Different Roles for AMPA and NMDA Receptors in Transmission at the Immature Retinogeniculate Synapse

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Liu X, Chen C. Different roles for AMPA and NMDA receptors in transmission at the immature retinogeniculate synapse. J Neurophysiol 99: 629–643, 2008. First published November 21, 2007; doi:10.1152/jn.01171.2007. The relay of information at the retinogeniculate synapse, the connection between retina and visual thalamus, begins days before eye opening and is thought to play an important role in the maturation of neural circuits in the thalamus and visual cortex. Remarkably, during this period of development, the retinogeniculate synapse is immature, with single retinal ganglion cell inputs evoking an average peak excitatory postsynaptic current (EPSC) of only about 40 pA compared with 800 pA in mature synapses. Yet, at the mature synapse, EPSCs >400 pA are needed to drive relay neuron firing. This raises the question of how small-amplitude EPSCs can drive transmission at the immature retinogeniculate synapse. Here we find that several features of the immature synapse, compared with the mature synapse, contribute to synaptic transmission. First, although the peak amplitude of EPSC is small, the decay time course of both α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) and N-methyl-D-aspartate receptor (NMDAR) currents is significantly slower. The prolonged time course of NMDAR currents is a result of the presence of both NR2B and NR2C/D subunits. In addition, the extended presence of neurotransmitter released prolongs the synaptic current time course. Second, reduced sensitivity to magnesium block results in significantly greater synaptic charge transfer through NMDAR. Third, AMPAR currents contribute to the spike latency, but not to temporal precision, at the immature synapse. Furthermore, intrinsic excitability is greater. These properties enable immature synapses with predominantly NMDARs and little or no AMPARs to contribute to the relay of information from retina to visual cortex.

INTRODUCTION

Over development, neuronal connections in many areas of the CNS remodel extensively. At the retinogeniculate synapse, the connection between retinal ganglion cells (RGCs) in the eye and thalamic relay neurons in the lateral geniculate nucleus (LGN), transmission is mediated via glutamatergic synapses containing both α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) and N-methyl-D-aspartate receptors (NMDARs) (Chen and Regehr 2000; Kemp et al. 1982; Molinar-Rode and Pasik 1992; Mooney et al. 1993; Salt 1986). Large-scale refinement of the retinogeniculate synapse has been demonstrated in the rodent over a developmental period spanning three postnatal weeks as synaptic strength increases, and the number of RGCs that innervate a given relay neuron is reduced from more than 10 inputs to 1–3 inputs (Chen and Regehr 2000; Hooks and Chen 2006; Jaubert-Miazza et al. 2005; Ziburkus and Guido 2006). Remarkably, during this period of robust synaptic remodeling, the retinogeniculate synapse remains effective in transmitting information from the retina to the visual cortex (Akerman et al. 2002; Hüttenlocher 1967; Krug et al. 2001; Moseley et al. 1988).

Before eye-opening, when the retinogeniculate synapse is immature [postnatal day (p) 9 to p11], the AMPAR/NMDAR ratio is about four times lower in immature synapses compared with mature synapses, p27–p32 (0.3 vs. 1.3). Consistent with this ratio, about 13% of retinal inputs at the immature synapse evoke NMDAR currents but not AMPAR currents (Chen and Regehr 2000; Hooks and Chen 2006; see Supplemental Fig. S3).1 If AMPAR current is needed to drive membrane depolarization to a threshold where NMDAR are activated, then are these inputs silent? The average immature single-fiber RGC input peak AMPAR amplitude, including inputs with and without AMPAR currents, is only about 40 pA compared with about 800 pA at mature synapses (Hooks and Chen 2006). In our previous studies of retinogeniculate synapses from mice older than p23, we observed that synaptic inputs with AMPAR excitatory postsynaptic currents (EPSCs) >400 pA are needed to elicit a relay neuron action potential (Seeburg et al. 2004). Thus if all other properties of the synapse were equal over development, individual immature RGC inputs may be too weak to reliably drive relay neuron firing. However, we know from in vivo studies during a time when the retinogeniculate synapse is immature that information is effectively transmitted from the retina to the visual cortex (Hanganu et al. 2006; Mooney et al. 1996). Several explanations may account for this apparent discrepancy. First, it has previously been shown that intrinsic membrane properties of relay neurons change over development, resulting in a decrease in input resistance (MacLeod et al. 1997; Pirchio et al. 1997; Ramoa and McCormick 1994a). A high-input resistance early in development will enable small retinal inputs to drive relay neuron firing. Second, synaptic properties at the immature synapse may be different from those at the mature synapse. In addition, the high degree of retinal convergence and synchronous firing of multiple RGC activated by retinal waves may contribute to a larger postsynaptic current. This last explanation, however, would still require the simultaneous activation of about 10 inputs (each with synaptic strength of 40 pA) to achieve a 400-pA postsynaptic current.

1 The online version of this article contains supplemental data.

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To gain insight into how immature retinogeniculate synapses transmit information to the visual cortex, we compared the synaptic properties over development. Here we show that distinct properties of immature synapses, compared with mature synapses, contribute to effective retinogeniculate transmission early in development. We find that although the amplitude of immature EPSCs is small, the kinetics of EPSC decay is much slower than that of the mature synapse. This slow component of the immature EPSC observed at negative membrane potentials is largely composed of inward current through NMDARs that contain both NR2B and NR2C/D subunits. Indeed, current influx through activated NMDARs accounts for the majority of synaptic charge transfer at immature synapses and is sufficient to drive immature relay neurons to fire. In addition, the kinetics of glutamate receptor currents at immature synapses is slower due to an extended time course of transmitter in the synaptic cleft. The added charge transfer via AMPARs helps drive the relay neuron membrane potential toward the threshold of action potential firing. In combination with differences in postsynaptic membrane excitability, these properties at immature synapses ensure a greater amount of synaptic charge transfer, contributing to the effective relay of information from retinal ganglion cells to the visual cortex at an early age in development.

Methods

Slice preparation

Parasagittal brain slices containing both the optic tract and the dorsal lateral geniculate nucleus were obtained as previously described (Chen and Regelr 2000) from p9–p11 and p26–p32 C57BL/6 mice (C57BL/6J from Jackson Laboratory, Bar Harbor, ME or C57BL/6NTac (B6) from Taconic Farms, Hudson, NY). Briefly, the brain was quickly removed and immersed into an oxygenated 4°C cutting solution. Slices (250 μm) in the young mice were allowed to recover for 22 min at 30°C in oxygenated saline containing (in mM): 125 NaCl, 26 NaHCO3, 37.5 choline chloride, 25 glucose, 2.5 KCl, 1.25 NaH2PO4, 7 MgCl2, and 0.5 CaCl2. The brain tissue was then mounted on the cutting stage of a vibratome (Leica VT1000S) and submerged into oxygenated 4°C cutting solution. Slices (250 μm) in the old mice and those (300 μm) in the young mice were allowed to recover for 22 min at 30°C in an oxygenated choline-based cutting solution, and then for another 22 min at 30°C in oxygenated saline containing (in mM): 125 NaCl, 26 NaHCO3, 1.25 NaH2PO4, 2.5 KCl, 1 MgCl2, 2 CaCl2, and 25 glucose.

