

Differential Regulation of Metabotropic Glutamate Receptor- and AMPA Receptor-Mediated Dendritic Ca²⁺ Signals by Presynaptic and Postsynaptic Activity in Hippocampal Interneurons

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Calcium plays a crucial role as a ubiquitous second messenger and has a key influence in many forms of synaptic plasticity in neurons. The spatiotemporal properties of dendritic Ca²⁺ signals in hippocampal interneurons are relatively unexplored. Here we use two-photon calcium imaging and whole-cell recordings to study properties of dendritic Ca²⁺ signals mediated by different glutamate receptors and their regulation by synaptic activity in oriens/alveus (O/A) interneurons of rat hippocampus. We demonstrate that O/A interneurons express Ca²⁺-permeable AMPA receptors (CP-AMPA receptors) providing fast Ca²⁺ signals. O/A cells can also coexpress CP-AMPA receptors, Ca²⁺-impermeable AMPARs (CI-AMPA receptors), and group I/II metabotropic glutamate receptors (mGluRs) (including mGluR1a), in the same cell. CI-AMPA receptors are often associated with mGluRs, resulting in longer-lasting Ca²⁺ signals than CP-AMPA receptor-mediated responses. Finally, CP-AMPA receptor- and mGluR-mediated Ca²⁺ signals demonstrate distinct voltage dependence and are differentially regulated by presynaptic and postsynaptic activity: weak synaptic stimulation produces Ca²⁺ signals mediated by CP-AMPA receptors, whereas stronger stimulation, or weak stimulation coupled with postsynaptic depolarization, recruits Ca²⁺ signals mediated by mGluRs. Our results suggest that differential activation of specific glutamate receptor-mediated Ca²⁺ signals within spatially restricted dendritic microdomains may serve distinct signaling functions and endow oriens/alveus interneurons with multiple forms of Ca²⁺-mediated synaptic plasticity. Specific activation of mGluR-mediated Ca²⁺ signals by coincident presynaptic and postsynaptic activity fulfills the conditions for Hebbian pairing and likely underlies their important role in long-term potentiation induction at O/A interneuron synapses.

Key words: Ca²⁺-permeable AMPA receptor; group I mGluR; Ca²⁺ microdomains; synaptic Ca²⁺ signals; two-photon confocal imaging; dendritic microdomains

Introduction

The cellular basis for information processing and storage in the brain is provided by long-lasting, activity-dependent changes in synaptic efficacy, such as long-term potentiation (LTP). Hippocampal CA1 interneurons in stratum oriens/alveus (O/A) demonstrate a Hebbian form of LTP that depends on the activation of the metabotropic glutamate receptor (mGluR) 1a subtype of mGluRs, occurs at synapses containing Ca²⁺-permeable (CP)-

AMPA receptors, and requires postsynaptic Ca²⁺ rises (Perez et al., 2001; Lapointe et al., 2004). This form of LTP does not depend on the activation of NMDA receptors (NMDARs).

AMPA receptors of interneurons, in contrast to those of pyramidal cells, are heteromers often characterized by an absence of GluR2 subunits that renders them Ca²⁺ permeable (Jonas and Burnashev, 1995). It is well established that CP-AMPA receptors participate in synaptic transmission in some inhibitory interneurons (McBain and Dingledine, 1993; Isa et al., 1996). Moreover, they endow interneuron synapses with short- and long-term plasticity (Mahanty and Sah, 1998; Rozov et al., 1998; Laezza et al., 1999; Rozov and Burnashev, 1999; Toth et al., 2000; Lei and McBain, 2002, 2004). In neocortical aspiny interneurons, CP-AMPA receptors mediate a spine-free mechanism of input-specific calcium compartmentalization (Goldberg et al., 2003c); however, in hippocampal O/A interneurons, the spatiotemporal properties of Ca²⁺ signals mediated by CP-AMPA receptors and their contribution to synaptic activity and LTP induction are presently unknown.

Group I mGluRs (specifically mGluR1a) are highly expressed in O/A interneurons (Masu et al., 1991; Baude et al., 1993; Lujan et al., 1996). It has been shown that these receptors can be acti-

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vated synaptically in O/A cells (Huang et al., 2004). Moreover, group I mGluR agonists produce intracellular Ca²⁺ rises in these cells (Carmant et al., 1997; Woodhall et al., 1999; Gee and Lacaille, 2004) that arise from a coupling of Ca²⁺ entry via voltage-gated calcium channels (VGCCs) and intracellular Ca²⁺ release (Woodhall et al., 1999); however, the properties and spatial localization of group I mGluR-mediated Ca²⁺ signals in interneuron dendrites are unknown. Because LTP in O/A interneurons requires postsynaptic Ca²⁺ rises, the relative contribution of different Ca²⁺ signals mediated by CP-AMPA and mGluRs to LTP induction also remains to be determined. To answer these questions we examined the hypotheses that in O/A interneurons, distinct dendritic Ca²⁺ signals produced by CP-AMPA and mGluRs (1) are localized to particular dendritic microdomains, (2) demonstrate specific properties, and (3) are differentially regulated by synaptic activity.

Using two-photon calcium imaging and whole-cell recordings in O/A interneurons, we identified three distinct types of NMDA- and VGCC-independent postsynaptic currents and Ca²⁺ transients that are localized within distinct dendritic microdomains and dependent on the glutamate receptor class involved (ionotropic versus metabotropic). Using different paradigms of synaptic stimulation, we found that both CP-AMPA- and mGluR-mediated Ca²⁺ signals were evoked within given dendritic microdomains, but they were differentially regulated by presynaptic and postsynaptic activity. Furthermore, the properties of mGluR-mediated Ca²⁺ signals were consistent with the Hebbian requirements for LTP induction at interneuron synapses.

Materials and Methods

Slice preparation. Transverse hippocampal slices were obtained from 15- to 23-d-old Sprague Dawley rats (Charles River, St. Laurent, Quebec, Canada). Animals were deeply anesthetized with halothane. After decapitation, the brain was rapidly removed into ice-cold, oxygenated "cutting" solution containing (in mM): 250 sucrose, 2 KCl, 1.25 NaH₂PO₄, 26 NaHCO₃, 7 MgSO₄, 0.5 CaCl₂, and 10 glucose, pH 7.4, 320–340 mOsm. Slices (300 μm thick) were cut with a Vibratome (Leica VT1000S; Leitz, Wetzlar, Germany), transferred to a heated (35°C) oxygenated solution containing (in mM): 124 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 26 NaHCO₃, 3 MgSO₄, 1 CaCl₂, and 10 glucose, and allowed to cool down to room temperature for 30 min. Slices were allowed to recover for at least 1 h before experiments.

Whole-cell recording. During experiments, slices were perfused continuously (2.5 ml/min) with artificial CSF (ACSF) containing (in mM): 124 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 26 NaHCO₃, 2 MgSO₄, 2 CaCl₂, and 10 glucose at 25°C. In some experiments (see Figs. 7, 8), ACSF contained lower Mg²⁺ (1 mM) and higher Ca²⁺ (3 mM) concentrations. CA1 interneurons of stratum oriens/alveus were identified with the aid of an infrared camera (70 Series; Dage-MTI, Michigan City, IN) mounted on an upright microscope (Axioskop 2FS; Carl Zeiss, Kirkland, Quebec, Canada) equipped with a long-range water-immersion objective. Whole-cell voltage-clamp recordings were made from somata using an Axopatch 200B amplifier (Axon Instruments, Foster City, CA). Recording pipettes (4–5 MΩ) were filled with a solution containing (in mM): 130 CsMeSO₃, 5 CsCl, 2 MgCl₂, 5 diNa-phosphocreatine, 10 HEPES, 2 ATP₃Na, 0.4 GTPNa, 0.1 spermine, 2 N-ethyl bromide quaternary salt, and 0.2 Oregon Green-488-BAPTA-1-hexapotassium salt (OGB-1) (K_D = 170 nM) (Molecular Probes, Eugene, OR) or 0.2 Fluo-5F (K_D = 2.3 μM) (Molecular Probes), pH 7.25–7.35, 275–285 mOsm. Cells were voltage clamped at –60 mV unless stated otherwise. Membrane currents were low-pass filtered at 2 kHz, digitized at 10 kHz, and stored on a microcomputer using a data acquisition board (Digidata1322A; Axon Instruments) and pClamp8 software (Axon Instruments).

Ca²⁺ imaging of interneuron dendrites. After obtaining the whole-cell configuration, 20–30 min were allowed for intracellular diffusion of the fluorophore. Imaging was performed using a multiphoton confocal laser

scanning microscope LSM 510 (Carl Zeiss) with a mode-locked Ti:Sapphire laser operated at 780 nm wavelength, 76 MHz pulse repeat, <200 fs pulse width and pumped by a solid-state source (Mira 900 and 5 W Verdi argon ion laser; Coherent, Santa Clara, CA). A long-range water-immersion objective (40×; numerical aperture, 0.8) was used. Fluorescence was detected through a long-pass filter (cutoff, 505 nm), and images were acquired using the LSM 510 software (Carl Zeiss). Fluorescence signals were collected by scanning a line along the dendrite of interest (total length, ~5–20 μm), resulting in a high temporal (3 ms per line) resolution (see Fig. 1). Fluorescence transients were measured at 30–100 μm from the soma. Recordings were not made from axon-bearing segments of dendrites. The image focus of the line was checked carefully and adjusted occasionally for possible drift. Electrophysiological recordings and Ca²⁺ imaging were typically stable for ~1 h.

Pharmacological and synaptic stimulation. Direct activation of dendritic postsynaptic receptors was achieved by local micropressure pulses of glutamate (1 mM; 5–10 psi; 3–30 ms) via a glass pipette (tip diameter of 2–3 μm) connected to a pressure application system (PicoSpritzer II; Parker Instrumentation, Fairfield, NJ) and positioned ~10 μm above the OGB-1-filled dendrite. Local synaptic stimulation of interneurons was performed using an extracellular pipette (2–3 MΩ) filled with ACSF and positioned in the immediate vicinity (<15 μm) of the dendrite of interest. To achieve the precise positioning of puff- and stimulation pipettes, tips of pipettes were marked with the concentrated water-insoluble dye DiI (Molecular Probes). Active dendritic spots were identified by imaging the frames containing the target dendritic region of interest before and during glutamate puff or electrical stimulation (see Fig. 1).

