# Modulation of excitatory synaptic transmission by low concentrations of glutamate in cultured rat hippocampal neurons

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1. The effects of low micromolar concentrations of glutamate on fast excitatory synaptic responses were studied in microcultures of postnatal rat hippocampal neurons using whole-cell patch clamp recordings.

2. Glutamate depressed the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor component of excitatory autaptic currents (EACs) with an $EC_{50}$ of 3·8 μM.

3. Both pre- and postsynaptic effects contributed to the depression of AMPA receptor-mediated EACs. Cyclothiazide and wheatgerm agglutinin, agents which inhibit AMPA receptor desensitization, partially reversed the depression produced by glutamate, as did pertussis toxin, an agent that blocks presynaptic inhibition mediated by metabotropic glutamate receptors.

4. In neurons in which both the AMPA and *N*-methyl-D-aspartate (NMDA) receptor components of EACs were examined, low concentrations of glutamate depressed the NMDA component of EACs to a greater extent. The $EC_{50}$ for inhibiting the NMDA component was 1·3 μM.

5. Calcium-dependent desensitization of postsynaptic NMDA receptors contributed to the depression of NMDA receptor-mediated synaptic responses. Both depolarization of postsynaptic neurons to +70 mV to decrease $Ca^{2+}$ influx via NMDA channels and inclusion of high concentrations of a calcium chelator in recording pipettes decreased the depression of NMDA receptor-mediated EACs.

6. Threo-3-hydroxy-aspartate (THA), an inhibitor of glutamate transport, depressed EACs by about 10% and increased the degree of depression produced by 2·5 μM glutamate, suggesting that glutamate transport in microcultures helps to control ambient glutamate levels.

7. Because the normal extracellular concentration of glutamate is about 1 μM, these results suggest that the ambient glutamate level is an important determinant of synaptic efficacy. Relatively small changes in extracellular glutamate can alter fast excitatory synaptic transmission by both presynaptic and postsynaptic mechanisms.

Under normal conditions, extracellular concentrations of glutamate in the mammalian CNS are about 1 μM (Benveniste, Drejer, Schousboe & Diemer, 1984; Lerma, Herranz, Herraras, Abraira & Martin del Rio, 1986). In contrast, free intracellular glutamate concentrations are about 10 mM with concentrations of 60–150 mM in synaptic vesicles (Burger *et al.* 1989). The low extracellular glutamate levels are maintained by electrogenic glutamate transporters that use the ionic gradients from $Na^+$ and $K^+$ to drive glutamate influx against a large concentration gradient (Danbolt, 1994). Because extracellular glutamate levels are likely to fluctuate during physiological and pathological processes (Bouvier, Szatkowski, Amato & Attwell, 1992), it is important to understand how changes in ambient glutamate levels influence excitatory synaptic transmission.

At concentrations near 1 μM, glutamate is likely to exert significant postsynaptic effects on synaptic transmission. Both the AMPA and NMDA receptors that participate in fast excitatory synaptic transmission are subject to desensitization, and low micromolar concentrations of glutamate are sufficient to desensitize a significant proportion of both receptor types. In studies using isolated membrane patches, 2–10 μM glutamate desensitizes 50% of AMPA receptors (Trussell & Fischbach, 1989; Colquhoun, Jonas & Sakmann, 1992) whereas concentrations $\leqslant$1 μM desensitize 50% of NMDA receptors (Sather, Dieudonne, MacDonald & Ascher,

1992). These concentrations are at the low end of the dose–response curve for glutamate activating macroscopic currents via these receptors, and both AMPA and NMDA receptors exhibit greater steady-state desensitization at agonist concentrations which gate macroscopic responses (Trussell & Fischbach, 1989; Lin & Stevens, 1994).

Low micromolar concentrations of glutamate could also alter synaptic transmission by activating presynaptic metabotropic receptors that inhibit glutamate release. Forsythe & Clements (1990) demonstrated that glutamate depresses fast excitatory synaptic responses presynaptically in cultured mouse hippocampal neurons at concentrations near 1 $\mu$M. This effect is mimicked by the metabotropic glutamate receptor agonists L-2-amino-4-phosphonobutyrate (L-AP4) (Forsythe & Clements, 1990) and 1*S*,3*R*-1-amino-cyclopentane-1,3-dicarboxylic acid (1*S*,3*R*-ACPD) (Baskys & Malenka, 1991). Recently, Maki *et al.* (Maki, Robinson & Dichter, 1994) observed that pyrrolidine dicarboxylic acid (PDC), an inhibitor of glutamate uptake, diminishes glutamate-mediated synaptic transmission presynaptically by presumably causing accumulation of extracellular glutamate and activating metabotropic receptors. The effects of PDC were mimicked by 2 $\mu$M glutamate.

Taken together, these results suggest that ambient glutamate levels have important effects on synaptic transmission by both pre- and postsynaptic mechanisms. To investigate the effects of changes in ambient glutamate levels on synaptic responses, we used a preparation of rat hippocampal neurons grown in a micro-island culture environment (Segal & Furshpan, 1990). This preparation allows study of glutamate-mediated synaptic transmission under voltage clamp conditions where there is no influence of polysynaptic activity and where glutamate concentrations can be controlled more precisely.

## METHODS

### Hippocampal cultures

Hippocampal neurons were dissociated from 1- to 3-day-old albino rat pups and grown as micro-island cultures using methods described previously (Segal & Furshpan, 1990; Mennerick & Zorumski, 1995*a*). Briefly, rat pups were anaesthetized with halothane (0·5–1·0%) and rapidly decapitated. The hippocampi were immediately dissected and dissociated using 1 mg ml$^{-1}$ papain in Leibovitz L-15 medium and mechanical trituration. Cells were plated in Eagle's minimal essential medium (MEM) supplemented with 5% horse serum, 5% fetal calf serum, 17 mM D-glucose, 0·4 mM glutamine, 50 u ml$^{-1}$ penicillin and 50 $\mu$g ml$^{-1}$ streptomycin at a density of 20000 cells ml$^{-1}$ in 35 mm plastic culture dishes. The culture dishes had previously been coated with a layer of 0·15% agarose and sprayed with droplets of 0·5 mg ml$^{-1}$ rat tail collagen (Type I) using a microatomizer. Glial proliferation was halted after 72 h in culture using 10 $\mu$M cytosine arabinoside. Cells were used for study between 8 and 14 days *in vitro*.

### Whole-cell recording

Autaptic and monosynaptic currents were obtained from visually identified single-neuron and two-neuron micro-islands, respectively, using whole-cell patch clamp recordings. Neurons were visualized using an inverted microscope equipped with phase-contrast optics. Patch pipettes were pulled from borosilicate glass and had resistances of 3–6 MΩ after fire polishing. For autaptic studies and for presynaptic recordings from pairs of neurons, the pipette solution contained (mM): 140 potassium gluconate, 4 NaCl, 5 EGTA, 0·5 $CaCl_2$, 10 Hepes, 2 Mg-ATP and 0·5 $Na_2$-GTP, at pH 7·3. In recordings from neuronal pairs, the pipette solution in the postsynaptic cell contained (mM): 140 caesium methanesulphonate, 5 NaCl, 5 EGTA, 0·5 $CaCl_2$, 2 Mg-ATP, 0·5 $Na_2$-GTP and 10 Hepes, at pH 7·3.

Autaptic currents were studied using an Axopatch-1D amplifier. This amplifier was also used for studying postsynaptic neurons during dual recordings while an Axoclamp-2A amplifier (both from Axon Instruments) was used to study presynaptic neurons. For these studies, series resistance was typically compensated by 70–90% using the circuitry of the Axopatch amplifier. Autaptic currents and presynaptic currents were evoked using 1·5 ms voltage steps from −70 to +20 mV. In most experiments, single synaptic responses were evoked every 15 s to avoid the depression and facilitation that can occur with more frequent synaptic activation (Mennerick & Zorumski, 1995*b*).

For evoked synaptic studies, the culture medium was replaced with a solution containing (mM): 140 NaCl, 4 KCl, 10 Hepes, 10 D-glucose, 2 $CaCl_2$, 1 $MgCl_2$ and 0·05–0·1 D-2-amino-5-phosphonovalerate (APV), at pH 7·3. APV was added to eliminate NMDA responses and to allow study of AMPA responses in isolation. For studies examining the NMDA component of synaptic responses, APV and $Mg^{2+}$ were removed from the extracellular solution and 5–20 $\mu$M glycine was added. Extracellular perfusion and drug applications were accomplished using a multi-barrel gravity-fed system that allows complete solution exchange over micro-islands in less than 1 s. For rapid exogenous glutamate applications, a gravity-driven flow-tube system with 100 $\mu$m diameter drug delivery pipettes positioned about 200 $\mu$m away from the recorded cell was used. In these flow-tube experiments, 0·5 $\mu$M tetrodotoxin (TTX) was added to the bath solution to diminish spontaneous activity.

