Module 3: Excitatory Synaptic Transmission at Glutamatergic Synapses

Theory: Overview of synaptic transmission.
The main function of the synapse is to transmit electrical signals from presynaptic neurons to their postsynaptic partners (neurons or muscles). Synaptic transmission occurs at two different types of synapses: the electrical synapse and the chemical synapse. The electrical synapse uses ‘gap junctions’ connecting the pre- and postsynaptic cells. Electrical signals (e.g. action potentials) passively invade the postsynaptic membrane when ions diffuse across the gap junction. The chemical synapse involves the conversion of an electrical signal into a chemical signal and then back to an electrical signal. An action potential arriving at the nerve terminal depolarizes the membrane and opens voltage-gated calcium channels. Once calcium rushes in, synaptic vesicles begin to release transmitter into the synaptic cleft via exocytosis. The released transmitter then binds to receptors causing changes of postsynaptic membrane potential. Both types of synapses co-exist in the nervous system. Most would agree that the elaborate synaptic organization and the rapid signaling at the synapse make neurons (and the nervous system) unique among all cell types.

The chemical synapse is more intensively studied by neuroscientists than the electrical synapse is, even though the latter is more numerous in the brain. A typical chemical synapse contains 1) transmitter-loaded synaptic vesicles (SVs), 2) mitochondria (which provide the energy for synaptic activity), 3) active zones (at which vesicle fusion takes place), 4) synaptic cleft (gap between pre- and post-synaptic membranes), and 5) the postsynaptic receptors. These features are illustrated in Figure 1. Most of these structures are consistently found at all synapses regardless what is used as transmitter. However, peptidergic synapses usually have large dense-core vesicles whereas the rest of the synapse has small, and clear-core vesicles. In addition, the number of SVs can differ dramatically among synapses. In general, neuromuscular junctions have more SVs than most synapses in the brain. This is part of the reason why there is no need for synaptic integration at NMJs. Through our discussions of the synapse, we specifically refer to those ‘fast synapses’ capable of causing immediate changes of postsynaptic potentials (i.e. EPSPs or IPSPs).
Figure 1. Synaptic Transmission at a Chemical Synapse.

(A). Transmission electron microscopic (TEM) micrograph of a synapse from the central nervous system. Synaptic vesicles (SVs), active zones (AZs), and mitochondria (mito) are labeled. Note the electron-dense materials present at the active zone and on the postsynaptic side (called PSD or postsynaptic density). Courtesy of Dr. John Heuser.

(B). A cartoon illustration of the synapse. From Neuroscience, Purves et al., editors, Sinauer.

(C). Schematic illustration of SVs and active zones in a frog neuromuscular junction. Note the small particles neatly lined up along the active zone. These particles are thought to represent voltage-gated calcium channels. From Harlow et al., 2001. Nature. 409:479-84.
II. BASIC FEATURES OF SYNAPTIC TRANSMISSION

Our current knowledge of the cellular mechanisms of synaptic transmission largely reflects the pioneering work conducted by the late Sir Bernard Katz and his colleagues during the 1950s and 1960s. The key concepts governing chemical transmission are briefly revisited here.

iiA. Spontaneous release of transmitter:

After the development of sharp microelectrodes, scientists were able to directly record membrane potentials from cells. The neuro-muscular preparation emerged as the favorite for most neurobiologists because it offered large muscle fibers. During the early 1950s, Katz and his colleagues used the frog neuromuscular junction to study synaptic transmission. They were surprised to learn that the nerve terminal was not ‘silent’ even though the motor nerve did not produce any action potentials! What they recorded were random and small depolarizations (< 0.5 mV) on the muscle fiber, as shown in Figure 2. These activities persisted even after the nerve was blocked with tetradotoxin (TTX). Hence, they called these responses ‘spontaneous end-plate potentials’ or MEPPs. Spontaneous synaptic potentials exist at all fast synapse and they are also often called ‘spontaneous miniature potentials’ or simply ‘minis’.

Figure 2. Miniature Postsynaptic Potentials.

