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Glutamate transporters bring competition to the synapse

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Glutamate transporters (GluTs) prevent the accumulation of glutamate and influence the occupancy of receptors at synapses. The ability of extrasynaptic NMDA receptors and metabotropic glutamate receptors to participate in signaling is tightly regulated by GluT activity. Astrocytes express the highest density of GluTs and dominate clearance away from these receptors; synapses that are not associated with astrocyte processes experience greater mGluR activation and can be exposed to glutamate released at adjacent synapses. Although less abundant, neuronal transporters residing in the postsynaptic membrane can also shield receptors from the glutamate that is released. The diversity in synaptic morphology suggests a correspondingly rich diversity of GluT function in excitatory transmission.

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Abbreviations

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate
CNS	central nervous system
D-AA	D-α-amino adipate
EPSC	excitatory postsynaptic current
GABA	γ-aminobutyric acid
GluT	glutamate transporter
LTP	long term potentiation
mGluR	metabotropic glutamate receptor
NMDA	*N*-methyl-D-aspartate

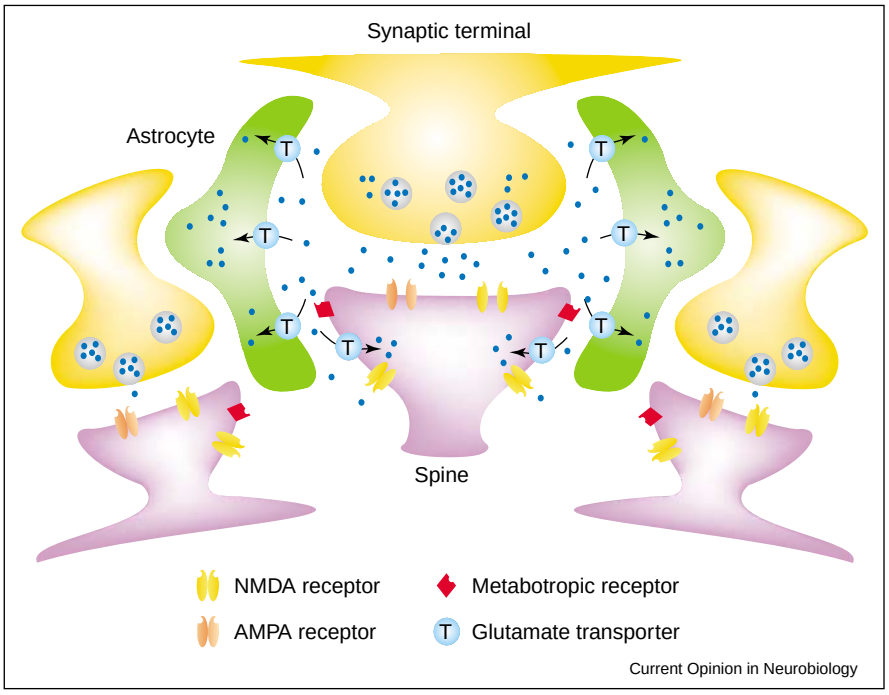
Introduction

Glutamate is a ubiquitous excitatory neurotransmitter and a potent neurotoxin, and its movement and lifetime in the extracellular space are tightly constrained. Glutamate transporters (GluTs) play a crucial part in preventing the accumulation of extracellular glutamate, which, apart from passive diffusion, represents the primary mechanism for glutamate clearance. Disrupting the expression or activity of these transporters results in excessive activation of glutamate receptors, abnormal neuronal activity, and eventual excitotoxic degeneration [1,2]. Considerable effort is being expended to understand the mechanisms responsible for transporter dysfunction in disease [3], and the contribution of reverse transport, the inappropriate transport of glutamate out of the cell, to glutamate release during acute ischemic episodes [4]. However, studies completed during the past ten years indicate that transporters do more than maintain low extracellular glutamate levels. Because these proteins are abundant near synapses and bind glutamate rapidly, they compete with receptors for glutamate that is released, and shape the concentration transient that receptors are exposed to. As a result, transporter activity influences receptor occupancy at individual synapses, and prevents promiscuous activation of receptors at neighboring synapses (see Figure 1). Although a general picture of GluT function has emerged from these studies, it is clear that the contribution of transporters to signaling varies considerably among synapses; it is highly dependent on their structure, their association with glial cells, and the properties and locations of their glutamate receptors. Furthermore, this interaction is not fixed, but often changes depending on the frequency of release, the local ionic environment, and the structure of the synapse. Recent studies have demonstrated that the activity of receptors located outside the synaptic cleft is highly influenced by GluTs. This review focuses on these and other recent studies that have begun to define the role of GluTs in shaping the activation of receptors, in particular extrasynaptic receptors in the postsynaptic membrane. Although regulation of the expression and activity of transporters undoubtedly impacts this signaling, the reader is directed to the reviews by Danbolt and Gonzalez and Robinson [5,6] for a discussion of these data.