Chemicals. Mechanisms of glutamate-evoked postsynaptic currents and associated Ca²⁺ transients were explored in the presence of tetrodotoxin (TTX) (0.5 μM), bicuculline (Bic) (10 μM), DL-2-amino-5-phosphonvaleric acid (AP-5) (50 μM) or (+)-5-methyl-10,11-dihydro-5H-dibenzo [a,d] cyclohepten-5,10-imine maleate (MK-801) (15 μM), NiCl₂ (50 μM), and CdCl₂ (100 μM) (Sigma, St. Louis, MO). All compounds were prepared in advance as stock solutions, frozen at –20°C, diluted on the day of experiment, and bath applied. In some experiments the AMPA/kainate receptor (KAR) antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (20 μM), the Ca²⁺-permeable AMPAR antagonist philanthotoxin-433 Tris-trifluoroacetate (PhTx) (10 μM) (RBI, Natick, MA), the group I/II mGluR antagonist (RS)-α-ethyl-4-carboxyphenylglycine (E4CPG) (500 μM) (Tocris, Ellisville, MO), or the mGluR1a antagonist (S)(+)-α-amino-4-carboxy-2-methylbenzeneacetic acid (LY367385) (100 μM) (Tocris) was added to the extracellular solution.

Data analysis. Calcium measurements and electrophysiological recordings were analyzed using the LSM 510, Clampfit 9.0 software (Axon Instruments), and IgorPro (Wavemetrics, Lake Oswego, OR). For analysis of calcium transients, the fluorescence background was subtracted from the fluorescence intensity averaged over the line. Changes in fluorescence were calculated relative to the baseline (from 1 s before stimulation) and expressed as %ΔF/F = [(F – F_{rest})/F_{rest}] × 100. For comparison of voltage dependence of postsynaptic currents and Ca²⁺ transients from different cells, data derived from a single neuron held at a different membrane potential (–80 to +60 mV) were normalized to values at –60 mV. The rectification index (RI) of the inwardly rectifying I–V relationship was defined as the ratio of the recorded current amplitude at +40 mV to the predicted linear value at +40 mV (extrapolated from linear fit of the currents at the negative potentials) (Liu and Cull-Candy, 2000; Lei and McBain, 2002, 2004). The time-to-peak of the calcium signal was calculated as the time from the stimulation to the peak amplitude. Decay kinetics of membrane currents and calcium transients were fitted using single or double exponential fitting algorithms of IgorPro. Summary data are expressed as mean ± SE. Statistical significance between groups was determined using a two-tailed Student's *t* test or ANOVA followed by appropriate *post hoc* tests (Student–Newman–Keuls, Dunnett, or Tukey–Kramer).

Histology. Biocytin (0.15–0.2%) (Sigma) was routinely added to the internal patch solution to allow cell labeling and reconstruction. Visualization of biocytin was performed as described previously (Perez et al., 2001). Three-dimensional light microscopic reconstructions were performed using an Eclipse E600W microscope (Nikon, Tokyo, Japan)

equipped with a digital camera (Retiga 1300; QImaging, Burnaby, British Columbia, Canada). Final stacks of cell images (see Fig. 1B) were merged in two-dimension in Adobe Photoshop 6.0 (Adobe Systems, San Jose, CA).

Results

Glutamate-evoked local dendritic Ca^{2+} transients in O/A interneurons

To investigate the relative contribution of ionotropic and metabotropic glutamate receptors to dendritic Ca^{2+} rises in O/A interneurons, we first examined postsynaptic currents and Ca^{2+} transients evoked by glutamate micropressure application to interneuron dendrites (Fig. 1). Interneurons were recorded in whole-cell voltage-clamp mode and filled with the fluorescent Ca^{2+} indicator OGB-1 (Fig. 1A). Biocytin cell labeling confirmed that all cells recorded were CA1 interneurons with their soma located in stratum oriens near the alveus and horizontally oriented sparsely spiny dendrites ($n = 52$). In 17 of these cells, the axonal arborization was stained and projected to stratum lacunosum-moleculare (Fig. 1B). Such cells are known to be somatostatin-positive interneurons (Freund and Buzsaki, 1996) that express high levels of mGluR1a extrasynaptically and perisynaptically (Masu et al., 1991; Baude et al., 1993). After an initial dye-loading period of 20–30 min, interneuron dendrites were identified with confocal imaging, and a puff-pipette was positioned $\sim 10 \mu\text{m}$ above the dendrite of interest (Fig. 1A). Micropressure pulses of glutamate (1 mM) evoked a local increase in fluorescence in the dendrite of interest (Fig. 1C1,C2). To measure Ca^{2+} transients in the region of interest, a line was positioned along the dendrite (Fig. 1C3), and Ca^{2+} signals were imaged in the line-scanning mode while glutamate-evoked membrane currents were recorded in the soma (Fig. 1D).

Ca^{2+} signals evoked by such discrete applications of glutamate were highly localized to individual dendritic compartments (Fig. 1C2). To quantitatively examine the spatial spread of Ca^{2+} signals, we plotted the amplitude of the Ca^{2+} signal ($\% \Delta F/F$) as a function of distance along the dendrite, at distinct times after glutamate application (Fig. 1E). These traces were well fitted by Gaussian curves (Fig. 1E2), and the spatial extent of a Ca^{2+} signal at a given time was determined from the SD of the Gaussian curve (Goldberg et al., 2003c). At the peak amplitude of Ca^{2+} transients, which on average occurred at ~ 100 ms after glutamate application, Ca^{2+} signals were highly localized ($\sigma_{\text{peak}} = 2.53 \pm 0.36 \mu\text{m}$). The maximal resolvable spatial extent of the Ca^{2+} signal (σ_{max}),

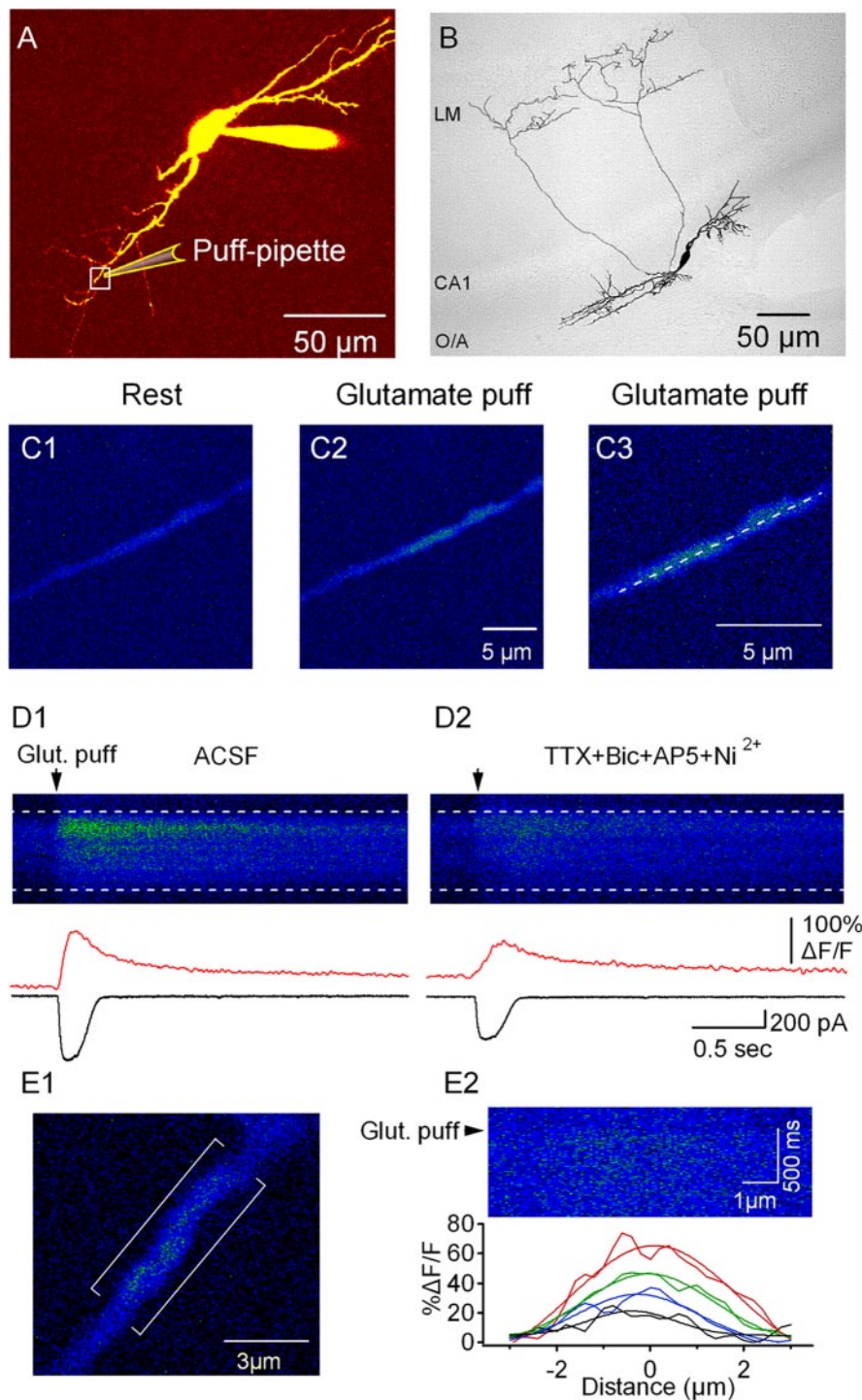


Figure 1. Micropressure application of glutamate to interneuron dendrites produces local Ca^{2+} signals via multiple mechanisms. *A*, Multiphoton image (z-stack) of 200 optical sections taken at $0.5 \mu\text{m}$ intervals of an Oregon Green-filled interneuron; dendritic region of interest (ROI) near a puff-pipette is indicated by the box. *B*, Reconstruction of the same biocytin-filled interneuron. *C*, Pseudocolor images of an enlarged ROI with basal dendritic fluorescence (*C1*) and after glutamate puff (*C2*). *C3*, Dashed line indicates position for the line scan shown in *D*. *D*, Line scan images of the responses to glutamate puff (Glut. puff; *C2*). (arrowhead) with dashed lines bordering the region of interest for fluorescence measurements. Traces below are glutamate-evoked dendritic Ca^{2+} transients (red) and postsynaptic currents recorded from soma (black) (average of 4 sweeps). *D2*, Residual responses are the Ca^{2+} transient and membrane current independent of NMDARs and VGCCs [elicited in the presence of TTX ($0.5 \mu\text{M}$), Bic ($10 \mu\text{M}$), AP-5 ($50 \mu\text{M}$), and Ni^{2+} ($50 \mu\text{M}$)]. *E*, Spatiotemporal pattern of glutamate-evoked Ca^{2+} signals from a different cell; a dendritic ROI (*E1*), through which a line scan was placed, is indicated by the brackets. The amplitude of the calcium signal ($\Delta F/F$) was measured from the corresponding line scan image (*E2*, top) and plotted as a function of distance along the dendrite for different times after glutamate application: 100 ms (red), 600 ms (green), 1100 ms (blue), and 1600 ms (black) (*E2*, bottom). These plots were well fitted by Gaussians. The x-axis of the plot (*E2*, bottom) corresponds to the length of the line scan image (*E2*, top). Note that in *E2* (top) only the initial 1000 ms of the line scan is illustrated.

which occurred at ~1600 ms after glutamate application, was $3.94 \pm 1.32 \mu\text{M}$ ($n = 10$). The spatiotemporal spread of Ca²⁺ signals in our experiments was larger than those associated with spontaneous CP-AMPA-mediated EPSPs ($<1 \mu\text{m}$) (Goldberg et al., 2003c), suggesting the activation of both synaptic and extrasynaptic receptors by glutamate application.

