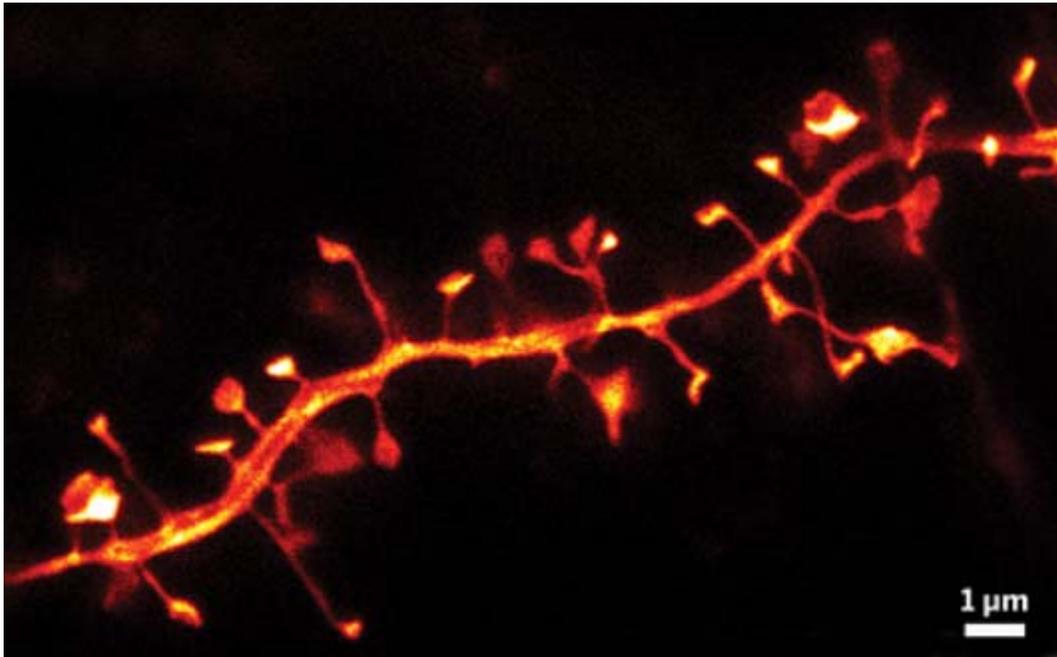


Neurobiology Laboratory

(BIO 365L)

Spring Semester 2010
Tuesdays 1-6 pm, PAI 1.04



Lab Manual: Module 3

Excitatory Synaptic Transmission

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 post-synaptic 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).