Escaping the transporter juggernaut

The influence of GluTs on excitatory transmission depends, in part, on the distribution and properties of glutamate receptors. Although α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and *N*-methyl-D-aspartate (NMDA) receptors are most often clustered in the postsynaptic membrane, at some synapses NMDA receptors appear to be exclusively extrasynaptic. Furthermore, metabotropic glutamate receptors (mGluRs) are enriched in an annulus surrounding the postsynaptic density [7] at the periphery of the synaptic junction, and are found on γ-aminobutyric acid (GABA)ergic terminals and astrocytes. This peri- or extrasynaptic localization suggests that postsynaptic signaling events might also occur outside the synaptic cleft. However, extrasynaptic signaling ('spillout') must contend with GluTs that are abundant in this region [8–10]. GluTs have an

Figure 1



The location of glutamate transporters and receptors at a typical excitatory synapse. Unlike receptors, glutamate transporters are excluded from the synaptic cleft. EAAT1 (GLAST) and EAAT2 (GLT-1) are present at a high density in the membranes of astrocytes that often ensheath synapses. EAAT3 (EAAC1) is found in the soma and dendrites of neurons, but is also found in GABAergic terminals (not shown). Glutamate transporters shield extrasynaptic NMDA receptors and mGluRs from glutamate as it diffuses from the cleft, and prevent glutamate from reaching receptors at nearby synapses. Inhibition of these transporters potentiates excitatory responses mediated by these receptors, and allows glutamate spillover, which suggests that transporter regulation might be used to regulate synaptic efficacy. Note that presynaptic mGluRs have been omitted from this diagram.

affinity comparable to NMDA receptors and mGluRs (GluT EC_{50}: 4 – 30 μM) [11–13]; and the rise times of glutamate-evoked transporter currents in outside-out patches [14,15] and in whole-cell recordings in response to photolysis [16] indicate that they bind glutamate as rapidly as AMPA receptors. The number of transporters is higher than the number of receptors in the extrasynaptic region [9], which suggests that they are likely to dominate the race for binding. In part, this could explain why activation of mGluRs and extrasynaptic NMDA receptors typically requires high frequency stimulation [17–19]. However, the slow nature of the transport process adds further complexity to signaling in this domain.

GluTs complete a reversible cycle in which $1H^+$ and $3Na^+$ ions are co-transported with glutamate, and the $1K^+$ ion is counter-transported [5,20]. This process requires 10–75 ms to complete, depending on the particular transporter [14,21–23], which suggests that sustained release could engage a significant fraction of the transporter pool and allow glutamate to avoid sequestration long enough to activate receptors. As transporter occupancy increases, glutamate will be allowed to diffuse further and gain access to additional receptors, possibly even those at neighboring synapses [24]. This model is supported by recent studies of excitatory synapses in the cerebellum, hippocampus, and retina. In the cerebellum, inhibition of GluTs potentiates the activity of postsynaptic mGluRs in Purkinje neurons in response to parallel fiber stimulation [25,26], and facilitates mGluR-mediated long-term depression (LTD) [25]. In the hippocampus, GluT inhibition similarly potentiates postsynaptic mGluR activation in interneurons, leading to enhanced inhibition of pyramidal neurons [27•]. This phenomenon is not restricted to mGluRs, as GluT inhibition also facilitates the recruitment of extrasynaptic NMDA receptors at parallel fiber–interneuron synapses in the cerebellum [17,28], as well as at ganglion cell synapses in the retina [18]. GluTs not only restrict 'spillout' of glutamate and activation of homosynaptic receptors, but also shield receptors from glutamate that is released at neighboring synapses ('spillover'). In hippocampal pyramidal neurons, GluT inhibition allows glutamate to diffuse from one set of synapses and activate NMDA receptors at adjacent synapses [24,29•,30]. As demonstrated by a recent study of long-term potentiation (LTP) in the lateral amygdala [31•], this loss of input specificity during GluT inhibition can allow induction of a heterosynaptic form of LTP. Most of these studies relied on field stimulation, which can cause greater activation of extrasynaptic receptors

than occurs *in vivo* by forcing summation of glutamate transients from adjacent synapses ('pooling'). Nevertheless, paired recordings from pyramidal neurons in hippocampal slice cultures that were connected by a small number of synapses [29•] indicate that, qualitatively, GluTs take a similar role at individual synapses.

