Chapter 3 subtitles – Action potentials

Introduction (3:15) This third chapter explains the calcium current triggered by the arrival of the action potential in the axon terminal. This calcium current enables calcium ions to enter the presynaptic terminal and to trigger the fusion of the vesicles with the membrane and the release of the neurotransmitter. What are the objectives of this chapter? At the end of the chapter, you will know how to record a calcium current through a single N-type voltage-gated calcium channel. You will be able to identify the various states of the calcium channel: closed, open, and inactivated. You will know how to build calcium current-voltage curves and how to draw information from them. You will understand how intracellular calcium ions trigger neurotransmitter release. Can you explain in simple terms this first phase of synaptic transmission? Information is transmitted between neurons in a chemical form. You will understand how axonal terminals translate the arrival of an action potential, which is an electric signal, into neurotransmitter release, a chemical signal. The action potential is like the electric control of a factory door which, once open, lets employees out... except that this electric control does not work very well, and sometimes, employees cannot go through. This means that even when there is a presynaptic action potential, neurotransmitter is not always released. What learning tools are provided in this chapter? In addition to the course videos, supplements, and quizzes, there is a video of an experiment staged at INMED.

Ch. 3-1 : Observation (7:38)

In this third chapter, we will study how the action potential arriving at the axon terminals evokes neurotransmitter release. Let's begin by observing synaptic transmission. Let's suppose we have two connected neurons: here the presynaptic neuron is in orange and the postsynaptic one in blue. The orange one is called presynaptic because it's located before the synapse, and the blue one is located after the synapse so it's called postsynaptic. We record both neurons in whole-cell configuration, to record the activity of the whole membrane, and in current-clamp mode, to record potential changes. If we stimulate here the orange neuron, we record, in response to the stimulation, an action potential. In response to this action potential, in the postsynaptic neuron, we record a small depolarization that, because it's a depolarization, we call "excitatory postsynaptic potential". Now, if we apply in the bath TT X, tetrodotoxin (this is a blocker of sodium channels), of course you don't get any action potential in response to the stimulation, because sodium channels are blocked and you don't record a postsynaptic response either. Now if we use a bath devoid of calcium ions (zero calcium), we still record the action potential in the presynaptic neuron because the action potential is due to sodium and potassium ions but we don't get a response here in the postsynaptic neuron. Why? Do calcium ions play a special part in synaptic transmission? If so, what do they act upon? To understand the role of calcium ions in synaptic transmission, we work with the squid giant synapse, which is here. In this giant synapse, we see here the presynaptic terminal, the axonal presynaptic terminal in blue-green, and underneath is the postsynaptic element, in white. The synapse is beneath the blue-green, in between the two. It's a view from above. We can load the presynaptic element with a calcium dye called aequorin. Aequorin binds calcium ions and in its bound form, becomes aequorin-calcium. This molecule, aequorin, fluoresces differently depending on whether it is bound or free. So using aequorin fluorescence, we can

find out whether calcium ions have entered the presynaptic terminal. The result is as follows. In "a," this is the basal fluorescence of the presynaptic element, that's why we put it in green, to show that it's the presynaptic element only. When we stimulate (here it's the stimulation), when we stimulate the presynaptic terminal, we trigger action potentials. In response to this action potential, we have image "b". To understand exactly what happened between the two, we subtract image "a" from image "b" and we see here what happens during action potential initiation. We see that calcium entered the presynaptic terminal because we have here the fluorescence that tells us that the bound form increased. It entered the presynaptic terminals in small micro domains. This is because it's a giant synapse, so you have many synaptic complexes. In this synapse, which is very different from the central synpases of the brain, where you have only one synaptic complex in general. It's a peculiarity of the giant synapses. This is the princeps experiment that allowed to characterize the role of calcium ions in synaptic transmission and to characterize that calcium enter the presynaptic terminal for the synaptic transmission to happen. The hypothesis that we have now is the following: the action potential triggers the opening of calcium channels in the axon terminals. But what type of calcium channels do we have in axon terminals and what type of calcium currents? We should patch clamp and record the activity of axon terminals. This is very difficult in the brain, for example with brain neurons, because the axon terminals are very small, about one micrometer in diameter. So we can use giant synapses, as we saw with the squid giant synapse, the calyx of Held, which is in the auditory system of the brain, or the neuromuscular synapse, which is between motor neurons and muscles. We can also (this is a third possibility) record calcium channels in the soma, because the somatic membrane contains the same calcium channels as the presynaptic terminals. It is is usually what we do in the brain. So we know that there are many (at least four) types of calcium channels that were called L, N, P/Q and T. Now they are named CaV, for voltage-gated calcium channels, and then they have a number after, which can be 1, 2 etc. To understand which channels are present in the presynaptic terminals, we label these channels with antibodies. What we see is that most of the presynaptic terminals contain N-type channels, sometimes also another type which is called P/Q, that we are not going to study. We are going to study the N-type, which is the more common form of calcium channels present in the presynaptic terminals. So we now have all these new hypotheses: the transient increase in in the intracellular concentration of calcium is due to an inward voltage-sensitive calcium current. "Voltage-sensitive" because we saw that we have to stimulate the presynaptic terminal to get this increase. This increase is due to calcium entry through N-type calcium channels that are called "high threshold", because they open for a strong depolarization of the membrane. So let's test this hypothesis.

