



3 Modifying Synapses: Central Concepts

The change in synaptic potentials that is produced in an LTP experiment is the product of hundreds of biochemical interactions. These interactions involve processes that modify and rearrange *existing* proteins contained in synapses as well as processes that generate *new* proteins.

The purpose of this chapter is to provide some general ideas that will facilitate an understanding of these processes and what they have to achieve. It begins with a functional description of the synapse as a biochemical factory. It then reviews the key elements of signaling cascades that modify synapses. The central role of glutamate receptors in the induction and expression of LTP is then discussed. The increase in the number of AMPA receptors in the dendritic-spine compartment of the neuron is identified as a fundamental outcome that mediates LTP. The chapter ends by providing an organizing framework for understanding the processes that increase and maintain these receptors in the synapse.

The Synapse as a Biochemical Factory

A standard view of a synapse is that it comprises three components: (1) a presynaptic component—a terminal ending of an axon of a sending neuron, (2) a postsynaptic component of the receiving neuron, and (3) a synaptic cleft that separates the two primary components. To understand how a synapse can be modified, however, it is useful to think of it as a biochemical factory with each component containing molecules needed to accomplish specific functions. Some of the components and processes involved in the release of neurotransmitters by the presynaptic neuron already have been described (see Figure 2.2). So the primary focus here is on postsynaptic processes.

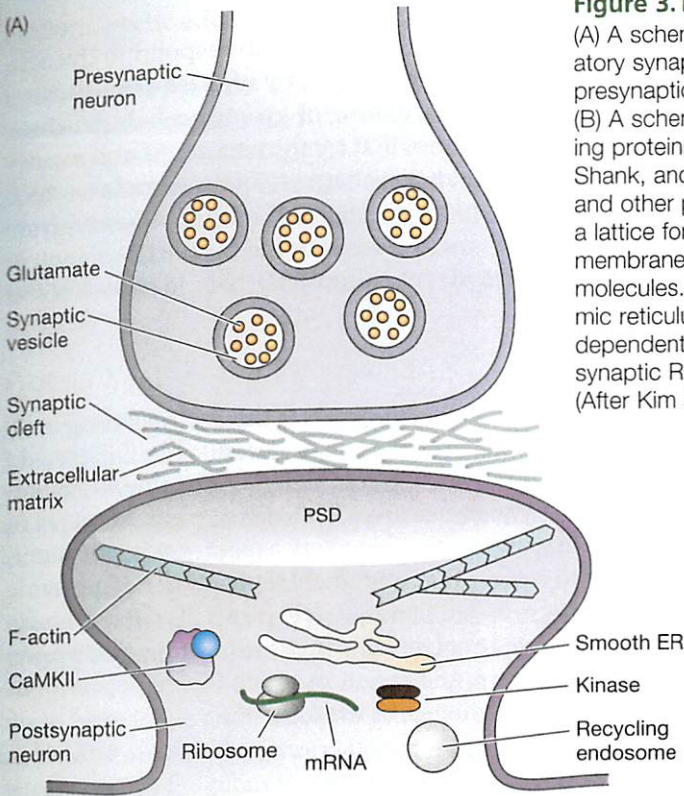
The synaptic cleft, which separates the pre and post components of the synapse, is occupied by the **extracellular matrix (ECM)**, illustrated in Figure 3.1. This matrix is composed of molecules synthesized and secreted by neurons and glial cells (Dityateve and Fellin, 2008). The ECM forms a bridge between the pre and postsynaptic neurons and the molecules it contains interact with the neurons to influence their function.

Changes in synaptic potentials produced by an LTP-inducing stimulus are primarily the result of modifying **excitatory synapses**—synapses whose postsynaptic component is a spiny-like protrusion called a dendritic spine that contains membrane-spanning receptors, called glutamate receptors, that respond to the excitatory neurotransmitter **glutamate**. Synapses that contain dendritic spines are organized both to participate in synaptic transmission and to be modified by synaptic activity. Some general features of an excitatory synapse are shown in Figure 3.1.

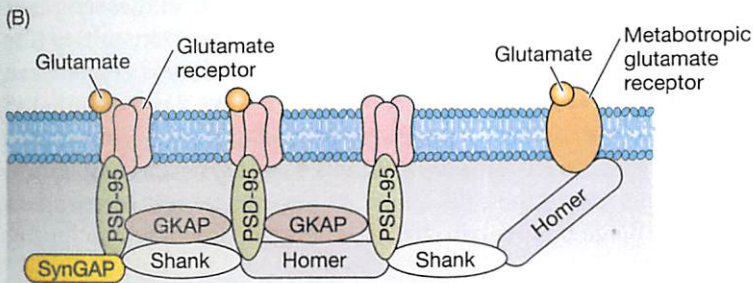
Postsynaptic Density

A major feature of excitatory synapses is a thickening of the postsynaptic membrane termed the **postsynaptic density (PSD)**. The PSD contains several hundred proteins that include glutamate receptors, ion channels, signaling enzymes, scaffolding proteins, and adhesion molecules (see Figure 3.1). Several core, scaffolding proteins (PSD-95, GKAP, Shank, and Homer) play a key role in organizing the postsynaptic density (Kim and Sheng, 2009; Sheng and Hoogenraad, 2007).