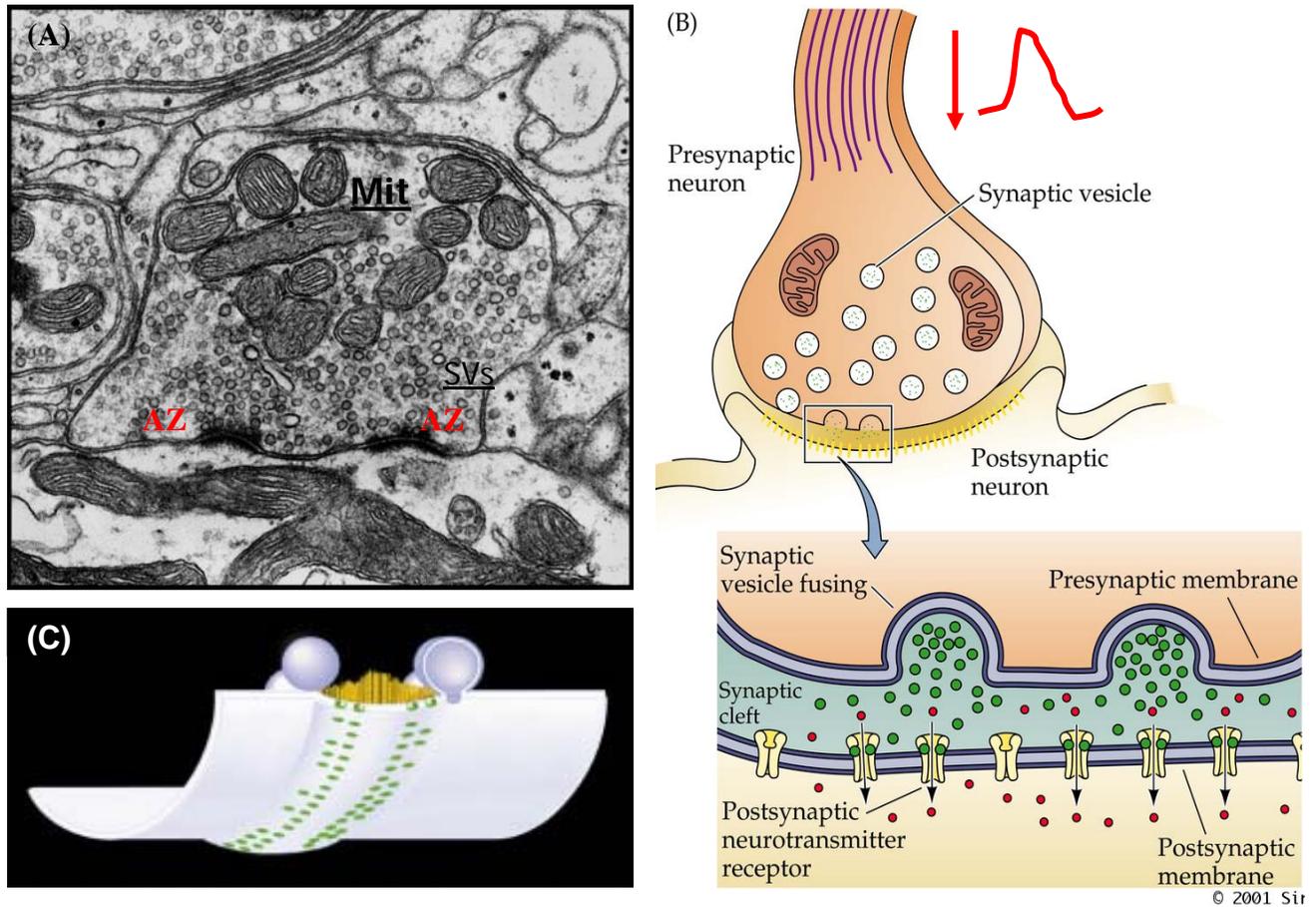


Figure 1. Synaptic Transmission at a Chemical Synapse.

(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

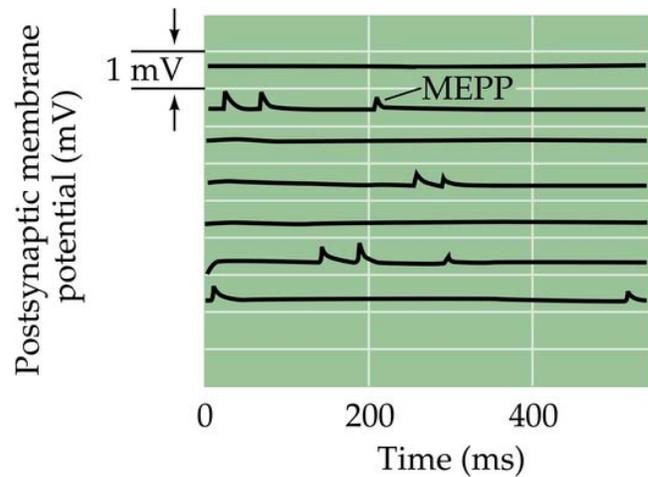
Our current knowledge of the cellular mechanisms of synaptic transmission largely reflects the pioneering work conducted by the late Sir Bernard Katz and his colleagues during the 1950s and 1960s. The key concepts governing chemical transmission are briefly revisited here.

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 tetrodotoxin (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 Castillo 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.

