughton & Nelson, 2000, *Journal of Physiology* 524, 135–146). (**D**) OFF-bipolar kainate responses and ON-bipolar APB responses were also recorded from bipolar cells in larval retinas (**C** and **D** reprinted from *Progress in Brain Research*, Vol. 131, Connaughton, V.P. Organization of ON- and OFF-pathways in the zebrafish retina: Neurotransmitter localization, electrophysiological responses of bipolar cells, and patterns of axon terminal stratification, 161–176, 2006, with permission from Elsevier.)

https://doi.org/10.1017/S0952523810000295 Published online by Cambridge University Press

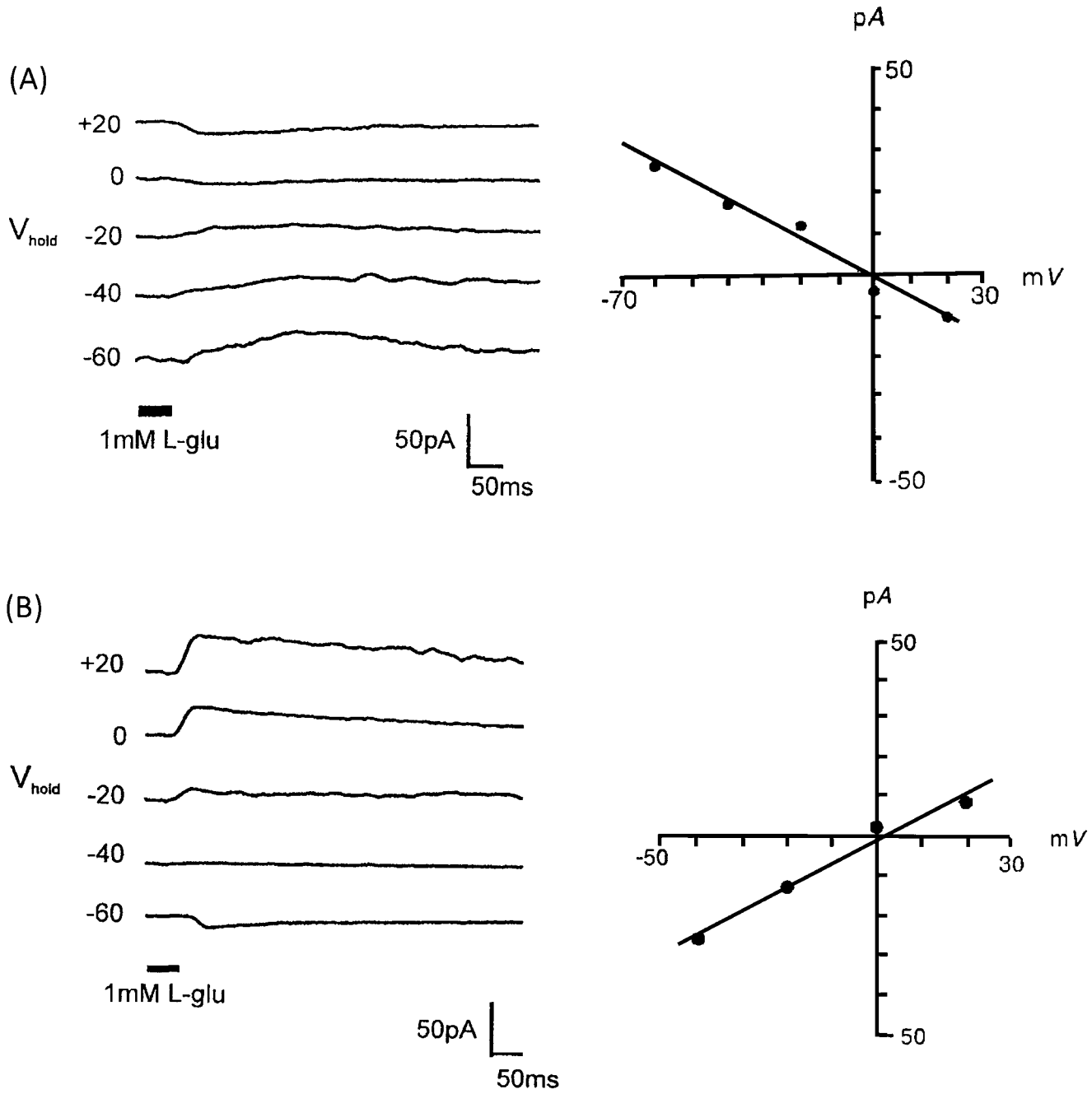


Fig. 4. ON-bipolar cells express two glutamate-gated conductance mechanisms. Whole-cell patch-clamp recordings of zebrafish bipolar cells in retinal slices identified two glutamate responses in ON-type bipolar cells. (**A**) In 4% of cells, glutamate application elicited a conductance decrease with a reversal potential ~0 mV. This response was also elicited by focal application of 2-amino-4-phosphonobutyric acid (APB or LAP4) onto the dendritic arbor, indicating that it is mediated by metabotropic glutamate receptors. (**B**) Most ON-cells (60%), however, responded to glutamate application with I_{glu}; the glutamate-gated chloride conductance mediated through a receptor with transporter-like pharmacology. Morphologically, recorded cells had axon terminals restricted to sublamina b (APB and I_{glu} responses); some recorded cells had terminals in both sublaminae (I_{glu}). For (**A**) and (**B**), each set of current traces (left) is accompanied by the corresponding current–voltage relationship. Taken together, these findings indicate that both glutamate transporters and metabotropic receptors are present on ON-type bipolar cells (Reprinted from Connaughton & Nelson, 2000, *Journal of Physiology* 524, 135–146).

some general characteristics similar to those reported in these species, though differences were also observed.

In retinal slices, focal application of GABA onto bipolar cell terminals elicited a current in both ON- and OFF-type monostratified and multistratified cells. Similarly, in isolated cells, GABA responses were recorded from ON cells hyperpolarized by glutamate and OFF cells depolarized by glutamate. In all cases, GABA-elicited responses were associated with a chloride conductance (Connaughton et al., 2008). GABA responses in zebrafish bipolar cells were partially blocked by the $GABA_A$ receptor antagonist bicuculline and sensitive to (though not always completely blocked by) the mixed $GABA_{A/C}$ antagonist picrotoxin

https://doi.org/10.1017/S0952523810000295 Published online by Cambridge University Press