Electrophysiology

Whole cell voltage-clamp synaptic recordings from geniculate neurons were obtained using glass pipettes (1–2.0 MΩ) filled with an internal solution consisting of (in mM): 35 CsF, 100 CsCl, 10 EGTA, 10 HEPES, and the L-type calcium channel antagonist, 0.1 methoxyverapamil (pH 7.3) for voltage-clamp experiments. This solution was designed to minimize the contributions from postsynaptic intrinsic membrane conductances and second-messenger systems. The AMPAR-mediated miniature (m)EPSCs at the retinogeniculate synapse were resolved in the presence of an oxygenated extracellular solution containing (in mM): 3 SrCl2, 2 MgCl2, 125 NaCl, 26 NaHCO3, 1.25 NaH2PO4, 2.5 KCl, and 25 glucose. For current-clamp experiments, the internal solution contained (in mM): 116 KMeSO4, 6 KCl, 2 NaCl, 20 HEPES, 0.5 EGTA, 4 MgATP, 0.3 NaGTP, and 10 Na phosphocreatine (pH 7.25 with KOH). The bath saline solution contained the γ-aminobutyric acid type A (GABA,γ)–receptor antagonist bicuculline (20 μM) and the GABA,γ-receptor antagonist 3-N-[1-(S)-(3,4-dichlorophenyl)ethyl]amino-2-(S)-hydroxypropyl-P-benzyl-phosphonic acid (CGP55845, 2 μM). 2,3-Dihydro-6-nitro-7-sulfamoyl-benzof[1]quinoline-2,3-dione (NBQX, 5 μM) and 3-[(R)-2-carboxypropionazin-4-yl]-propyl-1-phosphonic acid [(R)-CPP, ≤100 μM] were used to block AMPARs and NMDARs, respectively.

To monitor relay neuron spiking in response to optic nerve stimulation, voltage-clamp recordings were initially established. The optic tract was stimulated using a pair of pulled glass electrodes filled with saline in a bipolar configuration. We examined the response of evoked EPSCs from single fibers and from groups of fibers that were reproducible from trial to trial.

Single RGC fiber responses were obtained from minimal stimulation as previously described (Chen and Regelr 2000). Briefly, the stimulating electrode is positioned to obtain a reliable postsynaptic response. The stimulus intensity is then reduced to a level that does not evoke a synaptic current, after which the intensity is increased in 0.25–μA increments until a synaptic response is elicited.

To directly compare results from this study to published measurements of synaptic strength over development of the retinogeniculate synapse (Hooks and Chen 2006), peak EPSC amplitude measurements were performed at a holding potential of −70 mV for results shown in Figs. 1 and 2. This is possible because the relationship of peak EPSC amplitude measured at −70 versus −55 mV is not different between immature and mature synapses (see Supplemental Fig. S1A). Thus the relationship between the synaptic charge transfer (Q), calculated as the integral of the synaptic current, obtained from Vm of −70 mV could be linearly related to that obtained from Vm = −55 mV (Supplemental Fig. S1B).

Even with maximal stimulation (defined as the intensity at which the maximal synaptic current is evoked) the peak EPSC amplitude at immature synapses is <600 pA. Therefore to compare the firing response of relay neurons to synaptic inputs with similar strength over development, we adjusted stimulus intensity to obtain EPSCs <600 pA in mature neurons. Once a reliable synaptic response was elicited with optic nerve stimulation, recordings were switched to the current-clamp mode of the Multiclamp 700A (Axon Instruments, Foster City, CA) to examine the firing response to the same presynaptic stimulation.

The long- and short-ramp durations for current-injection experiments were chosen to mimic the time course of the immature and mature EPSC, respectively. Input resistance was measured in current-clamp mode.

Stock solutions of pharmacological agents were stored at −20°C and diluted according to the final concentrations into the bath reservoir immediately prior to application. Constant bath flow during application of drugs was ensured by a perfusion pump (Gilson, Medfield, MA). Dead space in the perfusion tubing was reduced to 1 ml, allowing rapid bath exchange of pharmacological agents. (R)-CPP, bicuculline, CGP55845, NBQX, ifenprodil hemitartrate, and [R-(R*,S*)]-α-(4-hydroxyphenyl)-β-methyl-4-(phenylmethyl)-1-piperidinone propanol (Ro 25-6981) maleate were all obtained from Tocris Bioscience (Ellisville, MO). All other chemicals were from Sigma (St. Louis, MO) and all experiments were performed at 33–36°C.

Data acquisition and analysis

Current- and voltage-clamp recordings were acquired with a Multiclamp 700A amplifier filtered at 1 kHz and digitized at 10–20 kHz with an ITC-16 interface (InstruTECH, Port Washington, NY). Data analysis was performed using Igor software (Wavemetrics, Portland, OR) and Excel (Microsoft, Redmond, WA).

Splice probability was obtained by summing the number of action potentials that occur over a 50-ms (for single stimulus) or 180-ms (for pairs of stimuli) period following the first stimulus (>3–30 trials) and dividing this value by the number of trials. Two methods were used to identify an action potential. The first counted any spike that peaked above −10 mV as an action potential. For the second method, the criterion for an action potential was a depolarizing change in mem-
brane potential \((\mathrm{dV/dt}) \approx 10 \, \text{V/s}^{-1}\). Synaptic charge \((Q)\) was calculated by integrating the evoked synaptic current.

Latency time to first spike was measured from the onset of the stimulus artifact (evoked experiments) or current injection (current injection experiments) to the beginning of the first action potential [defined as the time when \((\mathrm{dV/dt}) \approx 10 \, \text{V/s}^{-1}\)]. The spike threshold was measured as the membrane potential at which \((\mathrm{dV/dt}) \approx 10 \, \text{V/s}^{-1}\). For each experiment, average values were determined from results of 5–20 trials.

The decay \((\tau)\) of both AMPA- and NMDA-receptor–mediated EPSCs at the immature retinogeniculate synapse was best fitted with a double exponential, \(f(x) = y_0 + A_1 e^{-x/T_{fast}} + A_2 e^{-x/T_{slow}}\). The weighted decay \(\tau\) was calculated as \(\tau_{\text{weighted}} = [\tau_{\text{fast}} \times A_1/(A_1 + A_2)] + [\tau_{\text{slow}} \times A_2/(A_1 + A_2)]\).

To quantify the shift of the NMDAR EPSC current–voltage \((I–V)\) relationship between immature and mature synapses, we fitted the NMDAR \(I–V\) curve with the Boltzmann relationship, \(I = I_{\text{offset}} + I_{\text{max}}/[1 + e^{(V_{1/2} - V)/kT}]\), where \(I_{\text{offset}}\) is the baseline NMDAR EPSC that is still present at \(-100 \, \text{mV}, I_{\text{max}}\) is the amplitude of maximal inward NMDAR EPSC, and \(V_{1/2}\) is the half-maximal voltage at which the amplitude of the NMDAR EPSC reaches 50% of maximal inward current.

