Distinct AMPA-Type Glutamatergic Synapses in Developing Rat CA1 Hippocampus

Elizabeth A. Stubblefield and Tim A. Benke

Departments of Pharmacology, Pediatrics, Neurology, and Neuroscience Program, University of Colorado, Denver, School of Medicine, Aurora, Colorado

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Stubblefield EA, Benke TA. Distinct AMPA-type glutamatergic synapses in developing rat CA1 hippocampus. J Neurophysiol 104: 1899–1912, 2010. First published August 4, 2010; doi:10.1152/jn.00099.2010. We assessed synaptic α-amino-3-hydroxy-5-methyl-4-isoxazole propionate receptor (AMPA) properties during synaptogenesis to describe the development of individual glutamatergic synapses on rat hippocampal CA1 principal neurons. Pharmacologically isolated AMPA-mediated glutamatergic synaptic currents [evoked by stimulation of the Schaffer Col-lateral pathway, excitatory postsynaptic currents (EPSCs)], had significantly greater inward-rectification at ages P5–7 compared with P8–18. These inward rectifying EPSCs demonstrated paired-pulse dependent unblocking at positive holding potentials, consistent with voltage-dependent internal polyamine block. Measurements of paired-pulse facilitation did not support altered presynaptic properties associated with inward rectification. Using asynchronous EPSCs (aEPSCs) to analyze populations of individual synapses, we found that quantal amplitudes (Q) increased across early postnatal development (P5-P18) and were directly modulated by increases in the number of activated receptors. Quantal AMPAR decay kinetics (aEPSC τ\text{decay}) exhibited the highest coefficient of variation (CV) from P5 to 7 and became markedly less variable at P8–18. At P5–7, faster quantal kinetics coexisted with much slower kinetics; only slower quantal kinetics were found at P8–18. This supports diverse quantal synaptic properties limited to P5–7. Multivariate cluster analysis of Q, CV, τ\text{decay}, and median τ\text{decay} supported a segregation of neurons into two distinct age groups of P5–7 and P8–18, similar to the age-related segregation suggested by inward rectification. Taken together, these findings support synaptic, calcium permeable AMPARs at a subset of synapses onto CA1 pyramidal neurons exclusively at P5–7. These distinct synapses coexist with those sharing the properties of more mature synapses. These synapses disappear after P7 as activated receptor numbers increase with age.

INTRODUCTION

Changes in synaptic α-amino-3-hydroxy-5-methyl-4-isoxazole propionate receptor (AMPA) activation in the CA1 region of the hippocampus underlie important processes such as learning and memory formation. Thus a better understanding of how the biophysical properties of AMPARs change during synaptogenesis would give insight into the mechanisms for developmental regulation of synaptic maturation. The AMPAR forms as a tetramer composed of homo- and heteromeric combinations of four subunits (GluA1, -2, -3, and -4) (Dingledine et al. 1999) and functions as a synthetically activated channel after postnatal day (P) 0 in rat (Hanse and Gustafsson 2001; Tzyio et al. 1999). It is thought that hetero-meric assemblies of these subunits form preferentially in adults rats (P30-40) (Wenthold et al. 1996) and in heterologous expression systems (Mansour et al. 2001), but preferred GluA assemblies in postnatal development have not yet been fully established. Electrophysiological evidence suggests that heterogeneity in AMPAR subunit populations dominate at most developing rat synapses, in that AMPAR GluA1 homomers co-exist at the same synapses as GluA1/2 and/or GluA2/3 heteromeric combinations as well as different splice variants of GluA subunits (Seifert et al. 2000).

Biochemical evidence suggests that GluA subunit transcript levels (Ritter et al. 2002) and protein levels (Petralia et al. 2005) change drastically during synaptogenesis in rodents. For example, GluA4 levels increase from birth to P7 and then decrease thereafter to lower-than-birth levels in rat CA1. GluA1 levels appear to be higher than GluA2 during the first week of life (Monyer et al. 1991; Wisden and Seeburg 1993), but then GluA2 and GluA3 levels dramatically increase around P7-10 (Forti et al. 1997; Petralia et al. 2005; Pickard et al. 2000; Ritter et al. 2002). The total number of AMPARs at each synapse (N) is thought to increase during this period (Baudet et al. 1995; Pickard et al. 2000; Tzyio et al. 1999), and supporting this, spontaneous excitatory postsynaptic current (EPSC) amplitude has been shown to increase from P1 up to P8 (Groc et al. 2002). At rat thalamocortical synapses, it has been demonstrated that the number of activated AMPARs modulating quantal amplitude (Q) adjusts with the AMPAR single-channel conductance (γ) to result in a uniform Q during early development (Bannister et al. 2005).

A wide range of AMPAR activation and deactivation kinetics (EPSC τ\text{rise} and τ\text{decay}) and single-channel conductances (γ) have been previously reported (Cull-Candy and Usowicz 1987; Gardner et al. 2001; Mosbacher et al. 1994), and it is likely that these differences arise due to developmental differences in AMPAR expression (Cathala et al. 2005; Seifert et al. 2000). Evidence in the rat cerebellum suggests a speeding of AMPAR-mediated EPSC kinetics as age increases. For example, AMPAR kinetics are slower at ages ≤ P10 versus ages ≥ P40 (Cathala et al. 2005; Wall and Usowicz 1998). Previous studies in CA1 reveal faster τ\text{decay} at P45 compared with P15, supporting the idea that EPSC τ\text{decay} are faster in the adult (Seifert et al. 2000). Recombinant studies (Oh and Derkach 2005) and evidence in rat hippocampal culture (Thiagarajan et al. 2005) demonstrate much faster channel kinetics for GluA2-lacking receptors compared with channels that contain the GluA2 subunit, suggesting these changes are influenced by subunit composition. Hippocampal AMPAR-mediated γ values range from 2 to 20 pS (Gebhardt and Cull-Candy 2006). These values could vary depending on differing subunit populations of AMPAR chan-
nels because (recombinant) GluA2-lacking receptors have a higher γ compared with GluA2-containing AMPARs (Oh and Derkach 2005). GluA2-lacking AMPARs (or unedited GluA2) are calcium permeable (Bowie and Mayer 1995; Geiger et al. 1995; Kamboj et al. 1995) and highly developmentally regulated. Calcium permeable AMPARs have been found in the rat CA3 region mainly during the first 3 weeks of life (Ho et al. 2007), in neocortical pyramidal neurons until P16, coincident when GluA2s become more prevalent (Kumar et al. 2002), and transiently in primary auditory neurons before the onset of hearing (Eybalin et al. 2004). In CA1, the developmental expression of calcium permeable synaptic AMPARs has been explored, revealing the presence of inward rectifying channels mainly in interneurons (Isa et al. 1996). Detailed analysis of inward rectifying AMPAR on CA1 principal neurons has not been studied during early developmental ages except to note that they are typically not active around P14 (Plant et al. 2006).

