Coincident glutamatergic and cholinergic inputs transiently depress glutamate release at rat Schaffer collateral synapses

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ABSTRACT

The mammalian hippocampus, together with subcortical and cortical areas, is responsible for some forms of learning and memory. Proper hippocampal function depends on the highly dynamic nature of its circuitry, including the ability of synapses to change their strength for brief to long periods of time. In this study, we focused on a transient depression of glutamatergic synaptic transmission at Schaffer collateral synapses in acute hippocampal slices. The depression of evoked excitatory postsynaptic current (EPSC) amplitudes, herein called transient depression, follows brief trains of synaptic stimulation in stratum radiatum of CA1 and lasts for 2–3 minutes. Depression results from a decrease in presynaptic glutamate release, as NMDA receptor-mediated EPSCs and composite EPSCs are depressed similarly and depression is accompanied by an increase in the paired-pulse ratio. Transient depression is prevented by blockade of metabotropic glutamate and acetylcholine receptors, presumably located presynaptically. These two receptor types—acting together—cause depression. Blockade of a single receptor type necessitates significantly stronger conditioning trains for triggering depression. Addition of an acetylcholinesterase inhibitor enables depression from previously insufficient conditioning trains. Furthermore, a strong coincident, but not causal, relationship existed between presynaptic depression and postsynaptic internal Ca$^{2+}$ release, emphasizing the potential importance of functional interactions between presynaptic and postsynaptic effects of convergent cholinergic and glutamatergic inputs to CA1. These convergent afferents, one intrinsic to the hippocampus and the other likely originating in the medial septum, may regulate CA1 network activity, the induction of long-term synaptic plasticity, and ultimately hippocampal function.

**key words:** g-protein, acetylcholine, short-term synaptic plasticity, muscarine, mGluR
INTRODUCTION

The hippocampal formation, through its communication with a host of neural regions, contributes to long-term storage and retrieval of some forms of memory. The ability to carry out these functions depends on network activity both intrinsic and extrinsic to the hippocampus, which in turn depends on spatial and temporal patterns of synaptic activity, and the capacity for dynamic changes in synaptic strength (Martin et al. 2000; Yeckel and Berger 1998). There are a number of loci and time scales over which synaptic function can be altered, and myriad mechanisms have been shown to contribute to these processes. Although the properties of synaptic plasticity in the hippocampus have traditionally focused on long-term potentiation and long-term depression (LTP and LTD, respectively), many other forms of synaptic plasticity have been identified that have shorter durations (Zucker and Regehr 2002). Of particular interest are mechanisms of short-term synaptic plasticity at hippocampal synapses that may hold functional implications for the hippocampus, both directly by determining patterns of activity relevant to ongoing behavior and indirectly by modulating the ability to induce LTP and LTD (Abraham and Bear 1996; Zucker and Regehr 2002).

Short-term changes in synaptic transmission lasting from 2–10 min at hippocampal pyramidal neuron synapses have been reported to depend either on alteration of presynaptic glutamate release or on changes to postsynaptic glutamate receptor function (Fernandez de Sevilla and Buno 2003; Fernandez de Sevilla et al. 2002; Grishin et al. 2004; Grover and Teyler 1993b; Manzoni et al. 1994; Sekino and Koyama 1992). For example, trains of synaptic stimulation or application of Gq-coupled receptor agonists elicit a brief depression of synaptic transmission by directly or indirectly decreasing glutamate release from presynaptic terminals.
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(Fernandez de Sevilla and Buno 2003; Manzoni et al. 1994). In contrast to this presynaptic form of depression, others have reported a postsynaptic form of depression due to Ca$^{2+}$- and/or G-protein-dependent changes in postsynaptic NMDA receptor function triggered by rises in [Ca$^{2+}$]$_i$ (Grishin et al. 2004; Markram and Segal 1990). Common to both of these pre- and postsynaptic forms of short-term synaptic depression is their dependence on the activation of G-protein-coupled receptors, which can be activated by many neurotransmitters and neuromodulators (Gilman 1987; Hille 1992).

Based on the role of G-proteins in short-term synaptic depression and their responsiveness to multiple ligands, we examined whether coincident activation of different G$_q$-coupled receptors by convergent afferent inputs might act cooperatively to elicit synaptic depression at hippocampal CA1 synapses. Using evidence gathered from whole-cell patch-clamp recordings and Ca$^{2+}$ fluorescence imaging in acute hippocampal slices, we describe a short-lived (2–3 minutes) depression (transient depression) of evoked glutamatergic synaptic responses. This transient depression was elicited by brief trains (30 stimuli at 100 Hz) of stimulation in stratum radiatum and was expressed presynaptically. Despite its high correlation with postsynaptic internal Ca$^{2+}$ release, transient depression was independent of internal Ca$^{2+}$ release. Depression of test response amplitudes depended on coincident activation of presumed presynaptic metabotropic glutamate receptors (mGluRs) and muscarinic acetylcholine receptors (mAChRs). Our data raise the possibility that convergent afferent inputs arising in different brain regions, through the release of neurotransmitters that activate a common G-protein subtype, can act cooperatively to depress neurotransmission at glutamatergic synapses.
METHODS

Acute Hippocampal Slice Preparation

Hippocampal slices (350 µm) were prepared from 3–5 week-old male rats (Sprague-Dawley, Charles River) using experimental procedures consistent with those outlined in National Institutes of Health publication 91-3207, *Preparation and Maintenance of Higher Animals During Neuroscience Experiments*, and approved by the *Institutional Animal Care and Use Committee* at the Yale School of Medicine. An anesthetic consisting of ketamine (125 mg/kg), xylazine (6.25 mg/kg), and acepromazine (1.25 mg/kg) was injected intraperitoneally, and animals were decapitated after they showed no response to a foot pinch. Brains were removed in ice-cold dissecting solution containing (in mM): 87 NaCl, 58.5 sucrose, 2.5 KCl, 25 NaHCO₃, 1.25 NaH₂PO₄, 7.0 MgCl₂, 0.5 CaCl₂, and 10 dextrose; 296–301 mOsm, then blocked rapidly and glued to a cold slicing chamber insert. Horizontal slices were cut on a Vibratome 1500 (Vibratome Co.) using a freshly broken glass blade (Ralph Knife Breaker; Ted Pella, Inc.) in a peltier-cooled slicing chamber (model 900R, Vibratome Co.). The slicing chamber was filled with dissecting solution bubbled with 95% O₂/5% CO₂ and maintained at -1 to -2°C by a VS-35M power supply (Astron) and digital thermometer (Fisher Scientific). Trimmed slices including the hippocampus were incubated for 10–20 minutes at 35°C in dissecting solution before being transferred to 35°C recording solution containing (in mM): 126 NaCl, 2.5 KCl, 25 NaHCO₃, 1.25 NaH₂PO₄, 2 CaCl₂, 10 dextrose, and 0.01 glycine, bubbled with 95% O₂/5% CO₂, and placed at room temperature. The slices were allowed to stabilize for one hour prior to recording. Mg²⁺ was omitted from, and glycine was added to, the recording solution to facilitate the activation of NMDA receptors (NMDARs), except where otherwise indicated (Johnson and Ascher 1987; Nowak et al. 1984). Test EPSCs were recorded in the presence of GABAₐR and
GABA\(_{\text{B}}\)R antagonists, except where indicated, and the AMPA receptor (AMPAR) antagonist 20\(\mu\)M DNQX as needed to isolate NMDAR-mediated EPSCs.

