Neurofunctional Techniques

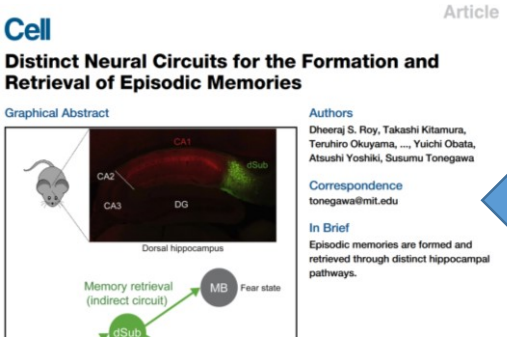
Lesson 8

28 October 2024

Review Ca^{2+} imaging

Calendar

- **M 30 Sept:** Course introduction
- **W 2 Oct:** Functional imaging
- **F 4 Oct:** Statistics (Cesca)
- **M 7 Oct:** Functional imaging
- **W 9 Oct:** Biophysics of diffusion
- **F 11 Oct:** Statistics (Cesca)
- **M 14 Oct:** Functional imaging
- **W 16 Oct:** General introduction to the papers for the presentations
- **F 18 Oct:** Statistics (Cesca)
- **M 21 Oct:** Modeling in neuroscience
- **W 23 Oct:** Molecular approaches in neuroscience
- **F 25 Oct:** Statistics (Cesca)
- **F 25 Oct:** Laboratory (14:00- 18:00)
- **M 28 Oct:** Practical exercises on the first part of the course
- **W 30 Oct:** Genome editing in neuroscience (Dr. Jaudon)
- **M 4 Nov:** Optogenetics
- **W 6 Nov:** Papers assignment to the groups; introductions to the specific papers
- **T 12 Nov:** X-genetics + Practical exercises on the second part of the course
- **W 13 Nov:** Introductions to the specific papers
- **M 18 Nov:** Introductions to the specific papers



- **9, 10, 11 Dic:** Paper presentation 15:00-19:00)
- **Tue 17 Dic:** Test (14:00 - 16:00 Room 3A, Building H2bis)

Structure of the exam

1. **Paper presentation in small groups (5 students 1 hour) Maximum score 20 (+1):**

   a) **Presentation (30 min)**
   - **What was known**
   - **What is the gap**
   - **What are the main findings**
   - **Are the techniques appropriate**
   - **What are the broader implications**

   b) **Questioning on individual figures and techniques used (30 min)**
   (9, 10, 11 December)

2. **Questionnaire on the Moodle platform. 10 multiple choice questions (also on statistics). Each question has only one correct answer, and each correct answer is awarded 1 point. Maximum score: 10.**
   (17 December Room 3A Building H2bis
   First turn: 14:00 - 15:00
   Second turn: 15:00 - 16:00

1) **Which is the approximate Ca^{2+} concentration in the cerebrospinal fluid ($[Ca^{2+}]_0$)**

   a) **20 mM**

   b) **2 mM**

   c) **200 µM**

   d) **20 µM**

2) Transient local Ca^{2+} concentrations in the cytosol, for example near the mouth of a voltage-gated Ca^{2+} channel, can reach values up to