Ch. 3-2 : Calcium channel and currents (7:28)

Here is the calcium channel Cav2.2, also called N-type. It looks like the sodium channel, because you can recognize here the four similar domains in Roman letters and the six transmembrane segments (1, 2, 3, 4, 5, 6), the P-loop (the pore loop), the S4 (segment 4), which is made up of a regular sequence of positively-charged amino acids which confers the voltage sensitivity to the protein. This protein folds, then when it's folded, you have in the middle a pore, an aqueous pore, where calcium ions can go through. The P-loop of each domain, you find it inside, at the entry of the pore, and it contains glutamate residues that are very important for the selectivity to calcium ions. To study the unitary calcium current, we

choose the cell- attached configuration, to have very few channels under the pipette, and we choose the voltage-clamp mode to study a current. Since we are in cell-attached mode, we put extracellular fluid in the pipette. Here we add 110 mM barium ions in this extracellular fluid instead of calcium ions. Why do we add barium ions to record unitary currents? Because calcium channels are very permeable to barium ions and this produces a unitary current of greater amplitude than that produced by calcium ions. We apply blockers of other calcium channels to record only N-type calcium currents. Since we are in voltage-clamp, we hold the membrane at -80 mV. At this potential, there are no calcium channels open; you see here: no current. We apply a depolarizing step to +20 mV during 200 ms. During this depolarizing step, we record unitary inward currents, sometimes no current, sometimes a lot of currents, and sometimes again here no current, lots of currents, and no current. I remind you that these are inward currents because they are negative. It's a weak unitary current, in the order of 0.5 pA. You see here that the channel opens, closes, opens, closes etc. To better understand the behavior of the channel, we are going to plot the i-V curve, which is the amplitude of the unitary current here as a function of membrane potential. At +20 mV, here, we saw that the amplitude is around 0.5 pA.If we now apply test potentials that are less depolarized than +20 mV, here 10, 0... And then they are negative: -10, -20, etc. The current amplitude increases. Why? Because we go further and further from the equilibrium potential of calcium ions. The further we are, the larger amplitude is the unitary current. Then we don't record anymore currents, because channels at these potentials are closed. What are the characteristics of the total N-type calcium current to study the total current, we record in whole-cell configuration, to record the activity of many calcium channels, and in voltage-clamp mode, because we want to record a current. In this experiment, we use calcium as the charge carrier, here 2 mM of calcium in the extracellular medium, and we add blockers of voltage-sensitive sodium and potassium channels and blockers of other calcium channels, because all these channels could respond to a depolarizing step. If we want to record the N-type calcium channel alone, we have to block all the others. Here is the depolarizing step that we apply to +20 mV. After an artifact, we see an inward current, a rising phase, a peak, a decay phase, and then the artifact again when we stop the depolarizing step. Note that the amplitude of the current decays though we are still at +20 mV: this indicates that the N-type calcium channels inactivate. The rising phase corresponds to the gradual opening of calcium channels: they open one after the other, but fairly quickly. We could see here little steps, and when they sum up, this gives the smooth curve. The peak corresponds to the amplitude of the current when all the calcium channels of the membrane that should open have opened. The decay phase correspond to the inactivation of the calcium channels. To better understand, let's build the I-V curve. We apply depolarizing steps of increasing amplitude and, on the top, we'll record the corresponding currents. The little arrow here indicates the peak and the value of the peak is plotted here against membrane potential. The unitary current-voltage curve was linear, this one is not: it's U-shaped. Why is it U-shaped? It reminds you of course of the sodium current, of the total sodium current. It's U-shaped because when the membrane is more hyperpolarized than -50 mV, the channels are closed, so there is no current. Then from -40, -30 mV, the channels begin to open. The more the membrane is depolarized by the step, the more channels open in the membrane. Because here we are far from the equilibrium potential of calcium, normally, at this value, the current should be very high: total current equals G, the conductance in the whole membrane for calcium channels, Vm - Ecalcium. So here the driving

force is very high, because we are far from the equilibrium potential, but the current is small because you don't have a lot of channels open. And then at the peak, here, you have the maximum channels open and the decay after is almost linear: it decays in relation with the driving force.