It has been shown that AMPAR subunits can be targeted to specific glutamatergic synapses (Craig et al. 1993; Harms et al. 2005; Rubio and Wenthold 1997), and in the cerebellum, this can be due to the activity of calcium permeable AMPARs that direct GluA2-containing receptors into synapses (Lin and Cull-Candy 2002). In CA1 hippocampus, it has been demonstrated that specific targeting of calcium permeable, GluA2-lacking AMPARs aids in the induction and expression of LTP (Plant et al. 2006; but see Adesnik and Nicoll 2007). Similar targeting also occurs in response to pharmacological blockade of AMPARs (Thiagarajan et al. 2005) or in response to hypoxia-induced seizures (Rakhade et al. 2008). This suggests that selective AMPAR properties including calcium permeability are intrinsically necessary in processes that involve precise strengthening or weakening of synaptic neurotransmission. However, it remains unknown if biophysically different AMPARs are segregated to different synapses during basal levels of neurotransmission. We therefore studied both multiquantal and quantal AMPAR-mediated synaptic responses during the formation of synapses in CA1 hippocampus when AMPAR-mediated calcium fluxes might affect synaptogenesis. We used whole cell electrophysiological recordings to investigate synaptic AMPAR-mediated multiquantal EPSCs to study inward rectification and quantal aEPSCs (in the presence of extracellular strontium) to study multiquantal EPSCs to study inward rectification and quantal inward rectifying AMPAR on CA1 principal neurons has not been studied during early developmental ages except to note that they are typically not active around P14 (Plant et al. 2006).

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\[ \text{Q, quantal } \tau_{\text{decay}}, \gamma, \text{ and number of activated receptors (N}^\text{P}_0) \text{.} \]

Our data suggest that neurons at P5–7 expressed two distinct types of synapses: one type expressed inward rectifying AMPARs associated with faster quantal kinetics, whereas the other type expressed nonrectifying (linear) AMPARs associated with slower quantal kinetics, similar to synapses at P8–18. The older neurons (P8–18) had more uniform biophysical properties, yet the number of activated AMPARs significantly increased at the synapse. We therefore speculate that there are AMPAR-mediated calcium fluxes at distinct synapses at P5–7 during early synaptogenesis; as these synapses mature, they then become more biophysically uniform.

**METHODS**

**Ethical approval**

All experiments were approved by the University of Colorado at Denver Health Science Center Institutional Animal Care and Use Committee. Data from 122 rat pups were used in this study.

Slice preparation

Sprague-Dawley rats, male and female, P5-18 days old, were decapitated, and the whole brain was rapidly removed and placed in ice-cold cutting solution comprising (in mM) 124 NaCl, 26 NaHCO₃, 3 KCl, 1.2 NaH₂PO₄, 5 MgSO₄, 10 ω-glucose, and 2 Na ascorbate and bubbled with 95% O₂-5% CO₂. Sagittal slices were made (300–400 μm thick) using a Leica VT-1200 microtome (Nussloch, Germany), and additional cuts were made with a scalpel to remove the CA3 area of the hippocampus. Hippocampal slices were transferred to a holding chamber and stored for ≥1 h prior to recording in recovery solution (as in preceding text except including 2 mM CaCl₂ and substituting 1 mM MgSO₄) at room temperature (18–22°C).

Electrophysiological recording

CA1 pyramidal neurons were visually identified by characteristic position and shape in submerged slices using infrared differential interference contrast (DIC) optics under magnification (×40; BX51-WI, Olympus) and patch-clamped in the whole cell configuration. Borosilicate glass (Harvard Instruments, Holliston, MA) recording electrodes were pulled with a Sutter P-97 electrode puller (Sutter Instruments, Novato, CA) with resistance 4–6 MΩ and filled with internal patch solution comprising (in mM) 135 CsMeSO₄, 10 HEPES, 0.5 EGTA, 0.3 Na-GTP, 5 QX314, 4 ATP-Na₂, 4 MgCl₂, and 0.1 spermine, pH 7.25 with 1 M CsOH. For recording, picrotoxin (100 μM), and d-APV (50 μM) were added to the recording solution to block γ-amino butyric acid (GABA) receptor and NMDA receptors, respectively (recording solution).

For rectification experiments, a platinum/iridium bipolar stimulating electrode (FHC, Bowdoin, ME) was placed in the stratum radiatum to evoke a near maximal, synchronous, multiquantal EPSC by using a constant voltage source (Digitimer, DS2A; 7–9 V, 100 μs) every 5 s. In the remainder of experiments, near minimal stimulation of axons in the stratum radiatum to evoke aEPSCs was performed with a glass stimulating electrode (resistance of 6–7 MΩ when filled with recording solution) and placed in a consistent location (30–50 μM distally from the soma and 15–30 μM laterally from the primary apical dendrite) to the recording electrode. A constant voltage (Digitimer, DS2A) or constant current source (WI, Sarasota, FL) source (1–3.0 V or 8.5–9.9 mA, 100 μs duration) was applied every 5 s. Calcium was replaced with 8 mM Sr²⁺ in the recording solution to cause asynchronous release of glutamate (Bekkers and Clements 1999; Goda and Stevens 1994) and was introduced following membrane rupture. Resulting asynchronous EPSCs (aEPSC) were collected at a holding potential of −70 mV in a 2.25 s time frame after each single stimulus. EPSCs and aEPSCs were confirmed to be mediated by AMPA receptors by addition of GYKI 52466 (100 μM) across all ages resulting in complete block of current (n = 8). Spontaneous events prior to stimulation were only seen in 2 of 122 recordings; these recordings were not analyzed further; this suggests that aEPSCs in the 2.25 s window after stimulation were not spontaneous events. Series resistance associated with the recording electrode was monitored in every sweep by a −2.0 mV voltage step; recordings were terminated if changes in series resistance of >10% of baseline occurred (see Supplemental Fig. S1).1 Cell capacitance was also determined from this voltage step (Bannister et al. 2005) (see Supplemental Fig. S2). Responses were amplified and filtered at 5 kHz (Axopatch 200B, MDS, Sunnyvale, CA) and digitized at 20 kHz (Digidata 1322A and Clampex 9.2, MDS). No series resistance cancellation was performed. All drugs were purchased from Sigma Aldrich (St Louis, MO) or Tocris Bioscience (Ellisville, MO).