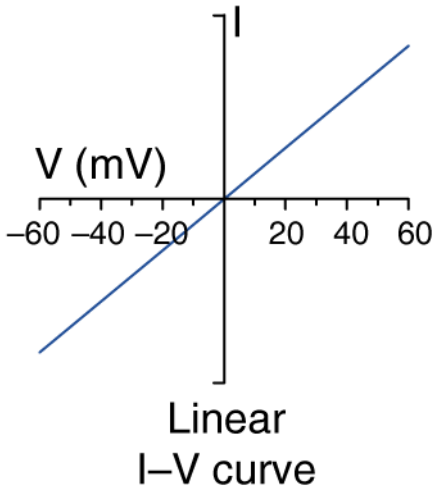
a) Hundreds molar

b) Hundreds millimolar

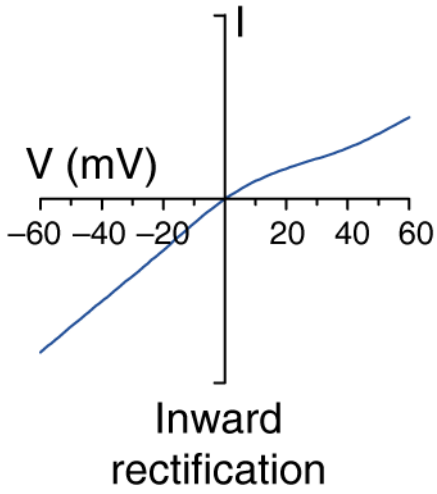
c) Hundreds micromolar

d) Hundreds nanomolar

3) **AMPA-type glutamate receptors (AMPARs) lacking the GluA2 subunit are Ca^{2+} permeable. The presence/absence of GluA2 in AMPARs is normally inferred by recording the rectification of AMPAR currents in the presence of spermine in the intracellular solution. Which of the two I/V plots below indicates GluA2-containing AMPARs? And why?**



Linear
I–V curve



Inward
rectification

4) **NMDA receptors (NMDARs) are permeable:**

   a) **Selectively to Ca^{2+}**

   b) **To Ca^{2+} but also to Na^{+}, K^{+}**

   c) **To Ca^{2+} and Mg^{2+} but also to Na^{+}, K^{+}**

5) **Most NMDARs are obligatory heterodimers between two GluN1 subunits and two GluN2 subunits (either GluN2A or GluN2B or GluN2C or GluN2D). Among other things, the type of GluN2 subunit affects….**

6) **Upon Ca^{2+} binding, Ca^{2+} sensors mediate biological responses. For example, Ca^{2+} binding to synaptotagmin triggers vesicle release. The contribution of Ca^{2+} sensors to the Ca^{2+}-binding ratio (κ_B) of a neuron is always negligible.**

TRUE

FALSE

7) **How can you load the Ca^{2+} indicator Fluo4 in one neuron only within a network of neurons?**

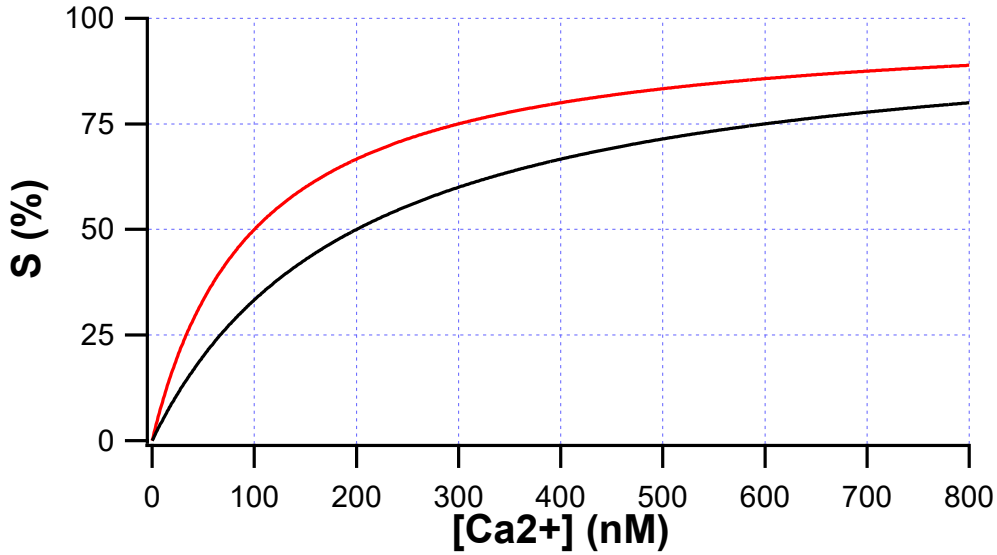
   a) **By patching the neuron of interest with an intracellular solution containing Fluo4**

   b) **By using the acetoxymethyl ester form of Fluo4 (Fluo4-AM), which is membrane permeable**

