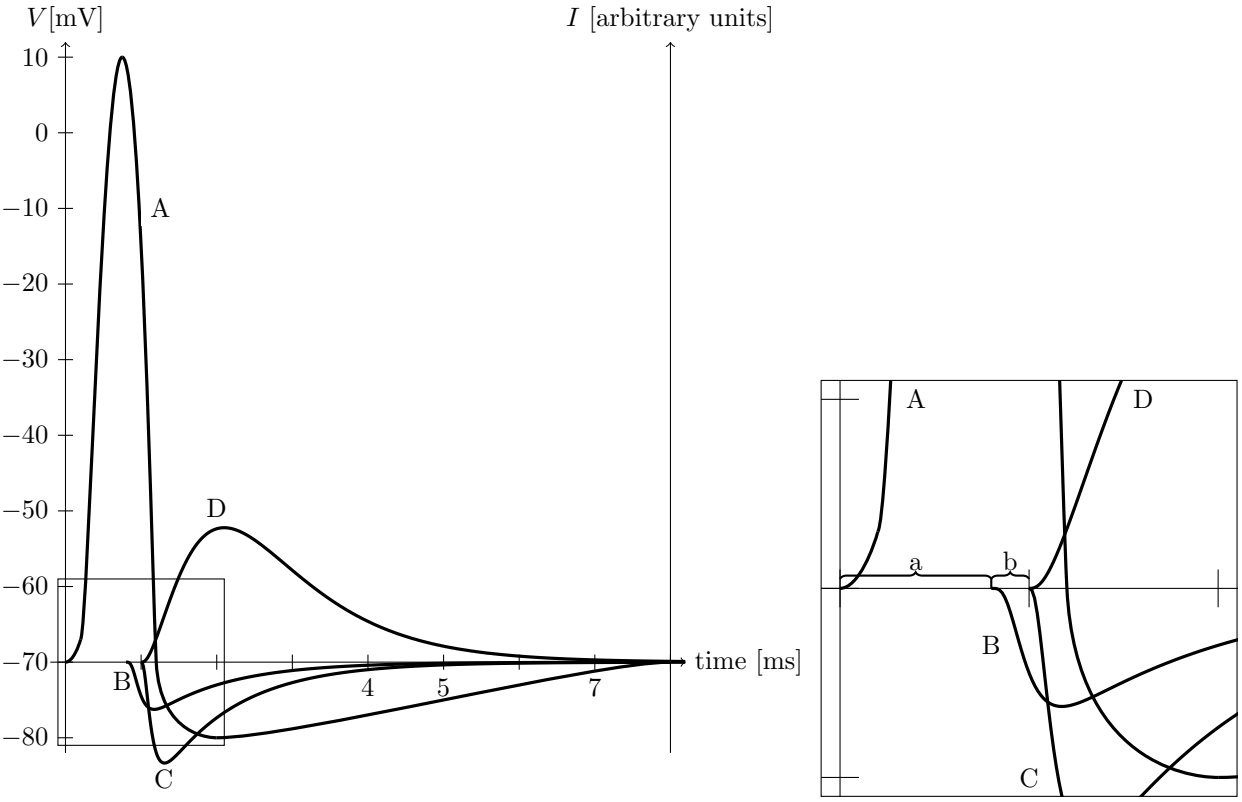
Benjamin Grewe, Matthew Cook, Giacomo Indiveri, Daniel Kiper, Wolfger von der Behrens, Valerio Mante
Lecture 8
Asst: Nicoletta Risi, Raphaela Kreiser, Ioana Calangiu {nicoletta, rakrei, calangiu}@ini.uzh.ch

Exercise 8.1: Components of the synaptic delay

In this exercise we look at some data measured on the giant squid synapse. The plot is adapted from a publication of Llinás *et al.* from 1981. It shows traces from a presynaptic cell and its postsynaptic cell. The scale for the two voltage traces is given on the left y-axis, the right y-axis for the two current traces is in arbitrary units. You may notice that the height of the EPSP does not correspond to the height observed in the mammalian CNS. There are two reasons for this: on the one hand we are looking at the giant squid synapse and on the other hand the height of the EPSP also depends on the extracellular calcium concentration, which was 10mM for this plot. All traces are plotted on the same time scale. Remember the convention that a membrane current from the intracellular to the extracellular compartment is defined as positive.



1. Identify which trace (A-D) is

   - the postsynaptic current (right y-axis)
   - the presynaptic action potential (left y-axis)
   - the postsynaptic potential (left y-axis)
   - the presynaptic Ca^{2+}-current (right y-axis)

   Explain how you came to your conclusion.

