Substrates for Coincidence Detection and Calcium Signaling for Induction of Synaptic Potentiation in the Neonatal Visual Cortex

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Submitted 16 September 2003; accepted in final form 12 February 2004

Schrader, Laura A., Stephen P. Perrett, Lan Ye, and Michael J. Friedlander. Substrates for coincidence detection and calcium signaling for induction of synaptic potentiation in the neonatal visual cortex. J Neurophysiol 91: 2747–2764, 2004. First published February 18, 2004; 10.1152/jn.00908.2003. Regulation of the efficacy of synaptic transmission by activity-dependent processes has been implicated in learning and memory as well as in developmental processes. We previously described transient potentiation of excitatory synapses onto layer 2/3 pyramidal neurons in the visual cortex that is induced by coincident presynaptic stimulation and postsynaptic depolarization. In the adult visual cortex, activation of N-methyl-D-aspartate (NMDA) glutamate receptors is necessary to induce this plasticity. These receptors act as coincidence detectors, sensing presynaptic glutamate release and postsynaptic depolarization, and cause an influx of Ca2+ that is necessary for the potentiation. In the neurons of the neonatal visual cortex, on the other hand, coincident presynaptic stimulation and postsynaptic depolarization induce stable long-term potentiation (LTP). In addition, reduced but significant LTP can be induced in many neurons in the presence of the NMDA receptor (NMDAR) antagonist, 2-amino-5-phosphonovaleric acid despite the Ca2+ requirement. Therefore there must be an alternative postsynaptic Ca2+ source and coincidence detection mechanism linked to the LTP induction mechanism in the neonatal cortex operating in addition to NMDARs. In this study, we find that in layer 2/3 pyramidal neurons, release of Ca2+ from inositol trisphosphate (InsP3) receptor-mediated intracellular stores and influx through voltage-gated Ca2+ channels (VGCCs) provide alternative postsynaptic Ca2+ sources. We hypothesize that InsP3 Rs are coincidence detectors, sensing presynaptic glutamate release through linkage with group I metabotropic glutamate receptors (mGlurRs), and depolarization, through VGCCs. We also find that the downstream protein kinases, PKA and PKC, have a role in potentiation in layer 2/3 pyramidal neurons of the neonatal visual cortex.

INTRODUCTION

In the visual cortex, the strength of synapses is rapidly modified by visual experience (Hubel and Wiesel 1970; Katz and Shatz 1996) as well as imposed activity regimes (Bear 1996; Fregnac et al. 1992; Shulz and Fregnac 1992) throughout postnatal development. These changes in synaptic strength may be the initial step in the activity-dependent anatomical organization of cortical circuits (Antonini and Stryker 1993; Engert and Bonhoeffer 1999; Friedlander et al. 1991; Harris 1999) as well as forming the basis for dynamic aspects of cortical processing of features and adaptive learning (Ahissar et al. 1992; Singer 1995). Thus elucidating the mechanisms underlying these initial activity-dependent changes in synaptic strength is important for understanding developmental processes, the adaptive recovery of function, and the ongoing balancing of the distribution of synaptic signaling during normal central processing of sensory input.

The theory of the “Hebbian” synapse (Hebb 1949), which states that the temporal correlation of pre- and postsynaptic activity induces an increase in synaptic efficacy, provides a cellular construct for synaptic plasticity. In this paradigm, the postsynaptic neuron must detect the temporal coincidence of presynaptic transmitter release and postsynaptic depolarization. For many forms of Hebbian plasticity, the N-methyl-D-aspartate (NMDA) type of glutamate receptors (NMDARs) serve as coincidence detectors on the postsynaptic neuron (reviewed by Kaczmarek et al. 1997). For example, we previously characterized a form of transient synaptic potentiation that is induced by a series of pairings of low-frequency (0.1 Hz) presynaptic stimulation with brief (80 ms) postsynaptic depolarizing pulses in the visual cortex of mature animals (Harsanyi and Friedlander 1997a). Induction of this potentiation requires activation of NMDARs in most cells and an increase in intracellular Ca2+ in the postsynaptic neuron in all cells (Harsanyi and Friedlander 1997a). Interestingly, when the same pairing protocol is applied to neurons in the neonatal visual cortex, a more persistent synaptic potentiation (long-term potentiation, LTP) is induced that has a small component that escapes NMDAR inhibition in over half of the cells tested but still requires an increase in postsynaptic calcium in all cells (Harsanyi and Friedlander 1997b). These results are consistent with a mechanism for regulating functional synaptic plasticity in neonatal cortex that partially relies on a combination of synaptic signaling pathways for detection of coincident activation of ligand and voltage sensors, including but not strictly limited to NMDARs. Therefore in this paper, we investigated the initial ligand and voltage-sensing limits of the induction pathway for this form of synaptic potentiation, their interaction with the important NMDAR pathway, and downstream signaling cascades as potential sites for combining these signals. The inositol trisphosphate receptor (InsP3R) is one candidate substrate for coincidence detection of ligand and voltage as well as a potential source of Ca2+ in addition to NMDARs. Ca2+ release from intracellular stores can be indirectly activated by metabotropic glutamate receptors (mGlurRs) and is amplified by Ca2+ (Berridge 1998; Bezprozvanny et al. 1991; Finch et al. 1991). Therefore in the neonatal visual cortex where NMDAR inhibition strongly reduces but does not entirely eliminate induction of synaptic potentiation, InsP3Rs could potentially contribute to this form of plasticity by detecting plasma mem-

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brane depolarization through activation of voltage-gated Ca\(^{2+}\) channels (VGCCs) and/or NMDARs and by sensing synaptic glutamate release through postsynaptic mGluRs coupled to InsP\(_3\) turnover (Courtney et al. 1990; Murphy and Miller 1989). In mature hippocampal neurons, it has been shown that mGluRs (Bashir et al. 1993; Bortolotto and Collingridge 1993; Little et al. 1995; Vickery et al. 1997; Wilsch et al. 1998) and VGCCs (Borroni et al. 2000; Cavus and Teyler 1996, 1998; Grover and Teyler 1990, 1992; Izumi and Zorumski 1998; Kapur et al. 1998; Shankar et al. 1998; Wang et al. 1997) both contribute to induction of certain forms of synaptic potentiation. Thus in the present study, we sought to determine whether InsP\(_3\)Rs act as downstream coincidence detectors after activation of mGluRs and VGCCs, potentially facilitating their calcium release in response to IP\(_3\) from mGluRs and to Ca\(^{2+}\) entering through NMDARs. This would provide an additional source of postsynaptic Ca\(^{2+}\) in supragranular pyramidal neurons of the neonatal visual cortex and contribute to induction of synaptic potentiation. In addition, we evaluated the potential contributions of downstream signaling cascades to induction of synaptic potentiation in the neonatal visual cortex that are known to be involved in LTP induction in the mature hippocampus [protein kinase C (PKC) and cAMP-activated protein kinase (PKA)] (reviewed by Shobe 2002; Silva 2003).

