Neurotransmission plays contrasting roles in the maturation of inhibitory synapses on axons and dendrites of retinal bipolar cells

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Neuronal output is modulated by inhibition onto both dendrites and axons. It is unknown whether inhibitory synapses at these two cellular compartments of an individual neuron are regulated coordinately or separately during in vivo development. Because neurotransmission influences synapse maturation and circuit development, we determined how loss of inhibition affects the expression of diverse types of inhibitory receptors on the axon and dendrites of mouse retinal bipolar cells. We found that axonal GABA but not glycine receptor expression depends on neurotransmission. Importantly, axonal and dendritic GABA_A receptors comprise distinct subunit compositions that are regulated differentially by GABA release: Axonal GABA_A receptors are down-regulated but dendritic receptors are up-regulated in the absence of inhibition. The homeostatic increase in GABA_A receptors on bipolar cell dendrites is pathway-specific: Cone but not rod bipolar cell dendrites maintain an up-regulation of receptors in the transmission deficient mutants. Furthermore, the bipolar cell GABA_A receptor alterations are a consequence of impaired vesicular GABA release from amacrine but not horizontal interneurons. Thus, inhibitory neurotransmission regulates in vivo postsynaptic maturation of inhibitory synapses with contrasting modes of action specific to synapse type and location.

GABA receptor | retina | synaptic inhibition | axon-dendrite

Interneurons of the CNS control neuronal excitability through release of γ-aminobutyric acid (GABA) and glycine. How inhibition modifies neuronal output depends largely on the types of presynaptic interneurons making synapses onto a postsynaptic cell, and the location and densities of these synapses (1–3). Moreover, inhibitory receptor types with distinct transmitter affinities and kinetics present on the axon or dendrites of an individual neuron can critically shape its output (3–6). Although much is known about how different inhibitory synapses shape the spatiotemporal activity patterns of mature neurons, it is less clear what factors regulate the expression of inhibitory receptors at these synapses during development in vivo. Is the expression of distinct inhibitory receptor types within a cellular compartment (axon or dendrite) regulated coordinately or independently? Conversely, is the expression of the same receptor type at different cellular compartments of an individual neuron regulated by common or separate factors?

To answer these questions, we assessed expression of inhibitory receptors on the axon and dendrites of individual glutamatergic retinal neurons in mice with genetically suppressed inhibition. We generated retina-specific knockouts of the vesicular inhibitory amino acid transporter (VIAAT), which mediates uptake of GABA or glycine into synaptic vesicles (7, 8). We perturbed inhibition because it has been found previously to influence pre- and postsynaptic maturation of GABAergic synapses (9–12). However, whether inhibitory receptor expression at the "input" and "output" compartments of an individual neuron

is coordinately regulated by activity remains unknown. We focused on retinal bipolar cells (BCs) because of the rich variety of inhibitory synapses found on these neurons. Moreover, the many types of BCs enabled us to determine whether inhibitory transmission plays a uniform or diverse role in regulating inhibitory synapses across cell types that signal in parallel. We compared the postsynaptic maturation of GABA- and glycinergic synapses on cone BCs (CBCs) versus rod BCs (RBCs) that operate at different light levels. Among CBCs, we analyzed both ON and OFF BC types, which depolarize or hyperpolarize to light increments, respectively (13).

Results

In the mouse retina, GABA_A (α1–3, β1–3 with γ2 or an auxiliary subunit), GABA_C (ρ1–3 subunits), and glycine receptors (α1–4 with a β subunit) (5, 14, 15) are present at nonoverlapping synapses. However, the complement of inhibitory receptor types and their relative expressions on the axons and dendrites of individual BCs has not been compared across BC types. We thus generated transgenic mouse lines to visualize ON and OFF BCs. We previously showed that ON BCs, especially type 6 CBCs and RBCs, express tdTomato in *Grm6-tdTomato* mice (ref. 16; *SI Appendix*, Fig. S1A). To label OFF CBCs, we cloned and used