Glutamate receptors located in dendritic spines respond to glutamate released from the presynaptic neuron. The postsynaptic density contributes to this process in two complementary ways. One of its functions is to localize both glutamate receptors and adhesion molecules in the postsynaptic membrane. In this way it facilitates the adhesion of the pre and postsynaptic components and aligns the glutamate receptors with the presynaptic neurotransmitter release zones. This alignment increases the likelihood that


Figure 3.1

(A) A schematic representation of an excitatory synapse. The PSD is opposed to the presynaptic active zone, attached to F-actin. (B) A schematic of the PSD. Core scaffolding proteins of the PSD—PSD-95, GKAP, Shank, and Homer—interact with each other and other proteins and are thought to form a lattice for the assembly of postsynaptic membrane glutamate receptors and signaling molecules. Smooth ER = smooth endoplasmic reticulum; CaMKII = calcium-calmodulin-dependent protein kinase II; SynGAP = synaptic Ras-GTPase-activating protein. (After Kim and Sheng, 2009.)



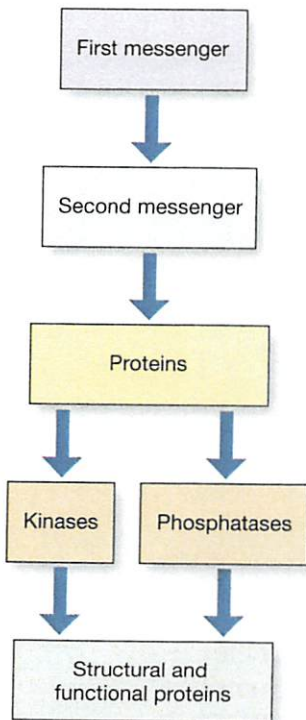
glutamate released into the extracellular space will bind to the receptors. Excitatory synapses are plastic—they can be modified when glutamate receptors are activated. This requires the activation of other signaling molecules by the glutamate receptors. A second function of the postsynaptic density is to position these signaling molecules near the glutamate receptors so they can be activated.

Other Synaptic Proteins

Many other proteins below the PSD are positioned to respond to the activation of glutamate receptors (see Figure 3.1). Many of these are structural proteins, such as **actin**, that provide scaffolding or give the cell its structure. Others are functional proteins—enzymes that catalyze reactions and modify the function of other proteins. In addition there are other complexes such as recycling **endosomes** that transport internalized receptors to and from the plasma membrane, **ribosomes** that are responsible for the translation of new protein, and smooth **endoplasmic reticulum (ER)** that can sequester and release calcium.

Signaling Cascades

Excitatory synapses are modified by synaptic activity that begins when glutamate is released by the presynaptic neuron. This activity initiates a set of events, signaling cascades that are involved in every aspect of synaptic modification. So it is important to review the fundamental properties of a generic signaling cascade as illustrated in Figure 3.2, which presents the components involved in the cascade—first messenger, second messenger, and target proteins (kinases, phosphatases)—and the sequence of the cascades.



First and Second Messengers

The signaling cascade is initiated when a **first messenger**—an extracellular substance, such as a neurotransmitter (for example, glutamate) or a hormone—binds to a cell-surface receptor and initiates intracellular activity. The second step in the cascade involves **second messengers**—molecules that relay signals from receptors on the cell surface to target molecules inside the cell. There are several second messengers (see Siegelbaum et al., 2000, for a detailed review), including

Figure 3.2

The signaling cascade is initiated when a first messenger—an extracellular substance, such as a neurotransmitter (for example, glutamate) or a hormone—binds to a cell-surface receptor and initiates intracellular activity. The second step in the cascade involves second messengers—molecules that relay signals from receptors on the cell surface to target intracellular protein kinases and phosphatases that then target other proteins.

calcium, cyclic adenosine monophosphates (cAMP), and inositol triphosphate (IP₃).

Second messengers are not proteins. So the number of second messenger molecules can be rapidly increased when a neurotransmitter or hormone binds to membrane receptors. This rapid increase is possible because their synthesis does not depend on the relatively slower transcription and translation processes (discussed in Chapter 5). The rapid production of second messenger molecules provides a way to amplify the effect of the first messenger inside the cell.

Protein Kinases and Phosphatases

Once generated, second messengers diffuse and target two classes of proteins, **kinases** and **phosphatases**. A protein kinase is an enzyme that modifies other proteins by chemically adding phosphate groups to them. This process, called **phosphorylation**, can change the protein's cellular location, its ability to associate with other proteins, or its enzyme activity.

A prototypical protein kinase is composed of two domains—an **inhibitory domain** and a **catalytic domain**. The catalytic domain carries out the phosphorylation function of the kinase. However, this activity is normally suppressed by the inhibitory domain. Thus, a kinase can be in two states: (1) it can be inactive and unable to phosphorylate other proteins or (2) it can be active and able to phosphorylate other proteins (including other kinases). To become active the catalytic unit must be released from the inhibitory unit. Second messengers target the inhibitory domain, unfold it, and thereby expose and activate the catalytic domain (see Figure 3.3). Dissociation of the second messenger can return the kinase to its inactive state. In this way, second messengers regulate the state (inactive or active) of kinases and thus their function. Second messengers also target proteins called phosphatases. These proteins are designed to remove phosphates from proteins and by doing so play an important role in regulating cellular activity.

