Glutamate receptor plasticity at excitatory synapses in the brain

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Summary

1. Synapse plasticity, defined as an activity-dependent change in the strength of synapses, was first described in 1973 by Tim Bliss and Terje Lømo.[1] Since these seminal experiments were reported, the field of synapse plasticity has expanded into one of the most widely studied areas in neuroscience.

2. Significant effort has been focussed on determining the expression mechanisms of the changes in synapse strength. This review will focus on the changes in the postsynaptic expression of glutamate receptors that have been shown to occur during the expression of synapse plasticity.

3. Biochemical studies of excitatory synapses in the central nervous system have revealed a high density of proteins concentrated at dendritic spines. These proteins appear to play critical roles in synaptic structure, plasticity and in trafficking receptors to synapses.

4. There is growing evidence that synapse plasticity could be the cellular basis of certain forms of learning and memory. Determining the behavioural correlates of this fundamental synaptic process will continue to be addressed in current and future research.

Introduction

Excitatory synapses of the mammalian central nervous system are asymmetric sites of neuron-neuron contact that enable the formation of neuronal networks within the brain. In response to depolarization of the presynaptic terminal, neurotransmitter is released into synaptic cleft where it binds specifically to postsynaptic receptors clustered on the postsynaptic dendritic spine (Figure 1). Neurotransmitter binding then triggers ion flow into the postsynaptic neuron. The majority of excitatory synapses are glutamatergic, meaning that they utilise the amino acid glutamate as the neurotransmitter. The primary subtypes of glutamate receptors expressed at glutamatergic synapses are the α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor (AMPA receptor) and N-methyl-D-aspartate receptor (NMDA receptor) subtypes. AMPA-type glutamate receptors are important in determining postsynaptic cell excitability, since they conduct the majority of the current flow at resting membrane potentials.[2] The NMDA-type glutamate receptor exhibits a distinct property of voltage-dependent magnesium blockade, enabling it to conduct current only at depolarised membrane potentials.[3,4] This receptor is also unique in its high calcium permeability, and slow activation and deactivation kinetics.[5,6] As discussed below, these properties allow highly regulated current flow in response to specific incoming synapse activity.

Glutamate receptors are targeted and anchored at excitatory synapses through a network of scaffolding proteins. These proteins are concentrated at the tip of the postsynaptic dendritic spine at a region termed the postsynaptic density (PSD; Figure 1). The PSD is estimated to contain more than 200 synaptic proteins which have a myriad of functions. Included in this group are the glutamate receptor binding Synapse Associated Proteins (SAPs) SAP97, SAP102 and SAP90 (also known as PSD95). These proteins are emerging as the central organisers of synapses: they are critical for synaptic structural integrity and for the trafficking of multi-component receptor complexes to synapses.[7,8]

Plasticity at excitatory synapses

Plasticity of the circuitry that wires the brain is a fundamental property of neurons that is thought to underlie behaviour, cognition, learning and memory.[9,10] The development of new synapses, the activity-dependent changes in the strength of existing synapses and the elimination of synapses have been proposed to form the basis of this plasticity. The NMDA-type glutamate receptor subtype is crucial for synapse plasticity[11] and for learning and memory.[12] The unique properties of the NMDA receptor play a key role in the cellular mechanisms thought to underlie learning and memory by defining the receptor as a ‘coincidence detector’ to initiate synapse plasticity and leading to the formation of new neural networks.[13] In response to afferent activity-induced depolarization of the postsynapse coincident with presynaptic transmitter release, calcium influx through the NMDA receptor triggers the active insertion or removal of AMPA-type glutamate receptors (Figure 1). Plasticity models that increase synaptic strength are termed long-term potentiation (LTP) while those that decrease synaptic strength are termed long-term depression (LTD). Thus AMPA receptors are thought responsible for the expression of synaptic plasticity, while NMDA receptors for its control.

