Published in final edited form as:

Eur J Neurosci. 2008 May ; 27(10): 2575–2586. doi:10.1111/j.1460-9568.2008.06226.x.

Quantal mEPSCs and residual glutamate: how horizontal cell responses are shaped at the photoreceptor ribbon synapse

Lucia Cadetti^{1,2}, Theodore M. Bartoletti^{1,3}, and Wallace B. Thoreson^{1,3}

¹Department of Ophthalmology and Visual Sciences, University of Nebraska Medical Center, Durham Research Center Room 4050, Omaha, NE 68198-5840, USA

²Laboratory of Cellular and Molecular Physiopathology of the Retina, National Institute for Health and Medical Research (INSERM Unité 592), Université Pierre et Marie Curie-Paris, Paris, France

³Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE, USA

Abstract

At the photoreceptor ribbon synapse, glutamate released from vesicles at different positions along the ribbon reaches the same postsynaptic receptors. Thus, vesicles may not exert entirely independent effects. We examined whether responses of salamander retinal horizontal cells evoked by light or direct depolarization during paired recordings could be predicted by summation of individual miniature excitatory postsynaptic currents (mEPSCs). For EPSCs evoked by depolarization of rods or cones, linear convolution of mEPSCs with photoreceptor release functions predicted EPSC waveforms and changes caused by inhibiting glutamate receptor desensitization. A low-affinity glutamate antagonist, kynurenic acid (KynA), preferentially reduced later components of rod-driven EPSCs, suggesting lower levels of glutamate are present during the later sustained component of the EPSC. A glutamate-scavenging enzyme, glutamic-pyruvic transaminase, did not inhibit mEPSCs or the initial component of rod-driven EPSCs, but reduced later components of the EPSC. Inhibiting glutamate uptake with a low concentration of DL-threo- β -benzoyloxyaspartate (TBOA) also did not alter mEPSCs or the initial component of rod-driven EPSCs, but enhanced later components of the EPSC. Low concentrations of TBOA and KynA did not affect the kinetics of fast cone-driven EPSCs. Under both rod- and cone-dominated conditions, light-evoked currents (LECs) were enhanced considerably by TBOA. LECs were more strongly inhibited than EPSCs by KynA, suggesting the presence of lower glutamate levels. Collectively, these results indicate that the initial EPSC component can be largely predicted from a linear sum of individual mEPSCs, but with sustained release, residual amounts of glutamate from multiple vesicles pool together, influencing LECs and later components of EPSCs.

Keywords

glutamate; light response; retinal photoreceptors; salamander; synaptic transmission

Introduction

At many synapses, postsynaptic responses appear to be constructed from a sum of quantal events reflecting the independent actions of individual synaptic vesicles (del Castillo &

Katz, 1954; Redman, 1990; Sakaba *et al.*, 2002). Many conventional synapses have a single presynaptic release site adjacent to a single postsynaptic density, and incoming action potentials stimulate release of only a few vesicles (Auger & Marty, 2000). Glutamate transporters surrounding the active zone at excitatory synapses limit the persistence of glutamate in the synapse and spillover onto neighboring synapses (Diamond & Jahr, 1997, 2000; Higgs & Lukasiewicz, 1999). Together, these factors help to keep the effects of vesicles independent from one another.

In contrast with conventional synapses, photoreceptor synapses have a deep invaginating cleft that envelopes multiple postsynaptic processes. This enlarges the synaptic cleft, yielding a volume at mammalian rod terminals that is 20-fold greater than conventional synapses (Rao-Mirotznik *et al.*, 1995). Vesicles are released continuously in darkness at rates of 20 - 100 vesicles/ribbon/s (Ashmore & Copenhagen, 1983; Rao-Mirotznik *et al.*, 1998; Berntson & Taylor, 2003), and each photoreceptor ribbon contains multiple release sites (~25/ribbon; Heidelberger *et al.*, 2005). The glutamate released from a single vesicle can diffuse to multiple postsynaptic processes (Rao-Mirotznik *et al.*, 1998; DeVries *et al.*, 2006). Thus, at high rates of release, glutamate released by different vesicles may reach the same postsynaptic receptors at the same time and obscure the independence of individual mEPSCs.

Glutamate transporters on photoreceptors (Eliasof & Werblin, 1993; Grant & Werblin, 1996; Rabl *et al.*, 2003; Hasegawa *et al.*, 2006) cluster at the base of the terminal, outside the cleft (Vandenbranden *et al.*, 2000; Hasegawa *et al.*, 2006). Transporters are also present on adjacent Müller cell processes (Sarantis & Mobbs, 1992; Burriss *et al.*, 2002). With few transporters within the synapse, the residual glutamate remaining after individual miniature excitatory postsynaptic currents (mEPSCs) can pool together during sustained release (Trussell *et al.*, 1993; Barbour *et al.*, 1994; Mennerick & Zorumski, 1995; Rossi *et al.*, 1995; Otis *et al.*, 1996). Consistent with pooling of glutamate at photoreceptor synapses, horizontal cell light responses can be modeled by ignoring individual mEPSCs and assuming that glutamate levels are spatially integrated throughout the synapse (Roska *et al.*, 1998).

Our goal was to test whether responses of second-order retinal neurons, horizontal cells, can be matched by a sum of individual quantal mEPSCs, or whether residual glutamate plays a significant role in shaping responses at rod and cone synapses. We defined 'residual glutamate' as glutamate in the synaptic cleft that produces postsynaptic effects that are not reflected in the mEPSC waveform. We analysed effects of glutamate-related drugs on mEPSCs and responses of horizontal cells evoked by light or depolarizing test steps applied to simultaneously recorded photoreceptors. The results indicate that when stimulated by rapid depolarization, the photoreceptor synapse produces transient EPSCs that can be largely predicted by summing a series of mEPSCs. However, responses of horizontal cells evoked by light or sustained depolarization during paired recording are strongly influenced by levels of residual glutamate in the synapse.

Materials and methods

Preparations

Experiments were performed using retinas from the aquatic tiger salamander (*Ambystoma tigrinum*, 18–25 cm). Care and handling procedures were approved by the UNMC Institutional Animal Care and Use Committee. The salamander was decapitated, and the brain and spinal cord were rapidly pithed. After enucleation, the front of the eye, including the lens, was removed. The resulting eyecup was cut into quarters, and a section of eyecup was placed vitreal side down on a piece of filter paper (2 × 5 mm, Type AAWP, 0.8 µm pores; Millipore, Bedford, MA, USA) and isolated in a cold amphibian saline solution (see

composition below). Retinal slices (125 μm) were cut with a razor blade tissue chopper (Stoelting, Wood Dale, IL, USA) and placed in a recording chamber to allow viewing of the retinal layers with an upright fixed-stage microscope (Olympus BHWI, Tokyo, Japan or Nikon E600 FN, Tokyo, Japan).

Slices were superfused at ~ 1 mL/min with an oxygenated amphibian saline solution containing (in mM): NaCl, 111; KCl, 2.5; CaCl_2 , 1.8; MgCl_2 , 0.5; HEPES, 10; glucose, 5; picrotoxin, 0.1; strychnine, 0.001 (pH 7.8). DL-threo- β -benzyloxyaspartate (TBOA) and 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(f)quinoxaline (NBQX) were obtained from Tocris Bioscience (Ellisville, MO, USA). Other chemicals were obtained from Sigma-Aldrich (St Louis, MO, USA).

Electrophysiology

Ruptured patch whole-cell recordings were obtained using 8–15 M Ω patch electrodes pulled from borosilicate glass (1.2 mm O.D., 0.95 mm I.D., with internal filament, World Precision Instruments, Sarasota, FL, USA) on a PP-830 micropipette puller (Narishige USA, East Meadow, NY, USA). The pipette solution contained (in mM): CsGluconate, 94; TEACl, 9.4; MgCl_2 , 1.9; MgATP, 9.4; GTP, 0.5; EGTA, 0.5; HEPES, 32.9 (pH 7.2). The osmolarity was measured with a vapor pressure osmometer (Wescor, Logan, UT, USA) and adjusted, if necessary, to ~ 245 mOsm.