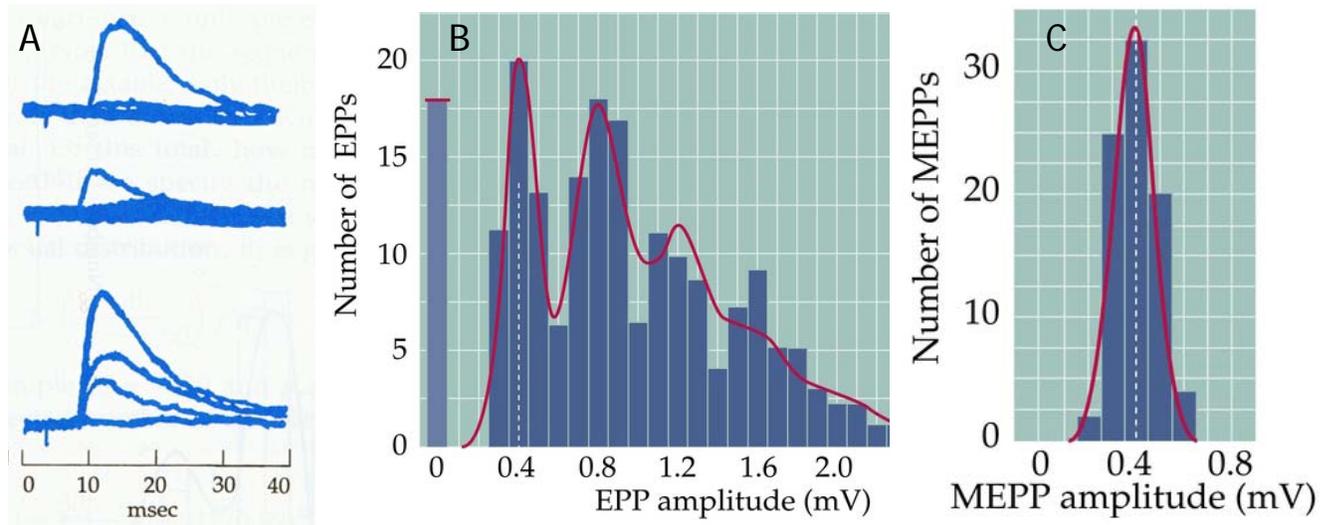


Figure 3. The Quantal Nature of Chemical Transmission.

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. Although both receptors are critical components of synaptic transmission, the exercises in this laboratory will focus on AMPA receptors, which mediate fast excitatory synaptic transmission in the majority of neurons in the brain. The key features of AMPA and NMDA receptors are in Figure 4 and the following sections.