Miniature excitatory postsynaptic currents (mEPSCs) were studied in the same extracellular solution containing 0·5–1 $\mu$M TTX, to block action potential-mediated synaptic transmission, and 25 $\mu$M bicuculline, to block GABA-mediated synaptic responses. The caesium methanesulphonate intracellular solution described above was used for all mEPSC studies. Continuous data segments were filtered at 2 kHz and stored on videotape for off-line analysis after digitization at a rate of 5–10 kHz. Miniature events were identified using a peak detection threshold of 2–3 standard deviations above the baseline noise and events detected automatically were visually confirmed to eliminate false positives caused by temporary changes in the baseline noise (Mennerick *et al.* 1995*b*).

Most salts and chemicals were obtained from Sigma. Exceptions to this are: 8-cyclopenty-1,3-dipropylxanthine (CPDPX) and (*RS*)-α-methyl-4-carboxyphenylglycine (MCPG) (Research Biochemicals Inc.), D-APV (Cambridge Research Biochemicals), 1*S*,3*R*-ACPD (Tocris Neuramin), cyclothiazide (gift from Eli Lilley), caesium methanesulphonate (Aldrich) and 6-nitro-7-sulphamoylbenzo(*f*)-quinoxaline-2,3-dione (NBQX) (gift from Dr John Olney, Washington University School of Medicine, St Louis, MO, USA).

### Data analysis

Evoked synaptic currents were filtered at 2 kHz and digitized at 5 kHz using routines written in the Axobasic language (Axon

Instruments). Evoked currents represent the average of between two and five individual responses. Because synaptic currents sometimes exhibited irreversible rundown over the course of an experiment, experimental results were compared with control responses averaged before and after the experimental manipulation. Data were analysed using routines written in Axobasic or using the pCLAMP software package (Axon Instruments). Currents were quantified in terms of the peak response (measured from the steady-state baseline either in control solution or in the presence of glutamate) and the charge transfer of isolated AMPA and NMDA receptor-mediated synaptic responses. Similar results were obtained with either method, so results are usually expressed in terms of peak autaptic currents. Measuring peak autaptic currents from the pre-stimulus holding current introduces a small error due to the summation of autaptic currents with decaying stimulus transients. Based on subtractions obtained in the presence of 3 $\mu$M NBQX from control autaptic responses, the overestimation of peak amplitude is $<5\%$ ($3{\cdot}9 \pm 0{\cdot}7\%$, $n = 12$).

In studies examining dual component synaptic responses, the NMDA component was measured as an average of 5 ms, beginning 50 ms after the peak of the AMPA response. Results are expressed as means $\pm$ S.E.M. Group means were compared by two-tailed Student's $t$ tests. Dose–response curves were fitted to data using a least-squares minimization routine (SigmaPlot, Jandel Scientific Inc.) with an equation of the form:

$$\text{Response} = 100 \times \{1 - ([\text{agonist}]^{N_H}/[\text{agonist}]^{N_H} + EC_{50}{}^{N_H})\},$$

where $EC_{50}$ is the half-maximal effective concentration and $N_H$ is the Hill coefficient.

### Kinetic modelling

A kinetic model of hippocampal AMPA receptors (Jonas, Major & Sakmann, 1993) was used to explore some features of receptor gating and desensitization. All rate constants were identical to those used by Jonas *et al.* (1993) in the unconstrained version of the model. The probability of a channel being open or desensitized was followed over time using the technique of Ambros-Ingerson & Lynch (1993) with a program written in Visual C. For calculation of the probability that a channel was desensitized, the probabilities of a channel being in each of the three desensitized states of the model (Jonas *et al.* 1993) were summed.

## RESULTS

### Glutamate depresses excitatory synaptic responses

At concentrations $\geqslant 0{\cdot}5$ $\mu$M, glutamate depressed evoked AMPA receptor-mediated excitatory autaptic currents (EACs) (Fig. 1). The effects of glutamate were consistent within single cells but showed considerable cell-to-cell variability. For example, the effects of 2·5 $\mu$M glutamate ranged from no inhibition to 94% depression of peak synaptic currents ($n = 52$). In a series of neurons in which glutamate was examined over the range of 0·1–10 $\mu$M, the depression of peak EACs was dose dependent with an $EC_{50}$ of 3·8 $\mu$M (Fig. 1). Similar effects were seen on the synaptic charge transfer ($EC_{50} = 3{\cdot}0$ $\mu$M).

### Postsynaptic contributions to glutamate-mediated depression

Under conditions in which AMPA receptor-mediated EACs were studied in isolation (by inclusion of 50 $\mu$M D-APV in the extracellular solution), glutamate activated inward currents at concentrations $\geqslant 1$ $\mu$M (Fig. 1). These currents ranged in steady-state amplitude from $<5$ pA to about 250 pA. Since AMPA receptors are subject to substantial desensitization at concentrations $\geqslant 1$ $\mu$M and desensitization is most prominent at agonist concentrations that activate macroscopic currents (Trussell & Fischbach, 1989; Colquhoun *et al.* 1992) this suggests that desensitization of postsynaptic AMPA receptors may play an important role in glutamate-mediated depression.

To examine the role of AMPA receptor desensitization in glutamate synaptic depression, we used cyclothiazide (CYZ), an agent that decreases AMPA receptor desensitization at concentrations $\geqslant 1$ $\mu$M (Yamada & Tang, 1993). A major difficulty in using CYZ for our studies was that in the presence of CYZ, low micromolar concentrations of glutamate activated large offset currents. In some instances these offset currents occluded the ability to record synaptic responses. Thus, both the concentration of CYZ and glutamate had to be titrated individually for these experiments. The combination of 2·5 $\mu$M CYZ and 2·5 $\mu$M glutamate produced offset currents that ranged from 13 to 115 pA ($n = 8$). In these cells, CYZ diminished the depression of peak EACs produced by glutamate from $-35 \pm 3$ to $-23 \pm 4\%$ ($P < 0{\cdot}02$) (Fig. 2). Although CYZ has been reported to have effects on metabotropic glutamate receptors (Sharp, Mayne & Burnett, 1994) and may augment glutamate release presynaptically (Diamond & Jahr, 1995; but see Mennerick & Zorumski, 1995*a*), we found that 2·5 $\mu$M CYZ diminished the inhibitory effects of 1·5 $\mu$M AMPA on EACs from $-27 \pm 4$ to $-12 \pm 4\%$ ($n = 16$, $P < 0{\cdot}005$), but did not alter the depression produced by 100 $\mu$M L-AP4 ($-20 \pm 4$ *vs.* $-23 \pm 5\%$, $n = 6$, $P > 0{\cdot}20$) or 100 $\mu$M 1*S*,3*R*-ACPD ($-35 \pm 10$ *vs.* $-36 \pm 10\%$, $n = 7$, $P > 0{\cdot}50$) (Fig. 2).

Wheatgerm agglutinin (WGA; 25 $\mu$g ml$^{-1}$), a lectin that diminishes AMPA receptor desensitization (Thio, Clark, Clifford & Zorumski, 1992) also decreased the depression produced by glutamate (Fig. 2). In eleven neurons, WGA diminished the depression of synaptic currents produced by 2·5 $\mu$M glutamate from $-36 \pm 7$ to $-21 \pm 7\%$ ($P < 0{\cdot}01$).

### Presynaptic contributions to glutamate-mediated depression

Glutamate has previously been shown to act at presynaptic metabotropic receptors to diminish its own release (Forsythe & Clements, 1990), making it possible that presynaptic factors contribute to the decrement in EACs. In some, but not all, preparations the presynaptic effects of glutamate are inhibited by MCPG, a weak but fairly selective metabotropic receptor antagonist (Baskys & Malenka, 1991; Maki *et al.* 1994). In our previous studies, we found that 500 $\mu$M MCPG was only weakly effective against presynaptic inhibitory effects of L-AP4 and ACPD, agonists at presynaptic metabotropic receptors in the hippocampus (Mennerick & Zorumski, 1995*b*). Similarly, we

found that 500 $\mu$M MCPG had no effect on the synaptic depression produced by 2·5 $\mu$M glutamate (−2 ± 4% change in depression, $n = 4$, $P > 0{\cdot}10$).

