Neurofunctional Techniques

Lesson 6

23 October 2026

Review Ca^{2+} imaging

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

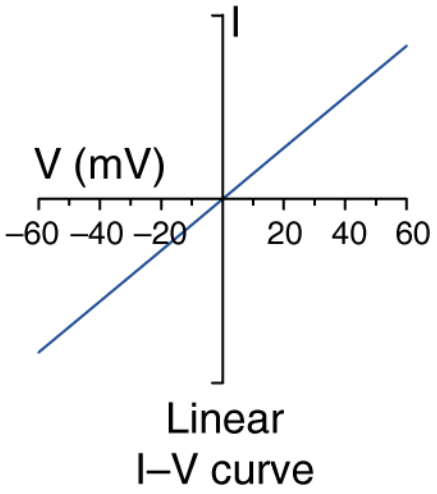
a) Hundreds molar

b) Hundreds millimolar

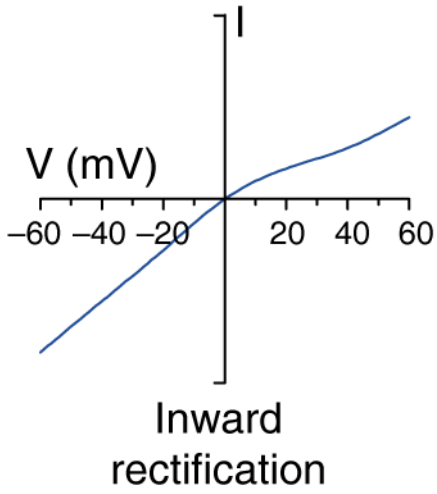
c) Hundreds micromolar

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?**

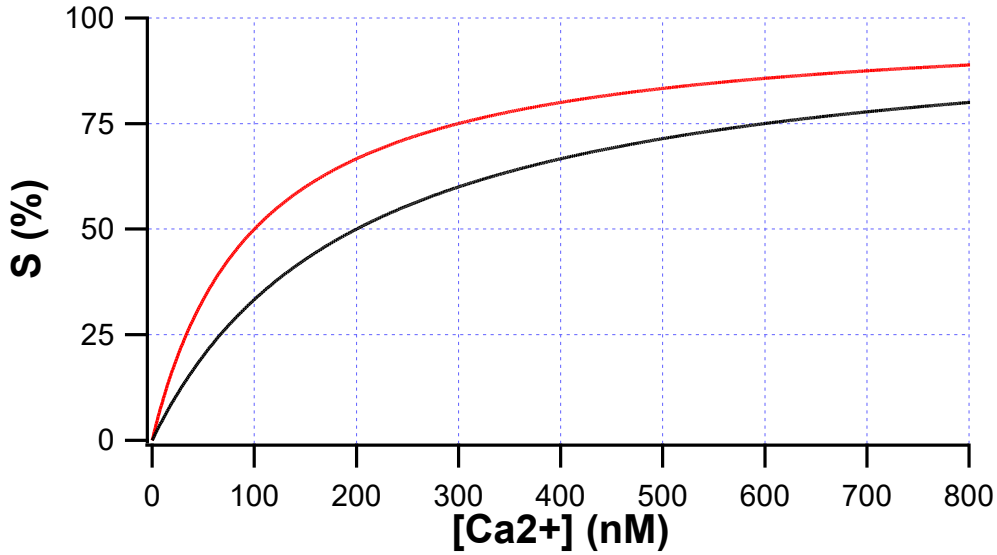
   a) **By patching the neuron of interest with an intracellular solution containing Fluo4**

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

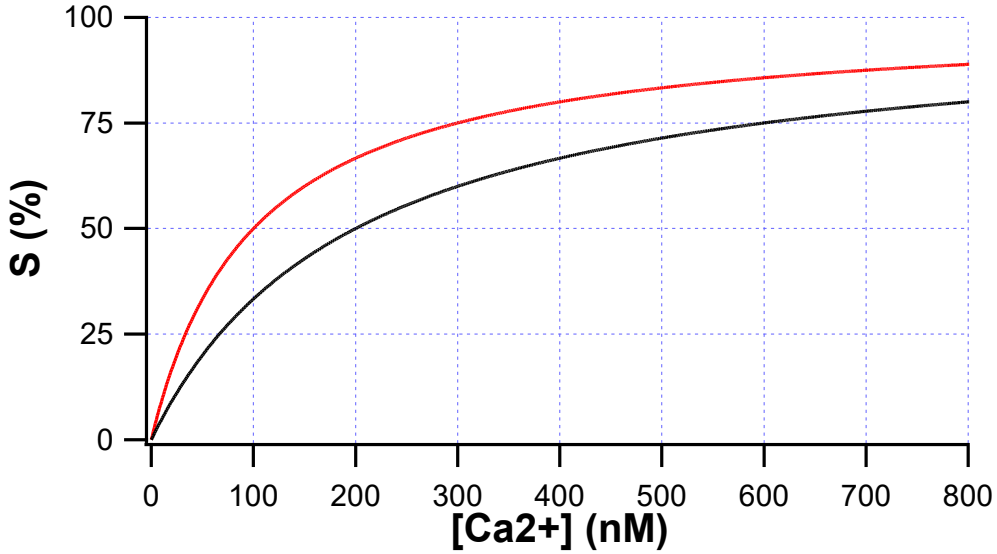
   c) **By using single-cell electroporation**