**Electrophysiological Recordings**

For recording, a slice was submerged in a recording chamber (bath volume \(\sim\)1ml) that was perfused continuously (\(\sim\)1ml/min) with recording solution saturated with 95% \(\text{O}_2\)/5% \(\text{CO}_2\). The slice was elevated off the chamber bottom using a circular platinum wire that supported a taut nylon mesh. Another, slightly smaller, circular platinum harp crossed by 4–5 recessed pairs of nylon strands stabilized the slice from above. An in-line flow heater (Warner Instruments) and a custom heated stage insert maintained chamber temperature at 32–35\(^\circ\)C. In an effort to limit outgassing, the recording solution reservoir was warmed to \(\sim\)30\(^\circ\)C with a band heater (Meriden Cooper Corp.) controlled by a VS-35M DC power supply (Astron). Slices were viewed through a Zeiss Axioskop 2 fitted with a 40X water-immersion objective and differential interference contrast optics (DIC). Whole-cell patch-clamp recording pipettes (3–5 M\(\Omega\) resistance) were pulled (model P-97 pipette puller, Sutter Instruments, Novato, CA) from thick-walled borosilicate glass (1.0 I.D., 2.0 O.D.) and firepolished. Recordings were made with a SEC 05L amplifier (npi, Tamm, Germany) in bridge mode or discontinuous voltage-clamp mode. Cells recorded in CA1 stratum pyramidale were identified morphologically as having a single large apical dendrite in stratum radiatum and smaller basal dendrites in stratum oriens, and electrophysiologically as displaying spike frequency adaptation and a hyperpolarization-activated current (\(I_h\)) (Magee 1998). Synaptic currents were recorded in voltage clamp at -63 mV (-73 mV if we account for a measured -10 mV junction potential). Series resistance was typically 10–20 M\(\Omega\). Whole-cell recording electrodes were filled with (in mM) 134 KMeSO\(_4\), 3
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KCl, 10 HEPES, 1.0 MgCl$_2$, 4 Mg-ATP, 0.5 Na-GTP, 5 K$_2$-creatine phosphate, 5 Na$_2$-creatine phosphate, 0.1 bis-fura-2, 0.015 Alexa 488, 50 units/ml creatine phosphokinase (pH 7.53 at 24°C, ~288 mOsm). KMeSO$_4$, Mg-ATP, Na-GTP, Na$_2$-creatine phosphate, and creatine phosphokinase were from Sigma Aldrich (St. Louis, MO). K$_2$-creatine phosphate was from Calbiochem (San Diego, CA). Bis-fura-2 and Alexa 488 were from Molecular Probes/Invitrogen, Carlsbad, CA.

**Afferent Stimulation**

Schaffer collateral axons were stimulated by applying brief unipolar currents (100–200 µs, 5–60 µA) through a glass microelectrode (3–10 µm tip diameter) with a fine tungsten rod glued to its side to provide for more local current flow. Stimulating electrodes were filled with extracellular solution and 2.5 µM Alexa 488 for visualization. Two stimulating electrodes were placed in stratum radiatum, each ~50 µm from the apical dendrite of the recorded pyramidal cell, one 50 and the other 100 µm from the cell body layer, to stimulate independent synaptic inputs onto the recorded cell. The independence of the two glutamatergic pathways was verified using a paired-pulse test (50 ms interval; (Barrionuevo and Brown 1983) in each experiment examining depression on an independent test pathway. Test synaptic currents (~500 pA in amplitude) were evoked separately on each stimulus pathway at 20-second intervals using single-, paired- (20 Hz), or triple-pulse (100 Hz) stimuli. Short conditioning trains (30 stimuli, 100 Hz) of synaptic stimulation on either or both pathways were used to elicit transient depression of test currents. This stimulus pattern was used because it elicited robust transient depression while being weaker than stimuli typically used to induce long-term changes in synaptic strength. No difference in test stimulus artifact amplitude was observed during depression, confirming proper
functioning of the stimulator after a conditioning train (model 2200 analog stimulus isolator, A-M Systems; artifact amplitude was 99.6 ± 0.4% of control at the peak of depression, n = 78).

**Ca\textsuperscript{2+} Fluorescence Imaging**

To measure relative changes in \([\text{Ca}\textsuperscript{2+}]_i\), the Ca\textsuperscript{2+} indicator dye bis-fura-2 (100 µM) was included in the pipette solution. Using a cooled CCD camera (Quantix 57; Photometrics, Tuscon, AZ) in a sequential frame transfer mode (50 Hz frame rate; binned in a 5 by 5 array), fluorescence images were recorded with single wavelength measurements (~380 nm excitation) from the soma and dendrites of filled cells. Synchronous electrical and optical data acquisition was performed with custom software written in IGOR Pro. Relative changes in \([\text{Ca}\textsuperscript{2+}]_i\) were quantified as changes in \(\Delta F/F\), where \(F\) is fluorescence intensity before stimulation and \(\Delta F\) is the relative reduction from this value during neuronal activity. Data representing tissue autofluorescence and dye bleaching were collected for each experiment (although not always applied to the analysis of imaging data). Tissue autofluorescence was determined with an equivalent fluorescence measurement at a parallel location in the slice that was away from the dye-filled cell. Dye bleaching was assessed as the change in fluorescence of the recorded cell observed during an imaging run of standard duration (4 s) with no stimulation of the cell.

**Pharmacology**

The following drugs were included in the recording pipette when needed to affect biochemical processes in the recorded neuron: 10 or 50 mM K\textsubscript{4}-BAPTA, 5 mM QX-314, 1 mM GDP-\(\beta\)-S. BAPTA and QX-314 were substituted for equiosmolar amounts of KMeSO\textsubscript{4}, and GDP-\(\beta\)-S replaced Na-GTP. BAPTA and GDP-\(\beta\)-S were confirmed to block internal Ca\textsuperscript{2+} release.
in each recorded cell using Ca$^{2+}$ imaging, and QX-314 blocked $I_h$. K$_4$-BAPTA and GDP-β-S trilithium salt were from Sigma. QX-314 bromide was from Tocris (Ellisville, MO).

