Neurofunctional Techniques

Lesson 8

30 October 2026

Single compartment model

Ca^{2+} imaging

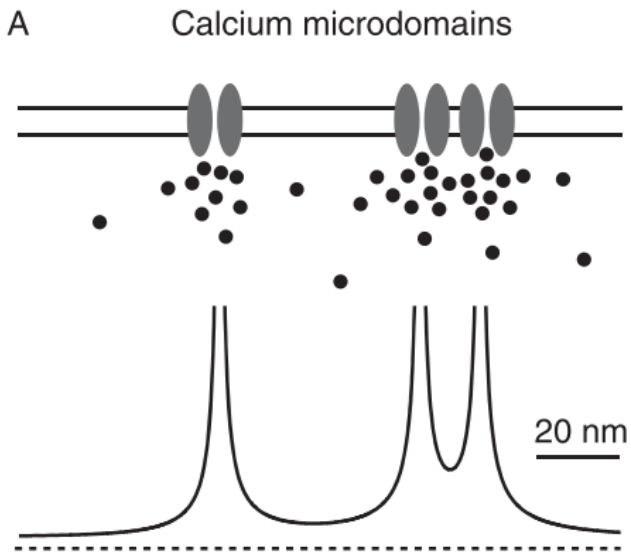
- Review Ca^{2+} signaling
- Ca^{2+} indicators
- Ca^{2+} binding
- Ca^{2+} diffusion
- Ca^{2+}-dependent fluorescence properties
- **Simplified models of Ca^{2+} dynamics**
- **Imaging and network activity**

Objectives

1. Integrate the concepts of Ca^{2+} influx, buffering and extrusion into a simple quantitative model.

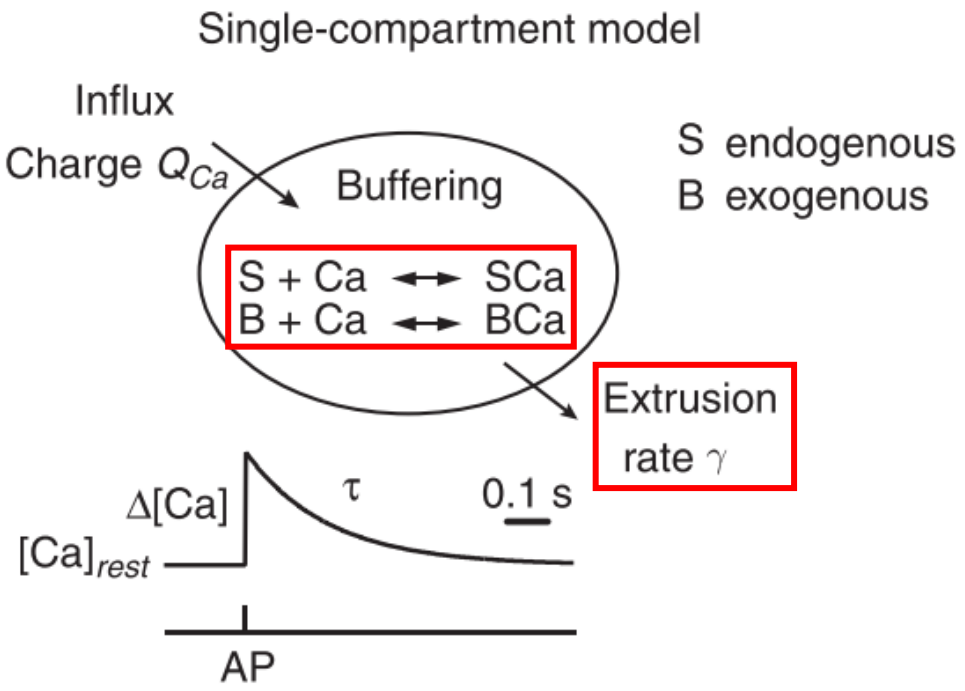
2. Understand how mathematical models and experimental measurements can be combined to estimate physiological parameters that cannot be measured directly

Simplified models of Ca^{2+} dynamics



Single-compartment model in nerve terminals and dendritic spines

- **It treats a cellular compartment as a homogeneous well-mixed compartment (diffusion is neglected),**
- **containing fast non-saturating buffers (including exogenous Ca^{2+} indicators)**
- **as well as a simple liner extrusion mechanism (γ)**



- **Rapid (< 1 ms) and local (< 100 nm) actions of Ca^{2+} near the site of entry are not addressed by this model; rather the model describes longer (>10 ms) homogeneous Ca^{2+} signal (the so called residual free Ca^{2+}), detected by standard Ca^{2+} imaging**

What happens to Ca^{2+} entering the compartment following the opening of Ca^{2+}-permeable channels?