Three other discoveries have a direct bearing on these results. First, transporters exhibit an efficiency close to 50% [32,33]; glutamate, once bound, is as likely to unbind as it is to be translocated. Thus, the decay of glutamate transients will depend on the rate of unbinding; if this rate is slow, transporters will continue to be effective at rapidly buffering extracellular glutamate transients [34]. Second, changes in Na^+, K^+, or H^+ gradients can have profound effects on cycling rates, efficiencies, and under extreme conditions can force transporters to cycle in reverse, releasing glutamate to the outside [35]. In particular, changes in extracellular K^+ that occur with high frequency activity might slow transporter cycling and promote extrasynaptic signaling. Third, release might occur 'ectopically' from outside the active zone at certain synapses [36•]. If release occurs adjacent to peri- or extrasynaptic receptors, higher occupancy might be achieved despite the presence of GluTs.

A giant sucking sound from astrocytes

The task of glutamate clearance in the central nervous system (CNS) is spread among six different GluTs, encoded by five distinct genes: EAAT1 (GLAST), EAAT2 (GLT-1), EAAT3 (EAAC1), EAAT4, and EAAT5. This elaboration of clearance pathways is greater for glutamate than for other neurotransmitters, as there are only two transporters for GABA (GAT-1,2), and only one for each of the monoamines, norepinephrine (NET), dopamine (DAT), and serotonin (SERT). Finer control over extracellular glutamate dynamics may be achieved by spreading the task of glutamate uptake among different transporters. Although it was initially proposed that glutamate was taken up back into the presynaptic terminal [37], anatomical studies suggest that EAAT1 (GLAST) and EAAT2 (GLT-1), the most abundant GluTs [1,38], are found in astrocyte membranes [9,39,40] that are in close association with, and sometimes ensheath synapses [41,42]. These results suggest a division of labor whereby glutamate is transported into astrocytes, converted to glutamine through glutamine synthetase and recycled back to neurons in the form of glutamine (the 'glutamate–glutamine cycle'). Indeed, astrocytes possess many features that create an environment optimized for efficient uptake, including a high resting potential, a high resting conductance, and a low intracellular concentration of glutamate. By contrast, transporters operating in neuronal membranes must fight against a higher electrochemical gradient, and a more depolarized constantly fluctuating membrane potential.

Despite the enrichment of GluTs in astrocyte membranes, the relative contributions of these different transporters to uptake near synapses remain poorly understood, in part, because selective antagonists for each transporter have yet to be developed. To overcome this limitation, both *in vivo* administration of antisense DNA [1] and generation of knockout mice [2,27,43,44,45•] have been used to address the role of different transporters. Consistent with the idea that astroglial uptake dominates clearance, disruption of GLT-1 or GLAST produces CNS pathologies, including seizures, elevated glutamate in the cerebrospinal fluid, and excitotoxicity [44,46], whereas knockout of EAAC1 or EAAT4 (or both concurrently) does not produce an obvious CNS phenotype [43,45•]. The crucial role for astrocyte transporters is further corroborated by the changes in receptor activation that occur when astrocytes are dissociated from synapses. The hypothalamus undergoes dramatic structural rearrangements during lactation, during which astrocytes retract their processes from excitatory synapses [47]. Following these morphological changes, activation of presynaptic mGluRs is enhanced [48] because of a lack of proximal GluTs. A similar phenomenon has been observed in the cerebellum. Viral mediated expression of GluR2 selectively in Bergmann glia, an astrocyte-like cell, renders their AMPA receptors impermeable to calcium, and causes these cells to retract their processes [49]. An analysis of parallel fiber and climbing fiber mediated excitatory postsynaptic currents (EPSCs) in Purkinje neurons under these conditions of reduced ensheathment revealed that glutamate clearance from synapses was dramatically impaired [49]. In addition, a recent analysis of mGluR signaling in hippocampal interneurons indicates that GLT-1 and GLAST play a prominent part in shielding these postsynaptic receptors [27•]. Taken together, these results indicate that astrocyte processes [50,51] are essential for delivering transporters to sites of release, and highlight the important contribution that these transporters make in removing glutamate that is released during excitatory transmission.