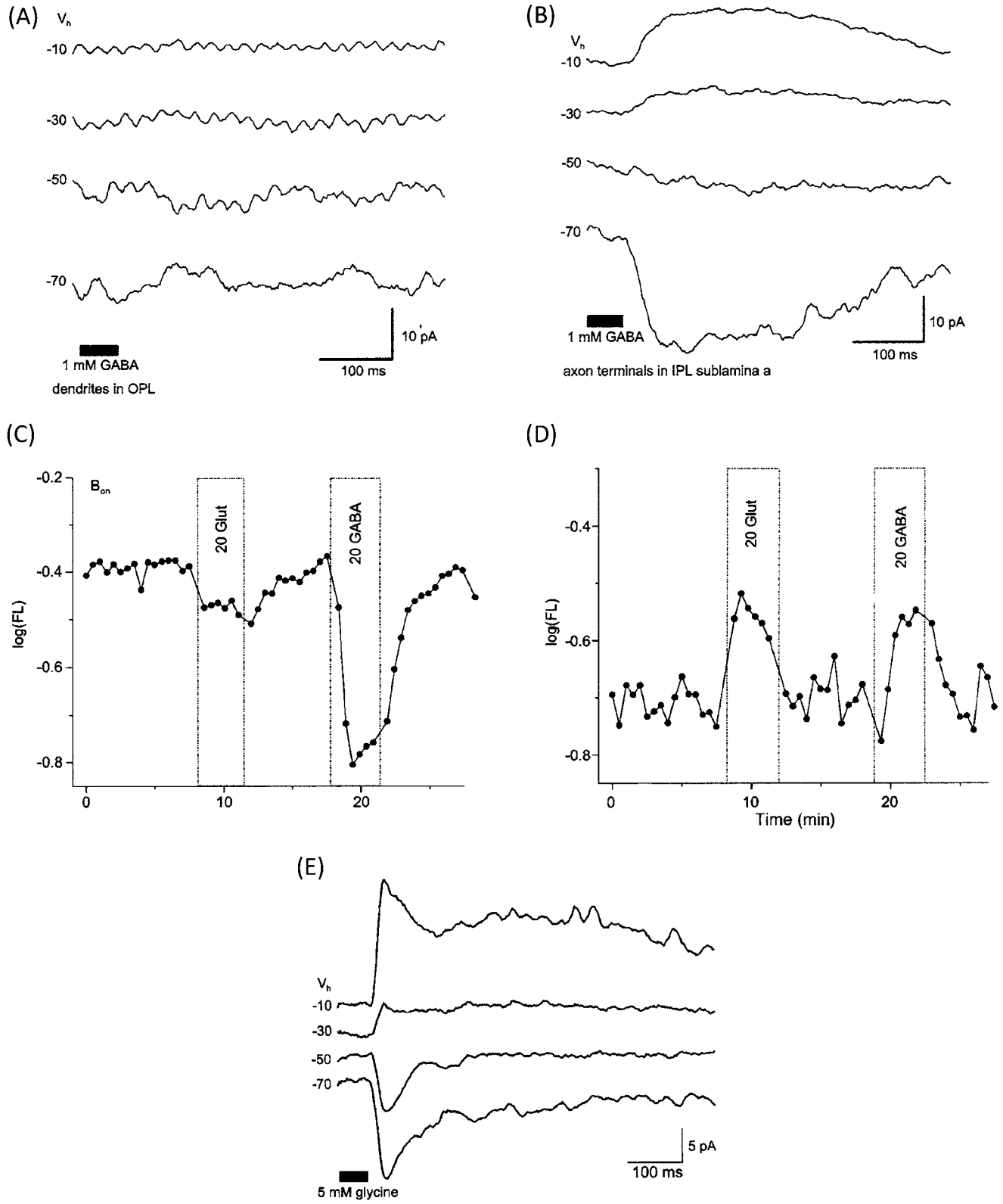


Fig. 5. Inhibitory GABA- and glycine-evoked currents in zebrafish bipolar cells. Focal application of 1 mM GABA onto bipolar cell dendrites (OPL) in retinal slices did not elicit a response (**A**); however, focal application onto axon terminals (IPL) evoked robust currents (**B**) with a negative reversal potential (between −30 and −50 mV) that was carried by chloride ions. GABA-evoked chloride currents were recorded from both ON- (terminals in sublamina b) and OFF- (terminals in sublamina a) cells in retinal slices. (**C** and **D**) GABA-elicited responses were also observed in voltage-probe recordings of isolated cells. In (**C**), an ON-bipolar cell hyperpolarized by glutamate (measured as a decrease in membrane fluorescence, log FL, following glutamate application, 20 Glut) was also hyperpolarized by GABA (20 GABA). OFF-type bipolar cells that increased membrane fluorescence during glutamate application (= depolarized by glutamate, **D**)

https://doi.org/10.1017/S0952523810000295 Published online by Cambridge University Press

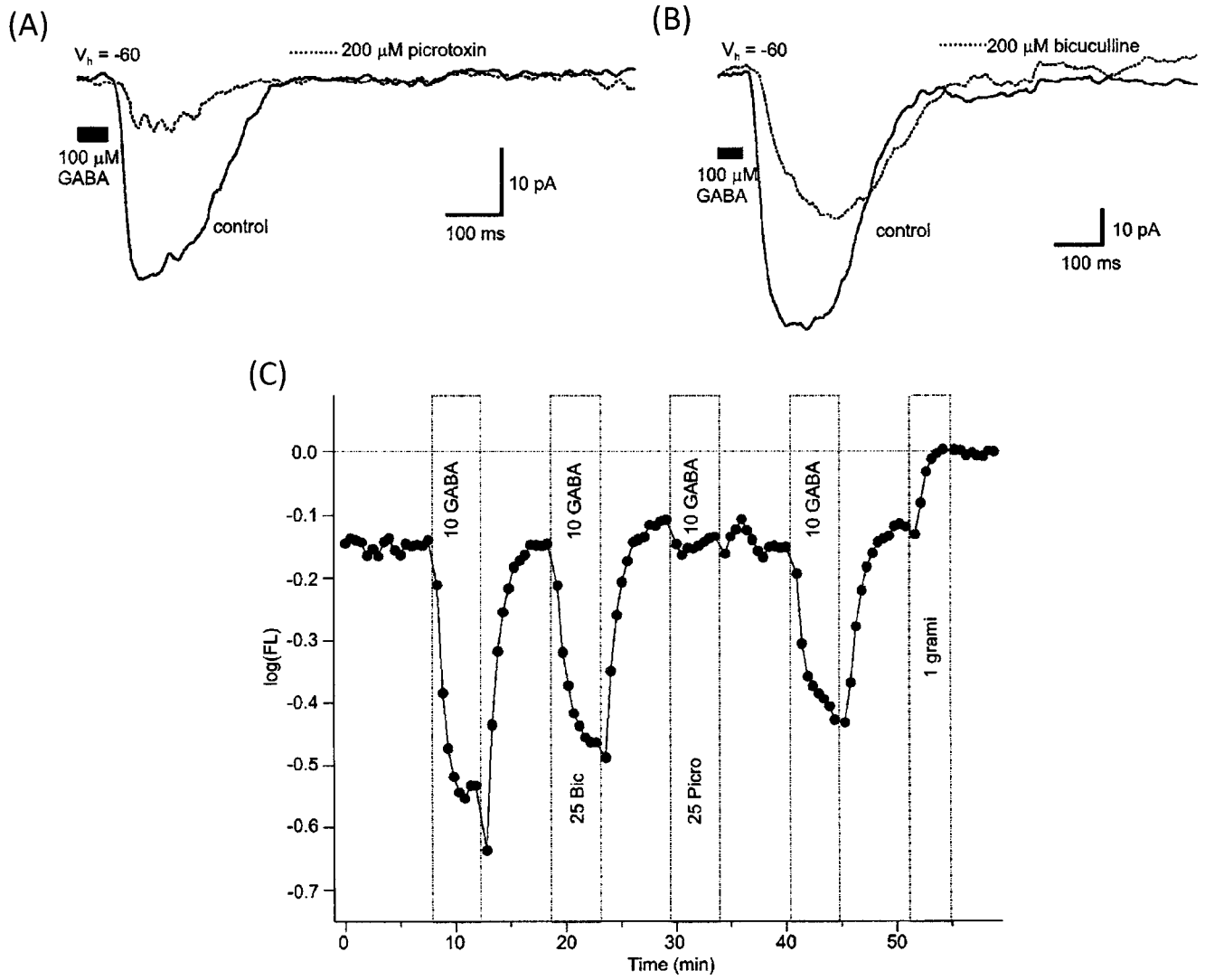


Fig. 6. $GABA_A$- and $GABA_C$-type receptors are expressed on bipolar cell processes. GABA-gated currents in zebrafish bipolar cells recorded in retinal slices (**A** and **B**) were partially blocked by picrotoxin (200 μM, **A**) and bicuculline (200 μM, **B**). Isolated bipolar cells recorded with the voltage-probe oxonol were also partially sensitive to bicuculline (25 Bic/10 GABA), while picrotoxin significantly reduced the GABA-evoked hyperpolarization (25 Picro/10 GABA). For (**A**) and (**B**), GABA responses were recorded at a holding potential (V_H) of −60 mV; black bar indicates duration of GABA application. For (**C**), log FL represents the background-subtracted voltage probe fluorescence. Decreases in fluorescence correspond to membrane hyperpolarizations. Concentrations are in μM (Reprinted from Connaughton et al., 2008, *Visual Neuroscience* 25, 139–153. Reprinted with permission).

(Fig. 6), indicating that zebrafish bipolar cells express both $GABA_A$ and $GABA_C$ type receptors. Furthermore, $GABA_C$ receptors on isolated zebrafish bipolar cells are antagonized by zinc, as reported in skate (Qian et al., 1997), hybrid bass (Qian & Dowling, 1995), and mouse (Kaneda et al., 2000) retinas, but insensitive to (1,2,5,6-tetra hydropyridine-4-yl) methyl phosphinic acid (TPMPA). TPMPA insensitivity was surprising as published reports have shown this compound to be a selective antagonist for $GABA_C$-type receptors (Ragozzino et al., 1996) and effective at blocking $GABA_C$ responses in retinal bipolar cells in both fish (Hull et al., 2006; Palmer, 2006; Jones & Palmer, 2009) and mammals (McGillem et al., 2000; Matsui et al., 2001). Overall, these observed pharmacological responses suggest that $GABA_C$ receptors expressed on zebrafish bipolar cells may have a different subunit composition than other species. The distribution of $GABA_A$- and $GABA_C$-type receptors varied between bipolar cell processes: dendrites were found to be picrotoxin sensitive and bicuculline insensitive, while axon terminals were both picrotoxin and bicuculline sensitive. Thus,

were also hyperpolarized by GABA, though the cell shown in (**D**) responded to GABA application with an increase in membrane fluorescence (= depolarized by GABA). (**E**) Focal application of glycine (5 mM) onto bipolar cell dendrites or axon terminals elicited a response in ~30% of recorded cells. Similar results were obtained in isolated cells (not shown). For (**A**), (**B**), and (**E**) holding potentials (V_H) are given to the left of each trace; the black bar at the bottom indicates duration of drug application. For (**C**) and (**D**), log FL (fluorescence) represents the background-subtracted voltage probe fluorescence (Reprinted from Connaughton et al., 2008, *Visual Neuroscience* 25, 139–153. Reprinted with permission).

https://doi.org/10.1017/S0952523810000295 Published online by Cambridge University Press

$GABA_C$-type receptors predominate on dendrites, and a combination of $GABA_A$- and $GABA_C$-type receptors are expressed on axon terminals (Connaughton et al., 2008). In other species, GABA receptors on bipolar cells mediate lateral inhibitory inputs, particularly from amacrine cells. For example, the large Mb axon terminals of goldfish also coexpress $GABA_A$- and $GABA_C$-type receptors (Vigh & von Gersdorff, 2005; Palmer, 2006; Jones & Palmer, 2009). In this species, $GABA_C$ receptors, working with GAT-1 GABA transporters, mediate the tonic inhibitory current recorded from terminals (Hull et al., 2006; Jones & Palmer, 2009), while $GABA_A$ receptors (and $GABA_C$ receptors) are stimulated by evoked release of GABA from amacrine cells (Vigh & von Gersdorff, 2005; Jones & Palmer, 2009). Glycine-elicited responses in zebrafish (Fig. 5) were recorded in ~30% of bipolar cells following exogenous application of glycine to either the dendritic arbor or the axon terminal (Connaughton et al., 2008). Differential sensitivity to glycine has also been reported in skate retina (Qian et al., 2001). Thus, zebrafish bipolar cells receive inhibitory glycinergic and GABAergic inputs. In contrast to glycine-evoked responses, all cells examined responded to GABA, indicating that the majority of inhibitory inputs to these cells originates from GABAergic neurons (Connaughton et al., 2008).