1 The online version of this article contains supplemental data.
Data analysis

For calculation of rectification index (RI), neurons were voltage clamped at -70 mV for a set of 10–12 EPSCs and then voltage-clamped at +40 mV for a set of 10–12 EPSCs. RI was calculated as the average of absolute peak current at -70 mV divided by absolute peak current at +40 mV for each neuron. In a subset of recordings, voltage was varied from -70 to -40, 0, +20, and +40 mV for ≥10–12 EPSCs each, to obtain peak current-voltage (I-V) relationship, verify minimal voltage offsets (no corrections were performed), and show separation of smaller EPSCs from stimulus artifacts. The voltage-dependent unblocking index (UBI) was calculated by obtaining the paired-pulse ratio (PPR) of averaged amplitudes (≥25 currents) EPSC2/EPSC1 (obtained within a 70 ms interstimulus interval) at +40 mV and divided by the PPR of averaged amplitudes at -70 mV for each neuron (Rozov and Burnashev). The averaged current for EPSC1 at -70 mV was normalized by the averaged amplitude of EPSC1 at +40 mV and compared (Fig. 2C, I and 2) to demonstrate degree of unblock for EPSC2 at +40 mV for inward rectifying AMPARs. Because EPSCs are potentially a mixture of inward rectifying and linear AMPARs, then RI is a reflection of that percentage (supplemental text). Because presynaptic-mediated paired pulse ratios will be the same at different postsynaptic holding potentials, UBI can be expressed in terms of the fractional percentage of inward rectifying AMPARs (PRI) as

\[
\text{UBI} = \frac{\text{PRI}(F_2 - F_3)/(\text{PRI}(F_1 - F_3) + F_3)}{\text{PRI}(F_4 - 1) + 1} \quad (1A)
\]

where, for a pure population of only inward rectifiers, F1 is the maximal block at +40 mV/EPSC1 at -70 mV) and F2 is the unblock at +40 mV/EPSC2 at -70 mV; unblock at -70 mV (F4 = EPSC2 at -70 mV/EPSC1 at -70 mV) was assumed to be near unity (see results, Fig. 2B). F1 was extrapolated to be ~0.05 (based on Rozov et al. 1998, their Fig. 4). For a linear AMPAR conductance, F3 is 0.57 (40/70). F1, F2, and F4 apply only to pure inward rectifiers; F3 applies only to linear AMPARs. The unknown F2 would be expected to be less than or equal to F3, based on the degree of unblock (Rozov et al. 1998). The unblock ratio, the amount of unblock of inward-rectifying AMPARs after single paired pulses, (F2/F3, where F3' = 40/70), would be expected to be less than unity for single paired pulses compared with a train of pulses when it would more closely approach unity (Rozov et al. 1998).

Furthermore, using

\[
\text{PRI} = (1 - \text{RI} \times F_3)/[\text{RI} \times (F_1 - F_3)] \quad (1B)
\]

allows an estimate of PRI from RI. This analysis assumes that presynaptic function, as it affects the activation of either pool of AMPARs, is equal.

AMPAR-mediated aEPSCs were visually detected as peaks arising ≥2.5 times the noise (σ_N, average variance 2.04 pA^2, range: 0.81–7.84 pA^2, n = 38). aEPSCs were analyzed for amplitude (Q) and quantal kinetics (τ_\text{rise} and τ_\text{decay}) using purpose-written software in which responses were identified based on the point of onset (Benke et al. 1998, 2001). Responses were selected as long as they were not responses were identified based on the point of onset (Benke et al. 1998, 2001). These limitations prevented analysis on some neurons due to an inadequate number of responses (Benke et al. 2001). Selected individual aEPSCs were averaged to obtain a mean response waveform. This mean aEPSC response waveform was then scaled to the peak of each individual aEPSC peak and subtracted and squared. The variance of fluctuation around the mean was calculated for 10–100 bins of equal current decline from the peak of the mean current until within a time frame five to six times τ_\text{decay}. The binned variance was plotted against the mean current amplitude and the single-channel current was estimated by fitting the data using a least-squares algorithm according to

\[
\sigma^2 = i \times I - F/(N \times P_o) + b_i \quad (2)
\]

where \(\sigma^2\) is the variance, I is the mean current, \(N \times P_o\) is the number of AMPAR activated at the peak of the mean current, i is the single-channel current, and \(b_i\) is the background variance (Sigworth 1980; Traynalis et al. 1993). The single-channel conductance, \(\gamma\), is then \(\gamma = i/V\) where \(V\) is the driving force (holding potential of -70 mV, assumed AMPAR reversal potential of 0 mV). To obtain the most accurate estimate for \(\gamma\), the data were fitted from 0 to 50% of the maximum current (Benke et al. 1998, 2001) with goodness of fit assessed with the Spearman rank-order correlation coefficient (\(R^2_{\text{sp}}\)) (Bannister et al. 2005; Press et al. 1992). Mean current (I) peak amplitude and single-channel conductance infer total AMPAR numbers and peak opening probability according to

\[
I = N \times P_o \times \gamma \times V = Q_{\text{calc}} \quad (3)
\]

where I is the mean current amplitude, \(N\) is the average total number of AMPARs across the population of AMPAR clusters activated, \(P_o\) is the peak open probability of AMPAR opening, \(\gamma\) is the single-channel conductance, and \(V\) was the holding potential. NSFA permits calculation of \(N \times P_o\) for individual neurons; peak-scaled NSFA does not allow direct calculation of \(P_o\). In the case of quantal aEPSCs, peak quantal current (\(Q_{\text{calc}}\)) and peak mean current (I) are theoretically equivalent.