Bath-applied drugs were typically present in the recording solution as soon as the slice was transferred to the recording chamber, usually 0.5–2 hours before establishing a recording. Sometimes drugs were applied as a wash-in during the experiment, as indicated. The following bath-applied drugs were from Tocris Cookson, Ellisville, MO (stock solution in parentheses): 100 µM DL-APV (25 mM in H$_2$O), 1–2 µM CGP55845 (20mM in DMSO), 50 µM cyclopiazonic acid (CPA, 100 mM in DMSO), 20 µM DNQX (40mM in DMSO), 0.5–5 µM DPCPX (5mM in DMSO, made fresh), 100 µM LY367385 (100 mM in 1.1 N NaOH), 20 µM MK-801 (10 mM in H$_2$O), 10 µM MPEP (100 mM in DMSO), 3 µM thapsigargin (30 mM in DMSO), 50 µM SR95531 (Gabazine, 10 mM in H$_2$O), 1 µM WIN-55 and 5 µM AM251 (both stored at -20°C as 100 mM DMSO stocks, then prediluted in DMSO to 0.5–2.5 mM in the presence of other drugs before addition to aqueous solution to prevent precipitate formation, final DMSO concentration 0.2%). In a control experiment, bath application of 5 µM AM251 was observed to antagonize the depression of evoked IPSCs produced by bath application of WIN-55,212-2 (data not shown). The following bath-applied drugs were from Sigma Aldrich, St. Louis, MO: 10 µM atropine (5 mM in H$_2$O), 10 µM bicuculline methiodide (2 mM in recording solution), 50 µM calmidazolium (50 mM in DMSO), 5 µM eserine (5 mM in H$_2$O, made fresh), 10 µM picrotoxin (50 mM in ethanol, made fresh). When atropine was present in the recording solution, GABA$_A$R antagonists were omitted to prevent slices from becoming hyperexcitable.
In control experiments to assess the efficacy of DPCPX, a pressure pipette was used to depress synaptic responses by applying 200 µM adenosine (from Sigma, freshly dissolved in puffer solution). The puffer solution contained (in mM): 124 NaCl, 2.5 KCl, 25 NaHCO$_3$, 1.25 NaH$_2$PO$_4$, 2 MgCl$_2$, 2 CaCl$_2$, 10 HEPES, pH 7.35 at 24ºC. The pressure pipette and stimulating electrode were placed 100 µm and 50 µm from the cell body layer in CA1, respectively, and 50 µm from the apical dendrite of the recorded cell. The effect of adenosine on NMDAR-mediated synaptic test current amplitudes was assessed in the absence and presence of 5 µM DPCPX.

Data Analysis

Electrical data were analyzed both on- and off-line using routines written to accompany our existing data collection and optical data analysis software. Current amplitudes were measured from a smoothed (10 point Gaussian) current trace to limit the contribution of noise to the current peak, and each measurement was confirmed visually. To assess the possibility that changes in resting dendritic conductances following conditioning trains may produce an apparent or real depression of synaptic current amplitudes measured at the soma, we followed the postsynaptic input resistance ($R_{IN}$) over time using a 5 mV hyperpolarizing voltage step every 20 seconds. In agreement with a previous report (Grover and Teyler 1993a), $R_{IN}$ was unchanged from baseline during transient depression in our whole-cell recordings (to 100.5 ± 1.2% of baseline 20 s after conditioning train, n = 54).

In experiments involving two stimulus pathways, data from trials in which depression occurred on a given test pathway were grouped into a data set. Data from trials within a data set were averaged, and then data were combined across data sets to generate summary data for a
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However, for simplicity we have reported N values as the number of cells recorded rather than the number of data sets. Current amplitude, R_in, stimulus artifact, and paired-pulse ratio (PPR) data were normalized for presentation so that the summary data represents variability of measurements within trials, rather than variability of baseline values between data sets or between cells. PPR data were averaged across trials in a data set as described (Kim and Alger 2001). Weighted exponential fits of summary data were performed in IGOR Pro.

Data from paired-pulse independence tests were analyzed by averaging raw traces from ten trials per condition. The first and second responses to a pair of stimuli on the test pathway were taken to be the baseline and potentiated responses, respectively. The second response to a pair of stimuli in which the first stimulus was delivered on the conditioning pathway and the second stimulus was delivered on the test pathway was taken as the test response. When a considerable difference between baseline and potentiated responses was present, and when the test response was not discernibly different than the baseline response, depression data from the independent pathway were included in the average (Figure 3C). Data describing depression on a separate test pathway not meeting these criteria were discarded.

Optical data from Ca^{2+} imaging were analyzed to determine the correlation between internal Ca^{2+} release events and transient depression, and to verify the effect of internal BAPTA and GDP-β-S, as well as bath applied CPA, thapsigargin, LY367385, MPEP, and atropine. Ca^{2+} transients elicited by synaptic trains included three components: 1) Ca^{2+} rises during the stimulus train associated with depolarization and the opening of voltage-gated Ca^{2+} channels
with fast exponential decay beginning at the end of the train (Christie et al. 1995), 2) \( \text{Ca}^{2+} \) rises due to influx through NMDARs with a slower exponential decay that begins at the end of the train and depends on NMDAR closure (Regehr and Tank 1992), and 3) \( \text{Ca}^{2+} \) rises due to IP\(_3\)-mediated \( \text{Ca}^{2+} \) release from internal stores, involving a continued \( \text{Ca}^{2+} \) rise after the end of the train, especially when involving an inflection point in the optical trace and an accelerating \( \text{Ca}^{2+} \) rise or propagation of a \( \text{Ca}^{2+} \) wave in the dendrites of the recorded cell (Kapur et al. 2001; Nakamura et al. 1999). Trials where \( \text{Ca}^{2+} \) transients had characteristics of this third component were considered to include an internal \( \text{Ca}^{2+} \) release event.

Conditioning train stimulus intensity data (Figure 7A) were gathered post hoc by examining the conditioning trains used in trials that are included in the summary data for each recording condition. We calculated the charge commanded (nC) through the stimulator during each stimulus of the 30-stimulus train as the product of the stimulus amplitude (µA) and duration (ms). Only experiments using NMDAR-mediated EPSCs, single or paired test stimuli, and depression on the conditioning pathway were used for comparison. We pooled stimulus intensity data (Figure 7A) from mGluR or mAChR blockade experiments (Figure 6A) with corresponding experiments that included adenosine receptor antagonism (Figure 6B) since the data were not significantly different.

