Synaptically Released Glutamate Does Not Overwhelm Transporters on Hippocampal Astrocytes During High-Frequency Stimulation

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Diamond, Jeffrey S. and Craig E. Jahr. Synaptically released glutamate does not overwhelm transporters on hippocampal astrocytes during high-frequency stimulation. J. Neurophysiol. 83: 2835–2843, 2000. In addition to maintaining the extracellular glutamate concentration at low ambient levels, high-affinity glutamate transporters play a direct role in synaptic transmission by speeding the clearance of glutamate from the synaptic cleft and limiting the extent to which transmitter spills over between synapses. Transporters are expressed in both neurons and glia, but glial transporters are likely to play the major role in removing synaptically released glutamate from the extracellular space. The role of transporters in synaptic transmission has been studied directly by measuring synaptically activated, transporter-mediated currents (STCs) in neurons and astrocytes. Here we record from astrocytes in the CA1 region of hippocampal slices and eliciting STCs with high-frequency (100 Hz) stimulus trains of varying length to determine whether transporters are overwhelmed by stimuli that induce long-term potentiation. We show that, at near-physiological temperatures (34°C), high-frequency stimulation (HFS) does not affect the rate at which transporters clear glutamate from the extrasynaptic space. Thus, although spillover between synapses during “normal” stimulation may compromise the absolute synaptic specificity of fast excitatory synaptic transmission, spillover is not exacerbated during HFS. Transporter capacity is diminished somewhat at room temperature (24°C), although transmitter released during brief, “theta burst” stimulation is still cleared as quickly as following a single stimulus, even when transport capacity is partially diminished by pharmacological means.

Although postsynaptic neuronal transporters remove a significant fraction of transmitter at the cerebellar climbing fiber-Purkinje cell synapse (Otis et al. 1997), several lines of evidence suggest that glutamate is transported primarily into glia (Bergles and Jahr 1998; de Barry et al. 1982; Koijima et al. 1999; McLennan 1976; Rothstein et al. 1994, 1996; Tanaka et al. 1997; Wilkin et al. 1982). The proposed buffering action of transporters would require a high density of transporters in glial processes, which has been demonstrated with both biochemical (Lehre and Danbolt 1998) and electrophysiological (Bergles and Jahr 1997) approaches.

By reducing glutamate spillover, glial transporters may help to isolate synapses, preserving the signal specificity thought to be required for efficient information processing. However, recent physiological evidence suggests that, in the hippocampus, such isolation may be incomplete (Asztely et al. 1997; Kullmann et al. 1996), perhaps due to the fact that many adjacent synapses in CA1 stratum radiatum have no glial processes between them (Harris and Ventura 1998; Lehre and Danbolt 1998). Moreover, it is not known to what degree glutamate transporters are occupied by synaptically released transmitter, or the extent to which this occupancy is affected by increased levels of synaptic activity, such as during bursts of high-frequency stimulation (HFS) that induce long-term potentiation (LTP) (Bliss and Lomo 1973). Synapse specificity is generally assumed to be preserved during HFS, even though the ability of glutamate transporters to accommodate the increased amounts of glutamate released during such episodes has not been tested. If transporters were overwhelmed by glutamate during HFS, transmitter might diffuse further from its point of release and activate extrasynaptic metabotropic receptors or even ionotropic glutamate receptors in neighboring, inactive synapses. The latter effect, which has become known as “spillover,” could lead to non-Hebbian changes in synaptic efficacy.