Significance

Neuronal output is modulated by inhibition onto axons and dendrites by diverse inhibitory synapses comprising distinct receptor subunits. Factors that regulate the in vivo maturation of these synapses across cell-compartments are not well understood. We discovered that axonal GABA_A receptors are down-regulated whereas dendritic GABA_A receptors are up-regulated on retinal bipolar cells in the absence of vesicular GABA release. Deleting the γ2 subunit of GABA_A receptors specifically in bipolar cells only alters axonal GABA_A receptor expression, suggesting that axonal and dendritic GABA_A receptors have distinct subunit compositions that are regulated independently. Moreover, vesicular GABA release from presynaptic amacrine but not horizontal interneurons is important. Thus, regulation of inhibitory synapse maturation across the bipolar cell is input-type, receptor-type, and cell-compartment-type specific.
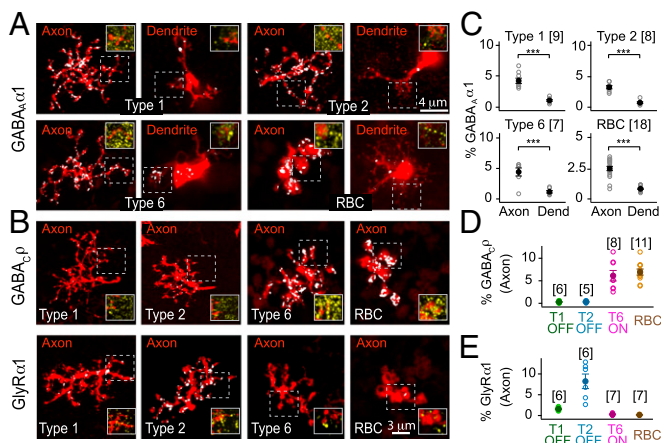
Fig. 1. Inhibitory receptor types expressed on the axons and dendrites of mouse retinal bipolar cells. (*A*) α1-subunit-containing GABA$_A$ (GABA$_A$α1) receptors (white) on the axons and dendrites (red) of types 1, 2, and 6 cone bipolar cells and rod bipolar cells (RBCs). Insets show raw images of receptor labeling (yellow) for axonal or dendritic processes within the boxed region. (*B*) ρ-subunit-containing GABA$_C$ receptors (GABA$_C$ρ) and α1-subunit-containing glycine receptors, GlyRα1, (white) on the axon terminals of OFF (type 1 and 2) and ON (type 6 and rod) bipolar cells (red). Axon terminals of ON rather than OFF bipolar cells robustly express GABA$_C$ receptors, whereas OFF bipolar cells abundantly express GlyRα1. (*C*) GABA$_A$α1 occupancy (%) at the axons and dendrites (Dend) of bipolar cells (see *SI Appendix, SI Materials and Methods* and text for definition of % occupancy). (*D* and *E*) GABA$_C$ρ and GlyRα1 receptor occupancy (%) in bipolar cell axon terminals. All error bars represent SEM. Number of cells is shown in parentheses; for all cell types, $n > 3$ animals. ***$P < 0.001$.

the *Vsx1* promoter to drive expression of cerulean fluorescent protein. In one *Vsx1-cerulean* mouse line, we found sparse labeling of OFF CBCs, particularly type 1 and 2 CBCs (*SI Appendix, Fig. S1A*). These transgenic lines enabled us to compare the inhibitory receptor types expressed in the dendrites and axons of four distinct BC types and to determine how this expression relied on the vesicular release of inhibitory neurotransmitters.

α1-Subunit Containing GABA$_A$ Receptors Are Expressed on Axons and Dendrites of Mouse Retinal Bipolar Cells. GABAergic and glycinergic amacrine cells innervate BC axon terminals, whereas BC dendrites are contacted by GABAergic horizontal cells and interplexiform amacrine cells (refs. 13, 17, and 18, and *SI Appendix, Fig. S1B*). Previous electrophysiological studies suggest that GABA$_A$ receptors are abundant on axon terminals of BCs (4, 19). Using subunit-specific antibodies, we determined the expression of GABA$_A$ α-subunits on the axons and dendrites of type 1 and 2 OFF CBCs, type 6 ON CBCs, and RBCs (Fig. 1 and *SI Appendix, Fig. S2*). For all BCs examined, we found α1-subunit-containing GABA$_A$ receptors (GABA$_A$α1) at both axon terminals and dendrites. We quantified receptor expression by calculating the % of the volume of the axon terminal or dendritic arbor occupied by immunolabeled receptors, and called the measure % occupancy (*SI Appendix, Fig. S1 C–F* and *SI Materials and Methods*). GABA$_A$α1 receptors were more abundant at BC axons relative to dendrites (Fig. 1*C*). GABA$_A$α3 receptor expression was low compared with GABA$_A$α1 expression at both ON and OFF BC axon terminals (*SI Appendix, Fig. S2B*). We did not immunolabel for GABA$_A$α2 subunits because they are not localized to BCs (15). As found previously (4, 19, 20), our immunolabeling analysis showed that ON, but not OFF BC axon terminals express substantial amounts of GABA$_C$ receptors (Fig. 1 *B* and *D*). Also OFF but not ON BC axons express abundant α1-subunit-containing glycine receptors (GlyRα1), with type 2 CBC axons expressing more GlyRα1 compared with type 1 CBCs ($P = 0.0035$, Fig. 1 *B* and *E*). Our immunolabeling thus reveals expression patterns of inhibitory receptors across BC axon types consistent with previous electrophysiology. Moreover, we discovered that although the axons of

different BC types express distinct combinations of GABA and glycine receptors, their dendrites all have GABA$_A$α1 receptors.

Perturbing GABA Release Exerts Cell-Type-Specific Effects on Bipolar Cell Morphology and Cone Connectivity. VIAAT is expressed by both mouse horizontal cells and amacrine cells (21). To assess the importance of GABA and glycine release on the formation and maintenance of inhibitory synapses on BCs, we perturbed vesicular release of inhibitory neurotransmitters specifically in the retina by abolishing VIAAT expression using *αPax6*-Cre (22) mice (VIAAT KO). In the KO, VIAAT immunoreactivity is virtually eliminated from both the outer and inner plexiform layer (OPL and IPL respectively, *SI Appendix, Fig. S3A*). To confirm the lack of inhibitory neurotransmission in the VIAAT KO, we performed whole-cell voltage clamp recordings from OFF BCs (*SI Appendix, Fig. S3B*). Indeed, OFF BCs in the VIAAT KO retina displayed a dramatically reduced frequency of spontaneous inhibitory postsynaptic currents (sIPSCs, *SI Appendix, Fig. S3B*), compared with BCs of the same type in littermate controls (control). Light-evoked inhibitory responses of OFF CBCs were also abolished in the VIAAT KO retina (*SI Appendix, Fig. S3C*).