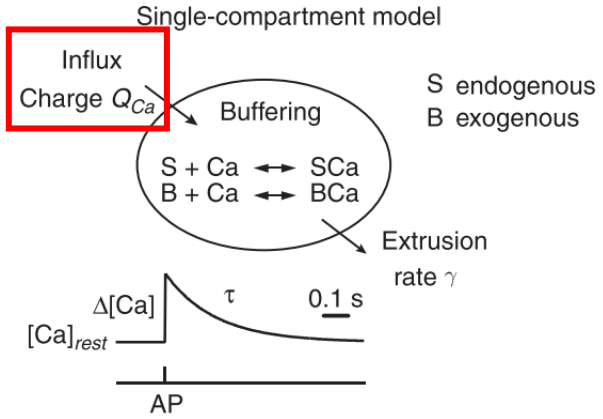
(We limit our attention to brief pulses of Ca^{2+} compared to Ca^{2+} clearance; e.g. action potentials ~1 ms, Ca^{2+} clearance is at least 10 ms)

?

$$\Delta [\mathrm{Ca}]_T = \Delta [\mathrm{Ca}] + \sum_i \Delta [\mathrm{CaB}_i]$$

?

$$\kappa_B = \frac{\partial [\mathrm{CaB}]}{\partial [\mathrm{Ca}]}$$



$$\Delta [\mathrm{Ca}] = \frac{\Delta [\mathrm{Ca}]_T}{(1 + \sum_i \kappa_{B,i})}$$

?

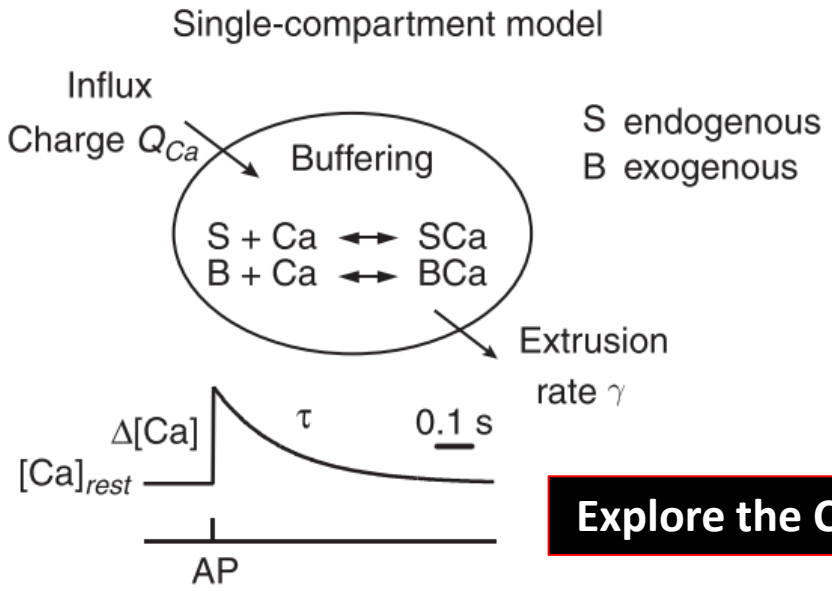
The change in free $[Ca^{2+}]$ is equal to the change in total $[Ca^{2+}]$ divided by 1 plus the sum of all Ca^{2+}-binding ratios

F=Faraday constant=electric charge per mole = $e*N_A$ = $1.60*10^{-19}C * 6.02*10^{23}mol^{-1}$ = 96485 C mol^{-1}

V = volume of the cellular compartment

Q_{Ca} = Ca^{2+} charge injected

Are Ca^{2+} indicators deforming Ca^{2+} signals?