Ca²⁺ transients, induced in ACSF at -60 mV (Fig. 1D1), consisted of NMDAR- and VGCC-mediated components, because they were significantly reduced in the presence of $50 \mu\text{M}$ AP-5 and $50 \mu\text{M}$ Ni²⁺ (Fig. 1D2) (control, $108 \pm 11\% \Delta F/F$; AP-5 + Ni²⁺, $50.3 \pm 6.9\% \Delta F/F$; $n = 25$; $p < 0.001$; paired *t* test). A significant component of Ca²⁺ signal was dependent on NMDARs ($43 \pm 14\%$ of total Ca²⁺ response; range, 25–75%; $n = 10$), whereas a smaller contribution was attributable to Ni²⁺-sensitive VGCCs ($10 \pm 2\%$ of total Ca²⁺ response; range, 6–15%; $n = 5$). Importantly, we observed a significant component of Ca²⁺ signals independent of NMDAR/VGCC ($47 \pm 7\%$ of total Ca²⁺ response; $n = 25$) (Fig. 1D2). To examine the mechanisms of these NMDAR- and VGCC-independent Ca²⁺ signals, all subsequent experiments were conducted in the presence of $50 \mu\text{M}$ AP-5 (or MK-801), $50 \mu\text{M}$ Ni²⁺, and $100 \mu\text{M}$ Cd²⁺. The combination of Ni²⁺ and Cd²⁺ completely blocked Ca²⁺ transients elicited by membrane depolarization (-20 mV , 50 ms; $n = 3$ cells; data not shown).

Dendritic Ca²⁺ signals arising from CP-AMPA activation

Activation of two types of glutamate receptors, apart from NMDARs, has been linked to Ca²⁺ transients in interneurons: CP-AMPA receptors (Isa et al., 1996) and group I mGluRs (Woodhall et al., 1999; Gee and Lacaille, 2004). It is well established that endogenous polyamines block CP-AMPA receptors and endow them with the property of inward rectification (Bowie and Mayer, 1995; Donevan and Rogawski, 1995; Kamboj et al., 1995; Koh et al., 1995; Washburn and Dingledine, 1996). To discriminate between responses mediated by CP-AMPA and mGluRs, we examined the *I*-*V* relationship of glutamate-evoked membrane currents (in the presence of TTX, Bic, AP-5, Ni²⁺, and Cd²⁺). We observed that membrane currents and their associated Ca²⁺ responses could be separated in three types based on the rectification index. The first type of response elicited by local glutamate application consisted of inwardly rectifying currents ($RI < 0.3$) with a reversal potential of 0 mV (Fig. 2A) ($n = 5$). Dendritic Ca²⁺ transients associated with this type of current declined steeply in amplitude with membrane depolarization (Fig. 2B). Comparison of peak Ca²⁺ transients at -60 and -20 mV showed that Ca²⁺ signals were significantly diminished at depolarized membrane potential (to $52 \pm 5.1\% \Delta F/F_{\text{norm}}$; $p < 0.05$; $n = 5$).

To verify that CP-AMPA receptors mediate those responses, we used the high-affinity and use-dependent antagonist of CP-AMPA receptors, PhTx ($10 \mu\text{M}$) (Toth and McBain, 1998). After an initial period of recording of control responses, the slice was incubated in a solution containing PhTx ($10 \mu\text{M}$) for 10 min. After this period, stimulation by glutamate was resumed, and current amplitude as well as peak Ca²⁺ transient were blocked in a use-dependent manner during the next 20 min (Fig. 2C,D). Thus, dendritic Ca²⁺ signals associated with inwardly rectifying glutamate currents are mediated by CP-AMPA receptors in O/A interneurons.

Dendritic Ca²⁺ signals mediated by mGluRs and CP-AMPA receptors

The second type of glutamate-evoked membrane currents consisted of responses with intermediate inward rectification ($0.3 <$

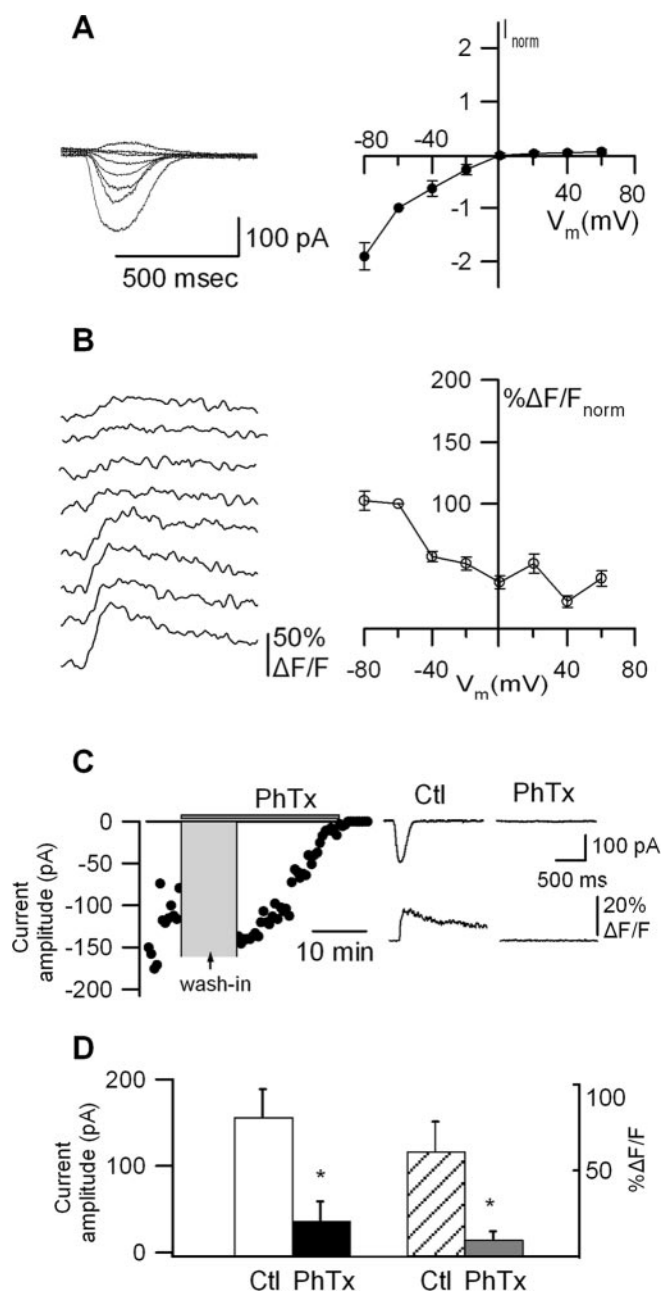


Figure 2. CP-AMPA receptors mediate dendritic Ca²⁺ transients associated with inwardly rectifying glutamate currents. *A*, Representative membrane currents (average of 4 sweeps) independent of NMDARs and VGCCs (in TTX, Bic, AP-5, Ni²⁺, and Cd²⁺) evoked by glutamate at different membrane potentials (V_m , -80 to $+60 \text{ mV}$; left) and average *I*-*V* relationship for all cells ($n = 5$) with inward rectification (right). Before averaging, data from individual cells were normalized to the current amplitude at -60 mV . *B*, Associated Ca²⁺ transients (average of 4 sweeps) at different V_m (left) and plots of average peak Ca²⁺ transients for all cells as a function of V_m , showing steeply declining voltage dependence of Ca²⁺ transients (right). Data at different voltages were also normalized to Ca²⁺ transients at -60 mV . *C*, An example of use-dependent block of inwardly rectifying currents (left graph) by PhTx in one cell. Representative traces of membrane currents and associated Ca²⁺ transients before (Ctl) and after PhTx block are shown at right. *D*, Bar graph of the average amplitude of current and Ca²⁺ transient for all cells in the same conditions, showing that CP-AMPA receptors mediated this type of current and Ca²⁺ transients ($n = 5$; $*p < 0.05$).