**METHODS**

**Slice and electrode preparation**

The basic experimental procedures have previously been described in detail (Fregnac et al. 1994; Harsanyi and Friedlander 1997a,b). Guinea pigs (7–17 days) of either sex (adult animals >40 days) were deeply anesthetized with ether and decapitated. The brain was rapidly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) consisting of (in mM) 124 NaCl, 2 KCl, 2 MgSO\(_4\), 2 CaCl\(_2\), 1.25 KH\(_2\)PO\(_4\), 26 NaHCO\(_3\), and 11 glucose, pH maintained at 7.4 by saturating the solution with 95% O\(_2\)-5% CO\(_2\). The occipital pole of one hemisphere of the brain was removed and blocked in the frontal plane, and coronal slices (400 \(\mu\)m) of the visual cortex were cut. Slices were transferred to a humidified interface-type chamber (Medical Systems) maintained at 35°C and allowed to stabilize for 1.5–2 h before recording. Microelectrodes were pulled from glass capillaries (1.5 mm OD, 0.86 mm ID; A-M Systems) with a horizontal puller (Sutter Instruments) and filled with 2 M potassium acetate. Electrode resistances ranged from 80 to 180 m\(\Omega\) (138 ± 5 m\(\Omega\); mean ± SE). In several experiments, micropipettes were also filled with a 2% biocytin solution (Sigma, St. Louis, MO), and subsequent to the electrophysiology experimental protocol (see following text), cells were filled with biocytin by application of 200-ms negative current pulses of ~0.5- to ~1.0-nA amplitude at 3 Hz for 10–15 min. In those cases, slices were fixed by immersion in 4% paraformaldehyde in 0.1 M phosphate buffer and stored overnight at 4°C. After overnight fixation, slices were re-imbedded in albumin/gelatin and sectioned at 100 \(\mu\)m on a vibratome. Sections were incubated in an avidin/biotin/horseradish peroxidase complex (Vector Elite, Burlingame, CA) and reacted using 3,3’-diaminobenzidine (DAB, Vector). Sections were counterstained with cresyl violet and cover-slipped with DPX (EM Sciences, Ft. Washington, PA) for subsequent microscopical examination of cellular morphology and somatic laminar location in the cortex.

In some experiments (Figs. 2, D–F, and 3, D–F, and 7), whole cell recording using the perforated-patch technique was used to compare the basic findings by evaluating PSCs (holding potentials were ~70 mV). Recordings were made using an Axopatch 1B amplifier and data collection was begun 20–30 min after patch formation when the series resistance had stabilized at 40 ~ 50 M\(\Omega\). Series resistance was continuously monitored. Patch pipettes were pulled on a Narashige PP-830 from 8250 capillary glass (596800 A-M Systems) to a resistance of 3–5 M\(\Omega\) and filled with (in mM): 125 K gluconate, 20 KCl, 10 HEPES, 4 NaCl, 1.3 Mg, 4 ATP, 3 Na, 3 GTP, and 240 \(\mu\)g/ml amphotericin B (Sigma).

**Synaptic activation and pairing protocol**

Recordings were made exclusively from layer 2/3 pyramidal neurons in primary visual cortex. This was determined by both morphological evaluation after biocytin filling in some cases (see preceding text) and/or by the cells’ electrophysiological characteristics such as spike duration >0.8 ms (at half-amplitude) and substantial spike frequency adaptation in response to application of 200-ms depolarizing pulses. In the perforated-patch experiments, cellular morphology and location were also apparent with direct visualization during patching. Compound postsynaptic potentials (PSPs) were evoked with constant current stimulation (50-\(\mu\)s square wave) with a bipolar electrode placed at the white matter/layer 6 border of the visual cortex on beam with the recording electrode. The input resistance of each cell was continuously monitored. In those cases, slices were filled with a 2% biocytin solution (Sigma, St. Louis, MO), and subsequent to the electrophysiology experimental protocol (see following text), cells were filled with biocytin by application of 200-ms negative current pulses of ~0.5- to ~1.0-nA amplitude at 3 Hz for 10–15 min. In those cases, slices were fixed by immersion in 4% paraformaldehyde in 0.1 M phosphate buffer and stored overnight at 4°C. After overnight fixation, slices were re-imbedded in albumin/gelatin and sectioned at 100 \(\mu\)m on a vibratome. Sections were incubated in an avidin/biotin/horseradish peroxidase complex (Vector Elite, Burlingame, CA) and reacted using 3,3’-diaminobenzidine (DAB, Vector). Sections were counterstained with cresyl violet and cover-slipped with DPX (EM Sciences, Ft. Washington, PA) for subsequent microscopical examination of cellular morphology and somatic laminar location in the cortex.

