Chapter 4 – Glutamatergic transmission

In this chapter, we offer you the following 6 videos:
- Observations and Glutamate Receptor Channels
- AMPA-Type Glutamate Unit Current
- Total AMPA Current
- NMDA-Type Glutamate Unit Current
- Total NMDA Current
- Conclusion

CHAPTER 4 INTRO (1:47)
In this chapter, you will learn how we get from a chemical signal in the form of glutamate to an electrical signal in the form of post-synaptic depolarization. More specifically, how the binding of glutamate at its receptor sites in the post-synaptic membrane creates a post-synaptic depolarizing ion current. This is referred as an excitatory transmission because it results in post-synaptic membrane depolarization. What are the objectives of this chapter? In this chapter, you will learn about the operation of two types of glutamate receptors which we refer to as AMPA-type and NMDA-type. You will understand how a current is created across a channel receptor. You will learn how unit currents add up to a total glutamate current and how this total glutamate current changes the membrane potential. And you will understand the difference between an AMPA and an NMDA-type glutamate current. Can you illustrate with an example from everyday life how a chemical molecule like glutamate is able to create a post-synaptic current? The binding of two glutamate molecules to their receptors and the creation of an ion current is a little like translating a text from Chinese into French. The Chinese source is the glutamate, the translator is the glutamate channel receptor, and the French target is the post-synaptic ion current. What do you propose as tools for this chapter? In addition to the course videos, quizzes, practical exercises, there are videos of lab experiments and an analysis of results.

CH. 4-1: OBSERVATIONS AND GLUTAMATE RECEPTOR CHANNELS (6:21)
In this chapter, we approach the second part of synaptic transmission, that is to say, the things that do on in the post-synaptic membrane of a post-synaptic neuron when a neurotransmitter binds to its post-synaptic receptors. In this chapter, we are going to study the glutamate neurotransmitter and the effects of glutamate on its receptors that are the glutamate channel receptors. To understand the operation of a glutamatergic synapse, we could stage the following experiment. We record connected neurons. The neuron here is called afferent to the neuron here which is called post-synaptic. Thus, this here is pre-synaptic and the other is post-synaptic. We record the activity of these neurons in whole-cell configuration and in current-patch mode to record changes in potential. This is a fairly complex experiment because, in fact, neurons that are connected to each other must be identified which is not obvious. But when we succeed, we are able to record a post-synaptic neuron response to the stimulation of a pre-synaptic neuron. What we do is send a current pulse across the pre-synaptic electrode with sufficient amplitude to generate a pre-synaptic action potential. This action potential created here, at the initial segment, propagates the entire length of the axon.
arriving at the axonal terminal and releasing glutamate. In this case, we record a post-synaptic response in the form of depolarization. We see that it is a low-amplitude depolarization if one were to compare the scale here to the scale of an action potential and that this depolarization is transient. We refer to it as an excitatory post-synaptic potential, or EPSP, because it is a depolarization; therefore, it has a tendency to mount to the sodium channel excitation threshold in this neuron here. That is why it is called excitatory. And this excitatory post-synaptic potential is short-lived. And we have come to understand that this EPSP is composite. We see it here. This is the one we recorded previously which is made of dots and which we refer to as control. It is composite because there are many known glutamate receptor blockers and we realized that if we added a blocker that we refer to here as APV we saw a change in the way EPSP declined over time. If we add another antagonist after APV here... Well, you have seen the previous trace, and we add a micromolar of a second blocker, NBQX, there is no longer anything at all. And in some cases, there is a small response that still persists. The interpretation is as follows. Here in the control, we have a response that is composite. This response is composed of the response of the receptors here, kainate, the AMPA receptors here, and NMDA, if we wanted to subtract. Thus, the glutamate EPSP is composed of EPSPs of several types because it is made up of currents of several types, and we are going to investigate those one by one. In conclusion, in this instance, there are two glutamate currents underlying this control EPSP. And in this case, there are three glutamate currents. So, the two glutamate currents in this case are called NMDA and AMPA. And in this case, they are called NMDA, AMPA, and kainate. We are going to investigate the NMDA and the AMPA currents but not kainate. If there are glutamate EPSPs of several types, this means there are several types of glutamate receptors. In fact, there are ionotrophic receptors, which we refer to as channel receptors, and