

Glutamate Release Monitored with Astrocyte Transporter Currents during LTP

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Summary

Long-term potentiation (LTP) of synaptic transmission in the CA1 region of the hippocampus is thought to result from either increased transmitter release, heightened postsynaptic sensitivity, or a combination of the two. We have measured evoked glutamate release from Schaffer collateral/commissural fiber terminals in CA1 by recording synaptically activated glutamate transporter currents in hippocampal astrocytes located in stratum radiatum. Although several manipulations of release probability caused parallel changes in extracellular field potentials and synaptically activated transporter current amplitudes, induction of LTP failed to alter transporter-mediated responses, suggesting that LTP does not alter the amount of glutamate released upon synaptic stimulation.

Introduction

The primary steps of LTP induction in the CA1 region of hippocampus are generally agreed to occur postsynaptically, but it is still debated whether the locus of LTP expression resides pre- or postsynaptically. Many different approaches have been taken to address this fundamental question, such as measuring the effects of LTP induction on (1) the relative amplitudes of NMDA and AMPA receptor-mediated excitatory postsynaptic currents (EPSCs), (2) paired-pulse facilitation, (3) the rate of block of the NMDA receptor EPSCs by the channel blocker MK-801, and (4) the quantal parameters of release, including the probability of release (p_r), the number of active synaptic contacts, and the size of the postsynaptic response to a single quantum of transmitter (reviewed by Bliss and Collingridge, 1993; Kullmann and Siegelbaum, 1995). The interpretations of these results, however, are complicated, because all of the methods attempt to extract information about changes in both transmitter release and postsynaptic sensitivity from a single measure—the postsynaptic response.

Recently, an independent method of monitoring synaptic transmitter release in hippocampal slices has been reported (Bergles and Jahr, 1997); glutamate released from Schaffer collateral/commissural fiber synaptic terminals can be detected by recording synaptically activated glutamate transporter currents (STCs) in astrocytes located in stratum radiatum. The fine processes of these astrocytes ramify extensively through the neuropil, where they come into close proximity with synaptic contacts (Spacek, 1985; Kosaka and Hama, 1986; Harris

et al., 1992). Astrocytes in the CA1 region of the hippocampus express high levels of glutamate transporters (Rothstein et al., 1994; Lehre et al., 1995) and are thought to take up much of the glutamate from these synapses (Rothstein et al., 1996; Tanaka et al., 1997). Because transport is electrogenic (Brew and Attwell, 1987), glutamate that escapes from the synaptic cleft binds to astrocytic transporters and activates a measurable current (Mennerick and Zorumski, 1994; Mennerick et al., 1996; Bergles and Jahr, 1997; Bergles et al., 1997; Clark and Barbour, 1997). The STC has been shown to report paired-pulse facilitation of synaptic transmission and has been suggested as a sensitive assay for measuring changes in p_r (Bergles and Jahr, 1997). Here, we report that the amplitude of the STC in astrocytes follows changes in p_r and the number of release sites (n) but is not altered by the induction of LTP. These results argue that expression of LTP in area CA1 does not involve an increase in the amount of glutamate released in response to synaptic stimulation.

Results

To measure whether transporter currents in astrocytes are sensitive to changes in glutamate release from Schaffer collateral/commissural terminals, we simultaneously recorded field excitatory postsynaptic potentials (fEPSPs) as well as STCs in whole-cell voltage clamp from astrocytes located in stratum radiatum. Field responses were recorded in voltage-clamp mode and then converted to units of potential through multiplication by the resistance of the field electrode (Figure 1A; see Experimental Procedures). In the presence of picrotoxin (0.1 mM) added to isolate excitatory responses, stimulation in stratum radiatum elicited a complex, biphasic response in astrocytes comprising a current due to the extracellular field potential, a long-lasting current presumably due to the slow reequilibration of the extracellular potassium concentration following stimulation, and a transporter-mediated current (Figure 1B₂; Mennerick and Zorumski, 1994; Mennerick et al., 1996; Bergles and Jahr, 1997; Clark and Barbour, 1997). The field component and much of the putative potassium current were eliminated by bath application of the glutamate receptor antagonist kynurenic acid (KYN, 5 mM; Figure 1B₂), which abolished the synaptic response measured with the field electrode (Figure 1B₁). The transient component of the remaining current was blocked by addition of the glutamate uptake blockers threo- β -hydroxyaspartate (THA, 300 μ M) and dihydrokainate (DHK, 300 μ M), confirming that it was mediated by glutamate transporters; this current has previously been shown to be unaffected by antagonists of GABA transporters and numerous metabotropic receptors (Bergles and Jahr, 1997). Analogous effects of glutamate uptake blockers on STCs have been reported in hippocampal astrocytes (Mennerick and Zorumski, 1994; Mennerick et al., 1996; Bergles and Jahr, 1997), as well as Bergmann glial cells (Bergles et al., 1997; Clark and Barbour, 1997) and Purkinje cells