All data are presented as mean ± SEM. Statistical significance \((p < 0.05)\) was tested using paired or unpaired two-tailed Student’s \( t \)-tests as appropriate, except for the series of experiments examining stimulation intensity requirements (Figure 7A), which used a one-way ANOVA with Dunnett’s multiple comparison post hoc analysis.
RESULTS

Brief trains of synaptic stimulation elicit a transient depression of CA1 glutamatergic synapses.

We describe a transient depression of glutamatergic neurotransmission at Schaffer collateral synapses using whole-cell recordings in acute hippocampal slices from 3–5 week-old male rats. NMDAR-mediated or composite (AMPA + NMDA) excitatory postsynaptic currents (EPSCs) were evoked in the recorded CA1 pyramidal cell by brief currents through a small (3–10 µm tip diameter) stimulation pipette placed in stratum radiatum. Short conditioning trains (30 stimuli at 100 Hz) of synaptic stimulation were followed by a transient reduction in EPSC amplitude (Figure 1A) in 86% of the cells tested (96 of 111 cells). This stimulus pattern was used because it elicited transient depression reliably but was not expected to induce long-term changes in synaptic strength. Depression was maximal within 40 seconds following the conditioning train (to 57.5 ± 1.0% of baseline 20 s after train, n = 83), and recovered monoexponentially (to 94.9 ± 1.0% of baseline after 180–200 s, τ = 76.5 s; Figure 1B). Depression could be elicited repeatedly through recordings of one hour or more (Figure 1C). Additional conditioning trains delivered while responses were depressed prolonged the duration of depression without increasing its amplitude (n = 3; data not shown). We frequently examined the transient depression of isolated NMDAR test currents because they are known targets for diffusible modulators of channel function that may be liberated during conditioning trains (e.g., nitric oxide; see below) (Yamakura and Shimoji 1999), and also targets for intracellular Ca$_{2+}$ and other postsynaptic effectors (Kotecha and MacDonald 2003). As such, most experiments were conducted without extracellular Mg$_{2+}$ and with GABA$_A$ receptor (GABA$_A$R) antagonists. However, transient depression was also observed in more physiologic conditions (Figure 1D).
**Transient depression results from a decrease in presynaptic glutamate release**

To begin to understand the mechanism of transient depression, we next examined whether it was expressed presynaptically or postsynaptically. One prediction about a presynaptic depression is that the associated reduction of glutamate release will reduce the activation of all types of glutamate receptors. Therefore we tested for transient depression of currents through two types of postsynaptic glutamate receptors and used paired test stimuli to assess relative changes in the probability of neurotransmitter release (P_{r}). Consistent with a previous report (Grover and Teyler 1993a), both NMDAR-mediated and composite EPSC amplitudes were depressed similarly following conditioning trains (composite depression to 55.8 ± 2.7% of baseline, $\tau = 79.4$ s, n = 19; NMDAR depression to 57.1 ± 1.1% of baseline, $\tau = 74.9$ s, n = 64; Figure 2A). Composite EPSC amplitudes mainly reflect the level of synaptic AMPAR activation (Collingridge et al. 1988; Hestrin et al. 1990). Transient depression of current amplitudes was accompanied by a transient increase in the paired-pulse ratio (PPR), and the two effects recovered with similar time courses (Figure 2B and 2C). The PPR was increased during depression for both composite (to 139.1 ± 7.9% of baseline 20 s after train, n = 19, $p < 0.001$) and isolated NMDAR EPSCs (to 110.2 ± 2.1% of baseline, n = 52, $p < 0.001$). To illustrate the time course similarity between amplitude depression and PPR increase, we fit each group of PPR data with an exponential constrained to the time course of depression ($\tau = 76.5$ s, Figure 2B, red lines). The similar depression of NMDAR- and AMPAR-mediated currents, and the coincidence of depression with a PPR increase, leads us to conclude that transient depression is mediated presynaptically through a reduction in P_{r}. The PPR increase for isolated NMDAR currents, although significant, was smaller than that observed with composite EPSCs ($p < 0.001$). This
difference may represent receptor-specific differences in PPR, like a reduction of the contribution of AMPAR desensitization during depression (Colquhoun et al. 1992) or different presynaptic properties of silent versus functional synapses (Cabezas and Buno 2006; Fernandez de Sevilla et al. 2002), but does not affect our conclusion that transient depression is expressed presynaptically.

**Depression is observed on an independent test pathway**

One possible mechanism for transient depression is a use-dependent depression of the presynaptic terminal, in which the action potentials of the conditioning train may exhaust neurotransmitter release by, for example, depleting the readily releasable pool of synaptic vesicles (Wang and Kaczmarek 1998; Zucker and Regehr 2002). To explore this possibility, we tested whether depression could be observed at test synapses that were not stimulated during the conditioning train. More specifically, two small stimulating electrodes were placed in stratum radiatum at different distances (50 and 100 µm) from the pyramidal cell layer, and response amplitudes were measured on a test pathway before and after a conditioning train of stimuli on an independent conditioning pathway (Figure 3A). The independence of the two pathways was verified using a paired-pulse test (50 ms interstimulus interval; Figure 3B) (Barrionuevo and Brown 1983) in each experiment. Depression was observed on an independent test pathway in 20–40% of such experiments (to 64.7 ± 3.6% of baseline, τ = 69.8 s, n = 11; Figure 3C), and was similar to depression observed on the conditioning pathway (described above). Either of the two stimulators could trigger transient depression on the other pathway, and conditioning stimuli were delivered on the proximal pathway (n = 6) or the distal pathway (n = 7) in these experiments. Our ability to observe synaptic depression on an independent test pathway was
inconsistent with a use-dependent mechanism intrinsic to the axon terminal, thus leading us to consider alternative traveling or diffusible signals capable of contributing to a presynaptic depression.