Cells were voltage-clamped using Multiclamp or Axopatch 200B (Axon Instruments, Foster City, CA, USA) patch-clamp amplifiers. For capacitance recordings, we used the phase tracking circuitry of an Optopatch amplifier (Cairn Instruments, Faversham, UK) as described by Rabl *et al.* (2005). Recording pipettes were positioned with Huxley-Wall micromanipulators (Sutter Instruments, Novato, CA, USA). Currents were acquired using a Digidata 1322 interface and pClamp 9.2 software (Axon Instruments). Photoreceptors were voltage clamped at -70 mV, and horizontal cells at -50 or -60 mV. Rods and cones were distinguished morphologically. Horizontal cells were distinguished from bipolar cells by the shape of their cell bodies, large outward light-induced currents and a strong inwardly rectifying current/voltage relationship (Thoreson *et al.*, 1997). For additional verification of cell type, the studied neurons were visualized with use of a fluorescent tracer, Lucifer Yellow (2 mg/mL) or sulfarhodamine B (0.5 mg/mL), added to the pipette solution. Passive cell properties of rods, cones and horizontal cells were similar to those reported by Cadetti *et al.* (2005). Charging curves for rods, cones and many horizontal cells could be fit by single exponentials, indicating compact electrotonic structures. The finding that a single exponential fitted the charging curves of most horizontal cells examined indicates that they were largely uncoupled from their neighbors in the retinal slice preparations used for these studies.

Randomly occurring mEPSCs in horizontal cells were analysed using Minianalysis v. 5.6 (Synaptosoft, Decatur, GA, USA). To create average mEPSC waveforms, a sample of 25–100 well-isolated, individual mEPSCs were aligned at their half-maximal rise times. For convolution, average mEPSCs were fit with a three-exponential function: $y = e^{(-t/\tau_1)} + b * e^{(-t/\tau_2)} - (1 + b) e^{(-t/\tau_3)}$, where b is a scaling factor (Cadetti *et al.*, 2005). The release kinetics were determined previously by measuring exocytotic capacitance changes evoked by test pulses of varying duration (Rabl *et al.*, 2005). The resulting cumulative release function was fit with a dual-exponential function: $\Delta C_m = A_1(1 - \exp(-t/\tau_1)) + A_2(1 - \exp(-t/\tau_2))$ using the following parameters: for cones: $\tau_1 = 3$ ms, $A_1 = 306$ fF, $\tau_2 = 1.4$ s, $A_2 = 474$ fF; for rods: $\tau_1 = 46$ ms, $A_1 = 129$ fF, $\tau_2 = 12$ s, $A_2 = 1036$ fF (Rabl *et al.*, 2005). These cumulative release functions were differentiated to obtain instantaneous release functions. The fitted function for the mEPSC waveform was convolved with the instantaneous release function for rods or cones, and the resulting waveform was scaled to minimize the sum of the square

differences between the actual and predicted responses, as described previously (Cadetti *et al.*, 2005).

Light stimuli for most experiments were generated by a tungsten light source; intensity was controlled by Wratten gel neutral density filters, and wavelength by 580-nm or 680-nm interference filters. For some experiments, red or green light-emitting diodes were used as the stimulus. Rod-dominated light responses were evoked by using green light flashes applied to dark-adapted retina. Spectral measurements indicate that horizontal cells in the amphibian retinal slice preparation receive predominantly rod inputs under these conditions (Thoreson *et al.*, 2002). Strong rod input was further indicated by the slow recovery of dark-adapted responses, which mirrors the slow recovery of rod responses at offset of the 580-nm stimulus. Cone-dominated light responses were evoked with red light flashes applied in the presence of a 480-nm adapting background that greatly inhibited rod responses (Thoreson *et al.*, 2003).

The criterion for statistical significance was chosen to be $P < 0.05$ and evaluated with paired or unpaired Student's *t*-test using GraphPad Prism 4 (San Diego, CA, USA). Variability is reported as $\pm$ SEM.

Results

Linear convolution of EPSCs from paired recordings

Individual mEPSCs arising from glutamate transients produced by the exocytosis of one or more vesicles were recorded from horizontal cells in the retinal slice preparation. Few of the spontaneously occurring mEPSCs observed during paired recordings are due to release from the voltage-clamped photoreceptor, which is held at -70 mV, below calcium current (I_{Ca}) threshold. Instead, most spontaneously occurring mEPSCs arise from neighboring presynaptic photoreceptors, which have more depolarized resting membrane potentials. However, for any given horizontal cell, the mEPSCs derived from rods and cones exhibit similar amplitude and kinetics (Cadetti *et al.*, 2005). Because mEPSCs derived from different photoreceptors exhibit similar properties, we assumed that spontaneously occurring mEPSCs are representative of mEPSCs released by the depolarization of voltage-clamped photoreceptors in paired recordings. To compare EPSC and mEPSC kinetics, we therefore generated an average mEPSC waveform from each horizontal cell using a sample of well-isolated, randomly occurring mEPSCs recorded from that cell (Fig. 1A and D).

The kinetics of mEPSCs in horizontal cells, which lack *N*-methyl-D-aspartate (NMDA) receptors (Thoreson & Witkovsky, 1999), are slower than the kinetics of non-NMDA mEPSCs at other CNS synapses. The mEPSC waveforms from 16 horizontal cells averaged 5.7 pA in amplitude, with a 10–90% rise time of 780 μ s and decay time constant of 2.4 ms. This contrasts with non-NMDA mEPSCs at other CNS synapses, with rise times averaging < 150 μ s and decay time constants < 1 ms (Silver *et al.*, 1992; Zhang & Trussell, 1994; Sahara & Takahashi, 2001; Carter & Regehr, 2002). The slow kinetics of mEPSCs in horizontal cells is consistent with the diffusion of vesicular glutamate through a large cleft volume at the photoreceptor synapse.

To test whether the waveform of an EPSC can be recreated by linear summation of a series of individual mEPSCs, the average mEPSC for a given horizontal cell was fit with a three-exponential function to generate a smooth waveform (Fig. 1A) and then linearly convolved with a presynaptic release function derived from earlier exocytotic capacitance measurements (Fig. 1B, Rabl *et al.*, 2005). Figure 1C shows the EPSC evoked in a horizontal cell by a depolarizing test step applied to a simultaneously recorded cone. The average mEPSC recorded from the same horizontal cell was convolved with the cone release

function to generate the smooth trace in Fig. 1C. Consistent with previous results (Cadetti *et al.*, 2005), linear convolution of the mEPSC waveform with the cone release function closely reproduced the waveform of the cone-driven EPSC (Fig. 1C).

As a further test of whether the EPSC waveform reflects a linear sum of individual quantal events, we broadened mEPSCs by blocking α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor desensitization with cyclothiazide (CTZ, 0.1 mM). Broadening the mEPSC waveform with CTZ was accompanied by a similar broadening of the depolarization-evoked EPSC that was predicted reasonably well by convolution of CTZ-treated mEPSCs with the cone release function (Fig. 1). Furthermore, the charge transfer of cone-driven EPSCs (recorded ~1 min after CTZ application and evoked by 200-ms test steps from -70 to -10 mV) increased 2.3 times (2190–5075 pC, $n = 6$), similar to the 2.2-fold increase in mEPSC charge transfer (15.5–34.5 pC, $n = 6$). Thus, CTZ causes a proportional increase in both mEPSCs and EPSCs consistent with the suggestion that the cone-driven EPSC reflects a linear sum of individual quanta.

Rod-driven EPSCs are slower than cone-driven EPSCs due to slower release kinetics of rods (Cadetti *et al.*, 2005; Rabl *et al.*, 2005). The waveform predicted by convolution of mEPSCs with the rod release function approximated the waveform of the actual rod-driven EPSC but, as illustrated by the example in Fig. 1F, the actual rod-driven EPSC typically attained its peak later than the EPSC predicted by convolution. This is probably due to delayed release from coupled rods that is not incorporated into the presynaptic release function (Cadetti *et al.*, 2005). To test whether rod-driven EPSCs reflect a linear sum of individual quanta, we broadened mEPSCs with CTZ (0.1 mM). This caused a proportional broadening of the depolarization-evoked EPSC (Fig. 1D–F), and this broadening was predicted by convolving mEPSCs obtained in CTZ with the rod release function. In addition, the charge transfer of rod-driven EPSCs (evoked by 200-ms test steps from -70 to -10 mV) increased 2.1 times (12 405–25 716 pC, $n = 6$), similar to the 2.2-fold increase in mEPSC charge transfer. The finding that changes in rod-driven EPSCs scale linearly with changes in mEPSCs is consistent with the hypothesis that much of the rod-driven EPSC during the first 50 ms reflects a quantal sum of mEPSCs, despite the presence of coupling that contributes slow release components not incorporated into the release function used for convolution.

