

## BJ Classics

Enhancing the Hodgkin-Huxley Equations: Simulations Based on the First Publication in the *Biophysical Journal*John W. Moore<sup>1,\*</sup><sup>1</sup>Department of Neurobiology, Duke University Medical Center, Durham, North Carolina

**ABSTRACT** The experiments in the Cole and Moore article in the first issue of the *Biophysical Journal* provided the first independent experimental confirmation of the Hodgkin-Huxley (HH) equations. A log-log plot of the K current versus time showed that raising the HH variable  $n$  to the sixth power provided the best fit to the data. Subsequent simulations using  $n^6$  and setting the resting potential at the in vivo value simplifies the HH equations by eliminating the leakage term. Our article also reported that the K current in response to a depolarizing step to ENa was delayed if the step was preceded by a hyperpolarization. While the interpretation of this phenomenon in the article was flawed, subsequent simulations show that the effect completely arises from the original HH equations.

**Retrospective**

In 1958, K.S. (Kacy) Cole helped to found the Biophysical Society, and two years later he was delighted to have our article (1) be the very first in the then-new *Biophysical Journal* established by the Society. Being the only lab at that time with a voltage-clamp (aside from that of Hodgkin and Huxley), we had undertaken these experiments to examine the Hodgkin and Huxley description of the K currents (2) in the squid giant axon as a power of their variable  $n$  (3).

Hodgkin and Huxley (2) had used a current-subtraction method to separate Na and K currents flowing in response to a depolarizing step; by substituting choline for Na they could isolate the K current at different membrane potentials. We used a different approach from theirs, isolating the K current by setting the test voltage-clamp pulse to ENa where no Na current would flow. Then a pretest pulse could be varied in amplitude and duration, and the K current in response to the test pulse could be observed under a wide range of clamping paradigms. I had joined Kacy's lab almost a decade earlier with a knowledge of circuitry and operational amplifiers learned at RCA from Art Vance, a master engineer (4). I had refined our voltage clamp over that time so that we had excellent voltage control without oscillations.

The Hodgkin-Huxley (HH) equation for the K current incorporated the parameter  $n$  to represent the probability of the K "gates" being open. In their quantitative article (3), Hodgkin and Huxley discuss their plots of K conductance versus time associated with different depolarizations and the fit of the calculated curves to the experimental observations when  $n$  is raised to the fourth power. They noted that "it will be seen that there is reasonable agreement be-

tween theoretical and experimental curves, except that the latter show more initial delay. Better agreement might have been obtained with a fifth or sixth power but the improvement was not considered to be worth the additional complication" (3). To appreciate their "additional complication," one must realize that the numerical integrations of the HH equations were done manually by Huxley—with pencil, paper, and a Brunsviga hand-cranked calculator; the Cambridge University computer was unavailable, undergoing major modifications (5).

Richard Fitzhugh, a sophisticated mathematician in our lab, suggested that we plot the log of the K current against the log of the time to determine whether the K current could truly be a function of the single parameter  $n$ . He argued that a fit to our data would both validate Hodgkin and Huxley's use of the variable  $n$  and indicate the power to which it should be raised. Fig. 1 shows our log-log plot of current versus time from Cole and Moore (1), a plot that indeed validated the use of the first-order equation raised to a power, satisfying Fitzhugh. Further, it showed that a good fit to our data points was obtained with  $n$  raised to the sixth power. In this figure we also plotted Hodgkin and Huxley's data as circles, and found that these points were even closer than ours to the theoretical line for  $n$  raised to the sixth power. In the Cole-Moore discussion, however, the reader finds Kacy's curious statement that "the approximation to a sixth power function is not particularly important" (1). Now that computer simulations can replace manual numerical integration, they show that replacing  $n^4$  with  $n^6$  indeed is important, and reveals the full power of the HH equations.

**Replacing  $n^4$  with  $n^6$  in simulations**

Hodgkin and Huxley, using manual numerical integration and limited to raising  $n$  to the fourth power, were able to fit experimental data surprisingly well for membrane

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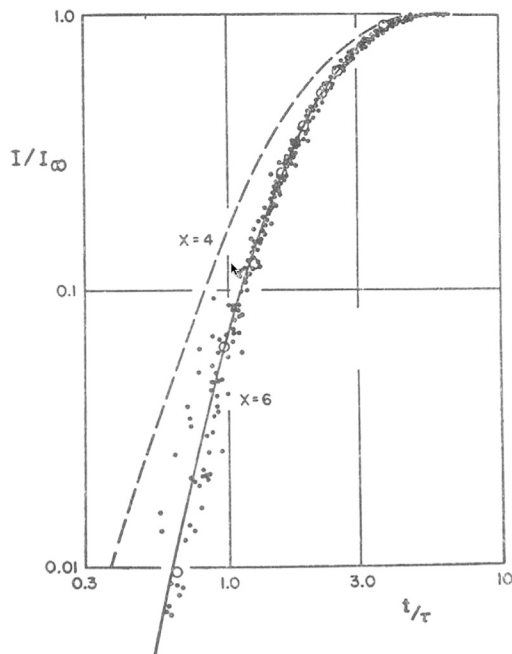


