The mode of retinal presynaptic inhibition switches with light intensity

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Excitatory amino acid transporters (EAATs) influence synaptic transmission by clearing glutamate from the synapse, limiting excitatory signals and restricting spillover (Higgs and Lukasiewicz, 1999; Matsui et al., 1999; Chen and Diamond, 2002; Rowan et al., 2010). EAATs are found on neurons and glia. Glial EAATs remove the bulk of released glutamate, while neuronal EAATs do not play a major role in glutamate clearance (Pow et al., 2000; Takayasu et al., 2005), with some exceptions, like EAATs on rod photoreceptors (Hasegawa et al., 2006). Glutamate also activates an EAAT-mediated Cl\(^{-}\) current that is independent of glutamate transport (Fairman et al., 1995; Wadiche et al., 1995). The Cl\(^{-}\) current magnitude varies with EAAT isoform. Large, glutamate-activated Cl\(^{-}\) currents are mediated by the neuronal EAAT4 and EAAT5 isoforms, found in the cerebellum and retina, respectively (Fairman et al., 1995; Arriza et al., 1997). These EAATs are located presynaptically and postsynaptically, but it is unclear whether they contribute to information processing within intact neural circuits.

The retina is an excellent model system for studying the roles of Cl\(^{-}\) currents mediated by neuronal EAATs. In fish retina, postsynaptic, EAAT-mediated Cl\(^{-}\) currents mediate cone input to depolarizing bipolar cells (BCs), while rod input is mediated by mGlu6 receptors (Grant and Dowling, 1995). This EAAT function may be limited to fish because both rod and cone inputs to mouse depolarizing BCs are mediated by mGluR6 (Masu et al., 1995; Gregg et al., 2007). In cerebellum, EAAT4 is found postsynaptically on Purkinje neurons (Takahashi et al., 1996; Otis et al., 1997), but its Cl\(^{-}\) current roles in information processing remain unknown. In retina, EAAT5 is presynaptically located on photoreceptors (Eliasof and Werblin, 1993; Hasegawa et al., 2006) and also on BC terminals (Palmer et al., 2003, Veruki et al., 2006; Wesinger et al., 2006). In cone photoreceptors, EAATs can either limit (Picaud et al., 1995) or enhance (Szmajda and Devries, 2011) transmitter release. In rod bipolar cells (RBCs), dynamic-clamp experiments suggest that EAAT-evoked Cl\(^{-}\) current limits transmitter release (Veruki et al., 2006). However, the roles of EAATs in visual processing are poorly understood.

Here, we show that EAAT-mediated Cl\(^{-}\) conductances in RBCs contribute to visual information processing. RBCs mediate rod signaling and receive GABA and glycine receptor-mediated inhibition that is evoked by dim light. We found that bright light suppressed receptor-mediated inhibition and activated EAAT-mediated inhibition in RBCs. This demonstrates that RBC terminals switch their mode of inhibition with light intensity. The two forms of inhibition are functionally distinct. GABAergic inhibition was mediated by wide-field amacrine cells, involved in rod signal processing. EAAT-mediated inhibition was caused by glutamate release from the RBCs, which activated a chloride conductance. The narrow dimensions of EAAT-mediated inhibition were attributed to the limited extent of glutamate spillover. EAAT-mediated inhibition complements transmitter-mediated inhibition to extend the dynamic range of inhibition and reduce rod pathway signaling in bright-light conditions, contributing to the transition from rod to cone signaling.
technological advances were similar to those described previously (Eggers and Lukasiewicz, 2006a). Mice of either sex (28–60 d of age; C57BL/6J strain; The Jackson Laboratory) were dark-adapted overnight and were killed using carbon dioxide. The retina was isolated, and either slice (250 μm thickness, for electrophysiological experiments) or whole-mount preparations (for FM1-43 experiments) were made. All dissection procedures were performed under infrared illumination. Dissection medium (see below, Solution and drugs) was cooled and continuously oxygenated. The retinal preparations were stored in an oxygenated dark box at room temperature.