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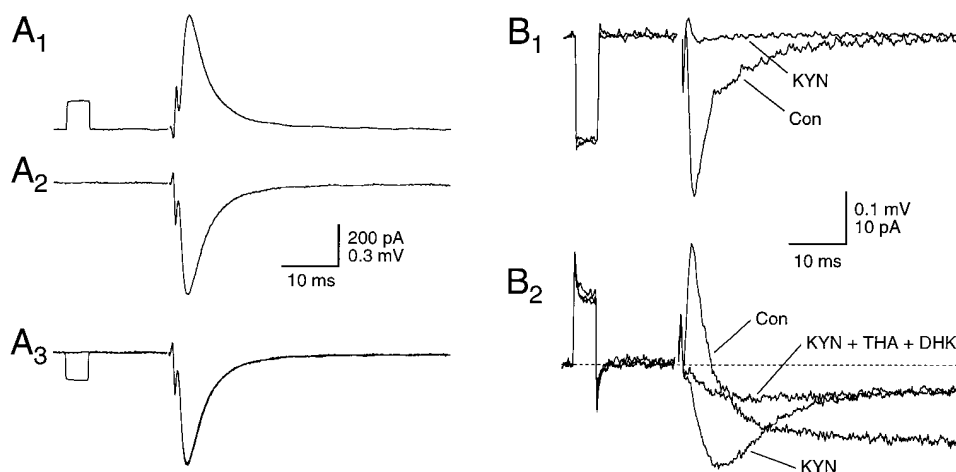


Figure 1. Evoked Synaptic Activity Monitored with Extracellular fEPSPs and Astrocyte STCs

(A) Extracellular fEPSPs recorded with a patch electrode in different recording modes.

(A₁) In voltage-clamp mode, stimulation was preceded by a 0.2 mV voltage step.

(A₂) fEPSP recorded in current clamp mode in the same slice. Responses in A₁ and A₂ are averages of 20 trials, which were interleaved.

(A₃) Traces from A₁ and A₂, superimposed, with the voltage-clamp trace scaled such that the amplitude of the conductance pulse equals 0.2 mV. Stimulus, 150 μ A.

(B) Comparison of evoked fEPSPs and astrocyte STCs. All traces are averages of 8–10 trials. Simultaneous recordings of field potentials (B₁) and whole-cell currents from an astrocyte (B₂) are shown. KYN (5 mM) blocked the field potential and reduced the long-lasting potassium current in the whole-cell recording. In the continued presence of KYN, 300 μ M THA and 300 μ M DHK blocked the STC. Stimulus, 100 μ A.

(Otis et al., 1997). fEPSPs exhibited repetitive firing due to reduced inhibition and high stimulus intensities used to elicit measurable STCs; however, the presence of population spikes did not affect the initial slope of the

fEPSP, which reflects postsynaptic current flow due to monosynaptic transmission.

Three standard manipulations that alter p_r were used to test if the STC was sensitive to changes in glutamate

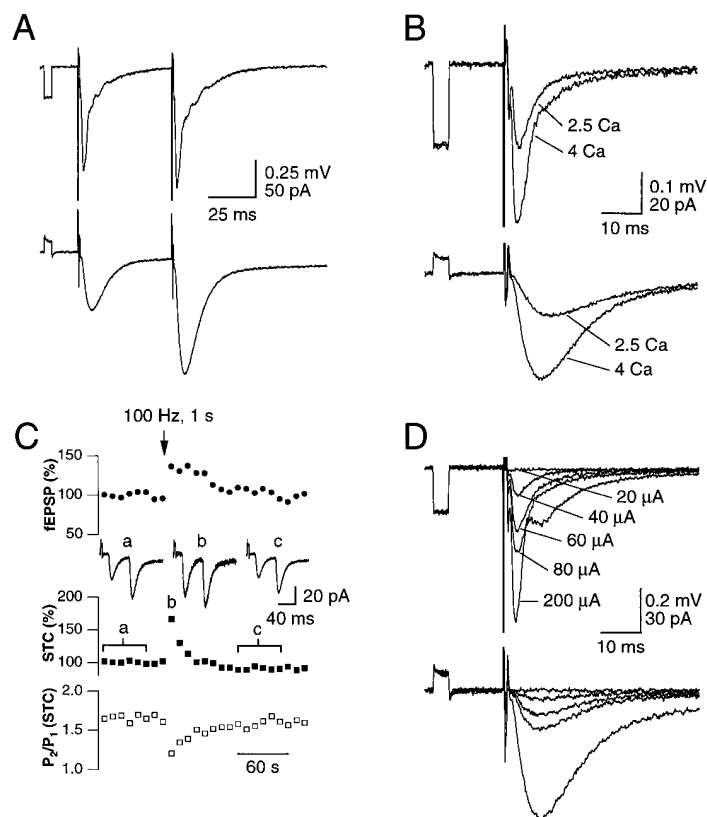


Figure 2. STCs Report Changes in Evoked Glutamate Release

(A) Paired-pulse facilitation of the field potential (top) and, after addition of 5 mM KYN, of the STC (bottom). Stimulus, 100 μ A.

(B) Effects of changing $\text{Ca}^{2+}:\text{Mg}^{2+}$ ratio from 4.0:4.0 to 2.5:5.5 on the field potential (top) and, in the presence of 5 mM KYN, the STC (bottom). Stimulus, 120 μ A.

(C) Effects of posttetanic potentiation on fEPSP slope (closed circles), STC amplitude (closed squares), and paired-pulse facilitation of the STC (open squares). Traces "a" and "c" between the top two panels illustrate averaged STCs before and >1 min after HFS (arrow, 100 Hz for 1 s), respectively. The middle trace ("b") shows a single response 10 s after HFS. Two separate HFS bursts were delivered: one in control solution (for fEPSPs) and one in 5 mM KYN (for STCs). Stimulus, 80 μ A.