Postsynaptic membranes are constantly depolarized by the nerve terminal. These miniature potentials (or minis) are caused by spontaneous release of transmitter. From Neuroscience, Purves et al., editors, Sinauer.

iiB. Quantal release of transmitter

The observation of minis prompted the hypothesis that transmitter is released in small packages (quanta) by the nerve terminal. We now know that SVs make it possible to release transmitter in a quantal fashion. Even before the discovery of SVs (1957), the idea of quantal release was firmly supported by other elegant experiments. One of such experiments was conducted by del Castello and Katz (1954) and by Boyd and Martin (1956), who developed the ‘failure analysis’ to probe the nature of transmitter release. In an NMJ bathed in a low calcium-containing saline, nerve stimulation led to most failures resulting in no synaptic response at all. However, the amplitude of synaptic potentials varied in a stepwise fashion when the muscle did show responses to the nerve stimulation. The histogram of the number of EPPs observed at the endplate was plotted. Interestingly, the peaks of the histogram occurred at one, two, three, and four times the mean amplitude of minis (Figure 3). These studies suggest that a mini is the smallest building block of evoked synaptic potential. The amplitude of average minis is often called ‘quantal size’, which reflects both the
amount of transmitter released by a vesicle (quantum) and the density and conductance of receptors on the postsynaptic side. Further, each vesicle has a low probability of being released in the terminal containing a large number of vesicles.

Figure 3. The Quantal Nature of Chemical Transmission.

A. Evoked end-plate potentials (EPPs) at the frog NMJ in low calcium. Note the amplitude of these EPPs. Adapted from *From Neuron to Brain*, Nicholis et al., editors, Sinauer.
B. Histograms of EPPs. Note that the smallest amplitude is similar to the average amplitude of the minis (C). These results demonstrate that the spontaneous mini is the basic unit of synaptic transmission and the large synaptic potential evoked by nerve action potentials is the integral multiple of minis. Adapted from *Neuroscience*, Purves et al., editors, Sinauer.

III. Glutamate Receptors: the postsynaptic side of synaptic excitation.

Overview of the glutamate receptors family

The glutamate receptors are a diverse family of *ionotropic receptors* (that is, the agonist directly gates an ion channel). Ionotropic receptors are readily distinguished from metabotropic receptors, where an agonist produces physiological effects indirectly, through intracellular messenger pathways. From a molecular biological perspective, the glutamate receptors comprise a superfamily of cation-selective channels that are the products of at least 15 different genes, depending on species. In addition, native glutamate channels may contain different combinations of subunits, and there may exist different splice variants of a particular subunit combination. As you might expect, this molecular diversity gives rise to a corresponding diversity of physiological properties, including, for example, channel kinetics, agonist selectivity and sensitivity.
Pharmacologically, there are 3 broad classes of glutamate receptors, named after the agonists that selectively activate each subtype: the AMPA receptor (α-amino-3-hydroxy-5-methyl-4-isoxalone propionic acid), the NMDA receptor (N-methyl-D-aspartate), and the kainate receptor. The key features of AMPA and NMDA receptors are in Figure 4 and the following sections.

Figure 4. NMDA and AMPA/kainate receptors. (A) NMDA receptors contain binding sites for glutamate and the co-activator glycine, as well as an Mg2+-binding site in the pore of the channel. At hyperpolarized potentials, the electrical driving force on Mg2+ drives this ion into the pore of the receptor and blocks it. (B) Current flow across NMDA receptors at a range of postsynaptic voltages, showing the requirement for glycine, and Mg2+ block at hyperpolarized potentials (dotted line). (C) The differing kinetics of NMDA and AMPA/kainate receptors can be directly observed by measuring synaptic currents at very positive membrane potentials, such as +50 mV, where the Mg2+ does not block NMDA receptors. Very fast EPSCs are due to the activation of AMPA or kainate receptors (top panel), somewhat slower EPSCs are due to the activation of NMDA receptors (middle panel), and mixed responses are due to the activation of both AMPA/kainate and NMDA receptors (bottom panel).

From *Neuroscience, 2nd edition*, Purves, Fitzpatrick, Augustine, Katz, and LaMantia.