$RI < 0.7$) (Fig. 3A). The mean reversal potential of these currents was $10.1 \pm 3.8 \text{ mV}$ ($n = 6$). These responses appeared to be composed of fast and slow components, but because both components overlapped to a large extent, it was difficult to separate

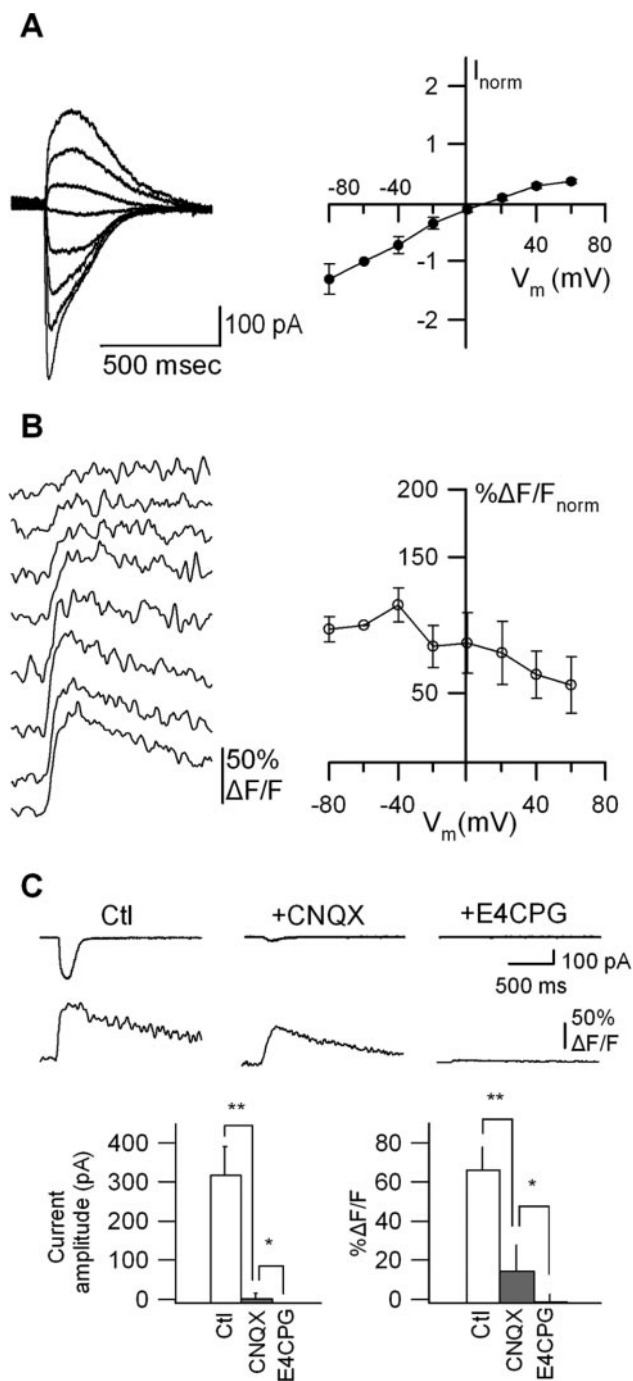


Figure 3. Dendritic Ca²⁺ signals produced by joint activation of AMPARs/KARs and group I/II mGluRs. *A*, Representative glutamate-evoked membrane currents (average of 4 sweeps) at different V_m (left) and average I - V relationship for all cells ($n = 6$) showing intermediate inward rectification (right). *B*, Associated Ca²⁺ transients (average of 4 sweeps) at different V_m (left) and plots of average peak Ca²⁺ transients as a function of V_m , indicating slightly declining voltage dependence of Ca²⁺ transients (right). *C*, Representative currents and Ca²⁺ transients (top) evoked by glutamate puffs in control (Ctl) and in the presence of the AMPA/KAR antagonist (CNQX) and the group I/II mGluR antagonist (E4CPG), and bar graphs (bottom) of the average amplitude of current (left) and Ca²⁺ transient (right) in the same conditions. Currents and Ca²⁺ transients were sensitive to CNQX and E4CPG, indicating that a combination of AMPA/KARs and group I/II mGluRs participate in these responses ($n = 6$; * $p < 0.05$; ** $p < 0.01$).

them (Fig. 3*A*, left). Dendritic Ca²⁺ transients associated with these currents showed a slightly declining voltage relationship (Fig. 3*B*), such that they did not show significant changes with membrane depolarization from -60 to -20 mV (decrease to

$84.6 \pm 15.6\% \Delta F/F_{\text{norm}}$; $p > 0.05$; $n = 6$). To test the idea that this type of response resulted from activation of mixed non-NMDARs and mGluRs, we examined their sensitivity to the AMPAR/KAR antagonist CNQX ($20 \mu\text{M}$) and the group I/II mGluR antagonist E4CPG ($500 \mu\text{M}$) (Fig. 3*C*). Bath application of CNQX significantly reduced the amplitude of membrane currents and Ca²⁺ transients, indicating that AMPARs/KARs partly mediated these responses. The residual membrane currents and Ca²⁺ transients observed in CNQX were fully blocked by addition of E4CPG ($p < 0.05$; $n = 5$) (Fig. 3*C*), showing that group I/II mGluRs also partly mediated these responses. Thus, a combination of AMPARs/KARs and group I/II mGluRs were involved in the Ca²⁺ transients associated with membrane currents showing intermediate inward rectification. The more pronounced block of these responses by CNQX indicates a larger contribution of AMPARs/KARs over mGluRs in the intermediate type of membrane currents and their associated Ca²⁺ signals.

Local dendritic Ca²⁺ signals via group I mGluRs

The third type of glutamate-evoked membrane currents consisted of linear and outwardly rectifying membrane currents ($RI > 0.7$). The mean reversal potential of these currents was 11.3 ± 3.5 mV (Fig. 4*A*) ($n = 14$). Occasionally, this type of current also appeared to consist of fast and slow components, but they were usually overlapping and undistinguishable. Ca²⁺ transients associated with this type of current demonstrated a bell-shaped relation with voltage reaching maximal values near -20 mV (Fig. 4*B*). Comparison of peak Ca²⁺ transients at -60 and -20 mV showed that Ca²⁺ transients significantly increase with membrane depolarization (to $156.2 \pm 22.5\% \Delta F/F_{\text{norm}}$; $p < 0.05$; $n = 14$). The Ca²⁺ transients associated with these membrane currents were recorded in the presence of Ni²⁺ and Cd²⁺ and thus did not likely implicate VGCCs. To test whether this type of current and Ca²⁺ transients were mediated by AMPARs/KARs and group I/II mGluRs, we examined their pharmacological sensitivity to CNQX and E4CPG (Fig. 4*C*). Bath application of CNQX inhibited membrane currents, indicating that they were mediated partly by AMPARs/KARs ($p < 0.05$; $n = 5$) (Fig. 4*C*); however, addition of CNQX did not significantly affect Ca²⁺ transients ($p = 0.275$; $n = 5$) (Fig. 4*C*). The residual current uncovered in the presence of CNQX demonstrated outward rectification and significantly slower rise time (control, 25.8 ± 2.4 ms; CNQX, 138.8 ± 28.8 ms; $p < 0.05$; $n = 5$) and decay (control, 189.5 ± 33.1 ms; CNQX, 749.0 ± 165.3 ms; $p < 0.05$; $n = 5$). The Ca²⁺ transient associated with this residual current significantly increased with membrane depolarization (to $143 \pm 17.9\% \Delta F/F_{\text{norm}}$; $p < 0.05$; $n = 5$). The residual currents and Ca²⁺ transients observed in CNQX were fully blocked by E4CPG, showing that these responses were mediated by the group I/II mGluRs ($p < 0.05$; $n = 5$) (Fig. 4*C*). Because O/A interneurons express high levels of the mGluR1a subtype of group I mGluRs (Masu et al., 1991; Baude et al., 1993), we also examined the effect of the selective mGluR1a antagonist LY367385. In the presence of LY367385, Ca²⁺ transients were reduced significantly (control, $37.5 \pm 10.3\% \Delta F/F$; LY367385, $12.5 \pm 5.8\% \Delta F/F$; $p < 0.05$, $n = 4$), whereas membrane currents were not affected (control, -35.3 ± 8.5 pA; LY367385, -39.8 ± 19.9 pA; $p = 0.749$; $n = 4$). These results indicate that AMPARs/KARs and group I/II mGluRs are differentially involved in outwardly rectifying currents and associated Ca²⁺ transients. Ca²⁺-impermeable AMPARs/KARs and group I/II mGluRs contribute to membrane currents, whereas only group I/II mGluRs (including mGluR1a) participate in their associated Ca²⁺ transients. Moreover, these

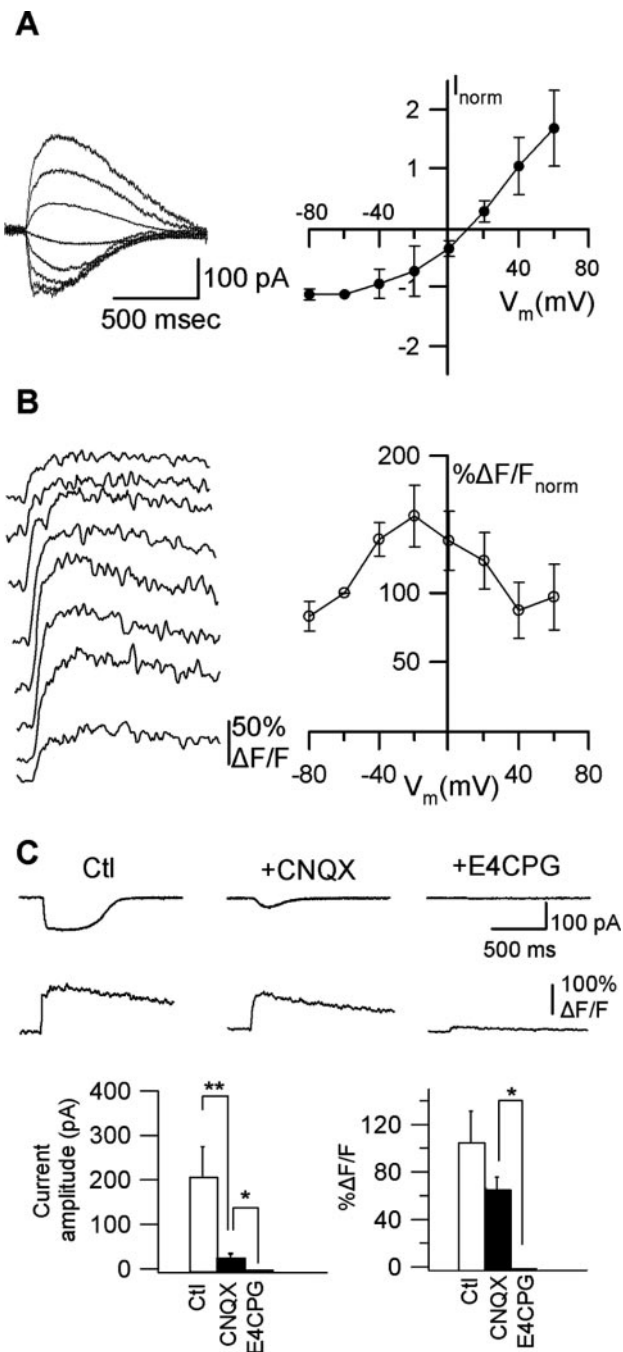


Figure 4. Dendritic Ca²⁺ signals mediated by group I/II mGluRs. *A*, Representative postsynaptic currents (average of 4 sweeps) at different V_m (left) and average I - V relationship for all cells ($n = 14$) with outward rectification (right). *B*, Associated Ca²⁺ transients (average of 4 sweeps) at different V_m (left) and plots of average peak Ca²⁺ transients for all cells as a function of V_m , showing bell-shaped voltage dependence of Ca²⁺ transients (right). *C*, Representative currents and Ca²⁺ transients evoked by glutamate puffs (top) in control (Ctl) and in the presence of the AMPA/KAR antagonist (CNQX) and the group I/II mGluR antagonist (E4CPG), and bar graphs (bottom) of the average amplitude of current (left) and Ca²⁺ transient (right) in the same conditions. Currents were sensitive to CNQX and E4CPG, whereas Ca²⁺ transients were significantly reduced only by E4CPG, showing that AMPA/KARs and group I/II mGluRs contribute to membrane currents, whereas only mGluRs mediate Ca²⁺ transients ($n = 5$; * $p < 0.05$; ** $p < 0.01$).

