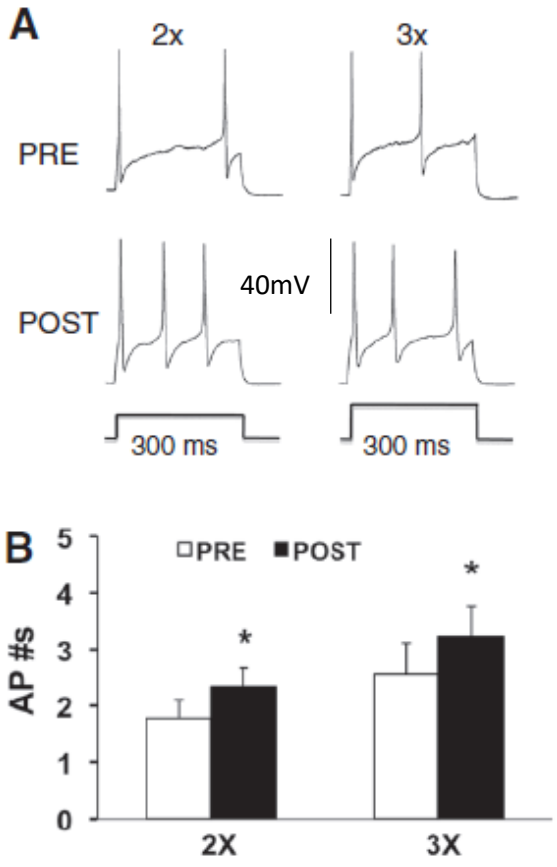
Supplementary Material

Schaefer J. Neurobiology 320

In class sessions preceding this Understanding Checkpoint, students discussed the following concepts, techniques, and publication:

- Membrane potentials
- Electrochemical gradients
- Action potentials
- Channels and gating
- Current and voltage clamp
- Immunostaining
- Hot plate test, electronic Von Frey
- Huang F, Wang X, Ostertag EM, Nuwal T, Huang B, Jan YN, Basbaum AI, Jan LY (2013) TMEM16C facilitates sodium-activated potassium currents in rat primary sensory neurons and regulates pain processing. Nat Neurosci 16:1284-1290.

Example: Understanding Checkpoint for cellular neurophysiology unit



The following figures and legends were modified from Feng X, Zhou YL, Meng X, Qui FH, Chen W, Jiang X, Xu GY (2013) Mol Pain 9:4.

Figure 1. The data shown at left were obtained from whole cell patch clamp recordings of cells cultured from the trigeminal ganglion of adult rats. The trigeminal nerve is a cranial nerve that carries sensory and motor axons between the face and brainstem. The trigeminal ganglion is the trigeminal nerve equivalent of the dorsal root ganglion in spinal nerves. The recordings at left are from trigeminal ganglion cell bodies before (pre) and after (post) application of a chemical suspected to induce inflammation and nociception (chemical Y).

A) Images of actual recordings. 2X and 3X refer to injected current amplitude (2X current required to elicit an action potential and 3X current required to elicit an action potential).

B) Summary data of recordings. (n = 6; *p < 0.05).

Question 1: Diagram at a cellular level the experimental setup used to obtain these recordings. Clearly label each component of the diagram and clearly indicate the stimulus required to generate the responses measured by the amplifier/electrode. Label your diagram as “current-clamp” or “voltage-clamp”.

Question 2: What effect did chemical Y have on neuron function? Explain your rationale.

Question 3: How would the amplitude of these action potentials change if the sodium concentration in the intracellular fluid were increased? Diagram the expected change on one of the recordings in figure 1. Then, explain your rationale based on electrochemical gradients below (a diagram of the relevant gradients is suggested for clarification of your explanation).

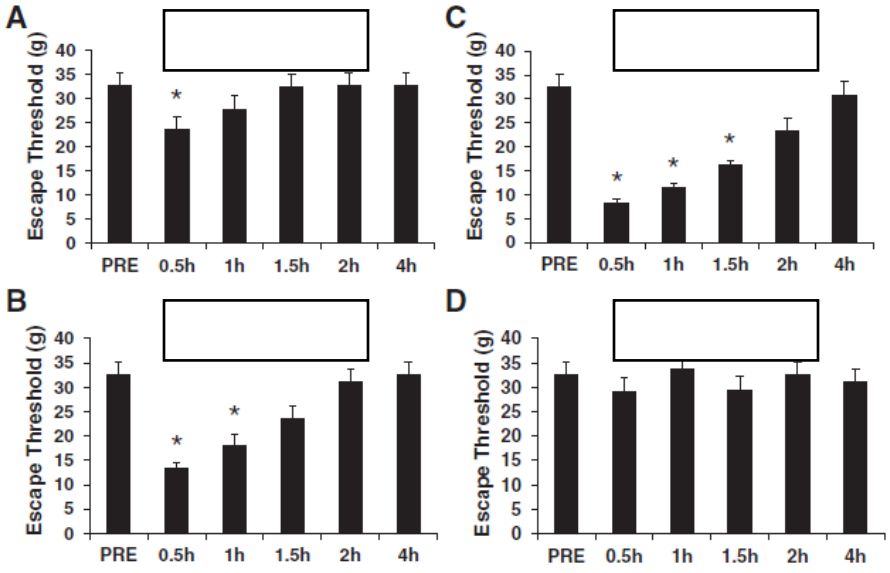


Figure 2. Subcutaneous (under skin) injections of normal saline, low-dose chemical Y, medium-dose chemical Y, and high-dose chemical Y were given to rats. Escape threshold against a painful mechanical stimulus was measured at various time points after injection.