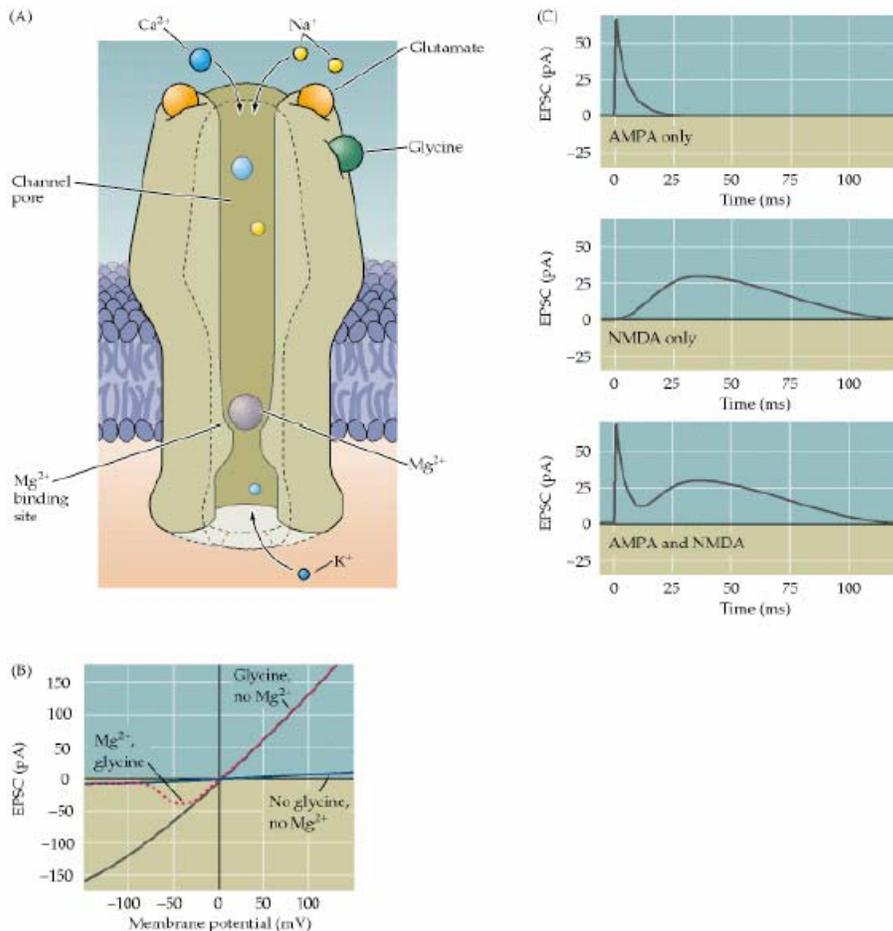


Figure 4. NMDA and AMPA/kainate receptors. (A) NMDA receptors contain binding sites for glutamate and the co-activator glycine, as well as an Mg^{2+} -binding site in the pore of the channel. At hyperpolarized potentials, the electrical driving force on Mg^{2+} drives this ion into the pore of the receptor and blocks it. (B) Current flow across NMDA receptors at a range of postsynaptic voltages, showing the requirement for glycine, and Mg^{2+} 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 Mg^{2+} 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_{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 considerable 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 of 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 laboratory 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.

V. Activation of synaptic inputs

Neurons are less active in brain slices than in the intact animal, largely because they no longer receive the normal pattern of synaptic input. In addition, neurons are severed from substantial portions of their neural networks.

All is not lost however. Some of the circuitry is left intact and functional. We will attempt to activate this circuitry artificially with electrical stimulation to the axons that are

running throughout the brain tissue. You will place a large bipolar metal electrode onto the surface of the tissue and activate these axons by delivering extracellular shocks. Due to the brevity of the stimulus (shock duration is 0.2 ms), the action potentials are generated synchronously. In intracellular recordings, the timing of the shock is detected by the presence of a brief electrical transient, called a “stimulus artifact”. It arises because your pipette remotely picks up the electrical field in the bath that is induced by the shock.

It is important to realize that this experimental arrangement is likely to activate dozens of synaptic inputs on your target cell. The stimulating electrode creates a large stimulation radius and therefore activates a large number of axons.

VI. Synaptic plasticity at glutamatergic synapses

A. Overview

One of the most critical properties of synapses is the ability to change and adapt. The ability to modify the strength of synaptic transmission is essential for diverse functions such as motor coordination, neural development, sensory perception, memory formation and our other diverse cognitive abilities. At the cellular level, the challenge to the nervous system is to change selectively only in response to functionally significant patterns of presynaptic action potential activity.

Hebb's Learning Rule

In the 1940's Donald Hebb developed a hypothesis and theoretical framework by which the strength, or “weight” of synapses could be adjusted by the pattern of activity. He hypothesized that learning and memory could be governed by a simple learning “rule”: that the strength of synapses would be increased when they are coactivated in close temporal coincidence, and decreased when they are activated in anti-coincidence. After many years of research, Hebb's postulate has been one of the most intensively investigated theories in Neuroscience, and many of the cellular details of his very broad framework have been elaborated. Hebb's conceptual framework has found wide applicability in the nervous system, and is often referred to as “Hebbian plasticity”.

“When an axon of cell A is near enough to excite cell B or repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased.”

Hebb, D. O. (1949).
*The organization of
behavior.*
Wiley, New York.



Many of the cell and molecular details of neural transmission were unknown in Hebb's time. However, the core of his hypothesis is that neurons adjust the strength of their connections through *correlations* in activity.