Statistics

For grouped comparisons, data are expressed as mean ± SE and \(n\) = number of recordings from individual neurons; Mann-Whitney Rank Sum tests were used for comparisons of two groups, with significance set at \(P \leq 0.05\) (SigmaPlot, Systat, Point Richmond, CA). Kruskal-Wallis one-way ANOVA on ranks was used to compare three groups with Dunn’s method for pair-wise testing of multiple comparisons. Significance was set at \(P \leq 0.05\). Linear interpolation was used to determine trends with \(r^2\) used to assess trend (1 = perfect) and \(r\) to assess significance. Median values were interpolated from binned cumulative data distributions as the point at which half of the distribution was above and half below that point. Grouped cumulative distributions were binned along the y axis for averaging. For comparisons of averaged cumulative distributions, the Kolmogorov-Smirnov (K-S) test was used with significance set at \(P \leq 0.05\) (Press et al. 1992). Hartigan’s dip test was used to test for multimodal distributions [significance set at \(P \leq 0.1\) (Hartigan and Hartigan 1985; Priebe et al. 2004; R Development Core Team 2010)]. Cluster analysis (JMP, SAS, Cary, NC) was used to suggest segregational break points for group comparisons. Levene’s test of equal variance (JMP, SAS, Cary, NC) was used for some group comparisons.
RESULTS

Inward rectifying AMPARs exist very early in development at CA1 synapses

To establish the existence of inward rectifying AMPARs during early development, we conducted whole cell recordings and obtained current-voltage relationships from electrically evoked EPSCs in individual pyramidal neurons. It is known that inward rectifying AMPARs exhibit a voltage-dependent decrease in open probability at positive holding potentials compared with negative holding potentials when in the presence of intracellular polyamines such as spermine (Bowie and Mayer 1995; Kamboj et al. 1995; Rozov et al. 1998). This means inward rectifying channels mediate less current at positive holding potentials compared with negative holding potentials. Figure 1 demonstrates the voltage-dependent properties of EPSCs in the presence of intracellular spermine. An example neuron at P6 (Fig. 1A1) demonstrated minimal outward EPSCs at +40 mV compared with the robust inward EPSCs at +70 mV to indicate the presence of inward rectifying, synaptic AMPARs. This was not always the case, however, because synaptic AMPARs also demonstrated robust current at both positive and negative holding potentials (Fig. 1A2, example neuron) at this age. I-V curves for neurons between ages P8 and 18 consistently revealed linear synaptic AMPAR conductances (Fig. 1A3, example neuron). Because the largest RI was found at P7 (RI = 21.0), all values of RI after P7 were <5.0, and cluster analysis of RI across all age groups segregated all RI > 6.0, we grouped all neurons at P5–7 and P8–18 (Fig. 1B). Mean RI at P8–18 (2.67 ± 0.15, n = 38) was significantly smaller than mean RI at P5–7 (5.39 ± 0.77, n = 38; P = 0.005, Mann-Whitney rank sum) suggesting the presence of inward rectifying, synaptic AMPARs restricted to P5–7.

Because this method of calculating RI can be biased toward higher RI values due to the presence of relatively small EPSCs in developing neurons, especially in the presence of small voltage offsets, full I-V relationships were obtained in a subset of neurons (Fig. 1A). At P5–7, we segregated RI > 6 from the

![Figure 1](jn.physiology.org/10.1152/jn.00570.2009)

**FIG. 1.** Inward rectifying AMPARs were only present in some P5–7 pyramidal neurons. A1: inward rectifying excitatory postsynaptic currents (EPSCs) at P6 showed less outward current at +40 mV holding potential (11 traces averaged) compared with inward currents at −70 mV (10 traces averaged) resulting in a high rectification index (RI). A2: nonrectifying EPSCs at P6 with greater outward current at +40 mV (10 traces averaged) and at +20 mV (10 traces) had a lower RI. A3: nonrectifying EPSCs at P15 with robust outward current at +40 mV (35 traces averaged) and at +20 mV (34 traces averaged). B: mean RI at P8–18 (2.67 ± 0.153, n = 38) was significantly smaller than mean RI at P5–7 (5.39 ± 0.77, n = 38; P = 0.005, Mann-Whitney rank sum). C: I-V plots averaged from P5–7 inward rectifiers (light gray triangles, n = 5, RI > 6.5) were significantly different from older P8–18 (black circles, n = 9; +40 mV, *P = 0.004; +20 mV, *P = 0.032 Kruskal-Wallis 1-way ANOVA on ranks, Dunn’s post hoc). The P5–7 inward rectifiers were also significantly different from their nonrectifying littermates (dark gray circles, n = 7, RI < 2.9; +40 mV, **P = 0.006; +20 mV *P = 0.032, Kruskal-Wallis 1-way ANOVA on ranks, Dunn’s post hoc). Linear P5–7 neurons were not statistically different at any holding potential compared with the older P8–18 neurons (P > 0.05 Kruskal-Wallis 1-way ANOVA on ranks, P > 0.05 Dunn’s method).
Isolation of quantal AMPAR-mediated EPSCs at immature CA1 hippocampal synapses

Because AMPAR rectification properties changed significantly at P8 in CA1 pyramidal neurons and to address whether AMPAR subtypes might be segregated or mixed at synapses, we further assessed synaptic AMPARs to elucidate other developmental differences to support our findings. We measured peak quantal current (\(Q\)), kinetics of deactivation (\(\tau_{\text{decay}}\)), and the underlying single-channel conductance (\(\gamma\)). Calcium was substituted with strontium in the extracellular recording medium to cause asynchronous release (i.e., 1 quantum at a time) of glutamatergic vesicles from presynaptic axonal terminals (Bekkers and Clemens 1999; Oliet et al. 1996; Xu-Friedman and Regehr 1999), (Fig. 3, A and B). Near-minimal stimulation allowed the individual quantal activation of a small population of synapses containing AMPARs. By stimulating at a uniform distance from the somatic recording electrode, we approximate that responses emanated from a similar position in the dendritic tree to minimize the contribution of different electrotonic filtering to measurements. It was previously demonstrated that eEPSCs, in contrast to mEPSCs, eliminated discrepancies associated with the activation of synapses throughout the dendritic tree that would likely be differentially filtered (Bekkers and Clemens 1999). eEPSCs were AMPAR-mediated responses because in the presence of d-2-amino-5-phosphono-pentanoic acid (d-AP5), they were completely blocked by the AMPAR-selective antagonist GYKI 52466 (100 \(\mu\)M) across all ages. (see METHODS). Response amplitudes of eEPSCs remained stable for \(\leq 35\) min (Fig. 3C) following membrane rupture. Response stability allowed us to apply both quantal analysis and peak-scaled nonstationary fluctuation analysis (NSFA) to eEPSCs to report a weighted mean estimate (Cull-Candy et al. 1988) of the underlying AMPAR \(\gamma\).
Quantal AMPA-mediated aEPSC amplitude during early development

We analyzed AMPAR-mediated aEPSC quantal amplitudes (Q) from each neuron across ages P5-18. We used density and cumulative distribution histograms to assess median Q, mean Q, and relative variability (CQV) for each neuron (Fig. 4, A and B). Q did not appear to be normally distributed in individual neurons at the younger ages (Fig. 4A, I and 2, P5 representative neuron), which necessitated analysis of cumulative distributions and median Q from each neuron. While suggestive, there was not a significant bimodal component for this distribution (Hartigan’s dip test, D = 0.030, 117 events, p = 0.7); pooled event data from all P5–7 neurons (not shown) also did not have a significant bimodal component (Hartigan’s dip test, D = 0.008, 732 events, p = 0.99). In comparison, distributions of Q from the older age group (P8–18; P17 representative neuron, Fig. 4B, I and 2) typically appeared more uniform (Hartigan’s dip test, D = 0.014, 290 events, p = 0.5); pooled event data from all P8–18 neurons (not shown) also did not have a significant bimodal component (Hartigan’s dip test, D = 0.005 1985 events, p = 0.99).