Because GABA can act as a trophic signal to influence morphogenesis (23, 24), we examined the axonal and dendritic morphologies of BCs in the VIAAT KO retina before eye-opening and at maturity (Fig. 2). In the mature retina [postnatal day (P) 30], the axonal and dendritic arbors of all BC types examined were unaffected in the VIAAT KO, with the exception that type 1 CBC dendritic arbors in the KO were smaller than normal (Fig. 2 *A* and *B*). Before eye-opening (P12), some BCs in the VIAAT KO retina exhibited transient differences in arbor sizes compared with controls (Fig. 2*B*). However, unlike in
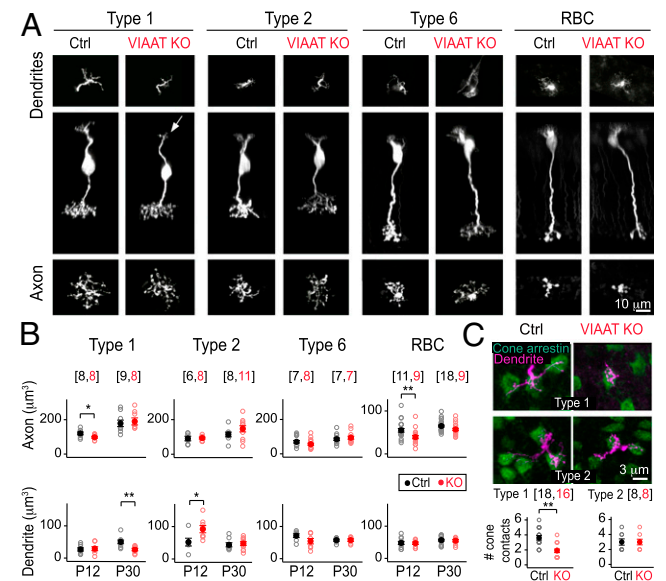

Fig. 2. Loss of VIAAT influences the morphology of ON and OFF bipolar cells in a cell-type-specific manner. (*A*) Types 1, 2, and 6 cone bipolar cells and rod bipolar cells (RBCs) in mature VIAAT knockout (KO) and littermate control (Ctrl) retinas. En face view of the dendrites and axon terminals of individual bipolar cells are displayed above and below the side view of the cell, respectively. Arrow points to the smaller dendritic arbor of a type 1 OFF cone bipolar cell in KO retina. (*B*) Quantification of the axonal (top plots) and dendritic (bottom plots) volumes of immature (postnatal day 12, P12) and mature (P30) bipolar cells in KO (red) and Ctrl (black) retinas. (*C, Upper*) Colabeling for cone arrestin (green) and dendrites of types 1 and 2 (magenta) OFF bipolar cells in adult retina. (*Lower*) Quantification of the number of cones contacted by type 1 and type 2 OFF bipolar cells in KO (red) and Ctrl (black) retinas. All error bars represent SEM. Number of cells in parentheses; for all cell types, $n > 3$ animals. Statistics for different cell types shown for Ctrl versus KO comparisons at each age. *$P < 0.05$; **$P < 0.01$.

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control retina, type 1 CBCs in the VIAAT KO did not increase their dendritic arbor size with maturation (control cells: $P = 0.007$, P12 versus P30; KO: $P = 0.75$, P12 versus P30; Fig. 2 B and C). Type 1 CBCs contacted fewer cone photoreceptors, as might be expected from their reduced dendritic territory ($P = 0.003$, Fig. 2C). Because type 1 dendritic arbors were normal in size early in development (Fig. 2B), the failure to contact more cones with maturation may be due to slowed dendritic growth and/or a failure to stabilize elaborating dendrites. Type 1 axon terminal sizes were normal in adult VIAAT KO retina (Fig. 2B), suggesting that their smaller dendritic arbors are not the result of a general impairment to cell growth. Together, these observations indicate a cell-type-specific role for VIAAT-mediated transmitter release in regulating the development and synaptic connectivity of BC dendrites.

GABA Receptor Expression on Axons and Dendrites of maturing Bipolar Cells Is Regulated Separately, and by Vesicular GABA Release from One Presynaptic Input Type. Does loss of VIAAT-mediated transmitter release perturb BC GABA or glycinergic synapse development in a receptor-type-specific and/or BC-type-specific manner? Could cell-compartment-specific alterations in receptor distribution occur on the axon and dendrites of BCs in VIAAT KO retina? To answer these questions, we analyzed GlyRα1 receptors on OFF BC (type 1 and 2) axons, GABA$_C$ receptors on ON BC (type 6 and RBC) axons, and GABA$_A$α1 receptors on the axon and dendrites of all four BC types in VIAAT KO and control retinas (Fig. 3). GlyRα1 receptor expression on the axon terminals of OFF CBCs was unaffected in the P30 VIAAT KO retina (Fig. 3 A and B). In contrast, GABA$_C$ receptors were reduced on type 6 CBC and RBC axon terminals in VIAAT KO retina at this age (Fig. 3 C and D). Similarly, GABA$_A$α1 receptor expression was diminished on the axon terminals of P30 RBCs and the CBC types studied in the VIAAT KO retina (Fig. 3 E and F, *Top*). However, CBC dendrites showed increased GABA$_A$α1 receptor expression in the P30 VIAAT KO retina (Fig. 3 E and F). In contrast, RBC dendrites in the KO maintained GABA$_A$α1 receptors at levels comparable to control (Fig. 3 E and F). Thus, impaired vesicular release of inhibitory neurotransmitters GABA and glycine in the retina causes disparate effects on GABA and glycine receptor distribution across BC types and cell compartments.