Data are summarized as means \(\pm\) SE, and statistics were calculated using the two-tailed Student’s \(t\)-test unless otherwise indicated.

**RESULTS**

**Relay neurons respond to small-amplitude EPSCs at the immature synapse**

Dramatic developmental changes occur at the mouse retinogeniculate synapse between p9 and p32. Over this period, the average peak inward EPSC current amplitude evoked by a retinal ganglion cell input increases from about 40 to 800 pA (Chen and Regehr 2000; Hooks and Chen 2006). To understand how these changes affect the transmission of information from retinal ganglion cells (RGCs) to LGN relay neurons, we examined relay neuron spiking in response to EPSCs during two age ranges: p9–p11 (immature) and p26–p32 (mature). Recording from brain slices of the mouse LGN, we obtained stable and reproducible EPSCs from relay neurons in response to optic tract stimulation, then switched to the current-clamp recording mode to examine postsynaptic firing driven by the synaptic current. The responses both to single-fiber inputs and to multiple inputs were examined. The amplitude of the maximal synaptic current we obtained at immature synapses is smaller than 600 pA. Thus, to better compare response properties of relay neurons over development, we adjusted stimulus intensities to obtain EPSCs with similar amplitudes in mature neurons. In addition, previous studies have demonstrated that immature mouse relay neurons have a more depolarized resting membrane potential than that of mature neurons, consistent

![Fig. 1](http://jn.physiology.org/). Comparison of relay neuron firing responses at immature [postnatal day (p) 9 to p11] and mature (p26–p32) synapses. A: relay neuron spiking from holding potential \((V_h) = -55 \, \text{mV}\) in response to paired presynaptic stimuli separated by an interstimulus interval (ISI) of 50 ms (left panels, voltage clamped at \(-70 \, \text{mV}\)). Immature relay neurons \((\text{top})\) are more likely to fire than mature relay neurons \((\text{bottom})\) in response to excitatory postsynaptic currents (EPSCs) with the same range of peak amplitudes. Arrows indicate time of optic tract stimulation and asterisk (*) indicates action potential. B: plot of average relay neuron spike probability as a function of peak EPSC amplitude in response to single (left) and pairs (right) of optic nerve stimuli. For each cell, the average spike probability was calculated from 3 to 30 trials. Firing responses to single-fiber stimulation at immature synapses are indicated by filled diamond symbols. C: same data as B plotted as a function of charge transfer \((Q)\) in response to single (left) and pairs (right) of optic nerve stimuli. D: summary of the spike probability averaged over many recorded neurons for immature \((n = 24)\) and mature \((n = 14)\) relay neurons in response to peak EPSC currents \(\leq 600 \, \text{pA}\).
with the observation that immature relay neurons exhibit only the tonic mode of firing (MacLeod et al. 1997; Pirchio et al. 1997; Ramoa and McCormick 1994a). Thus we focused on postsynaptic firing from a membrane potential ranging from −57 to −54 mV, a range that favors the tonic mode of firing in thalamic relay neurons (McCormick and Bal 1994; Steriade et al. 1993, 1997).

Figure 1A compares the synaptic and relay neuron firing responses to pairs of optic tract stimuli at the immature and mature synapses. To compare results from these experiments to published measurements of synaptic strength over development of the retinogeniculate synapse (Hooks and Chen 2006), peak EPSC amplitude measurements were performed at holding potential of −70 mV. The relationship between peak EPSC amplitude at −70 versus −55 mV is linear and does not change with development (see Supplemental Fig. S1A); thus we could compare relay neuron spiking at −55 mV over a developmental period when synaptic strength dramatically changes (also see METHODS).

A number of differences between immature and mature synapses are evident. First, the decay kinetics of the inward EPSC is slower at the immature synapse. In addition, consistent with previous studies, membrane potential responses at the immature synapse exhibit slower action potential (AP) kinetics and evidence of a calcium plateau compared with mature synapses (Jaubert-Miazza et al. 2005; Lo et al. 2002). The robust depolarization observed at immature synapses frequently resulted in inactivation of intrinsic conductances necessary for the generation of APs (depolarization blockade) (MacLeod et al. 1997; Ramoa and McCormick 1994a). Because the AP width and amplitude changes with development, we quantified the number of spikes using two methods. The first method counts any depolarization that crosses a threshold of −10 mV as an AP. The second method defines an AP as a depolarizing change in membrane potential (dV/dt) that is ≥10 V·s⁻¹. Average spike data in response to single and pairs of stimuli analyzed by the two methods were not significantly different; thus the second method for identifying APs was used.

The amplitude of synaptic inputs is highly variable; therefore the relationship between the peak EPSC amplitude and average number of spikes is shown as scatterplots in Fig. 1B for amplitudes ranging from 19 to 600 pA. The smallest peak EPSC amplitude to drive a relay neuron to fire is 120 pA at immature synapses and 380 pA at mature synapses. The average spike probability in immature relay neurons is 0.9 ± 0.1 and 1.4 ± 0.2 in response to single and paired stimuli, respectively (n = 24). In contrast, average spike probability of

FIG. 2. Activation of N-methyl-D-aspartate receptors (NMDARs) alone is sufficient to evoke action potentials in immature relay neurons. The synaptic currents recorded in voltage clamp at V_h = −70 mV (left), and the firing response of relay neurons recorded in current clamp (right) to optic nerve stimulation from the same immature (A) or mature (B) synapse. Responses are shown in control condition (top) and after bath application of 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione (NBQX; bottom). Arrows indicate time of optic tract stimulation and asterisks (*) indicate action potential.

C: plots of average spike probability of relay neurons elicited by pairs of stimuli vs. Q in the absence (black dots) and presence (gray triangles) of 5 μM NBQX, for immature (left) and mature (right) relay neurons. For each cell, the average spike probability was calculated from 3 to 30 trials.

D: summary of the average spike probability in response to presynaptic stimulation under different paradigms.

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mature neurons in response to small EPSCs (defined as <600 pA in amplitude) is significantly less frequent (0.2 ± 0.1 APs to both single and paired stimuli, n = 14, P < 0.0001). We found that the synaptic charge transferred (Q) between the RGC input and postsynaptic relay neuron, calculated as the integral of the synaptic current, is a better predictor of postsynaptic spiking. Figure 1C shows the relationship between Q and average spike probability for immature and mature synapses. The threshold Q for activating an AP is 1.89 and 2.3 pC, respectively, for immature and mature synapses. Thus even though the peak EPSC amplitude is small at immature synapses, the synaptic charge transfer is relatively large compared with that of a mature EPSC with the same peak amplitude. The larger synaptic charge transfer results in a higher likelihood of relay neuron firing at immature synapses (Fig. 1D).

NMDAR current can drive relay neuron firing at immature synapses

Two classes of glutamatergic receptors are found at the retinogeniculate synapse: AMPA and NMDA receptors (Chen and Regehr 2000). Because of the relatively small AMPAR currents present at retinogeniculate synapses early in development, we asked whether the slow immature EPSC kinetics reflected current through NMDAR by evaluating relay neuron spiking before and during bath application of the AMPAR inhibitor NBQX. In these experiments, stimulus intensities were adjusted to evoke sufficiently large EPSCs that could reliably drive AP firing.