The present experiments used synaptically activated, transporter-mediated currents (STCs) recorded in astrocytes located in CA1 stratum radiatum of hippocampal slices to study the time course of glutamate transport under different stimulus conditions. The results indicate that at physiological temperatures transporters are capable of clearing glutamate released during a burst of HFS nearly as quickly as after a single stimulus. At room temperature, transporters appear overwhelmed during longer bursts of HFS (e.g., “tetanic” stimulation), but not during shorter trains (e.g., “theta” stimulation).
METHODS

Slice preparation and extracellular solutions

Hippocampal slices (400 μm) were prepared from 13- to 15-day-old Sprague-Dawley rats, as described (Bergles and Jahr 1997) and in accordance with institutional guidelines. Slices were prepared in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM) 119 NaCl, 2.5 KCl, 1.3 MgCl₂, 2.5 CaCl₂, 26.2 NaHCO₃, and 11 glucose, bubbled with 95% O₂-5% CO₂ and delivered via a gravity-fed perfusion system (2–5 ml/min). Except where noted, the A₁ adenosine receptor antagonist 8-cyclopentyl-1,3-dimethylxanthine (8-CPT, 4 μM) was included in all solutions, to increase release probability (although see RESULTS) and also to reduce the possibly confounding effects of temperature on adenosine transport (Diao and Dunwiddie 1998). For astrocyte experiments, the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptor antagonists 6-nitro-7-sulfamoylbenzo[f]quinazoline-2,3-dione (NBQX, 5 μM) and (RS)-3-(2-carboxypropyl)aminopropyl-1-phosphonic acid (CPP, 5 μM) were added to the control ACSF to block the neuronal field potential that obscures the STC. For pyramidal cell experiments, 5 μM CPP was included to isolate the AMPA receptor-mediated component of the excitatory postsynaptic current (EPSC).

Electrophysiology

Whole cell (Axopatch 1D) recordings were made from astrocytes in stratum radiatum and from pyramidal cells in stratum pyramidale of the CA1 region. Whole cell electrodes (1.5–2.5 MΩ) were filled with (in mM) 120 K methane sulfonate (for astrocyte recordings) or Cs methane sulfonate (for pyramidal cell recordings), 10 EGTA, 20 HEPES, 2 MgATP, and 0.2 NaGTP (pH 7.4). Access resistance, estimated from the peak of the current transient elicited by a brief 1- to 2-mV test pulse preceding synaptic stimulation, was typically 5–15 MΩ and was not compensated.

Astrocytes were identified by their small cell bodies, low (∼10 MΩ) input resistance, and high resting potentials (approximately ∼95 mV). Astrocytes were held at their resting potential; pyramidal cells were held at −70 mV. Stimuli (60–200 μA, 100 μs duration) were delivered via a bipolar stimulating electrode placed in stratum radiatum ∼200 μm from the whole cell electrode.

Sahs and glucose were obtained from Mallinkrodt (Paris, KY), and all other reagents were obtained from Sigma (St. Louis, MO), except NBQX (RBI, Natick, MA), CPP (RBI), 8-CPT (RBI), (RS)-α-cyclopentyl-4-phosphonophenylglycine (CPPG, Tocris Cookson, St. Louis, MO), and (RS)-α-methyl-4-carboxyphenylglycine (MCPG, Tocris Cookson).

Analysis

Data acquisition and analysis was performed with custom macros written in Igor Pro (WaveMetrics). Data were sampled at 10–20 kHz and filtered at 2 kHz. In whole cell recordings from astrocytes, the amplitude of the stimulus-activated steady-state potassium current was subtracted from the peak current to obtain the STC amplitude. For coefficient of variation (CV) analysis (Fig. 1), the variance of the potassium current (measured 65 ms after stimulation) was subtracted from the variance of the peak amplitude before calculating the CV of the STC. In some experiments, responses elicited in the presence of 300 μM dihydrokainate (DHK) and 500 μM threo-β-hydroxyaspartic acid (THA), which completely blocked the STC, were subtracted from responses in 300 μM DHK + 100 μM THA to eliminate the potassium current. This operation did not affect the time course of the STC decay. Unless noted otherwise, all data are expressed as means ± SD and P values were calculated using a paired t-test.