The changes we observed in GABA receptor expression in the mature VIAAT KO retina could arise from a failure to localize receptors early in development and/or an impairment of their maintenance thereafter. To distinguish between these possibilities, we examined BC GABA receptor expression in the VIAAT KO retina at two developmental time points: P12, before eye-opening and P16 after eye-opening (Fig. 3 D and F and *SI Appendix*, Fig. S4). Axonal GABA$_C$ receptors on ON BCs were reduced in P16 VIAAT KO retina compared with controls (Fig. 3D and *SI Appendix*, Fig. S4). Similarly, axonal expression of GABA$_A$α1 receptors was reduced by P16 for all BC types studied in VIAAT KO compared with control (Fig. 3F and *SI Appendix*, Fig. S4). In control animals, axonal GABA$_A$α1 receptor expression increased significantly from P12 to P16 ($P < 0.03$ for all four BC types), but this developmental increase did not occur in the VIAAT KO. In fact, in the KO, axonal GABA$_A$α1 receptor expression decreased in type 1 and 6 CBCs and RBCs between P12 and P16 ($P < 0.009$ for the three BC types), whereas axonal receptor expression did not change between P12 and P16 in type 2 CBCs ($P = 0.128$). Together, these observations indicate that the initial accumulation of GABA receptors on BC axons is largely unaffected in the VIAAT KO, but the further localization and maintenance of these receptors after eye-opening is disrupted in the absence of vesicular GABA release.

GABA$_A$α1 receptor expression on BC dendrites in VIAAT KO retina also increased relative to control at P16 (Fig. 3F and *SI Appendix*, Fig. S4). At P16, dendritic expression of GABA$_A$α1 in the KO retina increased above normal values for the CBCs and RBCs, but remained elevated in the mature retina only at CBC but not RBC dendrites (P30, Fig. 3F). Thus, the RBC
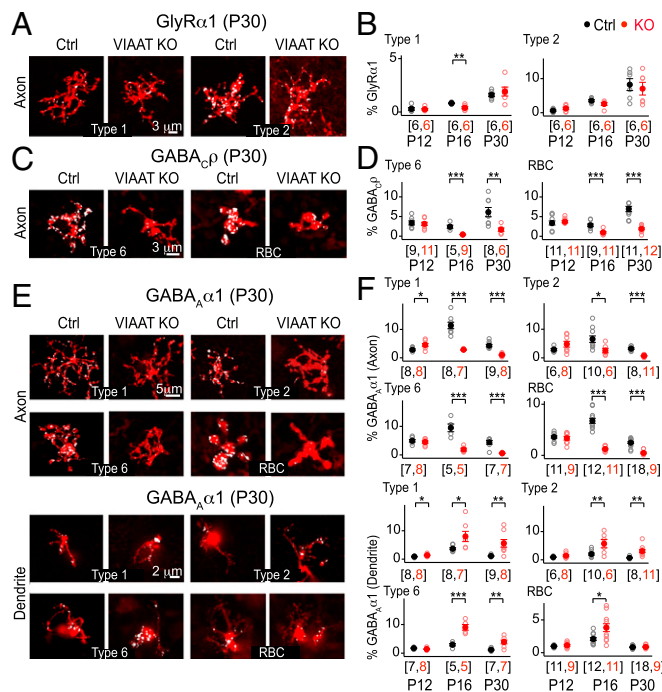

Fig. 3. Bipolar cell Glycine and GABA receptors are differentially altered in VIAAT KO retina. (*A*) α1-subunit-containing glycine receptors (GlyRα1, white) on the axon terminals (red) of type 1 and type 2 OFF cone bipolar cells in adult (postnatal day (P)30), VIAAT knockout (KO) and control (Ctrl) retina. (*B*) Quantification of GlyRα1 occupancy (%) in type 1 and type 2 terminals in Ctrl and KO retina before eye-opening (P12), just after eye-opening (P16), and in adult (P30) retinas. (*C*) ρ-subunit-containing GABA$_C$ receptors (white) on the axon terminals (red) of type 6 ON cone bipolar cells and rod bipolar cells (RBCs) in P30 VIAAT KO and Ctrl retinas. (*D*) Quantification of GABA$_C$ receptor occupancy (%) in type 6 and RBC terminals in Ctrl and KO retinas at various ages. (*E*) Examples of GABA$_A$α1 receptor immunoreactivity (white) on axons (*Upper*) and dendrites (*Lower*) of cone and rod bipolar cells (cell profile in red) in P30 Ctrl and VIAAT KO retinas. (*F*) Quantification of GABA$_A$α1 expression (%) on the axonal and dendritic arbors in Ctrl and KO retina at various ages. Alterations in bipolar cell axonal and dendritic GABA$_A$α1 receptor expression occur around eye-opening in the KO retina. Error bars represent SEM. Number of cells in parentheses; $n > 3$ animals for all cell types. For each cell type, Ctrl versus KO values are compared at each age, and statistical differences indicated. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

dendritic compartment has the capacity to reset its GABA$_A$α1 expression to wild-type levels by maturity.