Whole-cell recordings and perforated patch-clamp recordings. Whole-cell patch recordings were made from BC somas in retinal slices by viewing them with an upright microscope (Nikon Instrument) as described previously (Eggers and Lukasiewicz, 2006a). In some experiments for recording light-evoked voltage responses (see Fig. 6), perforated patch-clamp method was used. Amphotericin B solubilized (0.3 mg/ml) (A9528; Sigma-Aldrich) was dissolved in the intracellular solution. The series resistance of whole-cell clamp and perforated patch clamp was 9.6 ± 0.7 and 11.6 ± 0.3 MΩ (p < 0.05), respectively. Inhibitory currents were recorded from BCs voltage clamped at 0 mV, the reversal potential of GABA had been adjusted to 0 mV. The data were digitized and stored with a personal computer using a Labmaster DMA data acquisition board (Scientific Solutions). The retinal preparations were stored in an oxygenated dark box at room temperature. 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Image sometimes shifted by perfusion, which was corrected by Adobe Photoshop 7.0 software (Adobe Systems). However, if the focus shifted, we discarded the data. FM intensity was normalized to the terminal brightness level at either time 0 or 20 s (see Fig. 7E, F).

**Immunocytochemistry.** Anti-protein kinase Ca (PKCa) labeling was performed by using fixed tissue after FM1-43 was loaded. After fixation with 4% paraformaldehyde for 30 min, the tissue was washed with PBS and exposed to blocking solution (10% normal goat serum, 0.2% Triton X) with anti-PKCa (1:1000; Sigma-Aldrich) for 2 d at 4°C. Then primary antibody was washed with PBS with 10% normal goat serum, and Alexa 633-conjugated anti-mouse antibody (1:1000; Invitrogen) was applied for 2 h at 4°C. After secondary antibody was washed with PBS, tissue was mounted using SlowFade (Invitrogen) for confocal microscope observation.

**Data analysis.** For all of the experiments, charge transfer (Q) was measured and normalized using Tack analysis software (White Perch Software). Two-tailed, paired t test was used to determine whether responses to agonists or antagonists in the same cell were significantly different. Unpaired t tests were performed to compare means of two independent samples (see Fig. 2). Spearman’s rank test was used to test the correlation between FM1-43 unloading and CPPG concentration (see Fig. 7D). In the text, values are presented as mean ± SEM, and differences were considered significant if p < 0.05.

**Results**

**Bright light evoked a receptor-independent inhibitory current in rod bipolar cells**

Dim light stimuli evoked inhibitory Cl− currents in RBCs that are mediated by amacrine cell activation of GABA and glycine receptors on RBC axon terminals (Eggers and Lukasiewicz, 2006a). We confirmed these findings by showing that a mixture of glycine, GABA_A, and GABA_C receptor blockers eliminated inhibitory currents evoked by dim light (Fig. 1A), indicating that these currents were entirely mediated by inhibitory transmitter receptors. Unexpectedly, brighter light stimuli evoked a large inhibitory current (Fig. 1B, left) that was unaffected by GABA and glycine receptor antagonists (Fig. 1B, middle). We ruled out the possibility that the large inhibitory currents were caused by enhanced activation of GABA_C receptors that were inadequately blocked by 50 μM TPMPA, as previously reported (Hull et al., 2006). Bright light-evoked inhibitory currents were similar when either 50 or 150 μM TPMPA was in the inhibitory receptor blocker mixture (1.11 ± 0.38 and 1.03 ± 0.29 fraction of control, respectively; p = 0.56), indicating that the unblocked currents were not mediated by GABA and glycine receptors.