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FIG. 2. Pairing-induced potentiation in control artificial cerebrospinal fluid (ACSF) in neonatal animals. A: example time plot of normalized peak amplitudes of postsynaptic potentials (PSPs) from a neuron recorded with sharp electrodes in current-clamp mode in which the pairing protocol (10-min gap, postsynaptic depolarization paired with presynaptic stimulation) induces synaptic potentiation. This time plot shows the normalized peak amplitude of the PSPs prior to and after the pairing protocol. This neuron exhibits long-lasting potentiation (>40 min; 55% at 15–20 min). Inset: the PSP (average of 30) recorded 5 min pre- and 0–5 min postpairing from the neuron shown in A. B: mean ± SE time plot of the normalized peak amplitude of the PSPs recorded in all cells tested in control ACSF (n = 14). C: summary bar graph showing the mean amount of potentiation of PSPs in neurons recorded with intracellular electrodes at 5 min (n = 14; 46 ± 7%; P < 0.01) and at 15–20 min (n = 14; 35 ± 5%; P < 0.01). D: example time plot of normalized peak amplitudes of PSCs from a neuron recorded with perforated-patch technique in voltage-clamp mode in which the pairing protocol (10 min gap, postsynaptic depolarization paired with presynaptic stimulation) induces synaptic potentiation (+33%). This time plot shows the normalized peak amplitude of the evoked PSCs prior to and after the pairing protocol. Inset: the PSC (average of 30) recorded pre- and 5 min postpairing from the neuron shown in D. E: mean ± SE time plot of the normalized peak amplitude of the excitatory postsynaptic currents (EPSCs) recorded in all cells tested in control ACSF with the perforated-patch technique (n = 13). F: summary bar graph showing the mean ± SE amount of potentiation of EPSCs in neurons recorded in voltage clamp at 0–5 (n = 13; 20 ± 7%; P < 0.05) and at 15–20 min (n = 13; 18 ± 6%; P < 0.05) minutes postpairing, respectively.
the period when the amplitude of evoked responses was within 2 SD of the average baseline measurement. The conditioning protocol was 10 min (60 pairings) of 0.1-Hz stimulation paired with intracellular postsynaptic depolarizing current pulses (80-ms duration; +2.0 to +3.5 nA) adjusted to elicit 6–10 spikes. Afferent stimulation occurred 25 ms after the onset of the depolarizing current pulse. After pairing, PSPs continued to be evoked at 0.1 Hz for ≥20 min. The peak amplitudes of PSPs were analyzed using a
custom-made program (Signal Averager). In addition, initial slopes (1st millisecond) of PSPs were analyzed to selectively evaluate the monosynaptic component. There were no significant differences in changes in synaptic strength as evaluated by peak amplitude and initial slope criteria, suggesting that different outcomes (potentiation versus no change) induced by the pairing protocol were not due to differential effects on mono- and polysynaptic components of the PSPs. The statistics comparing the results for analysis of the PSP peak amplitudes and initial slopes for the 2-amino-5-phosphonovaleric acid (APV) versus control experiments are presented in RESULTS. Because these outcomes are similar for both types of analyses, for clarity's sake, the peak amplitude results only are presented for the remainder of the experiments. In keeping with our previous studies (Harsanyi et al. 1997a,b), the magnitude of potentiation was quantified by comparing the mean of the peak amplitudes (n = 30) of the PSPs at 5 min (an average of the 1st 5 min immediately after the pairing protocol) and at 20 min (an average of 15–20 min) postpairing to the last 5 min of the baseline period. The induction of statistically significant potentiation was evaluated with a paired t-test within individual experimental groups including the ACSF control group and the various groups where the effects of a single compound were tested (the 5-min control prepairing period was compared with the 5-min period immediately after pairing and to the 15– to 20-min period after pairing). Comparisons were also made between the ACSF control group (the results of which are illustrated in Fig. 2B) and the various experimental groups [e.g., the APV, (RS)-α-methyl-carboxyphenylglycine (MCPG), (RS)-1-aminoindan-1,5-dicarboxylic acid (AIDA), thapsigargin, heparin, with PKC activation/inhibition and with PKA activation/inhibition] whose results are illustrated in Figs. 3B, 4, B and E, 6, B and C, 9, A–D, and 10, A–D, respectively]. These comparisons were made for the first 5 min immediately after the pairing protocol and the 15– to 20-min period after the pairing protocol with statistical significance determined using a one-way ANOVA with a post hoc Dunnett's test to compare each experimental group to the ACSF control group. For the experiments where the effects of an additional compound was evaluated in conjunction with APV to determine its ability to eliminate the APV-resistant component of synaptic potentiation (e.g., APV + MCPG, APV + AIDA, and APV + nifedipine—Figs. 5, B, and E, and 6B, respectively), these results also were evaluated with a post hoc Dunnett's test and were compared with the APV alone results (Fig. 3B). For the patch recordings, APV was compared with ACSF control and APV + Xestospongin using an unpaired t-test.

**Drug application**

The broad-spectrum mGlur antagonist, MCPG (500 μM), and an antagonist specific for the group I receptors, AIDA (500 μM), were obtained from Tocris Cookson (St. Louis). APV (50 μM) and NiCl₂ (50 μM) were obtained from Sigma. Nifedipine (10 μM) and thapsigargin (1 μM) were obtained from RBL. All drugs were bath-applied for ≥10 min (thapsigargin: 15 min) prior to the control period for the pairing protocol. PMA and 8-Br-cAMP were obtained from Calbiochem (La Jolla, CA). Xestospongin C (a specific blocker of the InsP₃R) (Gafni et al. 1997), was obtained from Calbiochem and used in the patch pipettes at a concentration of 1 μM (Narasimhan et al. 1998). The PKC inhibitor peptide (PKC 19–36, 100 μM) was applied in the recording pipette. Protein kinase A inhibitor peptide (PKA 5–24, 20 μM) was also applied in the recording pipette. Both inhibitor peptides were obtained from Calbiochem.

**RESULTS**

**Summary**

Biocytin-filled cells always corresponded to spiny neurons with pyramidal morphology (Fig. 1) and were located in the middle of layers 2/3. The cell illustrated in Fig. 1 is typical of all of those injected with biocytin in having spiny dendrites, a pyramidal morphology with main apical dendrite, and branching secondary dendrites and basilar dendritic tree and distinct axon that contained local collaterals. Results were obtained only from neurons in layer 2/3 of visual cortex. All of these cells were characterized as regular-spiking neurons as described in McCormick et al. (1985). The occasionally recorded fast-spiking neurons were rejected from the study. The patch recordings were made under visual guidance, therefore pyramidal cells were chosen. Successful recordings from 175 neurons, including PSPs from 139 neurons obtained with intracellular sharp micropipettes under current clamp and PSCs from 36 neurons obtained under voltage clamp with perforated-patch recordings were analyzed and included in these results. The intracellular recordings (Figs. 2, A–C, 3, A–C, 4–6, and 8–11) were used for the bulk of the experiments because they generally elicited a stronger and more reliable potentiation (see following text) while the patch recordings (Figs. 2, D–F, 3, D–F, and 7) tended to elicit less potentiation. However, the patch recordings were useful to verify the control potentiation phenomenon and the ability of a component of the potentiation to persist during APV inhibition of NMDARs. They were particularly useful for the intracellular application of the InsP₃R-specific blocker, Xestospongin C, into the postsynaptic neuron. All neurons included for the intracellular recordings had membrane potentials less than −70 mV (−80 ± 2.4 mV) and input resistances >20 MΩ. The mean input resistance was 41 ± 1.3 MΩ and was monitored throughout the experiments and did not change significantly