(D) Effects of changing stimulus strength on fEPSPs (top) and STCs (bottom).

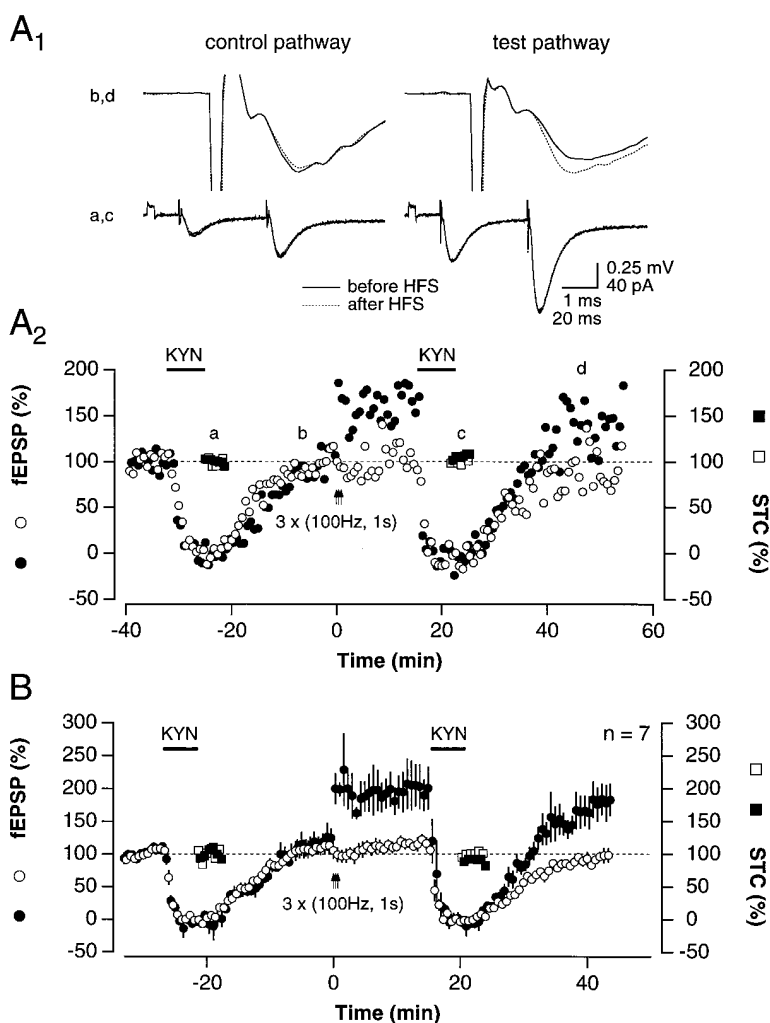


Figure 3. Effects of LTP Induction on fEPSP Slope and Astrocyte STC Amplitude

(A) Results from a single experiment. Stimulation, 150 μ A (control path) and 100 μ A (test path).

(A₁) fEPSPs (top) and STCs (bottom) are shown before (solid lines) and after (dotted lines) HFS for responses to stimulation of the control pathway (left) and the test (HFS) pathway (right). Traces are shown unscaled.

(A₂) fEPSP slopes (circles) and STC amplitudes (squares) over the course of the same experiment shown in A₁. Arrows, three bursts of HFS (100 Hz for 1 s) applied to test pathway. Responses to stimulation of the test (closed symbols) and control (open symbols) pathways are normalized (see Experimental Procedures).

(B) Summary of seven experiments. Experimental procedure and data display are the same as in (A₂). Error bars represent SEM and are obscured by the symbols in the STC data.

release. First, paired-pulse facilitation, which is thought to result from a residual elevation of intraterminal calcium (Zucker, 1996), was observed in both the fEPSP (1.4 ± 0.2 -fold, mean $\pm$ SD, $n = 8$) and, in the presence of 5 mM KYN applied to the same slices, the STC (1.9 ± 0.3 -fold, $n = 8$; Figure 2A). Second, decreasing release by lowering the $\text{Ca}^{2+}:\text{Mg}^{2+}$ ratio from 4.0:4.0 to 2.5:5.5 diminished both responses similarly (fEPSP, 0.6 ± 0.04 ; STC, 0.5 ± 0.2 ; $n = 4$; Figure 2B). Third, a burst of high frequency stimulation (HFS) resulted in posttetanic potentiation of both the fEPSP (1.5 ± 0.3 -fold, $n = 5$) and the STC (1.8 ± 0.2 -fold, $n = 5$). In many cases, a single burst of HFS resulted in a longer-lasting potentiation of the fEPSP; such experiments were excluded from this analysis in an effort to focus on the changes induced specifically by posttetanic potentiation. As expected during the elevated p_r of posttetanic potentiation, paired-pulse facilitation of the STC was decreased (Figure 2C). The STC was also sensitive to changes in n : activating more synapses by raising the stimulus strength increased the amplitude of both the STC and the fEPSP (Figure 2D). These results indicate that the amplitude of the STC is a reliable reporter of changes in the amount of glutamate released due to changes in either p_r or n .

