Chapter 5 subtitles – GABAergic synaptic transmission

INTRODUCTION (2:57)
In this fifth chapter, you will learn how the binding of the GABA neurotransmitter to GABA-A receptor-channels will produce a chloride current. What are the objectives of this chapter? At the end of the chapter, you will know how to record a GABAergic current through a GABA-A receptor-channel, which is a ligand-gated channel, and you will be able to identify the states of the channel: closed, open. You will know how to draw a GABAergic current-voltage curve and to draw information from it, and you will be able to predict how the GABAergic current is going to change membrane potential. This chapter is somewhat a little more complicated than the one on glutamate because GABA sometimes produces a hyperpolarization of the membrane (most often a hyperpolarization) but sometimes it produces a depolarization, and sometimes it produces no change of membrane potential. Can you explain in simple terms how GABA can affect the postsynaptic neuron membrane potential in all these different ways? It is completely different from what we have seen in the previous chapter on glutamate, where the different effects were produced by different receptor-channels. Here, there is only one type of receptor-channel, the GABA-A type. It is the difference of concentration of chloride ions on either side of the membrane which makes the difference. If we wanted to use an analogy, it would be like a balance with two weighing pans: depending on the relative charges on the pans, the balance leans on the right or on the left. We could say that the two pans would be the intracellular and the extracellular media, the charges would be the concentration of chloride ions in these media, and the leaning on the right would be a depolarization and the leaning on the left would be a hyperpolarization. When there is no leaning, the balance does not move, it would be no change of potential, what we call a silent inhibition. What learning tools are provided in this chapter? In addition to the course videos and quizzes, there will be videos of lab experiments with the analysis of the results.

CH. 5-1: GABA-A CURRENTS AND POTENTIAL CHANGES (3:20)
Today, we study the GABAergic synaptic transmission. This is another example of synaptic transmission, which uses GABA as a neurotransmitter. This transmission is a little difficult to grasp because it involves chloride ions, that are negatively-charged ions. Remember that all the currents are defined for positively-charged ions, so you have to reverse the logic a little. We will try to make this easy. Let’s record the response of a postsynaptic neuron to the activity of a GABAergic presynaptic neuron. So it’s a neuron here in blue which releases GABA as a neurotransmitter. We record both neurons in whole-cell configuration and in current-clamp mode. We use physiological concentrations of chloride ions: the intracellular concentration is around ten times lower than the extracellular one, which gives an equilibrium potential for chloride ions of -59 mV. When, in the presynaptic neuron through this electrode, we apply a depolarizing current step, to evoke an action potential, this action potential propagates along the axon down to the axon terminals and triggers the release of GABA. The response to GABA is the following. It’s a transient hyperpolarization of the
membrane called IPSP for "inhibitory postsynaptic potential". This inhibitory postsynaptic potential is of low amplitude. You have here the scale, which is very different from the scale of action potentials: it's very very small. So the stimulation of the presynaptic neuron causes a transient hyperpolarization of the postsynaptic membrane. Now, if we add in the bath a benzodiazepine such as diazepam. A benzodiazepine is a psychoactive drug used for instance in the treatment of anxiety. We record a much larger IPSP. This is exactly the role of a benzodiazepine: it's to increase the effect of GABA. To be sure that we record here an IPSP due to the release of GABA, we add in the extracellular bath a blocker of GABAergic transmission, a blocker of GABA receptor-channels, gabazine, and we see that the response totally disappears. What is the current underlying this IPSP? What is the channel involved? What is the mechanism of action of benzodiazepines?