The presynaptic inhibitory effects of adenosine, L-AP4 and ACPD are blocked by pretreating hippocampal cultures with 500 ng ml$^{-1}$ pertussis toxin (PTX) for 24 h (Mennerick & Zorumski, 1995*b*). Consistent with this, we found that PTX decreased the depression produced by 0·5 $\mu$M 2-chloro-adenosine (2-CA), a potent agonist at presynaptic adenosine A1 receptors (−69 ± 5% depression in control cultures, $n = 8$, *vs.* −3 ± 4% depression in PTX-treated cultures, $n = 11$, $P < 0{\cdot}001$) and 100 $\mu$M 1*S*,3*R*-ACPD (−42 ± 6%, $n = 12$, *vs.* −1 ± 1%, $n = 5$, $P < 0{\cdot}005$), but not that produced by 2·5 $\mu$M AMPA (−34 ± 7%, $n = 7$, *vs.* −39 ± 3%, $n = 5$, $P > 0{\cdot}5$) (Fig. 3). PTX also diminished the depression of peak EACs by both 1 and 2·5 $\mu$M glutamate (−41 ± 6% depression in 1 $\mu$M glutamate in control cultures, $n = 14$, *vs.* −10 ± 2% depression after PTX, $n = 14$, $P < 0{\cdot}01$; −48 ± 4% depression by 2·5 $\mu$M glutamate in control, $n = 17$, *vs.* −18 ± 6% depression after PTX, $n = 18$, $P < 0{\cdot}01$) (Fig. 3). Because the degree of depression induced by glutamate was somewhat greater in these control cultures compared with the data shown in Fig. 1, we also compared the degree of depression in PTX-treated cells with all cells exposed to 1 or 2·5 $\mu$M glutamate in the absence of other drug treatment (−25 ± 3% depression in 1 $\mu$M glutamate, $n = 69$, $P = 0{\cdot}01$ compared with PTX-treated cells; −41 ± 2% depression in 2·5 $\mu$M glutamate, $n = 82$, $P < 0{\cdot}001$ compared with PTX-treated cells).

PTX is a non-selective antagonist that inhibits the actions of several agents that depress synaptic transmission via certain G-proteins. It is thus possible that glutamate receptor activation could evoke release of adenosine which, in turn, exerts presynaptic inhibitory effects (Manzoni, Manabe & Nicoll, 1994). To exclude an effect of glutamate acting indirectly through adenosine $A_1$ receptors, we examined whether glutamate depression could be inhibited by the potent adenosine $A_1$ receptor antagonist CPDPX. At 1 $\mu$M, CPDPX had no effect on the depression of AMPA

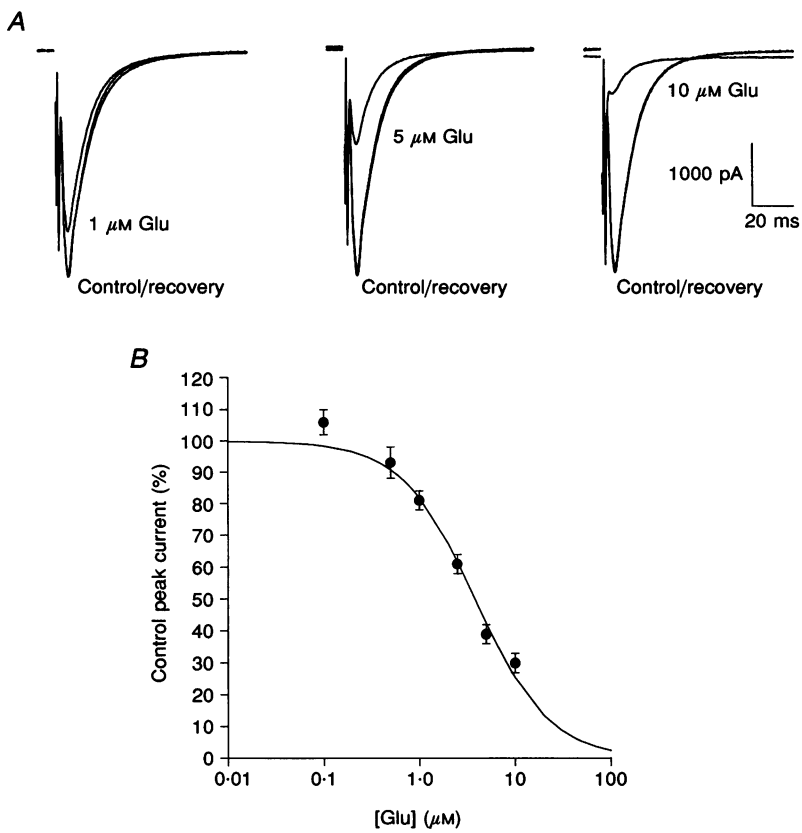


**Figure 1. Glutamate depresses excitatory synaptic transmission**

*A*, the effects of 1, 5 and 10 $\mu$M glutamate (Glu) on EACs in the same neuron. EACs were elicited by a 1·5 ms voltage jump from −70 to +20 mV as described in the Methods. For clarity, part of the stimulus artifact in this and other figures has been blanked. The shift in baseline current at 5 and 10 $\mu$M Glu reflects the steady-state offset current activated by glutamate. In this and all figures the displayed currents are the average of 2–5 individual traces. *B*, the dose dependence of glutamate-mediated depression of AMPA receptor EACs. Continuous lines represent the fit of the dose–response equation described in the Methods. $EC_{50} = 3{\cdot}8$ $\mu$M; $N_H = 1{\cdot}1$. Data points represent means ± S.E.M. of 7–39 cells.

receptor-mediated EACs produced by 2·5 μM glutamate (1 ± 3% increase in the depression produced by 2·5 μM glutamate, $n = 5$). However, 1 μM CPDPX completely blocked the presynaptic inhibitory effects of 0·5 μM 2-CA (−61 ± 6% depression in controls *vs.* +1 ± 2% change after CPDPX, $n = 5$, $P < 0{\cdot}001$).

To exclude an effect of PTX on postsynaptic AMPA receptor desensitization, we examined the desensitization produced by 200 ms flow-tube applications of 1 mM glutamate on neurons in control and PTX-treated cultures. In both sets of cultures, glutamate currents desensitized by >95% (98 ± 1% desensitization in control neurons, $n = 5$, *vs.* 97 ± 1% in PTX-treated neurons, $n = 5$, $P > 0{\cdot}10$) with time constants ranging from 10 to 20 ms.

Previous studies have shown that the degree of paired-pulse depression (PPD) exhibited by glutamate-mediated EACs in cultured hippocampal neurons depends on the initial probability of transmitter release (Mennerick & Zorumski, 1995*b*). Thus, it might be expected that glutamate would diminish the degree of paired-pulse depression by inhibiting glutamate release presynaptically. In control trials, EACs were depressed by −22 ± 3% at a paired-pulse interval of 100 ms in solutions containing 2 mM $Ca^{2+}$ and 1 mM $Mg^{2+}$ ($n = 69$). Over the range of 1–10 μM, glutamate