Bath application of 5 μM NBQX inhibits the fast transient component of the EPSC (Fig. 2, A and B, left panels). The remaining current seen at both immature and mature synapses exhibits slower kinetics and is completely blocked by 100 μM (R)-CPP, a specific antagonist for NMDARs (see Supplemental Fig. S2). The presence of 5 μM NBQX (Fig. 2A, bottom) did not significantly reduce the spike probability in response to the first (P = 0.2, n = 8) or to paired stimuli at the immature synapse (P = 0.7, n = 8, paired Student’s t-test). In contrast, consistent with previous reports (Augustinaite and Heggelund 2007; Blitz and Regehr 2003) at mature synapses, the presence of NBQX significantly reduced the spike probability in response to single and pairs of optic nerve stimulation (Fig. 2B, bottom, P ≤ 0.001 for both first and paired stimuli, n = 8; paired Student’s t-test). The effects of AMPAR inhibition on relay neuron spiking are summarized in Fig. 2, C and D. Plots of the spike probability versus Q reveal a reduction in firing in all mature relay neurons tested. In contrast, the firing of 2/8 immature neurons decreased, whereas 5/8 increased. Thus at the immature retinogeniculate synapse, evoked current via NMDAR, alone, is sufficient to drive relay neuron firing.

NMDAR currents contribute significantly to charge transfer at immature synapses

Our data demonstrate that significant inward NMDAR current is evoked by optic tract stimulation and that this current plays a major role in driving relay neuron firing at immature synapses. To further identify the features of this current that are important in relay neuron firing at the immature synapse, we examined developmental changes in the properties of NMDAR EPSC. Figure 3A compares the NMDAR EPSC evoked from a holding potential of −55 mV (inward current) and +40 mV (outward current) in the presence of the AMPAR blocker NBQX (5 μM), at immature (left) and mature (right) retinogeniculate synapses. Consistent with previous reports at many CNS synapses, the decay of both outward and inward NMDAR EPSCs is significantly slower at immature synapses than at mature synapses (Carmignoto and Vicini 1992; Hestrin 1992; Monyer et al. 1992, 1994; Ramoa and Prusky 1997). Inward NMDAR currents are present throughout development, although the amplitude is small relative to the outward current amplitude because of voltage-dependent magnesium blockade at negative potentials (Mayer et al. 1984; Nowak et al. 1984). Figure 3B compares the inward NMDAR current to the total EPSC evoked at −55 mV. Representative traces of the synaptic current before (Ctrl) and during bath application of NBQX (+NBQX) are shown for an immature (top) and mature (bottom) synapse. Superposition of the control and +NBQX traces (Overlay) reveals that the NMDAR current is the major contributor to the slow decay kinetics of immature EPSCs. Subtraction of the two traces yields the AMPAR contribution to the synaptic current; this component is the dominant current at mature synapses.

When compared with mature synapses, the absolute charge transfer via NMDAR at Vh = −55 mV is about fourfold greater for immature synapses (Fig. 3C). NMDAR-mediated current contributes 88.5 ± 4.3% of the total charge transfer at immature synapses (Fig. 3D). In contrast, NMDA receptors contribute significantly less to the total current of mature synapses (for Vh = −55 mV, 37.4 ± 3.1%).

Changes in NMDAR subunit composition over development

The greater synaptic charge transfer via NMDAR at immature versus mature synapses could be the result of a change in subunit composition of NMDAR, leading to changes in EPSC decay kinetics and/or differential sensitivity to magnesium block (Ben-Ari et al. 1988; Bowe and Nadler 1990; Clarke and Johnson 2006; Monyer et al. 1992, 1994; Morissett et al. 1990; Muller et al. 1989). Previous studies of CNS synapses have demonstrated a subunit switch from NR2B to NR2A containing NMDAR over development (Monyer et al. 1994; Quinlan et al. 1999; Ramoa and Prusky 1997; Sheng et al. 1994; Williams et al. 1993). To test whether this is also true at the mouse retinogeniculate synapse, we examined the effects of NMDAR antagonists that preferentially block receptors containing NR2B over NR2A or NR2C/2D subunits, ifenprodil and Ro 25-6981 (Bartlett et al. 2007; Cathala et al. 2000; Fischer et al. 1997; Joshi and Wang 2002; Morishita et al. 2007; Ramoa and Prusky 1997; Williams 1993, 2001; Williams et al. 1993). Figure 4A shows a representative example of the effects of Ro 25-6981 (0.5 μM). The time courses of the peak NMDAR EPSC (left) and the average NMDAR current traces (right) are plotted before and during bath application of the NMDAR antagonist. Consistent with reports from other CNS synapses, both 0.5 μM Ro 25-6981 and 3 μM ifenprodil inhibit significantly more of the amplitude of NMDAR EPSC at the immature synapse (Ro 25-6981: 57.1 ± 2.8%, n = 8; ifenprodil: 52.0 ± 3.6%, n = 4) compared with the mature synapse (Ro 25-6981: 42.9 ± 3.9%, n = 4, P < 0.05; ifenprodil: 35.8 ± 5.3%, n = 5, P < 0.05; Fig. 4, B and C).
To test whether preferential inhibition of NR2B over NR2A receptors accelerates the NMDAR decay kinetics at the immature synapse, we fit the NMDAR EPSC decay time course with a double exponential. Both $\tau_{\text{fast}}$ and $\tau_{\text{slow}}$ accelerate significantly over development (Fig. 4E). The NMDAR currents shown in Fig. 4B before and during bath application of ifenprodil or Ro 25-6981 are normalized and displayed on a log scale in Fig. 4D to compare the NMDAR current time courses. Contrary to similar experiments at other synapses, we found that preferential inhibition of NMDAR-containing NR2B subunits did not accelerate the NMDAR EPSC decay time course at either immature or mature retinogeniculate synapses. Rather, in the presence of ifenprodil or Ro25-6981, we found a significant slowing of $\tau_{\text{slow}}$ from 204.4 ms under control conditions to 298.1 ms in ifenprodil ($n = 4$, $P < 0.05$) and 603.2 ms in Ro 25-6981 ($n = 8$, $P < 0.05$; Fig. 4E) at immature synapses. In contrast, the NR2B-selective inhibitors did not significantly change $\tau_{\text{slow}}$ at mature synapses (ifenprodil: $n = 5$, $P = 0.59$; Ro 25-6981: $n = 4$, $P = 0.82$) or $\tau_{\text{fast}}$ at either immature (ifenprodil: $n = 4$, $P = 0.11$; Ro 25-6981: $n = 8$, $P = 0.37$) or mature (ifenprodil: $n = 5$, $P = 0.99$; Ro 25-6981: $n = 4$, $P = 0.36$) synapses. Thus inhibition of NR2B-containing NMDAR uncovers a component of NMDAR currents with even slower decay kinetics.