Due to the significant differences in rectification properties in neurons at ages P5–7 compared with P8–18 (see Fig. 1), we then grouped median Qs at the respective age groups accordingly for direct comparison (Fig. 4, C–E). Even though distributions of Q were (suggestively) more broadly distributed at

FIG. 2. Polyamine-mediated voltage dependence of paired-pulse stimulation was restricted to P5–7. A: paired-pulse ratio (PPR) at −70 mV at P5–7 (1.71 ± 0.18, n = 14) compared with P8–18 (1.85 ± 0.18, n = 18, P = 0.45, Mann-Whitney rank sum) were similar. B: at P5–7, there was no correlation of PPR at −70 mV with rectification index (solid line, r² = 0.009, 95% confidence intervals dashed lines). C: EPSCs evoked by paired-pulse stimulation at +40 and −70 mV were compared (top) in which (PPR at +40 mV)/(PPR at −70 mV) = the unblocking index. C1: inward rectifying AMPARs (P6, RI = 6.81 shown) demonstrated unblocked current in the 2nd pulse at +40 mV (peak current after 1st pulse = 10.54 pA, after 2nd pulse = 65.23 pA, 70 ms interstimulus interval, compared with the −70 mV peak current after 1st pulse = −79.54 pA, after 2nd pulse = −123.59 pA; unblocking index = 3.98). Bottom: the normalized currents from the top overlapped to reveal more unblock at +40 mV (vs. −70 mV) for inward rectifying AMPARs. C2: nonrectifying AMPARs (P15, RI = 1.12 shown) demonstrated little unblock on 2nd pulse at +40 mV, compared with C1 example (peak current after 1st pulse = 45.66 pA, after 2nd pulse = 60.92 pA, 70 ms interstimulus interval compared with the −70 mV peak current after 1st pulse = −53.80 pA, after 2nd pulse = −92.86 pA; unblocking index = 0.77). D: the degree of polyamine unblock correlated with the rectification index of neurons at P5–7 (Eq. 1A, n = 18, r² = 0.7, P < 0.0001). This correlation predicted an unblocking ratio (F2/F3 = 0.34/0.57) of less than unity (0.6). Minimal unblock was seen at P8–18 (n = 14, F2 = 0.09, r² = 0.03, P = 0.54, line not shown) suggesting a much smaller unblocking ratio (F2/F3 = 0.09/0.57 = 0.16). E: measurements of RI from D are replotted (Eq. 1B) to demonstrate the relationship of RI to the fraction of inward rectifying AMPARs activated; a fraction of 1 is equivalent to the synaptic activation of only inward rectifying AMPARs. Measurements of RI are relatively insensitive to changes in the fraction of inward rectifying AMPARs <0.5 (dotted line). Fractions >0.75 correlate with RI >6 (solid intersecting lines).
Fig. 3. AMPAR-mediated asynchronous EPSCs (aEPSCs) in the presence of extracellular Sr$^{2+}$. A, 1 and 2: successive traces of AMPAR-mediated aEPSC (a–d) evoking following an electrical stimulus (blanked at *) in the presence of 8 mM Sr$^{2+}$/0 added Ca$^{2+}$/1Mg$^{2+}$ in an example CA1 pyramidal neuron (P13). Series resistance and capacitance were monitored by the current response to the −2 mV voltage step. B: AMPAR-mediated aEPSCs (a–d from A) were distinguished from noise ($\sigma_n = 2.77$ pA, $\sigma_n^* = 7.67$ pA$^2$) and fitted with 2 exponentials (grey, superimposed, see METHODS); Bc: $\tau_{rise} = 2.1$ ms $\tau_{decay} = 8.5$ ms; Bb: $\tau_{rise} = 2.1$ ms $\tau_{decay} = 9.2$ ms; Bc: $\tau_{rise} = 1.5$ ms $\tau_{decay} = 10.2$ ms; Bd: $\tau_{rise} = 3.1$ ms $\tau_{decay} = 5.8$ ms. C: aEPSC amplitudes remained stable for the duration of data acquisition (data from neuron in A and B shown).

P5–7, when segregated into groups at P5–7 and P8–18, we found that group-averaged Q distributions for P8–18 were significantly shifted toward larger amplitudes compared with group-averaged Q distributions at P5–7 (Fig. 4C, **$P = 0.0026$, K-S test). Furthermore, median Qs from each individual neuron (cf. Fig. 4, A2 and B2, dotted lines) were found to be significantly increased between groups during postnatal development [Fig. 4D, P5–7 mean of median Qs = 8.41 ± 0.57 pA (n = 15); P8–18 mean of median Qs = 12.59 ± 0.66 pA (n = 23); **$P < 0.001$, Mann-Whitney rank sum test]. Also because there was great variability in the occurrence of inward rectifiers (Fig. 1B) and the suggestively broader distributions of Q (Fig. 4A, 1 and 2) at ages P5–7, we examined whether the relative variability of amplitudes ($CV_Q$) was higher at younger ages compared with the older ages. The suggestion of higher variability for AMPAR Q across all neurons at ages P5–7 compared with P8–18 was not significant [Fig. 4E, P5–7: mean of $CV_Q = 0.46 ± 0.065$ (n = 15), P8–18: mean of $CV_Q = 0.386 ± 0.031$ (n = 23) $P = 0.385$, Mann-Whitney rank sum].

