A Structured-Inquiry Approach to Teaching Neurophysiology Using Computer Simulation

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Computer simulation is a valuable tool for teaching the fundamentals of neurophysiology in undergraduate laboratories where time and equipment limitations restrict the amount of course content that can be delivered through hands-on interaction. However, students often find such exercises to be tedious and unstimulating. In an effort to engage students in the use of computational modeling while developing a deeper understanding of neurophysiology, an attempt was made to use an educational neurosimulation environment as the basis for a novel, inquiry-based research project. During the semester, students in the class wrote a research proposal, used the Neurodynamix II simulator to generate a large data set, analyzed their modeling results statistically, and presented their findings at the Midbrains Neuroscience Consortium undergraduate poster session. Learning was assessed in the form of a series of short term papers and two 10-min in-class writing responses to the open-ended question, “How do ion channels influence neuronal firing?”, which they completed on weeks 6 and 15 of the semester. Students’ answers to this question showed a deeper understanding of neuronal excitability after the project; their term papers revealed evidence of critical thinking about computational modeling and neuronal excitability. Suggestions for the adaptation of this structured-inquiry approach into shorter term lab experiences are discussed.

Key words: MetaNeuron; Neurodynamix; conductance-based models; neuron database

The principles of neurophysiology are challenging for undergraduates to master. Like other areas of physiology, neurophysiological concepts require students to consider causal mechanisms, dynamic systems, and multiple levels of organization. Students learn physiology from information that is presented to them in graphical and mathematical forms (Michael, 2007) as well as in text, diagrams and photographs. Often, theory is unpacked during laboratory exercises, to the degree which time, cost, equipment availability, student sophistication and faculty commitment afford. Computer simulation can be an effective means to introduce undergraduate students to the fundamental concepts of neurophysiology (for example, see Stuart, 2009). For the last six years, our introductory neuroscience course has used MetaNeuron, a graphic neural calculator with dozens of meticulously developed exercises (Leslie, 2004). Although such software packages deliver content in a manner that is both visual and interactive, students often complain that computer-based exercises are tedious, time-consuming and frustrating.

The goals in most of our introductory neuroscience lab activities are to introduce students to data collection from live material, to encourage them to pose sophisticated questions, and to teach them how such questions can be approached experimentally. While the wet lab experiments appear to promote a good deal of excitement, they tend to generate little data for analysis, and much of it noisy and difficult to analyze. These experiences focus on process rather than disciplinary knowledge, and emphasize thinking and doing physiology over content mastery. In contrast, the simulation exercises result in a phenomenal amount of “data” and illustrate numerous facts and relationships, but seem to promote little enthusiasm among the students.

From comments volunteered in the open-ended suggestions box on their course evaluation form, students highlighted a number of difficulties with the use of the MetaNeuron lab in our introductory course (see Table 1). The general means used by the MetaNeuron exercises are similar to those of other educational simulators, such as Neurons in Action (Stuart, 2009) and Neurodynamix (Friesen and Friesen, 2010), although it is the opinion of the author that MetaNeuron has the most intuitive interface for students. In order to teach students the causal basis for the Nernst potential, such software packages often require students to perform a series of simulations in which a single variable (e.g., extracellular potassium concentration) is iteratively altered and voltage repeatedly measured from a graph or output text box. Then, they graph their measurements and describe the relationship in their notebooks. Or, to teach the physical meaning of the time constant, students would measure the time constant of decay of the membrane potential over a series of current amplitude levels and repeat this series of measurements again on membranes with different resistances.

These systematic exercises allow an instructor to illustrate a much greater number neurophysiological concepts in a single laboratory period than would be deliverable if the pedagogy was founded solely on wet lab investigation. On the other hand, the exercises almost always seem to take on a less colorful character unless the instructor is extremely proactive in presenting the material and goals. These tutorials can, therefore, become busy work that is an effective teaching tool in this instructor’s opinion, but fail to excite students about the power and potential of computational methods in neuroscience. It is questionable whether they engage the student in the process of thinking and doing computational neuroscience.
"In general, the hands-on labs helped me much more than the computer simulations."
"I think it was a really valuable lab, but I think [splitting it into two labs would] make the experience less painful..."
"I can do computer work on my own time and not lab time."
"Some of the labs were a little boring because they were very hands off..."
"... after a while instead of learning from the computer simulation I found myself distracted while attempting to answer the questions as fast as possible."
"The Metaneuron activity was quite time-consuming and I didn’t really learn a lot from it."

Table 1. A sample of comments made by students concerning MetaNeuron exercises in the open-ended suggestions box of course evaluations for the introductory neuroscience course between 2006 and 2011. (This course was titled Neuroscience 234: Introduction to Neuroscience until 2011, when it was changed to Neuroscience 239: Cellular and Molecular Neuroscience.)