Though GABA hyperpolarized most bipolar cells, a minority of isolated cells (~5%) were depolarized by GABA (Nelson et al., 2008). GABA-elicited depolarizations were only observed in OFF-bipolar cells (unpublished observations). This depolarization was blocked by Li^+ substitution for Na^+ in the external media, suggesting that it is transporter mediated. GABA-evoked transporter currents have also been reported in some salamander bipolar cells (Yang, 1998), and ~13% (8 of 60) of isolated skate bipolar cells are depolarized by GABA (Lasater et al., 1984). GABA-elicited depolarization in zebrafish bipolar cells (Fig. 5D) was surprising, as GABA-positive bipolar cells were not previously observed in vibratome sections that were probed with an antibody to either GABA or GAD (Connaughton et al., 1999; Yazulla & Studholme, 2001). However, neurochemical analysis identified a subset of presumed bipolar cells that are GABA positive (Marc & Cameron, 2001), and punctate labeling with an antibody to the GABA transporter GAT-2 was identified in the mid- to distal-INL (Yazulla & Studholme, 2001), suggesting that some bipolar cells in the zebrafish retina may contain GABA and release it as their neurotransmitter.

Dopamine

Teleost retinas, including zebrafish, contain a single dopamine-positive cell type, the dopaminergic interplexiform cell, which has processes in both the IPL and the OPL (Kalloniatis & Marc, 1990; Yazulla & Studholme, 2001). Immunoreactivity for D1-type dopamine receptors has been identified on the terminals of some ON-bipolar cells in zebrafish (Yazulla & Studholme, 2001) and PKC(+) ON bipolars in goldfish (Mora-Ferrer et al., 1999), suggesting that these cells receive dopaminergic inputs. To that end, delayed rectifying I_K potassium currents in isolated zebrafish bipolar cells were decreased (Fig. 7; Table 1) following dopamine application (Yu & Li, 2005). This response was mimicked by applying the D1 receptor agonist SKF38393 and by activating components of the intracellular G-protein cascade in these cells (Yu & Li, 2005). These responses are similar to those found in goldfish, where application of either dopamine or ascorbate modulates I_K through the activation of D1-type dopamine receptors (Fan & Yazulla, 1999*a*). In contrast, chronic application of dopamine in the bath solution enhanced outward potassium currents in zebrafish retinal slices (Fig. 7), with activation of both D1- and D2-type dopamine receptors involved in the response (Connaughton & Warndorf, 2005). Exogenous dopamine application also modulates glutamate responses in isolated zebrafish bipolar cells (Asare et al., 2005).

Voltage-gated currents

In addition to ligand-gated responses, voltage-gated currents have been identified in zebrafish bipolar cells (Connaughton & Maguire, 1998). Recorded in retinal slices, these cells were found to differentially express voltage-gated potassium and calcium currents (Table 1). In particular, one population of bipolar cell expresses the delayed rectifying outward I_K current in response to membrane depolarizations and the slowly activating I_h-type current in response to membrane hyperpolarizations. These currents are similar to those reported in isolated goldfish bipolar cells (Kaneko & Tachibana, 1985), Mb-type cells in retinal slices (Fan & Yazulla, 1999*b*), and small bipolar cells in white bass (Lasater, 1988). The other population of zebrafish bipolar cells expresses a rapidly activating and inactivating I_A-type current when depolarized and a sustained inward current in response to membrane hyperpolarization (Connaughton & Maguire, 1998). I_A has also been recorded in the terminals of goldfish ON-bipolar cells after blockage of the calcium-dependent potassium current (Burrone & Lagnado, 1997) and in large bipolar cells in white bass (Lasater, 1988). These currents were carried by potassium ions, as indicated by their sensitivity to cesium substitution for potassium in the patch pipette and/or sensitivity to external application of the potassium channel blocker tetraethyl ammonium (TEA). When potassium channels were blocked, a depolarization-elicited inward calcium current was identified in all zebrafish bipolar cells. This inward current had characteristics of transient (T-type) and/or sustained (L-type) calcium currents, with response properties again varying among recorded cells. A calcium-dependent potassium current ($I_{K(Ca)}$) (Connaughton & Maguire, 1998) was also recorded in response to depolarizing voltage steps. Goldfish ON-bipolar cells also express $I_{K(Ca)}$ and L-type calcium channels (Burrone & Lagnado, 1997). In contrast, a transient calcium current has been identified in mouse (Kaneko et al., 1989), and both types of voltage-gated calcium currents were observed on rat rod bipolar cells (Pan, 2001).

Examination of recorded cells filled with Lucifer Yellow (1% solution, present in the patch pipette) indicated that some presumed ON-cells express I_K and others express I_A. A similar result was also found in cells with the morphology of OFF-type bipolar cells. In other words, the above two populations of bipolar cells (separated by expression of either I_K or I_A; Fig. 3) did not correspond to ON- and OFF-cell types in zebrafish. Kv1.1-IR (= I_K) is observed only on some ON-type bipolar cells in zebrafish (Yazulla & Studholme, 2001) consistent with physiological responses identifying I_K on a subset of ON cells. Kv1.4-IR (= I_A) is observed in the zebrafish OPL (Yazulla & Studholme, 2001). OFF- and some ON-type bipolars in zebrafish were also positively labeled with the PanNa sodium channel antibody (Yazulla & Studholme, 2001), though physiological recordings did not identify voltage-gated sodium currents on these cells (Connaughton & Maguire, 1998). In goldfish, PKC(+) bipolar cells display Kv1.1-IR (Yazulla & Studholme, 1998, 2001) and Kv1.4-IR colocalizes with GluR4-IR on OFF-bipolar dendrites (Yazulla & Studholme, 1998, 2001). Physiologically, voltage-gated sodium currents (I_{Na}) are not present on goldfish Mb cells (Fan & Yazulla, 1999*a*); however, I_{Na} has been recorded from ON-bipolar cells with small terminals (i.e., cone bipolar cells; Zenisek et al., 2001), and PanNaCh-IR identified voltage-gated sodium channels on most OFF- and half of the ON-type bipolar cells in goldfish (Zenisek et al., 2001). Calcium action

https://doi.org/10.1017/S0952523810000295 Published online by Cambridge University Press