**Depression does not require postsynaptic Ca$^{2+}$ or G-protein signalling**

Given the suggestion of a traveling signal extrinsic to the axon terminal, we examined the possible dependence of transient depression on some kinds of postsynaptic and network signaling. One candidate mechanism was internal Ca$^{2+}$ release or a propagating Ca$^{2+}$ wave in the recorded cell or in neighboring neurons and glia (Araque et al. 2002; Kapur et al. 2001; Nakamura et al. 1999). Using Ca$^{2+}$ fluorescence imaging of the recorded cell, we found a strong correlation between synaptic depression and postsynaptic internal Ca$^{2+}$ release following conditioning trains (Figure 4A,B). An internal Ca$^{2+}$ release event or propagating Ca$^{2+}$ wave followed 93% of conditioning trains that elicited transient depression (53 of 57 trials, n = 25 cells; Figure 4B), while only 25% of conditioning trains that failed to trigger depression were accompanied by internal Ca$^{2+}$ release (14 of 55 trials, n = 25 cells). In spite of this correlation, transient depression occurred normally when internal Ca$^{2+}$ release was blocked pharmacologically, either via the patch pipette or by bath application (Figure 4C). Internal Ca$^{2+}$ release was blocked effectively, as verified using Ca$^{2+}$ imaging, by including a Ca$^{2+}$ chelator (10 or 50 mM BAPTA, n = 24) and/or a G-protein antagonist (1 mM GDP-β-S, n = 14) in the recording pipette, or by bath application of an antagonist of the sarcoplasmic/endoplasmic Ca$^{2+}$ ATPase (SERCA, 50 µM cyclopiazonic acid (CPA) or 3 µM thapsigargin, n = 5; Figure 4D). The BAPTA data agree with a previous report (Grover and Teyler 1993a). Additionally, transient depression never followed trains of postsynaptic action potentials elicited by brief
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depolarizing pulses (2 nA steps lasting 2 ms; 30–100 action potentials at 100 Hz; n = 19, data not shown). The experiments performed in the presence of BAPTA, GDP-β-S, or SERCA antagonists demonstrate that the depression we observed does not require postsynaptic Ca\(^{2+}\) waves or internal Ca\(^{2+}\) release. Furthermore, these experiments suggest that no postsynaptic signal involving Ca\(^{2+}\) - or G-protein signaling is required for transient depression.

Evidence against the involvement of glia or neighboring dendrites in depression

In the experiments described above, SERCA antagonists were expected to block internal Ca\(^{2+}\) release not only in the recorded cell, but also in neighboring neurons and glia (Araque et al. 2002; Kapur et al. 2001; Nakamura et al. 1999; Yeckel et al. 1999). These data begin to address the possibility that Ca\(^{2+}\)-dependent release of modulatory factors from neighboring cells during conditioning trains may mediate the presynaptic depression (Araque et al. 1998; Kreitzer and Regehr 2002; Ledo et al. 2004; Lowenstein et al. 1994). To investigate this possibility further, we examined whether the retrograde messengers nitric oxide (Boulton et al. 1994) and endocannabinoids contributed to transient depression (Bernard et al. 2005; Domenici et al. 2006; Wilson and Nicoll 2001).

Because nitric oxide production is frequently triggered through NMDAR activation and the action of Ca\(^{2+}\)-calmodulin (Bredt and Snyder 1990; Garthwaite et al. 1988), we first examined whether antagonists expected to diminish the production of nitric oxide influenced transient depression. Bath application of NMDAR antagonists (100 µM DL-APV and 20 µM MK-801, n = 3; Figure 5A) had no effect on transient depression. NMDAR antagonists were effective in these experiments since they blocked a slow component of the composite EPSC
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(Figure 5B), and 100 µM dl-APV alone blocked NMDAR-mediated test currents in other experiments (to 4.6 ± 1.0% of baseline, n = 5). Similarly, the Ca²⁺-calmodulin antagonist calmidazolium (50 µM) did not prevent transient depression (n = 3 of 4; Figure 5A). Next, we blocked nitric oxide activation by pre-incubating slices with a combination of well-established nitric oxide antagonists, the nitric oxide synthase inhibitor L-NAME (100 µM) and the nitric oxide scavenger carboxy-PTIO (30 µM). Transient depression occurred normally in the presence of these antagonists (n = 5; Figure 5A). In another series of experiments we tested whether endocannabinoids released from neighboring neurons might be contributing to transient depression. Addition of the endocannabinoid receptor blocker AM251 (2–5 µM) failed to block transient depression (n=5; Figure 5C). AM251 (5 µM) was verified to antagonize the inhibition of evoked IPSCs produced by bath application of the endocannabinoid agonist WIN55,212-2 (1 µM) (n=1; data not shown). Together, these data are inconsistent with the hypothesis that Ca²⁺-dependent release of modulatory factors from neighboring neurons and glia mediates transient depression. Rather, they strengthen the case for a presynaptic mechanism of the transient depression we observe following brief trains of synaptic stimulation.

**Optimal transient depression depends on coincident synaptic activation of G_q-coupled receptors**

Because transient depression in our experiments resulted from a decrease in presynaptic glutamate release (Figure 2), we examined the role of receptors known to depress neurotransmitter release. More specifically, glutamate release at Schaffer collateral axon terminals can be depressed via activation of several types of presynaptic G-protein-coupled receptors, including Group I mGluRs (Baskys and Malenka 1991; Gereau and Conn 1995),
mACHRs (Fernandez de Sevilla and Buno 2003; Fernandez de Sevilla et al. 2002; Hounsgaard 1978; Sheridan and Sutor 1990; Valentino and Dingledine 1981), adenosine receptors (Dunwiddie and Hoffer 1980; Scholz and Miller 1992; Schubert and Mitzdorf 1979), and GABA_BRs (Ault and Nadler 1982; Bowery et al. 1980; Inoue et al. 1985; Isaacson et al. 1993; Lanthorn and Cotman 1981; Olpe et al. 1982). Consistent with previous reports and with the presence of presynaptic group I mGluRs at Schaffer collateral axon terminals, bath application of the specific group I mGluR agonist DHPG (50 µM) decreased the amplitude and increased the paired-pulse ratio of AMPAR-mediated EPSCs (amplitude to 53.9 ± 4.4% and PPR to 123.9 ± 3.9% of baseline 0–5 min after onset of DHPG effect, \( p < 0.001 \); Figure 6C) (Baskys and Malenka 1991; Gereau and Conn 1995). We investigated the contribution of presynaptic receptor types to transient depression, either alone or in combination, using receptor agonists and antagonists.

