ARTICLE
Teaching Neurophysiology to Undergraduates using *Neurons in Action*

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Neurons in Action, a set of 25 hyperlinked tutorials and interactive simulations on CD-ROM, provides the student with a completely different approach to neurophysiology from that of textbooks. Guided by the tutorials, by their professor, by the urge to test their understanding, or simply by curiosity, students specify the parameters of a patch of membrane, an axon, a postsynaptic membrane, or a cell and run virtual experiments. Parameters include geometry, the number and type of ion channels in the membrane, the number of myelin wraps of the axon, the ion concentrations inside and out, synaptic variables, and temperature. Hyperlinked explanations, historical information, and classic papers on the CD provide the "textbook" material. This article describes this learning tool and details several ways in which it is being used at the undergraduate level.

Key words: neuron, simulations, tutorials, virtual experiments, action potential, single channels, cardiac action potential, neuroscience, laboratory, software

INTRODUCTION
What is *Neurons in Action (NIA),* exactly?
In 1992 the first browser, Mozilla, appeared on the scene and the idea of *NIA* popped into the head of my co-author John W. Moore. At that time he had been teaching neurophysiology to graduate and medical students using the simulator, based on the Hodgkin-Huxley equations, that he and Michael Hines had been creating over the previous decade and that they called NEURON. John would drag his big desktop into the lecture hall and show an action potential zipping along a virtual axon, calculated and displayed by NEURON. With a sense of drama and suspense, he would ask, "Now what will happen to the speed of this impulse if I double the axon's diameter?" or "What will happen to its amplitude if the extracellular sodium concentration is halved?" He would have the students discuss it and vote, then he would change the parameter, re-run the simulation, and the students who voted correctly would cheer. The students who got it wrong now got a chance to correct their misunderstanding instead of leaving the lecture with the wrong idea.

John's Mozilla epiphany was this: he could frame his question-centered lectures as tutorials in HTML that Mozilla could display and link to NEURON simulations. It would be a new learning tool, a type of interactive text from which students could learn on their own as they played with NEURON's many parameters, unrestricted by class time. It would liberate the moving, propagating action potential, and even passively spreading voltages, from the static confines of the textbook figure.

Eventually, John persuaded me to help him develop his ideas into a set of tutorials that were organized enough to satisfy a publisher (Andy Sinauer). As we developed the first version of *NIA (NIA1),* I tested the simulations interactively in lectures to students at all levels, including undergraduates. I organized computer lab sessions for a large undergraduate class and shuffled the students through the lab in groups all afternoon. The feedback was positive. I was hooked on this engaging way of teaching and joined my co-author whole-heartedly in making it happen. *NIA1* was published in 2000 and then, with the help of an NSF grant, *NIA2* was published in 2007 with its many new tutorials and more user-friendly interface.
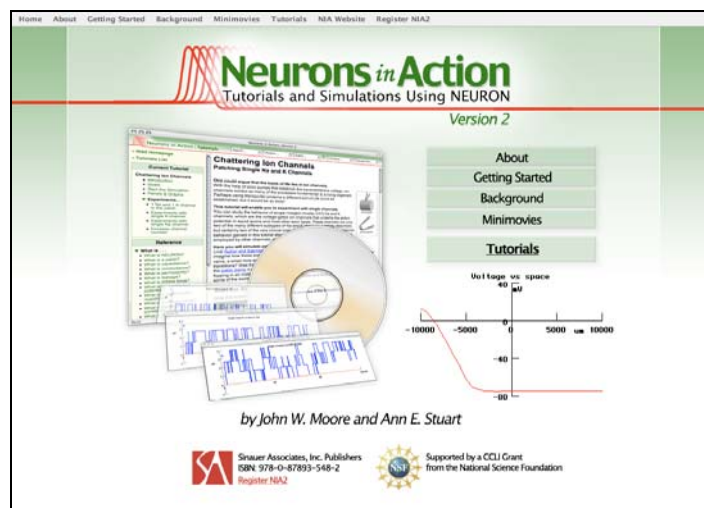

Figure 1. The home page of *NIA2*. Getting Started introduces the user quickly to the layout of *NIA's* panels and graphs and to its interface. Background contains detailed historical narrative. The Minimovies are grouped into Action Potential (with sound), Axon (movies of propagation in various conditions), and Synapse; they may be previewed before importing into a presentation.