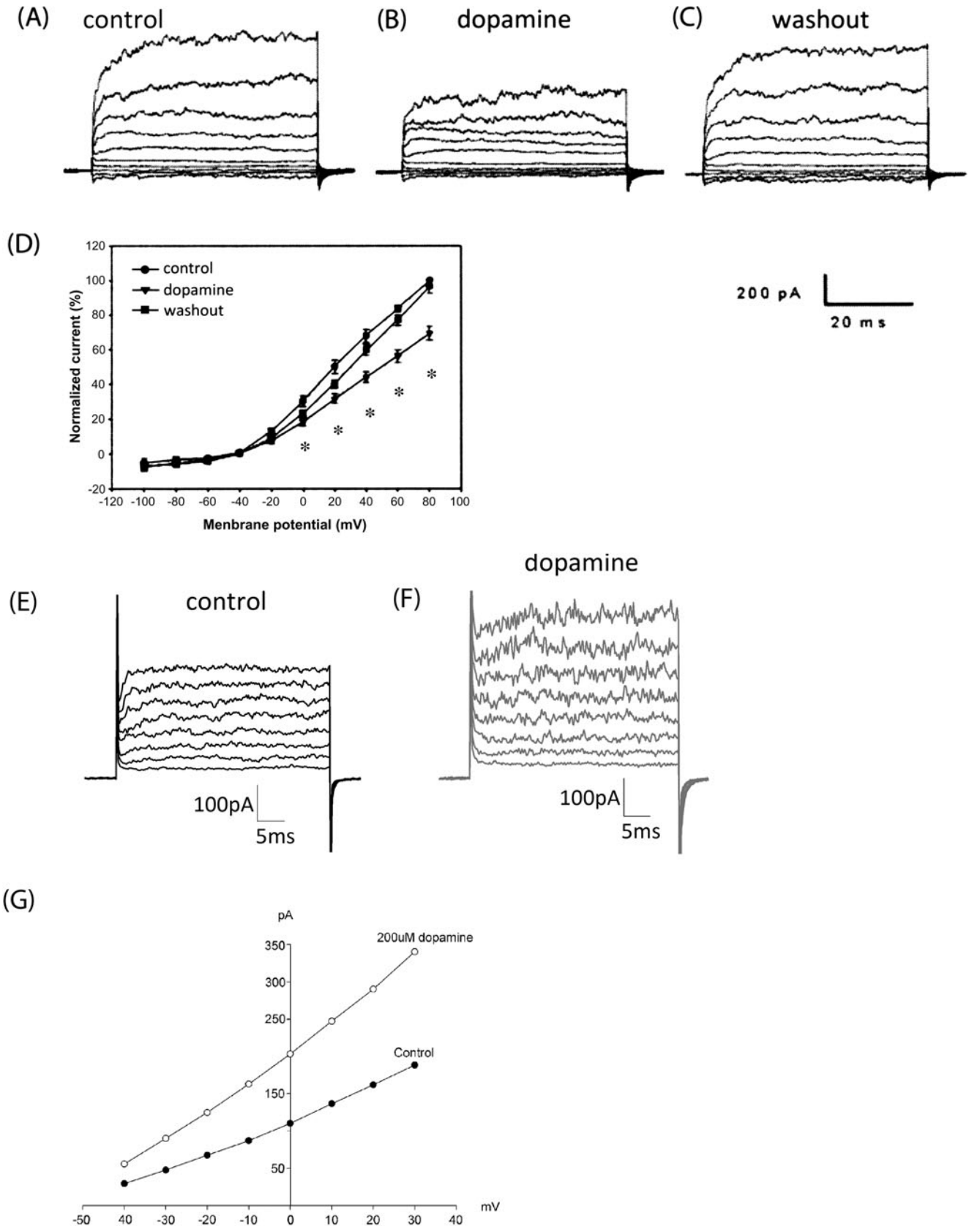


Fig. 7. Dopamine alters the amplitude of delayed rectifying potassium currents. Whole-cell patch recordings of delayed rectifying (I_K) currents in isolated zebrafish bipolar cells recorded (**A**) before/control, (**B**) during, and (**C**) after/washout of application of 50 μM dopamine show dopamine application decreases I_K. (**D**) Summary of current–voltage relationship. In retinal slices, however, chronic application of 200 μM dopamine in the bath solution enhanced I_K amplitude (**F**) compared to currents recorded in control/standard extracellular solution (**E**). (**G**) A representative current–voltage relationship for the effect of dopamine in retinal slices. (**A**–**D** reprinted from Yu & Li, 2005, *Journal of Neuroscience Research* 82, 368–376).

https://doi.org/10.1017/S0952523810000295 Published online by Cambridge University Press

potentials have also been recorded from the larger axon terminals in goldfish (Protti et al., 2000).

Genetic approaches allow the application of developmental and physiological data

A major strength of the zebrafish model is the ease with which mutant and transgenic animals can be generated (reviewed in Amsterdam & Becker, 2005) and the applicability of these animals to analysis of retinal development, function, or disease. Mutants are identified in large-scale behavioral screens (i.e., Brockerhoff et al., 1995; Neuhauss et al., 1999), while regions of interest in transgenic animals are detected by viewing fluorescently (green fluorescent protein and yellow fluorescent protein) labeled cells or structures (i.e., Schroeter et al., 2006; Zhao et al., 2009). These techniques have been applied to zebrafish retina, and some of the studies that impact retinal bipolar cells are highlighted below.

Mutant strains

Chemical mutagenesis, using ethyl nitrosourea (ENU) exposure, followed by third-generation screens (Mullins et al., 1994; Solnica-Krezel et al., 1994; Brockerhoff et al., 1995, 1997; Fadool et al., 1997) has identified zebrafish mutants with visual defects localized to the retina. Initial screening typically involves a behavioral assay, such as optokinetic response (OKR; Brockerhoff et al., 1995) or optomotor response (Neuhauss et al., 1999). Subsequent physiological (ERG), histological, and/or molecular analyses are employed to identify the specific mechanism(s) underlying the mutation. To date, there are no published reports describing a mutation that specifically targets zebrafish retinal bipolar cells. However, mutations to presynaptic photoreceptors and postsynaptic ganglion cells have been described, and these mutations have been shown to affect bipolar cell morphology and/or physiology.

For example, zebrafish *lakritz* (*lak*) mutants lack ganglion cells (Kay et al., 2001). At 7dpf, larval mutants displayed a ~2× increase in the number of PKC(+) ON-bipolar cells compared to wild-type animals and disrupted bipolar cell axon terminal ramification patterns in the IPL (Kay et al., 2001). The IPL, in general, is disorganized and formation is delayed (Kay et al., 2004). Interestingly, disrupted stratification of bipolar terminals was specific to ON cells, as patterns in the distal (OFF) IPL appeared normal, and was dependent on amacrine cell processes, as stratification of bipolar terminals was similar in wild-type and *lak* mutants in regions, where amacrine cell processes were not disrupted. Thus, amacrine cell processes are required for accurate ramification of ON-bipolar terminals (Kay et al., 2004). Disrupted retinal lamination patterns in *young* mutants, in contrast, decreases bipolar cell differentiation, as indicated by reduced *vsx2* expression and PKC-IR patterns (Link et al., 2000). Though degeneration of all cone types characterizes the phenotype of *pde6c* mutants, bipolar cell morphology is also unusual, with altered somal position in the INL and atypical process morphology in both plexiform layers (Stearns et al., 2007).

Mutants initially characterized by an abnormal OKR include *noa* (no optokinetic response a; Brockerhoff et al., 1995), *pob* (partial optokinetic response b; Brockerhoff et al., 1997), and *nrc* (no optokinetic response c; Allwardt et al., 2001). These mutants also display abnormal ERG responses characterized by altered b-waves (Brockerhoff et al., 1995; Allwardt et al., 2001) and d-waves (Allwardt et al., 2001), suggesting that ON- and/or OFF-bipolar cell activity may be altered. Interestingly, most of these mutations are localized to photoreceptors, suggesting that the abnormal ERG b- and d-waves are due to a change in synaptic transmission between photoreceptors and bipolar cells. In *nrc*, for example, photoreceptor terminals are altered structurally: invaginating processes are reduced, ribbons are misplaced and untethered, and the number of basal contacts is increased compared to wild-type animals (Allwardt et al., 2001). Molecular studies have identified *nrc* as a mutation in the *synj1* (*synaptojanin 1*) gene; Synj1 protein was not detected in *nrc* mutants, and knockdown of *synj1* mimicked the altered photoreceptor terminal morphology observed in *nrc* (Van Epps et al., 2004). In addition to the abnormal ON-bipolar b-wave, ON responses in ganglion cells are also altered in *nrc* mutants, indicating that the entire ON pathway is affected (Emran et al., 2007). *Noa* mutants have a mutation in the *dlat* (*dihydrolipoamide* s-*acetlytransferase*) gene, the protein product of which forms a subunit in the enzyme pyruvate dehydrogenase (PDH; Taylor et al., 2004). The PDH enzyme complex converts pyruvate, formed during glycolysis, to acetyl-CoA, which then enters the Krebs cycle during the aerobic production of ATP (Campbell et al., 2008). Thus, in addition to abnormal retinal ERG responses, this mutant also displays characteristics of a pyruvate dehydrogenase deficiency, as mutants could be rescued with a ketogenic diet, resulting in ERG and OKR responses comparable to wild-type animals (Taylor et al., 2004). The partial optokinetic response observed in *pob* mutants is due to the selective degeneration of red cones (Brockerhoff et al., 1997). Interestingly, pob protein is localized to inner segments and synapses of all photoreceptors, not just red cones (Taylor et al., 2005).

Altered ERG b-waves have also been observed in other mutants, including *bug eye* (Stujenske et al., 2010), *noir* and *dropje* (Neuhauss et al., 1999), and zvm7 larvae (Brockerhoff et al., 2010). However, these have not been characterized as extensively as those described above.

Transgenic lines

Transgenic zebrafish lines are developed by injecting DNA (plasmid or transposon containing GFP or other fluorescent marker) into one-cell stage embryos. Embryos are screened, raised to adulthood, and mated. Some of these adults will become founders consistently producing fluorescent transgenic embryos once the DNA is incorporated into the germ line (reviewed in Amsterdam & Becker, 2005; Kawakami, 2005; Higashijima, 2008). Though a wealth of transgenic lines have been developed in zebrafish, six have been used to examine retinal bipolar cells.