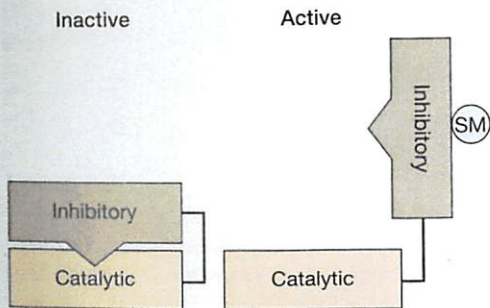


Figure 3.3

Kinases are composed of an inhibitory and a catalytic domain. In its normal inactive state the catalytic unit is unable to phosphorylate other proteins. The binding of a second messenger (SM) to the inhibitory domain exposes the catalytic unit and puts the kinase in an active state, enabling it to carry out its phosphorylation function.

Glutamate Receptors Are Critical to the Induction of LTP

Many of the signaling cascades that are required for long-term potentiation will be described in chapters that follow. It is useful at this point, however, to describe the critical events that initiate LTP—the activation of glutamate receptors. Glutamate is an excitatory neurotransmitter and a primary first messenger for the induction of LTP. It is released into the extracellular space from the presynaptic axon terminals when an LTP-inducing stimulus is applied to the axons of the sending neurons. Some glutamate receptors on the receiving dendritic spines are called **ionotropic receptors** or ligand-gated channels. They are constructed from four or five protein subunits that come together to form a potential channel or pore (Figure 3.4). These receptors span the cell membrane; they protrude outside the cell as well as inside the cell and are positioned to interact with signaling molecules present in both the extracellular space and the **cytosol** (the internal fluid of the neuron). They are called ionotropic receptors because, when their channels open, ions such as Na^+ (positive sodium ions) or Ca^{2+} (positive calcium ions) can enter the cell.

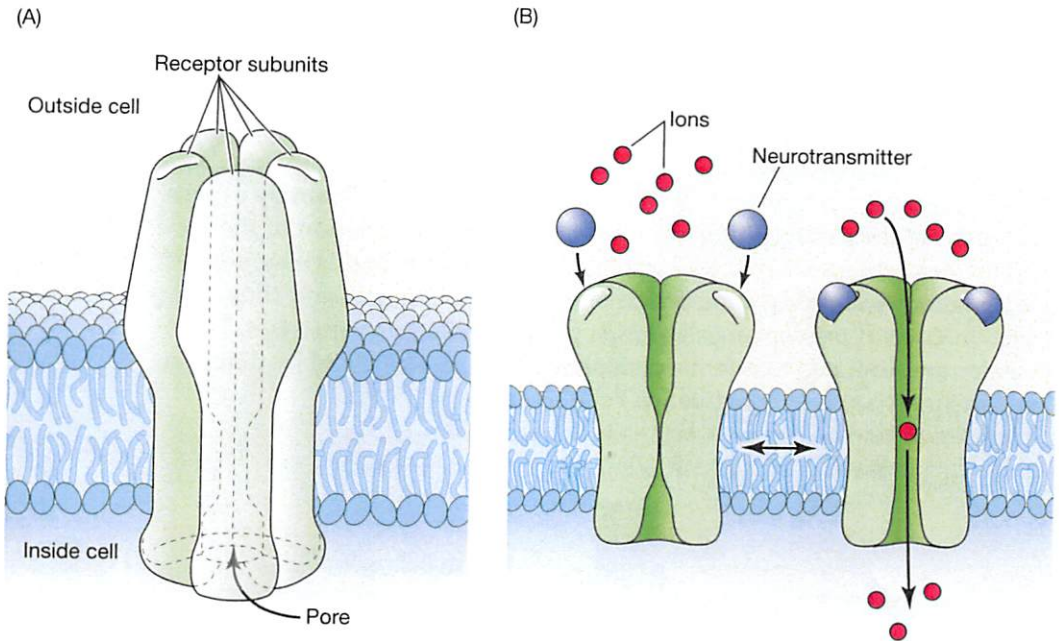


Figure 3.4

(A) Ionotropic receptors are located in the plasma membrane. (B) When a neurotransmitter binds to the receptor, the channel or pore opens and allows ions such as Na^+ and Ca^{2+} to enter the cell.

They are called ligand-gated channels because it is the binding of a **ligand** (an ion, molecule, or molecular group) that opens the channel. Thus, glutamate is a ligand that binds to the site on glutamate receptors and changes the conformation or shape of the receptor so that the channel is briefly open and ions can enter the cell (see Figure 3.4).

LTP Induction Requires Both NMDA and AMPA Receptors

Glutamate binds to three ionotropic receptors: (1) the α -amino-3-hydroxy-5-methyl-4-isoxazole-propionate (**AMPA**) **receptor**, (2) the N-methyl-D-aspartate (**NMDA**) **receptor**, and (3) the kainate receptor (Figure 3.5). However, only the AMPA and NMDA receptors are pertinent to this discussion because they are the primary contributors to long-term potentiation.