Agreement between the predicted and actual EPSC waveforms worsened after prolonged application of CTZ. Application of CTZ for > 2 min caused further increases in response amplitude and slowed the time to peak of rod- and cone-driven EPSCs, although it caused little additional change in mEPSC waveforms (not shown). Adding 1 mM kynurenic acid (KynA) to limit receptor saturation during prolonged application of CTZ did not improve the convolution, suggesting that this additional slowing of the peak current is not a consequence of receptor saturation at the peak of the EPSC. One possible explanation for these results is that CTZ may exert a presynaptic effect on synaptic transmission, as reported at other synapses (Diamond & Jahr, 1995; Ishikawa & Takahashi, 2001).

Effects of manipulating synaptic glutamate on EPSCs from paired recordings

If small amounts of glutamate remaining at the tail of each mEPSC accumulate during sustained release at the photoreceptor synapse, then glutamate levels late in the EPSC should be lower than those early in the EPSC, which are more directly determined by the high concentration of glutamate in synaptic vesicles. To test whether glutamate levels declined during the EPSC, we used the low-affinity, competitive GluR antagonist, KynA, which inhibits low glutamate concentrations more effectively than high glutamate concentrations (Clements, 1996). Application of a low concentration of KynA (0.25 mM) caused an outward shift in baseline current, and inhibited both mEPSCs and rod-driven EPSCs (Fig. 2A). The outward shift in baseline current produced by KynA is consistent with the presence of low

levels of glutamate in the synaptic cleft prior to the test step. KynA also significantly altered kinetics of rod-driven EPSCs, inhibiting later portions more strongly than the initial transient component (Fig. 2A). Preferential inhibition of the late component of the EPSC can be seen more clearly by rescaling the EPSC recorded in the presence of KynA to match the peak amplitude of the control EPSC (Fig. 2B). Effects of KynA recovered rapidly after washout (dashed line, Fig. 2A). Similar results were seen in 12 paired recordings from rods and horizontal cells. Changes in EPSC kinetics were not due to presynaptic effects, as KynA (0.25 mM) had no significant effect on I_{Ca} (test/control = 0.98 ± 0.07 , $n = 3$) or exocytotic capacitance changes (test/control = 0.97 ± 0.04 , $n = 7$) in rods. The changes in EPSC kinetics were also not due to changes in mEPSC kinetics. Figure 2C shows the average mEPSC waveform obtained in the presence of KynA scaled to match the amplitude of the mEPSC waveform obtained in control conditions from the same cell (same cell as Fig. 2A and B). Although the amplitude of the mEPSC was reduced by KynA (right axis, gray trace) compared with control (left axis, black trace), KynA had little effect on the kinetics of the average mEPSC.

Unlike KynA, the high-affinity antagonist NBQX blocks effects of low and high glutamate concentrations equally well. NBQX (0.1 μ M) inhibited both fast and slow components of rod-driven EPSCs equally well (Fig. 2D and E), and caused little change in mEPSC kinetics (Fig. 2F). Similar results were seen in 10 paired recordings. These results suggest that the preferential reduction of slow components by KynA is not simply a non-specific effect of blocking glutamate receptors. Instead, the results of KynA and NBQX suggest that average synaptic glutamate levels decline during later parts of the EPSC.

If the EPSC is determined by the sum of individual mEPSCs each reflecting a spatially discrete, transient elevation of glutamate, then the local concentration of glutamate attained during small depolarization-evoked EPSCs should be the same as the local concentration attained during large EPSCs. In contrast, if responses are determined by spatially integrated levels of glutamate in the synaptic cleft, then the concentration of glutamate at active receptors will be lower for small EPSCs and higher for large EPSCs. To distinguish these possibilities, we compared the effects of KynA on large and small EPSCs evoked by strong and weak depolarizing test steps, respectively. Figure 3A shows EPSCs evoked by depolarizing steps in control conditions (black trace) and after application of KynA (1 mM, gray trace). Figure 3B shows smaller EPSCs evoked by steps to -40 mV in control (black) and KynA (gray). As summarized in the bar graph in Fig. 3C, KynA inhibited the peak of the EPSC by a similar fraction in both cases. We also obtained similar results using a lower concentration of KynA (0.25 mM), in which the peak amplitudes of EPSCs evoked by steps to -10 mV was inhibited by $46.0 \pm 4.5\%$ ($n = 10$), and EPSCs evoked by steps to -40 mV were inhibited by $47.3 \pm 7.5\%$ ($n = 9$). The high-affinity glutamate antagonist, NBQX (0.1 mM), also caused a similar inhibition of large and small EPSCs (Fig. 3C). As a positive control, we applied a high concentration of TBOA (100 μ M) to slow glutamate removal from the cleft. Unlike the 10 μ M concentration used in later experiments, individual mEPSCs could not be detected in the presence of 100 μ M TBOA. In 100 μ M TBOA, EPSCs evoked by steps to -40 mV were inhibited to a significantly greater extent by 1 mM KynA ($80.1 \pm 2.2\%$, $n = 9$) than those evoked by steps to -10 mV ($67.2 \pm 3.9\%$, $P = 0.01$, paired t -test, $n = 9$), consistent with the buildup of higher concentrations of glutamate during stronger stimulation. These results suggest that the local concentration of glutamate attained at postsynaptic receptors remained the same when different numbers of vesicles were released by weak or strong stimulation. This further suggests that initially – at the peak of the EPSC – individual vesicles exert spatially segregated effects on the postsynaptic membrane.

The effects of KynA suggest that mean cleft glutamate levels decline during the EPSC. A decline in glutamate levels during the EPSC is consistent with the hypothesis that small

amounts of residual glutamate remaining at the tail of each mEPSC may accumulate to levels that exert physiologically significant effects with sustained release. However, a decline in the average cleft concentration is also consistent with the possibility that during slow, sustained release the local glutamate level may be low at one cluster of glutamate receptors (i.e. during the tail of a mEPSC) but high at another cluster of receptors (i.e. at the peak of another mEPSC). To distinguish these possibilities, we tested drugs that altered the levels of residual glutamate without affecting individual mEPSCs.

Glutamic-pyruvic transaminase (GPT) is a glutamate-scavenging enzyme that, in the presence of pyruvate, converts glutamate to alanine and alpha-ketoglutarate (O'Brien & Fischbach, 1986; Rossi & Slater, 1993; Min *et al.*, 1998; Turecek & Trussell, 2000). Unlike KynA, mEPSCs in horizontal cells were not significantly altered by GPT (10 U/mL plus 2 mM pyruvate), indicating that the rapid elevation of cleft glutamate produced by fusion of a single vesicle is not appreciably degraded by the slow catabolic actions of GPT (Fig. 4A). Consistent with the hypothesis that the initial transient component of the EPSC is constructed from a linear sum of individual mEPSCs, GPT also had no significant effect on the initial peak amplitude of rod-driven EPSCs (GPT/control = 1.05 ± 0.08 , $P = 0.79$). However, GPT caused an outward shift in baseline current (14.5 ± 5.4 pA, $n = 14$, $P = 0.02$), suggesting the presence of basal glutamate in the cleft. Consistent with the accumulation of residual glutamate during the EPSC, GPT also inhibited later components of rod-driven EPSCs (Fig. 4B), and thus accelerated decay of the EPSC in seven of nine rod–horizontal cell pairs. Changes in the EPSC waveform recovered after washout of the enzyme. Control experiments showed no effect of GPT plus pyruvate on rod I_{Ca} (0.97 ± 0.07 , $n = 3$) or pyruvate alone on depolarization-evoked EPSC (0.97 ± 0.04 , $n = 3$). GPT plus pyruvate did, however, alter photoreceptor light responses, so we did not use it for studies on light-evoked currents (LECs). The finding that GPT did not significantly alter mEPSC waveforms but nonetheless inhibited later components of the rod-driven EPSC is consistent with the hypothesis that the decline in mean cleft glutamate levels during the EPSC reflects an accumulation of residual glutamate.