**FIG. 3.** The N-methyl-d-aspartate receptor (NMDAR) antagonist, 2-amino-5-phosphonovaleric acid (APV), reduces the amount of pairing-induced potentiation, but significant potentiation still occurs. A: example time plot of normalized peak amplitudes of PSPs from a cell recorded with sharp electrodes whose PSPs potentiated (+22%) in the presence of the NMDAR antagonist, APV (50 μM). Insert: the average PSP recorded from the neuron in A 5 min pre- and 0.5 min postpairing. B: mean ± SE time plot of the normalized peak amplitude of the PSPs recorded in all cells tested in APV (n = 38). C: summary bar graph of the mean ± SE amount of potentiation obtained at 0–5 min postpairing in control ACSF (*) and APV (‡, 10 ± 2%) and at 15–20 min postpairing in control (*) and APV (‡, 7 ± 2%). The potentiation obtained in APV is significantly less than that obtained in control ACSF at both time points (*, P < 0.01) but still significant compared with baseline (*, P < 0.05). D: example time plot from a neuron recorded with the perforated-patch technique that showed potentiation in APV (+10%). Insert: the average PSC recorded from the neuron in 5 min pre- and 0–5 min postpairing. E: mean ± SE time plot of the normalized peak amplitude of the PSCs recorded from all cells tested in APV with the perforated-patch technique (n = 13). F: summary bar graph of the mean ± SE amount of potentiation obtained at 0–5 min postpairing in control (*) and APV (‡, n = 13; 9 ± 3%). The potentiation obtained in APV is significantly less than that obtained in control ACSF at 0–5 min postpairing (*, P < 0.05) but still significant compared with the prepairing baseline period (*, P < 0.05). At 15–20 min postpairing, the potentiation in APV also is significant compared with the prepairing baseline (‡, n = 13; 9 ± 2%; *, P < 0.05) but is not significantly different from the amount of potentiation in control ACSF (*) and P < 0.05).
(+0.9 ± 0.4%; n = 84; P > 0.25) after pairing. An additional 36 neurons were used for recording excitatory postsynaptic currents (EPSCs) with whole cell perforated-patch recording using amphotericin B in the micropipette. Membrane potentials in these cells were held at −70 mV for voltage-clamp recording while eliciting evoked PSCs in response to afferent stimulation. Input resistances were 361 ± 105 MΩ. Seal resistances were >1GΩ, and only data from electrodes with access resistances of 10–20 MΩ were used with <20% change during recording.

Potentiation of synaptic responses—controls

Pairing low-frequency afferent stimulation with coincident postsynaptic depolarization induces potentiation of PSPs recorded intracellularly with sharp micropipettes in the neonatal visual cortex. Figure 2A is a time plot of the normalized peak amplitudes of PSPs recorded from an example neuron that showed 55% potentiation at 15–20 min postpairing. Figure 2B is a summary time plot of the mean peak amplitudes of the sample (n = 14) of control PSPs in ACSF. The change in the
average peak amplitude of PSPs is +46 ± 7% at 0–5 min postpairing ($P < 0.01$) and +35 ± 5% at 15–20 min postpairing ($P < 0.01$). These results are summarized in Fig. 2C. Pairing also induces potentiation (albeit smaller and more variable) of PSCs recorded with the perforated patch method. Figure 2D is a time plot of the normalized peak amplitudes of PSCs from an example neuron that showed 33% potentiation at 15–20 min postpairing. Figure 2E is a summary time plot of the mean peak amplitudes of the sample of control ($n = 13$) PSCs in ACSF. The change in the average peak amplitude of the control PSCs is $+20 ± 7%$ at 0–5 min postpairing ($P < 0.05$) and $+18 ± 6%$ at 15–20 min postpairing ($P < 0.05$). These results are summarized in Fig. 2F.

Contribution of NMDARs to induction of synaptic potentiation

Figure 3, A and D, contains examples of individual records in the presence of 50 μM APV. These examples show 25% potentiation at 0–5 min after pairing and 22% potentiation at 15–20 min after pairing for the PSP (Fig. 3A) and 11% potentiation at 0–5 min after pairing and 10% potentiation at 15–20 min after pairing for the PSC (Fig. 3D). Figure 3, B and E, shows time plots of the normalized mean peak amplitudes for the entire sample of PSPs (Fig. 3B; $n = 38$) and PSCs (Fig. 3E; $n = 13$) tested in APV under current- and voltage-clamp conditions, respectively. APV substantially and significantly reduces (but does not eliminate) potentiation from that induced in control ACSF. For the PSPs at 0–5 min postpairing, the amount of potentiation is $+10 ± 2%$ in APV versus $+46 ± 7%$ in control ACSF ($P < 0.01$) and at 15–20 min postpairing, the amount of potentiation is $+7 ± 2%$ in APV versus $+35 ± 5%$ in control ACSF ($P < 0.01$). Although substantially reduced, the remaining potentiation in APV is significant ($P < 0.05$) at both 0–5 and 15–20 min postpairing compared with baseline. Similarly, the PSCs are also significantly potentiated in the presence of APV when evaluated by analyzing the initial slopes ($+8 ± 2%; P < 0.05$; $n = 38$; data not shown) at 15–20 min post pairing. The PSCs were affected similarly where the amount of potentiation at 0–5 min postpairing is $+9 ± 3%$ in APV versus $+20 ± 7%$ in control ACSF ($P < 0.05$), and the amount of potentiation at 15–20 min postpairing is $+9 ± 2%$ in APV versus $+18 ± 6%$ in control ACSF ($P > 0.05$). The potentiation of the PSCs in the presence of APV, although substantially reduced, is significant at both 0–5 ($P < 0.05$) and 15–20 ($P < 0.05$) minutes postpairing.