results uncover a characteristic voltage relationship of mGluR-mediated membrane currents and Ca²⁺ transients resulting in potentiation of these responses by membrane depolarization in the absence of Ca²⁺ influx through VGCCs.

The time course of dendritic Ca²⁺ signal depends on the glutamate receptor type involved

Having found that glutamate-evoked dendritic Ca²⁺ signals in O/A interneurons occur through multiple mechanisms, we next examined the time course of membrane currents and Ca²⁺ signals associated with each response type (Fig. 5). Inwardly rectifying membrane currents and associated Ca²⁺ signals (type I) demonstrated the fastest kinetics (current rise time, 19.1 ± 1.8 ms; current decay time, 85.2 ± 16.9 ms; Ca²⁺ transient time-to-peak, 62.6 ± 16.3 ms; Ca²⁺ transient decay time, 503.2 ± 75.5 ms; $n = 5$). In contrast, outwardly rectifying membrane currents and their Ca²⁺ transients (type III) had a significantly slower time course (current rise time, 44.5 ± 9.7 ms; current decay time, 197.5 ± 23.1 ms; Ca²⁺ transient time-to-peak, 191.6 ± 14.1 ms; Ca²⁺ transient decay, 1763.8 ± 386.9 ms; $n = 14$) than responses with inward rectification (type I) ($p < 0.05$; ANOVA). Responses with intermediate inward rectification (type II) also exhibited a fast rise time (current rise time, 14.1 ± 2.6 ms; Ca²⁺ transient time-to-peak, 66.3 ± 19.8 ms; $n = 6$), but their decay was intermediate (current decay time, 117.9 ± 34.9 ms; Ca²⁺ transient decay, 1323.8 ± 391.5 ms; $n = 6$) and not significantly different from that of type I and III responses. Because OGB-1, used in these experiments, is a relatively high-affinity Ca²⁺ indicator and could affect the kinetics of Ca²⁺ transients, we examined Ca²⁺ signals using a lower affinity Ca²⁺ indicator, Fluo-5F (Fig. 5C). Pharmacologically isolated (in Bic + AP-5 + E4CPG) CP-AMPA-mediated Ca²⁺ transients were significantly faster (time-to-peak, 52.6 ± 11.7 ms; decay, 443.3 ± 123.4 ms; $n = 5$) than those mediated by mGluRs (in Bic + AP-5 + CNQX) (time-to-peak, 220.6 ± 25.5 ms; decay, 1281.6 ± 93.4 ms; $n = 5$). Thus, membrane currents and Ca²⁺ signals with faster kinetics were associated with CP-AMPA, whereas those with slower kinetics were linked with mGluRs.

Distinct Ca²⁺ signals at different dendritic sites of single interneurons

To examine in more detail the spatial segregation of different Ca²⁺ signals, we measured Ca²⁺ transients at different dendritic sites of the same interneuron. First, distinct Ca²⁺ signals could arise at different dendritic sites separated by short distances ($< 5 \mu\text{m}$) in a given cell ($n = 3$ cells). As shown in Figure 6A, applying glutamate to a given dendritic region elicited different responses at two adjacent dendritic sites (Fig. 6A, sites 1 and 2). The Ca²⁺ signal measured within the first microdomain was unaffected by NMDAR, VGCC, and AMPAR/KAR antagonists and likely mediated by mGluRs (Fig. 6A, red trace). In contrast, the Ca²⁺ transient measured at the second site was decreased by AP-5 and Ni²⁺ and blocked by subsequent addition of CNQX (Fig. 6A, blue trace), suggesting that NMDAR, VGCC, and AMPAR/KAR activation contributed to this response. Thus, distinct Ca²⁺ microdomains involving ionotropic and metabotropic glutamate receptors can be closely localized but spatially segregated in interneuron dendrites.

To examine whether distinct Ca²⁺ signals mediated by different glutamate receptors can arise at distant dendritic sites in a given interneuron, we evoked membrane currents and Ca²⁺ transients with glutamate applications to two different dendritic branches of the same cell ($n = 5$ cells) (Fig. 6B). Ca²⁺ signals were measured in branches of the same diameter and at a similar distance from the soma (40 – $60 \mu\text{m}$). Membrane currents evoked by glutamate at different dendritic sites of the same cell displayed different rectification: (1) inward and intermediate rectification (type I/type II; $n = 2$ cells), (2) inward and linear/

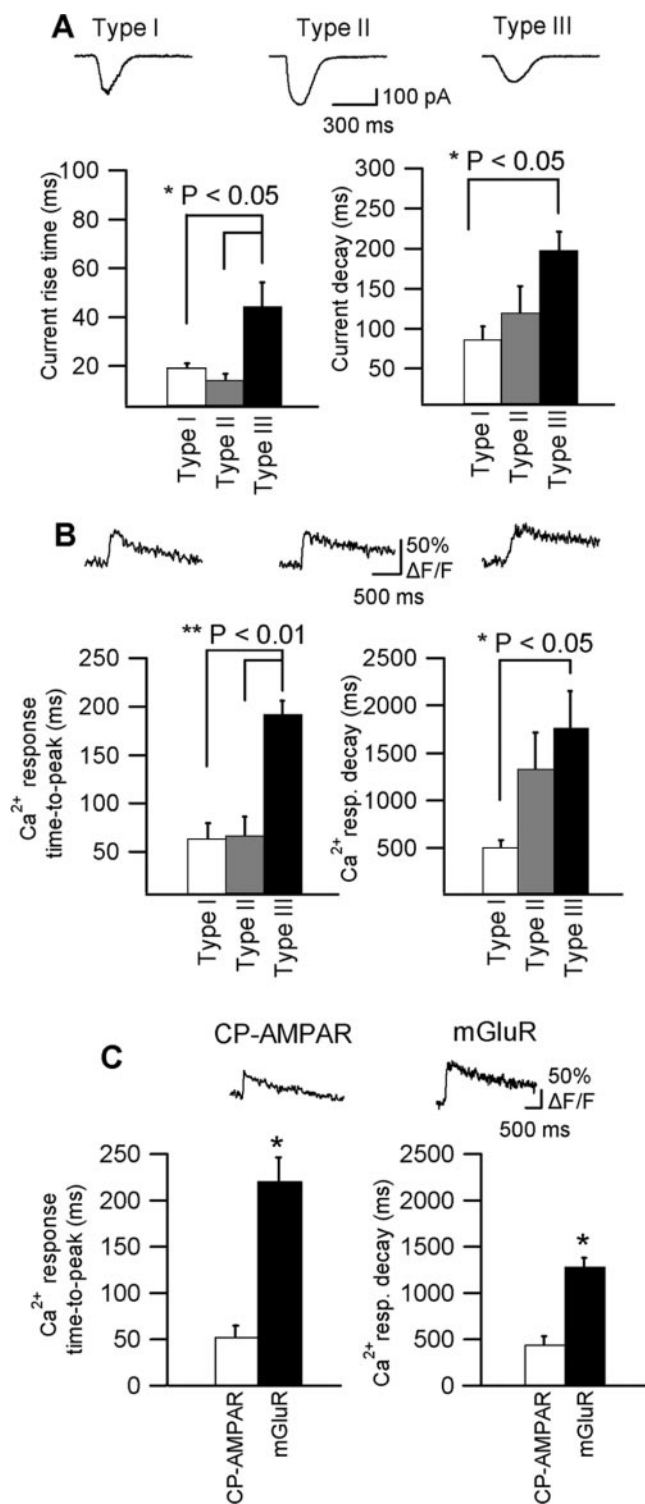


Figure 5. Response-specific time course. *A*, Representative examples of three types of glutamate-evoked currents ($V_h = -60$ mV): type I (inwardly rectifying), type II (intermediate), and type III (outwardly rectifying), and bar graphs of the mean rise (left) and decay times (right) of currents for the three response types for all cells. *B*, Ca²⁺ transients associated with the three types of membrane currents shown in *A* and bar graphs of the mean time-to-peak (left) and decay time (right) of Ca²⁺ transients for all cells. Membrane currents and Ca²⁺ transients of type I and II responses displayed significantly faster rise time than those of type III response. Type III response showed significantly slower decay than type I response (** $p < 0.01$; * $p < 0.05$; ANOVA). *C*, Representative examples of CP-AMPA-mediated (in AP-5 + E4CPG) and mGluR-mediated (in AP-5 + CNQX) Ca²⁺ transients measured with the low-affinity Ca²⁺ indicator Fluo-5F; bar graphs indicate the mean time-to-peak (left) and decay time (right) of Ca²⁺ transients for all cells (CP-AMPA, $n = 5$; mGluR, $n = 5$).

outward rectification (type I/type III; $n = 2$ cells) (Fig. 6*B*), or (3) only inward rectification at both sites (type I/type I; $n = 1$ cell). The distinct membrane currents were also associated with different Ca²⁺ signals. As illustrated in the representative cell in Figure 6, membrane currents evoked by glutamate at the first dendritic site showed inward rectification ($RI = 0.06$) (Fig. 6*B*). This response was associated with large Ca²⁺ signal at -60 mV (61% $\Delta F/F$) and no detectable transient at $+40$ mV. These response properties are consistent with the mediation of membrane currents and Ca²⁺ signals by CP-AMPA receptors. Currents evoked by glutamate at the second dendritic site showed an outward $I-V$ relation ($RI = 1.6$) (Fig. 6*B*). They were associated with Ca²⁺ signals of smaller amplitude at -60 mV (31% $\Delta F/F$) than at $+40$ mV (44% $\Delta F/F$). These response properties are consistent with a contribution of Ca²⁺-impermeable (CI)-AMPA/KARs and mGluRs to these membrane currents and Ca²⁺ signals. The elicitation of differently rectifying membrane currents and heterogeneous Ca²⁺ transients by glutamate application at different dendritic sites of single interneurons suggests a differential distribution of specific combination of ionotropic and metabotropic glutamate receptors at specific dendritic sites. This differential distribution of glutamate receptors suggests a dendritic site-specific Ca²⁺ signaling function in O/A interneurons.