By delaying the clearance of glutamate from the synaptic cleft, inhibition of glutamate uptake should enhance the effects of residual glutamate. We tested a low concentration (10 μ M) of the glutamate uptake inhibitor, TBOA, and found that it had little effect on mEPSCs (Fig. 5A). This low concentration of TBOA (10 μ M) also had no significant effect on the peak amplitude of rod-driven EPSCs (TBOA/control = $85.7 \pm 6.1\%$, $P = 0.86$, $n = 9$), consistent with the idea that the initial component is constructed from a sum of quantal mEPSCs. Although it had little effect on mEPSCs, TBOA nonetheless caused a pronounced broadening of rod-driven EPSCs (Fig. 5B). TBOA (10 μ M) also caused an inward shift in baseline current (-235 ± 71 pA, $n = 9$). We compensated for this baseline shift in Fig. 5 to facilitate comparisons of EPSC waveforms. Similar effects of TBOA were observed in nine rod–horizontal cell pairs. Increasing the concentration of TBOA to 100 μ M significantly increased EPSC amplitude (1.43 ± 0.17 , $P = 0.0004$, $n = 9$) and further broadened EPSC waveforms, but obscured individual mEPSCs. Control experiments showed no effect of TBOA (10 μ M) on rod I_{Ca} (TBOA/control = 0.96 ± 0.05 , $n = 3$). The ability of low concentrations of TBOA to broaden EPSCs even at concentrations that had little or no effect on mEPSCs is consistent with an accumulation of residual glutamate in the cleft late in the EPSC. In support of this, KynA (0.25 mM) preferentially inhibited increases in the late component produced by 10 μ M TBOA ($n = 9$; Fig. 5C).

Cone-driven EPSCs are considerably faster and more transient than rod-driven EPSCs due to their faster release kinetics (Cadetti *et al.*, 2005; Rabl *et al.*, 2005). Like rod-driven EPSCs, KynA inhibited cone-driven EPSCs, although a lower concentration of KynA (0.1 mM) was needed to produce ~50% inhibition of EPSCs at cone synapses (Fig. 6A) than at

rod synapses (0.25 mM). By rescaling the EPSC obtained in KynA to match the amplitude of that obtained in control (Fig. 6B), one can see that KynA had little effect on the kinetics of cone-driven EPSCs. Cone-driven EPSCs exhibit very little sustained current, making it difficult to detect any preferential inhibition of slow components of cone-driven EPSCs. Similar results were seen in 11 cone–horizontal cell pairs. As with rods, we also compared the effects of KynA on large and small EPSCs to test whether the local concentration of glutamate attained at glutamate receptors was similar. As can be seen by comparing the EPSCs in Fig. 6A and C, KynA (0.1 mM) produced a similar inhibition of large and small EPSCs. On average, KynA (0.1 mM) inhibited large EPSCs (evoked by steps to –10 mV) by $44.0 \pm 19.3\%$ ($n = 11$), and small EPSCs (evoked by steps to –30 mV) by $51.8 \pm 12.2\%$ ($n = 6$). The high-affinity antagonist, NBQX (0.1 μ M), inhibited EPSCs evoked by steps to –10 and –30 mV by $61.8 \pm 24.9\%$ ($n = 8$) and $50.7 \pm 25.8\%$ ($n = 5$), respectively.

TBOA (10 μ M) had little effect on the amplitude (TBOA/control = 0.88 ± 0.17 , $n = 3$, $P = 0.88$) or decay kinetics (5.5 ms in control vs. 5.0 ms in TBOA, $P = 0.33$) of cone-driven EPSCs (Fig. 6D). TBOA produced no clear enhancement of later parts of cone-driven EPSCs although, as described later, it did enhance cone-dominated LECs. The effects of TBOA and KynA support the hypothesis that the fast, transient EPSC evoked by depolarizing steps applied to simultaneously recorded cones can be constructed from a sum of individual mEPSCs.

LECs

Results from paired recording experiments indicate that the initial fast components of rod- and cone-driven EPSCs can largely be constructed by a linear sum of individual mEPSCs, but there is an increasing contribution of low, residual glutamate levels to rod-driven EPSCs when release is maintained by sustained depolarization. Under normal conditions in darkness, rods and cones maintain depolarized resting potentials of ~ 40 mV, allowing the continuous release of glutamate. We therefore tested the role of residual glutamate in shaping LECs.

As illustrated by the LECs shown in Fig. 7A and B, KynA strongly inhibited both rod- and cone-dominated LECs. Surprisingly, KynA inhibited LEC amplitude much more strongly than it inhibited the peak amplitude of EPSCs evoked during paired recordings (Fig. 7C and D). KynA (0.25 mM) reduced the amplitude of rod-dominated LECs by 93%, but inhibited the peak of rod-driven EPSCs evoked by test steps from –70 to –10 mV by only 46% (Fig. 7C). Similarly, cone-dominated, photopic light responses were inhibited 80% by 0.1 mM KynA, but cone-driven EPSCs were inhibited by only 44% (Fig. 7D). By contrast with the effects of KynA, the high-affinity antagonist NBQX (0.1 μ M) blocked depolarization-evoked EPSCs and LECs equally well (Fig. 7C and D). The stronger inhibition of LECs than EPSCs is not due to presynaptic effects of KynA on rod light responses (KynA/control = 0.99 ± 0.02 , $n = 4$), cone light responses (0.94 ± 0.14 , $n = 4$), rod I_{Ca} (0.98 ± 0.07 , $n = 3$) or cone I_{Ca} (0.99 ± 0.12 , $n = 4$). Thus, the strong block of LECs by KynA suggests that synaptic glutamate levels in darkness and light are lower than those attained at the peak of the EPSC, consistent with an accumulation of residual glutamate at the synapse when rods and cones are at their depolarized, dark resting potential.

TBOA (10 μ M) had little or no effect on the cumulative amplitude histogram of mEPSCs from a large, continuous sample of mEPSCs (Fig. 8A), consistent with the conclusion of Fig. 5A that this low concentration of TBOA had little effect on mEPSCs. Although TBOA did not alter mEPSC amplitude, it caused a large inward shift in baseline holding current and greatly increased the amplitude of LECs in both rod- and cone-dominated conditions (Fig. 8). The amplitude of LECs increased $480 \pm 172\%$ ($n = 7$) in rod-dominated conditions and $450 \pm 103\%$ ($n = 6$) in cone-dominated conditions. Consistent with earlier studies (Gaal *et*

et al., 1998; Thoreson *et al.*, 2003), application of a higher concentration of TBOA (100 μM) caused LECs to decrease, probably due to saturation and desensitization of postsynaptic receptors (data not shown). The large increase in dark currents and LECs produced by 10 μM TBOA despite little effect on mEPSCs is consistent with a substantial contribution by residual glutamate to the light response.

Discussion

The present results indicate that the initial transient component of horizontal cell EPSCs evoked by depolarizing steps applied to simultaneously recorded photoreceptors can be generally matched by summing a series of independent mEPSCs. However, with sustained release, small residual amounts of glutamate remaining in the cleft after each mEPSC pool together, accumulating sufficiently to contribute to synaptic responses. Therefore, synaptic responses evoked by sustained depolarization cannot be simply predicted by summing a series of mEPSCs. Contributions by residual glutamate to slow response components have also been observed at calycal or brush-type synapses which, like photoreceptor synapses, have a large extracellular volume and multiple release sites (Rossi *et al.*, 1995; Otis *et al.*, 1996). The continuous release of vesicles by photoreceptors in darkness allows the buildup of residual glutamate at multiple synapses on horizontal cells, and thus basal synaptic glutamate levels play a particularly important role in shaping LECs.