**Fast synaptic excitation through AMPA-type glutamate receptors**

**Ion selectivity**

AMPA receptors are broadly selective for monovalent cations (Na⁺ and K⁺), and thus exhibit a reversal potential between the equilibrium potentials for these two ions.
The reversal potential ($E_{\text{rev}}$) is typically close to 0 mV in central neurons, reflecting the fact that the permeability of the channel to Na and K is not identical.

**Kinetics**

AMPA receptors display fast kinetics relative to other ligand gated ion channels in the brain and spinal cord. There are 4 major gene products (alpha subunits) that can combine to form a functional receptor, and the specific combination can give rise to considerabe diversity in the speed of signaling at the synapse.

**Functional role**

AMPA receptors mediate the majority of fast excitation in the CNS. The diversity of kinetic properties of the receptor gives neurons a way to match the speed of their excitation to the requirements of the circuit in which they participate.

**Synaptic excitation through NMDA-type glutamate receptors**

**Ion selectivity**

NMDA receptors (NMDARs), like AMPA receptors display permeability to both Na$^+$ and K$^+$. However, unlike AMPARs, NMDARs are about 5-10 times as permeable to calcium ions than Na$^+$ and K$^+$. Also, the sensitivity of the NMDA receptor to glutamate is far higher than that of AMPA receptors. NMDAR-mediated currents, like those from AMPARs, reverse near 0 mV.

**Kinetics**

NMDA receptors exhibit longer open times than AMPARs, and thus the NMDAR component of synaptic excitation tends to be much longer lasting than the AMPAR component. Contributing to the longer time course is also the higher affinity of the channel to glutamate (it takes glutamate longer to unbind from the channel).

**Co-agonist required**

In addition to requiring bound glutamate for channel opening, NMDARs also require glycine to be bound for efficient channel opening. In this sense, glycine acts as an allosteric modulator. Fortunately for all of us, there is always free glycine present in the extracellular space of the brain, and even within an *in vitro* slice preparation.

**Voltage sensitivity**

The primary distinctive feature of the NMDA receptor is its voltage sensitivity. Channel conductance increases dramatically as the membrane potential becomes progressively depolarized. This voltage-dependent activation is the consequence of the blockade of the channel pore by free extracellular magnesium ions. At negative potentials, magnesium "senses" the negative charge of the inside of the cell, and plugs up the channel pore. However, when the inside of the cell is depolarized, the magnesium is electrostatically repelled from the pore, thus clearing the channel to conduct sodium, potassium and calcium ions.
**Functional role**

The role of the NMDA receptor is multifaceted. In terms of cell signaling, EPSPs mediated by this receptor are slower and longer lasting as compared to AMPA receptors (e.g. Fig. 4), and thus are well suited to drive long trains of action potentials in their host neurons. Since NMDA receptors show high permeability to calcium ions, they are also a key mechanistic trigger for calcium-dependent processes in neurons. Such calcium influx can participate in second messenger pathways, providing modulation of ion channels, receptors, and other proteins. Perhaps one the best known functions of the NMDAR-mediated calcium influx is in triggering long-term changes in synaptic efficacy, such as long-term potentiation.

**IV. Equilibrium potentials vs. reversal potentials**

You have learned in earlier sections of this class that current flow through channels permeable to one ion always proceeds in a direction that moves the membrane potential toward that ion’s equilibrium potential. We will now extend this concept to cover the instances where a channel or receptor protein is permeable to multiple ions at the same time. Because current flow is governed by two or more equilibrium potentials, there must exist a voltage at which there is no net inward or outward movement of ions. This is referred to as the reversal potential of the channel/receptor. The reversal potential is defined as the point at which there is no net inward or outward current flow. Importantly, the reversal potential will exist at a voltage in between the equilibrium potentials of the permeant ions.

In the case of AMPARs, which are permeable primarily to both sodium and potassium, the reversal potential is usually between −15 mV and 0 mV, depending on the subunit combination of the channel. This range of reversal potentials exists between the positive sodium equilibrium potential (around +50 mV), and the negative potassium equilibrium potential (around −90 mV). As before, we can use the Goldman-Hodgkin-Katz equation to calculate the reversal potential of any channel or receptor if the relative permeabilities and concentrations of the permeant ions are known.