Trafficking and plasticity of AMPA receptors

Most AMPA receptors are tetramers composed of a combination of GluR1, 2, 3 and 4 subunits[13] (for example, GluR1/GluR2 or GluR2/GluR3 heteromers). The subunit composition varies in a brain region-dependent manner. At hippocampal CA3-CA1 synapses, the synapse population

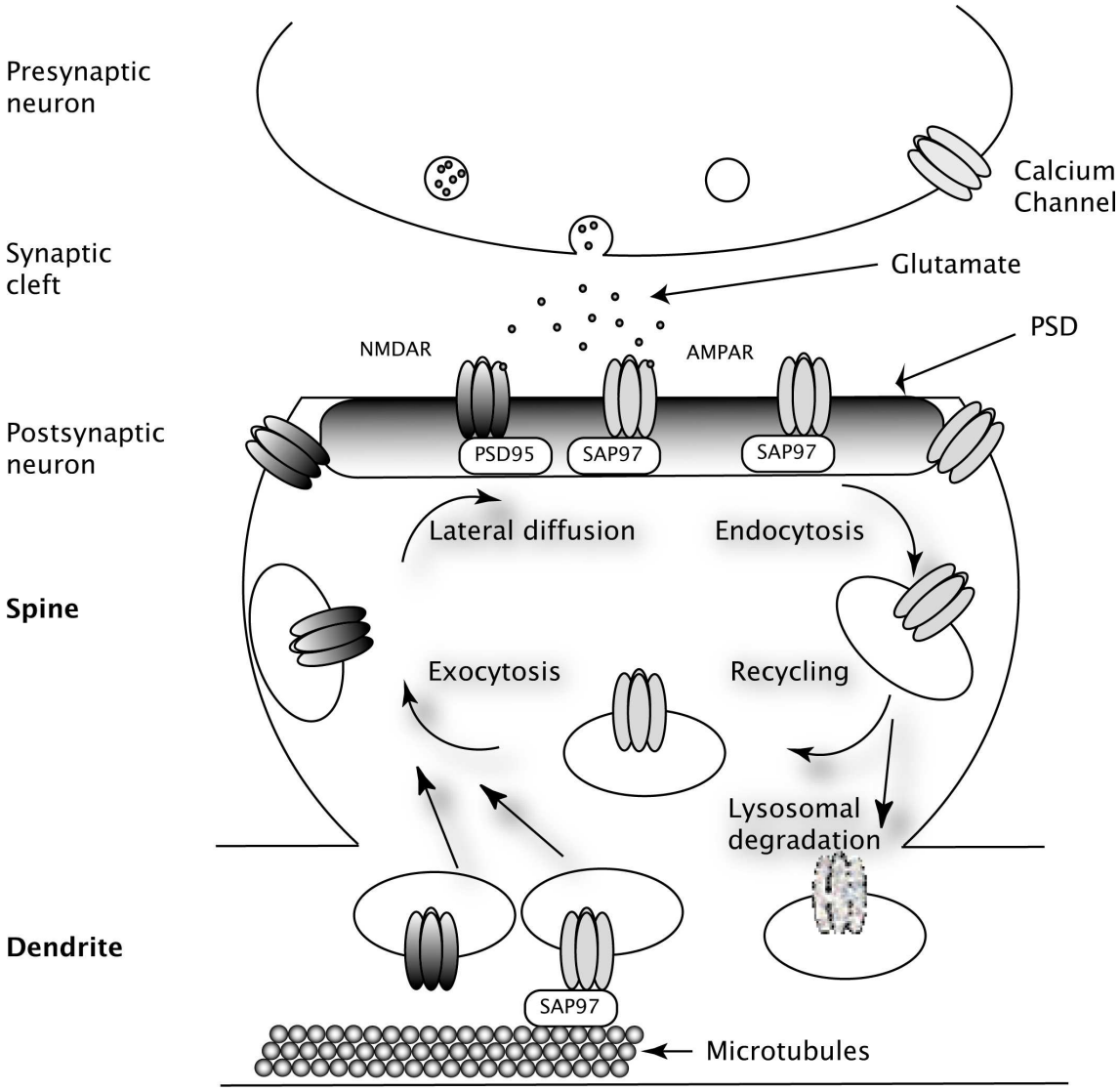

Figure 1. Plasticity at excitatory synapses in the central nervous system. *The two major subtypes of glutamate receptors, AMPA and NMDA, are localized in the electron-dense postsynaptic density where they bind glutamate released from the presynaptic terminal. In response to LTP-inducing stimuli, AMPA receptors are rapidly inserted into the synaptic membrane followed by lateral diffusion into the PSD. As a result, synapse strength is increased, as measured by an increase in the amplitude of synaptic currents. In response to LTD-inducing stimuli, both AMPA and NMDA receptors are thought to be removed from the synaptic membrane, potentially at designated endocytic zones. As a result, synapse strength is decreased, as measured by a decrease in the amplitude of synaptic currents. After removal from the synapse, receptors can be recycled back to the membrane or targeted for degradation.*