RESULTS

Whole cell recordings were made with patch electrodes in voltage-clamp mode from astrocytes located in stratum radiatum of the CA1 region of hippocampal slices, and synaptic responses were elicited by stimulating the Schaffer collateral/commissural fibers. AMPA and NMDA receptor antagonists (5 μM NBQX and 5 μM CPP) were applied to block the postsynaptic field depolarization generated in the surrounding neurons. Under these conditions, synthetically activated currents in astrocytes consisted of a small, long-lasting component, thought to arise from the slow reequilibration of extracellular potassium following stimulation, and a transient component (Fig. 1A). This transient current can be blocked by a cocktail of the glutamate transporter antagonists DHK and THA (Bergles and Jahr 1997; Diamond et al. 1998), identifying it as a STC (Otis et al. 1997). Although glutamate transport is associated with an anion conductance (Fairman et al. 1995; Wadiche et al. 1995), the STCs described here were recorded with an impermanent anion conductance (Fairman et al. 1995; Wadiche et al. 1995), which could account for the slow reequilibration of extracellular potassium. In addition to this slow component, the STC amplitude includes a fast component that is influenced by the decay of the stimulus-evoked excitatory postsynaptic current (EPSC) (Diamond and Jahr 1995) and also to reduce the possibly confounding effects of temperature on adenosine transport (Diao and Dunwiddie 1998). For astrocyte experiments, the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptor antagonists 6-nitro-7-sulfamoylbenzo[f]quinazoline-2,3-dione (NBQX, 5 μM) and (RS)-3-(2-carboxypropyl)aminopropyl-1-phosphonic acid (CPP, 5 μM) were added to the control ACSF to block the neuronal field potential that obscures the STC. For pyramidal cell experiments, 5 μM CPP was included to isolate the AMPA receptor-mediated component of the excitatory postsynaptic current (EPSC).
anion (methane sulfonate) in the patch pipette and therefore primarily reflect the charge flux arising from the electrogenic glutamate transport cycle.

**STCs reflect activation of a large number of synapses**

STCs recorded in astrocytes have been shown to follow changes in release probability (Diamond et al. 1998; Luscher et al. 1998), but the number of synapses contributing to an astrocyte STC (i.e., the quantal content, \( m \)) is unknown. Because postsynaptic responses to single-vesicle events cannot be resolved in astrocytic recordings, the coefficient of variation (CV = \( \sigma / \text{mean} \)) method (Clements 1990; del Castillo and Katz 1954; Faber and Korn 1991) was used to obtain a rough estimate of the quantal content of STCs relative to pyramidal cell EPSCs in the same slice.

Inspection of 50 consecutive STCs recorded in an astrocyte (Fig. 1A) indicated that the responses exhibited very little trial-to-trial variation. EPSCs elicited by identical stimulation in a nearby pyramidal cell in the same slice were significantly more variable (Fig. 1B). In four slices the CV of evoked STCs (0.006 ± 0.002, mean ± SD) was 15 times less than that of EPSCs (0.09 ± 0.03) recorded from pyramidal cells located equidistant from the stimulating electrode (\( P = 0.012 \)). If transmitter release is a Poisson process, then the CV of the response amplitude is inversely proportional to the square root of the mean quantal content (e.g., \( m = 1 / CV^2 \)), suggesting that an astrocyte STC may reflect release from several hundred times as many synapses as a pyramidal cell EPSC. Two factors complicate more precise quantitation of these results. First, release probability at excitatory synapses onto CA1 pyramidal cells is probably too great to be described adequately by Poisson statistics (Hjelmstad et al. 1997; Stevens and Wang 1995). Second, much of the STC is likely to be shunted by the low resistance astrocytic membrane, although this would probably affect the variance and mean to similar extents (see next section) and therefore exert little effect on the CV.

**STCs reliably report changes in response amplitude and time course**

The experiments described in this study rely on the ability to accurately record changes in the size and shape of the STC under different conditions. To test for this, STCs were elicited by a range of stimulus intensities, which caused broad changes in response amplitude (Fig. 2A1). Normalizing these responses indicated that large differences in amplitude did not affect the time course of the STC (Fig. 2A2).

Hippocampal astrocytes have extremely low input resistances (\( \# \approx 10 \text{ M\Omega} \)), which, although severely compromising space clamp by the recording electrode, would also limit the membrane depolarization caused by electrogenic transport (Hausser and Roth 1997; Spruston et al. 1993). Therefore, although the current measured at the soma reflects a large underestimate of synaptic conductance, proportional changes appear to be recorded faithfully.