metabotropic receptors. Ionotrophic receptors include AMPA receptors, NMDA receptors, and kainate receptors. These three ionotrophic receptors are located in the post-synaptic membrane. Sometimes, a post-synaptic membrane only has AMPA receptors. Sometimes, there are AMPA and NMDA receptors, other times all three. Here is a model of a subunit of a glutamate channel receptor. We see here that the protein is linear form. There are four transmembrane domains, an N-terminal and a C-terminal domain. Here is a subunit as it is believed to be when positioned in a membrane. There is an N-terminal domain followed by another N-terminal domain, but where does a glutamate agonist bind, for instance. Then, four transmembrane segments and a terminus, a C-terminus which is inside the membrane. It takes four subunits of this type to make a channel which we see here, and it takes two glutamate molecules to open a channel. So, if two glutamate molecules bind, the channel opens, and ions can cross. We will see which ions are transported depending on whether it is an AMPA or an NMDA channel. And we will see what makes the channel open. It is glutamate, no doubt, but on occasion it takes more than that. Each of the three types of glutamate channel receptors is made up of four subunits that have this general shape but also have unique features, of course, that make them AMPA, kainate, or NMDA. Therefore, these subunits are not identical even though that have the same general shape. They are referred to as GluA for AMPA, and there are 4 of them, 1 through 4, that have been described; GluK for kainate, and 1 through 5 have been described; and GluN for NMDA, and 1 through 2a, b, c, d have been described. These subunits do not mix which is to say that NMDA receptors are only made up of GluN subunits, AMPA receptors are only made up of GluA subunits, and kainate receptors are only made up of GluK subunits.
CH. 4-2: AMPA-TYPE GLUTAMATE UNIT CURRENT (4:19)
Here is the AMPA molecule which opens the AMPA channel receptors same as glutamate. Therefore, this is an AMPA receptor agonist. And when we want to investigate only the AMPA-type glutamate channel receptors, we use AMPA because otherwise, using glutamate, we would open all the glutamate channel receptors. To record AMPA-type glutamate unit current, we go to the outside-out configuration to capture very few membrane channels (so, this is about a very small piece of membrane) and to voltage-patch mode to record current. And we add AMPA to the extracellular environment to open the channels. If we keep the membrane at -60 mV here, we record very weak currents which are negative, therefore inward, currents. This are currents induced by the application of AMPA. If we change the holding potential to +60, we will now get outward currents whose amplitude increases as the membrane is depolarized. To find the reversal potential, we will stage the same experiment but with a lot more potential values to test here. When we change a membrane holding potential and record the current that results from the addition of AMPA, we get this i-V curve showing unit current as a function of voltage. We see that this plot reverses at 0 mV and that it is linear. It reverses at 0 mV, and that does not correspond to the reversal potential of any ion. Sodium reverses at +50, potassium at -90, etc. Therefore, we are dealing with a channel which is permeable to ions of several types. This is a cation channel which is to say that it is permeable to sodium, potassium, and sometimes calcium. If we do the math for the sodium and potassium ions inside and outside, we get a reversal potential of 0 mV. You can do this on your own. If the plot is linear, it means the current is ohmic; therefore, it is a current that is voltage-independent. To find out which types of ions the AMPA channel is permeable to, we could, for instance, change the extracellular sodium concentration by going from a control concentration of 140 mM to a concentration of 50 mM. And we see that we change the reversal potential. So, it shifts toward the more hyperpolarized potentials because there is less sodium outside. This means that sodium is involved in AMPA current. If we stage the same experiment with potassium by changing the potassium concentration inside the pipette which would then transfer to the inside of the neuron, if we reduced intracellular potassium, we would have shift towards more depolarized potentials. The AMPA channel is permeable to both sodium and potassium ions. We could, therefore, say this: we see that when two glutamate molecules bind, the channel opens, and sodium ions enter the neuron and potassium ions exit. This appears a little strange that there is a system like this that has currents traveling in different directions but that is not a problem as long as we always have to do with cations. What makes the channel open? We have seen that it was the binding of two glutamate molecules. So, here it is not the voltage which makes the channel open but glutamate binding. Therefore, we go from the closed to the open state thanks to the binding of two molecules which attach to two subunits, and you see here that a glutamate channel is a heterotetramer made up of four subunits that may be different.
