The Time Course of Signaling at Central Glutamatergic Synapses

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Glutamate is the main excitatory transmitter in the mammalian CNS, mediating fast synaptic transmission primarily by activation of AMPA-type glutamate receptor channels. Both synaptic structure and a cell-specific molecular switch in the AMPA receptor subunit expression are involved in the regulation of the synaptic signaling time course.

Chemical synaptic transmission involves the release of a transmitter into the synaptic cleft and the subsequent activation of postsynaptic receptors. The most extensively characterized synapse is the neuromuscular junction, formed between motor axons and skeletal muscle fibers. The transmitter acetylcholine (ACh) is released from synaptic vesicles in multivesicular packets, or quanta, and activates nicotinic ACh receptors (AChRs) in the postsynaptic membrane. This generates postsynaptic conductance changes of fast time course and large amplitude, which induce reliable activation of the muscle fiber.

In the mammalian central nervous system (CNS), glutamate is the main excitatory transmitter. The principles of synaptic communication at glutamatergic synapses, however, appear to be more complex than at the neuromuscular junction. First, glutamatergic synapses differ in morphological properties, such as number of release sites and presence of dendritic spines. Second, synapses in different circuitries differ substantially in impact and time course of synaptic signaling. Finally, glutamate activates several different types of ionotropic and metabotropic receptors: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptors (AMPARs), N-methyl-D-aspartate receptors (NMDARs), kainate receptors (KARs), and metabotropic glutamate receptors coupled to either inositol 1,4,5-trisphosphate (IP₃; class 1 mGlurS) or cAMP-signaling cascades (class 2 and 3 mGlurS) (10). On the basis of extensive work in the last ten years, we are beginning to understand how cellular and molecular factors shape functional differences between glutamatergic synapses.

The time course of the excitatory postsynaptic currents

The patch-clamp technique in brain slices developed by Bert Sakmann and co-workers allows us to record from neurons in the CNS with high resolution in an intact synaptic environment. Analysis of several synapses revealed that fast glutamate-mediated signaling is predominantly mediated by AMPARs (5, 9, 15). However, the time course of signaling differs substantially between synapses. This is illustrated in Fig. 1, which shows AMPAR-mediated evoked excitatory postsynaptic currents (EPSCs) at three well-characterized synapses: the mossy fiber-CA3 pyramidal cell synapse in the hippocampus (9), the granule cell-basket cell synapse in the dentate gyrus (5), and the calyx synapse on neurons in the medial nucleus of the trapezoid body (MNTB) in the brain stem (1). Throughout the CNS, slow AMPAR-mediated evoked EPSCs are generated at excitatory synapses on hippocampal and neocortical principal neurons (decay time constant ~5 ms at 22°C). In contrast, fast AMPAR-mediated evoked EPSCs are generated at glutamatergic synapses on hippocampal and neocortical inhibitory interneurons and on neurons in the auditory pathway (decay time constant ~1–2 ms).

Synchrony vs. asynchrony of quantal release

Which factors determine the time course of the AMPAR-mediated postsynaptic conductance change? First, asynchrony of quantal release needs to be considered (Fig. 2A; Refs. 3, 5, and 8). Evoked release of transmitter involves several steps: invasion of the action potential into the presynaptic terminal, activation of voltage-gated Ca²⁺ channels, Ca²⁺ entry into the
presynaptic terminal, and binding of $\text{Ca}^{2+}$ to the putative $\text{Ca}^{2+}$ sensor (for example see Ref. 1). Thus it is not surprising that the synaptic delay is somewhat variable from trial to trial at the same release site and between different release sites.

Similar to the neuromuscular junction, it appears that quantal release is highly synchronized at many glutamatergic synapses. The duration of the early release period at physiological temperature is only a few hundred microseconds at the granule cell-basket cell synapse (5) and the calyx synapse on cochlear nucleus neurons (8), despite the presence of multiple release sites at both synapses. Whether these results can be extrapolated to all glutamatergic synapses remains to be determined (see Ref. 3 for information on a higher asynchrony of release in cultured neurons).

**Time course of glutamate concentration in the synaptic cleft**

Second, the time course of transmitter concentration in the synaptic cleft is potentially important for the time course of the postsynaptic conductance change (Fig. 2B). At the neuromuscular junction, enzymatic transmitter degradation limits the lifetime of transmitter in the synaptic cleft. At glutamatergic synapses, the mechanisms of transmitter clearance must be different, because a high-turnover enzyme analogous to the ACh esterase is lacking.