CH. 5-2 : UNITARY GABA-A RECEPTOR MEDIATED CURRENTS (7:02)
There are two types of GABA receptors: ionotropic and metabotropic. Ionotropic receptors are receptor-channels but metabotropic receptors are not receptor-channels. We are going to study the GABA-A receptors, which are ionotropic receptor-channels. The GABA-A receptor-channel is made up of five subunits. Here on the right, you have the general organization of a GABA-A subunit with a large NH2 terminal domain, 1, 2, 3, 4 transmembrane segments, and a C-terminal domain, which is also extracellular. These subunits are called alpha, beta or gamma. There are also other subunits we are not going to study here. On the left here, you have two examples of GABA-A receptor-channel: one with 2 alpha subunits and 3 beta subunits, and one with 2 alphas, 2 betas and 1 gamma. The receptor site for GABA is located at the interface between one alpha and one beta subunit. We see here that we have two receptor sites in a GABA-A receptor-channel, and in this one too: it's always like that, there are two receptor sites for GABA. Two molecules of GABA bind to the GABA-A receptor-channel for this channel to open. So we can write it like that: two molecules of GABA (and here is the GABA-A receptor in the closed state) must bind to the receptor, which finally opens. This is the open state with the star. Then it closes back when GABA unbinds from its receptor sites. Which type of ions cross the GABA-A channel? To address this question, we record the unitary GABA-A current. We record in cell-attached configuration, to recall a single channel activity, and we record in voltage-clamp mode, to record a current. In this configuration, cell-attached, the pipette solution corresponds to the extracellular medium because it's extracellular to the recorded channel. So in this pipette, we add the extracellular concentration of sodium, of potassium, of chloride (here 146 mM), and we add also GABA to open the GABA channels. We hold the membrane potential at -60 mV and we record no current. If we hold the membrane potential at -30 mV, we record an outward current. At 0 mV, we record an outward current but larger, and at +20 mV, we still record an outward current and larger again. I want to remind you that an outward current corresponds to an exit of positive charges or an entry of negative charges. To understand the type of ions that carry the GABA-A current, we are going to do as usual, the i-V plot. Why? Because when we identify the reversal potential of the GABA-A current, it will give ideas on which type of ions are involved. The corresponding i-V curve, which is here the unitary current of GABA vs membrane potential. We see that the current is outward. This is what we have here, and larger when we depolarize the membrane. Now we have the impression that the current reverses at -60 mV. Here the possible reversal potential of the GABA-A current is
-60 mV. This suggests that chloride ions are involved. If this hypothesis is right, do chloride ions go in or out in the situations that we have studied here? Let’s have a look. Chloride ions, due to the concentration gradient, have a tendency to enter the neuron. At, for example here, +20 mV, when the membrane is more positive on the inner side than on the outside, of course chloride ions have also a tendency to enter due to the electrical gradient: negatively-charged ions are attracted by positive charges of the inner side of the membrane. So the net flux is an entry of chloride ions and an entry of chloride ions is an outward current of + charges. It’s exactly what we record, so it seems that our hypothesis that chloride ions are involved and maybe the only ions involved, is possible. To address this question and to get a definitive answer, we are going to change the chloride concentrations on either side of the membrane. This here is the previous situation, with physiological concentrations of chloride ions, and on the right, now, it’s what we call "equivalent chloride": we have the same concentration of chloride inside and outside, so there is no concentration gradient, there is only an electrical gradient that forces the chloride ions to move. The equilibrium potential for chloride ions is 0 mV. What we see here, if we redo the i-V curve, I remind you that this is the unitary GABA-A current, with a lowercase i, versus membrane potential (holding potential), we see that it reverses around 0, which fits with the hypothesis of chloride ions. It’s inward for hyperpolarized potentials and outward for depolarized potentials. Why? Because now the chloride ions follow the electrical gradient only and when the membrane is more positive on the inner side, the chloride ions will have a tendency to enter, so it corresponds to an outward current of + charges. We have an outward, positive current, and the contrary, of course, for hyperpolarized membrane potentials.