   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

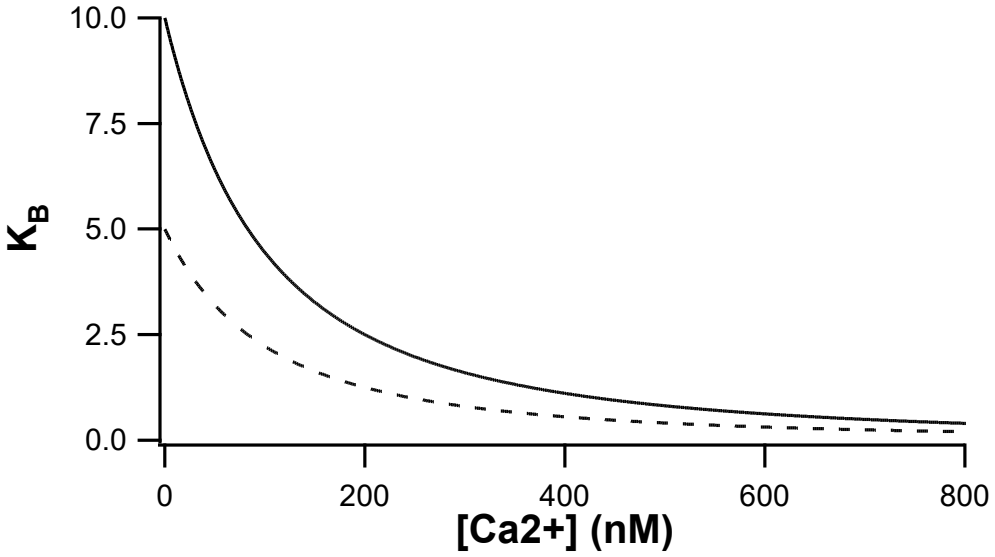


8) **The graph below represents the saturation curves for two Ca^{2+} indicators. Write the equations for the red and black curve**

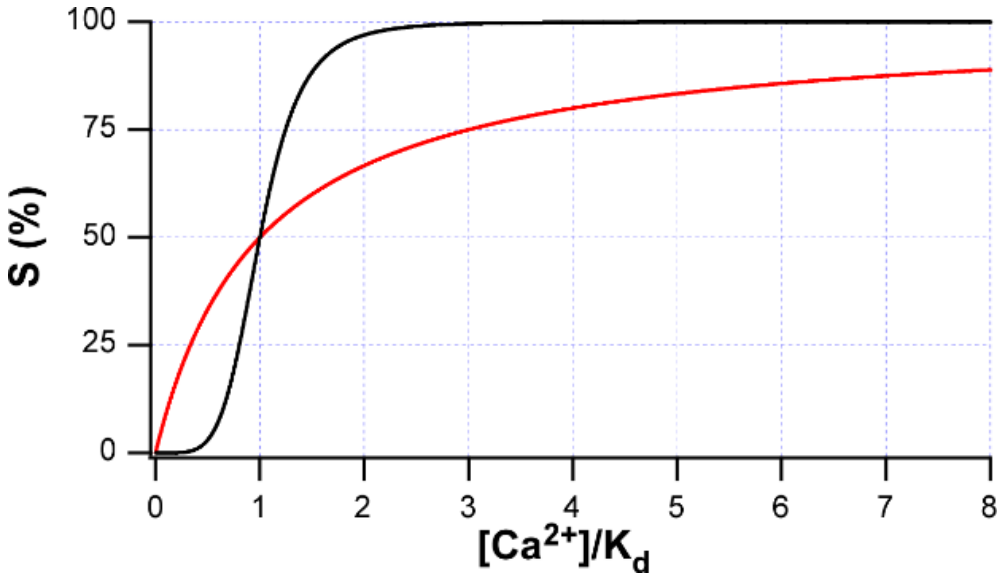


9) **You need to measure Ca^{2+} transients in the range 200-300 nM. Which Ca^{2+} indicator of the two above would you use? And why?**