![Fig. 2](https://example.com/fig2.png)  
**FIG. 2.** Astrocyte STCs recorded in voltage-clamp mode accurately reflect relative changes in the transporter-mediated conductance. Temperature, 24°C. A1: STCs elicited by 4 different stimulus intensities. A2: responses from A1 normalized to the same amplitude to allow comparison of time course. B1: responses from the same astrocyte as in A, but in the presence of 300 \( \mu \text{M} \) dihydrokainate (DHK). C1: comparison of the effects of DHK on STC amplitude (●, Fig. 2C1), charge transfer (○, Fig. 2C1), and decay (□, Fig. 2C2) over the same range of stimulus intensities as in A. The data (\( n = 6 \)) are plotted vs. the amplitude of the STC in control solution, normalized to the response to 150-\( \mu \text{A} \) stimulation. The charge transfer values (C1) were insignificantly different from unity (\( P > 0.05 \)) at all stimulation intensities.
Measuring the occupancy of transporters during synaptic transmission

Our strategy to determine the extent to which transporters are occupied by synaptically released glutamate was based on the following rationale: if glutamate released during bursts of high-frequency synaptic stimulation were sufficient to saturate transporters, then the glutamate released late in the burst would remain in the extrasynaptic space for a longer time than following a single stimulus because transporter binding capacity would be overwhelmed. This would result in a slowing of the STC decay. To test this prediction, STCs were elicited by single stimuli and 100-Hz trains of 3, 4, 9, or 10 stimuli (Fig. 3A). STCs elicited by single stimuli decayed exponentially (at 24°C, τ = 16.3 ± 2.9 ms, n = 25). To extract the response to the fourth stimulus, the response to a train of three stimuli was subtracted out in an analogous manner (Fig. 3B). The decay time courses of the subtracted responses could then be compared with the single-stimulus response by fitting the decays with a single-exponential function and calculating the ratios τ_d/τ_1 or τ_1/τ_d. In many cases, responses elicited 15–30 s after HFS were slightly enhanced, due to residual posttetanic potentiation. This often resulted in small differences in the amplitudes of responses in trials immediately following single-stimulus trials and responses in trials immediately following HFS trials (Fig. 3A).

Effect of transporter antagonists on STC elicited by stimulus trains

The above results indicate that the glutamate released in response to the fourth in a 100-Hz train of stimuli is cleared about as quickly as transmitter released in response to a single stimulus. This suggests that the capacity of the transporters is
not exceeded during a four-stimulus train. To determine how far transporters are from saturation under these stimulus conditions, we sought to decrease the transport capacity of the astrocyte until an increase in $t_4/t_1$ was observed. This was initially attempted by blocking a fraction of transporters with one of two competitive antagonists. At 300 mM, DHK blocks transport mediated specifically by the EAAT2/GLT-1 transporter subtype (Arriza et al. 1994), which makes up nearly 80% of the transporters in stratum radiatum (Lehre and Danbolt 1998). THA does not differentiate between transporter subtypes but, unlike DHK, also acts as a substrate (Arriza et al. 1994), reducing its efficacy because uptake lowers its concentration. STC amplitude was reduced by either 300 mM DHK (to 44 ± 4% of control; $n = 5$; Fig. 5A1) or 300 mM THA (to 41 ± 10% of control; $n = 6$; Fig. 5B1), in agreement with a previous report (Bergles and Jahr 1997). In addition, both drugs slowed the decay of the STC (Fig. 5, A1 and B1), possibly indicating that transporters near active synapses normally take up much of the synaptically released glutamate but are partially prevented from doing so in the presence of antagonist, allowing glutamate to diffuse a greater distance before being transported. It may be that transporters close to the release site actually are saturated by transmitter, whereas those further away remain unoccupied, a three-dimensional analogue of the “saturated disk” phenomenon described at the neuromuscular junction (Hartzell et al. 1975). The slowing of the STC in DHK or THA suggests that the size of the “saturated sphere” is inversely related to transport capacity. Expanding the dimensions of this region of saturation with transporter antagonists might cause neighboring “spheres” to overlap during high-frequency stimulation, leading to saturation of all the transporters between release sites and, as a result, slower clearance. However, neither DHK (Fig. 5A3) nor THA (Fig. 5B3) alone affected $t_4/t_1$ (DHK: $t_4/t_1 = 110 ± 24\%$ of control; $n = 5$, $P = 0.4$; THA: $t_4/t_1 = 108 ± 10\%$ of control, $n = 6$, $P = 0.1$; see Fig. 7). These results suggest that total transport capacity is not overwhelmed during a brief HFS train, even when a majority of the transporters are blocked by a competitive antagonist.