Synaptically activated Ca²⁺ signals mediated by CP-AMPA receptors and mGluRs

To investigate the mechanisms of synaptically activated dendritic Ca²⁺ signals, we examined Ca²⁺ transients associated with EPSCs evoked by low-intensity stimulation ($\sim 30 \mu A$; $50 \mu s$; five stimuli at 100 Hz; $V_h = -60$ mV). Such stimulation elicited local Ca²⁺ responses associated with compound EPSCs of large amplitude, suggesting that Ca²⁺ transients and synaptic currents were generated at overlapping dendritic sites.

Comparison of Ca²⁺ transients in the presence and absence of AP-5 indicated that synaptic Ca²⁺ transients included an NMDAR-dependent component ($43.3 \pm 11.5\% \Delta F/F$; $n = 6$). NMDAR-independent Ca²⁺ signals (in AP-5) (Fig. 7*A1*) were observed in 8 of 14 cells, and they were of small amplitude ($33.1 \pm 8.1\% \Delta F/F$), had relatively fast kinetics (time-to-peak, 70.4 ± 11.8 ms; decay time, 1418 ± 437 ms), and were fully blocked by PhTx ($n = 8$) (Fig. 7*A2*). PhTx significantly reduced EPSCs but did not block them completely, suggesting that CP-AMPA receptors and CI-AMPA receptors were involved in the generation of EPSCs; however, because synaptic activation of CI-AMPA receptors did not produce any detectable Ca²⁺ signals, low-intensity synaptic stimulation activated dendritic Ca²⁺ signals solely mediated by NMDARs and CP-AMPA receptors.

We next investigated in the same cells whether an increase in intensity and duration of synaptic stimulation could recruit group I/II mGluR-mediated EPSCs. In the absence of ionotropic glutamatergic transmission (in CNQX and AP-5), a gradual increase in stimulus strength (up to 150–200 μA) evoked EPSCs and Ca²⁺ transients that were sensitive to E4CPG in two of eight cells (data not shown). Thus, release of glutamate by a short burst of stimulation can occasionally be sufficient to activate mGluR-mediated postsynaptic currents and Ca²⁺ transients. Because increased presynaptic activity was required to activate group I mGluRs and elicit postsynaptic responses in CA1 pyramidal cells (Congar et al., 1997), we examined the effect of longer trains of stimuli. Tetanic stimulation (150 μA ; 0.3–1 s at 100 Hz; $V_h = -60$ mV) evoked slower EPSCs and Ca²⁺ transients (time-to-peak, 770.2 ± 51.7 ms; decay time, 9337 ± 1637 ms) (Fig. 7*A3*) that were blocked by E4CPG ($n = 8$) (Fig. 7*A4*). The amplitude of

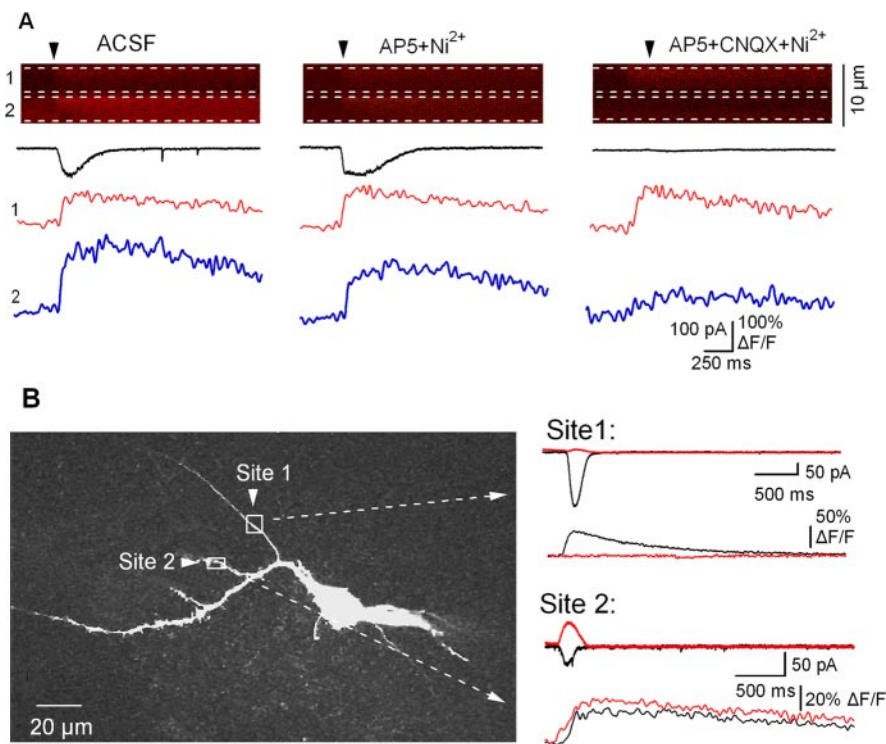


Figure 6. Distinct Ca²⁺ signals in different dendritic sites of single interneurons. *A*, Representative line scan images of responses to glutamate applications (arrowhead). Dashed lines indicate the regions of interest for fluorescence measurements. Traces below illustrate glutamate-evoked currents at -60 mV (black) and associated dendritic Ca²⁺ transients measured within microdomains 1 (red) and 2 (blue). Distinct Ca²⁺ signals were produced in the two adjacent microdomains. At site 1 (red trace) the Ca²⁺ response was independent of NMDARs, VGCCs, and AMPA/KARs and was likely mediated by mGluRs, whereas at site 2 (blue trace) the Ca²⁺ response consisted of components mediated by NMDARs/VGCCs and AMPARs/KARs. *B*, Z-stack of an Oregon Green-filled interneuron (left) showing the two different locations of the puff-pipette and two dendritic regions of interest (1 and 2) indicated by the boxes. The corresponding membrane currents and Ca²⁺ transients evoked in each dendritic region at two V_m [$V_h = -60$ mV (black) and +40 mV (red)] are illustrated at right. The membrane currents at site 1 showed inward rectification, whereas those at site 2 showed an outward $I-V$ relationship. The currents and Ca²⁺ transients are an average of four sweeps.

the synaptically activated mGluR-mediated Ca²⁺ signals was significantly larger than that mediated by CP-AMPA (mGluRs, $62.2 \pm 8.4\% \Delta F/F$; $n = 8$; $p < 0.05$). Thus, synaptically activated Ca²⁺ signals that are independent of NMDARs were mediated in part by CP-AMPA and group I/II mGluRs. Moreover, these different components were present in the same dendritic microdomains but were elicited by different stimulation paradigms. These results indicate that dendritic Ca²⁺ signaling in O/A interneurons is differentially regulated by a presynaptic activity pattern: short duration presynaptic activity involves only CP-AMPA, whereas longer-duration repetitive synaptic activity will lead to the additional recruitment of mGluRs. Moreover, the increase in presynaptic activity will change Ca²⁺ transients from small, fast signals to large, slow responses within given dendritic microdomains.

mGluR-mediated Ca²⁺ signals induced by pairing synaptic stimulation with postsynaptic depolarization

Because a stimulation protocol pairing repetitive stimulation with postsynaptic depolarization induces a mGluR1a- and Ca²⁺-dependent LTP in O/A interneurons (Perez et al., 2001; Lapointe et al., 2004), we examined whether CP-AMPA- and mGluR-mediated Ca²⁺ signals were differentially activated by pairing synaptic theta-burst stimulation (TBS) [five bursts at 200 ms intervals (5 Hz), each burst consisting of four pulses at 100 Hz] with depolarization (five 60-ms-long depolarizing steps to -20

mV). We measured Ca²⁺ signals activated by the pairing protocol in two recording conditions, using Ca²⁺ indicators with low-affinity Fluo-5F (Fig. 8*A–D,E1*) and high-affinity OGB-1 (Fig. 8*E2*). As expected from using a low-affinity indicator (Helmchen, 1999), we observed larger peak calcium signals that were not saturated during the pairing protocol with Fluo-5F (Fig. 8*A–D*). In the presence of Bic and AP-5 and at a holding potential of -60 mV, TBS alone produced Ca²⁺ transients that were fully blocked by CNQX and thus likely mediated by CP-AMPA (Fig. 8*A1,A2*). Postsynaptic depolarization alone produced Ca²⁺ spikes and Ca²⁺ transients that were not sensitive to CNQX but slightly reduced by the group I/II mGluR antagonist E4CPG (Fig. 8*B*). The pairing of TBS with depolarization evoked Ca²⁺ transients that summated sublinearly (Fig. 8*D*) and were not reduced in CNQX (Fig. 8*C1,C2*), demonstrating that CP-AMPA does not provide significant Ca²⁺ influx during the paired stimulation protocol; however, E4CPG significantly decreased Ca²⁺ signals induced by TBS paired with membrane depolarization (Fig. 8*C3*). Moreover, in AP-5 and CNQX, Ca²⁺ transients evoked by TBS paired with depolarization were significantly larger using both Ca²⁺ indicators [Fluo-5F area under curve, $634.9 \pm 45.1\% \Delta F/F \cdot s$ (Fig. 8*D2*); OGB-1, $486.9 \pm 43.8\% \Delta F/F \cdot s$] than the Ca²⁺ rises produced by postsynaptic depolarization alone [Fluo-5F area under curve, $358.6 \pm 37.1\% \Delta F/F \cdot s$ (Fig. 8*D2*); OGB-1, $281.3 \pm 58.2\% \Delta F/F \cdot s$; $n = 8$; $p < 0.05$; ANOVA]. Furthermore, with both indicators, the difference between Ca²⁺ signals induced by TBS paired with postsynaptic depolarization and those produced by the depolarization alone (in AP-5 and CNQX) was fully blocked by E4CPG ($n = 8$; $p < 0.05$; paired t test) (Fig. 8*E*). Because TBS alone did not produce any Ca²⁺ signals in CNQX, an additional component of Ca²⁺ transients was produced by TBS paired with postsynaptic depolarization and mediated by mGluRs. Thus, Ca²⁺ transients appearing to summate sublinearly during the pairing protocol (Fig. 8*D*) were not a result of summation of Ca²⁺ transients evoked by TBS alone and depolarization alone but were caused by another additional mGluR-mediated component recruited by the pairing paradigm. These results indicate that TBS paired with postsynaptic depolarization is an effective protocol to elicit dendritic Ca²⁺ signals mediated by mGluRs in O/A interneurons and that group I/II mGluR-mediated Ca²⁺ mechanisms may effectively signal coincident presynaptic and postsynaptic activity.