Different approaches have been used to determine the time course of glutamate concentration in the synaptic cleft. The first approach uses low-affinity competitive antagonists of AMPARs; the longer the transmitter is present, the less blockade of the EPSC by the competitive antagonist will occur, due to antagonist displacement (4). A second approach is based on a comparison of the time course of the quantal EPSC at a real synapse with that of currents evoked by glutamate pulses at a surrogate synapse where “presynaptic” parameters can be readily controlled. Such surrogate synapses are based on the technique of fast application of agonists to isolated outside-out patches introduced by Josef Dudel and colleagues (2, 5, 6, 7, 12, 15). Finally, computer simulation of transmitter diffusion and receptor activation has provided insights into the factors that determine the shape of the synaptic glutamate pulse.

The results converge on the conclusion that diffusion is the main factor that promotes transmitter elimination at glutamatergic synapses. If the instantaneous release of 5000 glutamate molecules into an infinite synaptic cleft is simulated (assuming that the diffusion coefficient for glutamate is half of that in aqueous solution), a complex time course of the synaptic glutamate pulse is predicted (Fig. 2B). The initial rate
of glutamate clearance (from millimolar to micromolar levels) is extremely rapid, but the later phases of elimination (from micromolar to submicromolar) are much slower (4).

The exact time course of the synaptic glutamate pulse may depend on microanatomical properties of the synapse and glutamate transporter density. Small synaptic contact size and simple synaptic morphology will facilitate rapid transmitter clearance. In contrast, large synaptic contact size, the presence of spines on the dendrite of the postsynaptic neuron, and glial wrapping of the contact will slow transmitter clearance. Glutamate transporters bind glutamate rapidly, whereas the subsequent translocation step appears to be slow; the cycling time is ~50 ms at 22°C. Thus binding of glutamate to transporters could shape the early phase of the synaptic glutamate pulse, whereas the subsequent translocation step could shape the late phase. Both the high density of transporters and the location in proximity to the release site (in both neuronal and glial membranes) would be consistent with the hypothesis that glutamate transporters act as buffers (4).

Interactions between release site-postsynaptic density units, referred to as cross talk (or spillover), complicate the picture. Cross talk was first described at the neuromuscular junction in the presence of ACh esterase inhibitors. If several vesicles fuse at neighboring release sites, the transmitter pulses in the synaptic cleft will summate. This may result in a gradual prolongation of transmitter clearance and evoked EPSC decay as the release probability is increased. In principle, cross talk could occur between release sites-postsynaptic density units of the same bouton or between those of different synapses. High probability of release and close spacing of release sites will favor cross talk, whereas low release probability and large distance between release sites will minimize cross talk.

At glutamatergic synapses, the effects of cross talk are much smaller than at the neuromuscular junction. Several glutamatergic synapses do not show significant cross talk (4, 5). However, at calyx synapses on auditory neurons that have several hundred release sites, the slight prolongation of the EPSC with increasing release probability and the presence of a slowly decaying component of the AMPAR-mediated evoked EPSC were attributed to cross talk (12, 15).

**Gating properties of postsynaptic AMPARs**

Gating of the postsynaptic receptors is a third factor that is likely to be relevant for the time course of the postsynaptic conductance change (Fig. 2C); at the neuromuscular junction, receptor gating is thought to be the main determinant of the decay of the synaptic current. Fast application experiments using a variety of paradigms indicate that AMPAR gating is complex. The agonist affinity of AMPARs is relatively low, with the half-maximal activating glutamate concentrations in the range of 500 μM–2 mM for the peak current. With saturating

