

**Homework 3 – Ca<sup>2+</sup> entry pathways (posted 4/6/06; due 4/14/06)**

1. Assume that 2000 Ca<sup>2+</sup> channels open in a cell with a volume of 10<sup>-9</sup> ml and a resting Ca<sup>2+</sup> concentration of 100 nM. Each Ca<sup>2+</sup> channel passes 10<sup>6</sup> Ca<sup>2+</sup> ions per second. For how long would the channels need to stay open in order to raise the cytosolic Ca<sup>2+</sup> concentration to 5 μM?
  
2.
  - (a) Describe the experiments that you would perform, in as much detail as necessary, to distinguish a low-threshold from a high-threshold Ca<sup>2+</sup> current.

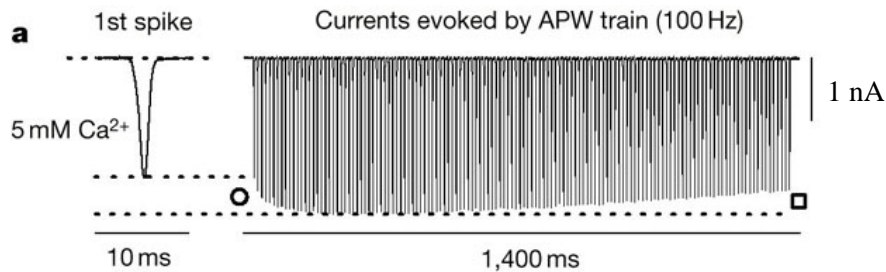
(b) Equations for three different Ca<sup>2+</sup> currents are given below:

$$I_{Ca1} = m^2 h_v h_{Ca} I_{Ca1}(V)$$

$$I_{Ca2} = m^2 h_v I_{Ca2}(V)$$

$$I_{Ca3} = m^2 h_{Ca} I_{Ca3}(V)$$

- (i) Briefly discuss each term in the above equations.
  - (ii) Which equations describe the two principal types of voltage-gated Ca<sup>2+</sup> channels found in canine atrial myocytes.
  - (iii) Describe how you would use ion substitution experiments to distinguish the three different types of channels in voltage clamp experiments.
  
3. The figure below shows two interesting forms of ionic current plasticity when recombinant P/Q type Ca<sup>2+</sup> channels are subject to a train of action potential waveforms (APW), with 5 mM Ca<sup>2+</sup> as charge carrier and minimal buffering capacity (0.5 mM EGTA) in the intracellular solution. The ionic currents first display an increase in current amplitude (facilitation) followed by a decrease (inactivation) at later time periods.

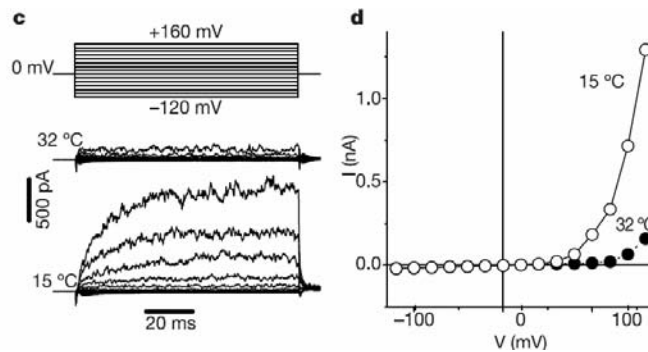


For each of the following conditions draw how you would expect the APW-evoked P/Q-type currents to change. Briefly explain your answers (in 1-2 sentences).

- (a) 5 mM Ba<sup>2+</sup> is used as charge carrier.

- (b) Cells are transfected with mutant CaM<sub>1234</sub>.
- (c) Cells are treated with saturating concentrations of calmidazolium, an inhibitor of Ca<sup>2+</sup>-CaM.
- (d) 10 mM BAPTA is included in the intracellular solution.
- (e) Cells are transfected with CaM<sub>34</sub>.

4. The figure below shows current records and *I-V* relations for TRPM8-currents measured in inside-out patches at two different temperatures.



- (a) What is the mechanism underlying the outward rectification seen with the TRPM8 channel?
- (b) Briefly outline and explain the experimental evidence for your answer in (a).
- (c) Propose another possible mechanism for the outward rectification and describe an experiment to test your proposal.
- (d) What is the mechanism underlying the thermo-sensitivity of afferent neurons expressing TRPM8 channels?