WHAT IS ON THE *NIA2* CD-ROM?
NIA does not require an Internet connection because it is self-contained. It runs on either a PC (even one running Vista!) or a Mac. The following explains what is on the CD.

The tutorials plus linked text and graphics.
On the CD are thousands of files of text and graphics that set forth the material of the tutorials and basic neurophysiology. Each tutorial enumerates a set of orderly steps that suggest stimulator and parameter settings for running simulations. Within a tutorial, the student might be asked a question about the results from a simulation; although the answer is provided in a link, the authors hope that the user will first attempt to figure out the answer and

then consult the link to make sure they are correct. Links on the interface to sections for "What is...?" and to Equations as well as Help and History menus (see Fig. 2) provide this material when the student wants it (rather than when the author wants to present it, as in textbooks).
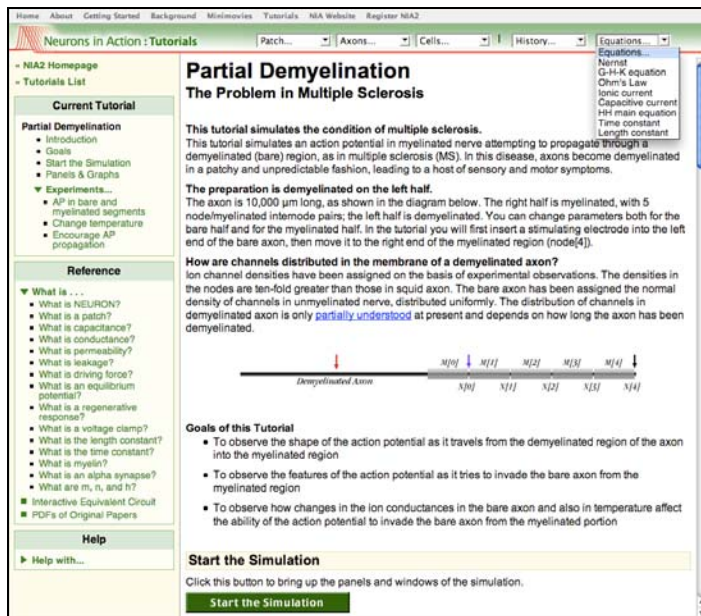

Figure 2. The interface for *NIA2.* Each tutorial appears in the main panel on the right. A graphic within the introduction illustrates whether the recordings are from a patch, an axon (in this case), or a cell, and where the recording electrodes are located. On the top bar, the Equations menu is shown here pulled down. The left side bar give links to "What is..?" fundamental concepts, to an Interactive Equivalent Circuit, and to PDFs of original classic papers. The Help section, when expanded, explains all aspects of the running the simulations.

The browser.
The very first version of *NIA, NIA1.0,* was written so that any browser, primarily Netscape or Internet Explorer at the time, could read the HTML files and instruct NEURON. What a nightmare! Different browsers read the HTML code differently and updates of the browsers became a problem. Starting with version 1.45 (2004) and continuing for later versions, we customized the browser Firefox and included it on the CD. This "*NIA* Firefox" has its own set of preferences; they will not interfere with users' preferences if they are using Firefox as a browser.