To reduce the transport capacity even further, 100 mM THA was added to the bath together with 300 mM DHK (Fig. 6). At 24°C this antagonist cocktail decreased the STC amplitude to 26 ± 7% ($n = 9$) of control and slowed $t_1$ by 277 ± 168% ($n = 9$; e.g., Fig. 6A). In addition, the DHK/THA cocktail increased $t_4/t_1$ to 161 ± 49% of control ($n = 8$, $P = 0.008$; Fig. 7). At 34°C the DHK/THA cocktail exerted similar effects on STCs elicited by single stimuli (Fig. 6B), decreasing the STC amplitude to 35 ± 10% ($n = 7$) of control and slowing $t_1$.
by 350 ± 77% (n = 7), yet the effect on $\tau_d/\tau_1$ was completely abolished (100 ± 19% of control, n = 7, $P = 0.97$; Fig. 7). These results indicate that blocking a large majority of transporters can cause a marked slowing of transmitter clearance at 24°C. At 34°C, however, it appears that transport capacity is so great that even a small fraction of transporters is able to take up transmitter released during a brief train of high-frequency stimulation.

**Effects of metabotropic glutamate receptor antagonists on STCs**

Exogenous activation of metabotropic glutamate receptors (mGluRs) decreases the probability of release at excitatory synapses onto many cell types in the brain, including CA1 pyramidal cells (Baskys and Malenka 1991; Forsythe and Clements 1990). Synaptic activation of mGluRs reduces transmitter release at CA3 mossy fiber synapses (Min et al. 1998; Scanziani et al. 1997) and, to a lesser extent, at a calyceal synapse in the brain stem (von Gersdorff et al. 1997), but effects in CA1 have not been reported. It is also unknown whether activation of presynaptic mGluRs in CA1 might be restricted by glutamate transporters, as in CA3 (Min et al. 1998; Scanziani et al. 1997), although DHK has been shown to increase activation of postsynaptic mGluRs elicited by very strong stimulation in CA1 pyramidal cells (Cognar et al. 1997).

Blocking the majority of transporters with the DHK/THA cocktail in the experiments described above (Fig. 6) may have elevated ambient levels of glutamate or extended the extrasynaptic diffusion of synaptically released glutamate sufficiently to increase activation of mGluRs and perhaps affect the shape of the STC during an HFS train. To test this possibility, the effects of the mGluR antagonists CPPG (300 $\mu$M) and MCPG (1 mM) were measured on STCs elicited by single stimuli and four-stimulus (100 Hz) trains. At 24°C in the presence of DHK and THA, the addition of MCPG and CPPG did not affect the response to a single stimulus (P, in MCPG/CPPG = 89 ± 11% of control, n = 4, $P = 0.2$). This result suggests that blocking...
transporters with the DHK/THA cocktail did not elevate ambient glutamate levels sufficiently to change release probability via activation of mGluRs. CPPG and MCPG caused only a small increase on $\tau_d/\tau_t$, either in the absence (110 ± 5% of control, $n = 4$, $P = 0.026$) or in the presence (107 ± 8% of control, $n = 5$, $P = 0.15$) of 300 $\mu$M DHK and 100 $\mu$M THA. Similar results were observed at 34°C in the presence of DHK and THA (114 ± 12% of control, $n = 5$, $P = 0.06$). The large increase in $\tau_d/\tau_t$ observed at 24°C in the presence of DHK and THA (Figs. 6 and 7) therefore appears not to be due to increased activation of mGluRs when glutamate uptake is reduced.