As described above, development and maturation of bipolar cells have been examined in the *nyx::MYFP* transgenic line (Schroeter et al., 2006) and in double transgenic *vsx2:dsRed; vsx1:GFP* embryos (Vitorino et al., 2009). Transgenic XOPS-mCFP zebrafish displays degeneration of rod photoreceptors (Morris et al., 2005). Double transgenic fish formed by mating fish from this line with *nyx::MYFP* fish or labeling XOPS-mCFP retinas with an antibody to PKCα allowed changes in ON-bipolar cell morphology to be observed following loss of rod photoreceptors (Saade & Fadool, 2010).

Specific bipolar cell types are labeled in *xfz3* and *xfz43* transgenic lines (Zhao et al., 2009), as already noted. However, these two lines have also been used to examine selective ablation and regeneration of bipolar cells. To do this, adults from both transgenic lines were mated to *UAS:nsfB-mCherry* transgenic fish (Zhao et al., 2009). The NTR-mCherry line expresses nitroreductase, a compound that becomes toxic to cells following application and conversion of metronidazole (Curado et al., 2008). Thus, bipolar cells coexpressing eGFP and mCherry could be selectively ablated by applying metronidazole to the retina. This was, in fact, observed

https://doi.org/10.1017/S0952523810000295 Published online by Cambridge University Press

in bipolar cells within the *xfz43* line. Furthermore, new eGFP-positive bipolar cells were formed within 7 days of metronidazole removal (Zhao et al., 2009). These findings indicate that zebrafish bipolar cells are capable of regeneration in larval retinas and that transgenic lines can be applied to studies of targeted cell loss.

Summary and future perspectives

Use of the zebrafish model in vision research increases each year. Existing data on zebrafish bipolar cells include physiology, morphology, ultrastructure, immunocytochemistry, and gene expression. Transgenic lines provide a powerful tool to examine bipolar cell development, regeneration, and response to presynaptic cell loss. Mutant strains identify the important roles that bipolar cells play in retinal processing, as their function and/or morphology can be altered by mutations localized to other cell types. Furthermore, toxicological studies (Fig. 8) report changes in bipolar cell physiology following developmental exposure to various compounds, such as methylmercury (Weber et al., 2008), and new imaging techniques, such as SyGCaMP2, a reporter molecule formed by tethering a genetically encoded calcium indicator to synaptophysin, are being used to study synaptic activity of bipolar axon terminals (Dreosti et al., 2009; Dorostkar et al., 2010). Combining existing data with the diversity of techniques available will further our knowledge of bipolar cell function, in general, but also position zebrafish bipolar cells to be a system in which to

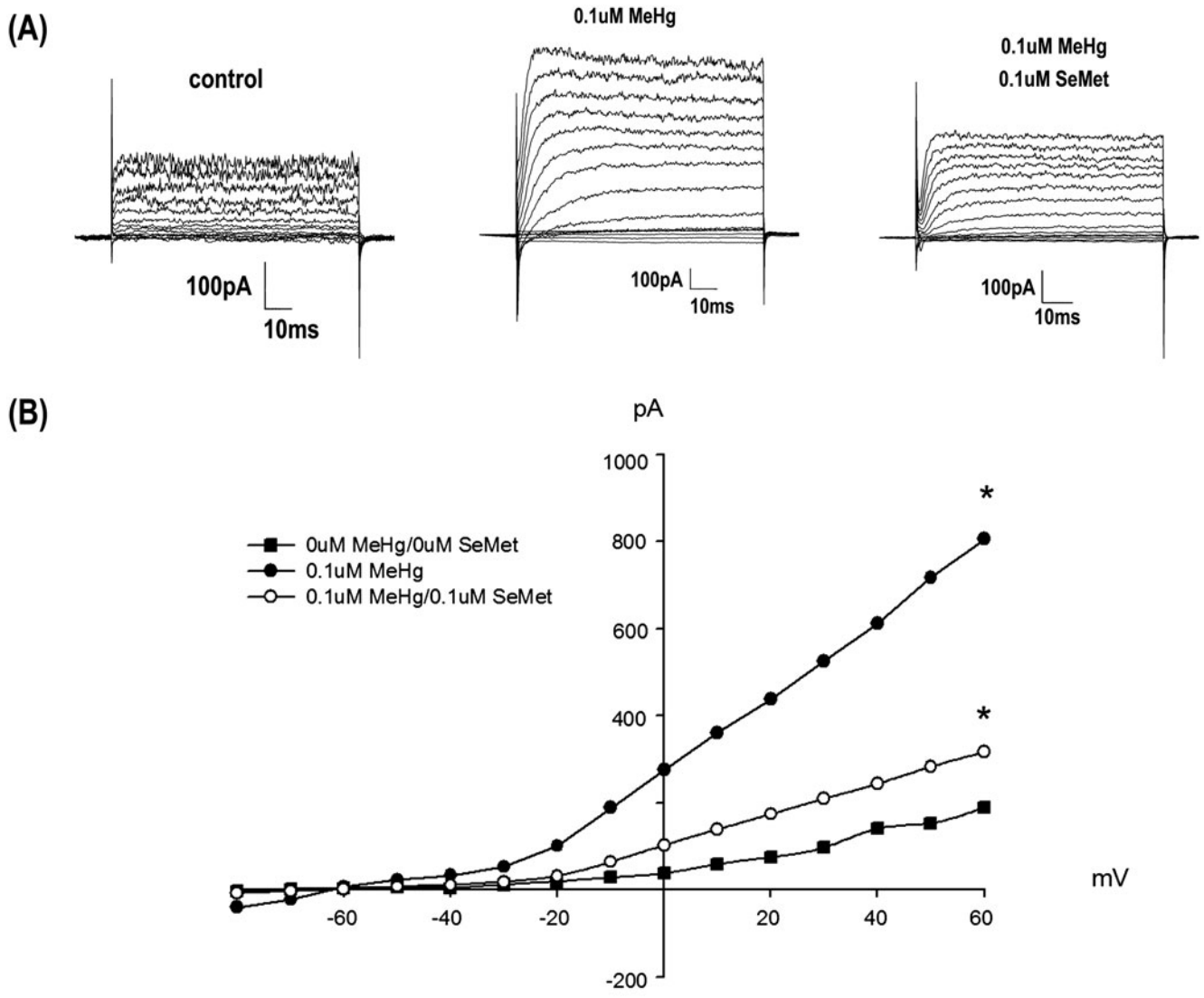


Fig. 8. Outward rectifying I_K currents in adult bipolar cells are enhanced following developmental exposure to methylmercury. (**A**) Representative whole-cell current traces showing the increase in the amplitude of the delayed rectifying I_K current recorded from bipolar cells in adult zebrafish that had been developmentally exposed to 0.1 μM methylmercury (MeHg) (middle traces). Coexposure to selenomethionine (SeMet) and methylmercury (MeHg) suppressed the increase in I_K amplitude (right traces). (**B**) Mean current–voltage relationship; asterisks indicate significant differences from control (Reprinted from *Physiology and Behavior*, Vol. 93, Weber, D.N., Connaughton, V.P., Dellinger, J.A., Klemer, D., Udvadia, A., Carvan, M.A. III. Selenomethionine reduces visual deficits due to developmental methylmercury exposures, 250–260, with permission from Elsevier).

https://doi.org/10.1017/S0952523810000295 Published online by Cambridge University Press

examine how this retinal cell type functions under and responds to a variety of conditions. Furthermore, many characteristics of zebrafish bipolar cells are similar to higher vertebrates, indicating that findings in zebrafish related to mutations, pharmacology, and disease have far reaching impacts.

Acknowledgments

This work was supported, in part, by the Mellon Research Fund and an American University Senate Research Award. Thanks to R. Nelson, R. Tarboush, and M. Connaughton for helpful comments on the manuscript.

References

Allwardt, B., Lall, A., Brockerhoff, W. & Dowling, J. (2001). Synapse formation is arrested in retinal photoreceptors of the zebrafish nrc mutant. *The Journal of Neuroscience* **21**, 2330–2342.

Amsterdam, A. & Becker, T. (2005). Transgenes as screening tools to probe and manipulate the zebrafish genome. *Developmental Dynamics* **234**, 255–268.

Asare, M., Nelson, R. & Connaughton, V. (2005). Effects of dopamine on glutamate responses in horizontal and bipolar cells isolated from zebrafish retina. ARVO e-abstracts 605.

Bahadori, R., Biehlmaier, O., Zeitz, C., Labhart, T., Makhankov, Y., Forster, U., Gesemann, M., Berger, W. & Neuhauss, S. (2006). Nyctalopin is essential for synaptic transmission in the cone dominated zebrafish retina. *The European Journal of Neuroscience* **24**, 1664–1674.

Bech-Hansen, N., Naylor, M., Maybaum, T., Sparkes, R., Koop, B., Birch, D., Bergen, A., Prinsen, C., Polomeno, R., Gal, A., Drack, A., Musarella, M., Jacobson, S., Young, R. & Weleber, R. (2000). Mutations in *NYX*, encoding the leucine-rick proteoglycan nyctalopin, cause X-linked complete congenital stationary night blindness. *Nature* **26**, 319–323.