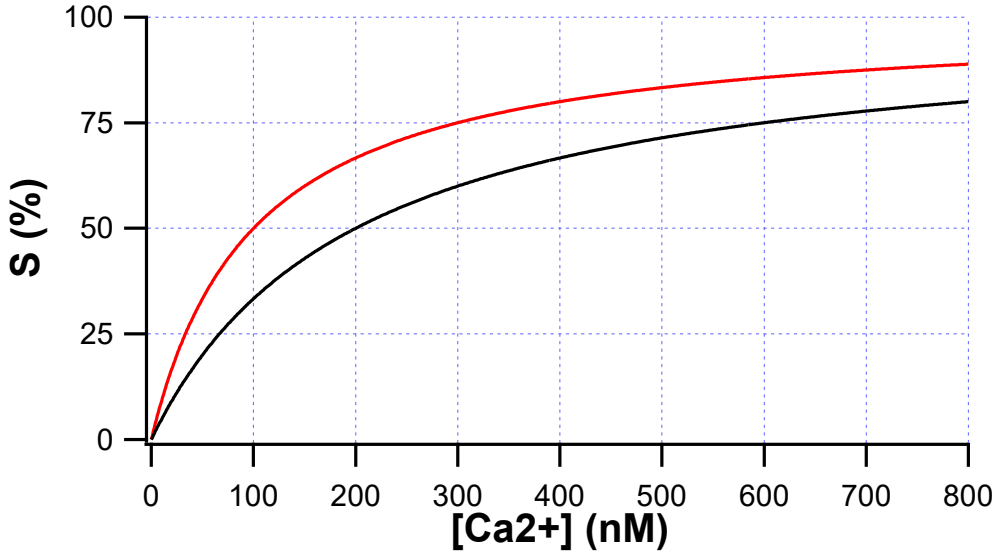
   c) **By using single-cell electroporation**

   d) **By bulk electroporation**

8) The graph below represents the saturation curves for two Ca^{2+} indicators. Write the equations for the red and black curve

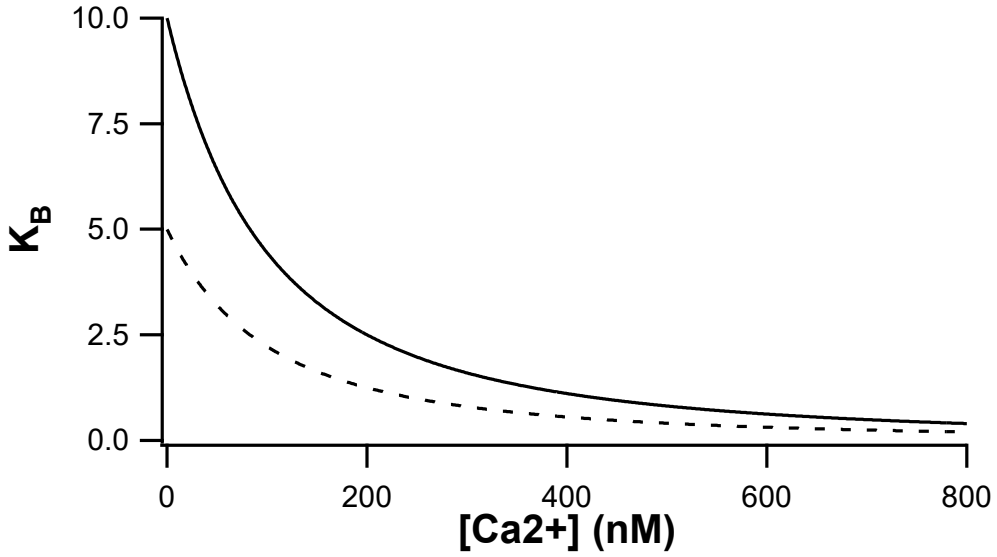


8) **The graph below represents the saturation curves for two Ca^{2+} indicators. Write the equations for the red and black curve**