CH. 4-3: TOTAL AMPA CURRENT (3:49)

If we want to record the total AMPA current now, the total post-synaptic current, we need to record a post-synaptic neuron in whole-cell configuration but in voltage-clamp mode to record the current. There are several ways and many types of post-synaptic currents that can be recorded. We can record a current by adding AMPA to the extracellular environment. This will be an AMPA current resulting from the application of AMPA. But we could also record an AMPA current resulting from the effect of afferent synapse activity on a post-synaptic neuron. This is more correct from the physiology standpoint because we record the activity of AMPA receptors to the release of glutamate from the terminals. This is a glutamate endogenous to the post-synaptic neurons. To do this, we add GABA synaptic blocker to stop this activity which blocks the GABA channels. We will see this in Chapter 5. We add a NMDA channel blocker not to have an NMDA current, and we add a kainate-specific channel blocker, if required, - I say "if required because some synapses do not have kainate channels. We register an inward current while at a holding potential of -60 millivolts. This inward current results from the entry of sodium and the exit of a small quantity of potassium. It is sodium that wins over the potassium; thus we have an inward current of + charges. This inward current has a rising phase, a peak, and a decay phase. The rising phase results from the fact that many AMPA channels open almost at once; therefore, it is the sum of all the unit currents that spring up almost, but not quite, at once because we see a certain small gradient in the rising phase. And the decay phase results from the fact that channels close one after another because glutamate unbinds from the receptors and subsequently removed from the synaptic gap. And at peak, here, we have the maximum number of AMPA channels open. This inward current depolarizes the membrane, and here we see, an EPSP. Why does it depolarize the membrane? In current-patch mode, we have a depolarization because the entry of + charges which is much stronger than the exit of potassium + charges, the entry of sodium + charges depolarizes the interior membrane with respect to the exterior one, and thus, there is a shift in potential toward a more depolarizing value. The AMPA receptor agonists, i.e. the molecules that will open the AMPA channel, the agonists are glutamate and AMPA; there is also quisqualate that we have not seen. The antagonist, i.e. a molecule that will position itself in the same place as the agonist but that does not open the channels, is NBQX here used at 1 micromolar. This AMPA receptor is voltage-independent, i.e. it does not have to depend on membrane potential to open. The AMPA channel is permeable to sodium and potassium ions, and we have seen that more sodium ions enter than potassium ions exit, thus, the net result, whenever the potential is at -60 mV, is an inward current; and its is sometimes permeable to calcium ions which we have not seen and which is explained in a supplement. This depends on whether Subunit GluA2 is present.
Let us now look at the NMDA channel receptors. So, these are glutamate channel receptors that are opened specifically by NMDA, which is N-methyl-D-aspartic acid. NMDA is a specific agonist for NMDA-type glutamate channel receptors. If we wish to record an NMDA unit current, we will select a configuration in which there are very few membrane channels that we can investigate, outside-out for example. And we add NMDA to the bath to open the channels. And it is only these channels here that will open. We have also realized in the course of these experiments that magnesium ions also blocked the NMDA channel. To investigate this simply, we take an extracellular environment devoid of magnesium ions. We hold the membrane at a potential of -60 mV, and while applying NMDA here, we record inward negative currents. If we change the membrane potential to +40 mV, we will get outward currents. This shows that the NMDA current reversal potential is to be found between -60 and +40 mV. To find this out precisely, we apply a holding potential selected from between these two values with a step of 10 or 20 mV. We record an I-V plot with no extracellular magnesium ions and find that it is linear and reverses at 0 mV. There again, we have a cation current and a channel that is voltage-independent with no extracellular magnesium ions. This has to be remembered well that this is only in the absence of extracellular magnesium ions. What do extracellular magnesium ions do? We study those using a single channel and a unit current. The holding potential is -60 mV. We are still in the outside-out configuration and we externally apply some NMDA. This channel opens in the absence of extracellular magnesium ions. Now, if we add 10 micromolars of magnesium ions to the extracellular solution which is very little because in general, there is about 1 mM of it there, we see the channel open, close, open, close... In fact, it fluctuates between the open and the blocked (bl) states. It opens, it is blocked. It unblocks, it re-blocks. It re-opens, it blocks after a very short while. Then, it will unblock, re-open, and block. Now, if we apply a holding potential of +40 mV in the presence of magnesium ions, there is no blockage even if we apply 1 mM of magnesium ions. Therefore, it is a blockage that is voltage-dependent. The NMDA receptor is closed. When two glutamate or NMDA molecules attach to the receptor sites, the channel opens and lets ions through. We now that, for the NMDA channel, these are cations. Sodium ions enter through the action of an electrochemical gradient, while potassium ions exit, and the channel is also permeable to calcium ions that enter by way of an electrochemical gradient. Therefore, we are dealing with a cation channel in broadest sense of the term: it lets through all cations. It even lets through magnesium ions and that is why it is blocked. Here, it is in its open state. Here, it is in its closed state. There is a balance between these two states resulting from glutamate or NMDA attachment (agonists). It is going to transition to a blocked state if magnesium ions enter the channel alongside others, and at that time, it is an ion that plugs up the channel here and prevents other ions from crossing. Thus, we are in a blocked state. This magnesium cork can pop from time to time which makes the channel unblock, and let through sodium, potassium, and calcium ions again. As we have seen previously, there is a fluctuation between these two states: open and blocked. Why do magnesium ions block while calcium ions get through? This is explained in the supplements. Magnesium ions enter the channel through the action of an electrochemical gradient. In fact, while the membrane is hyperpolarized, which means here that the interior is more negative than the exterior. We have a measurable potential, for instance, which is on the order of -60 mV. At that time, the + charged magnesium ions are attracted by the - charges here. Now, when the potential is reversed, i. e. is +40 mV, for
instance, and the + charges are here and here, we have more negative charges, this is relative, and the interior is more positively charged than the exterior at which time magnesium ions are no longer attracted to enter, the cork pops. And then, we can record the channel in this state all the time fluctuating between open and closed. We get rid of the blocked state either by adding 0 magnesium or by recording at depolarized potentials, such as + 40 mV was we will see in the experiment.

**CH. 4-5: TOTAL NMDA CURRENT (5:48)**

We just looked at the properties of unit NMDA current. What are the properties of the total NMDA current? To record the total current, we go to the whole-cell configuration and, as with the total AMPA current, there are two options: either we apply NMDA on the outside and register the current released by this exogenous NMDA, or we record spontaneous NMDA currents still in the whole-cell configuration. These are currents produced by the release of glutamate from pre-synaptic terminals which will open NMDA channels. In this case, to record NMDA current, we obviously block the other channels. And we record in whole-cell configuration channel activity throughout the entire membrane, including dendrites, in voltage-clamp mode to register current. This shows GABA synapses in blue and glutamate synapses in red. To record only the channels opened by glutamate, we block those opened by GABA using gabazine, and to record only NMDA-type channels, we block AMPA and kainate channels with some CNQX, this time around using a fairly high dosage of 10 micromolars. We also apply a depolarizing potential of + 40mV to get rid of the magnesium ion blockage. This is not at all a physiological potential but it helps us generate some good NMDA currents that are not blocked off by magnesium ions. We saw that a unit NMDA current is a cation current carried by sodium, potassium, and calcium ions. We saw that the NMDA channels was blocked by magnesium ions at a resting potential, i.e. at the membrane physiological potential. So, where is going on with the total current? Do we also this this calcium ion permeability? Presumably, yes, but how to prove it? And what happens to this magnesium ion blockage?