In fact, the STC was more sensitive than the fEPSP to changes brought about by paired-pulse facilitation, posttetanic potentiation, or by increasing stimulus intensity (Figure 7). This may result from the fact that field responses were contaminated by significant postsynaptic depolarization that reduced the driving force on the synaptic conductance and thereby diminished the effect of increased transmission. Because astrocytes have such low input resistances, they are depolarized only slightly during a synaptic response (Bergles and Jahr, 1997), causing little attenuation of the STC due to reduction in driving force. Control experiments showed that, when AMPA and NMDA receptors were blocked by 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide (NBQX, 10 μ M) and R(-)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (D-CPP, 5 μ M), subsequent application of 5 mM KYN did not change the STC (data not shown), suggesting that KYN itself does not affect p_r .

To test whether an increase in p_r or n occurs following induction of LTP, two independent pathways were stimulated, eliciting STCs and fEPSPs in each (Figure 3A; see Experimental Procedures). After obtaining a ≥ 5 min baseline of the field potential, 5 mM KYN was applied

to record the STC in both pathways. After recovery from KYN, three bursts of HFS (100 Hz for 1 s) were applied to the test pathway to induce LTP. A second application of KYN 15 min after HFS revealed that, although the fEPSP in the test pathway was increased 1.7 ± 0.3 -fold relative to the control pathway ($n = 7$, $p = 0.0002$), the STC was unchanged (0.9 ± 0.2 -fold, $n = 7$, $p = 0.4$; Figure 3B). In some cases, rundown in STC amplitude, due to increased access resistance (see Experimental Procedures), was observed over the ~ 40 min between KYN applications (control pathway STC amplitudes during the second KYN application were $80\% \pm 37\%$ of those during the first KYN application [$n = 8$]). To account for this, the amplitude of the STC evoked by the test pathway was normalized by that of the control pathway, even in experiments (5 of 8) in which rundown was $<12\%$ (Figure 3A₂). The pooled data (Figure 3B) illustrate only those experiments (7 of 8) in which robust ($\geq 30\%$) potentiation was observed. In the remaining experiment (fEPSP potentiated by 12%), no HFS-induced change in the STC was observed (97% of control).

Our interpretation of these data is that the induction of LTP does not significantly increase the probability of release or the number of active release sites. An alternative possibility is that any increase in the STC resulting from greater transmitter release is exactly offset by a concomitant downregulation of transport, even though this would seem to be a nonadaptive response to increased release since it could promote epileptiform activity and excitotoxicity. Either arachidonic acid (AA) or nitric oxide (NO) could mediate such a compensatory mechanism: both have been reported to be released upon activation of NMDA receptors (Dumuis et al., 1988; Garthwaite et al., 1988) and inhibit glutamate transporters (Barbour et al., 1989; Pogun et al., 1994; Trotti et al., 1995; Zerangue et al., 1995). AA inhibits directly at least one of the glutamate transporters expressed in astrocytes (Trotti et al., 1995; Zerangue et al., 1995), and NO decreases glutamate transport in hippocampal synaptosomes (Pogun et al., 1994). We investigated the effects of AA and NO on astrocyte transporters with outside-out patches excised from astrocytes in the CA1 region.

Rapid application of 10 mM glutamate elicited transporter-mediated currents in outside-out patches (Figure 4). When AA (0.2 mM) was included in the control and glutamate-containing solutions, the glutamate-elicited current was reduced (Figure 4A; % inhibition: peak, 11.2 ± 3.3 ; steady state, 58.2 ± 22.0 ; $n = 5$). However, this reduction was rapidly reversible, recovering in <1 min after washing out AA. The transient nature of AA inhibition of transporter function has been reported previously in flux studies (Trotti et al., 1995) and oocyte recordings (Zerangue et al., 1995) and is consistent with a direct action of AA on the transporter (Barbour et al., 1989; Zerangue et al., 1995). In retinal glial recordings (Barbour et al., 1989), the majority of the inhibition (80%–85%) was also transient. In the present study, the effects of LTP on the STC were measured >15 min following LTP induction (Figure 3). A compensatory role for AA, therefore, would require that induction of LTP result in a substantial, long-lasting increase in extracellular AA. The evidence for a sustained increase in AA (Lynch et al., 1989; Williams and Bliss, 1989) is controversial (Förstermann et al., 1988; O'Dell et al., 1991).

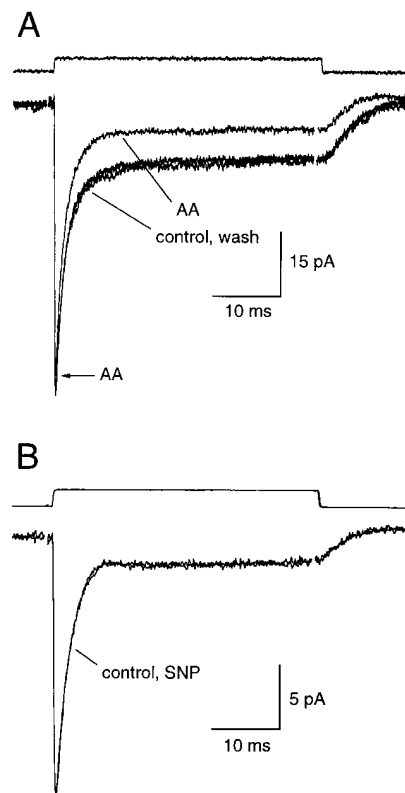


Figure 4. Effects of Candidate Diffusible Retrograde Messengers on Transporter Currents in Outside-Out Patches

(A) Arachidonic acid (AA) reversibly inhibits transporter currents. The "control" and "wash" traces were evoked by stepping into 10 mM glutamate. The "AA" trace was evoked by stepping into 10 mM glutamate plus 0.2 mM AA after equilibration in 0.2 mM AA.