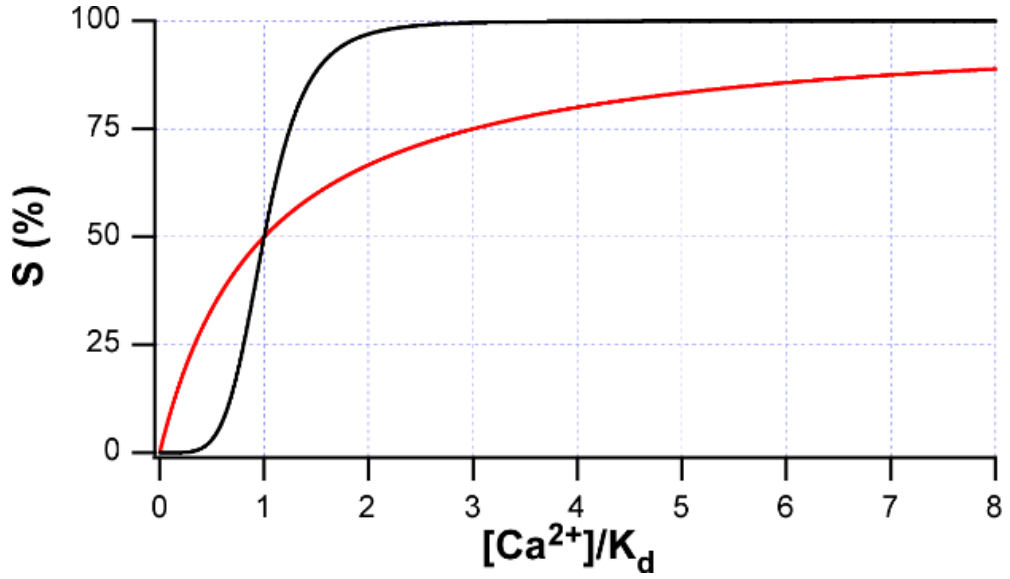


9) **You need to measure Ca^{2+} transients in the range 200-300 nM. Which Ca^{2+} indicator of the two above would you use? And why?**

10) The graph below plots the Ca^{2+} binding ratio (κ_B) for the Ca^{2+} indicator whose saturation plot is in black in question (8). Infer the concentration of the Ca^{2+} indicator in the case of (i) the continuous and (ii) dashed line



11) The graph below plots the saturation curves for the Ca^{2+} indicators Fluo4 and GCaMP6s. Which one is which? And why?



12) **You are interested in monitoring the activity of the medial prefrontal cortex in mouse in response to prolonged (2 months) social isolation as part of a study on the effects of social interaction on cortical activity. Which indicator of the two in question (11) would you pick up and why?**

13) The Ca^{2+} binding ratio (κ_B) of a neuron depends:

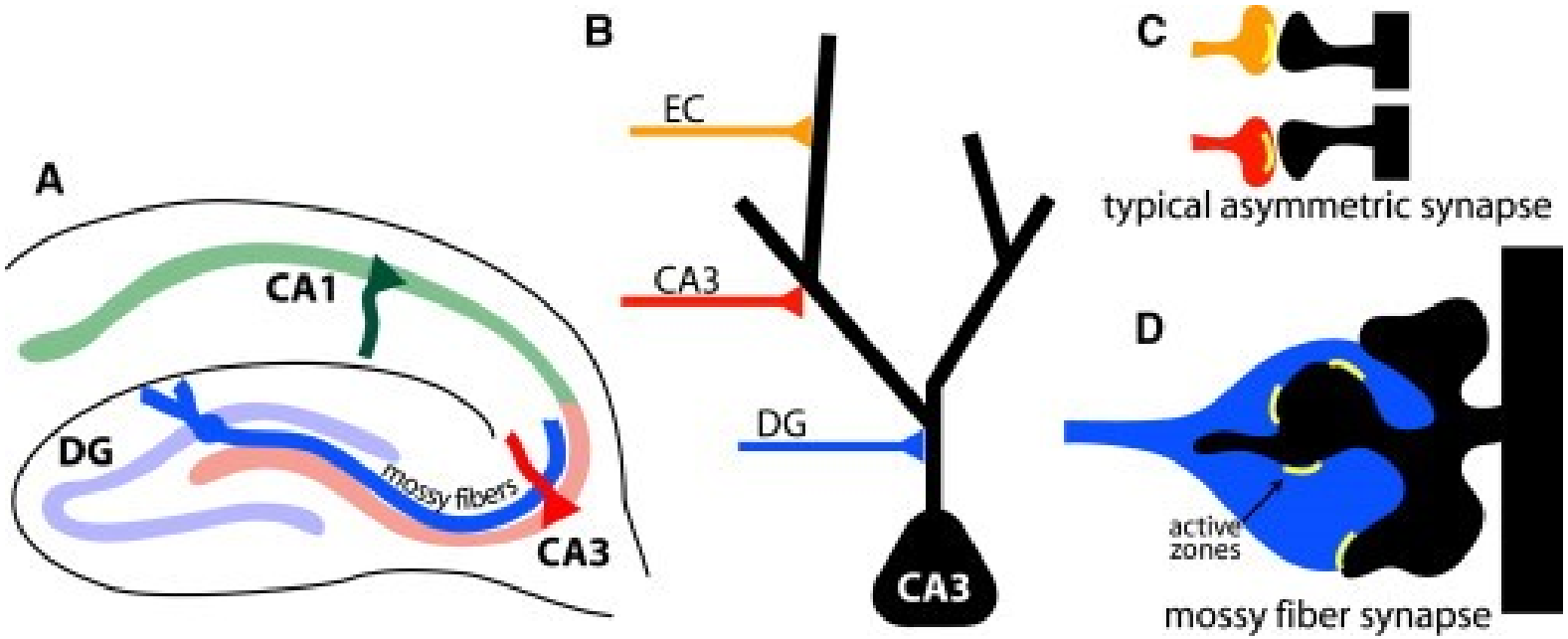
a) on the concentration of the various Ca^{2+} buffers

