# Chapter 4 Lab

**LAB 1: RECORDING SPONTANEOUS SYNAPTIC AMPA CURRENTS (4:18)**

*We always think that, in the absence of external stimulation, neurons are silent. This is not the case: the vast majority of networks have spontaneous synaptic activity at rest. In the experiment that you saw, we recorded postsynaptic currents emitted by a postsynaptic neuron in* ***response to the spontaneous activity of its glutamatergic afferent synapses****. What does this activity tell us?*

- *By using specific* ***blockers****, the* ***type of glutamate receptors*** *present in the postsynaptic membrane can be known: AMPA, kainate, NMDA.*
- *The analysis of the* ***frequency and amplitude*** *of the glutamatergic postsynaptic currents provides information about the* ***number, activity and strength of the afferent connections*** *in the recorded neuron.*

*Spontaneous synaptic activity is the activity of a network of afferent neurons. This is the background activity, also known as "synaptic noise", that provides information on the network status at the time of recording. It may vary (e.g. there may be changes in the type of glutamatergic connections) with the state of alertness (sleep-wake), degree of stress, or developmental stage.*

Neuronal networks are spontaneously active even in vitro in brain slices. When researchers record spontaneous glutamatergic currents from a single neuron, it gives them information on the activity of the glutamatergic neurons afferent to the one being recorded. I record AMPA currents in the whole-cell configuration and in voltage clamp mode. I add gabazine to the extracellular environment, to block GABA-A currents, as well as APV to block NMDA-type glutamate currents. In this experiment, the cation reversal potential is +10 mV. If I hold the membrane potential at -70 mV, AMPA currents will be negative. We see here currents that show a downward deflection of the baseline: these currents are negative and therefore inward. A time base of about 40 ms gives us an indication of AMPA currents duration and frequency of occurrence. We see a recording of spontaneous AMPA currents over time passing here on the screen. These currents have a variable amplitude on the order of about 10 pA, because we do not always have the same number of active glutamatergic synapses at the same time. AMPA synaptic currents result from a simultaneous activation of multiple postsynaptic AMPA receptor-channels by a glutamate release from the axonal terminals afferent to the neurons being recorded. To make sure that we are in fact dealing with an AMPA current, we add CNQX to the extracellular environment which is a specific antagonist for the AMPA-type receptor-channels. We then observe a gradual disappearance of AMPA currents. We launch automatic current analysis in the software and then validate this analysis manually. The parameters of interest to us for each current are the magnitude of the deflection here between the yellow point and the red cross, the time between the yellow point and the same red cross, and then duration between the red cross and the violent point. We are also interested in the AMPA currents recurrence frequency, which may be measured by the time between two yellow points. The diagram on the left shows us the AMPA current amplitude. Here, for each blue point, we have the measure of the mean AMPA current for a given cell or for an experiment. The red line indicates the mean

amplitude of all the currents, about -18 pA in this case, while the two green lines represent the variability i.e. the standard deviation. The diagram on the right represents the inter-event interval (IEI), that is to say the mean time elapsing between the appearance of two consecutive AMPA currents. Each point represents the mean IEI for a given cell and all the points represent the mean values that we recorded during our experiments. The red line is still representative of the mean, about 4 seconds, while the green lines inform us of the variability. This helps us understand the activity of AMPA-type glutamate synapses afferent to the neuron being recorded. This activity may vary with development or pathology.

**LAB 2: RECORDING UNITARY NMDA CURRENTS (7:33)**

*The experiment that you'll see is used to record the **activity of single NMDA channels**. This allows us to quantify the following parameters:*

- *the **amplitude** of the unitary NMDA current as a function of the membrane potential (i-V curve)*
- *the **conductance** of the NMDA channel, which is the slope of the i-V curve,*
- *the mean **opening time** of the NMDA channel,*
- *the channel **sensitivity to the extracellular concentration of magnesium ions**.*

*With these parameters, we can identify particular **subunits** that form the NMDA channel, or **mutations** of these subunits.*

*In the experiment presented by Roman Tyzio, the cell-attached configuration lets him know the **neuron resting potential**. This configuration has the advantage of maintaining the intracellular medium of the neuron; however, it has the disadvantage of not providing access to the intracellular side of the membrane, thus to the transmembrane potential. However, knowing that glutamatergic currents are reversed towards 0-10 mV, one can deduce the neuron resting potential: it corresponds to the membrane potential when no current is injected.*

*Note: In this video, $V_o$ (outside) is sometimes referred to as $V_e$ (external).*

The objective of the experiment is to demonstrate the recording of a unitary NMDA current, which is a glutamatergic NMDA current, i.e. a current across a single NMDA channel in a neuron of the central nervous system. We see here a recording of the opening of NMDA channels towards the top. These are very short openings. Here is one that is a little longer. These are spontaneous openings of this channel, because there is some NMDA in the pipette. We will see all of this later. Here are some more openings. Here we get the impression that it is not of the same amplitude while in fact it is: because the opening is very brief, the system really does not have enough time to record the correct amplitude. We see that these openings are sometimes very close together and at other times, separated. Thus they are of variable duration, and are square current steps, due to the fact that we are dealing with a single channel that opens from time to time. Here we have a burst of openings. How are they recorded? We record these unitary NMDA currents in the cell-attached configuration and in voltage-clamp mode. In this configuration, we record the activity in a channel or several channels in the little piece of membrane underneath the pipette. Recall that in the cell-attached configuration, the pipette contains the extracellular medium because it will be found on the outside of the channel being recorded, outside the membrane, and the intracellular medium is the neuron's intracellular physiological