Biehlmaier, O., Neuhauss, S. & Kohler, K. (2003). Synaptic plasticity and functionality at the cone terminal of the developing zebrafish retina. *Journal of Neurobiology* **56**, 222–236.

Bilotta, J., Saszik, S. & Sutherland, S. (2001). Rod contributions to the electroretinogram of the dark-adapted developing zebrafish. *Developmental Dynamics* **222**, 564–570.

Brockerhoff, S., Hurley, J., Janssen-Bienhold, U., Neuhauss, S., Driever, W. & Dowling, J. (1995). A behavioral screen for isolating zebrafish mutants with visual system defects. *Proceedings of the National Academy of Sciences of the United States of America* **92**, 10545–10549.

Brockerhoff, S., Hurley, J., Niemi, G. & Dowling, J. (1997). A new form of inherited red-blindness identified in zebrafish. *The Journal of Neuroscience* **17**, 4236–4242.

Brockerhoff, S., Wilson, N., Stearns, G. & Lewis, A. (2010). A zebrafish mutation identifies nonclassic cadherins as critical modulators of outer retina physiology. ARVO e-abstracts 4799.

Burrone, J. & Lagnado, L. (1997). Electrical resonance and Ca2+ influx in the synaptic terminal of depolarizing bipolar cells from the goldfish retina. *Journal of Physiology* **505**, 571–584.

Caminos, E., Velasco, A., Jarrin, M., Lillo, C., Jimeno, D., Aijon, J. & Lara, J. (2000). A comparative study of protein kinase C-like immunoreactive cells in the retina. *Brain, Behavior and Evolution* **56**, 330–339.

Campbell, N., Reece, J., Urry, L., Cain, M., Wasserman, S., Minorsky, P. & Jackson, R. (2008). *Biology* (8th ed.). San Francisco, CA: Pearson-Benjamin Cummings.

Chuang, J., Mathers, P. & Raymond, P. (1999). Expression of three Rx homeobox genes in embryonic and adult zebrafish. *Mechanisms of Development* **84**, 195–198.

Clark, A., Yun, S., Veien, E., Wu, Y., Chow, R., Dorsky, R. & Levine, E. (2008). Negative regulation of Vsx1 by its paralog Chx10/Vsx2 is conserved in the vertebrate retina. *Brain Research* **1192**, 99–113.

Connaughton, V. (2001). Organization of ON- and OFF-pathways in the zebrafish retina: Neurotransmitter localization, electrophysiological responses of bipolar cells, and patterns of axon terminal stratification. *Progress in Brain Research* **131**, 161–176.

Connaughton, V., Behar, T., Liu, W.-L. & Massey, S. (1999). Immunocytochemical localization of excitatory and inhibitory neurotransmitters in the zebrafish retina. *Visual Neuroscience* **16**, 483–490.

Connaughton, V., Graham, D. & Nelson, R. (2004). Identification and morphological classification of horizontal, bipolar, and amacrine cells within the zebrafish retina. *The Journal of Comparative Neurology* **477**, 371–385.

Connaughton, V. & Maguire, G. (1998). Differential expression of voltage-gated K+ and Ca2+ currents in bipolar cells in the zebrafish retinal slice. *The European Journal of Neuroscience* **10**, 1350–1362.

Connaughton, V. & Nelson, R. (2000). Axonal stratification patterns and glutamate-gated conductance mechanisms in zebrafish retinal bipolar cells. *Journal of Physiology* **524**, 135–146.

Connaughton, V., Nelson, R. & Bender, A. (2008). Electrophysiological evidence of GABAA and GABAC receptors on zebrafish retinal bipolar cells. *Visual Neuroscience* **25**, 139–153.

Connaughton, V. & Warndorf, M. (2005). D1- and D2-like dopamine receptor activity enhance outward K+ currents in zebrafish retinal bipolar cells. ARVO e-abstracts 1195.

Curado, S., Stainier, D. & Anderson, R. (2008). Nitroreductase-mediated cell/tissue ablation in zebrafish: A spatially and temporally controlled ablation method with applications in developmental and regeneration studies. *Nature Protocols* **3**, 948–954.

Dorostkar, M., Dreosti, E., Odermatt, B. & Lagnado, L. (2010). Computational processing of optical measurements of neuronal and synaptic activity in networks. *Journal of Neuroscience Methods* **188**, 141–150.

Dowling, J. & Werblin, F. (1969). Organization of retina of the mudpuppy, *Necturus maculosus.* I. Synaptic structure. *Journal of Neurophysiology* **32**, 315–338.

Dreosti, E., Odermatt, B., Dorostkar, M. & Lagnado, L. (2009). A genetically encoded reporter of synaptic activity in vivo. *Nature Methods* **6**, 883–891.

Emran, F., Rihel, J., Adolph, A., Wong, K., Kraves, S. & Dowling, J. (2007). OFF ganglion cells cannot drive the optokinetic reflex in zebrafish. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 19126–19131.

Euler, T. & Wassle, H. (1998). Different contributions of GABAA and GABAC receptors to rod and cone bipolar cells in a rat retinal slice preparation. *Journal of Neurophysiology* **79**, 1384–1395.

Fadool, J., Brockerhoff, S., Hyatt, G. & Dowling, J. (1997). Mutations affecting eye morphology in the developing zebrafish (Danio rerio). *Developmental Genetics* **20**, 288–295.

Fan, S. & Yazulla, S. (1999*a*). Modulation of voltage-dependent K+ currents (IK(V)) in retinal bipolar cells by ascorbate is mediated by dopamine D1 receptors. *Visual Neuroscience* **16**, 923–931.

Fan, S. & Yazulla, S. (1999*b*). Suppression of voltage-dependent K+ currents in retinal bipolar cells by ascorbate. *Visual Neuroscience* **16**, 141–148.

Gafka, A., Vogel, K. & Linn, C. (1999). Evidence of metabotropic glutamate receptor subtypes found on catfish horizontal and bipolar retinal neurons. *Neuroscience* **90**, 1403–1414.

Gan, W.-B., Grutzendler, J., Wong, W., Wong, R. & Lichtman, J. (2000). Multicolor "DiOlistic" labeling of the nervous system using lipophilic dye combinations. *Neuron* **27**, 219–225.

Grant, G. & Dowling, J. (1995). A glutamate-activated chloride current in the cone-driven on bipolar cells of the white perch retina. *The Journal of Neuroscience* **15**, 3852–3862.

Grant, G. & Dowling, J. (1996). ON bipolar cell responses in the teleost retina are generated by two distinct mechanisms. *Journal of Neurophysiology* **76**, 3842–3849.

Gregg, R., Kamermans, M., Klooster, J., Lukasiewicz, P., Peachey, N., Vessey, K. & McCall, M. (2007). Nyctalopin expression in retinal bipolar cells restores visual function in a mouse model of complete X-linked congenital stationary night blindness. *Journal of Neurophysiology* **98**, 3023–3033.

Gregg, R., Mukhopadhyay, S., Candille, S., Ball, S., Pardue, M., McCall, M. & Peachey, N. (2003). Identification of the gene and the mutation responsible for the mouse *nob* phenotype. *Investigative Ophthalmology and Visual Science* **44**, 378–384.

Han, M.-H., Li, Y. & Yang, X.-L. (1997). Desensitizing GABAC receptors on carp retinal bipolar cells. *Neuroreport* **8**, 1331–1335.

Heidelberger, R. & Matthews, G. (1991). Inhibition of calcium influx and calcium current by γ-aminobutyric acid in single synaptic terminals. *Proceedings of the National Academy of Sciences of the United States of America* **88**, 7135–7139.

https://doi.org/10.1017/S0952523810000295 Published online by Cambridge University Press

Heidelberger, R. & Matthews, G. (1992). Calcium influx and calcium current in single synaptic terminals of goldfish retinal bipolar neurons. *Journal of Physiology* **447**, 235–256.

Higashijima, S. (2008). Transgenic zebrafish expressing fluorescent proteins in central nervous system neurons. *Development, Growth and Differentiation* **50**, 407–413.

Huag, M., Huang, Y.-Y., Gesemann, M. & Neuhauss, S. (2010). Expression and function of mGluR6 in the zebrafish retina. ARVO e-abstracts 4137.

Hughes, A., Saszik, S., Bilotta, J., DeMarco, P. Jr. & Patterson, W.I. (1998). Cone contributions to the phototopic spectral sensitivity of the zebrafish ERG. *Visual Neuroscience* **15**, 1029–1037.

Hull, C., Li, G.-L. & Von Gersdorff, H. (2006). GABA transporters regulate a standing GABAC receptor-mediated current at a retinal presynaptic terminal. *The Journal of Neuroscience* **26**, 6979–6984.

Hull, C. & Von Gersdorff, H. (2004). Fast endocytosis is inhibited by GABA-mediated chloride influx at a presynaptic terminal. *Neuron* **44**, 469–482.

Jones, S. & Palmer, M. (2009). Activation of the tonic GABAC receptor current in retinal bipolar cell terminals by nonvesicular GABA release. *Journal of Neurophysiology* **102**, 691–699.