Blockade of individual receptor subtypes impeded but did not entirely prevent conditioning train-elicited transient depression (Figure 6A). Depression was not blocked during bath application of Group I mGluR antagonists (100 µM LY367385 and 10 µM MPEP, \( n = 5 \)), the mACHR antagonist atropine (10 µM, \( n = 3 \)), or the adenosine receptor antagonist DPCPX (0.5–5 µM, \( n = 7 \)). However, mGluR or mACHR antagonists did make depression more difficult to elicit (described below). In control experiments, DPCPX (5µM) blocked the depression of glutamatergic transmission produced by a pressure application of 200 µM adenosine (depression to 64.4 ± 3.8% of baseline in control 10–30 s after puff and to 96.9 ± 2.0% with DPCPX, \( n = 3 \), \( p < 0.001 \); Figure 6D). GABA_BRs were blocked in most experiments using CGP55845 (1–2 µM), and depression was observed with or without this antagonist (\( n = 14 \); see Figure 1D).
To evaluate the possibility that Group I mGluRs and mAChRs act together in triggering transient depression, we looked retrospectively at stimulus intensities used during conditioning trains in comparable experiments represented in Figures 6A and B. While neither mGluR nor mAChR activation alone was absolutely required for transient depression, mGluR or mAChR antagonists increased the stimulus intensity required during the conditioning train to trigger transient depression (14.6 ± 1.8 nC per stimulus during conditioning train with mGluR antagonists, n = 6; 9.6 ± 1.0 nC with mAChR antagonist, n = 6; vs. 5.2 ± 0.4 nC in control conditions, n = 17; p < 0.005; Figure 7A). Pharmacologic conditions that increased the stimulus requirement during the conditioning train, however, did not require more intense stimulation to elicit test synaptic responses (2.3 ± 0.4 nC per test stimulus with mGluR antagonists, n = 6; 3.1 ± 0.3 nC with mAChR antagonist, n = 6; vs. 3.7 ± 0.4 nC in control conditions; p > 0.15; n = 17).

In the course of increasing the conditioning train stimulation intensity during experiments we noted that depression tended to appear at a threshold level of intensity and rapidly approached a magnitude of depression ~40% below baseline (data not shown). The observation that mGluR or mAChR blockade made depression more difficult to elicit suggested the possibility that evoked glutamate and acetylcholine release might act cooperatively through activation of G_q protein-coupled receptors to mediate transient depression. Consistent with this possibility, depression was blocked following conditioning trains when both the Group I mGluR antagonists and the mAChR antagonist were present in the recording solution (agonists described above, n = 6; Figure 6B).
No significant increase in the stimulus intensity used during the conditioning train was observed when DPCPX (5 µM) antagonized the adenosine receptor, a G\textsubscript{i}-coupled receptor (6.7 ± 0.8 nC per stimulus during conditioning train, 2.8 ± 0.3 nC per test stimulus; \( p > 0.3 \)). However, the implication of adenosine receptor activation in presynaptic depression studied at this synapse (Grover and Teyler 1993b; Manzoni et al. 1994; Mitchell et al. 1993; Sekino and Koyama 1992), led us to test whether transient depression was prevented by a combination of DPCPX with either mGluR or mAChR antagonists. Neither of these combinations prevented transient depression (\( n = 3 \) for DPCPX plus mGluR antagonists; \( n = 3 \) of 4 for DPCPX plus atropine; Figure 6B), and there was no significant increase in conditioning train stimulus intensity compared with mGluR or mAChR antagonists alone (\( p > 0.1 \)). Stimulus intensity data from these combinations were thus pooled with data from mGluR or mAChR blockade for plotting in Figure 7A. Experiments using receptor antagonists suggest at most a small, insufficient role for G\textsubscript{i}-coupled adenosine receptors in transient depression under our recording conditions, but leave open the possibility of a greater contribution from this receptor type \textit{in vivo} or on a faster time scale (see discussion).

In an effort to confirm that glutamate and acetylcholine work together to trigger transient depression, we used the acetylcholinesterase inhibitor eserine to enhance the action of acetylcholine during conditioning trains. In experiments where transient depression was not observed following conditioning trains at baseline, we found that bath application of eserine (5 µM) enabled depression to occur (\( n = 3 \); Figure 7B). The effect of eserine was reversed by atropine (10 µM) in each experiment. Conditioning train stimulus intensities used during these experiments were similar to those used in control experiments in Figure 7A (5.3 ± 0.7 nC). These
findings, taken together with data presented in Figures 6 and 7A, support our conclusion that the combined action of evoked glutamate and acetylcholine release in stratum radiatum depresses glutamate release from Schaffer collateral synapses. The data raise the possibility that spatial and temporal convergence of glutamatergic and cholinergic inputs to CA1 may help to shape hippocampal network activity in vivo (see schematic, Figure 8).
DISCUSSION

We have studied a synaptically elicited transient depression of glutamatergic neurotransmission at Schaffer collateral synapses in area CA1 using acute slice electrophysiology and Ca$^{2+}$ fluorescence imaging. This depression was induced by a brief conditioning train of electrical stimulation in stratum radiatum and lasted 2–3 minutes. NMDAR- and composite EPSCs were depressed similarly, and depression coincided with an increase in the paired-pulse ratio (PPR), consistent with a presynaptic locus of depression. Eliciting transient depression was most easily achieved when both mGluRs and mAChRs were activated, suggesting a cooperative interaction involving a common G-protein subtype. Although transient depression correlates with postsynaptic Ca$^{2+}$ release from internal stores mediated by these same receptor types, it does not require increases in postsynaptic [Ca$^{2+}$] or other forms of postsynaptic or network signaling that we tested. The involvement of two neurotransmitters—elicited with modest synaptic activation—in triggering transient depression is consistent with numerous behavioral, systems, and theoretical reports suggesting a functional importance for convergent glutamatergic and cholinergic afferents to area CA1 (Hasselmo 1999; Hasselmo and Schnell 1994).

Although it is notoriously difficult to be certain whether changes in synaptic transmission are expressed presynaptically and/or postsynaptically, our data, in toto, are strongly consistent with a decrease in glutamate release from presynaptic terminals. First, the amplitude and time course of the depression were remarkably similar for two species of ionotropic glutamate receptors, consistent with an overall decrease of glutamate available to postsynaptic receptors. Second, there was a transient increase in the PPR with a time course that closely matched the
time course of the transient depression, consistent with a decrease in the probability of release of glutamate from the axon terminal. Slight deviations between the time courses of PPR increase and amplitude depression are likely due to factors other than P, that affect PPR measurements (e.g. AMPAR desensitization and differential sensitivity of silent synapses) (Colquhoun et al. 1992; Fernandez de Sevilla et al. 2002; Cabezas and Buno 2006). Finally, no changes were observed in postsynaptic cells during transient depression (e.g. R_IN, V_m), and a multitude of experimental manipulations known to block plasticity at postsynaptic sites or the release of retrograde messengers failed to prevent transient depression.