Quantal AMPAR-mediated aEPSC kinetics during early development

Inward rectifying AMPARs display faster kinetics ($\tau_{rise}$ and $\tau_{decay}$) than linear AMPARs (Oh and Derkach 2005). Collective activation time of an AMPAR synapse is represented in the $\tau_{rise}$ phase of an aEPSC, while the time it takes for AMPARs to deactivate and desensitize is evident in the $\tau_{decay}$ phase of an aEPSC. We measured quantal $\tau_{rise}$ and $\tau_{decay}$ for each aEPSC from each individual neuron across the developmental time points P5–18 and hypothesized the kinetics would be faster for P5–7 when grouped and compared with older animals. Density (Fig. 5, A1 and B1) and cumulative (A2 and B2) distributions of quantal $\tau_{decay}$ for each aEPSC (Fig. 3, A and B) from each neuron were analyzed. Analysis of $\tau_{rise}$ suggested that these distributions were potentially influenced by filtering while $\tau_{decay}$ was not (Supplemental Figs. S1 and S2). Similar to our findings of Q per neuron, aEPSC quantal $\tau_{decay}$ in each neuron demonstrated wider distributions at P5–7 (representative P7 neuron shown in Fig. 5A, 1 and 2) compared with individual neurons at P8–18 (representative P11 neuron shown in Fig. 5B, 1 and 2). While suggestive, there was not a significant bimodal component for this distribution at P7 (Hartigan’s dip test, $D = 0.022$, 83 events, $p = 0.9$); pooled event data from all neurons at P5–7 (see Supplemental Fig. S4F) also did not have a significant bimodal component (Hartigan’s dip test, $D = 0.011$, 732 events, $p = 0.9$). Given the variability of Q and aEPSC $\tau_{decay}$ at this age, we compared Q and aEPSC $\tau_{decay}$ for each aEPSC at both ages; no correlations were found (Supplemental Figs. S1 and S2). Similar to our findings of Q per neuron, $\tau_{rise}$ was not a significant bimodal component for this distribution at P7 (Hartigan’s dip test, $D = 0.022$, 83 events, $p = 0.9$); pooled event data (not shown) did not have a significant bimodal component (Hartigan’s dip test, $D = 0.007$, 1985 events, $p = 0.99$).

We averaged cumulative probability distributions of quantal $\tau_{decay}$ for all P5–7 neurons and compared them to all P8–18 neurons. This revealed that distributions of quantal $\tau_{decay}$ for all P5–7 neurons were significantly different from all P8–18 neurons (Fig. 5C, P5–7 n = 15, P8201318 n = 23, **$P = 0.0022$ K-S test), most likely due to significantly slower channels at P8–18. Median quantal $\tau_{decay}$ were much faster for all P5–7 [mean of median $\tau_{decay} = 3.48 ± 0.467$ ms (n = 15)] compared with all P8–18 [mean of median $\tau_{decay} = 7.03 ± 0.22$ ms (n = 23), **$P < 0.001$, Mann-Whitney rank sum test]. Median quantal $\tau_{decay}$ were clearly segregated into two groups at P5–7 (Hartigan’s dip test, $D = 0.097$, modal break point = 2.6 ms, $n = 15$, $P < 0.1$). To further determine the source of variability of quantal $\tau_{decay}$, we compared the $CV_{\tau_{decay}}$ of each neuron from the two age groups. Consistent with a greater degree of variability in inter-synaptic parameters at P5–7, neurons at P5–7 had a significantly higher $CV_{\tau_{decay}}$ when grouped together and compared with all neurons at P8–18 (Fig. 5E, *$P = 0.023$, Mann-Whitney rank sum). Multivariate cluster analysis of all neurons also segregated neurons into groups of P5–7 and P8–18, similar to the grouping suggested by inward rectification.

Single-channel conductance ($\gamma$) of AMPARs in developing CA1 neurons

It is known that inward rectifying AMPARs have a higher $\gamma$ compared with linear AMPARs (Oh and Derkach 2005). We
used peak-scaled nonstationary fluctuation analysis (NSFA) to estimate AMPAR $\gamma$ across P5–18 to determine if $\gamma$ might decrease during the developmental time points tested. To perform this analysis, a subset of the original aEPSCs (Fig. 6A, 1 and 2) was chosen from each neuron meeting the necessary criteria (see Methods; Fig. 6B, 1 and 2). The average $\gamma$ at P5–7 compared with P8–18 was not statistically different. Similar to rectification and quantal $\tau_{\text{decay}}$, the greatest differences in $\gamma$ occurred at P5–7 [Fig. 6C, P5–7: $\gamma = 9.46 \pm 3.10$ pS ($n = 6$); P8–18: $\gamma = 7.52 \pm 0.37$ pS ($n = 15$), Levene’s $F = 10.59$, $P < 0.0042$]. Independent of age, $\gamma$ was relatively uniform with the corresponding AMPAR median $Q$ for each neuron tested (Fig. 6D). At P6, one neuron demonstrated a very high $\gamma$ (24.3 pS) and correspondingly low $Q$ (5.0 pA). It is possible that neurons may, indeed, have AMPAR channels that conduct more single-channel current than this plot indicates. However, because only a weighted mean conductance per neuron is possible using peak-scaled NSFA, it is likely that if there are only a few high-conducting channels, then they are most likely obscured by a comparatively greater number of lower-conducting channels. Therefore AMPAR $\gamma$ was not likely the dominant mechanism regulating AMPAR $Q$; thus the modest increase in $Q$ during development (P5–18) cannot be explained by an increase in AMPAR single-channel conductance.

$N^*P_o$ increases to directly mediate larger AMPAR quantal amplitudes

At thalamocortical synapses, $Q$ is tightly controlled across development by regulation of $\gamma$ and the number of activated receptors ($N^*P_o$) (Bannister et al. 2005). Because AMPAR $\gamma$ demonstrated no direct correlation with $Q$ (Fig. 6D), it was possible that $Q$ was directly associated with the number of activated receptors ($N^*P_o$). This can also be determined from peak-scaled NSFA (Eq. 3). When $N^*P_o$ was examined over the developmental ages P5–18, there was no clearly defined correlation (Fig. 7A). However, $N^*P_o$ was directly correlated with $Q$ (Fig. 7B, linear regression, $R^2 = 0.74$, $P < 0.001$). Thus
regulation of AMPAR number and/or AMPAR activation most directly influenced $Q$ at these synapses during early postnatal development. Increasing AMPAR number and/or its activation best supports increases in $Q$ from P5–7 to P8–18.

**DISCUSSION**

Development of AMPAR synapses

In the CNS, the hallmark of synaptogenesis is the formation of contacts between presynaptic axons and postsynaptic densities to form a synapse (Cohen-Cory 2002). During this formative phase, which includes the ages studied here (P5-18), anatomical data and microscopy studies have demonstrated significant neuronal growth, both in size and shape (Fiala et al. 1998), as well as an increase in dendritic spine density (Cottrell et al. 2000) and total number of AMPAR clusters per spine (Tyzio et al. 1999). We hypothesized that synaptic AMPAR properties also change significantly during this time period. Under our experimental conditions, $Q$ increased across the developmental time points P5-18. This is an extension of the previous finding that spontaneous EPSCs recorded in this brain region also increase from birth up to P8 (Groc et al. 2002). We determined that these increases in amplitude are directly mediated by increases in the number of activated AMPARs ($N^*P_o$) across development. Importantly, our findings suggest that the increases in AMPAR numbers occur across development in a heterogeneous fashion. Inward rectification and faster channel kinetics exclusively at P5–7 suggest that AMPARs mediating these properties are restricted to this developmental window. Furthermore, our data suggest that heterogeneous AMPARs are segregated to distinct synapses prior to P8. After P8, AMPAR synaptic properties are more uniform. These findings have important and novel implications for understanding how AMPAR synapses develop and raise important questions about the role of these distinct synapses in early synaptogenesis.