CH. 5-3 : TOTAL GABA-A CURRENT (2:14)
To record the total GABA-A current, we choose here to record spontaneous synaptic GAAB-A currents. These currents are due to the spontaneous activity of presynaptic GABAergic neurons. Let’s understand why we record inward spontaneous currents. We have the same concentration of chloride ions on either side of the membrane. There is no concentration gradient, so the chloride ions are going to move only because of the electrical gradient. At -70 mV, the membrane is more negatively charged on the inside than on the outside, so chloride ions are attracted by the + charges outside and there is an exit of chloride ions. An exit of negatively-charged ions is the same as an entry of + charges, and an entry of + charges is an inward current of + charges. That’s why the chloride current here is inward. Here are some numerical data: the duration of GABA-A current is around 0.5-5 ms, it corresponds to the summation of the unitary GABA-A currents and, because of the GABA release and binding to GABA-A receptors, the receptor-channels open and there is a chloride current. The decay phase is longer: it’s around 1 to 10 milliseconds, it’s due to the successive closure of GABA-A receptor-channels, because GABA unbinds and is re-uptaken by transporters, so it has a low probability to bind again to GABA-A receptors.

CH. 5-4 : GABA-A RECEPTOR-CHANNEL AND BENZODIAZEPINES (2:42)
Now let’s go back to the benzodiazepines that we saw at the beginning. How do they work? Do they bind directly on the GABA-A receptors? Do they induce a similar chloride current? Many questions about the effect of benzodiazepines... To test how they work, we are going to record the unitary GABA-A current in the presence of GABA only. We are here in outside-
out configuration, to record the activity of a single channel, in voltage-clamp mode, and we are at -75 mV, with equivalent chloride concentrations on either side of the membrane. When we add GABA only, we have the openings of the GABA-A channel here at -75 with many many closures. This is a specificity of the channel. If we add, on top of GABA, diazepam, which is a benzodiazepine, we see immediately that the channel opens more frequently. If we increase the probability of opening of the channel, though the unitary current has the same amplitude (here they have the same amplitude), the opening frequency is higher, then the total current is larger. That’s why benzodiazepines increase the GABA-mediated current. Now again, if the opening frequency of GABA-A channels increases, then the intensity of the total GABA-A current increases. Where do benzodiazepines bind? They bind on the receptor-channel, at the interface between gamma and alpha subunits. This means that only channels that have the gamma subunit can bind benzodiazepine, so not all GABA-A receptors are sensitive to benzodiazepines. They need to have a gamma subunit in their structure.

CH. 5-5 : GABA-A RECEPTOR MEDIATED POSTSYNAPTIC POTENTIAL (5:04)
How does a chloride current change the membrane potential? We address this question of course in physiological conditions. It means that we have a physiological concentration of chloride ions inside the pipette, so that we have a physiological concentration of chloride ions inside the neuron, here, the postsynaptic neuron. We record the changes of membrane potential, so we are in current-clamp mode. We record the changes of membrane potential in response to GABA release. We also block the glutamatergic synapses and we record only the activity of these synapses, but in current-clamp mode. What we record is a small hyperpolarization of the membrane as we saw in the first slides. This slight hyperpolarization of the membrane is due to the chloride current. Why? Because we are here with an equilibrium potential of chloride ions of -59 mV, nearly -60 mV. Due to the concentration gradient, chloride ions have a tendency to enter the neuron, but due to the electrical gradient, chloride ions have a tendency to exit the neuron, because they are attracted by the + charges on the outer side of the membrane. The two forces are equivalent at ECl, so at -60 mV, there is no net flux of chloride ions, no current, no change of potential. Here we are at -50 mV, so what is going to happen? Chloride ions are going to enter and to hyperpolarize the membrane until the membrane gets to -60 mV. This is why sometimes there is a very small hyperpolarization, sometimes nothing, sometimes it’s a small depolarization. This response here, is not always the same, it depends of where the equilibrium potential of chloride ions is, and it’s not the same in all neurons of the brain. For example, we have neurons where the equilibrium potential is at -70 mV, other neurons where it is at -60 mV, and other neurons where it is at -50 mV. So on the left, here, when the equilibrium potential is at -70 mV, at -70 mV, of course, there is no current, because we are at the equilibrium potential of chloride ions. Now at -60 mV, we have a small hyperpolarization when GABA-A channels open, because the membrane has a tendency to go to the equilibrium potential of chloride ions. And the hyperpolarization is larger and larger as we depolarize the membrane. In the middle, when the membrane is as -60 mV, there is no current. When the membrane is at -70 mV, then the current is reversed because the membrane has a tendency to go to -60 mV, and you have a small depolarization. At -50, it’s the contrary: we have a small hyperpolarization because the membrane has a tendency to go to the equilibrium potential,
to ECl, which is -60, and also a hyperpolarization which is larger at -40 mV. Now, when the reversal potential is around -50 mV, GABA has a depolarizing action. At hyperpolarized potentials, more hyperpolarized than -50 at -50, it has no effect, and at -40 mV, it has a hyperpolarizing effect. So if we look for example at a membrane potential of, let's say, -60 mV. In some neurons, GABA will be hyperpolarizing. In some neurons, it will have no effect, no visible effect, because we will see that it has an effect but we will see it later. And sometimes, it has a depolarizing effect. So always remember that, when you want to know what is the effect of GABA, you have to know where the reversal potential of chloride ions is, and at which potential you are recording.