**EAA Ts have been shown to elicit Cl− currents in RBC terminals (Palmer et al., 2003; Veruki et al., 2006; Wersinger et al., 2006). We also evoked Cl− currents by puffing glutamate onto RBC terminals (Fig. 2A).** The puff-evoked currents reversed polarity near ECl and were blocked by the EAAT inhibitor TBOA (Shimamoto et al., 1998; Shigeri et al., 2001), confirming that EAATs were present on RBC terminals. We then tested whether the bright light-evoked, inhibitory receptor antagonist-resistant...
current was mediated by EAATs. TBOA abolished the light response that was resistant to the inhibitory receptor blockers (Fig. 1 B, right), suggesting that EAATs mediated bright light-evoked inhibitory currents in RBCs. The source of glutamate that mediates the light-evoked EAAT currents could be from either the recorded RBC and/or neighboring BCs, via spillover transmission (Veruki et al., 2006). However, here the RBC is voltage clamped and unable to release glutamate. Thus, for voltage-clamp experiments, the EAATs were activated by spillover transmission from neighboring BCs. However, for current-clamp experiments (see Fig. 6), EAATs may be activated by release from the recorded RBC and neighboring BCs. While previous work has shown that RBCs receive light-evoked GABAergic and glyciner-
rents, which are mainly mediated by inhibitory receptors (Fig. 1A). Intracellular TBOA did not reduce the dim light-evoked inhibitory currents (Fig. 2B-2), confirming that it did not affect GABA and glycine receptors. Together, these data support our findings that receptor- and EAAT-mediated inhibition largely operates over different light intensity ranges (Fig. 1C). Dim light evoked mainly GABA and glycine inputs, in agreement with earlier observations (Eggers and Lukasiewicz, 2006a). Bright, but not dim, light evoked mainly EAAT inputs, consistent with the higher levels of RBC depolarization and spillover transmission that are required to activate perisynaptic EAATs (Veruki et al., 2006).

**EAAT currents depend on the extent of RBC depolarization**

We tested whether the lower light sensitivity for EAAT-mediated responses was attributable to the amount of glutamate release in the IPL. We bypassed transmission from photoreceptors to RBC dendrites and directly depolarized RBCs with the mGluR6 antagonist CPPG, which opens nonspecific cation channels in RBCs (Snellman and Navy, 2002). CPPG-evoked depolarizations of RBCs mimic electrical (Snellman et al., 2009) and light stimuli (Kalbaugh et al., 2009), consistent with CPPG only acting at mGluR6 sites. CPPG-evoked glutamate release from BCs elicited inhibitory currents in RBCs that were attributed to the activation of EAATs and inhibitory transmitter receptors. Similar to the dim light-evoked inhibitory currents (Fig. 1), a lower concentration of CPPG elicited small inhibitory currents (Fig. 3A, left; C) that were mostly eliminated with the inhibitory receptor blockers (Fig. 3A, middle and right; C). By contrast, a higher concentration of CPPG elicited larger inhibitory currents that were only slightly reduced by the inhibitory receptor blockers (Fig. 3B). The inhibitory receptor blockers diminished the fast synaptic activity that is characteristic of GABA and glycine receptor activity (see below) (Fig. 3B, middle; C). The addition of TBOA abolished the remaining current (Fig. 3B, right; C), suggesting that EAATs in RBC terminals mediated the majority of the inhibitory current evoked by the higher concentration of CPPG.

These results showed that large and small depolarizations evoked by CPPG, elicited predominantly EAAT- or transmitter-mediated inhibition, respectively. EAATs were only activated by a strong depolarization, which likely resulted in more glutamate release and spillover (Veruki et al., 2006). These findings confirm that the relative contributions of receptors and EAATs to inhibition of the RBC terminals depend on stimulus intensity. We then examined whether the temporal and spatial properties of EAAT and receptor-mediated inhibition differed.