2. Based on these observations, we can state that there are two components of the synaptic delay: a, b as depicted in the figure. Give approximate values for the duration of a and b, respectively.
   Indicate for each of these four processes whether it is comprised in period a or in period b:

   - Gating of postsynaptic ligand-gated channels
   - Transmitter diffusion in the synaptic cleft
   - Gating of presynaptic Ca^{2+}-channels
   - Ca^{2+}-dependent vesicle fusion (exocytosis)

Exercise 8.2: Quantal release I

You are designing an experiment to test the probabilistic nature of neurotransmitter release. You know that when the probability of release is small (*e.g.* if you lower extracellular Ca^{++} in your preparation) and the number of synaptic vesicles available for release is large (as at the neuromuscular junction) then the expected number of vesicles released following a presynaptic action potential follows a Poisson distribution (the limit of a binomial distribution $B(n, p)$ if p is small and $n \cdot p$ finite). Therefore if m is the mean number of vesicles released per trial, then the probability P of observing a particular number x ($x = 0, 1, 2, 3$ ...) of vesicles released in a given trial is:

$$P[X = x] = \frac{m^x}{x!} e^{-m}$$

You stimulate a nerve 500 times. Assume that each trial is independent of the other trials performed. Given that the number of quanta released in one trial (=*quantal content*) follows Poisson statistics and the mean quantal content $m = 5$:

1. How many failures of transmission ($x = 0$) do you expect to observe in the 500 trials?
2. How often do you expect two quanta to be released ($x = 2$)?

Exercise 8.3: Quantal release II

Fig. 1 shows a histogram of end-plate potentials of a cat muscular fiber, intracellular recording. These data were recorded in 1956 by I. A. Boyd and A. R. Martin to study the release properties of neurotransmitter at the muscular end-plate. The recordings were made under low extracellular Ca^{++} and high Mg^{++} concentrations. These special conditions permit to reach extremely low release probabilities, so that the end-plate potential amplitude fluctuates following a Poisson distribution.

The histogram of Fig. 1 corresponds to the amplitude distribution of 200 events (evoked and spontaneous). The mean amplitude of all events is 0.93 mV.

(a) What do the different peaks labelled A, B, C, and D correspond to?

(b) From the graph, what is the quantal amplitude Q? The quantal amplitude (also known as the *quantal size*) is the amplitude of the response (EPP endplate potential) induced by transmitter from one vesicle.

(c) Using the result from (b) and the mean amplitude of all events calculate m, the mean number of vesicles released per observation. Can you imagine another way of calculating m assuming a Poisson distribution?

(d) Assume that m and Q are measured for a given experiment. In a subsequent measurement in which experimental parameters have changed, m and Q are measured again and they might have changed. How can you detect an increase in the release probability? How can you detect postsynaptic modifications?

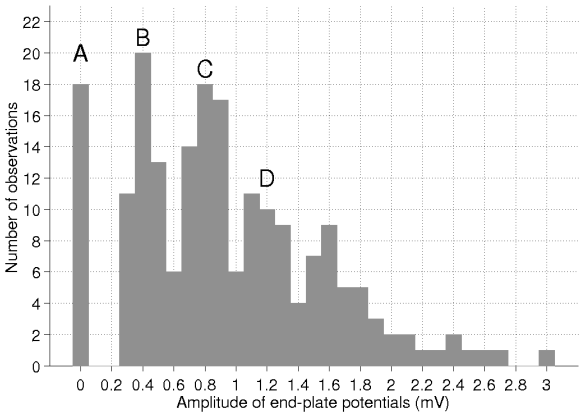


Figure 1: End-plate potential of a cat muscular fiber

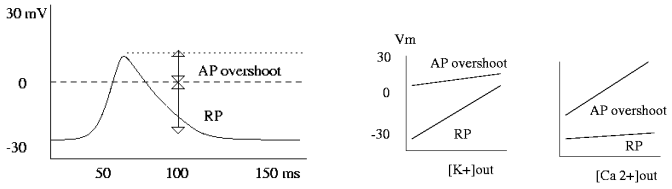
Exercise 8.4: Back to basics

Draw an action potential on a voltage-time graph. On the time-axis indicate where the Na and K channels open and close. On the voltage-axis mark the reversal potentials E_K, E_{Na}, and the threshold θ.

Exercise 6.5: Influence of Ion Concentrations

Assume that we have a cell whose membrane is permeable only to K^+ and Ca^{++} ions (and water). Such a cell shows a resting potential (RP) and an action potential (AP) as indicated in the Fig. 2(a).

If one varies the concentration of extracellular potassium $[K^+]_{out}$ only, or the concentration of extracellular Calcium $[Ca^{++}]_{out}$ only, one observes the changes shown in Fig. 2(b).



(a) (b)

Figure 2: An action potential and effect of single extracellular ion concentration changes

Answer the following questions:

1. Which of the following statements about the permeabilities is true in the resting state (P stands for permeability)?

   (a) $P_K > P_{\mathrm{Ca}}$

   (b) $P_K = P_{\mathrm{Ca}}$

   (c) $P_K < P_{\mathrm{Ca}}$

   Explain why.

2. Which of the previous statements is true for the peak of the AP?

3. Compare $[K^+]_{in}$ to $[K^+]_{out}$. Compare also $[Ca^{++}]_{in}$ with $[Ca^{++}]_{out}$. In which direction do more ions diffuse to? What is the resulting current flow? Discuss how potassium and calcium currents relate to each other.

4. When the cell is perturbed mechanically, the membrane transiently hyperpolarizes. What permeability changes might be responsible? What ionic currents typically lead to hyperpolarization at physiological concentrations?