NEURON.
Version 5.9 of the simulator NEURON (http://www.neuron.yale.edu/neuron/) is on the *NIA2* CD. NEURON presents vast possibilities from which the simulations of each tutorial have been customized. Thus, for each tutorial, "*NIA* Firefox" launches a screen-filling collection of selected graphs and panels from NEURON (e.g. Fig. 3). The graphs, typically for voltage, current, and conductance, will display the results of the simulation. One of the panels is a "stimulator," where the current clamp or voltage clamp pulse may be controlled. In the tutorials devoted to synaptic transmission, a special synapse panel controls

"transmitter release" from the virtual presynaptic neuron onto the virtual postsynaptic cell. Other panels control the parameters of the experiment: e.g. ion concentration, channel type and density, degree of myelination, geometry, or, for the synapse, the amount and reversal potential of the transmitter and the timing of its release. Finally, a RunControl panel controls the timing, speed and temperature of the simulation.

Although at this writing NEURON is in version 7.0, it is essential that the user not try to download or use later versions than the one on the CD as "*NIA* Firefox" has special instructions on where to find NEURON and how to launch the customized simulations.
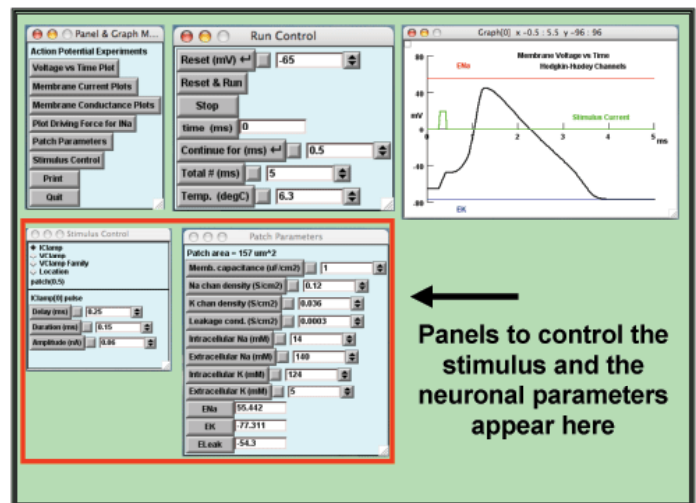

Figure 3. The basic layout of the panels and graphs of *NIA2,* taken from the Na Action Potential. In the upper left quadrant appears a panel that launches other panels and graphs and a panel that runs the simulations. The lower left quadrant contains the stimulus and parameters panels. Graphs (here voltage-vs-time) appear on the right half. Graphs of current- and conductance-vs-time can be launched in this tutorial and will appear below the voltage-vs-time graph.

Minimovies.
In undergraduate introductory lectures there is typically not enough time to run simulations. Yet showing action potentials moving along an axon, or having voltage converted to sound as it is plotted, can be an engaging method for presenting this mysterious material at this level. Consequently, we made 19 "canned" simulations as minimovies that illustrate basic principles of neuronal function. These movies can easily be imported into a PowerPoint or Keynote presentation.

PDFs.
Finally, on the CD is a collection of classic papers of neurophysiology including the Hodgkin-Huxley papers.

THE EVOLUTION OF NIA1 INTO NIA2
The first version of NIA.
NIA1 was an experiment. Comparing the interface of *NIA2* with that of *NIA1* should make that obvious to anyone who has both versions! *NIA1* was published just at the moment

when book publishers, such as Sinauer, were struggling with the new challenges of publishing CDs. By publishing *NIA1*, however, and having people find it useful despite its clunky style, we convinced the NSF to support a version 2. With resources that we did not have before, we completely restructured the files, made a new interface, added new channels from the NEURON database, rewrote old tutorials to incorporate user feedback, and added nine new tutorials (Fig. 4). Each tutorial was also associated with a relevant clinical condition.