Understanding of how LTP is induced took a giant step forward when Graham Collingridge (Collingridge et al., 1983) applied a pharmacological agent to the brain called amino-phosphono-valeric acid (abbreviated as APV), a competitive NMDA receptor **antagonist**. Competitive antagonists are molecules that have the property of occupying the receptor site so that the normal binding partner or ligand (in this case glutamate) cannot access

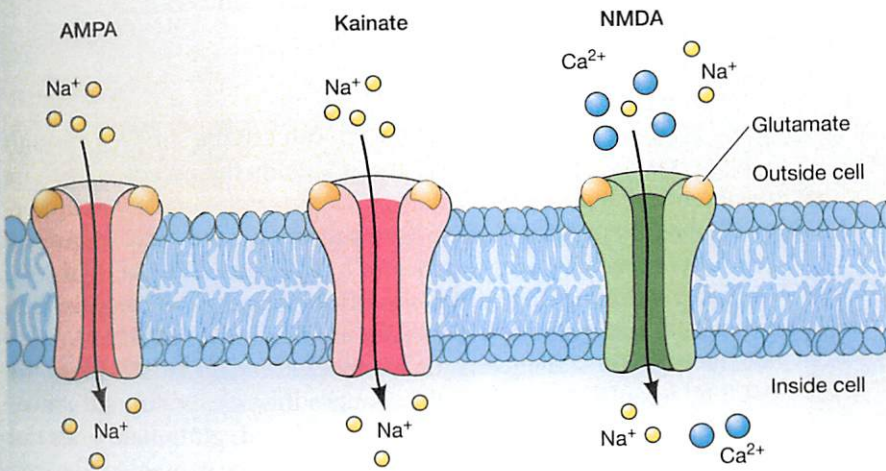


Figure 3.5

There are three types of glutamate receptors. AMPA and NMDA receptors, which are located in dendritic spines, play a major role in the induction and expression of LTP. When glutamate binds to these receptors their channels open and positively charged ions in the extracellular fluid (Na⁺ and Ca²⁺) enter the neuron.

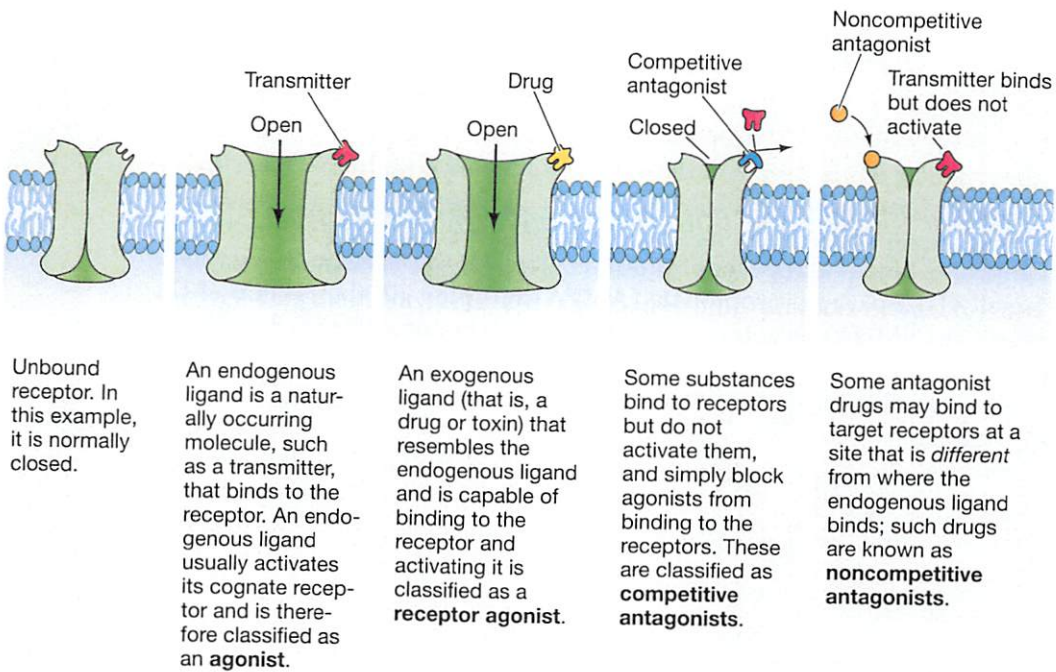


Figure 3.6

The agonistic and antagonistic actions of drugs.

the site (Figure 3.6). However, the antagonist doesn't fit the site well enough to change the conformation of the receptor: it shields the receptor from its endogenous ligand. Using this methodology, Collingridge made two important observations: (1) applying APV before presenting the high-frequency stimulus prevented the induction of LTP (Figure 3.7), but (2) if APV was applied after LTP had been induced, that is, during the test phase, the test stimulus still evoked a potentiated fEPSP.