**The temporal properties of receptor- and EAAT-mediated inhibition differ**

The time course of light-evoked GABA- and glycine-mediated inhibition to RBC terminals is largely determined by postsynaptic receptor properties (Frech and Backus, 2004; Eggers and Lukasiewicz, 2006a,b). Fast GABA<sub>A</sub> and glycine receptor-mediated spontaneous currents and slower GABA<sub>B</sub> and/or EAAT-mediated spontaneous currents were recorded in control solution (Fig. 4A, Control, 4.3 Hz; n = 4). After GABA and glycine receptors were pharmacologically blocked, we only observed relatively infrequent spontaneous currents with a slow time course (Fig. 4A, Inhibitory R blockers, 0.03 Hz; n = 4). The slow spontaneous currents were eliminated by TBOA (Fig. 4A, TBOA + Inhibitory R blockers), indicating that they were mediated by EAATs. The time course of spontaneous EAAT-mediated currents was dramatically slower than the GABA and glycine receptor-
mediated currents (Fig. 4B, Table 1). These findings show that inhibitory transmitter receptors and EAATs on RBC terminals each mediate responses with distinct time courses. The slow onset and lower frequency of the spontaneous EAAT-mediated current may be attributable to spillover and the effects of glutamate clearance, respectively (Veruki et al., 2006).

The spatial properties of light-evoked EAAT- and GABA-mediated inhibition differ

GABA mediates the predominant inhibitory input to RBCs and serves both feedback (Vigh and von Gersdorff, 2005; Chávez et al., 2006) and lateral signaling (Eggers and Lukasiewicz, 2006a; Vigh et al., 2011). The spatial dimensions of light-evoked EAAT-mediated inhibition are unknown, but are likely determined by the extent of spillover transmission from neighboring BCs (Veruki et al., 2006). The spatial dimensions of light-elicted GABA inhibition in the mouse retinal slice can be up to 800 μm (Eggers and Lukasiewicz, 2010). Using light stimuli of increasing size, we measured the spatial dimensions of EAAT-mediated inputs to RBCs. EAAT-mediated inputs were pharmacologically isolated by including a mixture of inhibitory receptor blockers in the bath. To enhance the signal-to-noise ratio and maintain long-lasting recordings of t-IPSCs, we voltage clamped RBCs to −60 mV. Because we excluded ATP and GTP in a recording pipette, excitatory currents rapidly ran down and did not contaminate the inhibitory responses (Fig. 5). Rundown of the excitatory currents was attributable to the washout of components of the mGluR6 signaling cascade. This was confirmed by showing that the inward current in Figure 5A was blocked by TBOA, demonstrating that it was mainly an EAAT-mediated current (data not shown). Figure 5A shows that the EAAT-mediated inhibitory current increased as a function of spot size and plateaued when the spot was 110 μm in diameter. Because we voltage clamped the RBC and prevented it from releasing glutamate, the CI− current was mediated by spillover from neighboring RBCs. The receptive field center of RBC is 68 μm in diameter (Bermint and Taylor, 2000), and RBCs within 15 μm of each other interact via glutamate spillover (Veruki et al., 2006). The combination of these two measurements gives an estimate of the spatial extent for EAAT-mediated inhibition of 100 μm, in good agreement with our measurement of 110 μm. The spatial extent of light-evoked, EAAT-mediated inhibition is significantly narrower than GABA-mediated inhibition.

Light intensity determines the spatial extent of inhibition in RBCs

Our findings show that GABA receptor-mediated inhibition is more sensitive to light compared with EAAT-mediated inhibition (Fig. 1C). The intracellular TBOA experiments indicate that inhibition switches from receptor-mediated to largely EAAT-mediated inhibition when the stimulus intensity was increased (Fig. 2B). We measured the spatial extent of inhibition to RBCs, using dim- and bright-light stimuli, to determine whether the mode of RBC inhibition depends on stimulus intensity. Because the spatial extents of GABA receptor- and EAAT-mediated inhibition vary so dramatically, the contributions of each form of light-evoked inhibition can be estimated from their distinct spatial properties. The spatial extents of each form of inhibition should depend on light stimulus intensity. Since the maximal GABA receptor-mediated inhibition was evoked by a 400 μm stimulus (Eggers and Lukasiewicz, 2010), while maximal EAAT-mediated inhibition was evoked by a 110 μm stimulus (Fig. 5A), we compared the inhibitory currents evoked by wide-field (400 μm) and narrow-field (150 μm) light stimuli. In the absence of any blockers, responses to dim light evoked by wide field stimuli (Fig. 5B) were always larger than those evoked by narrow-field stimuli (Fig. 5B). These findings show that inhibition occurred over a broad area, suggesting that wide-field GABAergic inputs mediated dim light-evoked inhibition. By contrast, when bright light stimuli were used, there were no differences in the inhibitory currents evoked by narrow- and wide-field bright-light stimuli (Fig. 5B, Bright 150 and 400 μm), indicating that inhibition occurred over a narrow area. These findings suggest that narrow-field EAAT-mediated inputs, but not wide-field GABAergic inputs, mediated bright-light-evoked inhibition. The switch in spatial profiles that occurred with light intensity demonstrates that inhibition switched from receptor-mediated to EAAT-mediated when intensity was increased.