New BASIC Level tutorials in NIA2.
An "Introduction to Neurons in Action" tutorial explains to the student how to use this tool by having them observe an action potential, then play with the Na and K concentrations to see the effect on the spike. A link allows the students to compare their simulations with the original observations of Hodgkin and Katz (1949) so that they may see that the simulations accurately reproduce reality; linking like this to original observations is done when possible throughout the tutorials. As with all of the tutorials, an outline of the sections of the tutorial in the side bar, shown in Fig. 2 and expanded from two other tutorials in Fig. 5, allows the user to see the suggested experiments at a glance and jump to any section of the tutorial with a click.

Figure 4. The set of tutorials in *NIA2*. Several of the new tutorials in this version are described here.

We imagine that the new Equilibrium Potentials, Chattering Ion Channels and Calcium Action Potential tutorials might be especially useful for undergraduates.

Equilibrium Potentials.   We ditched the Resting Potential Tutorial of *NIA1* in favor of one on Equilibrium Potentials. It seemed that the concept of the equilibrium potential was what the students needed to understand: the resting potential was simply a special application of that knowledge. The Equilibrium Potentials tutorial is the only one in *NIA2* where the conductances are expressed as ratios. When the students realize that the membrane voltage is trapped between ENa and EK, that its value depends on the ENa:EK ratio, and that one can get an action potential simply by making that ratio first favor ENa and then EK, a mysterious mechanism suddenly seems so

much simpler! As for the resting potential, it now can be set anywhere by specifying conductance ratios.   By changing ion concentrations from their default values students can see how ENa and EK are determined.

Chattering Ion Channels.   A single channel simulator underlies the new Chattering Ion Channels tutorial. The user inserts Na and/or K channels into a patch--individually or together, and in any number from one to ten thousands-- and records their single channel currents and conductances. Many principles and concepts emerge from the experiments suggested in this tutorial. Ohm's law becomes obvious from comparing the voltage, conductance, and current of a single channel opening. ("Wow--why is the K current suddenly smaller when the depolarizing clamp pulse ends but the channel has not closed? I thought there was only one K channel in the patch...Oh--I get it!") Long recordings of channel openings at different depolarizations drive home the message that depolarization favors the open state for voltage-gated channels. For final drama: When the student increases channel number in the patch from one to two, to five, then ten, hundreds and thousands, the channels indeed first chatter, then transition to smoother traces and eventually into the canonical macroscopic currents seen in all neuroscience texts.

Figure 5. Outlines of two BASIC Level tutorials as shown in their side bars. The student can see at a glance the experiments to be detailed in the tutorial.

The Calcium Action Potential.   This tutorial is subtitled "How to Make a Long, Cardiac-like Action Potential." It reveals how the prolonged action potentials, such as those that enable the heart to function, come about from deploying the differing properties of the Na, L-type Ca, and K channels to initiate the spike, then sustain its long plateau. As the Ca and K currents enter a war with one another over who is going to be in charge of the membrane potential, generating the action potentials' plateau, the perceptive student might suddenly remember another war-- that between the Na and K currents at threshold, when the question is whether the Na current will win and the spike will take off or the K current will win and quell the unrest. When the suspense of the cardiac plateau is finally resolved, and the K current wins, driving the voltage explosively back to its resting value, the student may be amazed to realize that the process is essentially the same

as that generating the rising phase of the action potential. It simply works in reverse!

Synaptic transmission.   For *NIA2* there are now three Basic Level patch tutorials: The Neuromuscular Junction (excitation), Postsynaptic Inhibition, and Interactions of Synaptic Potentials.   Following these tutorials with the motoneuron tutorials in Cells (Advanced Level) shows how synaptic potentials on dendrites can interact to elicit neuronal firing.