Many CNS synapses display an asymmetric distribution of astrocyte membranes [41,42]. A recent study performed a morphometric analysis of electron micrographs of hippocampal and cerebellar synapses, and revealed that the association between neuronal and astrocyte membranes on the postsynaptic side far exceeds that on the presynaptic side [52]. Although the significance of this asymmetry is not yet understood, a simple prediction is that glutamate will reach a higher effective concentration and diffuse further when moving in the presynaptic direction, an effect that might lead to preferential activation of presynaptic mGluRs.

Close, but not too close

The extreme heterogeneity exhibited by CNS synapses, particularly with regard to their association with astrocytes

[41,42], suggests that exceptions to this theme of astrocyte dominated glutamate uptake are likely. Cerebellar mossy fiber–granule cell synapses are situated in a glomerulus, in which 100–150 synaptic contacts are formed with ~50 granule cell dendrites, without intervening astroglial processes [53]. A recent study revealed that following stimulation of single mossy fibers as much as 48–70% of the total EPSC charge transfer was mediated by glutamate that spills over from neighboring synapses within the glomerulus. This spillover enhances synaptic efficacy and improves the reliability of transmission [54•]. The exclusion of astrocyte membranes from the interior of glomerulus permits extensive spillover to occur, while GluTs present in a sheet of astrocyte membrane that surrounds the glomerulus are presumably crucial for preventing interaction with extraglomerular synapses. Similar phenomena are likely to occur at other complex glomerular and calyceal synapses where release sites lack intervening astroglia.

The contribution of neuronal transporters

Antisense knockdown of EAAC1 produces epilepsy in adult mice [1], which suggests that neuronal transporters might also play an important part in clearance. Although this transporter is primarily localized to the soma and dendrites of neurons, it is also occasionally found GABAergic terminals [10,39]. Treatment of animals with antisense EAAC1 led to a decrease in the production of GABA and a reduction in miniature inhibitory postsynaptic current (IPSC) amplitude [55]. In addition, acute inhibition of GluTs in brain slices decreased the amplitude of both evoked and miniature IPSCs in pyramidal neurons [56•]. Thus, the seizures observed in animals treated with antisense EAAC1 could result from a decrease in GABAergic inhibition.

Several recent studies have provided compelling evidence that neurons contribute substantially to clearance of glutamate from postsynaptic receptors. To overcome the absence of selective antagonists, these studies took advantage of the dependence of transport on voltage and availability of intracellular K^+ to selectively inhibit uptake in the postsynaptic neurons. Complete replacement of K^+ with $Tris^+$, a cation that will not support GluT cycling, in Purkinje neurons resulted in a greater than twofold enhancement in the amplitude of parallel fiber evoked mGluR EPSCs, without altering the size of mGluR responses evoked through application of the mGluR agonist DHPG [25], and dramatically reduced the potentiation elicited by bath application of an antagonist that inhibits all GluTs. These results suggest that selective disruption of glutamate uptake into Purkinje cells results in higher occupancy of postsynaptic mGluRs. In the hippocampus, evoked NMDA EPSCs recorded in the presence of a low affinity antagonist (D-α-amino adipate, D-AA) decayed more rapidly than responses recorded in the presence of high affinity antagonist. This phenomenon could be explained if some of these receptors were located at synapses that are exposed to small slow glutamate transients indicative of spillover. However, D-AA only speeded the decay of EPSCs recorded at depolarized potentials, an effect attributed to the activity of postsynaptic transporters, which would be inhibited at depolarized potentials and promote spillover. In support of this conclusion, D-AA was able to speed the decay of NMDA EPSCs at negative potentials when recordings were made with an internal solution containing $NMDG^+$, a cation that also does not support transporter cycling. A confounding issue is the apparent low density of transporters in these cells [9,21,45•]; however, kinetic modeling suggests that a few strategically located transporters could effectively shield receptors [30]. Because removal of K^+ would be expected to prevent, or at least slow, transporter cycling, but leave the capacity for binding and translocation largely intact [32] (unless they become trapped at the K^+ binding step), these results suggest that cycling rather than simple binding to transporters [34] is essential for clearing glutamate away from extrasynaptic receptors [57]. In contrast to this evidence of postsynaptic uptake at pyramidal neuron synapses, an examination of mGluR EPSCs in interneurons indicates that EAAC1 contributes little to uptake near extrasynaptic mGluRs in these cells [27•].