$$\Delta[\mathrm{Ca}] = A = \frac{Q_{Ca}/(2FV)}{(1 + \kappa_S + \kappa_B)}$$

Explore the Ca^{2+} model

$$\tau = \frac{(1 + \kappa_S + \kappa_B)}{\gamma}$$

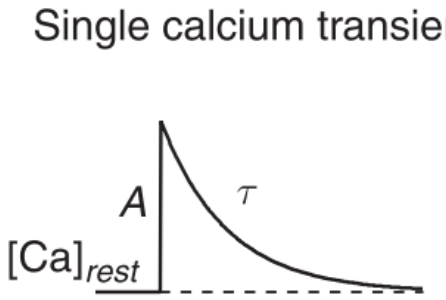
1) **A large total Ca^{2+}-binding ratio ($\kappa_B + \kappa_S$) reduces amplitude (A) and slows down decay time constant (τ) of $\Delta[Ca^{2+}]_{free}$**

2) **It is the relative amount of added buffering capacity (and not its absolute value) that determines how strongly the Ca^{2+} indicator will deform the Ca^{2+} signal. We want $\kappa_B << \kappa_S$**

Are Ca^{2+} indicators deforming Ca^{2+} signals?

(i) following a single AP

$$A = \frac{Q_{Ca}/(2FV)}{(1 + \kappa_S + \kappa_B)} \qquad \tau = \frac{(1 + \kappa_S + \kappa_B)}{\gamma}$$



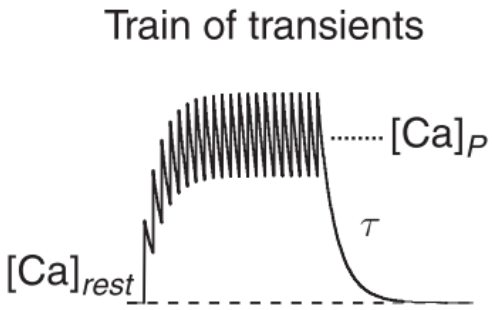


A*τ = the integral of Ca^{2+} transient (the area under the curve) does not depend on κ_B

Are Ca^{2+} indicators deforming Ca^{2+} signals?

(ii) following a train of APs

Because AP-evoked Ca^{2+} transients are slow (tens of ms), individual Ca^{2+} transients summate during trains of action potentials reaching a plateau with influx and extrusion balancing each other.



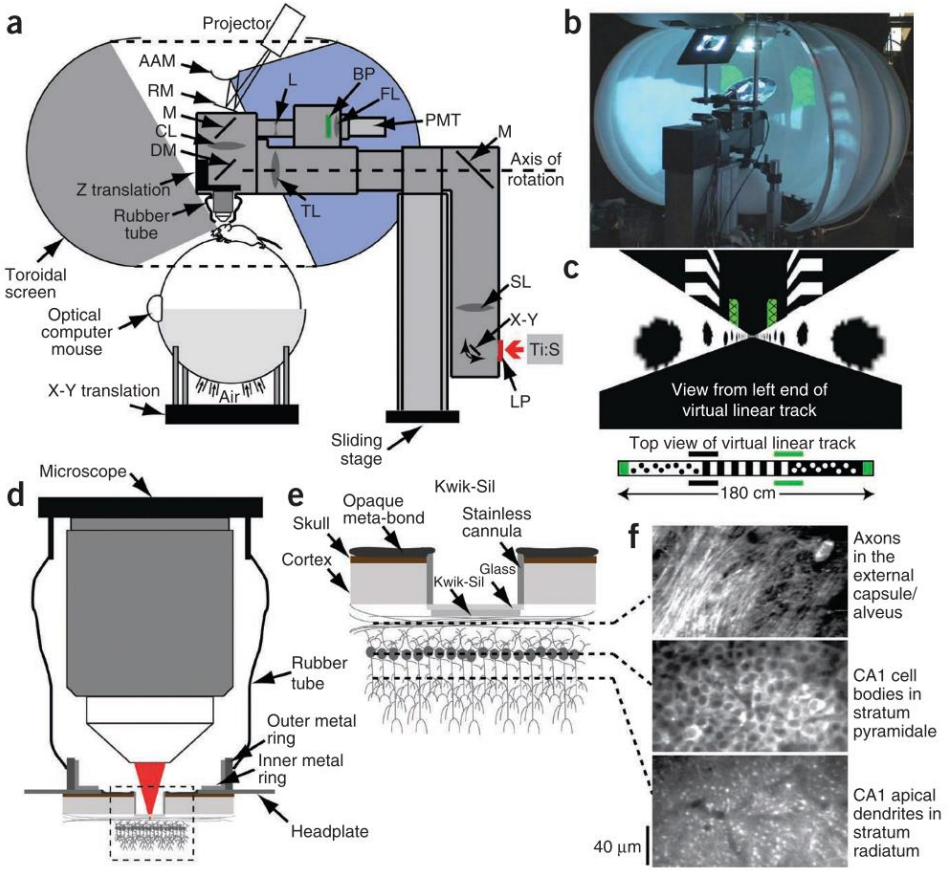
The plateau P is equal to AP frequency (f) times the integral of the single transient (A*τ).