Discussion

Our experiments led to four main findings regarding dendritic Ca²⁺ signaling via glutamate receptors in hippocampal O/A interneurons. First, these cells express CP-AMPA providing fast Ca²⁺ signals. Second, individual cells can coexpress CP-AMPA, CI-AMPA, and/or group I/II mGluRs (including

mGluR1a), producing heterogeneous Ca²⁺ signals in different dendritic sites. Third, activation of CI-AMPA receptors did not elicit detectable Ca²⁺ signals, but these receptors were often associated with mGluRs, resulting in longer-lasting Ca²⁺ signals. Fourth, CP-AMPA- and mGluR-mediated Ca²⁺ signals demonstrate distinct voltage dependence and are differentially regulated by presynaptic and postsynaptic activity within the same dendritic microdomain. These findings indicate that differential activation of specific glutamate receptor-mediated Ca²⁺ signals within spatially restricted dendritic microdomains serve distinct signaling functions and allow for multiple forms of Ca²⁺-mediated synaptic plasticity in these cells. The specific properties of mGluR-mediated Ca²⁺ signals are consistent with the Hebbian requirements for LTP induction in O/A interneurons.

Heterogeneity of Ca²⁺ signals in dendrites of O/A interneurons

Our findings and other recent studies provide new insights into the complexity of Ca²⁺ signaling in interneuron dendrites (Gee et al., 2001; Kaiser et al., 2001, 2004; Goldberg et al., 2003a,b,c). In neocortical interneurons, synaptic Ca²⁺ signals were highly localized within dendritic microdomains and mediated by NMDARs or CP-AMPA receptors (Goldberg et al., 2003a,b,c; Kaiser et al., 2004). We provide novel evidence that in hippocampal O/A interneurons, multiple Ca²⁺ signals mediated by NMDARs, CP-AMPA receptors, and mGluRs coexist within highly restricted dendritic compartments. NMDAR-mediated Ca²⁺ transients were variable in amplitude in O/A interneurons, consistent with their variable dendritic NMDAR distribution (Nyiri et al., 2003). In pyramidal cells, NMDAR-mediated Ca²⁺ signals underlie long-term potentiation (Malenka et al., 1988; Malenka and Nicoll, 1999), but this may not be the case in O/A interneurons (Maccaferri and McBain, 1996). Interestingly, in hippocampal lucidum interneurons, activation of NMDARs at synapses colocalizing CI-AMPA receptors leads to long-term depression (LTD) (Lei and McBain, 2002, 2004). For O/A interneurons, however, a role of NMDARs in such LTD remains to be determined.

Our data uncovered significant contributions of CP-AMPA receptors to dendritic calcium signaling in O/A interneurons. CP-AMPA-mediated Ca²⁺ transients exhibited fast kinetics, allowing for calcium compartmentalization in aspiny interneuron dendrites, as in neocortical fast-spiking cells (Goldberg et al., 2003c); however, this property of CP-AMPA receptors may be prominent mostly near resting membrane potentials, because CP-AMPA-mediated Ca²⁺ signaling diminished with depolarization. In contrast, mGluR-mediated Ca²⁺ transients were enhanced by membrane depolarization and demonstrated slower kinetics. Thus, the different properties of CP-AMPA- and mGluR-mediated Ca²⁺ signals suggest different signaling roles for these Ca²⁺ mechanisms in O/A interneurons. Together, these results highlight the heterogeneity of

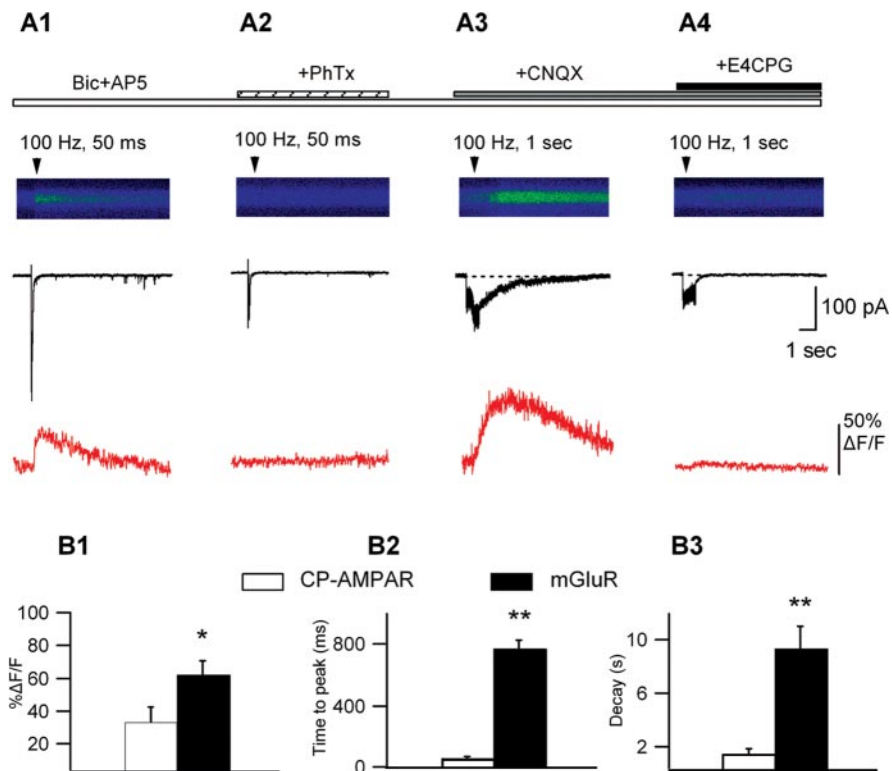


Figure 7. Differential regulation by presynaptic activity of synaptically elicited Ca²⁺ signals mediated by CP-AMPA receptors and mGluRs. *A*, Line scan images of synaptically evoked Ca²⁺ signals with representative currents (black) and associated Ca²⁺ transients (red) from the same cell in the presence of Bic and AP5 (*A1*), after PhTx block (*A2*), in the presence of Bic, AP5, and CNQX (*A3*), and after adding E4CPG (*A4*). Single bursts of high-frequency stimulation (30 μA; 5 stimuli at 100 Hz) produced summated EPSCs with appreciable Ca²⁺ influx (*A1*). These Ca²⁺ transients were completely blocked by PhTx (*A2*). In the absence of ionotropic glutamate transmission (in Bic, AP-5, and CNQX), repetitive synaptic stimulation (150 μA; 0.5–1 s at 100 Hz) evoked slower postsynaptic inward currents accompanied by slow Ca²⁺ transients (*A3*) that were both antagonized by E4CPG (*A4*). *B*, Bar graphs of the peak amplitude (*B1*), time-to-peak (*B2*), and decay time (*B3*) of synaptically evoked Ca²⁺ transients mediated by CP-AMPA receptors (PhTx sensitive; i.e., from *A1*) and by mGluRs (E4CPG sensitive; i.e., from *A3*) (*n* = 5; **p* < 0.05; ***p* < 0.01).

Ca²⁺ signals in O/A interneuron dendrites and suggest multiple distinct Ca²⁺ signaling functions at interneuron synapses.

Heterogeneous targeting of CP-AMPA receptors, CI-AMPA receptors, and mGluRs

Our findings indicate major differences in dendritic Ca²⁺ signals elicited by glutamate and synaptic stimulation. With glutamate application, we found three types of Ca²⁺ signals: (1) via CP-AMPA receptors, (2) via mixed activation of CP-AMPA receptors and mGluRs, and (3) via mGluRs. In contrast, synaptic Ca²⁺ responses were mediated by both CP-AMPA receptors and mGluRs within given dendritic microdomains. Because glutamate stimulation may involve synaptic and extrasynaptic receptors whereas synaptic stimulation may implicate only synaptic receptors, our observations suggest that CP-AMPA receptors and group I/II mGluRs (1) are colocalized at synaptic sites and (2) can be found independently at extrasynaptic sites. Heterogeneous glutamate-evoked currents and Ca²⁺ signals were observed in different dendritic microdomains of a given cell, indicating a heterogeneous distribution of AMPA receptors and mGluRs within individual cells. Interestingly, CI-AMPA receptors were more often associated with mGluRs than CP-AMPA receptors. Thus, longer-lasting Ca²⁺ signals may occur in dendritic microdomains colocalizing CI-AMPA receptors and mGluRs. Such heterogeneity in Ca²⁺ mechanisms suggests distinct, site-specific Ca²⁺ signaling roles in dendritic microdomains with different combinations of CP-AMPA receptors, CI-AMPA receptors, and mGluRs.