**NMDAR sensitivity to Mg$^{2+}$ block increases over development**

One possible explanation for the slower component of NMDAR current is the presence of NR2C and/or NR2D subunits at immature synapses. Receptors containing these subunits exhibit slower decay kinetics and lower sensitivity to Mg$^{2+}$ block compared with NMDARs containing NR2B or NR2A subunits (Kirson et al. 1999; Misra et al. 2000; Momiyama et al. 1996; Monyer et al. 1994). A highly selective pharmacological agent that can distinguish the presence of NR2C/2D versus NR2A or NR2B in slice recordings is currently not available. Thus to test for contributions of NR2C and/or NR2D subunits in shaping the NMDAR EPSC time course, we examined the sensitivity of NMDAR currents to extracellular Mg$^{2+}$ over development in the presence of 5 μM NBQX (Fig. 5). In 1 mM Mg$^{2+}$, the $I-V$ relationship of NMDAR EPSC is significantly shifted to more hyperpolarized potentials for $V_h$ negative to −40 mV at immature synapses compared with mature synapses (Fig. 5B, $P < 0.01$, $n = 6$, two-way ANOVA). Fitting the normalized $I-V$ to a Boltzmann function yielded a voltage of half-maximal activation ($V_{1/2}$) of $-60 \pm 4$ and $-52 \pm 4$ mV for immature and mature synapses ($P < 0.05$, $n = 6$), respectively (see METHODS). The difference in $I-V$ relationships becomes insignificant in a nominally Mg$^{2+}$ free external solution as the peak of the NMDAR $I-V$ relationship shifts to more hyperpolarized potentials for both immature and mature synapses (Fig. 5C, $P > 0.2$, $n = 6$, two-way ANOVA). The nonlinear $I-V$ relationship observed between $-70$ and $-100$ mV in 0 Mg$^{2+}$ is thought to be a result of intracellular Mg$^{2+}$ release into a nominally Mg$^{2+}$ free external solution (Perouansky et al. 2001). Thus at subthreshold potentials around $-60$ mV, the peak inward NMDAR current at immature synapses is about 1.5-fold greater than that at mature synapses (Fig. 5B, dashed lines), an increase that enhances the efficacy of NMDAR-mediated spiking.

**FIG. 3.** Significantly greater charge transfer occurs via NMDARs at negative holding potentials at immature synapses. A: NMDAR EPSCs evoked from $V_h = +40$ (outward current) and $-55$ mV (inward current) are shown for an immature (left) and mature (right) neuron. Currents are normalized to the peaks of the outward EPSCs. B: representative traces of EPSCs from $V_h = -55$ mV are shown for immature (top) and mature (bottom) synapses. From left to right: control traces, after bath application of 5 μM NBQX, overlay of control and NBQX traces, and the trace obtained by subtracting the − NBQX trace from the control trace (EPSC$_{AMPA}$). Traces are an average of 5–10 consecutive trials. C: average charge transfer at immature (gray) and mature (black) synapses mediated via NMDARs at $V_h = -55$ mV. Significantly greater charge transfer via NMDAR occurs at immature compared with mature synapses ($P < 0.05$, $n = 5–6$). Inward peak amplitude of total EPSC for these mature synapses ranged from 446 to 1582 pA. D: the relative contribution of the NMDAR-mediated current to total charge transfer at $V_h = -55$ mV is also significantly greater at immature (gray) synapses compared with mature (black) synapses ($P < 0.001$, $n = 5–6$).
Developmental changes in the AMPAR-mediated EPSC decay time course

The observation that slower NMDAR decay kinetics enhances spiking at immature synapses raises the question of whether there are also changes in AMPAR current kinetics over development. Figure 6A compares the immature (left) and mature (right) synaptic currents before and during bath application of (R)-CPP. In the presence of the NMDAR antagonist, the time course of the synaptic current accelerates significantly at the immature synapse ($P < 0.002$) but not at the mature synapse ($P = 0.35$). Figure 6B (left) compares the kinetics of the immature EPSCs to that of the mature synapse under control conditions. The decay time course ($\tau$) of the total EPSC is best fit with a double-exponential relationship (immature: $\tau_{\text{fast}} = 1.3 \pm 0.1 \text{ ms}$ and $\tau_{\text{slow}} = 122.0 \pm 60.0 \text{ ms}$, $n = 7$; mature: $\tau_{\text{fast}} = 1.3 \pm 0.2 \text{ ms}$ and $\tau_{\text{slow}} = 16.3 \pm 4.4 \text{ ms}$, $n = 14$). Blocking the NMDAR component of the synaptic current accelerates $\tau_{\text{slow}}$ at immature synapses ($\tau_{\text{fast}} = 2.0 \pm 0.1 \text{ ms}$; $\tau_{\text{slow}} = 55.3 \pm 7.5 \text{ ms}$, $n = 5$), whereas a single-exponential process is sufficient to approximate the $\tau$ of the mature synaptic current ($\tau = 2.2 \pm 0.3 \text{ ms}$, $n = 4$; Fig. 6B). Comparison of the weighted $\tau$ for the immature and mature synapse under control conditions and during NMDAR blockade reveals that the immature AMPAR current exhibits significantly slower decay kinetics than that at mature synapses (Fig. 6, B and D).

At the immature retinogeniculate synapse, multiple RGC inputs innervate a given relay neuron. To rule out the possibility that the slow AMPAR EPSC decay time course that we observe at immature synapses is secondary to asynchronous activation of multiple inputs, we also examined the decay time course of AMPAR EPSC evoked from a single RGC input. Single-fiber responses were elicited using a minimal stimulation protocol as previously described (Chen and Regehr 2000). We found that the single-fiber AMPAR EPSC kinetics was indistinguishable from the AMPAR currents obtained by activating many retinal axon fibers ($\tau_{\text{fast}} = 1.3 \pm 0.3 \text{ ms}$ and $\tau_{\text{slow}} = 62.2 \pm 12.8 \text{ ms}$, $n = 7$; Fig. 6, C and D) at immature synapses. Thus the observed slow AMPAR decay kinetics is an inherent property of the synaptic current.