**Discussion**

The experiments presented here used transporter-mediated synaptic responses in hippocampal CA1 astrocytes to explore whether glutamate transporters are overwhelmed by HFS. The results indicate that, at 24°C, transporters clear glutamate released during a brief (4-stimulus) burst of HFS quite easily. Significant slowing of transmitter clearance was observed only if a large majority of transporters were blocked with competitive antagonists. At 34°C, even the small remaining fraction of transporters appeared capable of clearing glutamate after a brief burst of HFS. In the absence of antagonists, transporters were overwhelmed by longer (10-stimulus) HFS bursts at 24°C but not at 34°C. Taken together, these results suggest that glutamate transporters do a remarkably good job of clearing synthetically released glutamate over a range of stimulus conditions, particularly at physiological temperatures.

**Mechanisms underlying the time course of the STC**

Implicit in this interpretation is the assumption that the decay of the STC reflects the decline in the extrasympathetic glutamate concentration following synaptic stimulation. Theoretical and experimental results suggest that the glutamate concentration inside the cleft decreases to ≤10 $\mu$M 5–10 ms after the release of a synaptic vesicle (Clements 1996; Clements et al. 1992; Diamond and Jahr 1997; Wahl et al. 1996), which is approximately the time required for the STC to reach a peak (Bergles and Jahr 1997; see also the present data). Thus even allowing for concomitant release from neighboring synapses in response to the same stimulus, the average extrasynaptic concentration of glutamate during the decay of the STC is likely to be below the EC$_{50}$ of glial transporters for glutamate (∼20 $\mu$M) (Arriza et al. 1994; Bergles and Jahr 1997; Klockner et al. 1994; Pines et al. 1992) and, in fact, well below the concentration of the transporters themselves (140–250 $\mu$M) (Lehre and Danbolt 1998). The decay of the STC is therefore likely to be proportional to the decrease in the number of extant glutamate molecules. Accordingly, the STC decay ($\tau \sim 16$ ms) is much slower than the decay of transporter-mediated currents in the continuous presence of glutamate ($\tau \sim 1$ ms) or on removal of glutamate ($\tau \sim 3$ ms) in excised patches (Bergles and Jahr 1997), as well as the decay of the AMPA receptor EPSC ($\tau \sim 7$ ms, data not shown), ruling out major roles for transporter kinetics and asynchronous release in shaping the STC decay (see also Bergles and Jahr 1997).

**Spatial extent of glutamate diffusion**

The STC is clearly slowed in the presence of DHK or THA (Figs. 2 and 5), yet transport is not slowed further under these conditions during a four-stimulus train (Fig. 5). This suggests that, in the presence of antagonist and perhaps even in control, transporters in the region closest to the release site are saturated by transmitter, forcing glutamate to diffuse farther to find available sites. Although DHK or THA would certainly expand regions of high occupancy [“saturated spheres,” analogous to the “saturated disk” at the neuromuscular junction (Hartzell et al. 1975)], neither DHK nor THA affect $\tau_d/\tau_t$, suggesting that there remain unoccupied transporters between active release sites during a brief train of HFS, even with transport capacity significantly diminished. It may be that electrical stimulation recruits only a small fraction of the Schaffer collateral fibers within a given volume and that release probability remains relatively low, despite our pharmacological efforts to increase it (see METHODS). As a result, active release sites may be well separated spatially, even during brief bursts of HFS, and glutamate may be permitted to diffuse a significant distance without overlapping significantly with transmitter released from another synapse. It would be desirable to use the kinetics of the STC decay to estimate the spatial extent of glutamate diffusion away from the release site, but such a calculation requires more information about the diffusion coefficient of glutamate in the neuropil (although see Rusakov and Kullmann 1998) and the profile of transporter concentration versus radial distance from the synaptic cleft.