Contribution of mGluRs to synaptic potentiation

To evaluate the role of mGluRs, we utilized bath application of the broad spectrum mGluR antagonist, MCPG (500 μM). Figure 4A shows an example from a neuron the PSPs of which were only transiently (<5 min) potentiated after the 0.1 Hz pairing protocol in the presence of MCPG. Figure 4B shows the time plot of the mean change in the normalized peak amplitude of PSPs induced by the pairing protocol in the presence of MCPG. These results are summarized in Fig. 4C for the entire sample ($n = 6$). Although MCPG does not significantly affect potentiation at 0–5 min postpairing ($+35 ± 6%$ in MCPG vs. $+46 ± 7%$ in ACSF control—also see Fig. 2B; $P > 0.05$), it does significantly reduce potentiation at 15–20 min postpairing ($+10 ± 4%$ in MCPG vs. $+35 ± 5%$ in ACSF control—also see Fig. 2B; $P < 0.05$). Bath application of MCPG does not affect baseline synaptic transmission (not shown). A similar trend is seen with a more specific blockade of group I mGluRs. Application of the specific group I mGluR antagonist, AIDA (500 μM) (Pellicari et al. 1995), also reduces the magnitude of potentiation (Fig. 4, D–F). An example of the effects of AIDA is illustrated in Fig. 4D where only a transient potentiation is induced. Figure 4E shows the time plot of the mean change in the normalized peak amplitude of PSPs induced by the pairing protocol in the presence of AIDA. These results are summarized in Fig. 4F for the entire sample ($n = 4$). Like MCPG, AIDA does not significantly affect the amount of potentiation at 0–5 min postpairing ($+33 ± 17%$ in AIDA vs. $+46 ± 7%$ in ACSF control—also see Fig. 2B; $P > 0.05$). At 15–20 min postpairing, the AIDA effects are similar to MCPG in reducing the amount of potentiation induced ($+20 ± 9%$ in AIDA vs. $+35 ± 5%$ in ACSF control—see also Fig. 2B), but these effects were not statistically significant ($P > 0.05$).

Blockade of mGluRs and NMDARs blocks potentiation

Because the mGluR blockers alone had significant (MCPG) and modest (AIDA) effects on the magnitude of potentiation induced, we evaluated the ability of these blockers to specifically inhibit the APV-resistant component of the potentiation by applying them in conjunction with APV. In combination with APV, MCPG eliminates potentiation. Figure 5A is a time plot of normalized PSPs recorded from an example neuron in which no potentiation occurs in the presence of MCPG + APV. Figure 5B is a time plot of the normalized mean PSP peak amplitudes of the entire MCPG + APV sample ($n = 13$) tested in AIDA (500 μM) under current- and voltage-clamp conditions.
that shows no significant potentiation ($P > 0.05$). Figure 5C compares the change in average peak PSP amplitudes at 15–20 min postpairing in control ACSF ($n = +35 \pm 5\%$; also see Fig. 2B), in APV alone ($+7 \pm 2\%$; also see Fig. 3, B and C), and in MCPG + APV ($-1 \pm 5\%$). These data suggest that activation of mGluRs and NMDARs is necessary for the full expression of this form of synaptic potentiation.

We also tested the effects of the more specific group I mGluR antagonist AIDA in combination with APV. Figure 5D illustrates an example cell that showed depression ($-35\%$) at
15–20 min postpairing in the presence of APV + AIDA. Figure 5E is a summary time plot of mean PSP amplitudes in the presence of AIDA + APV for the entire sample (n = 5), illustrating significant synaptic depression for the group at 15–20 min postpairing (−27 ± 12%; P < 0.01). Figure 5F compares the change in average PSP peak amplitudes at 15–20 min postpairing in control ACSF (+35 ± 5%; also see Fig. 2B), in APV alone (+7 ± 2%; also see Fig. 3, B and C), and in AIDA + APV (−27 ± 12%). The results in APV versus the results in AIDA + APV are significantly different (P < 0.01), suggesting that the group I mGluRs specifically play a role in potentiation in the cortex of the young animal.

**Contribution of release of calcium from intracellular stores to synaptic potentiation**

Activation of mGluRs (specifically Group I mGluRs) leads to the production of IP₃ and the subsequent release of Ca²⁺ from intracellular stores (see Fagni et al. 2000 for review). Either MCPG or AIDA alone reduce the strength and duration of synaptic potentiation. However, when applied in combination with APV, they completely eliminate induction of any potentiation. Thus Ca²⁺ release from IP₃-sensitive stores may, in addition to the NMDAR-mediated Ca²⁺ flux, contribute to the induction of potentiation in the neonatal visual cortex. We tested this hypothesis using two different approaches. In the first experiment, we tested the necessity of mobilization of intracellular Ca²⁺ alone for potentiation. We utilized bath application of thapsigargin (1 μM), which disrupts the release of Ca²⁺ from intracellular stores by blocking the SERCA pump that is necessary to refill the stores (Jackson et al. 1988).

Figure 6A shows a time plot of the peak amplitude of PSPs recorded from an example neuron tested with thapsigargin in the bath. The pairing protocol in the presence of thapsigargin induces a transient potentiation, qualitatively similar but not as rapidly decaying to that seen in the presence of MCPG or AIDA alone (see Fig. 4, A and D). Figure 6B is a time plot of the normalized mean peak amplitudes of PSPs in the presence of thapsigargin for the entire sample (n = 8). At 0–5 min postpairing, the amount of potentiation in thapsigargin is reduced but is not significantly less than that in control ACSF (+25 ± 11% in thapsigargin vs. +46 ± 7% in control ACSF—see also Fig. 2B; P > 0.05). However, at 15–20 min postpairing, the amount of potentiation in thapsigargin is significantly less than in control ACSF (+12 ± 8 vs. +35 ± 5%, respectively; P < 0.05). Thapsigargin has no effect on baseline synaptic transmission (96 ± 4% baseline transmission, P = 0.25; not shown). These data suggest that release of Ca²⁺ from intracellular stores is necessary for the later phases of potentiation. To further evaluate whether the thapsigargin effect is primarily postsynaptic, we utilized two additional approaches: application of heparin (0.1 μg/ml) in the sharp micropipette to block the release of calcium from InsP₃R-sensitive stores (Bezprozvanny et al. 1993; Ehrlich et al. 1994) and application of the specific inhibitor of InsP₃Rs, Xestospongin C (Gafni et al. 1997; Narasimham et al. 1998), in the patch pipettes in combination with bath-applied APV. Heparin (n = 6) reduces the amount of potentiation (+35 ± 18% potentiation with heparin vs. +46 ± 7% potentiation in control ACSF at 0–5 min postpairing and +23 ± 10% potentiation with heparin vs. +35 ± 5% potentiation in control ACSF at 20 min postpairing—see summary Fig. 6C and also compare with Fig. 2B) although this effect is not statistically significant (P > 0.05). Xestospongin C in the patch pipette in the presence of APV prevented potentiation. An example of a neuron that did not
potentiate with Xestospongin in the pipette is illustrated in Fig. 7A. This is indicative of the sample of cells (n = 10) tested with intracellular Xestospongin C as illustrated by the time plots of the normalized mean peak amplitudes in Fig. 7B. For the entire group, there was a small but significant depression (−7 ± 1% at 5 min post pairing; P < 0.05 and −11 ± 4% at 20 min post pairing; P < 0.05—Fig. 7C). These results with Xestospongin C plus APV are significantly different (P < 0.01 at 15–20 min post pairing) from those in APV alone where a small but significant potentiation persisted (Fig. 7C). Taken together, the thapsigargin, heparin and Xestospongin results are consistent with release of Ca^{2+} from IP_{3}R-mediated stores playing a role in NMDAR-independent potentiation in layer 2/3 pyramidal neurons in neonatal visual cortex.