CH. 5-6 : CONCLUSION (4:33)
In summary, the GABA-A receptor-channel is made up of five subunits, in general 2 alphas, 2 betas and one gamma, but there could be a lot of other combinations. Here, you have 2 alphas and 3 betas. The pore of the channel is permeable to chloride ions. It's an anionic channel. For the pore to open, it needs the binding of two molecules of GABA, and the GABA molecules bind at the interface between alpha and beta, and alpha and beta (alpha and beta, alpha and beta). There is also one receptor site for benzodiazepine, which is called an allosteric agonist of GABA-A receptor, because it increases the GABA-A current, and this binding is at the interface between alpha and gamma. So here in this receptor on the left, there is no receptor site for benzodiazepine. In general, when the channel opens, the chloride current hyperpolarizes the membrane, especially in adult brain neurons. Sometimes, this hyperpolarization is very small or even non-existent, and we speak of "silent inhibition". We will see in the next chapter the effect of such silent inhibition. But sometimes, the chloride current can also depolarize the membrane. This is a small depolarization. It depends on where the equilibrium potential of chloride ions is compared to the membrane potential. In developing immature neurons, there is usually a higher concentration of chloride inside in the cytoplasm and the equilibrium for chloride ions is very different from adult neurons, and we get more depolarizing currents. So remember that the effect of chloride current depends on the respective values of the equilibrium potential of chloride ions and of the membrane potential at which the current is recorded. The GABAergic synapse: you have here the presynaptic element, an axon terminal, the synaptic cleft, the postsynaptic element, which can be a dendritic spine or a patch of dendritic membrane, or somatic membrane also, and here a glial membrane. When an action potential arrives at the terminal, it opens the calcium channels. Calcium ions enter and there is a local and transient increase of calcium concentration. Because of calcium-sensitive proteins, it induces the fusion of the vesicles with the presynaptic membrane and the release of GABA in the synaptic cleft. GABA, once in the synaptic cleft, is going to bind to all its receptor sites. There are receptor sites on the GABA-A receptor and it opens the GABA-A receptor and chloride ions usually go in. It binds to transporters here in the glial membrane and transporters in the presynaptic element that help to re-uptake GABA and to clear it from the synaptic cleft. So when GABA unbinds from GABA-A receptors, it has a low probability to bind again, because it has disappeared from the synaptic cleft. GABA binds to the GABA-A receptors, the channels open. GABA binds to transporters, GABA is transported inside glial cells or back inside in the presynaptic element. And then GABA spontaneously unbinds from its receptor sites and GABA-A channels close.