Consistent with the presence of vesicular release from photoreceptors and results of previous studies (Hirasawa *et al.*, 2001; Cadetti *et al.*, 2005), we detected randomly occurring mEPSCs in horizontal cells. The ability to detect mEPSCs in horizontal cells is improved by the uncoupling (Hirasawa *et al.*, 2001) that occurs when the retina is sliced into thin sections. We assumed that randomly occurring mEPSCs were representative of mEPSCs evoked by depolarizing steps. Different pools of vesicles with different properties have been shown to participate in calcium-dependent evoked release and calcium-independent spontaneous release (Sara *et al.*, 2005). However, at photoreceptor synapses, most randomly occurring mEPSCs in horizontal cells are probably not due to calcium-independent release but rather to the calcium-dependent release of vesicles from nearby photoreceptors that have depolarized resting potentials at about -40 mV. The properties of mEPSCs released at rod and cone synapses are similar to one another (Cadetti *et al.*, 2005), consistent with the suggestion that randomly occurring mEPSCs in horizontal cells, although derived largely from neighboring photoreceptor cells, are nonetheless likely to be similar to mEPSCs evoked by depolarizing steps applied to voltage-clamped photoreceptors.

A number of findings support the conclusion that the initial fast component of the depolarization-evoked EPSC at both rod and cone synapses can be largely accounted for by linear summation of a series of independent mEPSCs. (1) Consistent with our previous results (Cadetti *et al.*, 2005), we found that linear convolution of the mEPSC waveform with an empirically determined presynaptic release function produced waveforms similar to those of actual EPSCs evoked by depolarizing test steps. (2) Blocking AMPA receptor desensitization with CTZ caused a similar broadening of both mEPSCs and depolarization-evoked EPSCs at rod and cone synapses. With fixed release rate parameters, much of the change in the EPSC waveform produced by CTZ could be predicted by convolving CTZ-treated mEPSCs with the presynaptic release function. (3) A low concentration of TBOA that did not alter mEPSC amplitude caused little change in the initial peak of rod- and cone-driven EPSCs. (4) A glutamate-scavenging enzyme, GPT, which had little effect on mEPSCs, also caused little effect on the initial peak of rod-driven EPSCs. (5) KynA inhibited large and small EPSCs equally well, consistent with the idea that during the initial peak of the EPSC vesicles act independently from one another, generating similar local glutamate levels.

Convolution predicted the EPSC waveform at cone synapses more accurately than at rod synapses. The EPSC waveform recorded at rod synapses was generally slower than the waveform predicted by convolution (Fig. 1F; Cadetti *et al.*, 2005). A number of different factors contribute to this mismatch. (1) Heterogeneity in processes regulating release among different rods, such as variable contributions from calcium-induced calcium release (Cadetti *et al.*, 2006; Suryanarayanan & Slaughter, 2006), can lead to differences between the average release rate used for convolution and the release rate for any specific synapse. While calcium-induced calcium release contributes to later components of synaptic release, it does so by triggering vesicular exocytosis and thus cannot readily account for the presence of lower glutamate levels late in the EPSC. (2) Because of low pass filtering by the rod network (Zhang & Wu, 2005), release from coupled rods contributes a slow response component to the EPSC (Cadetti *et al.*, 2006). Release from coupled rods is not detected by capacitance techniques used for measuring release (Rabl *et al.*, 2005) and was not therefore incorporated into the release rate used for convolution. Although the spread of current into neighboring rods contributes to later response components, the mechanisms of release employed by coupled rods are likely to be identical to those used by the voltage-clamped rod. Thus, contributions from coupled rods also cannot readily explain a decline in glutamate levels during the EPSC. (3) In addition to blocking desensitization, CTZ can have a presynaptic effect on transmitter release (Diamond & Jahr, 1995; Ishikawa & Takahashi, 2001). Consistent with presynaptic effects at photoreceptor synapses, we found that prolonged application of CTZ produced changes in EPSCs that were not accompanied by changes in mEPSCs. (4) Glutamate receptors may become saturated at the peak of the EPSC. However, adding KynA to limit receptor saturation during lengthy application of CTZ did not improve the convolution, suggesting that presynaptic actions of CTZ may be more important. (5) Postsynaptic effects of glutamate that are not reflected in the average mEPSC waveform (i.e. contributions by residual glutamate).

The contribution of residual glutamate to slower components of EPSCs at rod synapses may contribute to differences between the actual EPSC waveform and that predicted by convolution. Pharmacological experiments provided additional evidence for a contribution from residual glutamate. For example, the finding that KynA preferentially inhibited later parts of the rod-driven EPSC indicates that mean glutamate levels decline during the EPSC, consistent with an accumulation of low levels of glutamate in the synapse. An accumulation of residual glutamate is also supported by the findings that: (1) the glutamate scavenger, GPT, inhibited later components of rod-driven EPSCs without inhibiting mEPSCs; and (2) the glutamate uptake inhibitor, TBOA, enhanced later components of the EPSC, also at a concentration that did not affect mEPSCs. If glutamate was released by a non-vesicular mechanism, this would increase basal glutamate levels in the cleft. However, the correlation between EPSCs and exocytotic capacitance changes suggests that release from photoreceptors is vesicular in nature (Rabl *et al.*, 2005). Later components of rod-driven EPSCs involve contributions from calcium-induced calcium release as well as release triggered by the spread of depolarizing current through gap junctions into neighboring rods (Cadetti *et al.*, 2005, 2006; Suryanarayanan & Slaughter, 2006). However, like release triggered by calcium influx through calcium channels, these mechanisms appear to stimulate vesicular release and thus cannot by themselves account for lower glutamate levels late in the EPSC (Cadetti *et al.*, 2005, 2006; Suryanarayanan & Slaughter, 2006).

Although the effects of KynA are consistent with an accumulation of residual glutamate, there are at least two other possible explanations for a decline in mean cleft glutamate levels during the EPSC. (1) The simultaneous presence of a high glutamate concentration at one group of glutamate receptors (at the peak of the mEPSC) and a lower concentration at another group of receptors (during the tail of an mEPSC) would produce a lower mean average concentration than attained at the peak of the EPSC when local glutamate levels are

high at all receptor clusters. (2) A decline in mEPSC amplitude resulting from a decline in the amount of glutamate released from each vesicle (Neher & Sakaba, 2001) or a shift to more distant release sites. However, implicit in these possibilities is the assumption that the EPSC reflects a sum of individual mEPSCs. If so, then low concentrations of TBOA or GPT that do not affect individual mEPSCs should not affect the EPSC waveform, contrary to the experimental findings. Furthermore, mEPSCs do not diminish during sustained release, but appear to grow because of multivesicular release coordinated by calcium-induced calcium release (Suryanarayanan & Slaughter, 2006).

Cone-driven EPSCs are much faster than rod-driven EPSCs largely because of faster release kinetics at cone synapses (Cadetti *et al.*, 2005; Rabl *et al.*, 2005). As summarized previously, our evidence suggests that the initial fast component of cone-driven EPSCs can be constructed from a sum of individual mEPSCs. Because cone-driven EPSCs exhibit very little sustained current, it is not surprising that we found little evidence for residual glutamate during the later components of cone-driven EPSCs.

In darkness, rods and cones are continuously depolarized allowing sustained release of vesicles at rates of 20–100 vesicles/ribbons/s. The effects of residual glutamate on EPSCs during paired recordings were confined to sustained components of EPSCs at rod synapses and negligible at cone synapses. LECs arise from multiple synaptic inputs, and this may magnify the impact of residual glutamate seen during paired recordings between individual photoreceptors and horizontal cells. KynA inhibited LECs more strongly than it inhibited the amplitude of EPSCs evoked in paired recordings. This indicates that levels of glutamate attained in darkness and during LECs are lower than levels of glutamate attained at the peak of the depolarization-evoked EPSC, consistent with the hypothesis that LECs are shaped more strongly by low, basal levels of glutamate in the cleft than the high levels attained during individual mEPSCs. Also consistent with a significant contribution by residual glutamate to LECs is the finding that a low concentration of TBOA with little effect on mEPSC amplitude or kinetics nonetheless caused a substantial enhancement of LECs. These data support the hypothesis that changes in the levels of residual glutamate in the cleft are important for shaping LECs and are consistent with earlier studies showing that LECs can be simulated by assuming that glutamate levels are spatially integrated throughout the synapse (Roska *et al.*, 1998; Thoreson *et al.*, 2003).