Neuronal versus synaptic variability in early development

We questioned first whether developing neurons express uniform synaptic AMPAR properties and second if nonuni-
form, whether these were expressed distinctly or as mixtures across synapses. We suggest that during P5–7, developing hippocampal neurons segregate distinct AMPARs to different synapses leading to pronounced inter-synaptic variability. From P8, synaptic properties change with neuronal development but are more uniform across synapses. Synaptic variability prior to P8 was supported by several findings. First, measurement of the apparent rectification index demonstrated inward rectification only before P8 (Fig. 1B). Comparison with inward rectification after P8 suggested a cut-off value for better visualization (Fig. 1C). Analysis of apparent inward rectification compared with the unblocking index revealed that inward rectifying AMPARs are likely segregated to synapses across a continuum but with some synapses containing nearly all inward rectifying AMPARs. We approximated 26% of synapses contain ≥75% inward rectifying AMPARs at P5–7. We therefore investigated quantal aEPSCs to further quantify inter-synaptic variability. Second, the range of CVQ at P5–7 was greater than what would be expected for intra-synaptic variability (kinetic variability at an individual synapse) (Liu et al. 1999; McAllister and Stevens 2000). Therefore there was additional inter-synaptic variability in the quantal amplitude, indicating a great degree of variability across synapses. These findings of bimodal Q are reminiscent of those found in the developing Xenopus visual system (Pratt and Aizenman 2007). Prior studies have found that CVQ is low near birth but increases to similar levels to what we have found around P5–7 (Groc et al. 2002; Hanse and Gustafsson 2001); we have extended this to show that CVQ then suggestively decreases and stabilizes after P7. Third, median quantal τdecay was not correlated with CVQ (linear regression: n = 21, r² = 0.74, P < 0.001).
was greater at P5–7 than other ages (similar to increased CV\(_{Q}\)). This suggests that neurons at P5–7 can simultaneously contain two distinct populations of synapses. If synapses at P5–7 simply contained mixtures of functionally different AMPARs, it would be expected that the median quantal \(\tau_{\text{decay}}\) would be a weighted mean of the relative proportion of each without necessarily increased \(CV_{\tau_{\text{decay}}}\) (Supplemental Fig. S4). While statistically we did not find evidence of multimodal distributions with the use of Hartigan’s dip test, this test has low power for closely spaced modes or pronounced tails (Hartigan and Hartigan 1985) as seen with modeled data (Supplemental Fig. S4). We therefore suggest that analysis of \(CV_{\tau_{\text{decay}}}\) is more sensitive for detecting intersynaptic variability. The variability of quantal \(\tau_{\text{decay}}\) in individual neurons resulting in \(CV_{\tau_{\text{decay}}} > 1\) at P5–7 can most parsimoniously be explained by the co-existence of biophysically distinct AMPAR synapses; indeed, some mixture of different AMPARs at a synapse was also likely present to some degree. Nevertheless, the finding that there was markedly faster quantal \(\tau_{\text{decay}}\) at P5–7 suggested that younger synapses were biophysically different from older synapses. Taken together, this supports our finding that different AMPAR synapses co-exist at younger ages. Combining our similar estimates from inward rectification and pooled distribution of quantal \(\tau_{\text{decay}}\) (Supplemental Fig. S4F), we conservatively estimate that 30% of synapses prior to P8 may contain >75% inward rectifying AMPARs.

Functionally, inward rectification was not associated with differences in paired-pulse facilitation (Fig. 2, A and B). This suggested that presynaptic function did not contribute to intersynaptic variability and that each population of synapses, although different in numbers, was likely activated with equal probability. Our subsequent analysis to estimate the fraction of inward rectifying AMPARs relied on this assumption. A prior study found two populations of synapses at P6 with different release probabilities linked to different mechanisms of synaptic plasticity (Palmer et al. 2004). This study utilized a minimal stimulation protocol compared with our maximal stimulation protocol used to detect inward rectification. Maximal stimulation was necessary to minimize errors associated with the classification of synaptic failures as inward rectification. Thus the incidence of synapses with low release probability was potentially obscured by the protocol we employed. The prior study did not find that synapses with lower release probability contained higher conductance AMPARs (Palmer et al. 2004), which might have suggested that these synapses contained inward rectifiers, even rarely, as found here (Fig. 6). If taken on its own, however, our estimate from analysis of quantal \(\tau_{\text{decay}}\) at P5–7 suggesting 30% of synapses with inward rectifying AMPARs (Supplemental Fig. S4) could also be consistent with a lower probability of release at these synapses.

AMPARs can have a markedly faster \(\tau_{\text{decay}}\) at P5–7. While we have attributed this to the presence of inward rectifying AMPARs, this is potentially influenced by multiple factors (Conti and Weinberg 1999), such as subunit flip/flop variants (Partin et al. 1994; Quirk et al. 2004), glutamate release/uptake effects (Clements et al. 1992; Liu et al. 1999) on agonist-receptor desensitization/dissociation (Trussell and Fischbach 1989), and subunit composition (Oh and Derkach 2005). Previous studies demonstrated a uniform effect of cyclothiazide across the developmental ages studied here (Seifert et al. 2000), suggesting that flip/flop variability and desensitization effects are minimally contributing at these ages which has been supported by expression studies (Monyer et al. 1991). AMPAR-mediated EPSC \(CV_{\tau_{\text{decay}}}\) has not been previously examined during early development. A previous study at cerebellar inhibitory GABAergic synapses (Nusser et al. 2001), showed an increased \(CV_{\tau_{\text{decay}}} (0.1–0.3)\) that was associated, through modeling studies, with fluctuations in the time-course of GABA in the synaptic space of >55%. \(CV_{\tau_{\text{decay}}}\) measured here was substantially greater than this, supporting our conclusion that the variability was due to the simultaneous presence of functionally different receptor clusters and thus intersynaptic variability. It is unlikely that glutamate concentration cleft dynamics are fluctuating on a trial-to-trial basis to a degree to explain our findings. In support of this, strontium is thought to stabilize synaptic vesicle fusion to favor full, versus partial, release (Elmdandi et al. 2006). Correlation with inward rectification thus favors that faster kinetics are most likely due to inward rectifying AMPAR subunits at the synapse at P5–7 (Oh and Derkach 2005). While inward rectifiers were present at P5–7, they were never detected after P8 (Fig. 1, B and C).