(B) The nitric oxide donor sodium nitroprusside (SNP) does not affect transporter currents. The "SNP" trace was evoked by stepping into 10 mM glutamate plus 1 mM SNP after equilibration in 1 mM SNP. Traces in the upper panels of (A) and (B) illustrate the junction current that reflects the rapid solution exchange across the open tip of the electrode, following removal of the patch at the end of the experiment. Each trace is the average of 8–12 responses. Holding potential, -110 mV.

The NO donor sodium nitroprusside (SNP, 1 mM) had no effect on glutamate-elicited currents in patches pulled from astrocytes (Figure 4B; peak amplitude, $102\% \pm 7\%$ of control; $n = 3$; $p = 0.5$). It is possible that the previously described effects of SNP on transporters (Pogun et al., 1994) may require an intracellular, diffusible factor that was washed out from the patch in the present experiments. Similar washout, however, is likely to have occurred in the whole-cell experiments (Figure 3), as LTP was induced nearly 40 min following the establishment of a whole-cell recording from an astrocyte.

In contrast to LTP expression in area CA1, LTP at mossy fiber synapses onto CA3 pyramidal neurons is generally agreed to be expressed by an increase in glutamate release (reviewed by Nicoll and Malenka, 1995). Mossy fiber-evoked STCs from astrocytes in the stratum lucidum region of CA3, then, may be increased in size after induction of LTP. Stimulation in stratum lucidum within 100 μm of the astrocyte being recorded from

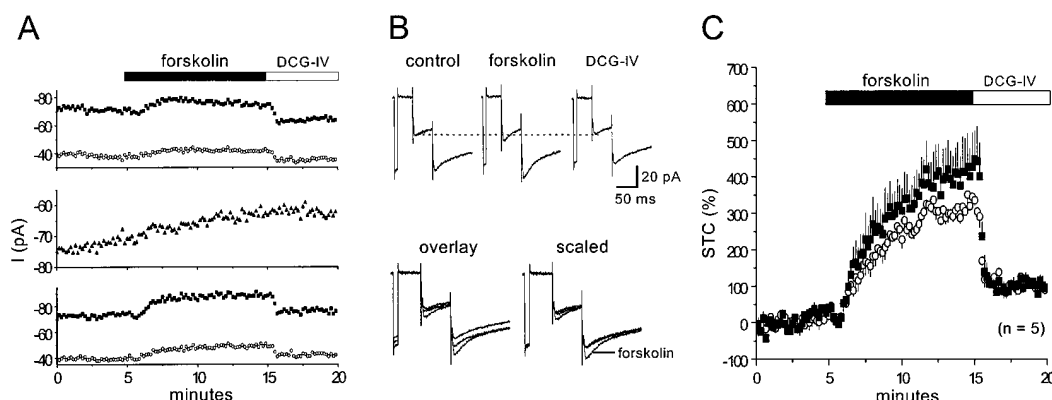


Figure 5. Forskolin Potentiates STCs in CA3 Astrocytes

(A) Forskolin (50 μ M) increased the amplitude of evoked responses recorded from astrocytes located in stratum lucidum of area CA3. Paired stimuli (50 ms interval) were applied to increase p_r . The amplitude of the first pulse is represented by the closed squares, and the second by the open circles.

(Top) The forskolin-induced increase in the transporter current was inhibited by the type II metabotropic glutamate receptor agonist, DCG-IV (1 μ M).

(Center) Plot of the amplitude of the conductance pulse (-1 mV, 8 ms) for this experiment.

(Bottom) Plot of the amplitudes of evoked responses after normalizing to the conductance pulse.

(B) Evoked responses to paired stimuli recorded from a CA3 astrocyte for the experiment shown in (A). Sweeps are averages of five consecutive responses. Stimulation intensity, 130 μ A, 100 μ s.

(C) Summary graph illustrating the potentiation of the transporter current by forskolin. The amplitude during the control period was subtracted from evoked responses to isolate the component of the response mediated by transporters; response amplitudes were then normalized to the amplitude of current in DCG-IV to allow comparison across cells (mean $\pm$ SEM, $n = 5$). Stimuli were applied at 0.1 Hz for all experiments, and drugs were applied for the time indicated by the bars.

elicited a long-lasting potassium component but little, if any, discernible transporter current. Even when paired stimuli were applied at 50 ms intervals, a manipulation that greatly enhances release from these terminals (Salin et al., 1996), a transporter current was rarely detected. This made measurements of HFS-induced changes in STC amplitudes problematic. However, activation of adenylyl cyclase by forskolin has been shown to potentiate mossy fiber input to CA3 pyramidal neurons 3- to 4-fold more than LTP induction (Weisskopf et al., 1994). In addition, forskolin occludes subsequent LTP induction, suggesting that the two manipulations share similar pathways (Weisskopf et al., 1994). Here, we show that forskolin (50 μ M) enhanced release sufficiently to reveal a transporter current component (Figure 5). The glutamate activating these transporters is likely to originate directly from mossy fiber terminals on pyramidal neurons, because (1) recurrent excitation of surrounding pyramidal cells was blocked by antagonists of AMPA and NMDA receptors; (2) responses enhanced by forskolin were inhibited by the group II metabotropic glutamate receptor agonist DCG-IV (1 μ M; Figure 5), which has been shown to inhibit release from mossy fibers but not associational-commissural fibers in the rat (Kamiya et al., 1996; Maccaferri et al., 1998), and (3) forskolin does not enhance release from mossy fiber endings on interneurons or from associational-commissural fibers (Weisskopf et al., 1994; Maccaferri et al., 1998). The incomplete blockade by DCG-IV of the mossy fiber-evoked response (Figure 5C) is consistent with the reported inhibition of DCG-IV action by forskolin (Maccaferri et al., 1998). The transient component of the mossy fiber-evoked response enhanced by forskolin was reversibly blocked by the addition of DHK (300 μ M) and

THA (300 μ M), confirming that it was the result of glutamate transporter activation (Figure 6).