most widely studied with respect to synapse plasticity, most AMPA receptors are GluR1/GluR2 or GluR2/GluR3 heteromers. The trafficking of AMPA receptors to the postsynaptic spine and subsequently to the postsynaptic membrane requires interactions between the AMPA receptor subunits and PSD proteins through their PDZ-domains[7,14] (postsynaptic density, discs large, zona occludens). These domains interact with the extreme C-termini of their binding partners, and with specific regards to AMPA receptor trafficking and synaptic localization include SAP97,[15] protein that interacts with C-kinase (PICK1),[16] and glutamate receptor interacting protein (GRIP).[17] SAP97 has been proposed to have a key role in directing AMPA receptors to synapses with myosin VI, a minus end, actin-dependent motor.[18] SAP97, myosin VI and GluR1 are thought to form a trimeric complex, such that SAP97 serves as an adaptor protein linking myosin VI to vesicular cargos carrying glutamate receptors from the soma to the synapse.

AMPA receptors can also be synthesized in the dendrites, independent of receptor trafficking from the soma. Live imaging of tetracysteine-tagged GluR1 and GluR2 subunits showed that both subunits are locally synthesized in the dendrites.[19] What the relative

contributions of local versus soma synthesized AMPA receptor subunits is not known, but dendritic synthesis may provide a synapse specific mechanism for more rapid changes in synapse strength that do not require long-term trafficking of AMPA receptors from the soma.[19]

Insertion of AMPA receptors into synapses

There is considerable evidence from many laboratories that AMPA receptors are inserted into the synaptic membrane in response to LTP induction. The process of synaptic insertion of AMPA receptors is a two step process, mediated by the 4-pass transmembrane protein Stargazin.[20] First, Stargazin recruits AMPA receptors to the surface membrane from a presumed intracellular pool. Then, *via* a protein kinase A-dependent interaction between the C-terminal tail of Stargazin and the first two PDZ domains of PSD95, AMPA receptors are recruited to the synaptic site.[21,22] Stargazin and the family of stargazin-related proteins TARPs (transmembrane AMPA receptor regulatory proteins) are also critical for maintaining the surface expression of AMPA receptors at synapses. TARPs are membrane stable proteins that turn over very slowly. The dependence of surface AMPA receptor expression on TARP proteins was first shown in stargazer knockout mice which exhibit a complete loss of surface AMPA receptors in cerebellar granule cells. Other members of this family (γ3, γ4 and γ8) are proposed to mediate surface AMPA receptor expression in the forebrain.[23]

Removal of AMPA receptors from the synapse

How glutamate receptors are removed from the synapse has been an area of intensive study and progress over the past 5 years, with multiple labs showing that AMPA receptors are rapidly recycled out of the synapse in the time course of minutes.[24-26] As a result, synapse strength is decreased and this weakening of synapses is proposed to initiate synapse elimination, although this has not been directly shown.

The process of AMPA receptor removal from CNS synapses is known to be intricately linked to the endocytic proteins clathrin and dynamin, and the PSD proteins GRIP and PICK.[25-27] The clathrin adaptor protein AP-2 binds the GluR2 subunit of the AMPA receptor and binding of AP-2 to AMPA receptors is required for NMDA-stimulated AMPA receptor endocytosis and LTD.[26]

Inhibition of GluR2/3 C-terminal tail interactions with the PSD proteins PICK and GRIP disrupts basal transmission and synaptic plasticity.[24,25] Specifically, the disruption of GluR2/3 binding interactions results in an increase in receptor expression at the synapse, and the inability to undergo LTD, suggestive of a role of PICK and GRIP in stabilizing an intracellular pool of AMPA receptors and regulating their reinsertion.[24] Interestingly, AMPA receptors can regulate whether GRIP or PICK binds to their C terminus through GluR2 phosphorylation, providing a mechanism to differentiate interactions of PICK1 or GRIP with GluR2 to regulate AMPA receptor surface expression.[27] Live imaging of neurons transfected with GFP-clathrin shows the existence of a specialized endocytic zone lateral to the PSD.[28] Membrane proteins such as AMPA receptors must therefore dissociate from TARPs and other PSD proteins and translocate to this extrasynaptic region to undergo internalization. After their removal from the postsynaptic membrane, AMPA receptors are thought to differentially sort between recycling pools and degradative pathways. Biochemical analysis has identified a light membrane fraction rich in AMPA receptors that corresponds to a population of tubular vesicles ranging in size from 50 to 300 nm.[29] This pool could serve as a dendritic recycling pool of AMPA receptors. AMPA receptors that have been endocytosed in an NMDA receptor-, calcium- and phosphatase-dependent manner have been shown to rapidly recycle back into the synaptic membrane; in contrast, those endocytosed independent of NMDA receptor activation are targeted to late endosomes and lysosomes.[30]