**VGCCs contribute to LTP**

Although group I mGluRs can detect glutamate release and initiate release of Ca^{2+} from intracellular stores in the presence of NMDAR inhibition by APV, there must be another mechanism to mediate the detection of the depolarization component of the pairing protocol for induction of potentiation. Thus we evaluated the possible contribution of VGCCs to the induction of the component of potentiation not blocked by APV. Figure 8, A and B, shows a time plot of PSPs from an example neuron in which pairing was applied in an attempt to induce potentiation in the presence of a VGCC blocker, nifedipine (10 μM) (Trombley and Westbrook 1991), + APV (50 μM) and a summary time plot of the normalized mean PSP amplitudes for the entire group of cells (n = 8) tested in this manner, respectively. The bar graph in Fig. 8C compares the mean changes in synaptic strength occurring 15–20 min after the pairing protocol in control ACSF (+35 ± 5% from Fig. 2B) versus in APV alone (+7 ± 2% from Fig. 3B) versus in nifedipine + APV (−6 ± 3%). The combination of APV + nifedipine completely prevents potentiation, although the results in APV + nifedipine versus in APV alone are not significantly different (P > 0.05).

**Contribution of the PKC and PKA signaling pathways to synaptic potentiation**

We also evaluated the potential contribution of two major downstream kinase signaling cascades the PKC and PKA pathways to induction of synaptic potentiation in layer 2/3 pyramidal neurons of the neonatal visual cortex. We first evaluated the effect of application of 0.1-Hz postsynaptic depolarizing pulses alone. Figure 9A illustrates that the depolarizing pulses, without coincident synaptic input, are not sufficient to induce synaptic potentiation at 15–20 min postpairing. We then tested the necessity and sufficiency of activation of these kinases for induction of potentiation. We used PMA, a phorbol ester, to activate PKC as phorbol esters bind to the region of the PKC enzyme to act by mimicking the action of DAG (Gschwendt et al. 1991). Application of the PKC activator alone, PMA (1 μM; Fig. 9B) in conjunction with 0.1-Hz synaptic activation, had no effect on the synaptic responses (n = 6). This effect is different from that seen in the hippocampus, where application of phorbol esters in conjunction with synaptic activation facilitates synaptic transmission (Malenka et al. 1986). However, when depolarizing pulses are applied in conjunction with application of the PKC activator, PMA (Fig. 9C), significant potentiation is induced even in the absence of synaptic input. These results are summarized in Fig. 9E (+46 ± 8%, P < 0.01, at 0–5 min post pairing and +39 ± 5%, P < 0.01 at 15–20 min post pairing), suggesting that PKC stimulation could substitute for the presynaptic stimulation. Interestingly, intracellular application of the PKC inhibitor peptide fragment 19–36 reduces the magnitude but does not
eliminate the potentiation. The grouped results for this experiment (n = 7), illustrated in Fig. 9D and are summarized in F (+34 ± 13%; at 5 min post pairing; +12 ± 10% at 15–20 min post pairing), which is not significantly different from the ACSF control group result at similar time periods (P > 0.05). These data imply that PKC is neither necessary nor sufficient to induce potentiation but that it may provide a signal representing one limb of the activation cascade (downstream from mGluR activation) that can contribute to the induction of potentiation.

Similar experiments were done with perturbation of the PKA pathway (Fig. 10). The effects of depolarization alone are replotted in Fig. 10A for comparison to the PKA data. Similar to the results where PKC was activated by PMA, the activation of PKA alone (Fig. 10B—by 8-Br-cAMP, 100 μM) (Uno et al. 1976) also has no effect on the synaptic response (n = 7), suggesting that activation of either kinase alone is not sufficient to trigger potentiation. The conjunction of PKA activation and depolarizing pulses (n = 6; Fig. 10C), unlike the conjunction of PKC activation and depolarizing pulses, does not induce potentiation (−1 ± 4% at 15–20 min after pairing compared with baseline; P > 0.05). However, intracellular application of the PKA inhibitor peptide fragment 5–24 (n = 6; Fig. 10D) prevents induction of potentiation at 0–5 min postpairing (+8 ± 4%) and at 15–20 min postpairing (+3 ± 7%) and is significantly different (P < 0.01) from the ACSF control. These data are summarized in Fig. 10, E and F. These results imply that PKA activation is necessary but not sufficient for the induction of potentiation in the neonatal cortex.

Comparison of potentiation in the neonate and in the adult

The mean amount of potentiation induced in the neonatal cortex at 5 min post pairing is not significantly different in the presence of control ACSF versus MCPG (+46 ± 7%, n = 14 vs. +35 ± 6%, n = 6; P > 0.05) but is significantly less in APV than in control ACSF (+10 ± 2%, n = 38, vs. +46 ± 7%, n = 14; P < 0.01; Fig. 11A), whereas at 15–20 min post pairing (Fig. 11B), it is significantly decreased in the presence of MCPG compared with ACSF controls (+10 ± 4%; n = 6, vs. +35 ± 5%; n = 14; P < 0.05) or in APV alone compared with ACSF controls (+7 ± 2%, n = 38, vs. +35 ± 5%, n = 14; P < 0.01). Figure 11, C and D, contains the corresponding bar graphs for the adult and illustrate three points: potentiation in control ACSF in the adult is more transient (+25 ± 8% at 5 min and +7 ± 5% at 20 min), similar to potentiation induced in the neonate in the presence of MCPG; MCPG does not affect this more transient synaptic potentiation in the adult (mean amount of potentiation in MCPG = +19 ± 5% at 5 min and +9 ± 4% at 20 min); and APV completely blocks synaptic potentiation in the adult (mean change = +0 ± 3% at 5 min and −1 ± 2% at 20 min; n = 14) while sparing some significant potentiation in the neonate (+10 ± 2% at 5 min and +9 ± 2% at 20 min; n = 38). These differences suggest that activation of mGluRs may play a role in the induction of the more sustained potentiation seen in the visual cortex of the young animal versus in the adult.