New ADVANCED Level tutorials in NIA2.
The tutorials at the Advanced level are meant to provide more "meat" for the engaged and stimulated undergraduate.   At this level, interest and background surely must guide the selection of tutorials that a student might take on. The premed student might be drawn to the Extracellular Ca Sensitivity of the Na Channel with its relevance to hypo- and hypercalcemia; to the Non-uniform Channel Density tutorial that explores the effect on action potential propagation of blocking channels with anesthetics; or to the Na and K Channel Kinetics tutorial that deals with the hyperexcitability underlying neuropathic pain as well as with toxins, such as the red tide, that kill by affecting channel kinetics.   The student with a strong grounding in physics might appreciate the phase-plane plot of the action potential in the Dynamical View of Threshold tutorial. The student tending more towards the brain and integrative mechanisms might gravitate towards the tutorials of the Cells section.

Finally, a student taking on a lab project or participating in research involving patch clamping in any preparation involving whole cells would be well advised to first run the simulations of the "Voltage Clamping" Intact Cells tutorial where he or she can see how geometry and electrode resistance might affect their results. To quote one of our local, enthusiastic instructors, "I actually think that no one should be allowed on a patch clamp rig without going through all of the tutorials!" (McLean Bolton, Duke University).

USES OF *NIA* IN LECTURE AND LAB
Using NIA in an introductory, didactic lecture.
Typically, the undergraduate first confronts the action potential in an introductory psychology or biology course. Like its very nature, the action potential sweeps into their lives and then it is gone as the material moves on. We made the minimovies for such lectures, hoping they would help cement action potential and synaptic mechanisms into student memory by grabbing attention through movement, sound, and suspense.

The movies are grouped into three categories: the stationary action potential (in a patch), the propagating action potential, and synapses.   The movies can be paused at any point for dramatic effect and suspense.

Minimovies of the action potential in a patch. As the voltage zooms up and back down, voltage is converted to frequency; in other words, the sound pitch tracks the spike. A subthreshold stimulus, causing the voltage to sag back to rest with a moan, is increased in amplitude for a second try, and the resulting suprathreshold stimulus kicks off the screaming action potential, introducing the concept of threshold.   Changing the external [Na] affects the amplitude of the spike; blocking the K channels ("What will happen, class?") sends the voltage up to ENa where it is pegged, declining only very slowly (due to leak channels). The movie showing the action potential prolonged as the patch is cooled can make the point that biochemical reactions (associated with channel openings, closings, and inactivation) must underlie its mechanism.   One movie shows the impulse's underlying conductances.

Minimovies of the propagating action potential. Students are so accustomed to the textbook action potential, a graph of voltage-vs-time, that understanding it stretched out in space takes some faculty patience and explaining, especially if time and space graphs are viewed together. But the reward is great as the false concept of an action potential "jumping" from node to node in myelin ("How could this be? What happens in between?) is replaced by the understanding that it simply spreads out farther and faster along the axon due to the myelin. Other movies show how a change in diameter affects the speed of propagation and how the impulse initiates in a motoneuron's axon in response to synaptic input. A more detailed description of the use of the axon minimovies in lecture may be found online at http://www.brains-minds-media.org/archive/1401 (Stuart, 2008).

Minimovies of synaptic transmission.   The four minimovies in this section illustrate two concepts fundamental to a lecture introducing synaptic transmission: (1) that the postsynaptic voltage change far outlasts the conductance change and current flow caused by the transmitter and (2) that the synaptic event has a reversal potential determined by the ion channels opened by the transmitter.   An inhibitory postsynaptic potential is shown reversing at the resting potential; thus it is essentially in hiding, and is only revealed by changing the voltage away from rest, here with injected current.

Using NIA interactively in lecture. It can be nerve-racking to use *NIA* interactively in lecture! Although it is easy to toggle between a PowerPoint presentation and *NIA*, the difficulty lies in the lecturer becoming familiar enough with the panels and graphs so as not to become confused in front of the students.   With practice and courage, using *NIA* interactively can be great fun for the teacher, as the author knows from personal experience with a class of over 100. To be able to set parameters--perhaps asking the students for suggestions, for expectations, or to vote on the expected outcome--creates an enlivening suspense.