Trafficking and plasticity of the NMDA receptor

Five NMDA receptor subunits are expressed in the brain.[31,32] The NR1 subunit is ubiquitously expressed and has 8 distinct splice isoforms. The four subtypes of the NR2 subunit are termed NR2A-NR2D (with each except for NR2A having several splice variants). NMDA receptors are tetramers composed of multiple NR1 subunits together with at least one NR2 type,[31,32] with the different combinations bestowing distinct functional properties onto the receptor.[31] The NR1 subunit is necessary for channel function and displays similar structure and sequence homology to subunits of other ion channels.[31] The NR2 subunits however are unique as they have long C-terminal tails serving as anchoring points for signal transduction enzymes.[33] Within the hippocampus, NR2A and NR2B subunits are most prominent. During synapse development and maturation, the subunit composition of the NMDA receptor switches from a heteromeric receptor composed of NR1 subunits together with NR2B subunits to one composed of NR1 with NR2A subunits.[6] This subunit replacement confers distinct kinetic properties on the receptor: replacement by 2A speeds the decay of the NMDA receptor-mediated EPSC, resulting in NMDA receptor-mediated synaptic currents of shorter duration. This change in channel properties may underlie experience-dependent plasticity.[34]

Trafficking and insertion of NMDA receptors

Live imaging of GFP-tagged NR1 has suggested that NMDA receptors traffic in mobile transport packets to developing synaptic sites.[35] However, timelapse imaging and FRAP (fluorescence recovery after photobleaching, a visual measure of protein turnover) of PSD proteins including NR1, showed gradual appearance of clusters, indicating that these proteins are recruited to new synapses in a gradual manner.[36] No postsynaptic vesicular transport packets of NR1 were evident. NMDA receptors are integral membrane proteins and therefore must be transported to the synaptic membrane *via* a vesicular intermediate. The above evidence suggests that this could be *via* packets[35] or by

vesicles too small to be detected at the light microscope level.[36]

Rapid delivery of NMDA receptors into the postsynaptic membrane has been shown to occur *via* PKC activated, SNARE-dependent exocytosis.[37] Live imaging of GFP-NMDA receptor subunit recombinant proteins have shown NMDA receptor insertion may be as complex as AMPA receptors. At the early postsynapse when NR2B-containing NMDA receptors are prevalent, GFP-tagged NR2B subunits were shown to be recruited in an activity-independent manner.[38] As development progresses, and synaptic activity begins to increase, NR2B-containing receptors are internalized and replaced by NR2A-containing receptors, with this switch requiring synaptic activity to occur.

The synaptic trafficking and the subsequent insertion of NMDA receptors into the synapse is tightly regulated. In the gene encoding NR1, exons 21 and 22a encode C1 and C2 cassettes in the intracellular domain of NR1 subunit. NR1 splice variants containing the C1 cassette have endoplasmic reticulum (ER) retention motifs that subsequently prevent surface expression of this splice variant.[39] Shielding of C1 cassette promotes forward trafficking to the synapse,[39] whereas the C2 cassette slows export from the ER.[40] In response to different levels of activity, neurons can control the level of NMDA receptor expression at the synapse through rapid translation of specific NR1A splice variants: chronic changes in synaptic activity control splicing at the C2/C2′ site to accelerate the trafficking of C2′ receptors to the synapse,[40] showing mRNA splicing as a novel mechanism to control NMDA receptor surface expression during activity-dependent changes in synaptic strength.