Joselevitch, C., Klooster, J. & Kamermans, M. (2007). Localization of metabotropic glutamate receptors in the outer plexiform layer of the goldfish retina. *Cell and Tissue Research* **330**, 389–403.

Kalloniatis, M. & Marc, R. (1990). Interplexiform cells of the goldfish retina. *The Journal of Comparative Neurology* **297**, 340–358.

Kaneda, M., Andrasfalvy, B. & Kaneko, A. (2000). Modulation by Zn2 + of GABA responses in bipolar cells of the mouse retina. *Visual Neuroscience* **17**, 273–281.

Kaneko, A., Pinto, L. & Tachibana, M. (1989). Transient calcium current of retinal bipolar cells of the mouse retina. *Journal of Physiology* **410**, 613–629.

Kaneko, A. & Tachibana, M. (1985). A voltage-clamp analysis of membrane currents in solitary bipolar cells dissociated from Carassius auratus. *Journal of Physiology* **358**, 131–152.

Kastenhuber, E., Maurer, C., Gesemann, M., Frueh, S., Renninger, S. & Neuhauss, S. (2010). Characterization of glutamate transporters (EAATs) in the zebrafish retina. ARVO e-abstracts 4118.

Kawakami, K. (2005). Transposon tools and methods in zebrafish. *Developmental Dynamics* **234**, 244–254.

Kay, J., Finger-Baier, K., Roeser, T., Staub, W. & Baier, H. (2001). Retinal ganglion cell genesis requires lakritz, a zebrafish atonal homolog. *Neuron* **30**, 725–736.

Kay, J., Roeser, T., Mumm, J., Godinho, L., Mrejeru, A., Wong, R. & Baier, H. (2004). Transient requirement for ganglion cells during assembly of retinal synaptic layers. *Development* **131**, 1331–1342.

Khan, N., Kondo, M., Hiriyanna, K., Jamison, J., Bush, R. & Sieving, P. (2005). Primate retinal signaling pathways: Suppressing ON-pathway activity in monkey with glutamate analogues mimics human CSNB1-*NYX* genetic night blindness. *Journal of Neurophysiology* **93**, 481–493.

Klooster, J., Yazulla, S. & Kamermans, M. (2009). Ultrastructural analysis of the glutamatergic system in the outer plexiform layer of zebrafish retina. *Journal of Chemical Neuroanatomy* **37**, 254–265.

Kolb, H. & Jones, J. (1984). Synaptic organization of the outer plexiform layer of the turtle retina: An electron microscope study of serial sections. *Journal of Neurocytology* **13**, 567–591.

Lakowski, J., Majumder, A. & Lauderdale, J. (2007). Mechanisms controlling Pax6 isoform expression in the retina have been conserved between teleosts and mammals. *Developmental Biology* **307**, 498–520.

Lasansky, A. (1973). Organization of the outer synaptic layer in the retina of the larval tiger salamander. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **265**, 471–489.

Lasater, E. (1988). Membrane currents of retinal bipolar cells in culture. *Journal of Neurophysiology* **60**, 1460–1480.

Lasater, E., Dowling, J. & Ripps, H. (1984). Pharmacological properties of isolated horizontal and bipolar cells from the skate retina. *The Journal of Neuroscience* **4**, 1966–1975.

Li, Y. & Dowling, J. (2010). Specificity in the bipolar cell-photoreceptor connections in the zebrafish retina. ARVO e-abstracts 4125.

Link, B., Fadool, J., Malicki, J. & Dowling, J. (2000). The zebrafish young mutation acts non-cell-autonomously to uncouple differentiation from specification for all retinal cells. *Development* **127**, 2177–2188.

Lukasiewicz, P. & Shields, C. (1998). Different contributions of GABAA and GABAC receptors confer distinct temporal properties to retinal synaptic responses. *Journal of Neurophysiology* **79**, 3157–3167.

Magnupalli, V., Schwarz, K., Alpadi, K., Natarajam, S., Seigel, G. & Schmitz, F. (2008). Multiple RIBEYE-RIBEYE interactions create a dynamic scaffold for the formation of synaptic ribbons. *The Journal of Neuroscience* **28**, 7954–7967.

Marc, R. & Cameron, D. (2001). A molecular phenotype atlas of the zebrafish retina. *Journal of Neurocytology* **30**, 593–654.

Marc, R. & Lam, D.-K. (1981). Glycinergic pathways in the goldfish retina. *The Journal of Neuroscience* **1**, 152–165.

Matsui, K., Hasegawa, J. & Tachibana, M. (2001). Modulation of excitatory synaptic transmission by GABAC receptor-mediated feedback in the mouse inner retina. *Journal of Neurophysiology* **86**, 2285–2298.

Matthews, G. (1999). Synaptic mechanisms of bipolar cell terminals. *Vision Research* **39**, 2469–2476.

Matthews, G., Ayoub, G. & Heidelberger, R. (1994). Presynaptic inhibition by GABA is mediated via two distinct GABA receptors with novel pharmacology. *The Journal of Neuroscience* **14**, 1079–1090.

McGillem, G., Rotolo, T. & Dacheux, R. (2000). GABA responses of rod bipolar cells in rabbit retinal slices. *Visual Neuroscience* **17**, 381–389.

Mora-Ferrer, C., Yazulla, S., Studholme, K. & Haak-Frendscho (1999). Dopamine D1-receptor immunolocalization in goldfish retina. *The Journal of Comparative Neurology* **411**, 705–714.

Morris, A., Schroeter, E., Bilotta, J., Wong, R. & Fadool, J. (2005). Cone survival despite rod degeneration in XOPS-mCFP transgenic zebrafish. *Investigative Ophthalmology and Visual Science* **46**, 4762–4771.

Mullins, M., Hammerschmidt, M., Haffter, P. & Nusslein-Volhard, C. (1994). Large-scale mutagenesis in the zebrafish: In search of genes controlling developing in a vertebrate. *Current Biology* **4**, 189–202.

Nelson, R., Bender, A. & Connaughton, V. (2008). Transporter-mediated GABA responses in horizontal and bipolar cells of zebrafish retina. *Visual Neuroscience* **25**, 155–165.

Nelson, R. & Singla, N. (2009). A spectral model for signal elements isolated from zebrafish photopic electroretinogram. *Visual Neuroscience* **26**, 349–363.

Neuhauss, S., Biehlmaier, O., Seeliger, M., Das, T., Kohler, K., Harris, W. & Baier, H. (1999). Genetic disorders of vision revealed by a behavioral screen of 400 essential loci in zebrafish. *The Journal of Neuroscience* **19**, 8603–8615.

Palmer, M. (2006). Functional segregation of synaptic GABAA and GABAC receptors in goldfish bipolar cell terminals. *Journal of Physiology* **577**, 45–53.

Pan, Z.-H. (2001). Voltage-activated Ca2+ channels and ionotropic GABA receptors localized to axon terminals of mammalian retinal bipolar cells. *Visual Neuroscience* **18**, 279–288.

Pang, J.-J., Gao, F. & Wu, S. (2004). Stratum-by-stratum projection of light response attributes by retinal bipolar cells of Ambystoma. *Journal of Physiology* **558**, 249–262.

Pardue, M., McCall, M., LaVail, M., Gregg, R. & Peachey, N. (1998). A naturally occurring mouse model of X-linked congenital stationary night blindness. *Investigative Ophthalmology and Visual Science* **39**, 2443–2449.

Passini, M., Levine, E., Canger, A., Raymond, P. & Schechter, N. (1997). Vsx-1 and Vsx-2: Differential expression of two paired-like homeobox genes during zebrafish and goldfish retinogenesis. *The Journal of Comparative Neurology* **388**, 495–505.

Patterson, W.I., McDowell, A., Hughes, A. & Bilotta, J. (2002). Opponent and nonopponent contributions to the zebrafish electroretinogram using heterochromatic flicker photometry. *Journal of Comparative Physiology A* **188**, 283–293.

Pesch, K., Zietz, C., Fries, J., Munscher, S., Pusch, C., Kohler, K., Berger, W. & Wissinger, B. (2003). Isolation of the mouse nyctalopin gene *nyx* and expression studies in mouse and rat retina. *Investigative Ophthalmology and Visual Science* **44**, 2260–2266.

Protti, D., Flores, H.N. & Von Gersdorff, H. (2000). Light evokes Ca2+ spikes in the axon terminal of a retinal bipolar cell. *Neuron* **25**, 215–227.

Qian, H. & Dowling, J. (1995). GABAA and GABAC receptors on hybrid bass retinal bipolar cells. *Journal of Neurophysiology* **74**, 1920–1928.

Qian, H., Li, L., Chappell, R. & Ripps, H. (1997). GABA receptors of bipolar cells from the skate retina: Actions of zinc on GABA-mediated membrane currents. *Journal of Neurophysiology* **78**, 2402–2412.

https://doi.org/10.1017/S0952523810000295 Published online by Cambridge University Press

Qian, H., Ripps, H., Schuette, E. & Chappell, R. (2001). Responses of small- and large-field bipolar cells to GABA and glycine. *Brain Research* **893**, 273–277.