EAATs regulate light-evoked EPSPs in RBCs

Does EAAT-mediated inhibition affect visual responses in the rod signaling pathway? Although EAATs evoke CI− currents in RBCs (Veruki et al., 2006; Wersinger et al., 2006), it is not known...
whether these currents affect light-evoked voltage responses in RBCs. To determine whether EAATs affect RBC light responses, we recorded bright-light-evoked EPSPs (L-EPSPs) in the presence or absence of TBOA that was puffed onto the RBC terminals. We pharmacologically isolated the EAAT-mediated current using a mixture of GABA and glycine receptor blockers. TBOA puffs enhanced the L-EPSPs (Fig. 6A, left), suggesting that glutamate released from the recorded RBC and neighboring BCs activated EAAT-mediated inhibition that reduced L-EPSPs in RBCs. TBOA had minimal effects on the resting membrane potential and caused, on average, a 1 mV depolarization. Similarly, Veruki et al. (2006) show that TBOA does not affect resting holding currents in rat RBCs. These findings suggest that little spillover activation of EAATs occurs at the RBC resting potential.

We also determined whether the selective activation of EAAT-mediated inhibition affected L-EPSPs recorded from RBCs. We activated EAATs on RBC terminals with focal puffs of D-aspartate, an EAAT agonist (Arriza et al., 1997). We used D-aspartate to activate EAATs in RBC terminals not only because it is an EAAT agonist but also, unlike glutamate, it does not affect mGluR6 in RBCs. Puffing D-aspartate reduced L-EPSPs in RBCs (Fig. 6A, middle), suggesting that EAAT-mediated inhibition reduces L-EPSPs. Puffs of D-aspartate slightly hyperpolarized the resting membrane potential, on average, by 2 mV. The slight hyperpolarization is attributed to the small driving force for Cl\(^-\) (\(-10 \text{ mV}\)). The interpretation of our results depends on the puffed ligands being confined to the RBC axon terminals and not spreading to the OPL where EAATs on photoreceptors could be affected, leading to indirect network effects (Hasegawa et al., 2006). OPL effects of puffs onto terminals were unlikely because cone BCs that do not have EAAT-mediated inhibition (Fig. 1D) were not affected by either aspartate or TBOA (Fig. 6B). These findings indicate that the puffs were confined to the RBC terminal area. Together, these results suggest that EAAT-mediated Cl\(^-\) currents in RBCs significantly reduce light-evoked excitation exclusively in the rod pathway.

**Exocytosis from RBC axon terminals is regulated by EAATs**

EAAT-mediated inhibition reduced light-evoked voltage responses in RBCs. Does this response reduction affect RBC output signaling? We were unable to evaluate the effect of EAATs on glutamate release by recording EPSCs from postsynaptic neurons because TBOA also reduces the clearance of glutamate, enhancing EPSCs in the postsynaptic neuron, in addition to blocking inhibition in RBC terminals. Furthermore, bath-applied TBOA may have network effects that complicate the interpretation of its direct effect on glutamate release. To
overcome these difficulties, we assessed the effects of TBOA on glutamate release by using the fluorescent styryl dye FM1-43 to directly visualize vesicular exocytosis at RBC axon terminals.