Activity-dependent regulation of NMDA receptor expression

For many years it was widely believed that NMDA receptors were not subject to activity-dependent regulation that has been reported for the AMPA receptor. For example, in contrast to AMPA receptors, NMDA receptors exhibit high resistance to detergent extraction from PSDs,[41] indicating that they are tightly anchored to the cytoskeleton at the synaptic site. In studies in dissociated neuronal cultures, field or pharmacological stimulation to induce AMPA receptor internalization resulted in no NR1 internalization.[42] In addition, there have been reports of a lack of activity-dependent up-regulation of NMDA receptors accompanying the up-regulation of AMPA receptors associated with the expression of LTP.[43-46]

Using the irreversible use-dependent NMDA receptor antagonist MK801, the movement of NMDA receptors into and out of the synaptic membrane was shown for the first time to occur through lateral diffusion between synaptic and extrasynaptic pools.[47] NMDA receptor movements occurred on the time scale of minutes. As many as 65% of synaptic NMDA receptors were calculated to be mobile. This study challenged the view that NMDA receptors are stable components, anchored to the PSD by PSD-associated proteins.

Recently it has been demonstrated that synaptic currents mediated by NMDA receptors can be regulated by synaptic activity, particularly in the negative direction. This evidence of activity-induced NMDA receptor downregulation has suggested that NMDA receptors are not static in the postsynaptic membrane, but may in fact be as dynamic as AMPA receptors following the induction of LTD. During synaptic depression, the amplitude of NMDA receptor-mediated currents is suppressed in an NMDA receptor-dependent manner.[48-50] This depression of the NMDA receptor component of the postsynaptic current has subsequently been linked to endocytic processes: evidence of NMDA receptor endocytosis following application of exogenous agonists has been shown in both heterologous and neuronal systems.[51-53] NMDA receptors undergo rapid dynamin-dependent endocytosis in response to the induction of LTD,[50] upon glycine priming,[53] and after repeated long-term agonist application.[52] In addition, NMDA receptors co-immunoprecipitate with the endocytic protein AP-2 that links internalized proteins to clathrin.[53] The NR2B subunit of the NMDA receptor contains an endocytic motif (YEKL) in its C-terminus that directly interacts with the endocytic AP-2 adaptor protein μ2.[54] The AP-2 binding site on NR2B is adjacent to but distinct from the PSD95 binding site of NR2B, with each site having opposing effects on surface NMDA receptor expression. The PSD proteins PSD95, SAP97 and PSD93 may control the availability of this endocytic motif for AP-2 binding and subsequent endocytosis of the NMDA receptor.[51] These recent studies can be consistent with earlier data suggesting NMDA receptors are fixed in the postsynaptic density by PSD proteins, by showing that NMDA receptors can be dynamic, but only following unbinding from PSD proteins and the subsequent binding of endocytic proteins.

Consequences of NMDA receptor plasticity: metaplasticity.

Activity-dependent regulation of the NMDA receptor influences the ability of the synapse to undergo further NMDA receptor-dependent plasticity, serving as a basis for some forms of metaplasticity. Activity-dependent regulation of NMDA receptor function and synaptic expression could be controlled by the PSD proteins it is bound to,[51] its location in the synaptic or extrasynaptic membrane,[47] and the activity state of the synapse.[49,50] Anchored NMDA receptors at the PSD that are only subject to downregulation under certain conditions would ensure that synapses in the brain protect their ability to undergo future NMDA receptor-dependent plasticity and subsequent NMDA-receptor dependent processes such as some forms of learning and memory.

Concluding remarks

Over the past 10 years, incredible progress has been made in our understanding of the molecular mechanisms of synapse function and plasticity in the central nervous system. The detailed analysis of the families of synaptic proteins localized to the PSD have provided fundamental information into how synapses are formed, how synaptic

proteins are targeted to synapses, and how synapses can change their strength. These processes are essential to our understanding of brain function at a behavioural level. Indeed, correlative studies of animal behaviour and synapse strength are revealing changes in glutamate receptor expression at synapses in response to visual changes, learning and drug addiction.[48,55,56] Moreover, recent advances are now enabling the measurement of synapse function in awake, behaving animals.[57] Such advances are critical to enable us to bridge the gap in our understanding of how cellular mechanisms translate to cognitive functions by providing powerful information on synapse physiology during natural behaviours.

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Received 11 November 2006, in revised form 3 January 2007. Accepted 8 March 2007.

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