DISCUSSION

In the present study, we found that low-frequency stimulation of afferents coincident with brief postsynaptic depolarization induces potentiation of synaptic responses recorded from supragranular (layers 2/3) pyramidal neurons in neonatal primary visual cortex. We have previously shown that this potentiation is synapse specific, not due to changes in inhibitory inputs, and dependent on an increase in intracellular Ca2+ in the postsynaptic neuron (Harsanyi and Friedlander 1997b). In addition, induction of this potentiation, although largely dependent on NMDARs, also contains a small but significant persistent component that manifests when induction occurs in the presence of NMDAR inhibition by APV. Therefore other mechanisms for the detection of coincident neurotransmitter release and postsynaptic depolarization, as well as alternate Ca2+ sources (other than those provided directly by NMDARs), contribute to the
induction of potentiation in the neonate. The apparent APV-resistant component of the potentiation that we found in the neonate but that is not seen in the adult might be due to the inability of the concentration of APV (50 μM) used in most of this study to fully block the receptors. However, we consider this unlikely for two reasons—we did several additional experiments with 100 μM APV (n = 3; data not shown) and obtained similar residual but significant potentiation and the addition of mGluR antagonists (either MCPG or AIDA) along with the 50 μM APV, completely eliminated all potentiation (Fig. 5).

We examined the contributions to the induction of potentiation of: mGluRs, VGCCs, intracellular Ca^{2+} stores, and downstream protein kinases. Blockade of mGluRs with MCPG reduces the duration of potentiation induced. In addition, the component of potentiation that persists in the
face of NMDAR inhibition by APV is completely blocked by MCPG or the group-I-specific antagonist, AIDA. These results, in conjunction with the ability of thapsigargin to reduce the duration of potentiation (similar to MCPG) and the prevention of induction of NMDAR-independent potentiation by inhibition of InsP3 Rs with Xestospongin C, are consistent with a model where the activation of group I mGluRs causes a release of Ca\(^{2+}\) from intracellular stores that in turn contributes to the induction of potentiation. However, although Ca\(^{2+}\) release from intracellular stores is necessary for the full manifestation of potentiation by presumably contributing to the NMDAR-independent component of potentiation, release of Ca\(^{2+}\) from intracellular stores is not sufficient for potentiation since antagonism of NMDARs and VGCCs together prevents any potentiation from being induced. These results are consistent with the hypothesis that InsP3 Rs act as coincidence detectors (in from being induced. These results are consistent with the developmentally regulated turn-on of InsP3 reported for the visual cortex (Dudek et al. 1994), suggesting that mGluRs may play a role in hippocampal LTP (Bashir et al. 1993; Bortolotto and Col ligndge 1993; Little et al. 1995; Vickers et al. 1997; Wilsch et al. 1998; but see Chinesta et al. 1993; Manzoni et al. 1994; Selig et al. 1995) and their contribution to induction is developmentally regulated (Izumi and Zorumski 1994). Interestingly, mGluR1 is essential for mediating changes in synapse efficiency and developmentally regulated progression of multiple innervation to Purkinje cells in the cerebellum (Ichise et al. 2000) and mGluR activation upregulates post synaptic protein synthesis both in cortex (Weiler and Greenough 1993) and in hippocampus area CA1, particularly after priming of LTP (Raymond et al. 2000), suggesting that mGluRs may play a role in structural organization of synapses.

An alternate explanation for the downregulation of the ability to induce potentiation in the adult cortex involves the “inhibitory gate” hypothesis (Kirkwood and Bear 1994; Kirk-
Wood et al. 1995; Rozas et al. 2001). One mechanism through which inhibitory inputs can limit LTP induction is through the shunting of back-propagating action potentials in the dendrites of adult animals. Back-propagating action potentials have been shown to be necessary for induction of LTP in hippocampal pyramidal neurons (Magee and Johnston 1997). This increase in inhibitory inputs may limit the amount of depolarization in the dendrites and, correspondingly, Ca\(^{2+}\) influx through VGCCs and NMDAR channels. In this case, the Ca\(^{2+}\) influx would not be sufficient to co-activate IP\(_3\)Rs thereby inhibiting release of Ca\(^{2+}\) from intracellular stores and the subsequent induction of potentiation in the adult.
**Downstream cascades in potentiation**

The contributions of PKC and PKA to LTP in the hippocampus are well characterized (reviewed in Dineley et al. 2001; Nelson et al. 2003; Silva 2003) with evidence in support of both pre- and postsynaptic actions (Carroll et al. 1998; Hori et al. 1999; Hu et al. 1987; Malenka et al. 1986; Wang and Feng 1992). Regardless of site of action, PKC enzymatic activity is persistently increased during early LTP (Klann et al. 1991, 1993; Sacktor et al. 1993). Interestingly, translocation of PKC occurs coincidentally with LTP (Akers and Routtenberg 1987) and is dependent on mGluRs (Angenstein et al. 1999).

We found that application of PMA alone had no effect on baseline synaptic transmission but that PKC activation can substitute for presynaptic stimulation when the postsynaptic cell is depolarized. The ability of PMA to substitute for synaptic activation and produce potentiation in conjunction with the depolarizing pulses argues for the sufficiency of PKC downstream from mGluR activation. However, the inability of the PKC inhibitor peptide fragment to completely prevent induction of potentiation suggests that the picture may be complicated. One interpretation is that PKC activation is not necessary, and although it may contribute to the induction process, other signaling pathways may also play this role. Alternatively, it is possible that the PKC peptide inhibitor fragment may not have effectively reached the synaptic sites due to limitations of the sharp micropipettes. The role of PKA in hippocampal LTP is well established (Abel et al. 1997; Huang et al. 1996; Roberson and Sweatt 1996) with evidence supporting both pre- and postsynaptic effects of PKA (Carroll et al. 1998; Chavez-Noriega and Stevens 1994; Duffy and Nguyen 2003; Frey et al. 1993). More applicable to the cortex, PKA-deficient mice lack LTP, long-term depression (LTD), or paired-pulse facilitation in layer 2/3 of the visual cortex with no effect on ocular dominance plasticity (Hensch et al. 1998). The ability of the PKA inhibitor peptide fragment to prevent induction of potentiation suggests that this pathway may play a role in potentiation in pyramidal cells of the cortex. The inability of 8-Br-cAMP to substitute for synaptic activation suggests that although PKA activation may be necessary, other parallel signaling pathways downstream from mGluR activation may also contribute to induction of potentiation. Interestingly, a recent report found that, similar to our results, PKA activation was necessary for induction of LTP in the neonatal hippocampus, whereas it is not required in the mature hippocampus (Yasuda et al. 2003). Thus the role of these kinases’ signaling effects in contributing to induction of synaptic plasticity may be under developmental regulation in multiple forebrain areas. Indeed, the peak of critical period for plasticity correlated with an increase in cAMP production in the visual cortex (Reid et al. 1996).

