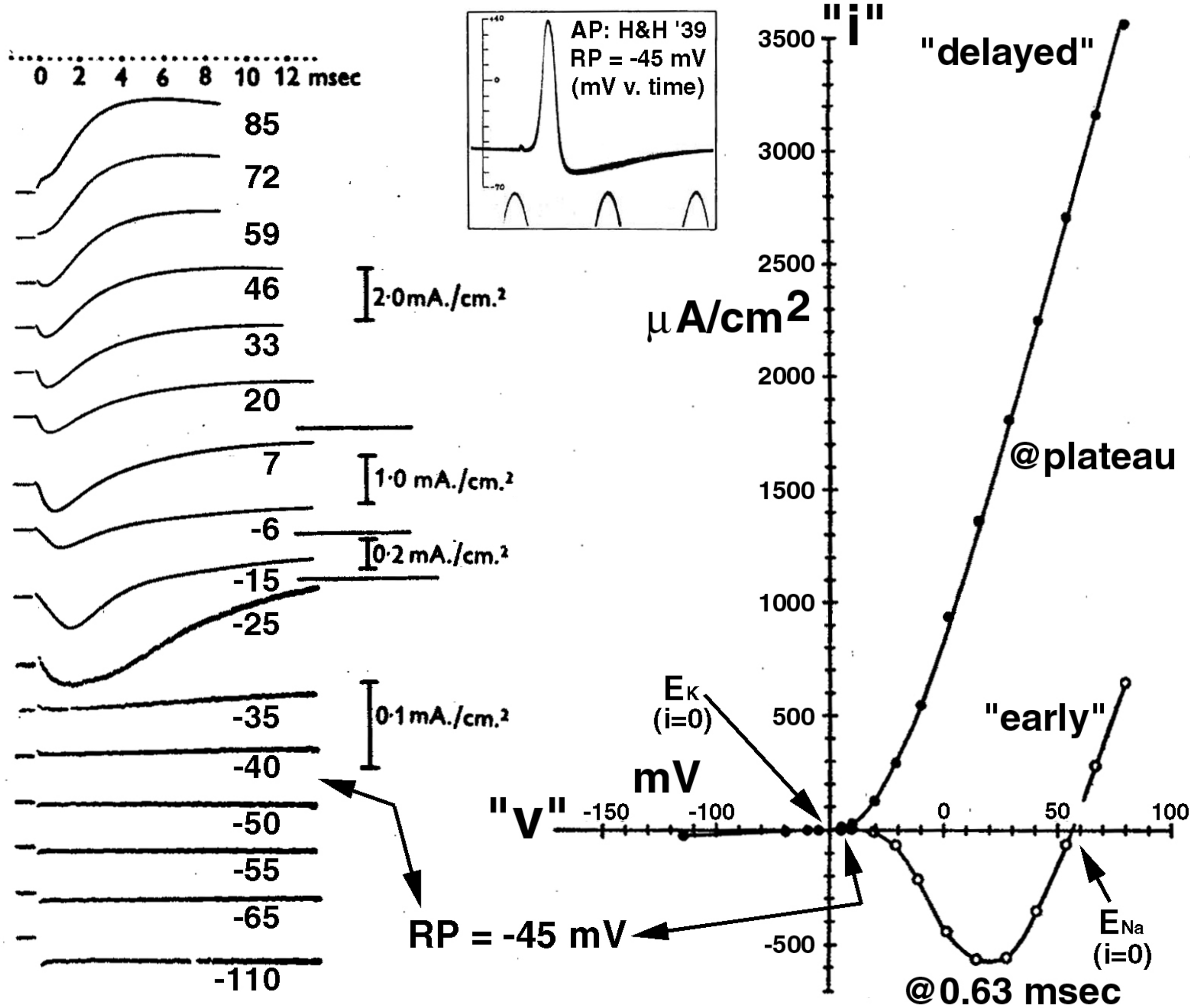
Voltage Clamp Experiments, Squid Giant Axon

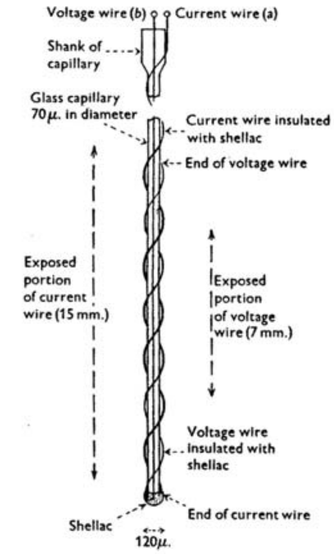
Hodgkin AL, AF Huxley & B Katz (1952) Measurement of current-voltage relations in the membrane of the giant axon of *Loligo. J. Physiol.* 116, 424-448.

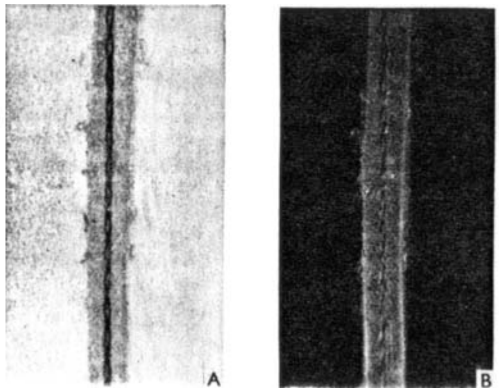


(A): Voltage clamp study of squid axon: record change in current v. time while clamping ("holding") Vm at the indicated voltages (mV). At Resting Potential (RP, -45mV) and more negative Vm ("hyperpolarized"), very slight inward current. With small depolarization (less negative Vm,-35mV), there was a slight outward current. At 20mV above RP (-25 mV), there was an "early" and large inward current followed by a "delayed" outward current (THRESHOLD for activating Action Potential (AP). As Vm increased (more positive), "early" current declined and then reversed; however, "delayed" current continued to increase.