Discussion

In previous reports on the locus of expression of LTP in the CA1 region of hippocampus, the postsynaptic response resulting from activation of AMPA and/or NMDA receptors has been used to monitor both p_r as well as changes in sensitivity of the postsynaptic receptors. In this study, we have used an independent sensor, glutamate transporter currents in astrocytes, to monitor glutamate release uncomplicated by the responses of the postsynaptic neurons. An analogous approach has been reported in cerebellar cultures, where AMPA receptor-mediated currents in glial cells were used to study a presynaptic form of LTP (Linden, 1997). We have found that several different methods of altering p_r , as well as varying the number of active synapses, all changed the fEPSP and STC in parallel (Figure 7). By contrast, induction of LTP, as measured by field potential recordings, had no effect on the amplitude of STCs recorded in astrocytes. The simplest interpretation of these results is that the expression of LTP does not result from an increase in p_r or n . The data illustrated in Figures 2, 5, 6, and 7 demonstrate that the STC provides a sensitive assay for changes in glutamate release. The increases in the fEPSP with paired-pulse facilitation and posttetanic potentiation were similar to the increase caused by induction of LTP (Figure 7); if p_r or n had increased with LTP, a corresponding increase in the STC current amplitude should have been observed. It seems unlikely that an increase in release was obscured

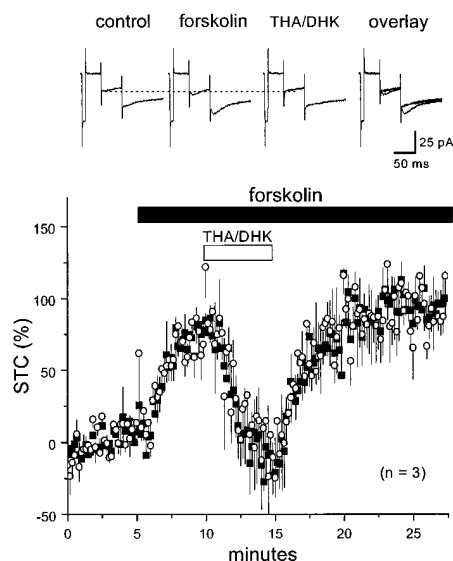


Figure 6. Glutamate Transporter Antagonists Block Mossy Fiber-Evoked Transporter Currents in CA3 Astrocytes

The glutamate transporter antagonists THA (300 μ M) and DHK (300 μ M) blocked the forskolin-induced increase in evoked responses. Sweeps are averages of eight consecutive responses. For the summary graph, the amplitude during the control period was subtracted from evoked responses to isolate the component of the current mediated by transporters; response amplitudes were then normalized to the amplitude of the current in forskolin (following THA/DHK wash) to allow comparison across cells (mean $\pm$ SEM; $n = 3$). Stimuli were applied at 0.1 Hz for all experiments. The open circles and closed squares correspond to the amplitudes of the first and second pulses, respectively.

because all of the glutamate transporters on the astrocyte were saturated by synaptically released transmitter, as paired-pulse facilitation of the STC in the test pathway persisted unchanged following induction of LTP (Figure 3A₁), analogous to results observed with EPSCs in CA1 pyramidal cells (Manabe et al., 1993).

One possible complication in comparing STCs with fEPSPs is that it is very unlikely that an astrocyte senses synaptic activity from the identical population of synapses as a field electrode. Focal application of glutamate elicits a response in a somatic recording from an astrocyte only when the puff occurs within 75 μ m of the cell body (Bergles and Jahr, 1997); a field electrode detects postsynaptic activity from a larger region of the slice (Llinás and Nicholson, 1974). Moreover, a field electrode reports the activity of every synapse within a region, while an astrocyte senses only some fraction. It is possible, therefore, that the synapses potentiated during LTP are sensed by the field electrode but not the astrocyte, so that an increase in transmitter release at the potentiated synapses is not reflected in the STC. We believe this scenario to be unlikely for several reasons. The STC detected with great sensitivity changes in release induced by every other presynaptic manipulation that was tested (Figures 2, 5, 6, and 7). Restricting potentiation to synapses from which glutamate release is not sensed by transporters on astrocyte processes would require an elaborate, precise exclusion mechanism for which there is no anatomical or physiological

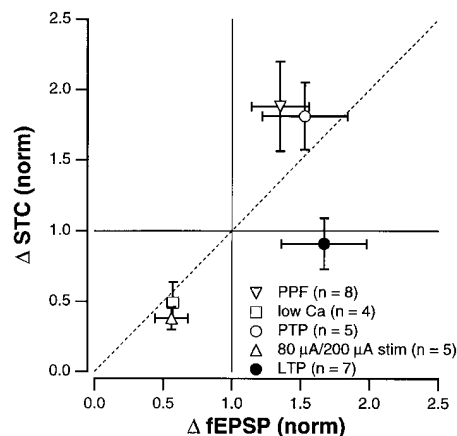


Figure 7. Summary of Synaptic Manipulations on the Astrocyte STC Amplitude and the fEPSP Slope