These two observations indicate that glutamate must bind to the NMDA receptor to produce the changes in synaptic strength, measured as LTP. But after LTP has been induced, glutamate does not have to bind to the NMDA receptor for LTP to be expressed. Experts say that the NMDA receptor is necessary for the induction of LTP but is not necessary for the expression of LTP. (The expression of LTP is measured as the enhanced field potential compared to baseline). The phrase "NMDA receptor-dependent LTP" is often used to denote the special importance of the NMDA receptor to the induction of LTP.



Graham Collingridge

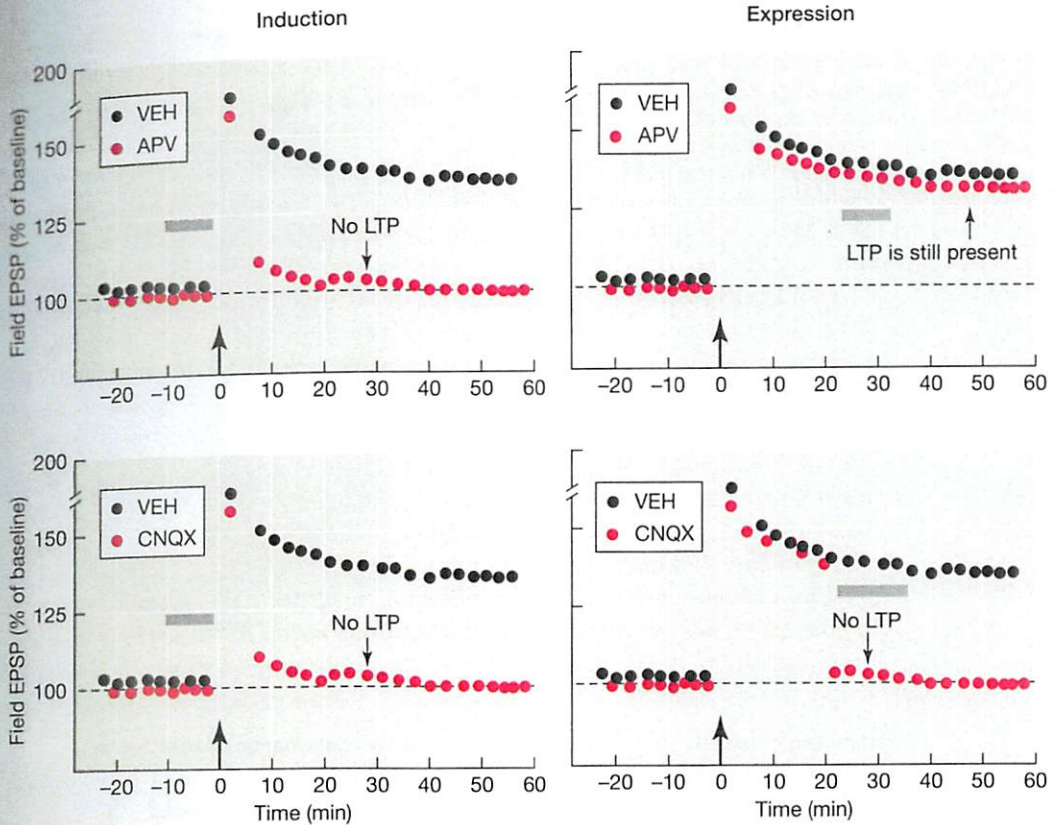


Figure 3.7

APV, an NMDA receptor antagonist, prevents the induction of LTP but has no effect on its expression. CNQX, an AMPA receptor antagonist, prevents both the induction and expression of LTP. The bar in each graph represents the application of the drugs—APV and CNQX—or the control vehicle (VEH).