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**FIGURE 2.** Factors that determine time course of postsynaptic conductance change at glutamatergic synapses. A: presynaptic action potential and normalized release probability distribution are shown superimposed at top. Individual EPSCs in conditions of reduced Ca²⁺/Mg²⁺ concentration ratio (1.5 mM Ca²⁺/3 mM Mg²⁺) are shown at bottom. Note high percentage of failures under these conditions. This indicates that majority of events were quantal EPSCs, caused by release of contents of a single synaptic vesicle. Recording temperature was 34°C. From Ref. 5 (copyright held by Cell press). B: time course of glutamate concentration in synaptic cleft. Glutamate concentration is plotted logarithmically against radial location (for times 0, 10, 50, 100, and 1000 μs; top) and against time (for center of synapse at release site and in periphery at 0.2 μm radial distance from release site; bottom). Curves were obtained by analytical solution of diffusion equations. Assumptions were 5000 glutamate molecules per vesicle, instantaneous release from a point source, infinite synaptic cleft of 20 nM width, and diffusion coefficient for glutamate D = 3 × 10⁻⁶ cm²·s⁻¹, approximately half of that tabulated for glutamine in aqueous solution. C: a hypothetical gating scheme of native AMPARs, with two binding sites for glutamate, is shown at top. C0, C1, and C2 are closed, available states, C3, C4, and C5 are closed, desensitized states, and O is the open state. From Ref. 9. Glutamate-activated currents in an outside-out patch isolated from a CA3 pyramidal neuron are illustrated at bottom. Currents evoked by 1- and 100-ms pulses of 1 mM glutamate from same patch are shown superimposed. Recording temperature was 22°C. From Ref. 5 (copyright held by Cell press).
concentrations of glutamate, AMPARs are activated within a few hundred microseconds. When the glutamate pulse is brief (e.g., 1 ms), AMPARs close rapidly after the end of the pulse because of AMPAR deactivation following removal of the agonist. When the glutamate pulse is more prolonged, AMPARs close more slowly because of AMPAR desensitization in the maintained presence of the agonist (Fig. 2C; Refs. 2, 6, 7, 12, and 15). The extent of desensitization is substantial (>90% with saturating glutamate concentrations). Somewhat unexpectedly, AMPARs enter desensitized states not only during prolonged applications of glutamate but also after brief glutamate pulses (a phenomenon referred to as “brief-pulse desensitization”; Refs. 2 and 7). Furthermore, low concentrations of glutamate (2–10 μM), when present for several seconds, can induce “equilibrium desensitization” without significant receptor activation (2). These gating properties can be accounted for by cyclic reaction schemes, similar to that shown in Fig. 2C (9).

The complex gating of AMPARs raises the question of which steps are limiting during the EPSC decay. Consistent with the rapid decline of glutamate concentration during the early phase of transmitter clearance, the decay of the quantal EPSC is very similar to the deactivation time constant at many glutamatergic synapses. The granule cell-basket cell synapse, where both time constants are identical, represents a particularly remarkable example (Fig. 1B; Ref. 5). However, in conditions that favor slow transmitter clearance (large contact area, spines, glial wrapping) or cross talk (high release probability, high number of closely spaced release sites), the synaptic glutamate pulse may be prolonged and the decay of the evoked EPSC may approach the desensitization time constant. The calyx synapse on auditory neurons (15) and the glutamatergic synapses on cerebellar Purkinje cells exemplify such a scenario. The low concentration of glutamate during the slow phase of transmitter clearance, although insufficient for receptor activation, may lead to receptor desensitization.

If AMPAR desensitization does not shape the EPSC directly at many synapses, one wonders whether it could have any other functional roles. One possibility is that desensitization contributes to paired-pulse depression during repetitive activation of a synapse. This may occur in auditory synapses, in which paired-pulse depression is sensitive to the desensitization inhibitor cyclothiazide (15). A second possibility is that desensitization induced by ambient glutamate regulates the amplitude of the EPSC. Ambient glutamate concentrations in the extracellular space are thought to be in the range of a few micromoles, which may be sufficiently high to induce equilibrium desensitization. Finally, AMPAR desensitization will minimize cross talk effects and thus ensure specificity of transmission at individual sites.

If gating kinetics shape the decay of the postsynaptic conductance change, regulation of gating kinetics may generate synapse-specific differences in signaling time course. Analysis by fast application techniques indicates that AMPARs expressed in different types of neurons differ substantially in their functional properties (Fig. 3A). Throughout the CNS, AMPARs with slow deactivation and desensitization are expressed in hippocampal and neocortical principal neurons (deactivation time constant ~3 ms and desensitization time constant ~15 ms at

![FIGURE 3. Regulation of AMPAR gating by subunit composition. A: currents evoked by 1- and 100-ms pulses of 1 mM glutamate in outside-out patches isolated from a CA3 pyramidal neuron, a dentate gyrus basket cell, and a neuron in medial nucleus of trapezoid body (MNTB). Mean values of deactivation and desensitization time constants are indicated below traces. All recordings were made at ~22°C. B: abundance bar graphs, indicating relative abundance of glutamate receptors (GluR)-A, -B, -C, and -D mRNA in 3 types of neurons. Data are averages from 10, 10, and 8 individual cells, respectively. Open portions of bars indicate flip splice variants, and filled portions depict flop splice variants. For GluR-C, splice variants were not analyzed. Putative subunit composition of GluR transcripts was determined by single-cell RT-PCR with AMPAR-specific primers. Quantitation of relative abundance of GluR-A, -B, -C, and -D transcripts in mixed PCR product was performed by subcloning and phage plaque counting. Modified from Ref. 5 (copyright held by Cell press).](http://physiologyonline.physiology.org/)

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22°C), whereas AMPARs with faster gating are present in inhibitory interneurons (2, 6, 7). The fastest AMPARs throughout the CNS are expressed in neurons of the MNTB and the cochlear nucleus (deactivation time constant 0.3–0.9 ms and desensitization time constant ~1.7 ms; Refs. 6, 12, and 15).