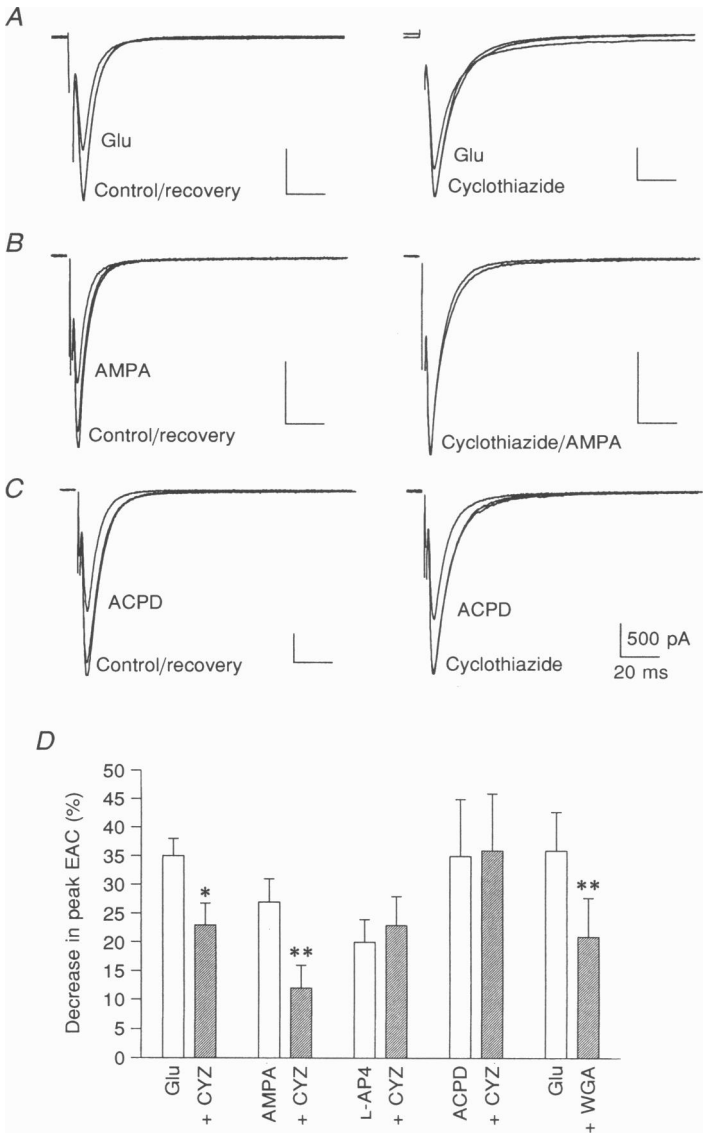


**Figure 2. Inhibition of AMPA receptor desensitization decreases glutamate-mediated depression**
Traces show the effects of 2·5 μM cyclothiazide (CYZ) on the depression of EACs in single neurons exposed to 2·5 μM Glu (*A*), 1·5 μM AMPA (*B*) and 100 μM 1*S*,3*R*-ACPD (*C*). *D*, a summary of the effects of 2·5 μM CYZ and 25 μg $ml^{-1}$ wheatgerm agglutinin (WGA) on the depression of peak EACs by 2·5 μM Glu. Effects of 2·5 μM CYZ on depression produced by 1·5 μM AMPA, 100 μM L-AP4 and 100 μM 1*S*,3*R*-ACPD are also shown. * $P < 0{\cdot}02$; ** $P < 0{\cdot}01$.

had variable effects on paired-pulse depression. At 1 $\mu$M, no effect was seen ($2 \pm 4\%$ increase in PPD, $n = 15$). At 2·5 $\mu$M, glutamate decreased the degree of PPD by $11 \pm 6\%$ compared with controls ($n = 14$, $P < 0{\cdot}05$), but no significant effects were observed at 5 or 10 $\mu$M glutamate. In contrast, 0·5 $\mu$M 2-CA, which inhibits EACs presynaptically, decreased the degree of PPD by $21 \pm 5\%$ ($n = 8$, $P < 0{\cdot}01$). Similarly, 1*S*,3*R*-ACPD (50–100 $\mu$M), an agonist at presynaptic metabotropic glutamate receptors, depressed baseline synaptic responses by $-38 \pm 3\%$ and decreased PPD by $19 \pm 4\%$ ($n = 8$, $P < 0{\cdot}01$).

The difficulty in obtaining significant effects of low glutamate concentrations upon PPD suggests that presynaptic effects may not be maximal at these concentrations. Although we were unable to examine presynaptic effects of glutamate in isolation because of the technical problems of using high CYZ concentrations to abolish receptor desensitization (see above), we performed a within-cell comparison of the depression elicited by 2·5 $\mu$M glutamate with the depression elicited by 100 $\mu$M 1*S*,3*R*-ACPD plus 100 $\mu$M L-AP4 to stimulate presynaptic receptors maximally. Despite postsynaptic contributions to depression with 2·5 $\mu$M glutamate (Fig. 2), the depression elicited by 2·5 $\mu$M glutamate was consistently less than that elicited by maximal metabotropic receptor stimulation ($-67 \pm 6\%$ by ACPD + L-AP4 *vs.* $-36 \pm 6\%$ by glutamate, $n = 8$, $P = 0{\cdot}01$). This indicates that despite the high agonist affinity of many G-protein-coupled receptors, presynaptic metabotropic receptors may require rather high concentrations of glutamate for maximal activation.

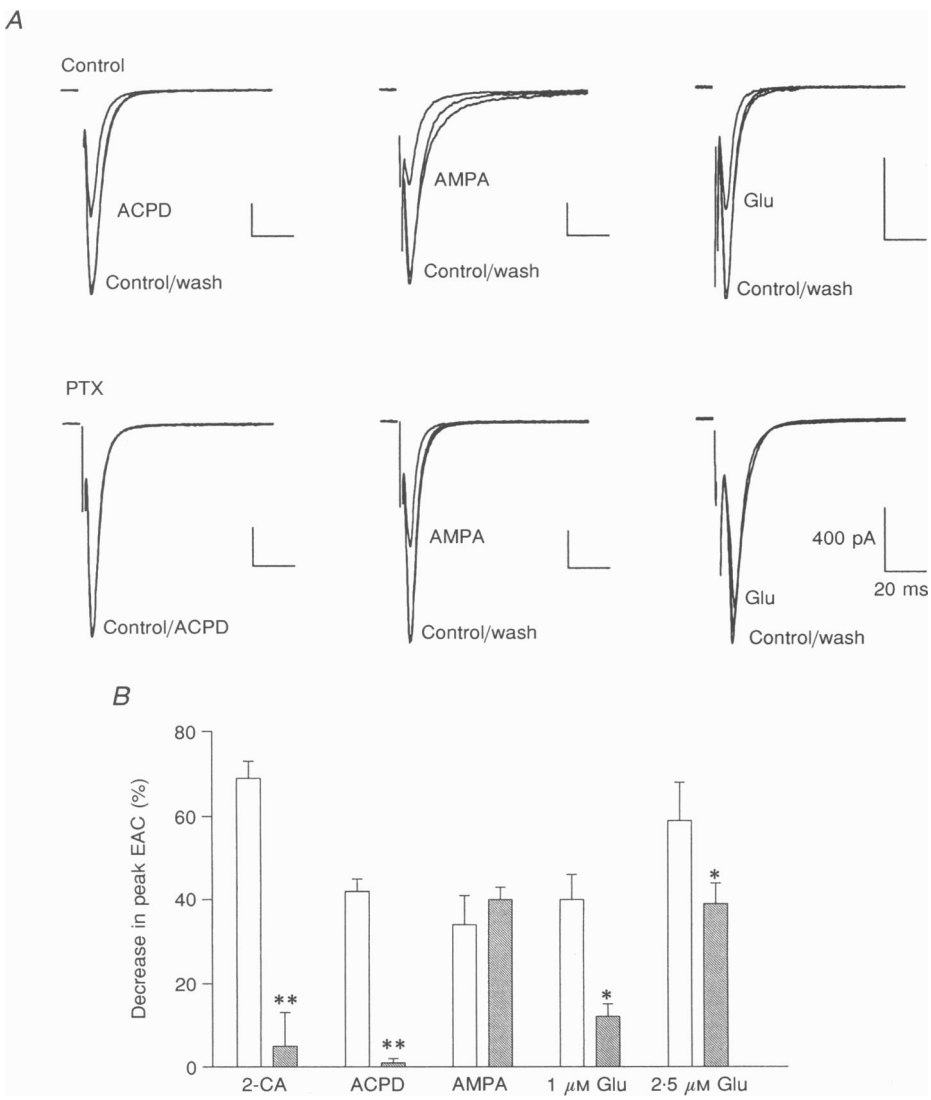


**Figure 3. PTX inhibits the depression of EACs by 2-CA, ACPD and Glu, but not AMPA**

*A*, traces show cells treated with 100 $\mu$M 1*S*,3*R*-ACPD, 2·5 $\mu$M AMPA and 2·5 $\mu$M Glu under control conditions and in cultures treated with 500 ng ml$^{-1}$ PTX for 24 h. The values of the calibration bar are the same for all traces. *B*, a summary of the effects of PTX. □, control; ▨, PTX. * $P < 0{\cdot}01$; ** $P < 0{\cdot}005$ compared with control.