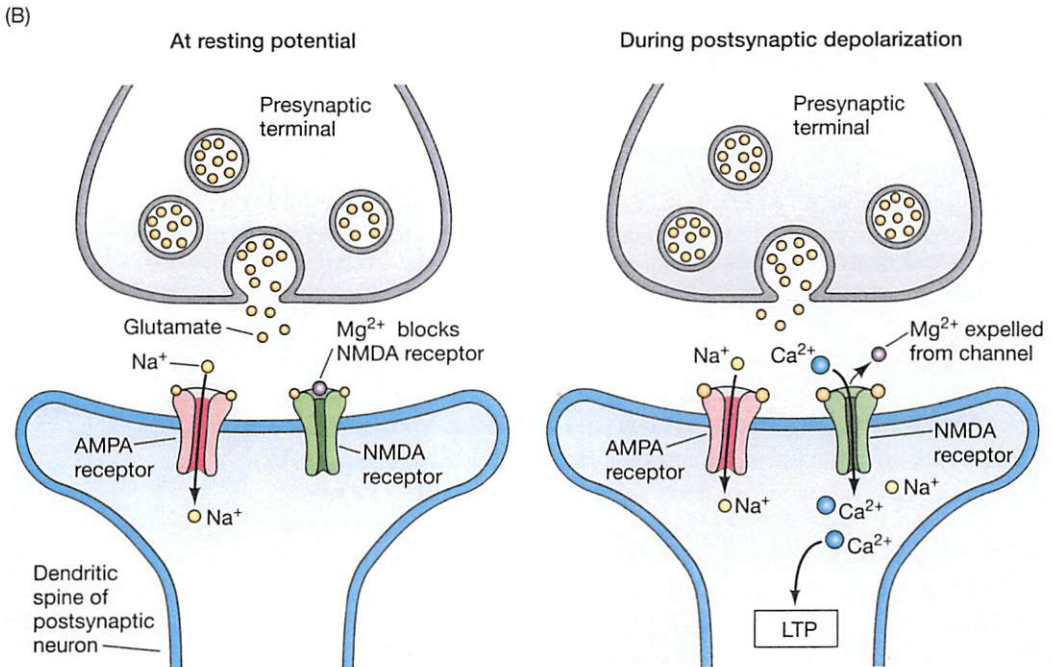
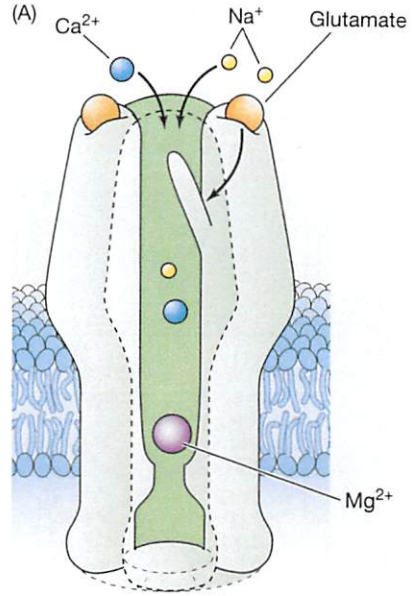
Thus, NMDA receptors are important for only the induction of LTP. In contrast, AMPA receptors are critical for both the induction and expression of LTP. So an AMPA receptor antagonist such as 6-cyano-7-nitroquinoxaline (CNQX) would block both the induction and expression of LTP (see Figure 3.7).

Two Events Open the NMDA Channel

The NMDA receptor channel is the gateway to the induction of LTP because it allows second-messenger Ca^{2+} into the spine (Dunwiddie and Lynch, 1978). This receptor has two critical binding sites. One site binds to glutamate and the other site binds to magnesium— Mg^{2+} . The binding site for

Figure 3.8

(A) The NMDA receptor binds to glutamate. It also binds to Mg^{2+} (sometimes called the magnesium plug) because Mg^{2+} binds to the NMDA channel. (B) The opening of the NMDA receptor requires two events: (1) glutamate must bind to the receptor and (2) the cell must depolarize. When this happens, the magnesium plug is removed and Ca^{2+} can enter the cell.



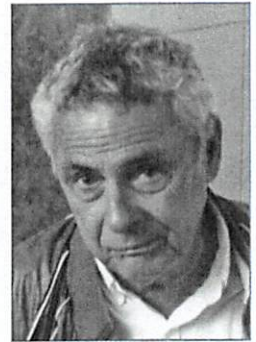
magnesium is in the pore formed by the protein subunits that make up the receptor. Because magnesium binds to a site in the pore, it is sometimes called the Mg^{2+} plug. Calcium cannot enter the cell unless magnesium is removed from the pore. The trick is how to remove the magnesium plug. This outcome requires two events: (1) glutamate has to bind to the NMDA receptor, and (2) the synapse has to depolarize (Figure 3.8). Thus, the binding of glutamate to the NMDA receptor is a necessary but not a sufficient condition to remove the Mg^{2+} plug; the synapse must also depolarize while glutamate is still bound to the receptor. AMPA receptors are critical for LTP induction because the binding of glutamate to these receptors opens the Na^+ channel and produces the synaptic depolarization needed to remove the Mg^{2+} plug.

In summary, the induction of LTP occurs when extracellular calcium enters the spine via the NMDA calcium channel. Opening of the calcium channel requires a change in the conformation of the NMDA receptor, produced when glutamate binds to it, coupled with synaptic depolarization that occurs when glutamate binds to AMPA receptors to open sodium channels. Although the opening of the NMDA receptor channel is critical for the induction of LTP, the calcium ions it can influx contribute little or nothing to the expression of LTP. In contrast, the expression of LTP depends completely on sodium ions entering the AMPA receptors.

Increasing AMPA Receptors Supports the Expression of LTP

The expression of LTP (the slope of the fEPSP) depends exclusively on an increase in the amount of Na^+ entering the spine and this depends on AMPA receptors. Consequently, one might infer that the signaling cascades that produce LTP do so by increasing the capacity of AMPA receptors to influx Na^+ . In fact, nearly 30 years ago, Gary Lynch and Michel Baudry (1984) proposed that LTP (and memory storage) was the result of an increase in the number of glutamate receptors in the spine.

This general idea has proven to be correct (Figure 3.9). Two prominent researchers, Robert Malenka and Mark Bear, concluded: "It now appears safe to state that a major mechanism for the expression of LTP involves increasing the number of AMPA receptors in the plasma membrane at synapses via activity-dependent changes in AMPA receptor trafficking" (Malenka and Bear, 2004, p. 7). This being true, then the fundamental challenge is to understand the biochemical-molecular processes that regulate AMPA receptor function. The following section provides a framework for understanding these processes.