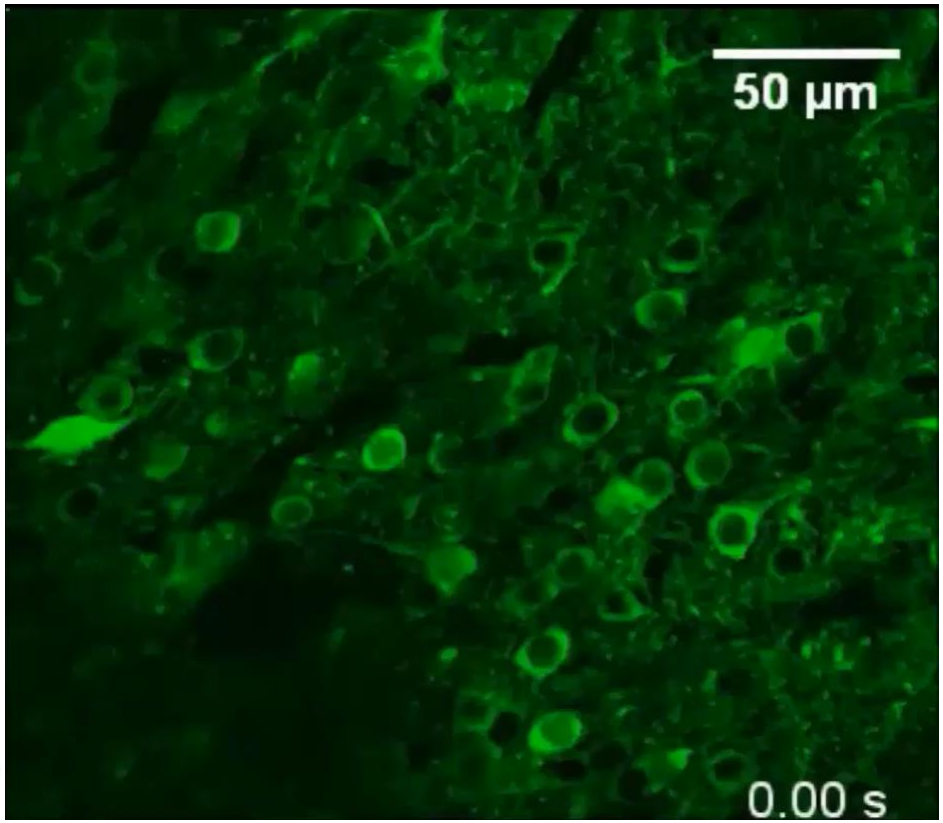
$$P = A\tau f$$

$$A = \frac{Q_{Ca}/(2FV)}{(1+\kappa_S+\kappa_B)}$$

$$\tau = \frac{(1+\kappa_S+\kappa_B)}{\gamma}$$

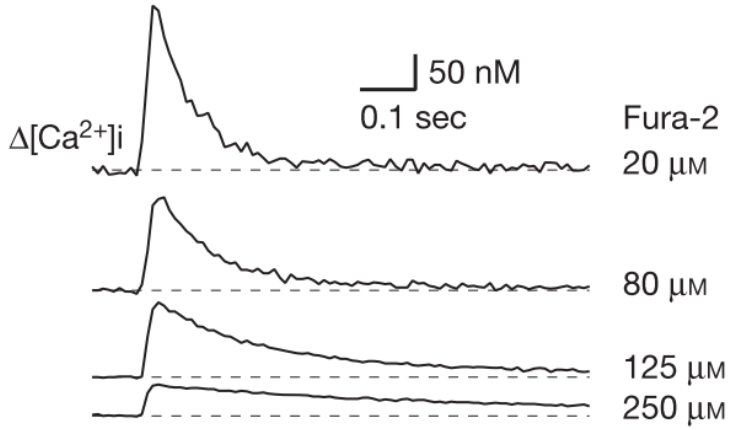