Using a whole-mount retinal preparation (Fig. 7A), we loaded FM1-43 into synaptic vesicles in RBC terminals by selectively depolarizing RBCs and ON CBCs with CPPG. Ionotropic glutamate receptor blockers (CNQX and D-AP5) were included in the bath to avoid depolarizing OFF BCs and amacrine cells whose terminals are also in the IPL. A confocal micrograph of the proximal region of the IPL, 10–20 µm below the surface of the ganglion cell layer, revealed spheres 2–3 µm in diameter that were tentatively identified as RBC axon terminals (Fig. 7B). This depth of the IPL corresponds to sublaminae 4–5, which are the strata where RBC terminals ramify (Ghosh et al., 2004; Pignatelli and Strettoi, 2004). We confirmed that these spheres were RBC terminals by demonstrating that the FM1-43-positive processes colocalized with an antibody for PKCoα, a specific marker for mouse RBCs (Fig. 7B) (Wässle et al., 1991, 2009). In addition to RBC terminals, small FM1-43-labeled puncta (<1 µm) were also observed in the same focal plane, which were most likely ON CBC release sites because they were PKCoα negative. Two control experiments also confirm that RBC labeling by FM1-43 was specific. First, when RBCs were maintained in a hyperpolarized state of the number of bright flashes and was not observed when RBCs were maintained in a hyperpolarized state with t-AP-4 (indicating that the reduction of FM1-43 fluorescence was not attributable to photo-bleaching). Thus, light-evoked and CPPG-evoked depolarizations both unload FM1-43. In the subsequent experiments, however, we used CPPG to monitor unloading because we could not use light to monitor real-time FM1-43 unloading from RBC terminals.

We tested whether EAATs affected exocytosis from RBC terminals by monitoring the effects of an EAAT agonist and an EAAT antagonist upon FM1-43 unloading. We applied the EAAT antagonist TBOA to determine whether EAAT-mediated inhibition modulated exocytosis from RBCs. We used a submaximal concentration of CPPG (200 µM) to elicit FM1-43 unloading. TBOA (100 µM) enhanced the CPPG-evoked FM1-43 unloading (Fig. 7F), suggesting that EAATs normally limit exocytosis from RBCs. We then determined the effects of activating EAATs upon RBC exocytosis by applying the EAAT agonist d-aspartate (1 mM). D-Aspartate reduced the magnitude of the CPPG-elicited FM1-43 unloading (Fig. 7F), suggesting that EAAT-mediated Cl− currents reduced exocytosis. Together, our findings show that the EAAT-mediated inhibition can regulate exocytosis from RBC terminals.

by including t-AP-4 in the bath, we never observed FM1-43 label in RBC terminals (data not shown). Second, when cobalt was present to block vesicular release and its subsequent endocytosis, CPPG-induced depolarization failed to label RBC terminals with FM1-43 (data not shown).

After loading RBC terminals with FM1-43, we monitored exocytosis by measuring FM1-43 unloading in response to a puff of light by including TBOA in the bath, we never observed FM1-43 label in RBC terminals (data not shown). Second, when cobalt was present to block vesicular release and its subsequent endocytosis, CPPG-induced depolarization failed to label RBC terminals with FM1-43 (data not shown).

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**Discussion**

EAAT5 is found on RBC axon terminals and mediates a large Cl⁻ current (Veruki et al., 2006; Wersinger et al., 2006). Here, we show that an EAAT-activated Cl⁻ current mediated light-evoked inhibition in RBCs. EAAT-mediated inhibition complemented conventional GABA-mediated inhibition in the RBC terminals. We found that the mode of presynaptic inhibition switched with light intensity. Dim light elicited GABAergic ama-
crine input to RBCs that was minimally activated by a bright light (see below). Bright light strongly depolarized RBCs and enhanced glutamate release, leading to spillover activation of EAAT-mediated Cl currents. EAAT-mediated inhibition contributed to retinal signaling by reducing RBC voltage responses to light, reducing RBC exocytosis, and extending the dynamic range of light-evoked inhibition. Light-evoked EAAT-mediated inhibition extends the dynamic range of inhibition by complementing receptor-mediated inhibition. This results in reduced rod pathway signaling in bright-light conditions, contributing to the transition from rod to cone signaling.