Previous studies have reported altered NMDAR function due to G_q-coupled receptor activation and rises in [Ca^{2+}]_i (Grishin et al. 2004; Markram and Segal 1990). In the present study we observed a G_q-coupled receptor-mediated depression of NMDAR and composite EPSCs that correlated with a robust rise in postsynaptic [Ca^{2+}]_i due to internal Ca^{2+} release. We demonstrate, however, that these two phenomena are not causally related in our experiments, because blocking rises in [Ca^{2+}]_i with high concentrations of BAPTA (50 mM), antagonizing postsynaptic G-proteins, or depleting intracellular Ca^{2+} stores failed to block transient depression. These previous studies, through direct activation of postsynaptic NMDARs by pressure application of agonists, may have unmasked a distinct postsynaptic effect of internal Ca^{2+} release on NMDARs by disengaging the presynapse. Also, the use of mGluR and mAChR agonist applications in these studies may have provided a stronger and more widespread stimulus of G_q-coupled receptors on the recorded cell than that achieved during our conditioning trains. The findings from these previous studies and the findings reported here suggest the possibility of interplay between presynaptic and postsynaptic effects of metabotropic receptor activation in the
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control of network activity in CA1, and the potential importance of factors that regulate these effects independently.

Others have reported a depression of glutamate release from Schaffer collateral nerve terminals following activation of presumed presynaptic G-protein-coupled receptors. Some showed that repeated trains of stimulation in the stratum oriens/alveus depress glutamate release through activation of presynaptic M1 acetylcholine receptors (Fernandez de Sevilla and Buno 2003; Fernandez de Sevilla et al. 2002). The present study builds on this work by describing a role for acetylcholine released during a single train and its cooperation with synaptically released glutamate. Other studies describe depression that depends partly on presynaptic adenosine receptor activation following conditioning stimulation (Grover and Teyler 1993b; Manzoni et al. 1994; Sekino and Koyama 1992), and one reported that adenosine release was dependent on NMDAR activation (Manzoni et al. 1994). In contrast, the transient depression we have studied occurs despite blockade of NMDARs or adenosine receptors. A likely explanation for this discrepancy is that we are studying the adenosine receptor-independent component of the depression they observed, and that the physiologic effects of adenosine on the presynapse occur on a shorter time scale than the effects of G_q-coupled receptor activation (Grover and Teyler 1993b; Mitchell et al. 1993). A greater or longer-lasting role for adenosine in previous studies may depend on a greater level of adenosine release achieved through stronger and spatially more extensive stimulation, or other difference in experimental conditions (Manzoni et al. 1994; Sekino and Koyama 1992). Similar to Manzoni et al., we found that transient depression was difficult to elicit after long-term blockade of NMDARs (antagonists present for 1–4 hours; n = 4; unpublished observations). This preliminary finding may indicate some role—perhaps a
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permissee one—of tonic or ongoing NMDAR activation for transient depression, and may warrant further study.

We favor a mechanism of transient depression wherein intrinsic glutamatergic signaling interacts with extrinsic cholinergic signaling, and the signals converge at the level of $G_q$ activation in the presynaptic terminal. This potential mechanism would be consistent with numerous studies demonstrating the importance of cholinergic input from the basal forebrain in hippocampal function (Aigner 1995; Dragoi et al. 1999; Hasselmo 1999), and with the capacity of G-protein-coupled receptors to converge on the same G-protein class and/or the same target molecule (Hille 1992). While our data indicate that evoked, rather than tonic, release of glutamate and acetylcholine trigger transient depression, they are not conclusive about the source of acetylcholine afferent input. More specifically, the source of acetylcholine axons might be cholinergic interneurons intrinsic to the hippocampus (Frotscher et al. 2000) or extrinsic inputs originating in the medial septum or diagonal band of Broca (Lewis and Shute 1967) (Frotscher and Leranth 1985). Finally, while we find that depression is presynaptic and can occur when certain types of signaling are antagonized in the recorded neuron and in neighboring cell types, we cannot exclude entirely that metabotropic receptors on neighboring pyramidal cells or other cell types may mediate this depression through a more complex mechanism.

**Functional Significance**

Our study reveals that the effects of glutamatergic and cholinergic signals in CA1 combine to depress EPSCs by attenuating neurotransmitter release. Cholinergic inputs from the basal forebrain have long been known to influence the ability of the hippocampal formation to
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participate in some forms of memory by affecting hippocampal network properties, hippocampal theta rhythm, and the induction of LTP (Bland et al. 1999; Louie and Wilson 2001; Otto et al. 1991; Pavlides et al. 1988; Winson and Abzug 1978). The transient depression we have studied may affect LTP induction by limiting ionotropic and metabotropic glutamatergic activation thereby limiting rises in \([\text{Ca}^{2+}]_i\) normally mediated by influx through NMDA channels and mGluR activated internal \(\text{Ca}^{2+}\) release (Morris et al. 1986; Yeckel et al. 1999). The possibility of cooperative action between acetylcholine and glutamate raises the possibility of depression at specific subsets of synapses with certain activity patterns or anatomical arrangements. Furthermore, were transient depression to be coincident with the potentially more general excitatory postsynaptic effects of acetylcholine (Cole and Nicoll 1983; Colino and Halliwell 1993; Dodd et al. 1981; Halliwell 1990), the combined effect could be to alter the distribution of synaptic weights along the dendrite, postsynaptic computations, and the induction of long-term synaptic plasticity at distant synapses as well.
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FIGURE LEGENDS

Figure 1. Transient depression of EPSCs follows conditioning trains in stratum radiatum.
A. EPSC amplitude is reduced following conditioning trains. Example traces are averages of 3 consecutive NMDAR-mediated test responses 20–60 s before (baseline), 20–60 s after, and 160–200 s following (recovery) a synaptic conditioning train. B. Data averaged across 83 experiments illustrate the magnitude and time-course of transient depression. Recovery is fit by a monoexponential curve (gray line, $\tau = 76.5$ s). SEM error bars are smaller than the plot symbols. C. Depression can be elicited repeatedly over a long experiment without extinction. This plot of raw EPSC amplitudes over time illustrates three consecutive trials of depression after 45 minutes of recording and 5 previous episodes of depression. D. While most experiments in this study were performed with GABA$_A$R antagonism and without extracellular Mg$^{2+}$, normal transient depression was also observed without GABA$_A$R antagonism (open symbols, $n = 17$) and with 2 mM Mg$^{2+}$ and no GABA$_A$R or GABA$_B$R antagonism (filled symbols, $n = 5$). All experiments in which GABA$_B$Rs were left unblocked ($n = 14$) are included in the two groups of data plotted here.