We have shown in previous studies (Frengac et al. 1994; Harsanyi and Friedlander 1997a,b) that potentiation of synaptic responses recorded from supragranular pyramidal neurons in primary visual cortex of both neonates and adults is synapse specific, not related to an increase in input resistance of the postsynaptic neuron, not due to changes in inhibitory inputs, and dependent on an increase in intracellular Ca\(^{2+}\) in the postsynaptic neuron in both immature and adult animals. The major differences between the two ages are that potentiation is more reliably and robustly induced in the neonatal than in the adult visual cortex and potentiation in the adult is entirely NMDAR-dependent, whereas a small component of potentiation in the young animal can be induced in the presence of APV. Interestingly, the time course and amplitude of the synaptic potentiation (robust initial potentiation followed by decline to baseline within 15–20 min) induced in the adult is strikingly similar to the synaptic potentiation induced in the neonate in the presence of MCPG. This led us to hypothesize that the contribution of the NMDARs to synaptic potentiation is similar in the neonate and the adult; however, the potentiation induced in the neonate is due to the contribution of the mGluR-linked pathway, which may be downregulated or dissociated from the plasticity cascade in the adult. We examined this issue more closely by comparing the effects of APV and MCPG on potentiation in the adult and neonate at 5 and 20 min postpairing. Figure 11, E and F, schematically illustrates these points as a developmental shift. “Immature” neurons possess two pathways for Ca\(^{2+}\)-dependent induction of potentiation, the NMDAR-mediated pathway and the mGluR-activated release of Ca\(^{2+}\) from intracellular stores. PKA activation is necessary downstream of these cascades. In addition to the NMDARs, the InsPP\(_R\) serves as an additional coincidence detector, converged on by Ca\(^{2+}\) through VGCCs that sense voltage and InsPP\(_R\) activated by group I mGluRs. The NMDAR pathway appears to contribute most to a transient robust component of the potentiation, while the mGluR pathway contributes a smaller amplitude, yet more persistent component of the potentiation. Conversely, in “mature” neurons in the adult visual cortex, only the very transient synaptic potentiation is induced and the mGluR pathway, if present, does not contribute at all as this potentiation is completely blocked by APV, and PKA inhibition in the mature cortex has no effect. This suggests that during development, the mGluR pathway, or release of Ca\(^{2+}\) from intracellular stores, may become functionally uncoupled from a signaling cascade that induces synaptic potentiation.

**FIG. 10.** Postsynaptic protein kinase A (PKA) activation is necessary for pairing-induced potentiation. **A**: replot of Fig. 9A for comparison to PKA data (response of PSPs to depolarization alone). **B**: time plot of the normalized mean ± SE peak amplitudes of PSPs in response to bath application of 8-Br-cAMP (100 µM) alone (n = 7) with no effect on synaptic responses. **C**: time plot of the normalized mean peak amplitudes of the PSPs in response to bath application of 8-Br-cAMP (100 µM) and postsynaptic depolarizing pulses (n = 6) does not result in significant potentiation (−1 ± 4% at 15–20 min postpairing; P > 0.05). **D**: time plot of the normalized mean peak amplitudes of the PSPs recorded with the PKA inhibitor peptide (5–24; 20 µM) in the pipette in response to pairing (n = 6). The PKA inhibitor blocked pairing-induced potentiation and was significantly different from potentiation in control ACSF (+8 ± 4% at 0–5 min postpairing; **P < 0.01). +3 ± 7% at 15–20 min postpairing; **P < 0.01). **E**: summary bar graph of potentiation obtained in control ACSF (●) and during bath application of 8-Br-cAMP and postsynaptic depolarization (●). **F**: summary bar graph comparing potentiation obtained in control ACSF (●) and during postsynaptic inhibition of PKA (○).
FIG. 11. Comparison of potentiation in the neonate and in the adult. A: summary bar graph showing the mean ± SE amount of potentiation induced in the neonatal cortex at 0–5 min postpairing in the presence of control ACSF (●), MCPG (○), and APV (■). The mean amount of potentiation in APV (+10 ± 2%; n = 38) is significantly less than in control ACSF (+46 ± 7%; n = 14; **; P < 0.01), but the mean amount of potentiation in MCPG (+35 ± 6%; n = 6) is not significantly different (P > 0.05) than control. B: summary bar graph showing the mean ± SE amount of potentiation induced in the neonatal cortex at 15–20 min postpairing in control ACSF (●) MCPG (○), and APV (■). At 15–20 min, the amounts of potentiation (+10 ± 4%) obtained in MCPG and APV (+7 ± 2%) are significantly different relative to control ACSF (*, P < 0.05 and **; P < 0.01, respectively). C: summary bar graph showing the mean ± SE amount of potentiation in the adult animal at 0–5 min postpairing in control ACSF (●), MCPG (○), and APV (■). The mean amount of potentiation in APV is significantly different from control ACSF (**; P < 0.01), but the mean amount of potentiation in MCPG is not significantly different from control ACSF (P > 0.05). D: summary bar graph showing the mean ± SE amount of potentiation seen in the adults 15–20 min postpairing in control ACSF (●; +7 ± 3%; n = 7), MCPG (○; +9 ± 4%; n = 7), and APV (■; −1 ± 2%). The mean amount of potentiation in control ACSF is significantly different from APV at 15–20 min (**, P < 0.01). E and F: schematic diagram of plasticity mechanisms in young and adult cortex. E: in the neonatal cortex, NMDARs, mGluRs, coupled to intracellular Ca²⁺ release, and VGCCs all provide a Ca²⁺ signal for plasticity induction. Activation of the downstream kinase, PKA is necessary for pairing-induced potentiation and PKC plays a role in the longer duration plasticity. F: in neurons of the adult, or neurons that have already matured in the young animal, the NMDARs provide the only coincidence detection mechanism coupled to the plasticity cascade. Metabotropic glutamate receptors are still present in the adult animal, and their activation can cause release of Ca²⁺ from intracellular stores; however, that release is not coupled to the plasticity cascade.
ACKNOWLEDGMENTS

We thank F. Hester for excellent technical assistance and D. Sweett for comments on the manuscript.

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GRANTS

This work was supported by National Eye Institute Grants EY-12782 and EY-06877.

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