However, not all P5–7 neurons exhibited inward rectification, as some behaved like P8–18 neurons with nonrectifying (linear) AMPARs (Fig. 1C) and slower channel kinetics (Fig. 3, C and D). AMPAR single-channel conductance (\(\gamma\)) was not significantly higher at the younger P5–7 ages (Fig. 3C), though, to support inward rectification at the younger ages. The variance of \(\gamma\) at P5–7 was significantly higher than at P8–18 (Fig. 3C), consistent with the presence of inward rectifying AMPAR channels in some but not all of the P5–7 neurons. As noted, \(CV_{\tau_{\text{decay}}}\) was also comparatively much higher at P5–7 (Fig. 5E) to support the wide variability of rectification at these ages both across neurons as well as synapses within a neuron. Quantal amplitude significantly increased from P5–7 compared with P8–18 (Fig. 3, C and D). This was most consistent, overall, with the developmental increase in AMPAR number (\(N_{\text{P}_{2}}\); Fig. 6B). This increase likely overshadowed any differences in \(CV_{Q}\) that might have been expected due to the presence of different AMPAR subtypes. Taken together, while two very different AMPAR synapses exist at younger ages (P5–7), the number of active AMPARs that increase at the synapse up to P18 are less variable in their biophysical properties (\(CV_{\tau_{\text{decay}}}\) and \(\gamma\)), slower, (\(\tau_{\text{decay}}\)) and are never inward rectifying (lower RI). Thus the biophysical properties of increasing numbers of AMPARs across neurons at P8–18 are much more uniform across synapses compared with the younger P5–7 age group.

Inward rectification, because of its association with calcium permeability of AMPARs (Bowie and Mayer 1995; Geiger et al. 1995; Kamboj et al. 1995), and faster kinetics, suggests that younger synapses have uniquely different electrical and chemical signaling properties compared with older synapses. AMPARs lacking GluA2 uniquely have faster kinetics (Oh and Derkach 2005) and greater relative calcium permeability/inward rectification (Geiger et al. 1995; Kamboj et al. 1995; Oh and Derkach 2005). These properties matched the features of synapses seen only at P5–7. Therefore we speculate that prior to P8, many synapses completely lack GluA2, but other synapses prior to P8 seem to contain GluA2; after P8, synapses generally contain GluA2. Immature synapses are thought to initially develop as “silent” synapses, containing relatively calcium permeable...
NMDA receptors (Liao et al. 1999). AMPAR are acquired through calcium-mediated, activity-dependent signaling (Harms et al. 2005; Isaac 2003; McAllister 2007; Petralia et al. 1999). Our findings temporally link calcium-permeable AMPARs with the synaptic development of principal neurons in hippocampal CA1. However, our findings could be explained by synapse specific clustering of transmembrane AMPAR regulatory proteins (TARPs) to achieve a similar clustering of AMPAR function (Kott et al. 2007; Soto et al. 2007).

Developmental increase in size of AMPAR synapses

Although the total number of activated receptors at the peak \( N^o P_o \) of quantal amplitude \( Q \) was greater to modulate a larger \( Q \), it was not possible to determine which factor of \( N^o P_o \) contributed to the increases in median amplitude with age over the developmental range tested (Fig. 6). Either receptor numbers \( N \) and/or peak-open probability \( P_o \) as influenced by such factors as cleft glutamate concentration (Liu et al. 1999), could be changing with development. This is due to the nature of peak-scaled NSFA, in that receptor number \( N \) and peak-open probability \( P_o \) cannot be directly calculated and thus cannot be separated. However, studies suggest that greater numbers of AMPARs are inserted postsynthetically with developmental age (Cottrell et al. 2000; Petralia et al. 1999; Pickard et al. 2000) favoring that increases in \( Q \) were primarily mediated by increases in \( N \).

It has been shown that channel conductance can depend on glutamate concentration (Gehrmann and Cull-Candy 2006; Rosenmund et al. 1998; Smith and Howe 2000) although under conditions in which desensitization is minimized. Glutamate concentrations in the synaptic cleft may increase during synaptogenesis (Renger et al. 2001); this would also cause an increase in the quantal amplitude (Franks et al. 2003) via increased \( P_o \) (Momiymama et al. 2003) if AMPARs were not already saturated. Under our conditions of presumed monovesicular, full release in strontium (Elhamdani et al. 2006), this would have to occur by either greater vesicular filling with transmitter or alterations in cleft volume and geometry. If this was the case, then increases in cleft glutamate levels could also then lead to an increase in the number of glutamate molecules bound to AMPAR and thus influence and favor higher AMPAR conductance levels (Cathala et al. 2005). Therefore if our measurements of relatively stable \( \gamma \) across development were an indication of stable cleft glutamate concentrations, this would suggest that \( P_o \) did not change significantly during the developmental range tested.

Significance of distinctly different AMPAR synapses at very early postnatal ages

Developmental changes in AMPAR subunit composition (Monyer et al. 1991; Pickard et al. 2000) have been demonstrated in the neocortex (Kumar et al. 2002; Shin et al. 2005) and CA3 hippocampus (Ho et al. 2007), but developmental regulation in which these subunits are segregated to different synapses has not been reported. Transient AMPAR subunit composition changes have been described with LTP (Plant et al. 2006), following hypoxia induced seizures (Rakhade et al. 2008), and with synaptic inactivity (Thiagarajan et al. 2005). Developmental changes in AMPAR subunits have been associated with dendritic branch-point maturation in the spinal cord (Inglis et al. 2002). It is assumed that these composition changes result in mixtures of AMPARs at synapses. Therefore and in contrast, the transient expression of different AMPAR at distinct synapses suggested here from P5–7 likely underlies an important developmental process of network maturation in CA1 hippocampus. Indeed the expression mechanisms of synaptic plasticity have been reported to change around P5–7 (Palmer et al. 2004). Further studies will be necessary to determine the exact impact at P5–7 of the faster, inward-rectifying AMPAR synapses that exist with the slower, non-rectifying, mature AMPAR synapses. We speculate in the developing hippocampus that the faster synapses are both GluA2-lacking and calcium permeable, leading to greater calcium accumulations and possibly more finely tuned dynamic signaling, compared with synapses with mixtures of AMPARs. This could have a substantial impact on not only our understanding of synaptic development but also disorders primarily affecting immature synapses (Cornejo et al. 2007).

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