Gary Lynch

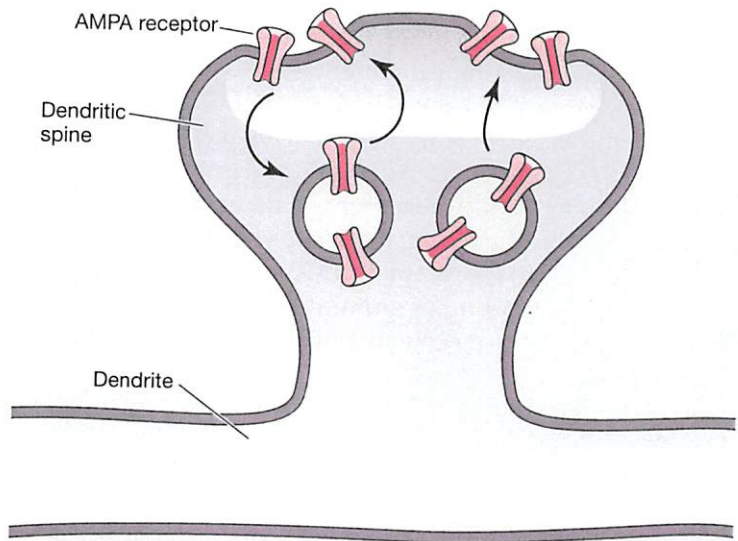


Figure 3.9

The complementary ideas that (a) an LTP-inducing stimulus can rapidly increase the number of AMPA receptors in the spines and (b) this is the fundamental outcome that supports the expression of LTP are now central principles of the field. AMPA receptors traffic into and out of dendritic spines. AMPA trafficking is regulated constitutively (double arrows) and by synaptic activity (single arrow). Constitutive trafficking routinely cycles AMPA receptors into and out of dendritic spines, while synaptic activity is thought to deliver new AMPA receptors to them.

An Organizing Framework: Three Principles

The number of molecules and biochemical interactions that contribute to AMPA receptor regulation is bewildering, and it is far beyond the scope of this book to provide a complete account of what is known. A more modest goal is to identify some of the key outcomes and processes and provide a coherent framework for understanding how this happens.

Three principles help to organize the complexities of the field:

1. The duration of LTP can vary.
2. The duration of LTP is dependent on the extent to which the inducing stimulus initiates the fundamental molecular processes that modify and rearrange existing protein as well as the processes needed to generate new protein.

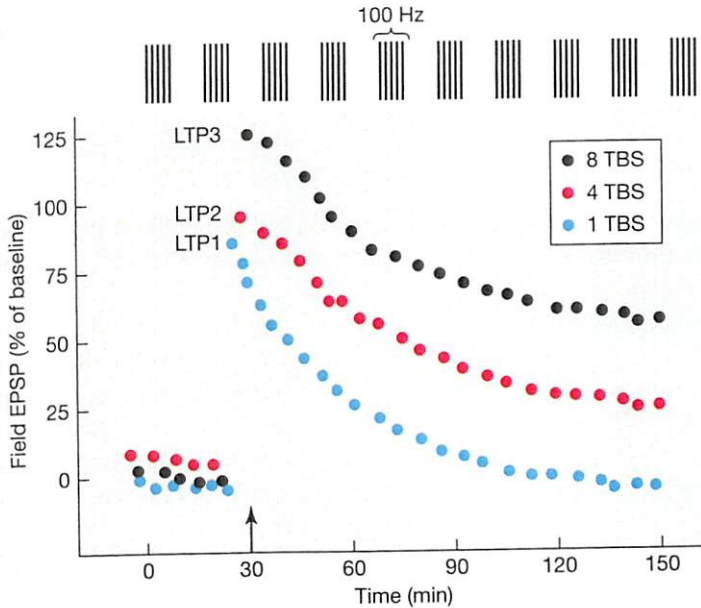


Figure 3.10

The number of theta-burst stimuli (TBS) determines the duration of LTP. This family of LTP functions implies that changes in synaptic strength can vary in duration and may have different molecular bases. A fundamental goal of neurobiologists is to understand these differences.

3. Synapses are strengthened and maintained in a sequence of temporal, distinct but overlapping processes.

The Duration of LTP Can Vary

The duration of long-term potentiation depends on the parameters of the inducing stimulus. Generally speaking, as the intensity of the high-frequency inducing stimulus increases so does the duration of LTP. For example, a **theta-burst stimulation (TBS)** protocol has been used in many important experiments to induce LTP. This protocol is modeled after an increased rate of pyramidal neuronal firing that occurs when a rodent is exploring a novel environment (Larson et al., 1993). One theta burst consists of trains of 10×100 -Hz bursts (5 pulses per burst) with a 200-millisecond interval between bursts. Figure 3.10 illustrates a family of LTP functions that can be produced by varying the number of theta bursts. Note that the duration of LTP increases with the number of theta bursts. The data illustrated in this figure have two implications: (1) changes in synaptic strength can vary in duration and (2) the

molecular basis of these synaptic changes is likely different. This being the case, then an important goal is to discover the different associated molecular processes that are needed to produce this variation.