Ca^{2+} imaging as a proxy for activity patterns



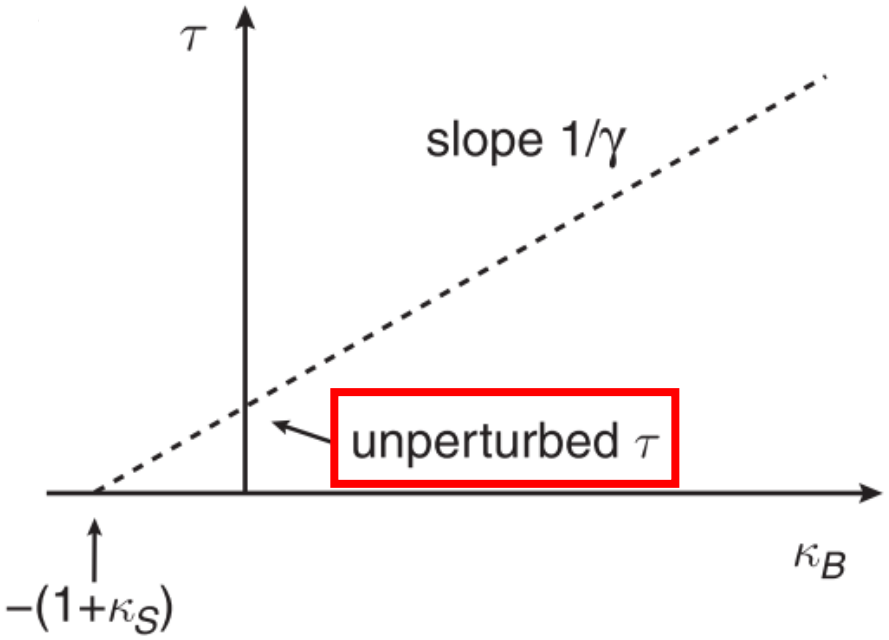


How to estimate (i) extrusion rate γ, (ii) endogenous κ_s and (iii) unperturbed τ for free Ca^{2+}