Ch. 3-3 : Calcium and neurotransmitter release (9:37)

We know now that in response to the arrival of an action potential, N-type calcium channels open, then calcium ions enter the axon terminal. Calcium ions that enter a presynaptic terminal cause neurotransmitter release. What are the connections between calcium ions and neurotransmitter release? Since the delay between calcium entry and neurotransmitter release is extremely short, on the order of hundreds of microseconds, it suggests that there is a physical link between calcium channels and the vesicles docked to the presynaptic membrane. This diagram shows an axon terminal, which we call a presynaptic element, with the presynaptic membrane. This terminal contains synaptic vesicles, which contain the neurotransmitter. Here is the synaptic cleft and across it, the postsynaptic membrane of the postsynaptic dendrite. Calcium channels are located very close to the vesicles docked to the presynaptic membrane. How are they docked to the presynaptic membrane? Now let's zoom on the presynaptic element, only the presynaptic element, here we see only the presynaptic membrane and the vesicles with the membrane around it. There is a physical connection between each vescile and the presynaptic membrane via proteins, for example here syntaxin, which is an integral protein of the presynaptic membrane. There are also connections between syntaxin and the proteins of the vesicles here, that are called v-SNARE. There is also here an integral protein in the presynaptic membrane called t-SNARE: v is for vesicle and t is for target. The entire complex here makes the vesicle attached to the presynaptic membrane and accounts for the fact that the calcium entrance is very close to the vesicle and very local. When calcium ions enter the intracellular medium of an axon terminal, they bind to protein here called synaptotagmin. It's a calcium sensor. What does it mean? There are sites where calcium binds and, when calcium binds, there is a change of conformation of the whole system: now the vesicle comes very close to the presynaptic membrane and the two membranes fuse. This phenomenon is known as exocytosis. The two membranes merge at a certain point and create a hole, referred to as a fusion pore. The neurotransmitter molecules exit through the fusion pore. All these stages between vesicle docking and exocytosis involve a large number of proteins. These are complex phases which are not yet fully understood, because they involve a change in protein conformation. We are not going to explain them all. Afterwards, these vesicles are recycled through endocytosis, then refilled with neurotransmitter, which enables them to participate in another neurotransmitter release cycle. To summarize, an action potential arrives at an axon terminal. It depolarizes the membrane up to around 20 mV, causes the opening of high-threshold voltage-dependent calcium channels. Calcium ions enter and trigger exocytosis and the release of neurotransmitter. It should be noted that even when all the conditions are there, for example there are several action potentials arriving at the axon terminals, neurotransmitter release does not always occur. This is a relatively inefficient process and only one action potential out of 2 to 20 will trigger exocytosis. How does neurotransmitter release stop? Once calcium ions are inside, how do vesicle fusion and exocytosis stop? This is due to multiple processes that allow the presynaptic terminal to return to a quiescent state. There is inactivation of sodium,

as we saw sodium channels of the action potential, but also of calcium channels. We have seen that these channels inactivate. This stops calcium entry. To repolarize the membrane, we have voltage-sensitive potassium channels of the sodium action potential: it allows the repolarization of the membrane after the action potential. We also have, in the membrane of the presynaptic terminal, calcium-sensitive potassium channels. It means that these channels are opened by calcium ions. When the intracellular concentration of calcium ions increases, it triggers the opening of these potassium channels and we say that it repolarizes calcium action potential. We did not say before that we have a "calcium action potential". It's because when calcium ions go in, they depolarize the membrane and this can be called a calcium action potential. These potassium channels that are calcium- sensitive are not explained here, they are explained in a supplement. What about the calcium ions that are already inside: where do they go? They are ejected from the cytoplasm via are multiple processes: pumps that use ATP energy to eject calcium channels (I remind you that it is against the electrochemical force so they go outside), you have transporters that exchange sodium with calcium, and you have here also a pump that put calcium ions in stocks, like in the endoplasmic reticulum, and you have calcium binding proteins that bind calcium and, like that, calcium is no more active, because the active form is the ionic form. All these mechanisms are called the clearance mechanisms for calcium ions. All this makes the increase in intracellular calcium transient, very brief, which means that neurotransmitter release is also brief. In summary, let's look at some quantitative data. The depolarizing phase of the sodium action potential opens voltage-sensitive calcium channels present in the axon terminals. As a result, calcium enters the presynaptic terminal. This entry is very localized (here you see 0.3 m2) and transient, because calcium channels inactivate and the membrane quickly repolarizes. When you have an increase of the intracellular calcium in the presynaptic terminal that reaches around 0.5-40 micromolar, then this triggers exocytosis, because they bind to a sensitive protein, and very quickly, in 200 microsecond, they induce exocytosis. This means that the entry of calcium ions and the binding to calcium-sensitive proteins and the change of conformation are very quick. The repolarization of the membrane terminal is due to potassium currents, either activated by the depolarization, like potassium currents of the action potential, they are called delayed potassium currents, or calcium-sensitive potassium currents. We now know that the action potential is created by ion currents because sodium and potassium ions are passively transported across the membrane. This electrical signal, the action potential, created at the initial segment of the axon and traveling along the axon, arrives at axon terminals. At the axon terminals, it depolarizes the membrane of the axon terminals, open calcium channels and causes calcium ions to enter. This inward transport of calcium ions is detected by calcium sensor proteins and results in the exocytosis of synaptic vesicles containing a neurotransmitter. This way, the neurotransmitter is released into the synaptic cleft. In the following courses, we will see how the neurotransmitter recreates an electrical signal, which is called a postsynaptic potential.