To confirm that alterations in GABA$_A$α1 receptor expression reflect functional changes, we recorded GABA-evoked responses from adult retinal BCs by puffing GABA onto their axon terminals or dendrites (Fig. 4). Indeed, puffing GABA onto axons of RBCs in the KO elicited a much-reduced response compared with cells in control retina (Fig. 4A). Because RBC terminals have both GABA$_A$ and GABA$_C$ receptors, we isolated the GABA$_A$ component by blocking GABA$_C$ receptors with TPMPA. The GABA$_A$-evoked response was significantly reduced at RBC terminals in VIAAT KO retinas (Fig. 4A), corroborating the immunolabeling results. Consistent with the GABA$_C$ immunostaining, the GABA$_C$ component of the RBC terminal was also significantly reduced in the VIAAT KO (mean peak amplitudes: control = 100 ± 11 pA, KO = 48 ± 9 pA, $P = 0.007$). GABA application at RBC dendrites generated equivalent responses in KO and control retinas, also consistent with the immunostaining (Fig. 4B, *Left*). TPMPA did not reduce the dendritic GABA-evoked PSC amplitude in RBCs in the KO retina (Fig. 4B, *Right*), suggesting that RBC dendritic receptors are predominantly GABA$_A$ receptors. In keeping with these observations, puffing GABA onto dendrites of isolated mouse RBCs elicits GABA$_A$- and not GABA$_C$-mediated currents (25).
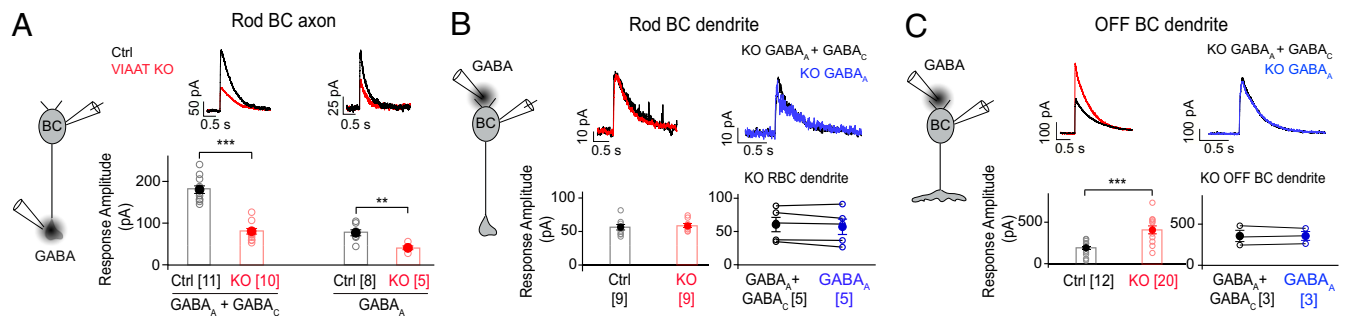
Fig. 4. Bipolar cell axonal and dendritic alterations of GABA receptor expression in VIAAT KO retina correspond to functional changes. (*A*) GABA application onto axon terminals of adult rod bipolar cells (BCs) in VIAAT knockout (KO) and littermate control (Ctrl) retinas before (GABA$_A$ + GABA$_C$) and after (GABA$_A$) application of the GABA$_C$ receptor antagonist, TPMPA. Examples of the evoked currents (cells held at 0 mV) are displayed above the population peak response amplitude plots. (*B*) Outward currents evoked upon puffing GABA onto rod BC dendrites. (*Left*) GABA puff responses of Ctrl and KO rod BCs. (*Right*) GABA-evoked responses in a subset of rod BCs in the KO retina were measured before and after TPMPA application. (*C*) Responses from adult OFF cone bipolar cells (sampled across type 1 and type 2 BCs) evoked by puffing GABA onto their dendrites. (*Left*) OFF BCs in KO retina compared with Ctrl. (*Right*) For a subset of OFF BCs in the KO retina, GABA-evoked dendritic responses were measured before and after TPMPA application. All error bars indicate SEM. Number of cells is shown in parentheses; for all recordings, *n* > 3 animals. **P < 0.01; ***P < 0.001.

In contrast to RBC responses, OFF CBC dendritic responses to puffed GABA were enhanced in the VIAAT KO retina compared with control (Fig. 4*C*, *Left*). The OFF CBC dendritic response in KO retina is mediated primarily by GABA$_A$ receptors (Fig. 4*C*, *Right*).

Collectively, our immunolabeling and electrophysiological experiments demonstrate that GABA$_C$ receptors at ON BC terminals and GABA$_A$α1 receptors at ON and OFF BC axons are sensitive to loss of vesicular transmitter release. In addition, GABA$_A$α1 receptors at BC dendrites increase in CBCs but not RBCs when vesicular release of GABA is impaired, suggesting rod/cone pathway-specific alterations in the outer retina.