Our Ca²⁺ imaging and electrophysiological results also show

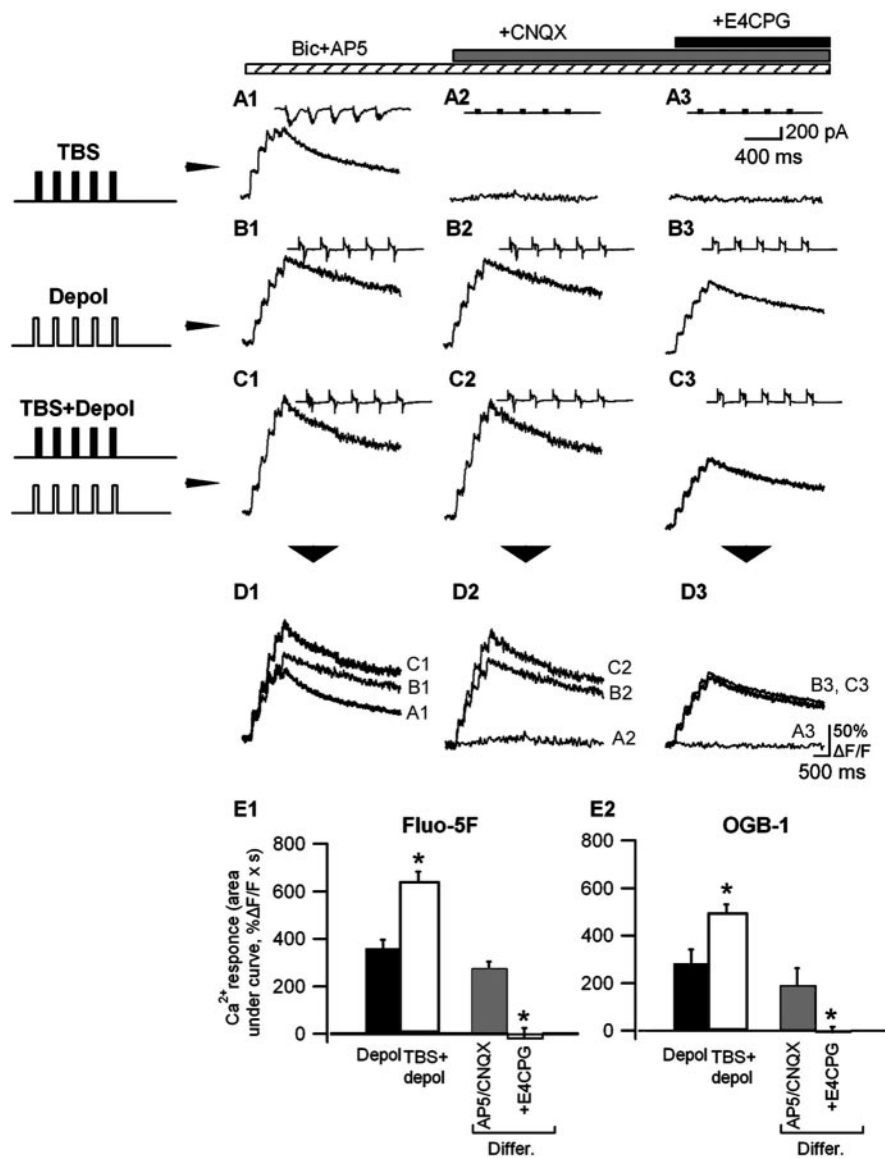


Figure 8. Regulation of Ca²⁺ signals mediated by mGluRs by postsynaptic depolarization. *A1–C3*, Representative Ca²⁺ transients measured with Fluo-5F and associated currents (insets) evoked by TBS at -60 mV (*A1, A2*), postsynaptic depolarization (-20 mV) (*B1, B2*), and TBS paired with depolarization (*C1, C2*) in different pharmacological conditions. *D*, Superimposed Ca²⁺ transients elicited in the same cell by the three stimulation protocols, showing that Ca²⁺ transients associated with TBS plus depolarization (*C1, C2*) were larger than those elicited by TBS (*A1, A2*) or depolarization (*B1, B2*) alone and that this difference was blocked by the mGluR antagonist E4CPG (*B3, C3*). *E*, Bar graphs of mean Ca²⁺ responses measured with Fluo-5F (*E1*) ($n = 4$ cells) and OGB-1 (*E2*) ($n = 4$ cells) evoked by depolarization alone (Depol), pairing TBS and depolarization (TBS + depol), and the difference in response between the two conditions (Differ.), showing that this difference was completely blocked by E4CPG ($*p < 0.05$).

that CP-AMPA and CI-AMPA can be (1) colocalized at some dendritic microdomains or (2) targeted independently at others, indicating a differential targeting of AMPARs with variable GluR2 composition to specific postsynaptic sites within individual cells. This is consistent with preliminary evidence that AMPAR-mediated synaptic currents have different types of $I-V$ relationships in O/A interneurons (Croce and Lacaille, 2004), as reported in CA3 stratum lucidum interneurons (Toth and McBain, 1998; Lei and McBain, 2002). Surprisingly, we found that at synapses composed of both CP-AMPA and CI-AMPA, Ca²⁺ signals were mediated solely by CP-AMPA. This observation is at odds with the partial Ca²⁺ permeability of CI-AMPA reported previously ($P_{Ca}/P_{Na} > 0$) (Geiger et al.,

1995). Our results suggest that the Ca²⁺ permeability of synaptically activated CI-AMPA *in situ* is negligible or below our detection threshold.

Differential regulation of CP-AMPA and mGluR-mediated Ca²⁺ transients

We found that each synaptic component of Ca²⁺ transients could be uncovered with different stimulation. Weak stimulation produced dendritic Ca²⁺ signals via CP-AMPA, whereas higher intensity or repetitive stimulation recruited group I/II mGluRs. Thus, synaptic CP-AMPA and mGluR-mediated Ca²⁺ signals are differentially regulated by presynaptic activity. The recruitment of group I/II mGluRs by intense and repetitive synaptic stimulation is consistent with their perisynaptic localization (Baude et al., 1993; Lujan et al., 1996). In cerebellar stellate cells, high presynaptic activity produced downregulation of CP-AMPA (Liu and Cull-Candy, 2000, 2002). Such AMPAR regulation by presynaptic activity has not been reported in O/A interneurons, but because they receive intense synaptic activity (McBain, 1995; Maccaferri and Lacaille, 2003), it represents another possible regulatory mechanism at these synapses.

Our finding that synaptic stimulation paired with depolarization results in group I/II mGluR-dependent Ca²⁺ transients provides clear evidence of a regulation of mGluR-mediated Ca²⁺ signal by postsynaptic activity. Enhancement of mGluR-mediated currents and Ca²⁺ signals by membrane depolarization was reported for pyramidal cells (Luthi et al., 1997; Chuang et al., 2000; Rae et al., 2000; Rae and Irving, 2004). Recently, mGluR1a-mediated EPSCs in O/A interneurons were reported to depend on depolarization causing Ca²⁺ entry via VGCCs (Huang et al., 2004); however, in our experiments, the bell-shaped voltage relationship of glutamate-evoked Ca²⁺ transients mediated by mGluRs was observed in Ni²⁺ and Cd²⁺ and thus did not implicate Ca²⁺ influx via VGCCs. Alternatively, mGluR-

mediated currents may result from activation of nonselective cation channels (Congar et al., 1997; Gee et al., 2003). These currents are modulated by divalent cations and demonstrate outward rectification (Congar et al., 1997). Interestingly, in heterologous systems, currents mediated by heteromultimeric channels consisting of transient-receptor-potential (TRP) subunits, TRPC1 and TRPC5, demonstrate properties similar to those of mGluR-mediated currents in our conditions (Strübing et al., 2001). Given that a TRPC1–mGluR1a interaction underlies mGluR-mediated EPSCs in Purkinje cells (Kim et al., 2003), a similar TRPC–mGluR1a relation may be present in O/A interneurons. Preliminary evidence suggests that Ca²⁺ signals in O/A interneurons involve multiple mechanisms differentially coupled to mGluR1

and mGluR5 as well as to Ca²⁺ influx via TRPCs and intracellular Ca²⁺ release (Topolnik et al., 2004). Interestingly, our observation that depolarization-induced Ca²⁺ signals were partly inhibited by mGluR antagonists suggests that, in addition, VGCCs may be modulated positively by basal mGluR activation.

Implication for long-term synaptic plasticity in interneurons

CP-AMPA receptors are prevalent in interneurons (McBain and Dingleline, 1993; Jonas et al., 1994; Geiger et al., 1995; Koh et al., 1995; Isa et al., 1996). Ca²⁺ influx via these receptors is involved in multiple forms of interneuron synaptic plasticity (Mahanty and Sah, 1998; Laezza et al., 1999; Liu and Cull-Candy, 2000, 2002; Toth et al., 2000; Ross and Soltesz, 2001; Lei and McBain, 2002). Short-term facilitation of CP-AMPA-mediated currents results from the use- and voltage-dependent relief of the polyamine block (Rozov et al., 1998; Toth et al., 2000). CP-AMPA-dependent LTD in hippocampal interneurons is a form of plasticity expressed presynaptically but induced postsynaptically. Moreover, it is induced at near resting membrane potentials but not at depolarized potentials (Laezza et al., 1999; Lei and McBain, 2002, 2004). The negative voltage relationship of CP-AMPA-mediated Ca²⁺ signals found in the present study is consistent with these LTD induction requirements because these responses were reduced in amplitude by membrane depolarization. These LTD induction mechanisms are in contrast to those of mGluR-dependent LTP induction in interneurons, which are Hebbian in nature and require presynaptic activation and postsynaptic depolarization (Perez et al., 2001). The bell-shaped voltage relationship of mGluR-dependent Ca²⁺ responses revealed here is compatible with these LTP induction requisites because these signals were uncovered by depolarization paired with stimulation. Thus, the LTD induction protocol is optimal for eliciting CP-AMPA-mediated Ca²⁺ signals, whereas the LTP induction protocol is best for evoking mGluR-dependent Ca²⁺ transients. Ca²⁺-dependent LTD- versus LTP-induction mechanisms in interneurons therefore may be determined by the specific CP-AMPA- versus mGluR-mediated Ca²⁺ mechanisms associated with each stimulation paradigm. Thus, the direction of synaptic plasticity appears to be a function of the specific Ca²⁺ signaling mechanism activated in interneuron dendritic microdomains. Our findings uncover heterogeneous computational capabilities of local dendritic Ca²⁺ signaling with fast CP-AMPA-mediated Ca²⁺ transients playing a role as coincidence detectors of synaptic inputs (Goldberg et al., 2003c) and slow mGluR-mediated Ca²⁺ responses signaling coincident presynaptic and postsynaptic activity in O/A interneurons.

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