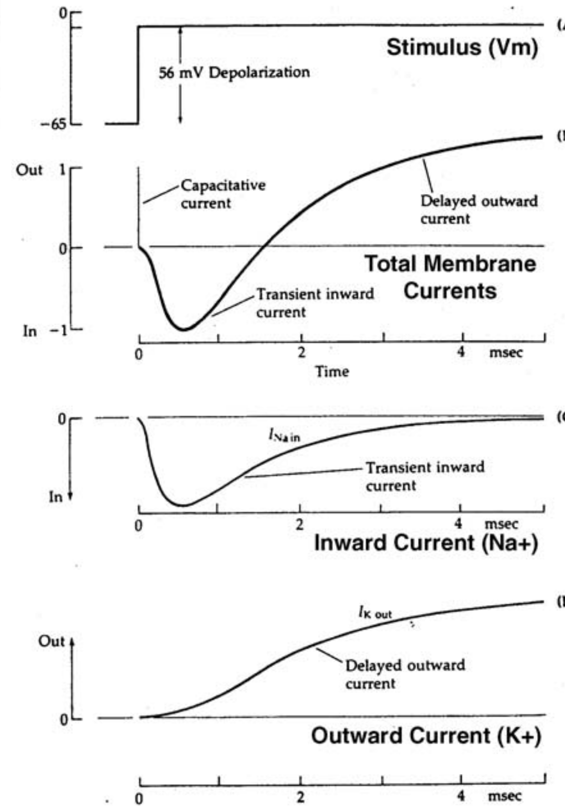
(B): Analysis of data using "i-v" plot (i=current, y-axis; v-=volts, x-axis). "Early" currents (@ 0.63 msec) are inward, increasing to max at 10mV, decreasing to zero at +54mV and reversing (outward) above +54mV. "Delayed" currents increase with Vm. The "reversal potential" for the "early" current equals the Nernst potential for Na^+; the "early" current was predicted to be a Na^+ current. The Vm where the "delayed" current is zero equals the Nernst potential for K^+. A device with unidirectional current flow is called a "rectifier"; the ion channels responsible for the "delayed" current are known as "delayed rectifier", or "K^+_{DR}". Note, both currents are activated at the same threshold voltage. Note, the "early" current (Na^+) spontaneously inactivates, while the "delayed" current does not.

HH&K predicted the existence of Na^+ and K^+ channels based on these studies, plus studies where ions were replaced. Hille confirmed these predictions using specific channel blockers (see next page).



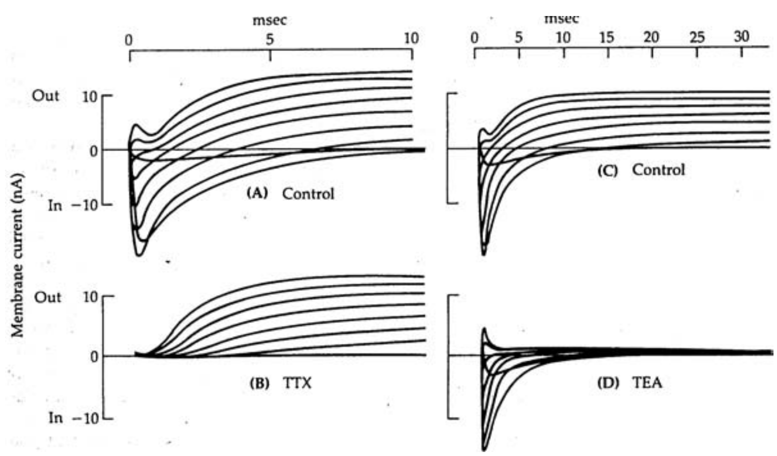


[1 - left] HH&K inserted this voltage clamp electrode into the giant axon of the nerve. Nerve contains many axons (see Young, 1936 paper for nerve profiles). Both axon and electrode were about 0.1 mm diameter.



[2 - right] The result of HH&K's studies are summarized at right. Depolarizing the membrane induces a transient current consisting of two components, and early, inward and self inactivating Na^+ current and a delayed, outward K^+ that only inactivates once the depolarizing stimulus (A) is turned off.

[3 - below] The predictions of HH&K were confirmed by B.Hille (1970, Ionic channels in nerve membranes. *Prog. Biophys. Mol. Biol.* 21, 1-32). By then it was known that TTX (pufferfish tetrodotoxin) blocked Na^+ conductance and TEA (Triethylamonium ion) blocked K^+ conductance. Hille performed voltage clamp experiments demonstrating that axons treated with TTX only displayed the delayed outward current and axons treated with TEA only displayed the self inactivating inward current. Notice the difference between the control (upper) and experimental (lower) experiments. Normal for a voltage clamp experiment, Hille observed the behavior of the membrane (current) over a series of voltages.



[4 - right] Armstrong and Bezanilla (1977, Inactivation of the sodium channel II. Gating current experiments *J. Gen. Physiol.* 70, 567-590) proposed a model of how the sodium channel might work (7 years before one was cloned). They proposed a protein that passed through multiple conformational (and energy) states, including a scheme where part of the channel protein plugs the actual channel, inactivating it while still open, a mechanism shown later to be largely true.