Molecular Processes Contribute to LTP Durability

Three general sets of molecular processes contribute to the durability of LTP: (1) post-translation processes, (2) transcription processes, and (3) translation processes (Figure 3.11).

The component proteins needed to change the synapse are already in place at the time glutamate is released. Thus, synaptic activity that induces LTP initiates biochemical processes that work on the already existing parts to modify and assemble them to strengthen synaptic connections. These are post-translation processes because they involve only the modification and rearrangement of existing protein.

Post-translation processes can initially change the strength of synapses. However, the generation of new protein is necessary to ensure these changes will endure. If synaptic activity is sufficiently strong it can initiate transcription processes that generate the messenger ribonucleic acid (mRNA) needed for creating new protein and/or the translation of mRNA into protein, also called protein synthesis. The goal of these processes is to increase the complement of AMPA receptors in the synapses activated by the release of glutamates and to ensure that they will remain there.

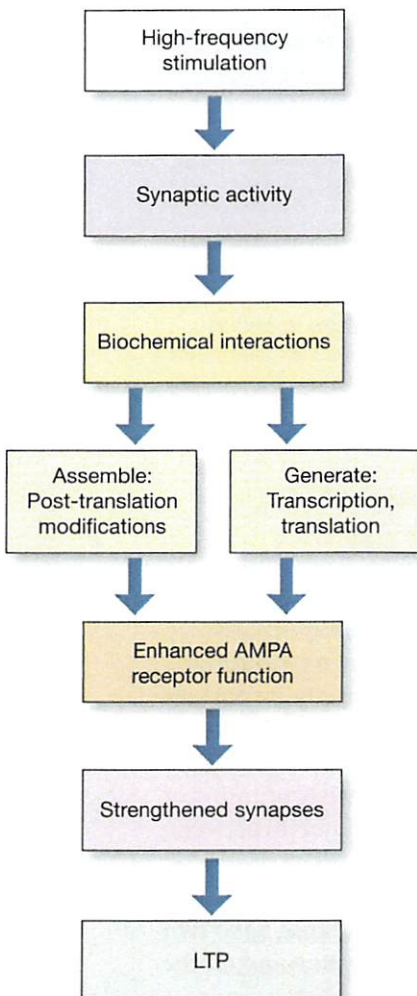


Figure 3.11

When high-frequency stimulation generates synaptic activity, biochemical interactions are initiated that lead to several functional outcomes that are critical to the induction of LTP. Post-translation modification processes assemble and rearrange existing proteins. Transcription and translation processes generate new proteins. A major consequence of these processes is that the contribution of AMPA receptors to synaptic depolarization is enhanced, that is, synapses are strengthened.

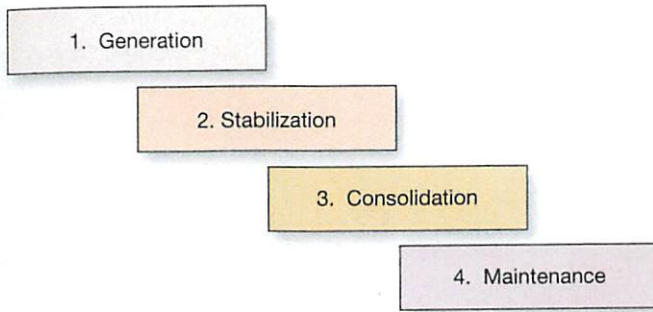


Figure 3.12

Changes in synaptic strength that support LTP evolve in stages that can be identified by the unique molecular processes that support each stage.

Synapses Are Strengthened and Maintained in Stages

Changes in synaptic strength can occur within a minute or so following the induction stimulus. However, these potentiated synapses are not stable and will revert back to their pre-potentiated state unless the inducing stimulus also initiates additional sequences of molecular events (Lynch et al., 2007). This leads to the idea that synapses and memory traces are constructed in stages (as hypothesized by William James; see Chapter 1). Thus, the next chapters in this book will be organized around this idea, and these stages will be referred to as the (a) generation, (b) stabilization, (c) consolidation, and (d) maintenance phases (Figure 3.12). These chapters will reveal that each stage depends on unique molecular processes and that the primary target of these processes is the reorganization of AMPA receptor trafficking and actin cytoskeleton proteins that provide the structural and functional scaffolding needed to maintain the increased number of AMPA receptors.

Summary

It is useful to think of the components of a synapse as biochemical factors, with each component containing molecules needed to accomplish specific functions. Signaling cascades can modify and rearrange the existing molecules and can generate new molecules through activating translation and transcription processes. The activation of NMDA and AMPA receptors by the release of the first messenger glutamate initiates the processes that potentiate synapses. An end product of the signaling cascades initiated by glutamate binding to NMDA and AMPA receptors is an increase in the number of AMPA

receptors in activated synapses. Potentiated synapses can have different durations, which are determined by the molecular processes activated by the inducing stimulus. These processes will be described in the next four chapters within a framework that assumes that synaptic changes evolve in overlapping stages—generation, stabilization, consolidation, and maintenance.

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