**Astrocytes take up a large majority of synaptically released glutamate**

STCs recorded in CA1 stratum radiatum have been used to monitor changes in synthetically released glutamate under different experimental conditions (Diamond et al. 1998; Luscher et al. 1998). STCs reliably reported changes caused by numerous manipulations of release probability but were unaffected by the induction of LTP, leading to the conclusion that LTP is not expressed via any change in the amount of glutamate released in response to synaptic stimulation (Diamond et al. 1998; Luscher et al. 1998). However, many excitatory synapses in CA1 stratum radiatum are not immediately adjacent to an astrocytic process (Harris and Ventura 1998; Lehre and Danbolt 1998), suggesting that astrocytes might not detect glutamate release from all synapses. One possibility is that astrocytes might take up glutamate released from only a particular fraction of synapses, perhaps a subset that does not undergo LTP. Given the density and affinity of astrocytic transporters in the CA1 neuropil, however, it seems likely that transmitter released from all synapses, even those located some distance from an astrocyte process, could be taken up by transporters on an astrocyte and contribute to the STC. However, some other “glutamate sink,” including the superfusion saline above the slice, might prevent transmitter released at some synapses from reaching astrocyte transporters. We would argue against this possibility based on the data presented in Fig. 2. At 300 $\mu$M, DHK is a selective and nearly saturating antagonist for the GLT-1 transporter subtype (Arriza et al. 1994), which is expressed exclusively in astrocytes (Rothstein et al. 1994). Although DHK decreased the amplitude and
sloved the decay of the STC, it did not significantly reduce the amount of glutamate transported by the astrocyte, as indicated by the total charge transfer of the STC (see Fig. 2C1, legend), despite the fact that in hippocampus ∼80% of astrocytic transporters are GLT-1 (Lehre and Danbolt 1998). If another significant source of glutamate uptake besides the GLAST transporter subtype is present, it would have removed some of the glutamate normally transported by GLT-1, and the charge transfer of the STC in DHK will have been significantly less than in control. Notwithstanding the possibility of a novel, nonastroglial, DHK-sensitive transporter subtype, we interpret this result to indicate that astrocytes take up nearly all of the glutamate released in a region of CA1 stratum radiatum, and that the STC provides a proportional measure of this uptake. This conclusion is consistent with recent optical (Kojima et al. 1999) and physiological (Bergles and Jahr 1998) data indicating that neuronal transporters play, at best, a minor role in glutamate uptake in the hippocampus.

Implications for the input-specificity of LTP

One of the hallmarks of LTP, its input specificity (Andersen et al. 1977; Lynch et al. 1977), seems at odds with the evidence for spillover (Asztely et al. 1997; Kullmann and Asztely 1998; Kullmann et al. 1996) and the extrasynaptic diffusion of glutamate implicit in STCs. However, if the spillover said to occur following a single-shock stimulation is not significantly enhanced during HFS required for LTP induction, then whatever input specificity existed before LTP induction would be largely preserved afterward. Therefore, although evidence for spillover may color our interpretation of silent synapses in the hippocampus (Isaac et al. 1995; Kullmann 1994; Liao et al. 1995), the input-specific nature of LTP (if not its absolute synapse specificity) might not be compromised unless spillover were exacerbated during periods of intense stimulation. Previous work has suggested that transporters restrict spillover (Asztely et al. 1997); the present data suggest that, at physiological temperatures, transport capacity is not challenged during HFS.

At room temperature, the ability of transporters to limit increased spillover during induction of LTP may depend on the protocol used. The four-stimulus, 100-Hz trains used above correspond to theta-burst stimulation, a potent LTP induction protocol in which such trains are delivered at 200-ms intervals (e.g., Larsson et al. 1986). The results presented here indicate that a four-stimulus train does not result in slowed glutamate clearance, and the 200-ms interval clearly provides enough time for transporters to return the glutamate concentration back to resting levels. Induction protocols comprising longer trains may, however, overwhelm transport at 24°C, possibly leading to a loss of input specificity. Given the remarkable capacity of transporters at higher temperatures, however, input specificity seems likely to be preserved in vivo, regardless of stimulus pattern.

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