**EAAT-mediated inhibition is physiologically activated by light**

We found that inhibitory currents in RBCs terminals, evoked by bright light, were relatively insensitive to inhibitory receptor blockers, but reduced by the EAAT antagonist TBOA. We ruled out network effects of bath-applied TBOA by obtaining similar results using intracellularly applied TBOA to block EAATs (Fig. 2). EAAT-mediated Cl⁻ currents were evoked in RBC axon terminals, in agreement with Palmer et al. (2003) and Veruki et al. (2006). Although Wersinger et al. (2006) suggest that a small number of EAATs are also localized to the dendrites, they are unlikely to contribute to light responses because light- and glutamate-evoked responses at RBC dendrites are eliminated in mGluR6 knock-out and NOB mice (Masu et al., 1995; Gregg et al., 2007). Activation of EAATs reduced both the light-evoked voltage responses and exocytosis in RBCs. Furthermore, blocking EAATs on RBCs enhanced their light-evoked voltage responses and exocytosis (Figs. 6, 7). These findings suggest that strong depolarization of RBCs by bright light results in sufficient glutamate release to activate EAATs in RBC terminals.

The source of glutamate activating EAATs might be either from the same BC terminal (Palmer et al., 2003), or from neighboring BCs via spillover transmission (Veruki et al., 2006). When recording EAAT-mediated Cl⁻ currents in voltage-clamp mode, light-elicited glutamate release did not occur from the RBC, but by spillover transmission from neighboring BCs. Bright, but not dim, lights elicited EAAT-mediated currents, consistent with greater glutamate release and spillover. The magnitude of the EAAT-mediated current is probably larger in physiological conditions when RBCs are not voltage clamped and can respond to their own glutamate release.

The properties of EAAT- and receptor-mediated inhibition differ

EAAT-mediated spontaneous currents were significantly slower than spontaneous currents mediated by GABA_A, GABA_C, and glycine receptors (Fig. 4, Table 1). The kinetics of spontaneous GABA and glycine currents mostly reflects receptor properties and is largely independent of transmitter release mechanisms. Veruki et al. (2006) show that EAAT-mediated spontaneous currents in RBCs are slower than EAAT-mediated currents evoked by glutamate application to excised patches, suggesting that spillover transmission shapes the slow spontaneous responses of EAATs. Spontaneous EAAT-mediated currents decayed almost fourfold slower than GABA_A,R-mediated currents (Fig. 4B), attributable to the time course of glutamate clearance (Otis and Jahn, 1998; Wadiche et al., 2006). However, the latency of light-evoked, EAAT-mediated inhibition was 66.7 ± 16 ms (n = 3) shorter than that of receptor-mediated inhibition in RBCs. Latency differences were likely attributable to the bright- and dim-light intensities used to evoke EAAT- and receptor-mediated inhibition, respectively. Both types of inhibition are driven by RBCs. The RBC excitatory light response latency is ~70 ms shorter for bright stimuli compared with dim stimuli (Euler and Masland, 2000; Trexler et al., 2005). The similar onset differences for excitatory and inhibitory RBC responses suggest that light intensity was the major determinant of latency differences between EAAT- and receptor-mediated inhibition. Synaptic delay differences for EAAT- and receptor-mediated inhibition may also contribute to the latency differences.

The spatial extent of inhibition varies in the retina. Retinal inhibition is classified into narrow-field and wide-field types (Wassle, 2004). Glycinergic amacrine cells mediate narrow field inhibition and signal between different sublaminae within the IPL, while GABAergic amacrine cells mediate wide-field inhibition and signal within given sublaminae in the IPL (Roska and Werblin, 2001). Furthermore, mouse RBCs and goldfish MB1 BCs receive distinct GABAergic inputs via two retinal circuits. One circuit mediates reciprocal feedback, whereas another circuit mediates nonreciprocal lateral inhibition (Chavez et al., 2010; Vigh et al., 2011). In mouse, the former is narrow field (up to 50 μm) and the latter is wide field (up to 800 μm). We found that the spatial extent of light-evoked, EAAT-mediated inhibition was intermediate to these dimensions (~110 μm) (Fig. 5A) and in good agreement with previous RBC receptive field and spillover measurements (Bernston and Taylor, 2000; Veruki et al., 2006). The narrow extent of spillover is likely limited by Muller cell EAATs that are mostly responsible for glutamate clearance (Higgs and Lukasiewicz, 1999).