FIGURE 1 Fig. 2 from Cole and Moore (1) reprinted with the permission of the author. The K current,  $I$ , is plotted as a function of time,  $t$ , after a step from near the resting potential to ENa.  $I_{\infty}$  is the steady-state current and  $\tau$  is the time constant of the process. (Points are from four 1956 axons; circles are from Fig. 3 in Hodgkin and Huxley (3).) (Solid line)  $I = I_{\infty} [1 - \exp(-t/\tau)]^6$ ; (broken line)  $I = I_{\infty} [1 - \exp(-t/\tau)]^4$ .

voltages well above the resting level. However, calculations with  $n^4$  severely reduced the accuracy of the equations near the resting level, causing the K current to be much larger than the Na current. This forced Hodgkin and Huxley to add the leakage term to balance the currents at rest. Now, with computers of increasing speed and multiple processors, and with sophisticated software, one can use high-speed numerical integration and easily test the result of increasing  $n$ 's exponent, as Hodgkin and Huxley suggested. To do this I employed NEURON (6), the free simulation environment used widely by computational neuroscientists. NEURON, developed in my lab at Duke University by Michael Hines, has the HH channel types built in and can easily incorporate descriptions of other channels and mechanisms, such as a different exponent for  $n$ . My simulations show that direct substitution of  $n^6$  for  $n^4$  will reduce the amplitude of the K current significantly at  $-65$  mV, the HH resting potential. What does this reduction imply for the leakage current term? To answer this question, I carried out simulations at the in vivo resting potential measured both by Moore and Cole (7) and Hodgkin (8).

### Measuring the in vivo resting potential of the squid axon

In extended voltage-clamp experiments on dissected and cleaned axons, I consistently observed declining amplitudes

of the Na current over time. This decline always required increasing the balancing current to maintain a steady holding potential. This was bothersome, and I was desperate to measure the in vivo resting potential and succeeded in the second summer of trying. I split open the squid's mantle from the jet orifice to the tail and pinned it down so thoroughly on a Sylgard base that the muscle contraction after an action potential no longer broke the microelectrode in the axon. Using this preparation, we found the resting potential to be  $-70$  mV; after an action potential, the membrane repolarized to EK, where the potential remained steady for some tens of milliseconds (7). Later, I found that Hodgkin (8) with Keynes had earlier observed  $-70$  mV as the in vivo resting potential before their microelectrode snapped when the mantle contracted. So I chose to use the  $-70$  mV value in further simulations with  $n^6$ .

### Simulations at the in vivo resting potential and using $n^6$ do not require a leakage term

In simulations with NEURON, I sought to balance the Na and K currents at  $-70$  mV without any additional balancing leakage current by setting the leakage conductance to zero. Then, adjusting the conductance for the K channel, I found that a value of  $0.175$  S/cm<sup>2</sup> allows the new K and Na currents to achieve a precise balance at surprisingly small values ( $26$  pA/cm<sup>2</sup> each). Consequently, using  $n^6$  for the K current renders the leakage term unnecessary, and it can simply be eliminated from the HH equations, making them significantly simpler. Furthermore, given these values of conductance, an action potential calculated with NEURON (Fig. 2) and without the leakage term is remarkably similar to records observed in vivo by both Moore and Cole (7) and by Hodgkin (8). Both the Na and K currents during an action potential are similar in shape but  $\sim 60\%$  larger than with  $n^4$ .

### The original HH equations predict the Cole-Moore effect

The second major finding described in the Cole-Moore article was that the K current was increasingly delayed in onset with longer and stronger prehyperpolarizations (Fig. 5 a of Cole and Moore (1); and see Fig. 3 A). This was certainly a new observation at the time, described by others as the Cole-Moore effect, delay, or shift. Kacy, really a mathematician and rather fixated on curve-matching, was confused about how to describe this observation mathematically. He found that if he assumed that  $n$  had decreased to zero at the end of each prehyperpolarization, then he could fit the different curves in our data (Fig. 7 in Cole and Moore (1)) by raising  $n$  to higher and higher powers as the hyperpolarization was increased. He emphasized this escalation of the power of  $n$  in that article's abstract, where  $n$  is raised to the extraordinary power of 25, a value chosen to fit a

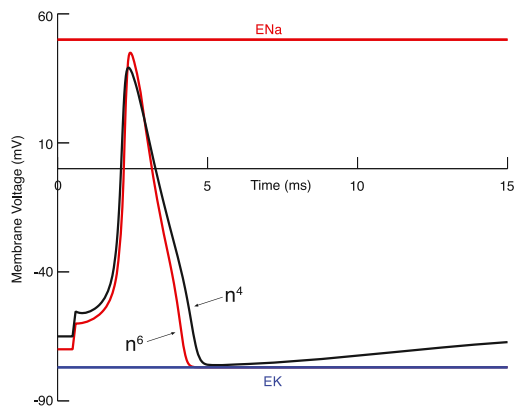


FIGURE 2 Comparison of simulated action potentials using the two powers of  $n$  in the HH equation for the K current. (Black trace) Using  $n^4$  and a resting potential of  $-65$  mV; (red trace) using  $n^6$  and a resting potential of  $-70$  mV. At the end of the downstroke, the  $n^6$  trace hits EK ( $-77$  mV) and continues for the 15-ms duration of the trace. The  $n^4$  trace shows a low of  $-76$  mV and the conventional rapid return to the resting level. The  $n^6$  depolarization eventually has to return to the original resting level of  $-70$  mV after some 200 ms; this is driven by a tiny Na current, which, in turn, causes the K current to follow until they balance. The temperature is the standard HH  $6.3^\circ\text{C}$ .

single record, that for the strongest hyperpolarization,  $-212$  mV.