Figure 2. Transient depression results from a decrease in presynaptic glutamate release.
A. Transient depression affects AMPAR- and NMDAR- mediated postsynaptic currents similarly. The plot shows depression of composite (open symbols, $n = 19$) and NMDAR-mediated EPSCs (filled symbols, $n = 64$). Together, these data are the same as Figure 1A, and the gray line is an exponential fit to the combined data ($\tau = 76.5$ s). B. The paired-pulse ratio (PPR) increases during depression for both composite ($n = 19$) and NMDAR-mediated EPSCs ($n = 52$), and the time course of the two phenomena is similar. Gray lines illustrate the time-course
similarity between amplitude depression and PPR increase, and are exponential fits to PPR data that are constrained to the time course of recovery from depression ($\tau = 76.5 \text{ s}$). C. Example traces are averages of 3 consecutive responses to paired test stimulation (50 ms interval) before and after a synaptic conditioning train, illustrating depression of composite EPSCs and the change in paired-pulse ratio (PPR) that accompanies depression. Right, the “after train” trace (gray) is scaled and overlayed on the baseline trace to show the relative difference in the amplitude of the second response.

**Figure 3. Neurotransmission on an independent test pathway can also be depressed.**

A. Two stimulating electrodes were placed in stratum radiatum (s.r.) at 50 and 100 $\mu$m from stratum pyramidale (s.p.) to stimulate independent glutamatergic inputs to the recorded cell. A conditioning train (30 stimuli at 100 Hz) was delivered on the conditioning pathway while test responses were followed over time on each pathway. s.o. = stratum oriens B. Test for independence of the stimulus pathways. Traces are averages of 10 responses where a paired stimulus (50 ms interval) on the test pathway was used to establish naïve and potentiated responses (black traces). A test response (gray) was preceded by a stimulus on the conditioning pathway. The test response was not distinguishable from the naïve response, consistent with non-overlapping axonal pathways. C. Summary plot of depression observed on an independent test pathway (open symbols, n = 11) and on the conditioning pathway (filled symbols, n = 78).

**Figure 4. Transient depression does not require postsynaptic internal Ca$^{2+}$ release.**

A. Conditioning trains frequently elicit postsynaptic Ca$^{2+}$ waves. Left, Image of a CA1 pyramidal neuron filled with bis-fura-2 (100 $\mu$M) and Alexa 488 (15 $\mu$M), plus colored regions
of interest (ROIs). A stimulating electrode filled with Alexa 488 (2.5 µM) is visible in the upper left. Right, Ca\(^{2+}\) imaging: colored traces correspond to relative [Ca\(^{2+}\)]\(_i\) changes in ROIs. Current trace (black): a train (30 stimuli at 100 Hz) of synaptic stimulation elicits an intracellular Ca\(^{2+}\) wave. B. The occurrence of transient depression correlates with postsynaptic internal Ca\(^{2+}\) release. The bar widths represent the number of trials in 25 recorded neurons with depression (57 trials, top bar) or no depression (55 trials, bottom bar). The placement of the bar along the abscissa indicates the fraction of trials in which internal Ca\(^{2+}\) release was observed (left of center) or not observed (right of center). C. Depression was observed in experiments where postsynaptic Ca\(^{2+}\) waves were blocked verifiably. The Ca\(^{2+}\) chelator BAPTA (black symbols, 10 or 50 mM, n = 24) and/or G-protein antagonist GDP-β-S (red symbols, 1 mM, n = 14) was included in the patch pipette for 30–75 minutes of recording, or SERCA antagonists were bath applied (blue symbols, wash-in of 50 µM CPA or preincubation with 3 µM thapsigargin, n = 5). D. The SERCA antagonist CPA (50 µM) blocks internal Ca release. A conditioning train evokes a propagating Ca\(^{2+}\) wave in control conditions (top optical and current traces) but not when CPA is present. During this experiment AMPAR-mediated responses were isolated with antagonists of GABARs (50 µM Gabazine, 1 µM CGP55845) and NMDARs (100 µM DL-APV).

Figure 5. The diffusible messengers nitric oxide and endocannabinoids do not mediate transient depression.

A. Nitric oxide (NO) antagonists do not prevent transient depression. NMDAR antagonists 100 µM D.L-APV and 20 µM MK-801 were bath applied to inhibit NMDAR activation during the conditioning train and NO production (open symbols, n = 5). The Ca\(^{2+}\)-calmodulin antagonist 50 µM Calmidazolium was applied by bath or by preincubation to inhibit NO production (black symbols, n = 3). The nitric oxide synthase inhibitor 100 µM L-NAME and NO scavenger 30 µM
carboxy-PTIO were applied together as a preincubation (gray symbols, n = 5). B. NMDAR antagonists block a slow component of composite EPSCs. Traces are averages of 10 consecutive responses before (black) and after (gray) APV and MK-801 application. Traces are scaled to illustrate the difference in time course, and stimulus intensity was different between the two conditions. C. The endocannabinoid CB1R antagonist AM251, applied as a preincubation at 2 µM (n = 1) or 5 µM (n = 4) does not prevent transient depression.

Figure 6. Both mGluRs and mAChRs participate in transient depression.

A. Depression was not blocked following preincubation with the adenosine receptor antagonist DPCPX (0.5–5 µM, black symbols, n = 7), mGluR antagonists (100 µM LY367385 and 10 µM MPEP, open symbols, n = 5), or the mAChR antagonist atropine (10 µM, gray symbols, n = 3).

B. Preincubation with combinations of the antagonists in A had different effects on transient depression. Depression was blocked when mGluR and mAChR activation were blocked simultaneously (black symbols, n = 6), but depression was observed during blockade of adenosine receptors (5 µM DPCPX) plus either mGluRs (open symbols, n = 3) or mAChRs (gray symbols, n = 3). C. An increase in the paired-pulse ratio of AMPAR EPSCs accompanied bath application of the group I mGluR agonist 50 µM DHPG, consistent with a presynaptic location of this receptor type (n = 3). Measurements were combined in 1 min bins for plotting, and data were aligned according to the onset of DHPG action. D. 5 µM DPCPX blocks the synaptic depression induced by a pressure application (puff) of 200 µM adenosine, verifying that DPCPX blocks adenosine receptors in our experiments (n = 3).
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**Figure 8. Schematic of convergent afferents to CA1.**

Our data support a model in which cholinergic and glutamatergic afferents converge in area CA1 to produce a transient presynaptic depression and postsynaptic Ca waves. Stratum radiatum stimulation elicits release of acetylcholine by axons from basal forebrain nuclei or from hippocampal cholinergic interneurons. The same stimuli evoke glutamate release from Schaffer collateral axons originating in CA3 and perhaps from axons from basal forebrain nuclei (Allen et al. 2006; Colom et al. 2005). Released acetylcholine and glutamate act in unison on presynaptic and postsynaptic Gq-coupled receptors.
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