Another factor that may contribute to the sensitivity of LECs to KynA is that the receptors immediately beneath release sites may become desensitized with the sustained release of vesicles in darkness. This would increase the fraction of the LEC that is carried by more remote receptors and hence activated by lower concentrations of glutamate. The enhancement of spontaneously occurring mEPSCs by CTZ is consistent with the hypothesis that some glutamate receptors are tonically desensitized by cleft glutamate levels in darkness. However, CTZ produced a far greater increase in LECs (Yang *et al.*, 1998) than predicted by a twofold enhancement in mEPSC charge transfer (present study). Consistent with GPT and TBOA experiments, this implies that glutamate transients spread far enough throughout the cleft that their contribution to individual mEPSCs is no longer discernable. Preferential desensitization of receptors immediately beneath release sites would enhance the impact of residual glutamate on more remote receptors by allowing glutamate release from different vesicles to pool together at high rates of release. Desensitization may also account for the finding that EPSCs evoked by weak test steps to –40 mV were blocked more strongly by KynA after inhibition of glutamate uptake (Fig. 3C). The concentration of TBOA used in these experiments (100 μM) obscured individual mEPSCs, suggesting that the glutamate receptors immediately below the release sites were mostly desensitized and thus the EPSC evoked by the test step largely reflects the activation of more remote receptors exposed to lower glutamate levels.

The concentration of a low-affinity antagonist needed to block responses provides an estimate of synaptic glutamate levels (Clements, 1996). The nearly complete block of rod-dominated LECs by 0.25 mM KynA and cone-dominated LECs by 0.1 mM KynA suggest that the sustained release of vesicles in darkness elevates basal glutamate to high micromolar levels, consistent with a previous estimate (Gaal *et al.*, 1998). The greater sensitivity of cone synapses to blockade by KynA is consistent with the results of Kim & Miller (1991). High basal levels of glutamate at photoreceptor synapses in darkness contrast with exceedingly low basal levels of glutamate at conventional synapses (Herman & Jahr, 2007).

Together, the present results show that the initial fast EPSC component evoked by depolarizing steps at photoreceptor synapses can be largely predicted by summing a series of quantal mEPSCs, particularly at cone synapses. Abrupt depolarization causes a burst of release and the amplitude of the resulting EPSC is determined mostly by the peak amplitudes of the underlying mEPSCs. With maintained depolarization, postsynaptic responses are less accurately predicted by a sum of quantal mEPSCs because of the spread and pooling of glutamate transients throughout the synaptic cleft. We defined residual glutamate as that which remains in the cleft at the tail of each glutamate transient but at a concentration that is too low to contribute appreciably to individual mEPSCs. Our results support the hypothesis that residual glutamate from multiple vesicles can pool together and accumulate to levels sufficient to contribute to postsynaptic responses. In darkness, horizontal cells receive continuous synaptic input from multiple photoreceptors, making the contribution of residual glutamate to LECs especially prominent, perhaps further amplified by the desensitization of glutamate receptors closer to release sites.

Our results indicate that horizontal cell responses evoked by rapid presynaptic depolarization are strongly shaped by the kinetics of individual synaptic events, but that horizontal cell light responses are shaped more by basal levels of glutamate in the cleft. The ability of the photoreceptor synapse to operate in two different modes provides valuable flexibility. The slow rate at which levels of basal glutamate change during light and dark reduces the impact of synaptic noise, but photoreceptor synapses nonetheless retain the ability to briefly transmit higher frequency responses (e.g. produced by the depolarization of photoreceptors at light offset).

Acknowledgments

This study was supported by NEI (EY-10542), Fight for Sight, and Research to Prevent Blindness. We thank Dr Paul Witkovsky for helpful comments on the manuscript.

Abbreviations

AMPA	α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
CTZ	cyclothiazide
EPSC	excitatory postsynaptic current
GPT	glutamic-pyruvic transaminase
KynA	kynurenic acid
LEC	light-evoked current
mEPSC	miniature excitatory postsynaptic potential
NBQX	2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(f)quinoxaline
NMDA	<i>N</i> -methyl-d-aspartate

TBOA DL-threo-β-benzoyloxyaspartate

References

- Ashmore JF, Copenhagen DR. An analysis of transmission from cones to hyperpolarizing bipolar cells in the retina of the turtle. *J. Physiol. (Lond.)*. 1983; 340:569–597. [PubMed: 6310101]
- Auger C, Marty A. Quantal currents at single-site central synapses. *J. Physiol. (Lond.)*. 2000; 526:3–11. [PubMed: 10878094]
- Barbour B, Keller BU, Llano I, Marty A. Prolonged presence of glutamate during excitatory synaptic transmission to cerebellar Purkinje cells. *Neuron*. 1994; 12:1331–1343. [PubMed: 7912092]
- Berntson A, Taylor WR. The unitary event amplitude of mouse retinal on-cone bipolar cells. *Vis. Neurosci.* 2003; 20:621–626. [PubMed: 15088715]
- Burris C, Klug K, Ngo IT, Sterling P, Schein S. How Muller glial cells in macaque fovea coat and isolate the synaptic terminals of cone photoreceptors. *J. Comp. Neurol.* 2002; 453:100–111. [PubMed: 12357435]
- Cadetti L, Tranchina D, Thoreson WB. A comparison of release kinetics and glutamate receptor properties in shaping rod-cone differences in EPSC kinetics in the salamander retina. *J. Physiol. (Lond.)*. 2005; 569:773–788. [PubMed: 16223761]
- Cadetti L, Bryson EJ, Ciccone CA, Rabl K, Thoreson WB. Calcium-induced calcium release in rod photoreceptor terminals boosts synaptic transmission during maintained depolarization. *Eur. J. Neurosci.* 2006; 23:2983–2990. [PubMed: 16819987]
- Carter AG, Regehr WG. Quantal events shape cerebellar interneuron firing. *Nat. Neurosci.* 2002; 5:1309–1318. [PubMed: 12411959]
- del Castillo J, Katz B. Statistical factors involved in neuromuscular facilitation and depression. *J. Physiol. (Lond.)*. 1954; 124:574–585. [PubMed: 13175200]
- Clements JD. Transmitter timecourse in the synaptic cleft: its role in central synaptic function. *Trends Neurosci.* 1996; 19:163–171. [PubMed: 8723198]
- DeVries SH, Li W, Saszik S. Parallel processing in two transmitter microenvironments at the cone photoreceptor synapse. *Neuron*. 2006; 50:735–748. [PubMed: 16731512]
- Diamond JS, Jahr CE. Asynchronous release of synaptic vesicles determines the time course of the AMPA receptor-mediated EPSC. *Neuron*. 1995; 15:1097–1107. [PubMed: 7576653]
- Diamond JS, Jahr CE. Transporters buffer synaptically released glutamate on a submillisecond time scale. *J. Neurosci.* 1997; 17:4672–4687. [PubMed: 9169528]
- Diamond JS, Jahr CE. Synaptically released glutamate does not overwhelm transporters on hippocampal astrocytes during high-frequency stimulation. *J. Neurophysiol.* 2000; 83:2835–2843. [PubMed: 10805681]
- Eliasof S, Werblin F. Characterization of the glutamate transporter in retinal cones of the tiger salamander. *J. Neurosci.* 1993; 13:402–411. [PubMed: 8093715]
- Gaal L, Roska B, Picaud SA, Wu SM, Marc R, Werblin F. Postsynaptic response kinetics are controlled by a glutamate transporter at cone photoreceptors. *J. Neurophysiol.* 1998; 79:190–196. [PubMed: 9425190]
- Grant GB, Werblin FS. A glutamate-elicited chloride current with transporter-like properties in rod photoreceptors of the tiger salamander. *Vis. Neurosci.* 1996; 13:135–144. [PubMed: 8730995]
- Hasegawa J, Obara T, Tanaka K, Tachibana M. High-density presynaptic transporters are required for glutamate removal from the first visual synapse. *Neuron*. 2006; 50:63–74. [PubMed: 16600856]
- Heidelberger R, Thoreson WB, Witkovsky P. Synaptic transmission at retinal ribbon synapses. *Prog. Retin. Eye Res.* 2005; 24:682–720. [PubMed: 16027025]
- Herman MA, Jahr CE. Extracellular glutamate concentration in the hippocampal slice. *J. Neurosci.* 2007; 27:9736–9741. [PubMed: 17804634]
- Higgs MH, Lukasiewicz PD. Glutamate uptake limits synaptic excitation of retinal ganglion cells. *J. Neurosci.* 1999; 19:3691–3700. [PubMed: 10234001]