Molecular mechanisms underlying differences between synapses

The substantial differences between AMPARs expressed in different types of neurons raise the question of the molecular basis of these differences. Native AMPARs are heteromers assembled from GluR-A to -D subunits. Additional structural diversity is created by alternative splicing of the flip/flop module and by RNA editing at different sites (10).

Studies on recombinant AMPARs expressed in host cells show that gating kinetics are regulated by subunit composition. In oocytes and mammalian cell lines, both homomeric and heteromeric channels can be expressed heterologously, indicating a high degree of flexibility in AMPAR assembly. This distinguishes AMPARs from nicotinic AChRs in skeletal muscle, which are assembled in constant subunit stoichiometry. In homomeric AMPARs, the desensitization time constant follows the sequence: GluR-Ai ~ GluR-Ao ~ GluR-Ci ~ GluR-Di > GluR-Co > GluR-Do, with 'o' designating the flop and 'i' the flip splice form (11, 13). Furthermore, heteromeric AMPARs formed by GluR-Bi in combination with other subunits have slower desensitization than the respective homomers, whereas GluR-Bo has little effect (11, 13). Thus GluR-Bi is a determinant of slow desensitization, whereas GluR-Co and GluR-Do are determinants of fast desensitization of recombinant AMPARs.

Similar principles of regulation may apply to native AMPARs (Fig. 3B). Correlated analysis by fast application and single-cell RT-PCR (a technique introduced by Jean Rossier and colleagues) has shed light onto the molecular determinants of native receptor function. Analysis in different types of cells revealed that the desensitization time constant is positively correlated with the relative abundance of GluR-Bi mRNA and negatively correlated with the relative abundance of GluR-D mRNA (6). Similar correlations are found for the deactivation time constant. Thus the results from both recombinant and native AMPARs indicate that AMPAR gating (deactivation and desensitization) is regulated reciprocally by GluR-Bi and GluR-D subunits. Other molecular factors, such as phosphorylation, may also affect AMPAR gating, but direct experimental evidence is not yet available.

Different AMPAR subunit composition may not only generate functionally distinct AMPARs in different types of neurons but also at different synapses on the same neuron. In bipolar cells of the cochlear nucleus, GluR-D subunit protein is targeted specifically to the basal dendrites, the region where the auditory afferents terminate (14). This could imply that glutamatergic synapses established on different dendrites of these neurons generate postsynaptic conductance changes with very different time courses. Whether sorting also occurs in other types of neurons and for AMPAR subunits other than GluR-D remains to be elucidated.

Functional significance

In the past few years, our understanding of glutamatergic synaptic transmission has gradually improved, reaching a level of depth comparable to that at the neuromuscular junction. Several links between the time course of synaptic signaling, receptor function, and receptor subunit composition have been discovered. First, AMPAR gating is the primary determinant of the decay of the postsynaptic conductance change, but synchrony of release and time course of transmitter concentration in the synaptic cleft are also relevant at certain synapses. Second, AMPAR gating is regulated by the subunit composition, with certain subunits determining slow gating and others conferring fast gating. Differential AMPAR subunit expression not only generates differences between AMPARs expressed in different regions of the brain but also between those in different types of neurons of the same circuitry.

Why do central neurons put effort into the generation of synapse-specific postsynaptic conductance changes by running distinct genetic programs? The answer is probably that the time course of the postsynaptic conductance change at glutamatergic synapses is of critical importance for neuronal function. One aspect is that the time course of the postsynaptic conductance change shapes the rising phase of the excitatory postsynaptic potential (EPSP). This could be of critical importance for the timing of action potentials evoked in the postsynaptic neuron, since activation of voltage-gated Na+ channels requires fast voltage changes. Furthermore, the time course of the postsynaptic conductance change shapes the decay phase of the local dendritic EPSP and, if the synapse is located close to the soma, the somatic EPSP (5). This could determine the extent of the temporal and spatial summation of synaptic signals.

What is the functional significance of the synapse-specific differences in the time course of the postsynaptic conductance change for the operation of neuronal networks? A final conclusion cannot be reached yet. It is remarkable, however, that slow AMPAR-mediated EPSCs are generated in neurons specialized to integrate synaptic signals, such as cortical principal neurons, whereas fast EPSCs are generated in neurons that operate as coincidence detectors, such as MNTB neurons that are specialized on sound localization. This may suggest that the time course of the AMPAR-mediated conductance change determines whether a neuron operates as an integrator, coincidence detector, or fast relay of synaptic input signals.