Long-term potentiation (LTP)

LTP is one of the most well documented forms of synaptic plasticity, and refers to long lasting changes in synaptic strength that last hours, and in some cases up to months. The mechanism underlying LTP most commonly requires postsynaptic calcium influx through the activation of NMDA receptors.

Induction of LTP proceeds in two phases:

1. Early-phase LTP: Increases in synaptic efficacy can be the result of an increase in the open probability of existing AMPA receptors through phosphorylation, or the insertion of additional AMPA receptors in the postsynaptic membrane.
2. Late-phase LTP: long lasting changes in synaptic strength result from changes in protein synthesis and gene transcription that produce long-term changes in synaptic strength.

Recall that activation of NMDA receptors depends on depolarizing the dendritic membrane sufficiently to relieve the Mg^{++} block of the receptor's pore. Some disagreement remains over exactly which events are necessary and sufficient to remove the Mg^{++} block and enable calcium influx via NMDA receptors.

Perhaps the best studied condition that has been shown to activate NMDA receptors and potentiate LTP is high-frequency synaptic activity. The high frequency EPSPs summate and depolarize the dendrites sufficiently to activate NMDA receptors. Another situation that produces NMDA receptor activation is the coincident occurrence of excitatory synaptic currents in the dendrite and arrival of a backpropagating dendritic action potential. We can test both arrangements during the Module 3 experiments.

Module 3 Experiments: Excitatory synaptic transmission

Experiment 1: Properties of synaptically evoked glutamatergic EPSPs

PreLab

The point of this experiment is simply to observe the basic characteristics of excitatory synaptic transmission at the glutamatergic synapses from the Schaffer collaterals onto CA1 pyramidal neurons. You will activate these axons with brief (100 μ s) shocks, which will evoke glutamate release from the terminals and cause postsynaptic voltage changes in your recorded cell.

InLab

1. Set capacitance compensation and bridge balance appropriately.
2. In the ADC_DAC Control Panel: Check the box next to "TTL0" and select Synpulse_TTL in the drop down menu to the right of the checkbox. This will send a command pulse to activate the stimulator at the beginning of the data sweep.

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3. In the Data Acquisition panel, check the “Stim on” box at the bottom then set the stimulus current to 0 so that no current is delivered via your patch pipette.
4. Set the stimulus current on the stimulus isolation unit to its lowest setting.
5. Press “Get data” and look for the stimulus artifact (a thin vertical line where the shock is delivered to the incoming axons). You may have to turn the current up some before you can see the stimulus artifact.
6. Increase the stimulus current on the stimulus isolation unit until it evokes an EPSP, then adjust the current until it evokes an EPSPs of 3-5 mV. Get help from your instructor or TA the first time you need to do this.
7. Once you have set your current amplitude, get 15 sweeps of data, waiting about 10 seconds between each sweep. Use the GetAverage macro to automate this process.

Record the sweep numbers here: _____ to _____

8. Observe whether EPSP amplitude and shape depends on membrane voltage. Using the same stimulus amplitude as in #1, Record EPSPs at -80 mV (negative to rest). Once you have adjusted the membrane potential to -80 mV using the “Holding Current” dial, get 15 sweeps of data, waiting about 10 seconds between each sweep.

Record the sweep numbers here: _____ to _____

9. Using the same stimulus amplitude, Record EPSPs at -95 mV. Once you have adjusted the membrane potential to -95 mV using the “Holding Current” dial, get 15 sweeps of data, waiting about 10 seconds between each sweep.

Record the sweep numbers here: _____ to _____

10. If possible, record EPSPs at 5-10 mV above rest (as depolarized as you can go without triggering action potentials). Record 15 sweeps of data, waiting about 10 seconds between each sweep.

Record the sweep numbers here: _____ to _____

PostLab

For your results

1. Superimpose all 15 responses recorded at the normal resting potential in one color. Add the average wave in a second color.
2. Superimpose the average synaptic responses at the different membrane potentials. Superimpose these average waves in a new graph, and artificially align their baselines so that you may compare their amplitudes relative to their baselines.