Compartment (axon versus dendrite) specific alterations of GABA$_A$α1 expression on BCs raised an important question: Does loss of VIAAT in both major inhibitory presynaptic cell classes, amacrine and horizontal interneurons, contribute to the GABA$_A$α1 expression changes? To answer this question, we examined GABA$_A$α1 immunoreactivity in retinas in which VIAAT was selectively eliminated from horizontal cells (HC VIAAT KO, Fig. 5). The pan-VIAAT KO line, generated by crossing with the αPax6-Cre line, showed the expected overall reduction of GABA$_A$α1 immunoreactivity in the IPL and elevation of the signal in the OPL relative to control (Fig. 5*A*). In the HC VIAAT KO, however, GABA$_A$α1 labeling in the IPL and OPL appeared normal (Fig. 5 *B* and *C*). Thus, GABA$_A$α1 expression in the IPL and OPL depends on vesicular GABA release from amacrine cells but not horizontal cells. Our results, however, cannot exclude the possibility that nonvesicular release of GABA from horizontal cells could contribute to GABA$_A$α1 regulation.

Differential Regulation of Axonal and Dendritic GABA$_A$α1 Subunits May Depend on Coassembly with GABA$_A$γ2 Subunit. GABA$_A$α1 expression on BC axons and dendrites may be regulated differentially if GABA$_A$ receptors in these separate compartments have different subunit compositions. Alternatively, GABA$_A$ receptors on the axons and dendrites could have the same subunit composition, but their regulation by activity could depend on their subcellular location. To distinguish between these possibilities, we immunolabeled adult wild-type retina for the γ2 subunit of the GABA$_A$ receptor (GABA$_A$γ2) known to be abundant in the retina (15), and localized at the majority of CNS GABAergic synapses (26). We found that type 6 CBC axon terminals robustly express GABA$_A$γ2, but surprisingly, their dendrites are deficient in this subunit (*SI Appendix*, Fig. S5*A*). In fact there was very little GABA$_A$γ2 immunoreactivity in the OPL (*SI Appendix*, Fig. S5*A*). Using cross-correlation analysis, we confirmed that GABA$_A$α1 and γ2 immunolabeling overlapped highly at type 6 CBC axon terminals (*SI Appendix*, Fig. S5 *B* and *C*).

These observations raise the possibility that GABA$_A$ receptors on BC axon and dendrites may not have the same subunit composition. This also makes the prediction that loss of axonal GABA$_A$α1 in the VIAAT KO retina should be accompanied by a loss of axonal GABA$_A$γ2. Indeed, we found that axonal GABA$_A$γ2 in type 6 CBCs was reduced in VIAAT KO retina compared with control but dendritic GABA$_A$γ2 expression was not altered (Fig. 6 *A* and *B*). We focused on type 6 CBCs because their morphology remains unaltered in the VIAAT KO (Fig. 2). To further examine whether GABA$_A$γ2 coassembles with axonal but not dendritic GABA$_A$α1, we genetically deleted GABA$_A$γ2 specifically in ON BCs by crossing *GABA$_A$γ2* conditional KO mice (27) with a transgenic line we created in which Cre-recombinase is expressed by ON BCs shortly after their differentiation (*Grm6*–Cre mice; Fig. 6*C*, *SI Appendix*, Fig. S6 and *SI Materials and Methods*; ref. 28). As expected, we found that a loss of GABA$_A$γ2 in type 6 BCs caused a reduction in axonal but not dendritic GABA$_A$α1 receptors (Fig. 6 *C* and *D*). Together, these observations demonstrate that axonal but not dendritic GABA$_A$α1 receptors on BCs coassemble with the γ2 subunit, and lend support to the possibility that GABA$_A$ receptors with distinct compositions, rather than the same receptors at separate cell compartments, are differentially regulated by vesicular GABA release.

Discussion

Our observations corroborate previous findings showing that inhibitory neurotransmission is not essential for synapse formation but is necessary for synaptic maturation (8–11, 29). However, our findings here add significantly to our current understanding of
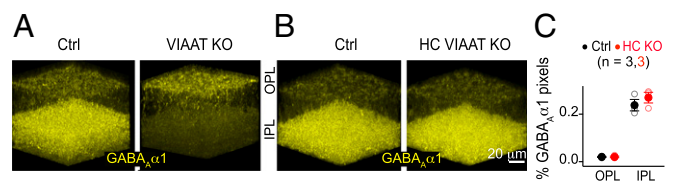