We record in whole-cell configuration and voltage-clamp mode again. Here, the total current is at -5 mV, i.e. when the membrane is held at -5 mV, we are close to the reversal potential which is 0 in this case. So, we see that we have a total inward current which results from multiple NMDA channels opening very quickly and re-closing little by little because of NMDA or glutamate detachment. Then it reverses, and we see that it is positive here. Now, to find out whether this current is also carried by calcium ions, we change the extracellular concentration of calcium ions by increasing it 20 times which should normally affect the reversal potential. And here, we see that the reversal potential has, in fact, shifted to +17 mV. Thus, there is a shift of about 17 mV in the reversal potential. This means that calcium ions are normally involved. Still with the physiological calcium of about 1 mM, we apply a whole range of holding potentials. We are going to build a current-voltage plot for the total current. We can build this plot in the absence of extracellular magnesium ions, and here, we have a plot that is practically ohmic; so, the channel is found to be voltage-independent. And then, with magnesium and at a concentration that is not yet the physiological one, we see that the current is very weak at hyperpolarized potentials. You see the large difference in currents for hyperpolarized potentials. For depolarized potentials, on the other hand, it is the same thing: we get the same current for the same increase in voltage. In fact, here, we know that magnesium ions are not blocking the channel for depolarized potentials, only for
hyperpolarized ones. So, under physiological conditions, you are going to ask me, when is there going to be an NMDA current? There is an NMDA current when the post-synaptic membrane is depolarized. What causes it to depolarize? It is depolarized by the AMPA current. This means that the glutamate released by the axonal terminals attached to both the AMPA and the NMDA receptors at the same time. But as long as the membrane is not depolarized by the AMPA current, the NMDA current is very weak. Then, when the membrane is good and depolarized by the AMPA current, NMDA receptors unblock, and at that time, an NMDA current springs up. NMDA receptors are opened by agonists, such as glutamate or NMDA. NMDA is a specific agonist that only opens NMDA channels. NMDA receptors are blocked by antagonists, such as APV which attach to the same receptor sites as glutamate, thereby preventing glutamate or NMDA from attaching. NMDA current is a cation current carried by sodium, potassium, and calcium ions at the same time. Calcium entry through NMDA channels is far from negligible. NMDA current is voltage-dependent because of magnesium ion blockage. It is not really the channel receptor molecule that is voltage-dependent as was the case for sodium or potassium channels. In this case, there is no special charge that the molecule carries that makes the channel voltage-dependent. This is voltage dependence consequential to the magnesium ion blockage. And since there are always magnesium ions under physiological conditions, at resting potentials or potentials that are close to resting, the NMDA channel is blocked by magnesium ions. Thus, there is no NMDA current unless the post-synaptic membrane becomes previously depolarized.

CH. 4-6: CONCLUSION (04:09)
Let us look at a glutamatergic synapse. We recognize an axonal terminal here which is a pre-synaptic element, the synaptic cleft, and here the post-synaptic element which is a piece of dendrite; so, let this here be a dendritic spine or a piece of dendritic trunk or somatic membrane. Here is a piece of a glial cell membrane. In the pre-synaptic element, we recognize some calcium channels, vesicles containing glutamate. And in the post-synaptic element, we recognize the presence of glutamate receptors. We understand that synaptic transmission is unidirectional, always from a pre-synaptic to a post-synaptic element because receptors are on one side only and vesicles also on one side only. Whenever action potentials descend to an axonal terminal, they depolarize the membrane to a potential that causes calcium channels to open and calcium ions to enter resulting in the merging of the vesicular and the pre-synaptic membranes and the release of a neurotransmitter. The neurotransmitter, once in the synaptic cleft, attaches to all the receptor sites accessible to it. Among these receptor sites, there are some that are located on channel, or AMPA, receptors, kainate receptors which we have not looked at but which are very close to AMPA receptors, and NMDA receptors. These NMDA receptors, as we see here, are normally blocked by magnesium ions, and we have seen that it takes an AMPA current depolarizing the membrane for them to become unblocked. Other receptors sites are located on glutamate transporters which help evacuate the glutamate from the synaptic gap. When there is glutamate in the synaptic gap either because it has just been released or because it has detached from post-synaptic receptors, it is recaptured by axonal terminals using transporters here or by glial cells. This helps remove the glutamate from the synaptic cleft and make synaptic transmission short. In summary, there a three types of glutamate receptors: AMPA, kainate, NMDA named for the agonists that cause them to open selectively. Either all three, or two, or only one of these receptors is present in the post-
synaptic membrane. In general, AMPA only, or AMPA/NMDA, or AMPA/kainate, or AMPA/kainate/NMDA. These receptors are made up of four subunits. AMPA receptors are made of four subunits referred to as GluA. Kainate receptors are made up of four subunits called GluK, and NMDA receptors are made of four subunits referred to as GluN. There are two major types of AMPA receptors: those containing the GluA2 subunits and those that do not have it. NMDA receptors open by the combined action of attached glutamate or NMDA and glycine. We have not mentioned it at all until now. This is handled in the supplements. But glycine is a co-agonist, and there is a special receptor site for glycine on one of the NMDA receptor subunits. This channel is permeable to sodium, potassium, and calcium ions. This calcium permeability is very important letting calcium ions enter and subsequently perform a physiological function. And these are voltage-gated channels since they are blocked by magnesium ions at hyperpolarized potentials, and NMDA current is peculiar insofar as it has a slow dynamic. Now, in the following course, we are going to look at GABAergic synaptic transmission where GABA is the neurotransmitter, and the post-synaptic receptors are GABA-A receptors.