Simulations show that his assumption was incorrect and that  $n$  does not reach zero, even for the strongest hyperpolarization (Fig. 3 B). Although Kacy and I did the experiments together, and he certainly relied on my instrumentation, he did not involve me in the analysis. Consequently, as junior author, I have never been comfortable with the article's emphasis on the escalating power of  $n$ , taken to an egregious extreme of 25 in that abstract and elsewhere. It was especially disturbing because it was clearly at odds with the power of 6 at all voltages demonstrated in our log-log plot in the very same article (Fig. 1). The term "Cole Moore effect" in squid giant axons should apply only to the experimental observations, not to the equation highlighted in that abstract.

## Summary

The Cole-Moore article was the first experimental confirmation of the HH equations. A log-log plot of the K current versus time indicated that raising the HH variable  $n$  to the sixth power would provide a much better fit than the fourth power to both our data and the HH data. Subsequently, I have found that replacement of  $n^4$  with  $n^6$ , and setting the resting potential at the in vivo value, allows the HH equations to be simplified because the leakage term is eliminated. Furthermore, with  $n^6$ , the shape of the action potential closely approaches that measured in vivo.

The Cole-Moore article also described a delay in the onset of the K current with hyperpolarization preceding a depolarizing voltage pulse. While the observation was cor-

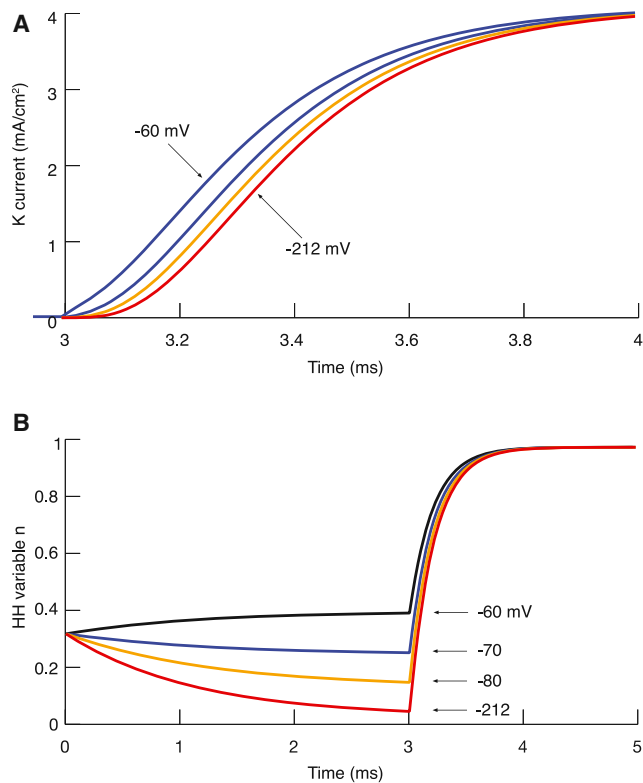


FIGURE 3 The dependence of  $n$  on voltage and time underlies the Cole-Moore shift and is predicted by the original HH equations (using  $n^4$ ). (A) Plots of K currents versus time in response to a voltage step to ENa after the four 3-ms prepulse steps shown in (B). The timescale of (B) is expanded in (A) to 1 ms (between 3 and 4 ms) to show the increasing delay in the onset of the current with increasing prepulse hyperpolarization, known as the Cole-Moore effect, or shift. These currents, plotted by NEURON, are similar to those in Fig. 5 a of the original article (1). (B). A plot of  $n$  versus time during the 3-ms steps preceding the step to ENa that elicited the currents shown in (A). The figure shows that the value of  $n$  increases with depolarization and decreases dramatically with the duration and amplitude of hyperpolarization. The four steps shown were from the resting value of  $-65$  mV to  $-60$ ,  $-70$ ,  $-80$ , and  $-212$  mV.

rect, the article mistakenly proposed that this shift was due to the power of  $n$  increasing with the increasing hyperpolarization. However, using simulations, I have found that this delay is completely incorporated in the original HH equations. I am deeply satisfied to find that the Cole-Moore effect arises from the stalwart, ingenious HH equations without any need for their modification.

I regret that I had not explored these possibilities while Sir Andrew Huxley and I were still corresponding on other issues before his death. It would have been so gratifying to both of us to show him this new level of elegance in their 1952 Opus.

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