### Effects of glutamate on mEPSCs

To examine pre- and postsynaptic contributions to glutamate's effects further we examined spontaneous mEPSCs in micro-island cultures. In control neurons, mEPSCs had peak amplitudes of 11·1 ± 0·8 pA, rise times (10–90%) of 0·9 ± 0·04 ms, half-decay times of 2·2 ± 0·1 ms and occurred at a frequency of 1·8 ± 0·3 mEPSCs $s^{-1}$ ($n = 21$) (Fig. 4*A*). At 1 μM, glutamate depressed mEPSC peak amplitudes and frequency by −11 ± 7% ($n = 11$, $P = 0·15$) and −11 ± 7%, respectively ($n = 11$, $P = 0·03$) (Fig. 4*A* and *C*). Greater effects on peak amplitude and frequency were observed at 2·5 μM (amplitude, −39 ± 7% change, $P = 0·004$; frequency, −36 ± 9% change, $P = 0·03$, $n = 10$) (Fig. 4*A–C*). Neither 1 nor 2·5 μM glutamate altered the rise time of mEPSCs (1 μM, −2 ± 3% change, $n = 11$; 2·5 μM, +8 ± 6% change, $n = 10$; $P > 0·20$ for each). The increase in membrane noise produced by glutamate made it difficult to record mEPSCs at concentrations >2·5 μM. Similarly, it was difficult to examine the effects of CYZ on mEPSCs in the presence of 1–2·5 μM glutamate because of the offset currents and increase in noise. However, the decrease in mEPSC frequency, but not the decrease in

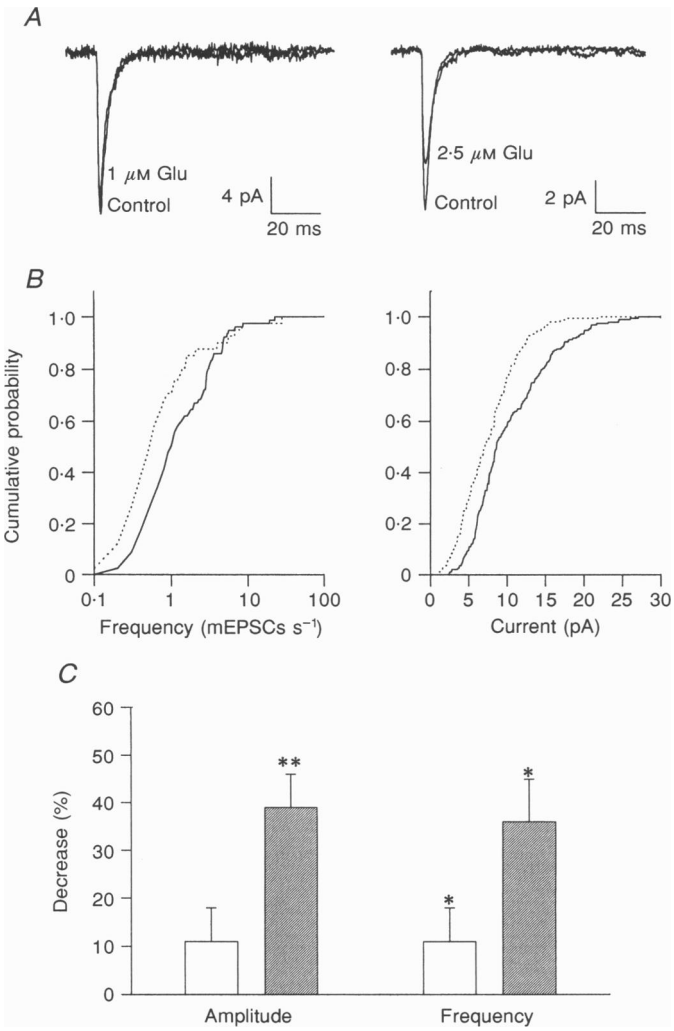


**Figure 4. Glutamate depresses spontaneous mEPSCs**

*A*, the effects of 1 and 2·5 μM Glu on averaged mEPSCs from two neurons voltage clamped at −70 mV. For averaging, mEPSCs were aligned at the onset of the response. The displayed traces are the average of 21 (left control), 30 (1 μM Glu), 178 (right control) and 136 (2·5 μM Glu) events. At 2·5 μM, glutamate clearly depressed the peak mEPSC amplitude, but had little effect on the time course of the events. *B*, cumulative probability plots showing the effects of 2·5 μM glutamate (dotted lines) on the frequency (left) and amplitude (right) of mEPSCs in separate neurons compared with control (continuous lines). In both cases, the decrease was significant by two-tailed Kolmogorov–Smirnov test ($P < 0·05$ (left), $P < 0·01$ (right)). The cell shown on the left exhibited a 15% decrease in mEPSC amplitude. The cell shown on the right is the same as that shown in *A*. This cell exhibited a 10% decrease in mEPSC frequency. *C*, a summary of the effects of 1 μM (□, $n = 11$) and 2·5 μM glutamate (▨, $n = 10$) on mEPSC amplitude and frequency. * $P < 0·05$; ** $P < 0·005$ compared with control by paired $t$ test.

mEPSC amplitude, produced by 2·5 μM glutamate was attenuated by treatment of cultures with PTX (frequency, −36 ± 9% change in control, $n = 10$, *vs.* +28 ± 17% change in PTX, $n = 7$, $P = 0{\cdot}003$; amplitude, −39 ± 7% change in control, $n = 10$, *vs.* −25 ± 6% change in PTX, $n = 7$, $P > 0{\cdot}10$).

**Effects of glutamate on NMDA synaptic currents**

The experiments described above indicate that both pre- and postsynaptic factors contribute to the depression of AMPA receptor-mediated synaptic responses by low concentrations of glutamate. These experiments were conducted under conditions that minimized the contributions of NMDA receptors (no added glycine, 1 mM $Mg^{2+}$ and −70 mV holding potential, with and without D-APV). To examine effects on NMDA receptors, we removed $Mg^{2+}$ and added 5–20 μM glycine to the extracellular solution. Under these conditions, low micromolar concentrations of glutamate gated larger offset currents than seen in the presence of $Mg^{2+}$ (Fig. 5*A*) and both the AMPA and NMDA components of EACs were depressed. The glutamate-mediated depression of the AMPA component was similar to that shown in Fig. 1, with an $EC_{50}$ of 2·7 μM (Fig. 5*B*). At concentrations of 1–10 μM, glutamate consistently depressed NMDA components to a greater degree than AMPA components. The $EC_{50}$ for depression of the NMDA component of EACs was 1·3 μM.

The additional depression of NMDA synaptic responses by glutamate cannot be explained by the release of adenosine, since 1 μM CPDPX failed to alter the depression of NMDA synaptic responses (1 ± 5% decrease in the depression produced by 2·5 μM glutamate, $n = 5$, $P > 0{\cdot}10$).

Because previous studies have indicated that the NMDA and AMPA receptor components of synaptic responses are modulated in parallel by presynaptic inhibitors (Perkel & Nicoll, 1994; Tong & Jahr, 1994*a*), we reasoned that the greater degree of depression of the NMDA component may result from postsynaptic factors, particularly desensitization of NMDA receptors. In the presence of glycine, desensitization of NMDA receptors is $Ca^{2+}$ dependent and more prominent at negative membrane potentials (Clark, Clifford & Zorumski, 1990; Legendre, Rosenmund & Westbrook, 1993). To examine the role of desensitization in