For your discussion

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1. What factor(s) contribute to the trial-to-trial variability of the EPSPs?
2. Are the amplitudes of the EPSP (relative to baseline) the same when membrane potential is manipulated? Explain in detail why or why not.
3. If there is an inhibitory component to the PSP, does it change when you change the membrane potential? Explain in detail why or why not
3. Explain what makes a neurotransmitter excitatory. Based on what you know about driving forces and reversal potentials, is it possible for activation of AMPA receptors to be inhibitory?

Experiment 2: Dynamics of synaptic transmission: Long Term Potentiation

Prelab

For the final experiment, you will induce long-term potentiation in Schaeffer collateral synapses. This requires an experimental protocol that is much more complicated than earlier experiments. Your attention to detail therefore will be critical for success of these experiments.

InLab

Experimental preparation before beginning your recordings.

First load the protocol files you will need. Navigate to the “LTP Files” folder within the “Protocols” folder. Load the macro file “LTP_iClampCA1.ipf” then load all of the stimulus waves in the folder (*.ibw files).

Patch a cell, select an effective synaptic stimulation intensity, and set the magnitude of current steps delivered via the patch pipette

This protocol requires initiating action potentials by delivering current steps via the patch pipette (as you did in Module 2), while at the same time eliciting EPSPs by stimulating the Schaeffer collaterals. Before beginning the experiment, you must set the appropriate amplitude for both stimuli.

1. Once you have patched a cell and have a stable recording, set capacitance compensation and bridge balance very carefully.
2. Set the stimulus magnitude in the Data Acquisition Panel to 0. This ensures that no current is delivered through your patch pipette. Using the same process as in Experiment 1, find the appropriate synaptic stimulus intensity to elicit an EPSP of 3-5 mV.
3. Uncheck the box next to “TTL0” in the ADC_DAC panel. This is IMPORTANT. If you leave this stimulus on, then you run the risk of inducing LTP before your experiment starts.
4. Select the “theta_5x_DAC” stimulus wave from the drop down menu for the analog current injection waves. Make sure that the This will deliver a rapid series of current

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steps to the patch pipette. We want to set the intensity of this stimulus so that each current step elicits an action potential.

5. Using the multiplier for the theta_5x_DAC wave, increase the current intensity until this stimulus elicits an action potential with each current step. It could require stimuli in excess of 1000 pA. *Make sure to note the stimulus magnitude required to elicit action potentials.* You will need this information later!

Collect your baseline data

Now collect several minutes of baseline recordings where you only elicit synaptic EPSPs while measuring the amplitude and rise slope of those EPSPs.

6. Set the multiplier for “theta_5x_DAC” to 0. IMPORTANT! This ensures that the stimulus train is not delivered to your patch pipette during baseline recordings.
7. Change the wave for TTL0 to “SynPulse” if it is not selected already.
8. Run the macro “Monitor_LTP_0.1Hz” This macro will elicit an EPSP every 10 seconds and display a set of running graphs that record resting potential, EPSP amplitude, and EPSP rise slope.
9. Let this macro run until you see that the amplitude and slope of the EPSPs is stable.

Run the LTP induction protocol

10. Stop the “Monitor LTP” macro by selecting “Abort Procedure Execution” in the macros menu.
11. In the ADC_DAC control panel, set the multiplier for “theta_5x_DAC” to the value you determined in #5 above.
12. Change the stimulus wave for TTL0 to “theta_5x_ttl”
13. Run the macro “Stim_LTP_Protocol” which will deliver four stimulation bursts.

Collect your post-induction data

14. Set the multiplier for “theta_5x_DAC” to 0. IMPORTANT! This ensures that the stimulus train is not delivered to your patch pipette during this part of the experiment.
15. Change the wave for TTL0 to “SynPulse” if it is not selected already.
16. Run the macro “Monitor_LTP_0.1Hz” just as you did during baseline conditions.
17. Let this macro run until you see that the amplitude and slope of the EPSPs is stable.
18. If you do not observe an increase in EPSP amplitude and rise slope, the induction was unsuccessful. Try repeating steps 1-18 once or twice more. If you still do not see evidence for LTP induction, you can try another induction protocol or try delivering more than one bout of LTP induction at a time. Consult with your instructor or TA for ideas and guidance.
19. Once you have successfully induced LTP, continue collecting post-induction data as long as possible until the quality of the recording declines.