We found that the type and spatial extent of RBC inhibition depended on light stimulus intensity. The light sensitivities of the two inhibitory inputs were distinct and largely non-overlapping (Fig. 1C), suggesting that each input is activated by different light intensities. Dim stimuli elicited wide-field, GABA receptor-mediated inhibition. GABAergic amacrine cells are components of the rod signaling pathway (Nelson and Kolb, 1985), accounting for the higher light sensitivity of this inhibition. Bright stimuli elicited narrow-field, EAAT-mediated inhibition that was less light sensitive. The lower light sensitivity is attributable to the larger depolarization and greater glutamate release necessary to activate EAATs on RBC terminals (Veruki et al., 2006). Because of these sensitivity differences, EAAT-mediated inhibition is only half activated at rod saturation, while GABA-mediated inhibition is almost saturated (Fig. 1C). Our findings show that the type and spatial extent of inhibition in RBCs changes with light intensity, attributable to a switch from GABA receptor-mediated to EAAT-mediated inhibition. The wide-field GABAergic inhibition contributes to surround inhibition in RBC (Bloomfield and Xin, 2000) and the narrow-field EAAT-mediated inhibition extends the dynamic range of presynaptic inhibition to limit rod signaling in bright-light conditions.

**Function of EAATs in the rod signaling pathway**

Bright light near rod saturation only minimally activated GABAergic and glycineric inhibition in RBCs (Figs. 2, 5B). Similarly, for AII amacrine cells in the rod-signaling pathway, GABA-mediated surround inhibition was activated by dim-light conditions, but not by bright light (Xin and Bloomfield, 1999). The mechanism by which GABA signaling is suppressed in bright-light conditions is not known and beyond the scope of the present study. Our findings suggest that this suppression is not attributable to inhibitory receptor saturation because GABA and glycine antagonists do not affect the holding current in bright-light conditions. Furthermore, Eggers and Klein (2010) show that
light-evoked inhibition is suppressed and the frequency of spontaneous IPSCs is lowered in bright-light conditions, consistent with reduced transmitter release. However, the RBC is still activated beyond rod saturation and exhibits a prolonged depolarization even after the light stimulation is terminated (Dacheux and Raviola, 1986; Euler and Masland, 2000). We found that bright light switches the mode of RBC inhibition to the EAAT-mediated type. EAAT-mediated inhibition is absent in dim light and does not interfere with GABA-mediated surround inhibition. When surround inhibition is absent in bright light, EAAT-mediated inhibition becomes apparent and suppresses rod pathway signaling.

High-sensitivity rod photoreceptors and low-sensitivity cone photoreceptors operate over dim and bright light intensity ranges, respectively. In mesopic light conditions, both rod and cone signaling pathways mediate visual information. Over the mesopic intensity range, signaling from both pathways is not always additive (Enroth-Cugell et al., 1977). Instead, rod and cone signals are more likely to be mutually suppressive (Gouras and Link, 1966; Arden and Hogg, 1985; Buck, 2004). Light stimulation near rod saturation evokes a prolonged depolarization in RBCs (Dacheux and Raviola, 1986). However, this prolonged RBC signal is not observed in ganglion cells, suggesting that it is either not transmitted or it is cancelled by suppressive, cone-generated signals (Gouras and Link, 1966; Steinberg, 1969). Since EAAT-mediated inhibition in the RBC terminals is activated at this light level, it might play a role in the suppression of rod signaling in mesopic conditions, contributing to the rod–cone signaling transitions.

References