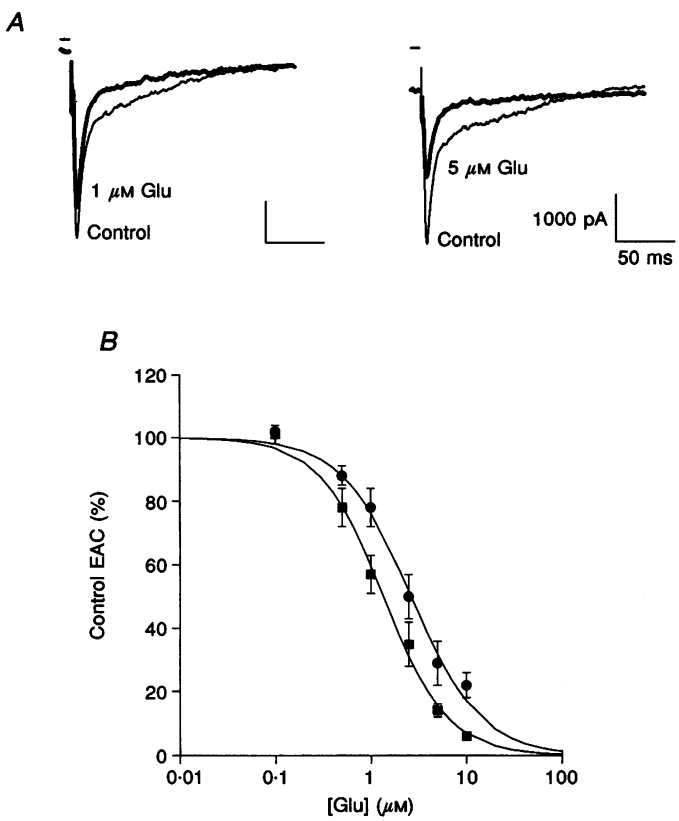


**Figure 5. Glutamate depresses both AMPA and NMDA receptor-mediated synaptic events**

*A*, the effects of 1 and 5 μM Glu on cells recorded in an extracellular solution containing no added $Mg^{2+}$ and 5 μM glycine at a holding potential of −70 mV. Note that the offset currents are larger than those shown in Fig. 1*B*. *B*, dose–response data for the depression of peak (AMPA, ●) EACs and NMDA components (■, measured 50 ms after the AMPA peak) by glutamate. Points represent the means ± S.E.M. of 3–9 cells. Continuous lines were fitted using the dose–response equation described in Methods. $EC_{50} = 2{\cdot}7$ μM, $N_H = 1{\cdot}2$ for AMPA components and $EC_{50} = 1{\cdot}3$ μM, $N_H = 1{\cdot}3$ for NMDA components.

the depression of NMDA responses by low concentrations of glutamate, we used dual recordings from monosynaptically connected pairs of neurons in two-neuron microcultures that had been treated with PTX to diminish presynaptic inhibition by glutamate acting at metabotropic receptors. In these recordings, presynaptic neurons were held at −70 mV while postsynaptic cells were held at +70 mV. The autaptic response of the presynaptic cell was used as a control for the effect of the positive holding potential in the postsynaptic neuron. Glutamate (1 μM) depressed NMDA autaptic responses at −70 mV, but had little effect on postsynaptic responses at +70 mV (Fig. 6*A* and *B*). This suggests that $Ca^{2+}$ influx into postsynaptic cells contributes to the depression of NMDA responses at −70 mV. To test this more directly, we examined isolated NMDA autaptic currents in PTX-treated neurons using an intracellular solution containing 20 mM BAPTA, a rapid $Ca^{2+}$ chelator (Tong, Shepherd & Jahr, 1995). In these neurons, the depression of NMDA responses was −19 ± 4% ($n = 15$) compared with −49 ± 5% ($n = 16$) in control neurons recorded using an intracellular solution containing 5 mM EGTA (Fig. 6*C*).

These results on NMDA responses were obtained under conditions that favoured current flow through NMDA ion channels. To determine whether depression of NMDA responses occurs in the presence of physiological concentrations of $Mg^{2+}$ (1 mM), we isolated the NMDA component of EACs by blocking the AMPA receptor component with 1 μM NBQX and recording at −50 mV. In these cells, 1 and 2·5 μM glutamate depressed NMDA receptor-mediated EACs by −31 ± 3% ($n = 11$) and −58 ± 4% ($n = 15$), respectively. These values are similar to the degree of depression produced by 1 and 2·5 μM glutamate in the absence of $Mg^{2+}$ at −70 mV (−43 ± 7%, $n = 18$, and −65 ± 7%, $n = 12$, respectively, $P > 0{\cdot}10$ for each).

**Effects of uptake inhibition on glutamate depression**

Extracellular glutamate is normally maintained at low concentrations by $Na^+$-dependent glutamate transporters

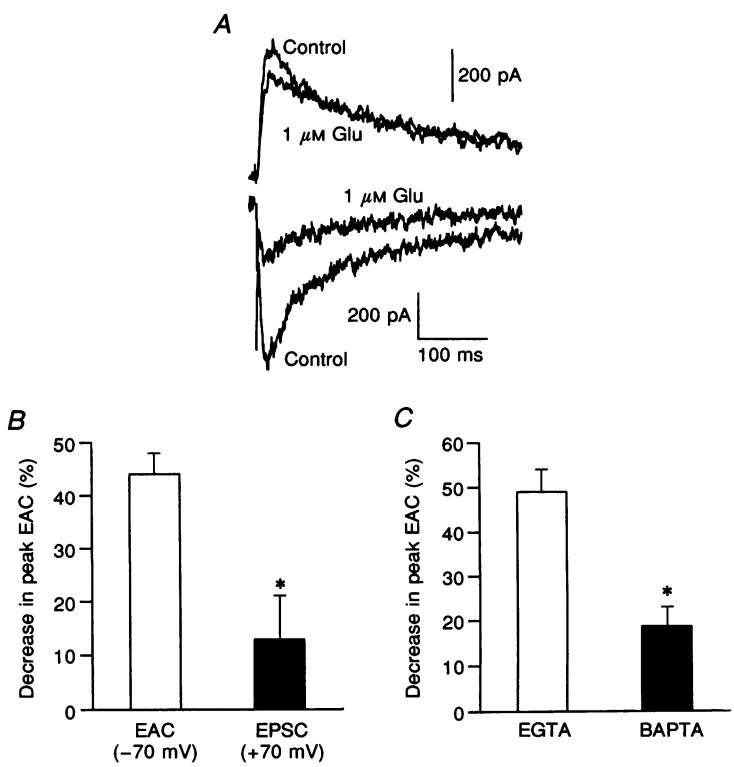


**Figure 6. Desensitization of NMDA receptors contributes to glutamate-mediated depression**

*A*, traces show the effect of 1 μM Glu upon autaptic NMDA responses (bottom) and conventional postsynaptic responses (top) from a two-neuron micro-island. The presynaptic cell was maintained at −70 mV. The bath solution contained 3 mM $Ca^{2+}$, 0 $Mg^{2+}$, 20 μM glycine and 1 μM NBQX. Part of the presynaptic neuron's stimulus transient has been subtracted. *B*, the degree of depression of autaptic and postsynaptic responses in 12 pairs of neurons studied as in *A*. * $P < 0{\cdot}005$, unpaired *t* test. *C*, the effects of different $Ca^{2+}$ chelators upon the degree of depression of NMDA EACs caused by 1 μM glutamate. Sixteen cells were studied using the standard potassium gluconate pipette solution, except that no free $Ca^{2+}$ was added (□, EGTA). Fifteen cells were studied using the same solution but with replacement of EGTA with 20 mM BAPTA (■). For all cells, 5–6 min elapsed following rupture of the cell membrane before commencing the experiment to allow diffusion of the pipette contents into the cell. * $P < 0{\cdot}001$, unpaired *t* test.

(Bouvier *et al.* 1992; Danbolt, 1994) and uptake inhibition should allow accumulation of extracellular glutamate that could contribute to synaptic depression. To examine the effects of glutamate transport inhibition we used threo-3-hydroxyaspartate (THA), a broad spectrum glutamate uptake inhibitor. In preliminary experiments we found that THA activates electrogenic glutamate transporter currents with an $EC_{50}$ of 4 $\mu$M in micro-island hippocampal glia (Mennerick, Benz & Zorumski, 1996). Similarly, micro-island glial uptake currents induced by synaptically released glutamate are inhibited by THA with an $EC_{50}$ of 5 $\mu$M (Mennerick & Zorumski, 1994). In the presence of D-APV, to inhibit the actions of THA at NMDA receptors (Tong & Jahr, 1994*b*), 50–100 $\mu$M THA produced small (1–2 pA) offset currents in hippocampal neurons and depressed AMPA-mediated EACs by $-10 \pm 4\%$ ($n = 15$) (Fig. 7). Based on the dose–response data shown in Fig. 2, this suggests that if the THA-induced depression results solely from the extracellular accumulation of glutamate, THA allows the accumulation of only 0·5 $\mu$M glutamate or less under our experimental conditions. When combined with 2·5 $\mu$M glutamate, THA more than doubled the glutamate-gated offset currents ($244 \pm 45\%$ of glutamate alone, $n = 14$), indicating the presence of more glutamate extracellularly following uptake inhibition. THA also increased the degree of synaptic depression produced by 2·5 $\mu$M glutamate from $-38 \pm 5\%$ to $-55 \pm 5\%$ ($n = 15$, $P = 0{\cdot}016$) (Fig. 7). The observed degree of depression produced by THA + glutamate ($-55 \pm 5\%$) was not significantly different from that calculated by summing the depression produced by THA and glutamate alone in single neurons ($-48 \pm 6\%$). The slightly greater degree of depression may have resulted from some exogenously applied glutamate being transported under control conditions.