- Hirasawa H, Shiells R, Yamada M. Analysis of spontaneous EPSCs in retinal horizontal cells of the carp. *Neurosci. Res.* 2001; 40:75–86. [PubMed: 11311408]
- Ishikawa T, Takahashi T. Mechanisms underlying presynaptic facilitatory effect of cyclothiazide at the calyx of Held of juvenile rats. *J. Physiol. (Lond.)*. 2001; 533:423–431. [PubMed: 11389202]
- Kim HG, Miller RF. Rods and cones activate different excitatory amino acid receptors on the mudpuppy retinal horizontal cell. *Brain Res.* 1991; 538:141–146. [PubMed: 1673359]
- Mennerick S, Zorumski CF. Presynaptic influence on the time course of fast excitatory synaptic currents in cultured hippocampal cells. *J. Neurosci.* 1995; 15:3178–3192. [PubMed: 7722655]
- Min M-Y, Rusakov DA, Kullmann DM. Activation of AMPA, kainate, and metabotropic receptors at hippocampal mossy fiber synapses: role of glutamate diffusion. *Neuron.* 1998; 21:561–570. [PubMed: 9768842]
- Neher E, Sakaba T. Combining deconvolution and noise analysis for the estimation of transmitter release rates at the calyx of held. *J. Neurosci.* 2001; 21:444–461. [PubMed: 11160425]
- O'Brien RJ, Fischbach GD. Modulation of embryonic chick motoneuron glutamate sensitivity by interneurons and agonists. *J. Neurosci.* 1986; 6:3290–3296. [PubMed: 3021928]
- Otis TS, Wu YC, Trussell LO. Delayed clearance of transmitter and the role of glutamate transporters at synapses with multiple release sites. *J. Neurosci.* 1996; 16:1634–1644. [PubMed: 8774432]
- Rabl K, Bryson EJ, Thoreson WB. Activation of glutamate transporters in rods inhibits presynaptic calcium currents. *Vis. Neurosci.* 2003; 20:557–566. [PubMed: 14977334]
- Rabl K, Cadetti L, Thoreson WB. Kinetics of exocytosis is faster in cones than rods. *J. Neurosci.* 2005; 25:4633–4640. [PubMed: 15872111]
- Rao-Mirotnik R, Harkins AB, Buchsbaum G, Sterling P. Mammalian rod terminal: architecture of a binary synapse. *Neuron.* 1995; 14:561–569. [PubMed: 7695902]
- Rao-Mirotnik R, Buchsbaum G, Sterling P. Transmitter concentration at a three-dimensional synapse. *J. Neurophysiol.* 1998; 80:3163–3172. [PubMed: 9862914]
- Redman S. Quantal analysis of synaptic potentials in neurons of the central nervous system. *Physiol. Rev.* 1990; 70:165–198. [PubMed: 2404288]
- Roska B, Gaal L, Werblin FS. Voltage-dependent uptake is a major determinant of glutamate concentration at the cone synapse: an analytical study. *J. Neurophysiol.* 1998; 80:1951–1960. [PubMed: 9772252]
- Rossi DJ, Slater NT. The developmental onset of NMDA receptor-channel activity during neuronal migration. *Neuropharmacology.* 1993; 32:1239–1248. [PubMed: 7509049]
- Rossi DJ, Alford S, Magnini E, Slater NT. Properties of transmission at a giant glutamatergic synapse in cerebellum: the mossy fiberunipolar brush cell synapse. *J. Neurophysiol.* 1995; 74:24–42. [PubMed: 7472327]
- Sahara Y, Takahashi T. Quantal components of the excitatory postsynaptic currents at a rat central auditory synapse. *J. Physiol. (Lond.)*. 2001; 536:189–197. [PubMed: 11579168]
- Sakaba T, Schneggenburger R, Neher E. Estimation of quantal parameters at the calyx of Held synapse. *Neurosci. Res.* 2002; 44:343–356. [PubMed: 12445623]
- Sara Y, Virmani T, Deak F, Liu X, Kavalali ET. An isolated pool of vesicles recycles at rest and drives spontaneous neurotransmission. *Neuron.* 2005; 45:563–573. [PubMed: 15721242]
- Sarantis M, Mobbs P. The spatial relationship between Muller cell processes and the photoreceptor output synapse. *Brain Res.* 1992; 584:299–304. [PubMed: 1325248]
- Silver RA, Traynelis SF, Cull-Candy SG. Rapid-time-course miniature and evoked excitatory currents at cerebellar synapses in situ. *Nature.* 1992; 355:163–166. [PubMed: 1370344]
- Suryanarayanan A, Slaughter MM. Synaptic transmission mediated by internal calcium stores in rod photoreceptors. *J. Neurosci.* 2006; 26:1759–1766. [PubMed: 16467524]
- Thoreson WB, Witkovsky P. Glutamate receptors and circuits in the vertebrate retina. *Prog. Retin. Eye Res.* 1999; 18:765–810. [PubMed: 10530751]
- Thoreson WB, Nitzan R, Miller RF. Reducing extracellular chloride suppresses dihydropyridine-sensitive calcium currents and synaptic transmission in amphibian photoreceptors. *J. Neurophysiol.* 1997; 77:2175–2190. [PubMed: 9114264]

- Thoreson WB, Stella SL Jr, Bryson EJ, Clements J, Witkovsky P. D₂-like dopamine receptors promote interactions between calcium and chloride channels that diminish rod synaptic transfer in the salamander retina. *Vis. Neurosci.* 2002; 19:235–247. [PubMed: 12392173]
- Thoreson WB, Tranchina D, Witkovsky P. Kinetics of synaptic transfer from rods and cones to horizontal cells in the salamander retina. *Neuroscience.* 2003; 122:785–798. [PubMed: 14622921]
- Trussell LO, Zhang S, Raman IM. Desensitization of AMPA receptors upon multiquantal neurotransmitter release. *Neuron.* 1993; 10:1185–1196. [PubMed: 7686382]
- Turecek R, Trussell LO. Control of synaptic depression by glutamate transporters. *J. Neurosci.* 2000; 20:2054–2063. [PubMed: 10684906]
- Vandenbranden CA, Yazulla S, Studholme KM, Kamphuis W, Kamermans M. Immunocytochemical localization of the glutamate transporter GLT-1 in goldfish (*Carassius auratus*) retina. *J. Comp. Neurol.* 2000; 423:440–451. [PubMed: 10870084]
- Yang JH, Maple B, Gao F, Maguire G, Wu SM. Postsynaptic responses of horizontal cells in the tiger salamander retina are mediated by AMPA-preferring receptors. *Brain Res.* 1998; 797:125–134. [PubMed: 9630565]
- Zhang S, Trussell LO. A characterization of excitatory postsynaptic potentials in the avian nucleus magnocellularis. *J. Neurophysiol.* 1994; 72:705–718. [PubMed: 7983529]
- Zhang J, Wu SM. Physiological properties of rod photoreceptor electrical coupling in the tiger salamander retina. *J. Physiol. (Lond.).* 2005; 564:849–862. [PubMed: 15746168]

Fig. 1.

Convolution of miniature excitatory postsynaptic currents (mEPSCs) with empirically derived photoreceptor release functions approximated the waveforms of actual EPSCs, even after broadening the mEPSC waveform by blocking glutamate receptor desensitization with cyclothiazide (CTZ). (A) Averaged mEPSCs from a horizontal cell during paired recording with a cone. mEPSCs were obtained in control conditions (red trace) and after application of CTZ (0.1 mM; blue trace), aligned at the half-maximal points of their rising phases, and fit with a three-exponential function as described in Materials and methods. (B) Cone release function derived from earlier measurements of exocytotic capacitance changes evoked by test pulses of varying duration (Rabl *et al.*, 2005). For purposes of illustration, the

instantaneous release function was scaled by 57 aF/vesicle. However, the convolution was scaled to minimize the sum of the square differences between the actual and predicted responses. (C) EPSCs evoked in the horizontal cell by depolarizing test steps applied to a cone in control conditions (red) and after 2 min application of CTZ (blue). Overlaid on these traces are the waveforms predicted by convolution of the mEPSCs shown in (A), with the cone release function shown in (B) (smooth trace). The waveform predicted by convolution was scaled to minimize the sum of the square differences between actual and predicted responses. (D) Average mEPSCs from a different horizontal cell obtained during paired recording with a rod, obtained in control (red trace) and CTZ (blue) conditions. (E) Rod release function. (F) Convolution of average mEPSCs in (D) with the release function (smooth trace) overlaid on actual EPSCs evoked by depolarizing steps applied to a simultaneously recorded rod in both control (red) and CTZ (blue) conditions.