environment. To activate NMDA channels and obtain a recording of unitary NMDA currents only, we apply NMDA and its co-agonist, glycine, to the extracellular environment. Most importantly, this extracellular environment has no magnesium ions, such that there will be no blockage of NMDA channels at certain potentials. In this experiment, we see a voltage trace at the top, with a current trace below. The voltage trace is in fact the voltage applied to the pipette. We can make it vary between depolarized or more hyperpolarized potentials. Underneath, there are current traces or recordings of individually open NMDA channels. Since the time base here is very slow, each opening is a small black line, hence they run together and we cannot see them very well. We see that there are openings towards the top, which subsequently disappear, and we find them again here, towards the top, becoming increasingly large. What we have towards the bottom we need not take into account. The large ones, like this one, here are artifacts associated with voltage changes. We can change the potential on either side of the channel being recorded. We can therefore build what we call an i-V plot, i.e. current as a function of changes in potential. What is the potential applied to the little piece of membrane? To make membrane potential change, we apply a potential to the extracellular surface of the little piece of membrane underneath the pipette. This potential is called Vp, for V pipette. Thus the patch's transmembrane potential is by definition Vm = Vi -Ve and, given that here Ve is Vpipette, we write Vi - Vpipette. Vp is the imposed potential that we know well. Vi is an unknown potential, because we do not have access to the intracellular environment in the cell- attached configuration. Changing Vp helps us change Vm, hence, as we saw previously and will see again, we can change the amplitude and even the direction of these current steps. When Vm is equal to Ecations i.e. the cation reversal potential (I remind you that the NMDA channel is permeable to cations), the unitary NMDA current is null at that time. In this experiment, iNMDA disappears whenever Vp is equal to -60 mV. We see here that the amplitude of the current changes as a function of the Vp potential, and that opening durations are very variable from one opening to the next, same as opening frequency . We see this current disappear at Vp equals -60 mV. Given that Ecations is close to 0 mV, we can make the conclusion that Vi should also be -60 mV. This way, Vm becomes 0 mV, Ecations is at 0 mV. We are going to look at these openings at different potentials. Here at Vp equals +80 mV, for instance, if we zoom in, we see that these openings exhibit a great amplitude and sometimes even appear in bursts. I will remind you that there is zero magnesium in the pipette here, hence there can be no blockage of the NMDA channel by magnesium ions. If we look at all of these openings, now we can see them and see that they always have a constant amplitude, whereas the duration is variable. Now if Vp changes, amplitude is going to grow smaller little by little as Vp approaches 0. We see here square openings still but of a smaller amplitude. Then we're going to get all the way to the reversal potential. Here for instance, we see that there are practically no openings. We see openings with a reverse direction. See: these are openings that are very small in amplitude and are now negative. Let us go back to potentials that are on the order of +20. Again the currents are upward. You see that they are of variable duration. However, for each given Vp, the amplitude is constant. I am not going to explain the current direction to you now: it goes down or up, but I am not going to tell you whether it corresponds to an inward or an outward current, because in a cell- attached configuration, it is fairly difficult to understand due to the fact that everything is inverted with respect to what you have learned in the whole- cell configuration. The cell-attached configuration helps

record unitary NMDA currents without changing the composition of the intracellular medium.

**LAB 3: RECORDING SPONTANEOUS SYNAPTIC NMDA CURRENTS (3:50)**

I record NMDA currents in the whole-cell configuration and in voltage-clamp mode. I add gabazine to the extracellular environment to block GABA-A currents, as well as CNQX to block AMPA and kainate glutamate currents. In this experiment, the cation reversal potential is +10 mV. First I hold the membrane potential at +40 mV. At this holding potential, NMDA current are represented by the upward or positive deflection of the baseline and are therefore outward. Holding the membrane at +40 mV helps avoid NMDA channel blockage by extracellular magnesium ions. A time base of about 250 ms provides an indication as to how long the durations of the NMDA currents are, and their recurrence frequency. We see a recording of spontaneous NMDA currents over time. These currents have a variable amplitude, on the order of about 10 pA, because we do not always have the same number of active glutamatergic synapses: the more active glutamatergic synapses there are at the same time, the stronger the NMDA spontaneous current. To make sure that we are in fact dealing with an NMDA current, we add APV to the extracellular environment, which is a specific antagonist for the NMDA type receptor-channels. After applying the APV, we observe a gradual disappearance of NMDA currents. We launch automatic current analysis in the software and then validate this analysis manually. The parameters of interest to us for each current are the amplitude, i.e. the magnitude of the upward deflection that we record between the yellow point and the red cross, the current rising duration, which we will measure between the yellow point and the red cross, and the current decay duration, which we measure between the red cross and the violet point. We can also measure the time elapsing between the appearance of two consecutive NMDA currents. This diagram shows you, on the left, NMDA current amplitude, and, on the right, the inter-event interval, i.e. the time interval between two consecutive NMDA currents. The set of blue points corresponds to all of the recordings that we have made, the red line found near 29 pA represents the mean amplitude of synaptic NMDA currents, the green lines exhibit the variability represented by the standard deviation. We can see the inter-event interval. The set of blue points corresponds to all of the recordings that we have made. The red line represents the mean inter-event interval. In conclusion, spontaneous synaptic NMDA currents provide information on the glutamatergic connection rate of the neurons being recorded. This helps us understand the activity of NMDA glutamatergic synapses afferent to the neuron being recorded. This activity may vary with development or pathology.