Data, normalized to control, are plotted as mean $\pm$ SD.

evidence. It is also possible that the STC reflects primarily glutamate released at synapses onto interneurons in stratum radiatum, which may undergo no change in release probability in response to HFS. However, because pyramidal cells greatly outnumber interneurons in the hippocampus (Brown and Zador, 1990) and individual interneurons in stratum radiatum do not appear to receive more synaptic inputs than pyramidal cells upon stimulation of Schaffer collateral fibers (Perouansky and Yaari, 1993), it is likely that the large majority of excitatory synapses in stratum radiatum are made onto pyramidal cells. Even if glutamate released onto interneurons were more accessible to astrocyte transporters, it remains improbable that such a small fraction of synapses could completely obscure changes at synapses onto pyramidal cells.

Determining the locus of LTP expression is a crucial step toward identifying the cellular processes that underlie synaptic plasticity. A number of studies have reported that, upon induction of LTP in CA1, an AMPA receptor-mediated component emerges at previously NMDA-only, "silent" synapses (Kullmann, 1994; Isaac et al., 1995; Liao et al., 1995; Durand et al., 1996). Based on these reports, it has been proposed that LTP is expressed, in large part, by the addition of functional AMPA receptors to previously "silent" synapses expressing only functional NMDA receptors. An alternative model contends that NMDA-only responses result from "spillover" of glutamate from neighboring synapses, activating only the more sensitive NMDA receptors at presynaptically quiescent synapses (Kullmann et al., 1996; Asztély et al., 1997). This indirect activation would then induce a retrograde signal to "turn on" the presynaptic terminal, explaining the increase in postsynaptic response. Recent evidence that synaptically released glutamate can activate transporters on surrounding glia (Mennerick and Zorumski, 1994; Mennerick et al., 1996; Bergles and Jahr, 1997; Bergles et al., 1997; Clark and Barbour, 1997; Linden, 1997) demonstrates that glutamate can "spill out" of the synaptic cleft. However, activation of receptors at neighboring synapses has not been shown directly. While the present data confirm

that glutamate "spillover" can be altered by changing release conditions, they argue against an increase in transmitter release resulting from induction of LTP.

Experimental Procedures

Hippocampal slices (400 μm) were prepared from 13- to 15-day-old Sprague-Dawley rats as previously described (Bergles and Jahr, 1997) and in accordance with institutional guidelines. Slices were prepared in ice-cold artificial cerebrospinal fluid (ACSF) containing 119 mM NaCl, 2.5 mM KCl, 1.3 mM MgCl_2 , 2.5 mM CaCl_2 , 26.2 mM NaHCO_3 , and 11 mM glucose, bubbled with 95% O_2 /5% CO_2 , and were transferred to identical solution at 34°C for 30 min and room temperature thereafter. Experiments were performed at room temperature with control ACSF containing 119 mM NaCl, 2.5 mM KCl, 4 mM MgCl_2 , 4 mM CaCl_2 , 26.2 mM NaHCO_3 , 11 mM glucose, and 0.1 mM picrotoxin, equilibrated with 95% O_2 /5% CO_2 and delivered via a gravity-fed perfusion system (2–5 ml min^{-1}). NaOH (2 mM) was added to all KYN-containing solutions to offset acidification caused by addition of the drug. Field (Axopatch 1B) and whole-cell (Axopatch 1D) recordings were made in stratum radiatum of the CA1 region. Field electrodes (1.5–5 M Ω) were filled with HEPES-buffered, glucose-free extracellular solution (pH 7.4); whole-cell electrodes (1.5–3 M Ω) were filled with 120 mM K methane sulfonate, 10 mM EGTA, 20 mM HEPES, 2 mM Mg-ATP and 0.2 mM Na-GTP (pH 7.4). While other anions more readily permeate the anion pore associated with the transporter (Wadiche et al., 1995), methane sulfonate was used to prolong the duration of the whole-cell recordings (see Bergles et al., 1997).