b) on their dissociation constants (K_d)

c) on the Ca^{2+} concentration

d) on all of the above

14) **Ca^{2+} in the giant presynaptic boutons (diameter = 5 µm) of the mossy fibers has an effective diffusional constant for Ca^{2+} (D_{Ca}) equal to 20 $\mu m^2 s^{-1}$. How many ms does it take for Ca^{2+} gradients to dissipate in a mossy fiber bouton?**



15) In an approximately spherical subcellular structure of 1 µm in diameter, Ca^{2+} gradients dissipate within 1 ms. How long would it take for the Ca^{2+} gradients to dissipate if the same subcellular structure had a diameter of:

a) **10 µm**

b) **100 µm**

c) **1000 µm**

16) **The fluorescent signal (F) of a fluorophore X is directly proportional to the fluorophore concentration ([X]). The proportionality constant (S) depends:**

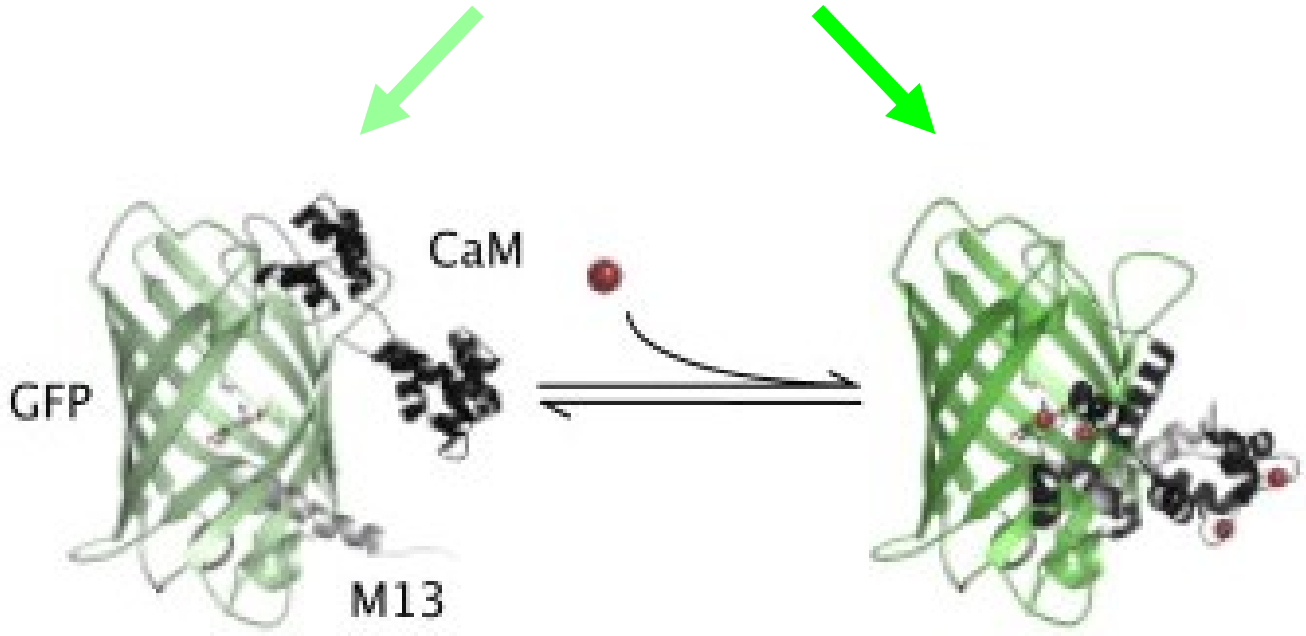
a) **On the properties of the fluorophore**