Fig. 2.

Effects of low- and high-affinity antagonists, kynurenic acid (KynA) and 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(f)quinoxaline (NBQX), on rod-driven excitatory postsynaptic currents (EPSCs) and miniature (m)EPSCs in horizontal cells. (A) EPSCs evoked in a horizontal cell by depolarizing steps from -70 to -10 mV applied to a simultaneously recorded rod. Control: black trace. KynA: gray trace. Wash: light gray trace. (B) Control and KynA traces rescaled to match their peak amplitudes. (C) Average mEPSCs recorded from the same horizontal cell in control conditions (black trace) and after application of KynA (dark gray trace, 0.25 mM). (D) EPSCs evoked in a horizontal cell by depolarizing steps from -70 to -10 mV applied to a simultaneously recorded rod. Control: black trace. NBQX: gray trace. Wash: light gray trace. (E) Control and NBQX traces rescaled to match their peak amplitudes. (F) Average mEPSCs recorded from the same horizontal cell in control conditions (black trace) and after application of NBQX (gray trace, 0.1 μ M).

Fig. 3.

The initial peak amplitudes of large and small EPSCs were inhibited similarly by kynurenic acid (KynA). EPSCs were evoked in a horizontal cell by depolarizing steps from -70 to -10 mV (A) and -40 mV (B) applied to a simultaneously recorded rod. Both the large (A) and small (B) EPSCs were inhibited to a similar degree by KynA (1 mM). (C) For the bar graph in (C), the amplitudes of test responses were divided by control responses to show the average changes in the peak amplitude of EPSCs evoked by test steps to -10 and -40 mV that were produced by KynA (1 mM; $n = 14$), 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(f)quinoxaline (NBQX; 0.1 μ M; step to -10 mV, $n = 10$; step to -40 mV, $n = 4$), and a combination of KynA (1 mM) and DL-threo- β -benzoyloxyaspartate (TBOA; 0.1 mM). There was no significant difference in the inhibition of large and small EPSCs by KynA or NBQX, but smaller EPSCs evoked by steps to -40 mV were inhibited significantly more than large EPSCs evoked by steps to -10 mV by KynA when applied in the presence of the glutamate uptake inhibitor, TBOA ($P = 0.01$, paired t -test, $n = 9$). * $P < 0.01$.

Fig. 4.

The glutamate scavenging enzyme, glutamic-pyruvic transaminase (GPT; 10 U/mL), had little effect on miniature excitatory postsynaptic currents (mEPSCs) but preferentially inhibited slower components of rod-driven EPSCs. GPT was applied in the presence of pyruvate (2 mM). (A) Average mEPSCs obtained in control conditions (black trace) and after application of GPT (gray trace). (B) EPSCs evoked in the same horizontal cell by depolarizing steps from -70 to -10 mV applied to a presynaptic rod. Control: black trace. GPT: dark gray trace. Wash: light gray trace. The outward shift in baseline current produced by GPT was compensated so as to compare the traces more easily.

Fig. 5.

The glutamate uptake inhibitor, DL-threo- β -benzyloxyaspartate (TBOA; 10 μ M), had little effect on mEPSCs, but nonetheless broadened rod-driven EPSCs. (A) Average mEPSCs obtained before (black trace) and after application of TBOA (gray trace). (B) EPSCs evoked in the same horizontal cell by 60-mV steps from -70 to -10 mV applied to a presynaptic rod. Control: black trace. TBOA: gray trace. Wash: light gray trace. (C) TBOA broadened rod-driven EPSCs recorded from a different horizontal cell. Kynurenic acid (KynA; 0.25 mM) inhibited and accelerated decay of the EPSC. The preferential inhibition by KynA of later parts of the EPSC is more evident after rescaling the trace to match the amplitude of the trace obtained in TBOA prior to KynA application. By accelerating response decay, KynA restored the kinetics of the EPSC closer to those of the control response. Baseline current shifts produced by TBOA and KynA were removed from (B) and (C) to compare the traces more easily.

Fig. 6.

Effects of kynurenic acid (KynA; 0.1 mM) and DL-threo-β-benzoyloxyaspartate (TBOA; 10 μM) on cone-driven EPSCs. (A) Inhibition by KynA of EPSCs evoked by depolarizing steps from -70 to -10 mV applied to a simultaneously recorded cone. Control: black trace. KynA: dark gray trace. (B) To compare waveforms more easily, the trace obtained in KynA was rescaled (light gray trace) to match the amplitude of the control trace. (C) The inhibition by KynA of EPSCs evoked in the same cell by steps to -30 mV was similar to the inhibition of larger EPSCs evoked by steps to -10 mV (A). (D) TBOA (10 μM) had little effect on cone-driven EPSCs. Control: black trace. TBOA: gray trace. The inward shift in baseline current produced by TBOA was compensated to better compare the traces.

Fig. 7.

Kynurenic acid (KynA) inhibited horizontal cell light-evoked currents (LECs) more strongly than EPSCs. (A) Rod-dominated LECs evoked by a saturating 1-s, 580-nm light flash applied in scotopic conditions. KynA (dark gray trace) reversibly inhibited the LEC. (B) Cone-dominated LECs evoked by a bright 1-s, 680-nm light flash applied in the presence of a 480-nm adapting background. KynA (dark gray trace) also reversibly inhibited the cone-dominated LEC. (C) Inhibition by KynA (0.25 mM) and 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(f)quinoxaline (NBQX; 0.1 μ M) of EPSCs recorded from rod-horizontal cell pairs and LECs recorded from horizontal cells under rod-dominated conditions (580 nm light applied to dark-adapted retina). KynA inhibited rod-dominated LECs ($n = 7$) significantly more than it inhibited EPSCs evoked by depolarizing steps applied to -10 mV ($n = 10$; $P < 0.0001$) or -40 mV ($n = 9$; $P = 0.0017$; not shown). NBQX inhibited LECs ($n = 7$) and EPSCs evoked by depolarizing steps applied to -10 mV ($n = 10$) or -40 mV ($n = 4$; data shown in Fig. 3) equally well. (D) Inhibition by KynA (0.1 mM) and NBQX (0.1 μ M) of EPSCs recorded from cone-horizontal cell pairs and LECs recorded from horizontal cells under cone-dominated conditions (680 nm light applied to light-adapted retina). Like rod-driven synapses, KynA inhibited cone-dominated LECs ($n = 5$) significantly more than it inhibited EPSCs evoked by depolarizing steps applied to -10 mV ($n = 11$; $P = 0.0013$) or -30 mV ($n = 6$; $P = 0.0011$; not shown). NBQX inhibited LECs ($n = 7$) and EPSCs evoked by depolarizing steps applied to -10 mV ($n = 8$) or -40 mV ($n = 8$; not shown) equally well. The data in (C) and (D) are plotted as fractional response, wherein the LECs or EPSCs recorded in KynA or NBQX were divided by the amplitude of responses obtained in control conditions. * $P < 0.01$.

Fig. 8.

DL-threo- β -benzyloxyaspartate (TBOA; 10 μM) enhanced LECs but not mEPSCs. (A) Cumulative amplitude histogram of mEPSCs obtained in control conditions (filled circles; $n = 426$ events) and after application of TBOA (open circles, $n = 419$). (B) Rod-dominated LECs evoked by a bright green light flash applied with an LED in scotopic conditions. TBOA (dark gray trace) reversibly enhanced the LEC. Control: black trace. Wash: light gray trace. (C) Cone-dominated LECs evoked by a bright red light flash from an LED applied in the presence of a 480-nm adapting background. TBOA (dark gray trace) also reversibly enhanced the cone-dominated LEC. Same cell in all three panels.