11) 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



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



13) **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 (12) would you pick up and why?**

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

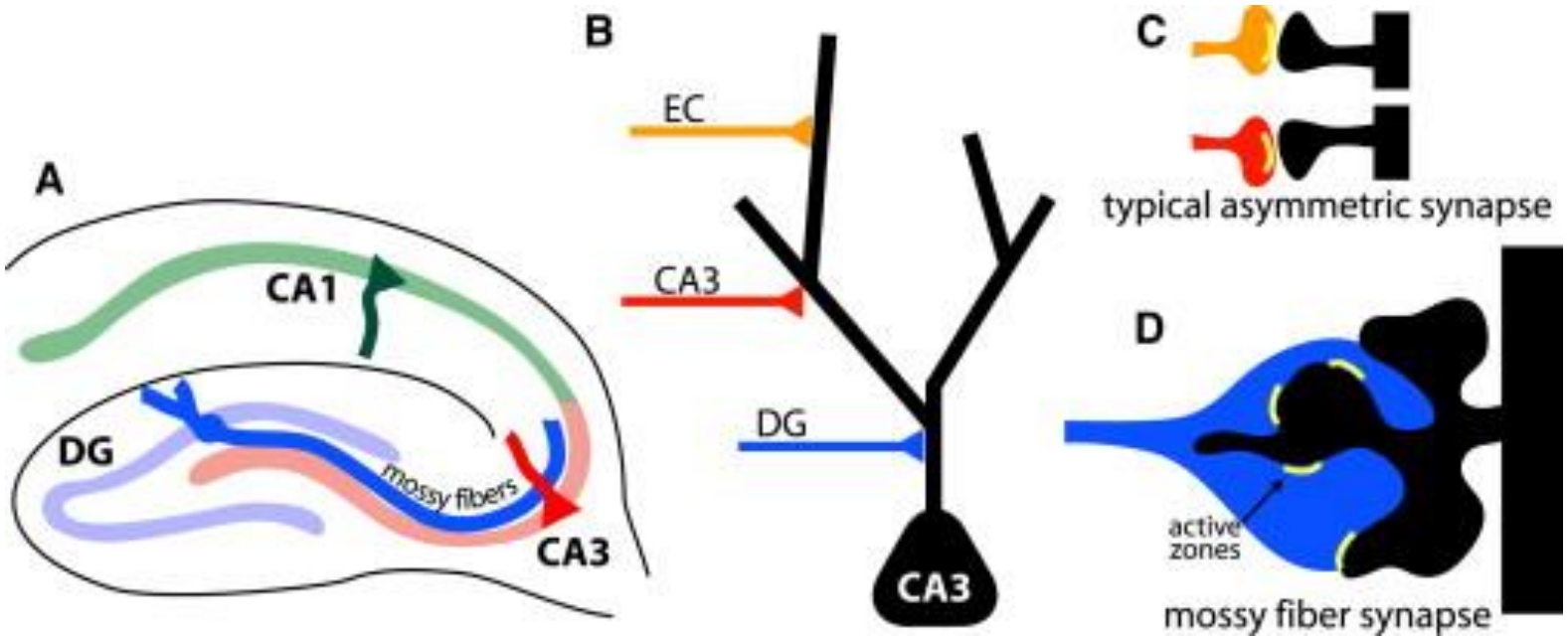
a) on the concentration of the various Ca^{2+} buffers

b) on their dissociation constants (K_d)