Asynchronous release contributes to the slow AMPAR decay kinetics at immature synapses

Two possible mechanisms may account for the developmental change in AMPAR decay kinetics at the retinogeniculate synapse. First, there could be a change in AMPAR subunit composition over development, as has been described for

**FIG. 4.** Subunit composition of NMDA receptors at the retinogeniculate synapse. A: time course of the peak NMDAR EPSC (left, filled circles, $V_h = +40 \text{ mV}$, in the presence of NBQX) and access resistance ($R_R$, open circles) and average traces (right) are shown before (black trace) and during bath application of 0.5 $\mu M$ [(R)-(S)]-4-(4-hydroxyphenyl)-2-methyl-4(phenylmethyl)-1-piperidine propanol (Ro 25-6981, gray trace). Traces are an average of 5–10 consecutive trials. B: average NMDAR EPSC traces are shown from an immature (left) and mature (right) synapse in control conditions (black traces), and during bath application of ifenprodil (3 $\mu M$, Bi, gray traces) and Ro 25-6981 (0.5 $\mu M$, Bii, gray traces). Currents are normalized to the peak NMDAR current under control conditions. C: summary of the relative inhibition of NMDAR EPSC peak amplitudes. D: current traces from B are normalized to their respective peaks and plotted on a log scale. E: summary of the changes in the decay time course by ifenprodil ($n = 4–5$) or Ro 25-6981 ($n = 4–8$).
that at other synapses (Lawrence and Trussell 2000; Lomeli et al. 1994; Takahashi 2005). Alternatively, there could be a change in the time course of neurotransmitter in the synaptic cleft (Cathala et al. 2005) as a result of changes in asynchronous release of synaptic vesicles (Atluri and Regehr 1998; Diamond and Jahr 1995; Sakaba 2006; Wadel et al. 2007) or the location and identity of transporters (Diamond and Jahr 1997; Otis et al. 1996). To distinguish between these possibilities, we compared the evoked mEPSC waveforms from immature and mature synapses. Because thalamic relay neurons receive glutamatergic innervation from cortical and thalamic and retinal inputs, we could not simply record spontaneous mEPSCs. Instead, we examined desynchronized quantal events evoked by optic tract stimulation in the presence of 3 mM Sr2+ in the presence of (R)-CPP (Chen and Regehr 2000; Dodge et al. 1969).

Figure 7A shows representative traces of the quantal events evoked from a p10 (top) and a p31 (bottom) mouse. The average mEPSC waveform, obtained from multiple trials recorded from the same cell, is shown on the right panel. An overlay of the normalized average mEPSC waveforms, obtained from recordings from different animals, is shown in Fig. 7Bi for immature (left) and mature (middle) synapses. The normalized averages of the waveforms from all recordings of immature (gray trace) and mature (black trace) synapses are superimposed in Fig. 7Bii (left). The mEPSC decay time course can be fit to a double-exponential relationship. Although the fast component of the decay is similar for the immature and mature mEPSCs, there is a trend for a larger contribution of $\tau_{\text{slow}}$ in the immature mEPSC, although this difference did not reach statistical significance ($n = 6–7$, $P > 0.23$).

Comparison of the normalized mEPSC waveform to the evoked EPSC waveform in the presence of (R)-CPP reveals that the evoked EPSC exhibits slower decay kinetics than that in the quantal event (Fig. 7C). The difference between the quantal and evoked waveforms is particularly striking at the immature synapse, where the slow component of the AMPAR EPSC decays over 810 ms after the peak of the current. In contrast, the evoked AMPAR EPSC at the mature synapse decays back to baseline in <20 ms and has a more similar waveform compared with the mEPSC. Comparison of Figs. 7Bii (right) to Fig. 6D reveals that the weighted decay time course of AMPAR mEPSC is significantly different from evoked EPSC at immature synapses ($n = 4–6$, $P < 0.001$), but not at mature synapses ($n = 6–7$, $P > 0.16$).

Developmental changes in the decay kinetics of miniature EPSCs at other synapses in the nervous system vary depending on the specific connection. Decay kinetics decreases at the mouse cochlear nucleus neurons and zebrafish Mauthner cells (Lu et al. 2007; McKay and Oleskevich 2007; Patten and Ali 2007), increases in rat spinal cord neurons (Gao et al. 1998), and remains unchanged in hippocampal CA1 pyramidal cells (Echegoyen 2007). Our results at the retinogeniculate synapse suggest that although we cannot completely rule out a developmental switch in AMPAR subunits, this switch would not fully account for the slow decay kinetics of the immature evoked EPSC. The evoked EPSC reflects the sum response to vesicles activated by a single retinal ganglion cell at many release sites, whereas the quantal event reflects the response to a single vesicle. Thus the difference in the waveform of the evoked and quantal EPSC suggests that asynchronous vesicular release or delayed glutamate clearance contributes prominently to the slow decay component of the immature AMPAR EPSC (Atluri and Regehr 1998; Diamond and Jahr 1995, 1997; Otis et al. 1996; Sakaba 2006; Wadel et al. 2007) and that this contribution decreases with development.
AMPAR currents play a role in spike latency at the immature synapse

At the mature retinogeniculate synapse, previous studies have shown that visual information is encoded in the timing of spikes (Butts et al. 2007b) and that activation of AMPAR is necessary for precise spike timing (Augustinaite and Hegge-lund 2007; Blitz and Regge 2003). To assess the role of synaptic currents on the timing of spikes over development and whether AMPARs play a similar role at an earlier developmental stage, we first examined the latency to first spike at immature relay neurons (Fig. 8; see METHODS). Latency is significantly longer at immature than that at mature neurons (Fig. 8A, \( P < 0.0001 \), \( n = 8–19 \), one-way ANOVA). Moreover, application of NBQX further prolongs the latency of the first spike in both immature (\( P < 0.01 \), \( n = 7–19 \), one-way ANOVA) and mature neurons (\( P < 0.05 \), \( n = 3–8 \), one-way ANOVA). Thus AMPAR currents play a role in the timing of the first spike at all ages by contributing to the total synaptic charge transferred.

We next assessed the precision of spike timing over repeated trials. Temporal jitter of postsynaptic APs for each experiment was quantified by examining the SD of the time of first AP peak over 5–20 repeated trials. Figure 8B shows that the SD of the time of first AP peak is significantly greater at immature than that at mature synapses (\( P < 0.001 \), \( n = 9–15 \), one-way ANOVA). Inhibition of AMPAR does not further degrade the precision of AP timing at the immature synapse (\( P = 0.79 \), \( n = 9–15 \), one-way ANOVA). However, blocking AMPAR significantly enhances jitter at the mature synapse (\( P < 0.01 \), \( n = 3–8 \), one-way ANOVA). Therefore whereas AMPAR currents contribute to the timing of the initial spike transmitted, the slower kinetics of the glutamatergic currents reduces the precision of AP generation at immature synapses.

Intrinsic excitability contributes to enhanced transmission at the immature synapse

In addition to changes in synaptic properties, developmental changes in the intrinsic properties of relay neurons have been reported (MacLeod et al. 1997; Pirchio et al. 1997; Ramoa and McCormick 1994a). Consistent with these reports, we found that immature mouse relay neurons have significantly higher input resistance than that of mature relay neurons (574.6 ± 32.6 vs. 161.5 ± 2.1 MΩ, \( n = 11–21 \), \( P < 0.01 \)). Thus both synaptic and intrinsic properties are likely to influence the response of relay neurons to synaptic input from LGN during development.

Next, to assess the contributions of synaptic and intrinsic properties to transmission at the retinogeniculate synapse, we examined the firing response of relay neurons to somatic current injections. Two durations of depolarizing current ramps were injected to mimic the immature (long ramp, Fig. 9Ai) and
plained by a difference in spike threshold. In response to the same 200-pA current injection, the spike threshold is 41.8 ± 1.8 mV (n = 5) and 41.5 ± 1.2 mV (n = 5), for immature and mature relay neurons, respectively (P = 0.87). A plot of the average number of spikes versus peak injected current reveals that the relationship is significantly shifted to the left for immature compared with mature relay neurons for both long- (Fig. 9Ci, P < 0.0001, n = 5, two-way ANOVA) and short-ramp injections (Fig. 9Ci, P < 0.0001, n = 5, two-way ANOVA). Moreover, at both mature and immature synapses, long-ramp injections consistently elicited more spikes compared with shorter-duration injections with identical peak amplitudes (P < 0.0001, n = 5, two-way ANOVA).