## DISCUSSION

Based on the properties of glutamate transporters, Bouvier *et al.* (1992) calculated a lower limit of 0·6 $\mu$M for the basal extracellular concentration of glutamate. Several studies have directly measured extracellular glutamate levels and found about 1 $\mu$M under baseline conditions (Benveniste *et al.* 1984; Lerma *et al.* 1986). At membrane potentials near rest and at concentrations in the range of 0·5–5 $\mu$M, glutamate activates AMPA, NMDA and metabotropic

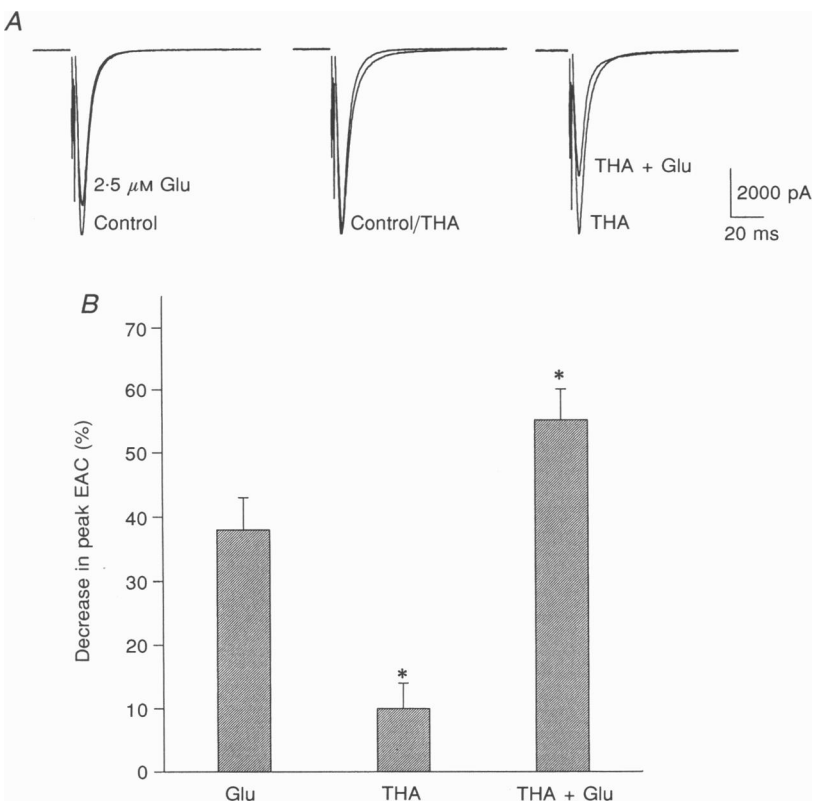


**Figure 7. A glutamate-uptake inhibitor increases glutamate-mediated depression**

*A*, the effects of 2·5 $\mu$M Glu (left), 100 $\mu$M THA (middle) and 100 $\mu$M THA + 2·5 $\mu$M Glu (right) on EACs from the same neuron. Note the prolongation of the EAC following THA treatment (see also Mennerick & Zorumski, 1995*a*). *B*, a summary of the effects of 50 and 100 $\mu$M THA with and without 2·5 $\mu$M Glu on peak EACs. To eliminate effects of THA on NMDA receptors, these experiments were conducted in the presence of 50–100 $\mu$M D-APV. * $P < 0{\cdot}01$ compared with glutamate alone.

receptors. Based on work in chick spinal cord and rat hippocampal neurons, concentrations >1 μM also promote substantial steady-state desensitization of AMPA receptors (Trussell & Fischbach, 1989; Colquhoun *et al.* 1992). NMDA receptors have a higher apparent affinity for glutamate than AMPA receptors (Patneau & Mayer, 1990) and are also likely to be desensitized by low concentrations of glutamate, particularly when the neuronal membrane is depolarized to relieve the $Mg^{2+}$ block of NMDA ion channels and allow $Ca^{2+}$ influx into neurons. All of these factors conspire to diminish the baseline efficacy of glutamate-mediated synaptic transmission in the presence of ambient concentrations of glutamate.

The present results indicate that both pre- and postsynaptic mechanisms contribute to glutamate-mediated depression at hippocampal micro-island synapses. For reasons that are presently unclear, the effects of glutamate show considerable cell-to-cell variability within the same culture. Some of this variability is likely to result from the presynaptic effects of glutamate, since the effects of selective metabotropic agonists vary from no effect to >60% depression of EPSCs (Forsythe & Clements, 1990; Mennerick & Zorumski, 1995*b*). It is unknown whether this variability in the presynaptic effects of glutamate arises from differences in receptor number, in receptor composition, or in the coupling of receptors to intracellular effectors.

Part of the variability may also reflect postsynaptic factors. Some of our data suggest that postsynaptic variability may arise from differences in the investment of synapses by micro-island glia and the density of glutamate transporters, since glutamate transport, which shows $EC_{50}$ values for glutamate of ~20 μM (see Danbolt, 1994, for review) will be activated in the range of glutamate concentrations where AMPA receptor desensitization begins to make a strong contribution to the depression of fast EPSCs. The ability of THA to more than double offset currents in response to application of 2·5 μM glutamate suggests that transporters limit the amount of glutamate that actually reaches neuronal receptors.

We estimated the average amount of glutamate that might actually be lost to transporters in order to explain the approximately 2·5-fold increase in current gated by 2·5 μM glutamate in the presence of THA. To achieve this estimate, we employed a model of AMPA receptor kinetics that describes many of the features of AMPA receptor function, including dose–response relations for macroscopic currents and receptor desensitization (Jonas *et al.* 1993). Use of the kinetic model allowed us to explore the effects of small changes in glutamate concentration upon steady-state glutamate currents and desensitization. To explain the ~2·5-fold increase in holding current with the application of THA (where the concentration of glutamate reaching receptors presumably accurately reflects the full 2·5 μM), the model estimates that true glutamate levels were decreased to ~1·5 μM in the absence of THA. The same simulated concentration change that produced the 2·5-fold increase in open channels, however, produced a much smaller change in the percentage of desensitized receptors: from 11·1% (1·5 μM) to 17·6% (2·5 μM). This smaller change in the number of desensitized receptors could explain the inability to detect a significant increase in the depression induced by a combination of THA and glutamate over the sum of the effect of each agent independently administered (Fig. 7). It is possible that local concentrations of glutamate in areas heavily invested by glia may have been even lower than 1·5 μM since some receptors, like those on neuronal somata, were likely to have received the full (2·5 μM) concentration of glutamate both in the presence and absence of THA.

The present results are consistent with studies demonstrating that desensitization of both AMPA and NMDA receptors is important in regulating excitatory synaptic transmission. Previous studies using CYZ and WGA found that inhibition of AMPA receptor desensitization augments the peak amplitude of fast synaptic responses under some experimental conditions (Vyklicky, Patneau & Mayer, 1991; Thio *et al.* 1992; Yamada & Tang, 1993). This suggests that either AMPA receptor desensitization is fast enough to limit peak responses or that there is some baseline level of desensitized synaptic receptors. Depending on whether an unliganded desensitized state is included, current models of AMPA receptor kinetics suggest that 0–30% of receptors are desensitized at rest (Vyklicky *et al.* 1991; Ambros-Ingerson & Lynch, 1993; Jonas *et al.* 1993). In the presence of micromolar concentrations of glutamate that activate macroscopic offset currents, a higher percentage of receptors would be desensitized. Other studies have demonstrated that $Ca^{2+}$-dependent desensitization of NMDA receptors contributes to the depression of NMDA receptor-mediated synaptic events during trains of stimuli (Rosenmund, Feltz & Westbrook, 1995; Tong *et al.* 1995) or in response to single synaptic stimuli (Mennerick & Zorumski, 1996). Our results indicate that steady-state desensitization of both AMPA and NMDA receptors plays an important role in regulating the availability of postsynaptic receptors for synaptic transmission. Furthermore, relatively small changes in extracellular glutamate levels over the range of 0·5–5 μM can have large effects on peak synaptic responses. Under conditions that produce neurodegeneration, extracellular glutamate levels are likely to rise to levels near 100 μM (Bouvier *et al.* 1992). In the presence of these toxic concentrations, excitatory synaptic transmission will be severely compromised well before the onset of neuronal death.