$$\tau = \frac{(1 + \kappa_S + \kappa_B)}{\gamma}$$



$$\tau = \frac{1}{\gamma}\kappa_B + \frac{1 + \kappa_S}{\gamma}$$

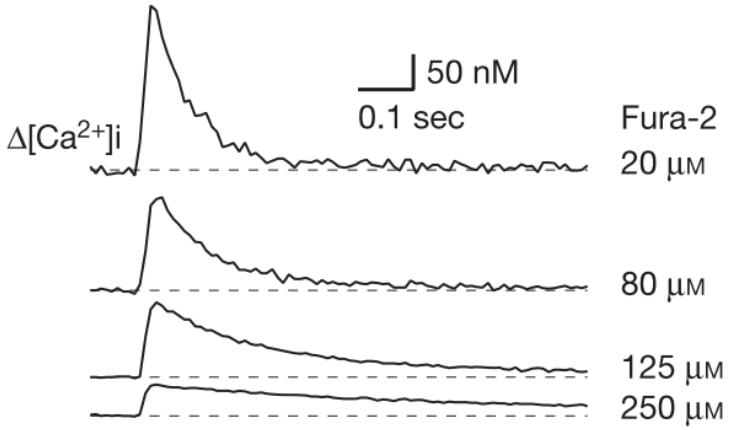


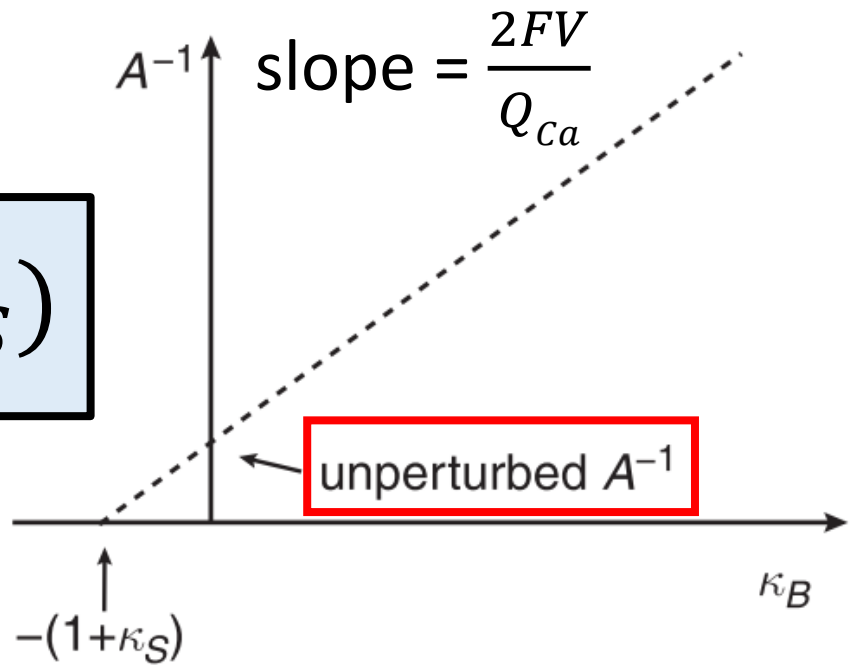
How to estimate (i) total Ca^{2+} influx, (ii) endogenous κ_s and (iii) unperturbed A for free Ca^{2+}

$$A = \frac{Q_{Ca}/(2FV)}{(1 + \kappa_S + \kappa_B)}$$

$$\frac{1}{A} = \frac{1 + \kappa_S + \kappa_B}{Q_{Ca}/2FV}$$

$$\frac{1}{A} = \frac{2FV}{Q_{Ca}}\kappa_B + \frac{2FV}{Q_{Ca}}(1 + \kappa_S)$$





Conclusions

- **Ca^{2+} signals reflect the interplay between influx, buffering and extrusion**
  - **Increasing Ca^{2+} buffering reduces the amplitude and prolongs the decay of single-AP Ca^{2+} transients**
  - **The integral of the transient ($A*\tau$) is independent of buffering**

- **Ca^{2+} imaging provides an indirect readout of neuronal activity.**
  - **Ca^{2+} signals summate during AP trains**
  - **The mean steady-state Ca^{2+} elevation depends on firing frequency, Ca^{2+} influx and extrusion, but not on buffering in this model**

- **Quantitative models help us interpret experimental measurements**
  - **Ca^{2+} indicators perturb the signals they measure.**
  - **By varying indicator concentration and extrapolating to zero added buffering, we can estimate**
    - **endogenous buffering (κ_s)**
    - **total Ca^{2+} influx**
    - **extrusion rate (γ)**
    - **unperturbed free Ca^{2+} transients**

Some examples….

1) Layer V pyramidal neurons have an endogenous Ca^{2+}-binding ratio (κ_s) of 120. Immediately after 1 action potential (before extrusion mechanisms are significantly activated) the total increase in Ca^{2+} concentration ($[Ca^{2+}]_T$) is 1.2 µM. How much is the concentration increase in free Ca^{2+} ($[Ca^{2+}]_f$)?

a) 1.2 µM

b) 100 nM

c) 10 nM

d) 1 nM

1) Layer V pyramidal neurons have an endogenous Ca^{2+}-binding ratio (κ_s) of 120. Immediately after 1 action potential (before extrusion mechanisms are significantly activated) the total increase in Ca^{2+} concentration ($[Ca^{2+}]_T$) is 1.2 µM. How much is the concentration increase in free Ca^{2+} ($[Ca^{2+}]_f$)?