Comparison of the first spike latency as a function of the injected current shows a statistically significant difference between immature and mature relay neurons (Fig. 9D, P < 0.0001, n = 5, two-way ANOVA). First spike latency is shorter for immature than that for mature relay neurons, consistent with the higher input resistance of immature neurons. Notably, the latency response to injected currents differs from that observed in response to synaptic currents, where spike latency is significantly shorter for mature than that for immature synapses (Fig. 8A). Thus the observed developmental differences in the synaptically evoked first spike latencies (Fig. 8A) are influenced by distinct amplitudes and kinetics of synaptic currents between immature and mature synapses, and not simply dictated by developmental differences in intrinsic excitability. Taken together, our studies demonstrate that a combination of slow synaptic kinetics and intrinsic excitability enable the relay of information at the immature retinogeniculate synapse.

**DISCUSSION**

At the retinogeniculate synapse, major changes in synaptic function occur over development, yet transmission is maintained even early in development (Akerman et al. 2002; Huttenlocher 1967; Krug et al. 2001; Moseley et al. 1988). Here we show that a number of features of the immature retinogeniculate synapse ensure the ability of small RGC inputs to transmit information to thalamocortical relay neurons that, in turn, project to the visual cortex. The slower kinetics of decay of the AMPA and NMDA receptors and decreased sensitivity of NMDA receptors to magnesium block at negative membrane potentials, in combination with the high-input resistance of immature relay neurons, result in postsynaptic firing in response to small-amplitude synaptic currents. These prop-
Properties allow RGC inputs containing synapses with NMDARs but few functional AMPARs to contribute to transmission of information from the retina to the visual cortex.

**Properties of NMDARs are significantly different at immature synapses**

Current through NMDARs contributes significantly to the synaptic charge transfer of immature retinogeniculate synapses for a number of reasons. First, the resting membrane potential has been observed to be relatively depolarized early in postnatal development and to hyperpolarize with age around p20 (MacLeod et al. 1997; Pirchio et al. 1997; Ramoa and McCormick 1994a). The resting membrane potentials of relay neurons of p10–p11 mice range between −35 and −65 mV (MacLeod et al. 1997). Within this voltage range, reduced magnesium block can lead to significant current influx through NMDARs (Mayer et al. 1984; Nowak et al. 1984). The underlying conductance changes that mediate the developmental hyperpolarization of resting membrane potential in thalamic relay neurons is not known, although similar depolarized membrane potentials during early development in neurons from other regions of the nervous system have been attributed to a low potassium conductance (Zanzouri et al. 2006) and high chloride conductance (Ben-Ari et al. 2007).
Another factor that contributes to transmission at immature synapses is the substantially slower NMDAR decay kinetics compared with that of the mature synapse, resulting in a greater synaptic charge transfer. At other synapses, a developmental switch from NR2B to NR2A subunits has been shown to underlie the acceleration of NMDAR decay kinetics (Monyer et al. 1994; Quinlan et al. 1999; Sheng et al. 1994; Williams et al. 1993). Our studies using selective inhibitors of NMDAR-containing NR2B subunits, ifenprodil and Ro 25-6981, demonstrate that this subunit switch also occurs over development of the mouse retinogeniculate synapse.

However, the relative change in contribution of this subunit over development, as measured by percentage block by the selective inhibitors, is surprisingly small compared with the developmental changes in sensitivity to ifenprodil at other synapses. In rat hippocampus, ifenprodil inhibits the NMDAR current by 80% at p9 and by 20% at p21 (Kirson et al. 1999), whereas the NMDAR current is inhibited by 38% (p12) and 18% (p21) in cerebellum (Cathala et al. 2000) and by 38% (p7–p9) and 13% (p20–p24) in the mouse ventral basal thalamus (Arsenault and Zhang 2006). Ifenprodil also has a larger differential effect over development at the ferret retinogeniculate synapse. [1 μM ifenprodil inhibited by ~45% at <p25 vs. about 10% at p40 (Ramoa and Prusky 1997)], suggesting that there could be distinct developmental profiles of NMDARs among different synapses and species. Notably, NMDAR currents do not accelerate significantly in the presence of the selective NR2B inhibitors, as one would predict if the unblocked NMDAR contained predominantly NR2A subunits. In fact, in the presence of ifenprodil or Ro 25-6981, we see a further slowing of the slow component of the NMDAR decay kinetics.

Our data suggest two possible explanations for the slow component of NMDAR current kinetics. First, similar to what we observe for AMPAR currents, asynchronous vesicular release or delayed glutamate clearance at the immature synapse could result in a prolonged NMDAR current decay kinetics. Second, the slow kinetics could reflect the presence of subunit subtypes other than NR2A and NR2B, such as NR2C/2D. It is likely that both processes contribute to the NMDAR EPSC time course at the immature synapse.

Support for the role of NR2C/2D subunits comes from in situ hybridization studies that demonstrated that mRNAs of NR2A, NR2B, and NR2D are present in the LGN of a number of species (Ibrahim et al. 2000; Monyer et al. 1994). Moreover, both protein and mRNA levels of NR2D are significantly enriched in LGN during early development, and then the levels decrease between p12 and adulthood (Dunah et al. 1996; Monyer et al. 1994; Wenzel et al. 1996). There are, unfortunately, only moderately selective pharmacological agents available for NR2C/2D subunits (Feng et al. 2004); these drugs would not be useful in evaluating the relative contribution of NR2B versus NR2C/2D subunits in LGN brain slices. However, our observed change in sensitivity of the NMDAR current–voltage relationship to magnesium block over development further supports the presence of NR2C/2D subunits at immature synapses. NMDAR-containing NR2A and NR2B subunits exhibit similar sensitivity to magnesium block, whereas those containing NR2C and NR2D subunits show significantly weaker block by the cation (Clarke and Johnson 2006; Monyer et al. 1992, 1994). Thus our data suggest that both NR2B and NR2C/2D subunits contribute to the relatively large and slow inward NMDAR current at the immature retinogeniculate synapses. The presence of these subunits and the extended neurotransmitter time course in combination with the depolarized resting membrane potential and high $R_{\text{input}}$ at immature retinogeniculate synapse facilitate transmission of retinal activity to the visual cortex.