Fig. 5. Bipolar cell GABA$_A$ receptor alterations in VIAAT deficient retina are due to impaired vesicular GABA release from amacrine but not horizontal cells. (*A*) α1-subunit-containing GABA$_A$ (GABA$_A$α1) receptor immunolabeling in outer (OPL) and inner (IPL) plexiform layers of adult VIAAT knockout (KO) and littermate control (Ctrl) retinas (*n* = 10 KO–Ctrl pairs). (*B*) GABA$_A$α1 immunoreactivity when VIAAT is selectively deleted in horizontal cells (HCs) using Cx57-Cre mice (HC VIAAT KO; *n* = 3 KO–Ctrl pairs). (*C*) Quantification of the % of pixels in the GABA$_A$α1 receptor channel above background in OPL and IPL of Ctrl and HC VIAAT KO retinas. Number of animals is shown in parentheses.
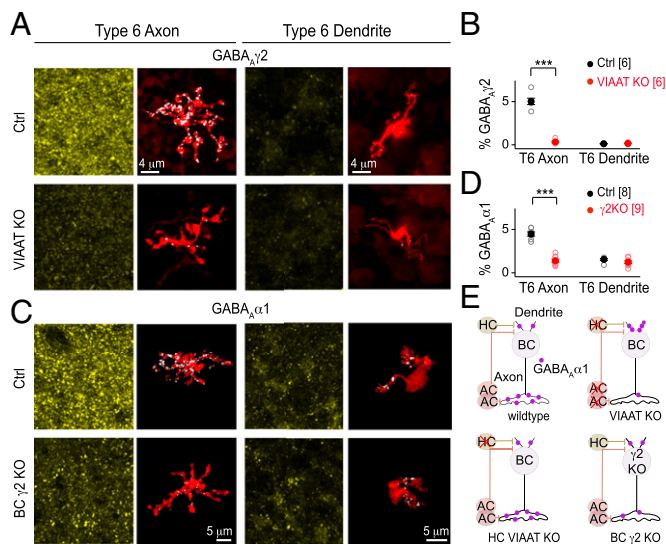
Fig. 6. Bipolar cell axonal and dendritic GABA$_A$ receptors have different subunit compositions. (*A*) γ2-subunit-containing GABA$_A$ (GABA$_A$γ2) receptor immunolabeling (yellow) in VIAAT knockout (KO) and littermate control (Ctrl) retinas in the same field as the labeled bipolar cell (BC). GABA$_A$γ2 receptors (white) on a type 6 cone BC (red). (*B*) GABA$_A$γ2% occupancy on the axonal and dendritic arbors of type 6 BCs in VIAAT KO and Ctrl. (*C*) GABA$_A$α1 receptor immunolabeling (yellow) in Ctrl retinas and retinas with ON BC-specific GABA$_A$γ2 deletion [BC γ2 KO: *GABA$_A$γ2* conditional knockout (cKO)/ *Grm6*-Cre mice]. Examples of GABA$_A$α1 receptors (white) on type 6 BC axons and dendrites (red). (*D*) Quantification of GABA$_A$α1 receptor occupancy (%) on type 6 BCs in γ2 KO and Ctrl. Error bars represent SEM. Number of cells is shown in parentheses; all genotypes, $n > 3$ animals. ***$P < 0.001$. (*E*) Schematic summarizing compartment-specific changes in the expression of BC axonal and dendritic GABA$_A$α1 receptors in pan-VIAAT KO (both horizontal cell (HC) and amacrine cell (AC) affected), HC-specific VIAAT KO (HC VIAAT KO), and BC-specific γ2 KO.

how neurotransmission influences the in vivo maturation of inhibitory circuits. We found that alterations in inhibitory receptor expression are not simply a general and uniform response to an overall loss of inhibitory drive in the network. Instead, (*i*) inhibitory transmitter release plays a central role in dictating the levels of GABA but not glycine receptor expression on retinal BCs, and (*ii*) activity-dependent regulation of GABA receptor expression is cell-compartment (axon versus dendrite) and input-type specific (summarized in Fig. 6*E*).

Activity-Dependent Regulation of Inhibitory Receptor Expression Varies with Presynaptic Input Type. The diversity of inhibitory connections onto retinal BCs enabled us to investigate the dependence of postsynaptic expression of inhibitory receptors on presynaptic input type. We found that glycine receptor expression on OFF CBC terminals is unchanged in VIAAT KOs even though GABA$_A$ receptors on the same axons are reduced. Spinal cord cultures from VIAAT KO animals also show no alteration in the density of glycine receptors (8). Even among GABAergic connections, the effects of neurotransmission on GABAergic synapse development and maturation have been found to vary with input types (30–32). After visual deprivation, transmission from basket interneurons onto dendrites of Layer 4 pyramidal cortical neurons in the visual cortex is reduced whereas input from regular spiking nonpyramidal interneuron is increased (31). Our study further uncovered distinct requirements for vesicular GABA release in regulating the maturation of synaptic contact from separate presynaptic GABAergic cell types on the axon and dendrite of an individual neuron. Comparing pan-VIAAT and HC-VIAAT KO retinas, we discovered that vesicular GABA release from amacrine but not horizontal cells regulates BC GABA receptors. Moreover, even among amacrine cells, expression of

GABA$_A$ receptors at amacrine synapses within the IPL versus the OPL are regulated in distinct ways. In the VIAAT KO, GABA$_A$α1 at synapses onto BC axons is down-regulated whereas its expression on the dendrites is up-regulated. Furthermore, different ionotropic GABA receptor types (GABA$_A$ and GABA$_C$) clustered at nonoverlapping sites (33, 34) but opposite the same presynaptic A17 amacrine cell bouton (35) are similarly down-regulated in the VIAAT KO. Together, these observations imply that activity-dependent regulation of inhibitory receptors is input type-specific.