These studies have implications for the involvement of glutamate receptors in synaptic plasticity. Previous studies have shown that activation of NMDA receptors by ambient

concentrations of excitatory amino acids enhances action potential firing in response to depolarizing inputs (Sah, Hestrin & Nicoll, 1989). However, untimely activation of NMDA receptors (Coan, Irving & Collingridge, 1989; Huang, Colino, Selig & Malenka, 1992) diminishes the ability of tetanic stimuli to induce long-term potentiation. Since both AMPA and NMDA receptors can be desensitized by low concentrations of glutamate and by synaptic activation, changes in local concentrations of glutamate could be important in altering the threshold for inducing synaptic plasticity.


Ambros-Ingerson, J. & Lynch, G. (1993). Channel gating kinetics and synaptic efficacy: hypothesis for expression of long-term potentiation. *Proceedings of the National Academy Sciences of the USA* **90**, 7903–7907.

Baskys, A. & Malenka, R. C. (1991). Agonists at metabotropic glutamate receptors presynaptically inhibit EPSCs in neonatal rat hippocampus. *Journal of Physiology* **444**, 687–701.

Benvenisite, H., Drejer, J., Schousboe, A. & Diemer, N. H. (1984). Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. *Journal of Neurochemistry* **43**, 1369–1374.

Bouvier, M., Szatkowski, M., Amato, A. & Attwell, D. (1992). The glial cell glutamate uptake carrier countertransports pH-changing anions. *Nature* **360**, 471–474.

Burger, P. M., Mehl, E., Cameron, P. L., Maycox, P. R., Baumert, M., Lottspeich, F., de Camilli, P. & Jahn, R. (1989). Synaptic vesicles immunoisolated from rat cerebral cortex contain high levels of glutamate. *Neuron* **3**, 715–720.

Clark, G. D., Clifford, D. B. & Zorumski, C. F. (1990). The effect of agonist concentration, membrane voltage, and calcium on *N*-methyl-D-aspartate receptor desensitization. *Neuroscience* **39**, 787–797.

Coan, E. J., Irving, A. J. & Collingridge, G. L. (1989). Low-frequency activation of the NMDA receptor system can prevent the induction of LTP. *Neuroscience Letters* **105**, 205–210.

Colquhoun, D., Jonas, P. & Sakmann, B. (1992). Action of brief pulses of glutamate on AMPA/kainate receptors in patches from different neurones of rat hippocampal slices. *Journal of Physiology* **458**, 261–287.

Danbolt, N. C. (1994). The high affinity uptake system for excitatory amino acids in the brain. *Progress in Neurobiology* **44**, 377–396.

Diamond, J. S. & Jahr, C. E. (1995). Asynchronous release of synaptic vesicles determines the time course of AMPA receptor-mediated EPSC. *Neuron* **15**, 1097–1107.

Forsythe, I. D. & Clements, J. D. (1990). Presynaptic glutamate receptors depress excitatory monosynaptic transmission between mouse hippocampal neurones. *Journal of Physiology* **429**, 1–16.

Huang, Y.-Y., Colino, A., Selig, D. K. & Malenka, R. C. (1992). The influence of prior synaptic activity on the induction of long-term potentiation. *Science* **255**, 730–733.

Jonas, P., Major, G. & Sakmann, B. (1993). Quantal components of unitary EPSCs at the mossy fibre synapse on CA3 pyramidal cells of rat hippocampus. *Journal of Physiology* **472**, 615–663.

Legendre, P., Rosenmund, C. & Westbrook, G. L. (1993). Inactivation of NMDA channels in cultured hippocampal neurons by intracellular calcium. *Journal of Neuroscience* **13**, 674–684.

Lerma, J., Herranz, A. S., Herraras, O., Abraira, V. & Martin del Rio, R. (1986). *In vivo* determination of extracellular concentration of amino acids in the rat hippocampus. A method based on brain dialysis and computerized analysis. *Brain Research* **384**, 145–155.

Lin, F. & Stevens, C. F. (1994). Both open and closed NMDA receptor channels desensitize. *Journal of Neuroscience* **14**, 2153–2160.

Maki, R., Robinson, M. B. & Dichter, M. A. (1994). The glutamate uptake inhibitor L-*trans*-pyrrolidine-2,4-dicarboxylate depresses excitatory synaptic transmission via a presynaptic mechanism in cultured hippocampal neurons. *Journal of Neuroscience* **14**, 6754–6762.

Manzoni, O. J., Manabe, T. & Nicoll, R. A. (1994). Release of adenosine by activation of NMDA receptors in the hippocampus. *Science* **265**, 2098–2101.

Mennerick, S., Benz, A. & Zorumski, C. F. (1996). Components of glial responses to exogenous and synaptic glutamate in rat hippocampal microcultures. *Journal of Neuroscience* **16**, 55–64.

Mennerick, S. & Zorumski, C. F. (1994). Glial contributions to excitatory neurotransmission in cultured hippocampal cells. *Nature* **368**, 59–62.

Mennerick, S. & Zorumski, C. F. (1995*a*). Presynaptic influence upon the time course of fast excitatory synaptic currents in cultured hippocampal cells. *Journal of Neuroscience* **15**, 3178–3192.

Mennerick, S. & Zorumski, C. F. (1995*b*). Paired-pulse modulation of fast excitatory synaptic currents in microcultures of rat hippocampal neurones. *Journal of Physiology* **488**, 85–101.

Mennerick, S. & Zorumski, C. F. (1996). Postsynaptic modulation of NMDA synaptic currents in rat hippocampal microcultures by paired-pulse stimulation. *Journal of Physiology* **490**, 405–417.

Patneau, D. K. & Mayer, M. L. (1990). Structure-activity relationships for amino acid transmitter candidates acting at *N*-methyl-D-aspartate and quisqualate receptors. *Journal of Neuroscience* **10**, 2385–2399.

Perkel, D. J. & Nicoll, R. A. (1994). Evidence for all-or-none regulation of neurotransmitter release: implications for long-term potentiation. *Journal of Physiology* **471**, 481–500.

Rosenmund, C., Feltz, A. & Westbrook, G. L. (1995). Calcium-dependent inactivation of synaptic NMDA receptors in hippocampal neurons. *Journal of Neurophysiology* **73**, 427–430.

Sah, P., Hestrin, S. & Nicoll, R. A. (1989). Tonic activation of NMDA receptors by ambient glutamate enhances excitability of neurons. *Science* **246**, 815–818.

Sather, W., Dieudonne, S., Macdonald, J. F. & Ascher, P. (1992). Activation and desensitization of *N*-methyl-D-aspartate receptors in nucleated outside-out patches from mouse neurones. *Journal of Physiology* **450**, 643–672.

Segal, M. M. & Furshpan, E. J. (1990). Epileptiform activity in microcultures containing small numbers of hippocampal neurons. *Journal of Neurophysiology* **64**, 1390–1399.

Sharp, R. L., Mayne, N. G. & Burnett, J. P. (1994). Cyclothiazide differentially modulates human metabotropic glutamate receptors linked to phosphoinositide hydrolysis stimulation in oocytes. *European Journal of Pharmacology* **269**, R5–7.

THIO, L. L., CLARK, G. D., CLIFFORD, D. B. & ZORUMSKI, C. F. (1992). Wheat germ agglutinin enhances EPSCs in cultured postnatal rat hippocampal neurons by blocking ionotropic quisqualate receptor desensitization. *Journal of Neurophysiology* **68**, 1930–1938.

TONG, G. & JAHR, C. E. (1994*a*). Multivesicular release from excitatory synapses of cultured hippocampal neurons. *Neuron* **12**, 51–59.

TONG, G. & JAHR, C. E. (1994*b*). Block of glutamate transporters potentiates postsynaptic excitation. *Neuron* **13**, 1195–1203.

TONG, G. SHEPHERD, D. & JAHR, C. E. (1995). Synaptic desensitization of NMDA receptors by calcineurin. *Science* **267**, 1510–1512.

TRUSSELL, L. O. & FISCHBACH, G. D. (1989). Glutamate receptor desensitization and its role in synaptic transmission. *Neuron* **3**, 209–218.

VYKLICKY, L. JR, PATNEAU, D. K. & MAYER, M. L. (1991). Modulation of excitatory synaptic transmission by drugs that reduce desensitization at AMPA/kainate receptors. *Neuron* **7**, 971–984.

YAMADA, K. A. & TANG, C.-M. (1993). Benzothiadiazides inhibit rapid glutamate receptor desensitization and enhance glutamatergic synaptic currents. *Journal of Neuroscience* **13**, 3904–3915.

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