c) on the Ca^{2+} concentration

d) on all of the above

15) **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?**



16) 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**

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

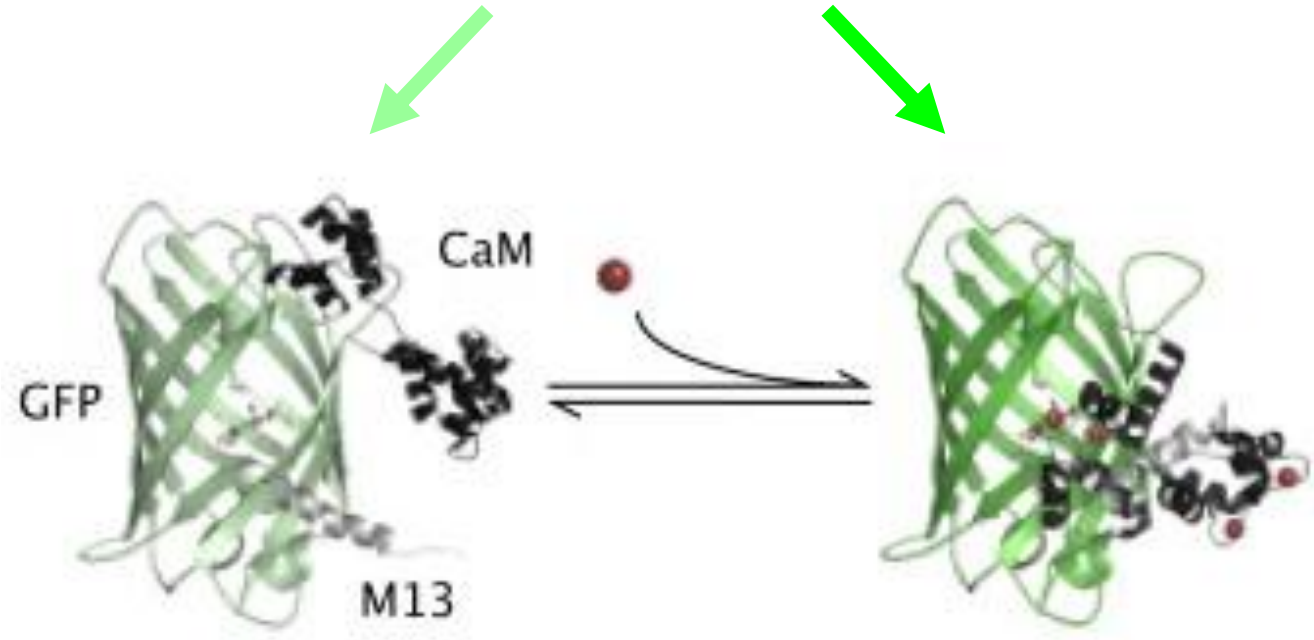
a) On the properties of the fluorophore

b) On the properties of the imaging set up

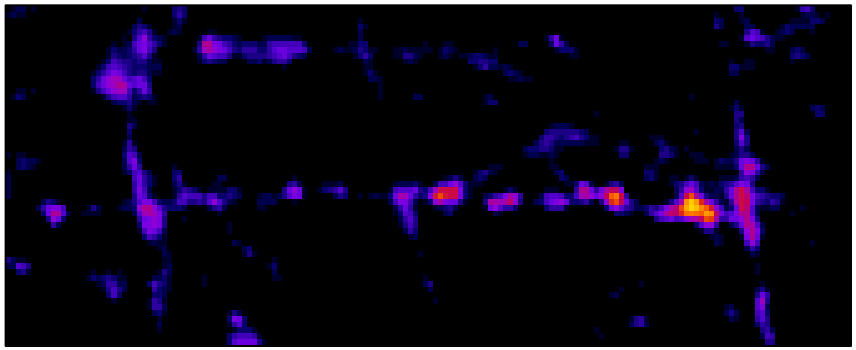
c) On the properties of both the fluorophore and the imaging set up

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

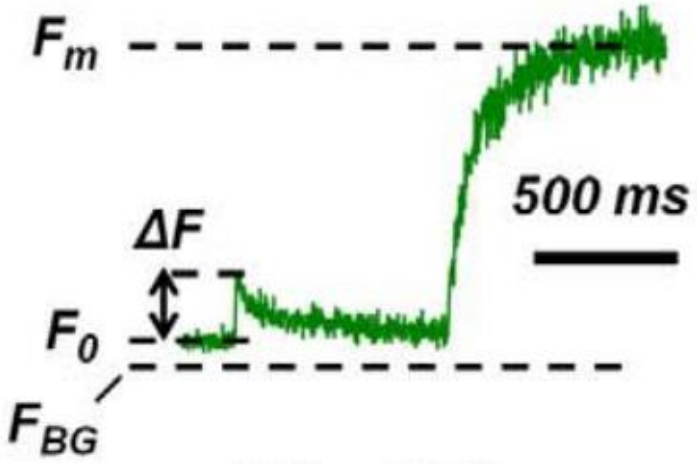
$$F = S_f\ [\mathrm{B}] + S_b\ [\mathrm{CaB}]$$



19) **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.**



20) **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?**



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

22) 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 choose 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?