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d) 1 nM

2) To monitor the firing properties of a layer V pyramidal neuron (exhibiting the same characteristics as in question (1)), you use Fluo-4 (K_d = 350 nM) at a concentration of 250 µM. According to the single compartment model, how large do you expect the Ca^{2+} signal to be in response to 1 action potential?

3) **According to the single compartment model, which of the following factors determine the decay time constant (τ) of a Ca^{2+} transient recorded in a dendritic spine in response to a single action potential?**

a) **The K_d of the Ca^{2+} indicator used to monitor the Ca^{2+} transient**

b) **The volume of the spine**

c) **The number of voltage-gated Ca^{2+} channels (VGCCs) in the spine**

d) **The open probability of these VGCCs**

e) **The number of plasma membrane Ca^{2+} ATPases (PMCAs) in the spine**

f) **The level of activity of these PMCAs**

g) **The concentration of calbidin in the spine**

4) According to the single compartment model, which of the following factors determine the Ca^{2+} plateau level reached in a dendritic spine in response to 20 action potentials delivered at 200 Hz?

a) The K_d of the Ca^{2+} indicator used to monitor the Ca^{2+} transient

b) The volume of the spine

c) The number of voltage-gated Ca^{2+} channels (VGCCs) in the spine

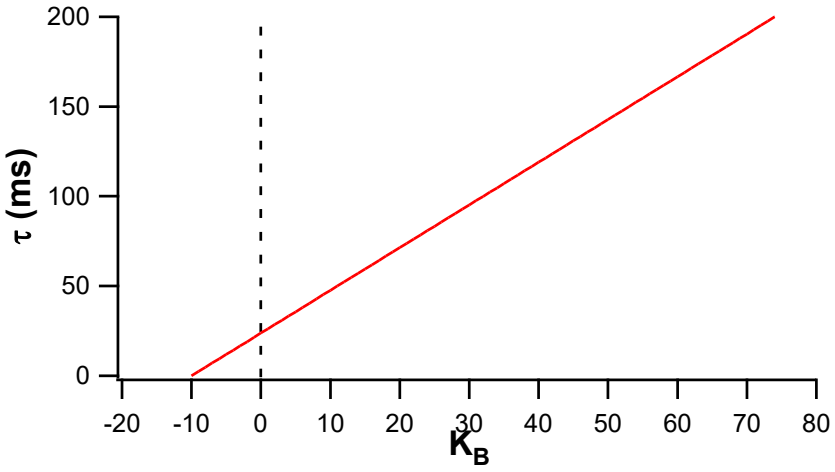
d) The open probability of these VGCCs

e) The number of plasma membrane Ca^{2+} ATPases (PMCAs) in the spine

f) The level of activity of these PMCAs

g) The concentration of calbidin in the spine

5) To determine the endogenous Ca^{2+}-binding ratio (κ_s) in dendritic spines of CA3 hippocampal pyramidal neurons, Ca^{2+} imaging experiments are performed with progressively lower concentrations of Fura-2 so to build a plot of the decay time constant (τ) of the Ca^{2+} signal vs. the exogenous Ca^{2+}-binding ratio (κ_B). Can you determine the endogenous Ca^{2+}-binding ratio (κ_s) of dendritic spines in CA3 hippocampal pyramidal neurons from the plot below?



6) To compare the unperturbed Ca^{2+} signal in the presynaptic boutons of dentate gyrus granule cells (red in the plot) to those of CA1 pyramidal neurons (black in the plot), Ca^{2+} imaging experiments were conducted with progressively lower concentrations of Fura-2 so to build a plot of the reciprocal of the Ca^{2+} signal amplitude (A^{-1}) vs. the exogenous Ca^{2+}-binding ratio (κ_B) for the two bouton types.

a) Which bouton type has the highest unperturbed Ca^{2+} signal?

b) Assuming that the two bouton types have the same volume, which one experiences the larger total Ca^{2+} increase?

c) Can you estimate the total Ca^{2+} concentration ($[Ca^{2+}]_T$) reached in the two bouton types?