Astrocytes were identified as described (Bergles and Jahr, 1997), by their small cell bodies, low (~ 10 M Ω) input resistance, and high resting potentials (in the present study, astrocyte resting potentials were -96 ± 7 mV [$n = 40$]). By contrast, interneurons, which were, on occasion, patched inadvertently, exhibited input resistances of >500 M Ω and resting potentials near -70 mV. Astrocytes were held at their resting potential; the field electrode was maintained at 0 mV. For the experiments in CA1, stimuli (40–200 μA , 100 μs) were delivered via bipolar stimulating electrodes placed in stratum radiatum ~ 200 μm from the whole-cell electrode. In two-pathway experiments, the stimulating electrodes were placed on opposite sides of the recording electrodes, with the test pathway stimulator closer to the whole-cell (astrocyte) electrode. As a result, the fEPSP elicited in the test pathway was $74\% \pm 58\%$ of the fEPSP in the control pathway (range, 16%–209%; $n = 8$). Conversely, the STC in the test pathway was $211\% \pm 155\%$ of the STC in the control pathway (range, 57%–531%; $n = 8$). Whole-cell and field electrodes were placed within 50–100 μm of each other in stratum radiatum, at roughly equal distances from stratum pyramidale. Stimuli were delivered at 0.05–0.1 Hz; in LTP experiments, stimuli were alternated between test and control pathways and were always delivered at 0.05 Hz. Whole-cell data were sampled at 10 kHz and filtered at 1 kHz. Picrotoxin (0.1 mM) was added to block contamination by GABAergic synaptic inputs. Even though a cut was made between CA1 and CA3 (for the CA1 experiments) and elevated divalent cations were present, some epileptiform activity was observed in the field potentials. To prevent contamination of the measurements from this activity, fEPSPs were quantified by measuring the initial slope (by linear regression) following the fiber volley. In some cases, an averaged field potential recorded in the presence of receptor blockers that consisted only of the fiber volley was subtracted from control and test fEPSPs before the initial slope was measured. This procedure did not significantly alter the percentage changes in synaptic strength, indicating that under these conditions the fiber volley did not significantly contaminate the field response. In whole-cell recordings from astrocytes, the amplitude of the stimulus-activated steady-state current (e.g., Figure 2A), which is probably due to activity-dependent changes in extracellular potassium concentration (Bergles and Jahr, 1997; Clark and Barbour, 1997), was subtracted from the peak current to obtain the STC amplitude. Unless noted otherwise, all data are expressed as mean $\pm$ SD.

To test for independent pathways, both pathways were stimulated in the same trial, 50 ms apart. The order of stimulation was alternated

from trial to trial (1–2, 2–1, 1–2, etc.). Independence was concluded if the response in either pathway was the same regardless of whether it was the first or second response in a trial. Each pathway, stimulated alone (1–1, 2–2, 1–1, etc.), exhibited paired-pulse facilitation. LTP of the fEPSP was quantified by calculating the ratio of the fEPSP slope 40 min after and 2 min before HFS. The calculated percent change in the test pathway was normalized to any change in the control pathway.

fEPSPs in each pathway were scaled such that the baseline response preceding the first KYN application averaged 100%. STCs were normalized such that the responses during the first application of KYN averaged 100% for each pathway. We believe that rundown of the STC seen in some cells (see Results) was due to increasing access resistance over the course of the experiment. In experiments in which rundown occurred, the decrease in the STC amplitude was proportional to the decrease in the amplitude of the 0.2 mV conductance test pulse that preceded synaptic stimulation (data not shown). In addition, the STC and the potassium current always ran down to the same extent. As this rundown affected the responses in both pathways equally, it was accounted for by scaling the control STCs recorded during the second KYN application so that they averaged 100% and then scaling the corresponding test pathway STCs by the same factor.

Field potentials were recorded in voltage-clamp mode. The waveform of these responses was indistinguishable from those recorded with the same pipette in current clamp (Figure 1A). By scaling the current by the pipette resistance, the field response could be expressed in units of potential. This nonstandard recording procedure was adopted with the original intent of obtaining a continuous record of the STC by subtracting the field response from the astrocyte response. However, this approach was abandoned because the stimulus-evoked potassium current in the astrocyte recording and differences in the field potentials recorded by the two electrodes resulted in unreliable subtractions. In all field recordings made under voltage clamp, stimulation was preceded by a 0.2 mV test pulse. Voltage-clamped field responses were converted to potential by multiplying the current trace by the resistance of the field electrode (i.e., 0.2 mV divided by the amplitude of the current response during the test pulse). In control experiments, when suction was applied to the field electrode (between field responses) to clog the tip and increase its electrical resistance, the absolute amplitude of the fiber volley was proportional to the conductance of the field electrode, indicating that this method accounted for changes in electrode resistance.

For CA3 experiments, astrocytes located in stratum lucidum within 30 μm of the pyramidal cell layer were chosen for recordings. Mossy fibers were stimulated by placing the cathode pole of a bipolar stimulating electrode in stratum lucidum ~ 100 μm from the astrocyte. Forskolin was dissolved in DMSO and diluted 1:1000 in ACSF. All experiments were performed in the presence of NBQX (5 μM), CPP (5 μM), SR-95531 (5 μM), and picrotoxin (100 μM). Evoked responses in CA3 consisted of a large, slowly decaying inward current presumably reflecting the redistribution of extracellular potassium, with little or no transporter component. To allow for the comparison of the small transporter current revealed by forskolin, the amplitude of the response during the control period was subtracted from all responses. The amplitudes of the remaining transporter currents were then normalized either to the response remaining in DCG-IV (Figure 5) or to the response following washout of THA/DHK (Figure 6), to allow between-cell comparisons. Due to the small size of electrodes used to patch CA3 astrocytes, these electrodes were more susceptible to increases in access resistance, causing a slow rundown in the amplitude of evoked responses. To account for this rundown in the summary data, response amplitudes were scaled to the amplitude of the conductance pulse applied before each evoked response. In control experiments where no pharmacological manipulations were made, the size of the evoked responses was directly proportional to the size of the conductance pulse.

For patch experiments, K methane sulfonate was replaced in the intracellular solution with KSCN to increase the anionic conductance associated with glutamate transport (Fairman et al., 1995; Wadiche et al., 1995; Eliasof and Jahr, 1996). Data were filtered at 5 kHz and sampled at 50 kHz. Solution exchanges were effected with a piezoelectric-driven multi-barreled flow pipe (Lester and Jahr, 1992).

SNP was added to solution from powder ~5 min prior to application to the patch.

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