Ragozzino, D., Woodward, R., Murata, Y., Eusebi, F., Overman, L. & Miledi, R. (1996). Design and in vitro pharmacology of a selective γ-aminobutyric acidC receptor antagonist. *Molecular Pharmacology* **50**, 1024–1030.

Ren, J. & Li, L. (2004). Rod and cone signaling transmission in the retina of zebrafish: An ERG study. *The International Journal of Neuroscience* **114**, 259–270.

Saade, C. & Fadool, J. (2010). Remodeling of bipolar cell morphology in response to photoreceptor dystrophies in the retina of the zebrafish (Danio rerio). ARVO e-abstracts 2487.

Saszik, S., Alexander, A., Lawrence, T. & Bilotta, J. (2002). APB differentially affects the cone contributions to the zebrafish ERG. *Visual Neuroscience* **19**, 521–529.

Schmitt, E. & Dowling, J. (1999). Early retinal development in the zebrafish, Danio rerio: Light and electron microscopic analysis. *The Journal of Comparative Neurology* **404**, 515–536.

Schmitz, F., Konigstorfer, A. & Sudhof, T. (2000). RIBEYE, a component of synaptic ribbons: A protein's journey through evolution provides insight into synaptic ribbon function. *Neuron* **28**, 857–872.

Scholes, J. (1975). Colour receptors, and their synaptic connections, in the retina of a cyprinid fish. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **270**, 61–115.

Schroeter, E., Wong, R. & Gregg, R. (2006). In vivo development of retinal ON-bipolar cell axon terminals visualized in nyx::MYFP transgenic zebrafish. *Visual Neuroscience* **23**, 833–843.

Seeliger, M., Rilk, A. & Neuhauss, S. (2002). Ganzfeld ERG in zebrafish larvae. *Documenta Ophthalmologica* **104**, 57–68.

Sherry, D., Micich, A. & Yazulla, S. (1993). Glycine in the lizard retina: Comparison to the GABA system. *Visual Neuroscience* **10**, 693–702.

Sherry, D. & Yazulla, S. (1993). Goldfish bipolar cells and axon terminal patterns: A Golgi study. *The Journal of Comparative Neurology* **329**, 188–200.

Solnica-Krezel, L., Schier, A. & Driever, W. (1994). Efficient recovery of ENU-induced mutations from the zebrafish germline. *Genetics* **136**, 1401–1420.

Stearns, G., Evangelista, M., Fadool, J. & Brockerhoff, S. (2007). A mutation in the cone-specific pde6 gene causes rapid cone photoreceptor degeneration in zebrafish. *The Journal of Neuroscience* **27**, 13866–13874.

Stockton, R. & Slaughter, M. (1989). B-wave of the electoretinogram. A reflection of ON bipolar cell activity. *Journal of Physiology* **95**, 101–122.

Stujenske, J., Emran, F. & Dowling, J. (2010). The bug eye mutant zebrafish exhibits visual deficits that arise with the onset of an enlarged eye phenotype. ARVO e-abstracts 5578.

Tachibana, M. & Kaneko, A. (1987). γ−Aminobutyric acid exerts a local inhibitory action on the axon terminal of bipolar cells: Evidence for negative feedback from amacrine cells. *Proceedings of the National Academy of Sciences of the United States of America* **84**, 3501–3505.

Taylor, M., Hurley, J., Van Epps, H. & Brockerhoff, S. (2004). A zebrafish model for pyruvate dehydrogenase deficiency: Rescue of neurological dysfunction and embryonic lethality using a ketogenic diet. *Proceedings of the National Academy of Sciences of United States of America* **101**, 4584–4589.

Taylor, M., Kikkawa, S., Diez-Juan, A., Ramamurthy, V., Kawakami, K., Carmeliet, P. & Brockerhoff, S. (2005). The zebrafish pob gene encodes a novel protein required for survival of red cone photoreceptor cells. *Genetics* **170**, 263–273.

Van Epps, H., Hayashi, M., Lucast, L., Stearns, G., Hurley, J., De Camilli, P. & Brockerhoff, S. (2004). The zebrafish nrc mutant reveals a role for the polyphosphoinositide phosphatase synaptojanin 1 in cone photoreceptor ribbon anchoring. *The Journal of Neuroscience* **24**, 8641–8650.

Vandenbranden, C., Kamphuis, W., Nunes Cardozo, B. & Kamermans, M. (2000). Expression and localization of ionotropic glutamate receptor subunits in the goldfish retina—An in situ hybridization and immunocytochemical study. *Journal of Neurocytology* **29**, 729–742.

Vigh, J. & von Gersdorff, H. (2005). Prolonged reciprocal signaling via NMDA and GABA receptors at a retinal ribbon synapse. *The Journal of Neuroscience* **25**, 11412–11423.

Vitorino, M., Jusuf, P., Maurus, D., Kimura, Y., Higashijima, S. & Harris, W. (2009). VSX2 in the zebrafish retina: Restricted lineages through derepression. *Neural Development* **4**, 14.

Wan, L., Almers, W. & Chen, W. (2005). Two ribeye genes in teleosts: The role of ribeye in ribbon formation and bipolar cell development. *The Journal of Neuroscience* **25**, 941–949.

Weber, D., Connaughton, V., Dellinger, J., Klemer, D., Udvadia, A. & Carvan, M.I. (2008). Selenomethionine reduces visual deficits due to developmental methylmercury exposure. *Physiology and Behavior* **93**, 250–260.

Wei, X., Luo, Y. & Hyde, D. (2006). Molecular cloning of three zebrafish lin7 genes and their expression patterns in the retina. *Experimental Eye Research* **82**, 122–131.

Wong, K., Adolph, A. & Dowling, J. (2005). Retinal bipolar cell input mechanisms in giant danio I. Electroretinographic analysis. *Journal of Neurophysiology* **93**, 84–93.

Wong, K., Cohen, E. & Dowling, J. (2005). Retinal bipolar cell input mechanisms in giant danio II. Patch-clamp analysis of ON bipolar cells. *Journal of Neurophysiology* **93**, 94–107.

Wong, K. & Dowling, J. (2005). Retinal bipolar cell input mechanisms in giant danio III. ON-OFF bipolar cells and their color-opponent mechanisms. *Journal of Neurophysiology* **94**, 265–272.

Wong, K., Gray, J., Hayward, C., Adolph, A. & Dowling, J. (2004). Glutamatergic mechanisms in the outer retina of larval zebrafish: Analysis of electroretinogram b- and d-waves using anovel preparation. *Zebrafish* **1**, 121–131.

Wu, S., Gao, F. & Maple, B. (2000). Functional architecture of synapses in the inner retina: Segregation of visual signals by stratification of bipolar cell axon terminals. *The Journal of Neuroscience* **20**, 4462–4470.

Xu, X. & Karwoski, C. (1995). Current source density analysis of the electrographic d wave of frog retina. *Journal of Neurophysiology* **73**, 2459–2469.

Yang, C.-Y. (1998). γ-Aminobutyric acid transporter-mediated current from bipolar cells in tiger salamander retinal slices. *Vision Research* **38**, 2521–2526.

Yazulla, S. & Studholme, K. (1990). Multiple subtypes of glycine-immunoreactive neurons in the goldfish retina: Single- and double-label studies. *Visual Neuroscience* **4**, 299–309.

Yazulla, S. & Studholme, K. (1991). Glycine-receptor immunoreactivity in retinal bipolar cells is postsynaptic to glycinergic and GABAergic amacrine cell synapses. *The Journal of Comparative Neurology* **310**, 11–20.

Yazulla, S. & Studholme, K. (1998). Differential distribution of *Shaker*-like and *Shab*-like K+-channel subunits in goldfish retina and retinal bipolar cells. *The Journal of Comparative Neurology* **396**, 131–140.

Yazulla, S. & Studholme, K. (2001). Neurochemical anatomy of the zebrafish retina as determined by immunocytochemistry. *Journal of Neurocytology* **30**, 551–592.

Yazulla, S., Studholme, K., Fan, S. & Mora-Ferrer, C. (2001). Neuromodulation of voltage-dependent K+ channels in bipolar cells: Immunocytochemical and electrophysiological studies. *Progress in Brain Research* **131**, 201–213.

Yu, C.-J. & Li, L. (2005). Dopamine modulates voltage-activated potassium currents in zebrafish retinal ON bipolar cells. *Journal of Neuroscience Research* **82**, 368–376.

Zenisek, D., Henry, D., Studholme, K., Yazulla, S. & Matthews, G. (2001). Voltage-dependent sodium channels are expressed in nonspiking retinal bipolar neurons. *The Journal of Neuroscience* **21**, 4543–4550.

Zhao, X.-F., Ellingsen, S. & Fjose, A. (2009). Labelling and targeted ablation of specific bipolar cell types in the zebrafish retina. *BMC Neuroscience* **10**, 107.

https://doi.org/10.1017/S0952523810000295 Published online by Cambridge University Press