PostLab

For your results

1. Create an average wave for 10 EPSPs from your baseline recordings and create a second average wave for 10 EPSPs after induction of LTP. Superimpose these average EPSPs on one graph.
2. Plot EPSP amplitude over time with indications of where the Induction protocols were delivered. You will use data waves generated by the experimental macros to produce this graph. To do this, create a new graph and select both “*epsp_ampl_soma*” and “*buzzmark*” as the Y waves. Select “*timer*” as the X-wave. Modify the graph so that *epsp* amplitude and *buzzmark* are plotted with different symbols, with no connecting lines.
3. Create a new plot of “*epsp_slope_soma*” as you did in step #2.

For your discussion

1. What changes do you observe in the amplitude and shape of the EPSPs after LTP induction?
2. If you observed any changes in the shape of the EPSP waveform, what does that tell you about the mechanisms underlying increases in EPSP amplitude?
3. Describe the timecourse of changes in EPSP amplitude and slope after induction of LTP. Is the timing of these changes indicative of underlying LTP mechanisms?.
4. What conditions were sufficient to induce LTP in your experiment?

Optional Experiment: Dynamics of synaptic transmission: short-term plasticity

PreLab

After performing experiment 1, you will have observed that synapses do not provide perfectly reproducible postsynaptic voltage changes. Here, you will discover further that synapses are dynamic structures whose output is highly sensitive to the recent history, or context, in which they are activated. You will deliver paired synaptic stimuli to observe the dynamic nature of synaptic transmission under controlled and reproducible conditions. You will then try to understand your observations in the context of what is known about the cellular mechanisms of synaptic transmission.

InLab

1. Deliver pairs of synaptic stimuli using the “Paired Evoked EPSPs” macro (you will need to load the Paired Pulse macro file to do this. This program will prompt you for the interval between stimuli (in ms). For this experiment, adjust the stimulation amplitude to evoke EPSPs of about 3-5 mV on average (the amplitude will vary considerably from trial to trial). Deliver pairs of pulses at four different interstimulus intervals (20, 50, 100, and 200 ms). Get an average of 15 sweeps at each interval with 10 seconds between

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each sweep, being sure to wait 10 seconds or so between each sweep. You can use the "GetAverage" macro to run and average multiple sweeps once you have run the Paired Evoked EPSP macro once to set the proper interval)

Take appropriate notes:

Interval (ms)	Sweeps
20	--
50	--
100	--
200	--

Perform this experiment on more than one cell if possible

PostLab

For your results

1. Superimpose the unaveraged, evoked single responses onto one another in one graph.
2. Average your paired pulse responses for each interstimulus interval. Superimpose these average traces over one another in a graph. Discuss with your instructors how you might do this...there are several equally effective ways.
3. Construct a bar graph of the plasticity index for these intervals (see below).

Graphing notes: calculating a plasticity index.

Creation of a plasticity index will provide you with a quick and easy measure of the direction and magnitude of short-term synaptic plasticity. The formula:

$$P.I. = [(EPSP2 - EPSP1) / EPSP1] * 100$$

Thus a plasticity index of 0 indicates no change, positive and negative values indicate the percentage change in paired-pulse potentiation and depression, respectively.

For your discussion

1. Discuss the results you found in terms of known mechanisms governing synaptic release from presynaptic terminals.
2. Does the direction and magnitude of short-term plasticity you recorded suggest a high or low probability of vesicle release?
3. You have already seen that single EPSPs are highly variable in amplitude. In paired pulse experiments, is there any relationship between the initial amplitude of the first EPSP and the magnitude of short-term plasticity? Would you expect there to be one?