Basics of glutamatergic synaptic transmission in the CNS

AMPAR: The main type of glutamate receptor that mediates fast excitatory synaptic transmission. As the name indicates, it is selectively activated by the agonist a-amino-3-
hydroxy-5-methyl-4-isoxazolepropionate. Some authors designate this receptor as the AMPA/kainate receptor, since it is also activated by kainate. Unlike the responses to AMPA and glutamate, those to kainate do not desensitize. Several years ago this receptor had been referred to as the “quisqualate receptor” but was renamed because quisqualate turned out to be a nonselective agonist, also activating class 1 mGluRs.

Cross talk: When transmitter is released simultaneously at closely spaced sites, the transmitter pulses in the synaptic cleft summate. This may lead to a slowing in transmitter clearance, which would result in a prolongation of the post-synaptic current.

Desensitization: Closure of AMPAR channels after removal of the agonist.

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Desensitization: Closure of AMPAR channels in the maintained presence of the agonist, which is approximately threefold slower than deactivation. Although desensitization is a general characteristic of ligand-gated ion channels, it is particularly fast for AMPARs.

EPSC, evoked: EPSC evoked by stimulation of presynaptic neurons. If a single neuron is stimulated (for example, in a paired recording configuration), the EPSC would be denoted as “unitary.” If several presynaptic neurons are stimulated (by extracellular stimulation of axonal tracts), the EPSC would be referred to as “composite.”

EPSC, quantal: EPSC generated by the release of the contents of a single synaptic vesicle. The term “quantum” was introduced by Bernard Katz for events at the neuromuscular junction, and is also used at glutamatergic synapses. One approach to isolating quantal events is to record evoked EPSCs in conditions of low release probability. An alternative approach is to record miniature EPSCs, i.e., spontaneously occurring EPSCs after suppression of presynaptic action potential activity (with tetrodotoxin and/or blockers of voltage-gated Ca$^{2+}$ channels).

Failure: Evoked EPSCs show statistical fluctuation in peak amplitude from trial to trial. Occasionally the presynaptic action potential does not evoke an EPSC in the postsynaptic neuron at all. This is denoted as failure of synaptic transmission.

GluR-A to -D: AMPAR subunits identified by molecular cloning. In an alternative nomenclature, these subunits are referred to as GluR1 to 4. Each of the subunits consists of ~900 amino acids (sequence identity between subunits ~70%). It is thought that each subunit has four membrane segments (three transmembrane segments and a loop that forms the aqueous pore). The functional channel appears to be an oligomer assembled from four or five subunits.

Flip, Flop: AMPAR subunit variants generated by alternative splicing of the flip/flop module. This module is comprised of 115 base pairs (corresponding to 38 amino acids, 9-11 of which are different between flip and flop variants).

RNA editing: Form of posttranscriptional modification of AMPAR RNA, discovered by Peter Seeburg and colleagues. Desamination of adenosine to inosine is thought to be the underlying molecular mechanism. Two editing sites of AMPAR subunit mRNA have been identified: the Q/R site in the pore-forming region of the GluR-B subunit and the R/G site preceding the flip/flop module of GluR-B, -C, and -D subunits. Q/R-site editing changes the Ca$^{2+}$ permeability of AMPARs by more than an order of magnitude, whereas R/G-site editing is involved in fine tuning of AMPAR gating.

Paired-pulse facilitation or depression: When two action potentials are elicited in a presynaptic neuron, separated by a short time interval, the amplitude of the second EPSC may be either enhanced or reduced as compared to that of the first, depending on the type of synapse and the probability of release. Paired-pulse modulation appears to be generated mainly by presynaptic mechanisms, but postsynaptic factors (such as desensitization) may also contribute at certain synapses.

Post-synaptic density: Specialized postsynaptic, electron-dense site that presumably contains the transmitter receptors.

Release site: Specialized presynaptic site where vesicle fusion is thought to occur.

Spine: Small, thorn-like protrusion of dendritic membrane, which may subserve the purpose of increasing surface area and producing compartmentalization. In the hippocampus and the neocortex, glutamatergic synapses between principal neurons are mainly formed on spines, whereas principal neuron-interneuron synapses are established on dendritic shafts (inhibitory interneurons characteristically lack spines). Narrow spine necks may delay the escape of glutamate from the synaptic cleft.

Synaptic delay: Time interval from the steepest point in the rising phase of the presynaptic action potential (recorded close to the site of release) to the beginning of the evoked EPSC.

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Molecular Mechanisms in Bile Formation