Immature synapses with predominantly NMDARs can contribute to information transmission

In this study, we also demonstrate that transmission between immature RGC and relay neurons can occur even in the presence of an AMPAR antagonist. In contrast to immature synapses, AMPARs contribute significantly to the response of relay neurons at mature synapses, consistent with previous findings in the LGN (Augustinaiite and Heggelund 2007; Blitz and Regehr 2003; Kwon et al. 1991; Ramoa and McCormick 1994b). Previous studies show that immature relay neurons receive a significant number of inputs that evoke NMDAR currents but not AMPAR currents (defined as “silent” inputs; Chen and Regehr 2000; Hooks and Chen 2006; see also Supplemental Fig. 3). Because each individual RGC input has multiple release sites associated with the axon arbor (Chen and Regehr 2000; Hamos et al. 1987), the occurrence of silent inputs suggests a significant presence of release sites containing NMDARs but no functioning AMPARs (“silent” synapses). Here we show that immature synapses with relatively few or no AMPARs can still contribute to information relay from RGCs to the visual cortex. Our findings are consistent with previous studies in ferret demonstrating that application of the NMDAR antagonist $\alpha$-2-amino-5-phosphonovaleric acid ($\alpha$-APV) significantly modified the amplitude and duration of EPSPs during the first two postnatal weeks (Ramoa and McCormick 1994b). Similar results have also been reported in the response of neurons of rat stratum griseum intermediale of superior colliculus to optic tract stimulation. $\alpha$-APV completely abolished synaptically induced burst of APs in the p17–p22 rat, whereas it had a lesser effect in adult rat (Saito and Isa 2003). Interestingly, NMDARs with reduced Mg$^{2+}$ sensitivity have been shown to play a significant role in synaptic transmission of adult radiatum giant cell in rat hippocampus (Kirson and Yaari 2000). Taken together with our results, these studies suggest that NMDAR-containing NR2C/D subunits can play a large role in driving postsynaptic firing.

AMPAR currents influence the latency to fire

Many intrinsic ionic mechanisms contribute to AP generation, temporal jitter, and latency of spikes, including the density and function of Na and K conductances (Jackson and Bean 2007; Klug and Trussell 2006; Raman and Bean 2001). Previous studies have attributed changes in intrinsic conductances, such as the A-type K current, to developmental differences in spike latency (Deng et al. 2004; MacLeod et al. 1997). Here we show that in addition to intrinsic conductances, synaptic conductances contribute to the latency to first spike. Inhibition of AMPAR current prolongs spike latency at both immature and mature synapses. Our results show that although current through AMPARs contributes to a relatively small
fraction of the total synaptic charge transfer early in development (~12% of $Q_{\text{total}}$ at $V_h = -55$ mV; see Fig. 3B), it plays a role in the latency to first spike.

At the mature retinogeniculate synapse, the efficacy of transmission in the tonic mode of firing has been shown to depend on temporal summation of synaptic charge transfer (Blitz and Regehr 2003; Mukherjee and Kaplan 1995; Seeberg et al. 2004). Thus addition of the fast activating AMPAR current, regardless of the size, enhances the probability of reaching AP threshold, thus shortening the time to first spike. Unlike the mature synapse, however, AMPAR currents do not contribute significantly to the precise timing of APs at the immature synapse (Augustinaitaite and Heggelund 2007; Blitz and Regehr 2003). AP jitter, measured as the SD of the time of first spike over many trials, is sensitive to the inhibition of AMPAR currents at the mature but not the immature synapse. The small contribution to the total synaptic charge and slower decay kinetics of the immature AMPAR current likely contribute to the different role of this glutamate receptor to timing. Thus AP firing is preserved at immature synapses by the slower glutamatergic currents and larger inward NMDAR currents, at the expense of precision. The timing between bursts of RGC APs and postsynaptic firing has been shown to be important for the strengthening or weakening of immature retinogeniculate synapses (Butts et al. 2007a); thus individual spike precision may be less important early in development of this synapse.

Functional role of small synaptic inputs over development

The results from this study also provide insight into whether single immature retinal ganglion cells are capable of driving relay neuron firing. Previous work from the laboratory have shown that the amplitude of single-fiber inputs at ages p9–11, measured at $-70$ mV, range from 0 pA (silent input) to 302 pA, with an average value of $43 \pm 10$ pA and a median of 22.8 pA ($n = 31$; see Supplemental Fig. 3) (Hooks and Chen 2006). Of this data set, 4/31 inputs (13%) have a peak EPSC amplitude of >120 pA, the smallest value that we found could drive relay neurons to fire with a single stimulus. Thus for the majority of RGCs, single presynaptic APs cannot drive postsynaptic relay neuron firing alone at the immature synapse. Instead, our data suggest that during early development, relay neurons in LGN may preferentially respond to trains of APs from single RGCs or by coactivation of multiple RGC inputs that innervate a given relay neuron (Usrey and Reid 1999).

Correlated retinal ganglion cell activity, in the form of retinal waves, is a likely source for these forms of presynaptic activity (Galli and Maffei 1988; Maffei and Galli-Resta 1990; Meister et al. 1991; Wong et al. 1993).

Recordings from retinal waves have demonstrated that the onset of firing of each retinal neuron can be staggered over several seconds during a wave, and neuronal firing can last >2–4 s (Meister et al. 1991). Staggered activation of multiple small-amplitude retinal inputs over the time course of a wave, aided by the prolonged decay time course of glutamatergic synaptic currents at immature synapses, would increase the probability for temporal summation of synaptic currents in postsynaptic relay neurons. Thus the small RGC inputs can transmit synchronous retinal activity to thalamic relay neurons in the form of periodic bursts (Mooney et al. 1996) and, subsequently, to the contralateral visual cortex as spindles (Hangau et al. 2006). This information conveyed by correlated RGC activity is thought to play an important role in coordinating the maturation of neuronal circuits in the LGN (Butts et al. 2007a; Galli and Maffei 1988; Meister et al. 1991; Penn et al. 1998; Torborg et al. 2005) and visual cortex (Cang et al. 2005; Huberman et al. 2006). The findings at the retinogeniculate synapse are consistent with findings in other regions of the CNS, where weak but synchronous activity from convergent thalamocortical inputs has also been shown to activate the somatosensory cortex (Bruno and Sakmann 2006).

In contrast to the function of the immature retinogeniculate synapse, the mature synapse transmits visually evoked activity from the retina to the cortex. At the mature retinogeniculate synapse, the synaptic strength increases significantly, such that the average peak EPSC amplitude is 800 pA at p27–p32. Typically, we find that a mature relay neuron is innervated by one very strong input (>800 pA) and one or two smaller inputs. Notably, about 30% (14/48) of single-fiber inputs at mature synapses have peak EPSC amplitudes <200 pA and 62.5% (30/48) have amplitudes <600 pA (measured at $V_h = -70$ mV; Supplemental Fig. 3; Hooks and Chen 2006). Our results suggest that a single presynaptic AP from small mature inputs cannot drive relay neuron firing. Instead, these small inputs may augment relay neuron firing when another input is activated simultaneously (Alonso et al. 2006) or activate the postsynaptic cell only with bursts of presynaptic activity. Alternatively, these inputs may be maintained as a reservoir of potential connections that are recruited in response to changes in visual experience (Hooks and Chen 2006). Future experiments will be necessary to distinguish between potential functional roles for these small inputs at the mature retinogeniculate connection.

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