For a detailed description of the author's interactive use of one of the most popular tutorials, Partial Demyelination, go to: http://www.brains-minds-media.org/archive/1401 (Stuart, 2008).

Using NIA in upper level undergraduate courses.
It seems to us that for upper level courses there are as many different uses of this tool as there are courses.   In part this reflects the backgrounds of the faculty, ranging from physics and engineering through computer science to biology, and in part the students' program.

Using NIA2 as a text. Yes, *NIA2* can be and is being used as the sole text for advanced undergraduate courses in the cellular basis of neuronal function (examples: Steve Zottoli's course at Williams College and Bruce Johnson's course at Cornell.) In authoring *NIA,* we envisioned a new type of text: one that provided structured information but when it was needed or desired rather than in the standard, linear, subject-oriented fashion. Hyperlinks made this "3D textbook" possible*.* Steve Zottoli eloquently expresses how liberation from a textbook powers his course. He says, "The advantage of having a "text" such as *NIA* is enormous in that questions can be answered by doing experiments. My goal is to transition students from a typical read, memorize and regurgitate mode to a scientific method mode of asking a question, making a hypothesis and testing it with *NIA*. This transition requires slow weaning from the student's prior educational experiences by using chalk board lectures combined with *NIA*. *NIA* is always projected so that when students question me during a lecture, we can do an experiment rather than me giving the answer (in fact, *NIA* answers have surprised me at times allowing me to grow with the students!). Gradually most of the students realize that they do not have to ask me a question but rather they can ask the question of *NIA* either in or outside of class."

Coupling NIA simulations with wet labs. Both Bruce Johnson's and Steve Zottoli's courses couple *NIA* tutorials with wet labs guided by Johnson's coauthored *Crawdad* tutorials (Wyttenbach et al., 1999). For their midterm and final "exams" in Johnson's course, students must design an original project that couples simulations with wet experiments. A more detailed description of this course, and examples of student projects, may be found in http://www.brains-minds-media.org/archive/1401   (Stuart, 2008).

We are aware of other colleagues who have assigned independent projects to students based on the *NIA* simulations.   While the tutorials suggest certain "experiments" and the parameter settings to carry out those experiments, the real test of the student's understanding, and indeed the fun, might come when the they are asked to design their own experiments.

NIA simulations as the basis of dry lab exercises, homework, and exam problems. Some colleagues introduce the program and the material, often using the minimovies, then have the students do selected tutorials on their own and present them in class. For his course at New York University, John Rinzel has students summarize their presented tutorial on a course website and add at least one additional question of their own.

Whether the instructor assigns the written *NIA* tutorials or ones he or she has custom-abbreviated, and whether they are assigned in lab, where the student can get help from the instructor, or for homework, there is the matter of final evaluation. Did the student really understand the material from doing these tutorials?

Problem sets and exams are the traditional method of checking understanding and it is the same with *NIA,* only more graphical. We, and others, have captured plots on the *NIA* graphs and simply put them into Word to make problem sets or exams, some of which are posted on the *NIA* website.   We encourage colleagues to send us problem sets or exams to post. In giving their answers, students can, of course, capture plots in a similar fashion or they can refer to the instructions for "Printing" or "Exporting" in the Help menu.

THE NIA WEBSITE: *http://neuronsinaction.com/*
We maintain a website where we post material for those colleagues thinking of whether to use *NIA* (e.g. sample tutorial texts, introductory videos, FAQs) as well as for those already using either an *NIA1* subversion or *NIA2* (e.g. changes made for each version, problem sets, links to articles such as this one discussing the use of *NIA*). As we ponder whether and how to make this site interactive, we welcome input from our colleagues.

As well, we are continuing to develop new Basic and Advanced Level tutorials that could perhaps be added one by one to *NIA2* in the future*,* and we welcome feedback from users regarding subjects they would like to see addressed. We welcome email at: stuart@med.unc.edu or moore@neuro.duke.edu.

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