Revisiting the question of presynaptic uptake

Studies of glutamate uptake into synaptosomes and brain slices suggest that GluTs are present in the nerve terminal membrane [5]. If present, how significant is this uptake to clearance? Taking advantage of the unique accessibility offered by two giant nerve terminals, a recent study showed that transporter-associated currents could be recorded from bipolar cell terminals in the retina, but not from the calyx of Held in brainstem slices, in response to both stimulation-induced release and exogenous glutamate [58•]. A comparison of the quanta released and the number of transporters activated indicates that GluTs in bipolar cell terminals capture much less than 1% of the glutamate released. It seems unlikely that this small contribution would influence glutamate dynamics sufficiently to alter receptor occupancy. Notably, the transporter responsible for uptake in bipolar cell terminals, EAAT5, exhibits an unusually high anion permeability [13,59], which might allow this transporter to act primarily as a glutamate-gated Cl^- channel with the ability to dampen terminal excitability.

The identity of the transporter responsible for glutamate uptake in nerve terminals in the brain remains elusive. Further analysis of glutamate uptake into synaptosomes and brain slices prepared from transporter knockout mice might resolve this question. Although immunocytochemical studies suggest that GLT-1 is expressed at a high density in astrocytes [39,40], *in situ* hybridization revealed the presence of GLT-1 RNA in many neurons, including

CA3 pyramidal neurons [60,61]. However, previous immunocytochemical and functional studies have failed to detect functional GLT-1 transporter expression in neurons [39,40,62], although GLT-1 expression is detected in the soma and dendrites of hippocampal neurons *in vitro* [61,63]. Two recent studies have re-evaluated the expression of GLT-1 by neurons in the hippocampus. Although initial results suggested that GLT-1b, a carboxy-terminal splice variant [64], was found in nerve terminals [65], this labeling was subsequently shown to be artifactual, as a similar pattern of labeling was observed in tissue taken from GLT-1 knockout mice [66]. However, subsequent studies using peroxidase-enhanced immuno-electron microscopy suggest that full-length GLT-1 is present in a subset of nerve terminals in area CA1 [66] that presumably arise from CA3 Schaffer collateral-commissural fibers. Precise localization and quantification of this GLT-1 expression will require further functional immunogold-level analysis.

Conclusions

GluTs are abundant in extrasynaptic membranes and bind glutamate rapidly. Recent studies of excitatory synapses indicate that these transporters restrict activation of mGluR and NMDA receptors in this region and prevent glutamate from diffusing between synapses. At synapses devoid of astrocyte processes, and at complex synapses that consist of multiple active zones with no intervening astroglia, the paucity of GluTs can lead to spillover and enhanced synaptic efficacy. Although less numerous, neuronal transporters also have been shown to influence clearance and receptor occupancy in this domain, presumably by being localized near receptors. However, these GluTs are present in the postsynaptic membrane rather than the nerve terminal. A prominent role for presynaptic GluTs has not been found, except in the case of GABAergic terminals that use glutamate to synthesize GABA. The extreme diversity in synaptic morphology indicates that caution should be exercised when generalizing results from a few model synapses. Nevertheless, these studies suggest that acute regulation of GluT activity could represent a widespread mechanism for influencing synaptic signaling. Although information about the individual roles of different GluTs is emerging, the apparent promiscuous expression of some transporters, in particular EAAT2/GLT-1, suggests that more refined cell-type selective knockout approaches might be necessary to create a complete picture of GluT involvement in excitatory transmission.

Update

A recent paper by Brasnjo and Otis [67] examined GluT currents at climbing fiber–Purkinje cell synapses in the cerebellum, and currents elicited in Purkinje cells in response to photolysis of caged L-glutamate. These authors conclude that Purkinje cells remove approximately 17% of the glutamate that is released at these synapses, and that an ionotropic receptor other than AMPA or NMDA receptors contributes to climbing fiber EPSCs. These results are in general agreement with those reported by Huang *et al.* [45•].

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