Question 4: Using your knowledge from Figure 1, predict whether A, B, C, and D are normal saline, low dose, medium dose, or high dose injections. Write your predictions in the open boxes above the data. Explain your rationale below, referencing data from Figure 1.

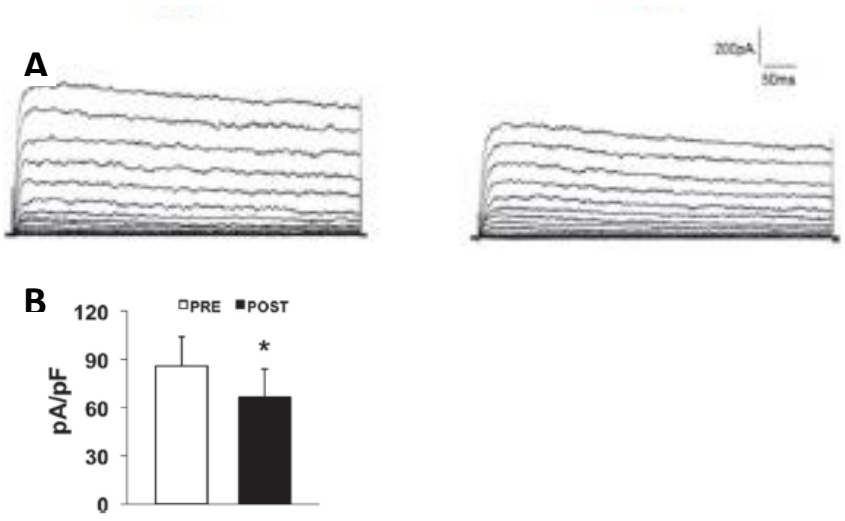


Figure 3. Currents were measured in response to 400msec voltage steps from -40mV to +30mV in increments of 5mV.
A) Example recordings. Scale inset indicates magnitude of a 200pA y-axis and a 50msec x-axis.
B) Current density was calculated by dividing the peak current measured in each cell by the cell's membrane capacitance (estimate of membrane surface area, measured in picofarads, pF)

Question 5: Do these data support or contradict the previous data? Your answer should include a clear explanation of the findings of figure 3, including identification of the ionic current being measured.

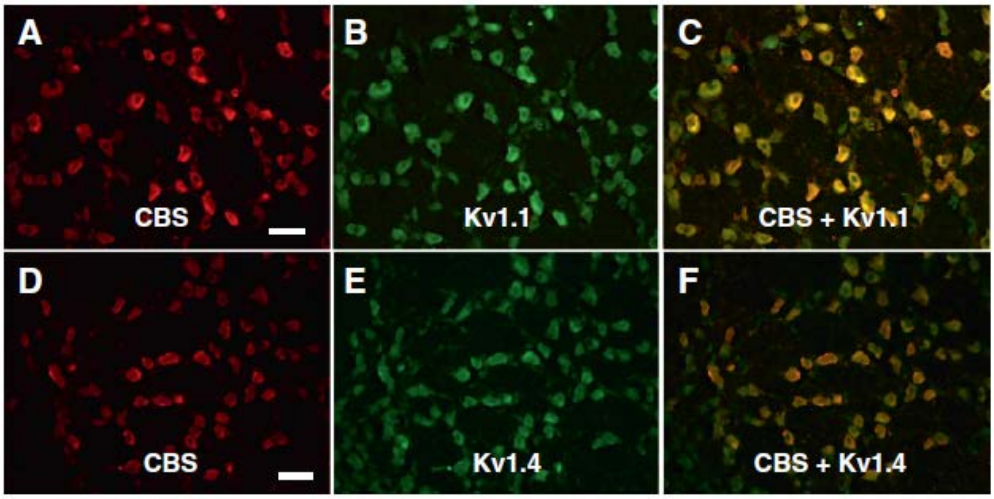


Figure 4. Antibodies to the enzyme that produces chemical Y (CBS--red) and to voltage-gated potassium channels (Kv--green) were applied to the trigeminal ganglion.

Question 6: What do the data in figure 4 tell you? Explain your rationale.

Question 7: Propose a treatment for hyperalgesia based on the data presented above. Explain your rationale for the proposed treatment citing data from figures 1-4.