b) **On the properties of the imaging set up**

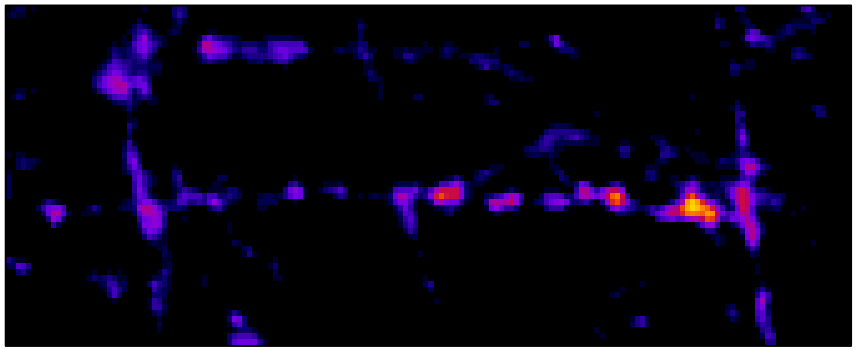
c) **On the properties of both the fluorophore and the imaging set up**

17) Can you define the dynamic range of a fluorophore? How could you improve the dynamic range of a fluorophore?

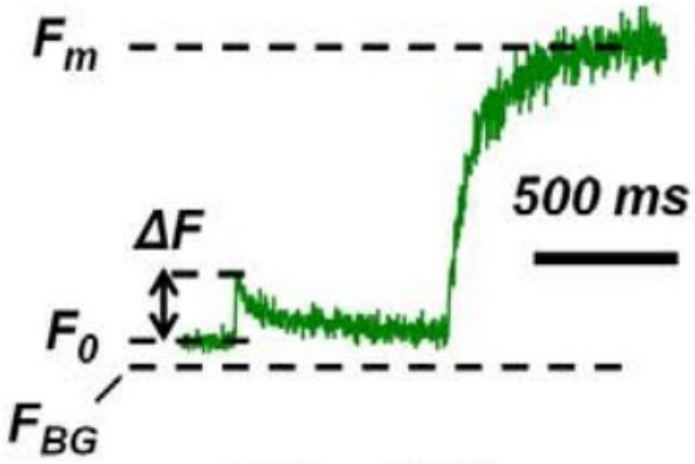
$$F = S_f\ [\mathrm{B}] + S_b\ [\mathrm{CaB}]$$



18) Practically, how do you calculate/measure $\Delta F/F_0$? You can use this snapshot of a time-lapse video of presynaptic boutons labeled with Fluo4 to help yourself in the explanation.



19) **The figure below shows the fluorescence response of Fluo4 (K_D = 350 nM) in a presynaptic bouton following first a single action potential (AP; $\Delta F/F_0$ = 1.2) and then 100 APs ($\Delta F/F_0$ = 12). Can you estimate the presynaptic $\Delta[Ca^{2+}]$ in response to one AP?**



20) What Fura2 and Cameleon indicators have in common? In what they differ?

21) You are interested in monitoring Ca^{2+} transients in the nucleus as part of a project on the effects of the transcription factor REST on gene expression. You have a mouse line where the gene for REST is floxed. You prepare primary hippocampal cultures from these mice and you infect them with a lentivirus expressing the recombinase Cre in order to knock out REST in the infected neurons. Cre is fused to EGFP (to highlight the infected neurons) and to a nuclear localization signal (NLS; to increase the accumulation of Cre-EGFP in the nucleus). You also have indication from the literate that Ca^{2+} transients in the nucleus are higher than those in the soma (in the range: 5-10 µM).

a) Would you use a small molecule indicator or a genetically encoded indicator? Why?

b) Would you choose a blue, green, yellow or red indicator? Why?

c) Would you chose a high affinity (K_d in the hundreds nanomolar range) or a low affinity indicator (K_d in the low micromolar range)? Why?

d) You have a molecular biology facility at your disposal. What could you do to increase the localization of the chosen indicator in the nucleus?