Activity Differentially Controls α1-Subunit Containing GABA$_A$ Receptor Expression on Bipolar Cell Axons and Dendrites. A differential effect on axonal and dendritic GABA$_A$ receptors has been observed in hippocampal cell cultures in which chronic depolarization increased the GABA$_A$ receptor mobility in the axon initial segment but not in dendrites (36). We found that in vivo GABA$_A$α1 expression at input and output compartments of individual BCs are altered differently in the absence of GABA-mediated transmission. In VIAAT KO retina, cone bipolar axonal GABA$_A$α1 is lost whereas dendritic GABA$_A$α1 is up-regulated. The homeostatic increase of dendritic GABA$_A$α1 receptors in the absence of GABA release is reminiscent of the increase in glutamate receptors found in other brain regions when excitatory transmission is suppressed or blocked (37, 38). Homeostatic regulation of dendritic GABA$_A$α1 receptor levels in BCs, however, is pathway specific: Cone but not rod BCs maintain elevated GABA$_A$α1 levels in VIAAT KO retina at maturity. Differential effects on transmitter receptors on rod versus cone bipolar dendrites have also been observed in visually deprived mice where metabotropic glutamate receptors on cone but not rod BC dendrites are up-regulated (39).

What factors could be responsible for the distinct activity-dependent changes of receptor expression in BC axons and dendrites? Although contact with different amacrine subtypes could be responsible for the opposite outcomes, it is also possible that "postsynaptic" factors play a role. We found that GABA$_A$ receptors on the axons but not dendrites of BCs are composed of GABA$_A$γ2 subunit. Thus, differences in receptor subunit composition may explain why axonal and dendritic GABA$_A$ receptors of the same BC are regulated in opposite directions by GABA release. Our observations also raise the possibility that GABA$_A$ receptors with α1γ2 subunit composition could be highly susceptible to perturbed neurotransmission. The γ2 subunit is necessary for synaptic localization of GABA$_A$ receptors (3). Thus, α1γ2 receptors may be sensitive to loss of GABA release because they are clustered directly opposite transmitter release sites, whereas receptors further away may be less susceptible. GABA$_A$ receptors on BC axons are localized at synapses (11), but the location of BC dendritic GABA$_A$ receptors relative to GABA release sites in the OPL is not yet known. It is also possible that regardless of their location, α1γ2-subunit-containing GABA$_A$ receptors at all synapses require presynaptic GABA release for their maintenance. If so, one may find that such receptors on other neurons or at other cellular compartments exhibit the same form of regulation by GABAergic transmission.

A final factor to consider is that the effects of GABA release on synaptic development or maintenance could be "dose dependent." For example, reducing GABA synthesis by deleting one of the GABA synthetic enzymes, *GAD1*, from basket interneurons decreases synapse size and density, as well as axonal arbor size (9). In contrast, complete blockade of inhibitory transmission (VIAAT KO) yields opposite effects comprising overproliferation of small synapses and overgrowth of the axonal arbor (10). We also found that in retinas lacking *GAD1*, GABA$_A$α1 but not GABA$_C$ receptor expression on RBC terminals is diminished (11). However, our current observations show a loss of both receptor types in VIAAT KO retina. The disparate observations from *GAD1* and VIAAT retinal KOs may be explained by GABA$_C$ receptors having a higher affinity for GABA compared with GABA$_A$α1 (40). Lower levels of

GABA in the *GAD1* mutant could thus be sufficient to maintain GABA$_C$ receptors, but already trigger loss of GABA$_A$α1 receptors.

In summary, our study has distinguished the role of vesicular release of inhibitory transmitters per se from the effects produced by overall changes in network activity on the postsynaptic expression of inhibitory receptors in vivo. This regulation is specific to presynaptic input type, and varies with receptor subunit composition, cellular compartment, and the levels of transmitter release. Although complex, such diverse roles for inhibitory transmitter release provide a rich platform from which pre- and postsynaptic mechanisms can be selected to control the maturation and maintenance of distinct inhibitory connections within the network.

Materials and Methods

Transgenic Mouse Lines. All animal experiments were carried out in accordance with the Institutional Animal Care and Use Committee at the University of Washington and University of California, Los Angeles. *Vsx1-cerulean*, *Grm6-tdTomato*, *αPax6*-Cre/VIAAT KO, *Cx57*-Cre/VIAAT KO, and Ai9/ *GABA$_A$γ2* conditional KO/*Grm6*-Cre mouse lines were used in this study. Detailed information on the transgenic lines is provided in *SI Appendix, SI Materials and Methods*.

Immunolabeling. Retinas were isolated in cold oxygenated mouse artificial cerebrospinal fluid and fixed in 4% (wt/vol) paraformaldehyde. Primary antibodies were directed against: VIAAT, GFP, Synaptotagmin-2, GlyRα1-subunit, GABA$_A$α1-subunit, GABA$_A$α3-subunit, GABA$_A$γ2-subunit, GABA$_C$ρ-subunit, RFP, DsRed, PKC, and cone arrestin. Detailed information on immunohistochemistry is provided in *SI Appendix, SI Materials and Methods*.

Image Acquisition and Analysis. Samples were imaged using an Olympus FV 1000 laser scanning confocal microscope and images were processed using MetaMorph (Molecular Devices) and Amira (FEI Visualization Sciences Group) software. Detailed information on image analysis routines is provided in *SI Appendix, SI Materials and Methods*.

Functional Recordings and Data Analysis. Retinal slices (200 μm thick) were prepared from dark-adapted VIAAT KO and control mice as described (41). Detailed information on functional recordings and analyses is provided in *SI Appendix, SI Materials and Methods*.

Statistics. As the data passed the normality test, for control–KO comparisons, a two-tailed unpaired T test was used. For comparing cone contact numbers, Wilcoxon–Mann–Whitney rank sum test was used. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

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