For example, while students develop experience in the use of simulation environments, it is rare that they are afforded the opportunity to pose a novel question or design an approach to answering it using the simulator. These simulation exercises, therefore, fall short of the expectations to which instructors hold their wet laboratory exercises.

The author endeavored to develop an introductory computational neuroscience experience for undergraduates in which student inquiry, rather than content mastery, was the central objective. A structured-inquiry based approach (Banchi and Bell, 2008) was adopted to promote student engagement while exploiting the advantages of neurosimulation. This approach was centered around a long-term project in which students were given a research question that could only be answered through computer simulation. They were informed that the project was novel and that the nature of the experiment was such that they would have data to present at the Midbrains Consortium (http://www.macalester.edu/midbrainconference/) undergraduate poster session no matter what they found. It was anticipated that student ownership over the project, and the group accountability afforded by a guaranteed public presentation, would encourage the students to use the simulator as a research tool, rather than an interactive workbook, as simulators are frequently used in undergraduate neuroscience laboratories.

The specific goals of this research-focused exercise were to teach students that: (1) ion channels are diverse; (2) ion currents (including voltage-gated and voltage-insensitive types) interact; (3) excitability is an emergent property related to these interactions; (4) the effect of an ion current on excitability depends on the context of other currents in the cell; and, (5) computer modeling permits analysis of complex systems that would be otherwise unpredictable. While this project was executed with a small group of students in a partial credit course, it is thought to be scalable, and was recently developed it into a 3-hour laboratory activity for our introductory neuroscience course.

MATERIALS AND METHODS

The Course: Biology 291: Computational Neuroscience met once a week for an hour during a single semester, and counted as a quarter credit for enrolled students. The class meetings were informal and tutorial-style, with assigned readings and in-class exercises from Friesen and Friesen’s Neurodynamix II.

The Students: The class began with seven registered students and one auditing student and concluded with six registered students, including one first-year, two juniors and three seniors. The students’ majors were in diverse disciplines, including biology, chemistry, physics, and psychology; five were neuroscience concentrators.

The Class Research Project: The structured-inquiry class project began with an in-class discussion of the research project concept and a 10-min writing exercise on week 6 and continued as an online discussion of experimental design on week 7. The project concept was pitched to the class as a central question (“How do ion currents interact to control excitability?”) and a general approach for answering the question. This approach was to generate many models (each based on Neurodynamix II’s Neuron_impulse_frequency.nld lesson model) with different magnitude conductances for five currents and to analyze the population of models for trends in excitability. The currents modified did not include the canonical fast sodium or delayed rectifier potassium currents that underlie the action potential. Students would classify each model according to the simulation results as quiescent, low or high frequency firing and determine the average magnitude for each conductance across these sub-populations. They would also look for correlations between conductances within these sub-populations, and build a statistical model that attempted to predict excitability based on a function of the magnitudes of the five conductances. These methods were inspired by Prinz et al. (2003) and Taylor et al. (2006). Importantly, it was impressed upon the students that this was pioneering work, that no one knew what they would find, and that no matter what happened they would have something novel to present at the Midbrains Conference.

Experimental Procedures: Students used the Neurodynamix II simulation environment (http://www.neurodynamix.net/) to model neurons in which somatic membrane potential was described by the equation:

$$-C_s \frac{\partial V}{\partial t} = \sum g_x (V_x - E_x) - I_{inj} - I_{dc} + I_{ac}$$  

(1)
where \( C_s \) is somatic membrane capacitance, \( V_s \) is the somatic membrane potential, \( g_x \) is the conductance of ion current \( x \), \( E_x \) is the Nernst equilibrium potential of ion \( x \), \( I_{inj} \) is current injected into the soma, \( I_{ac} \) is current influx from the dendritic compartment and \( I_{ac} \) is current efflux to the axonal compartment. The distribution of conductances and currents differed between compartments; for example, there were no voltage-gated conductances or current injection(s) into the dendritic compartment. Membrane currents were described by the equation:

\[
I_x = g_x(V - E_x)
\]  

(2)

However, impulses were modeled more simply, after the fashion of integrate-and-fire neurons. When membrane potential reached a voltage threshold of -69 mV (default setting in Neurodynamix II), the fast sodium conductance would increase to 600 nS for 2 ms and then change back to zero. At this point, the delayed rectifier potassium conductance would change to 200 nS, and decay back to zero with a time constant of 10 ms, resulting in the impulse undershoot. Unlike the overlapping time courses of these currents in more realistic simulations, the spike generation here is fashioned after integrate-and-fire neurons.

Other conductances were described by the equation:

\[
g = \frac{g_{\text{max}}}{1 + e^{(V - V_{\text{half}})/V_{\text{slope}}}}
\]  

(3)

where \( g_{\text{max}} \) is the peak conductance, \( V_{\text{half}} \) is the membrane potential at which the conductance is \( g_{\text{max}}/2 \), and \( V_{\text{slope}} \) is the rate of change of the conductance as a function of membrane potential. The leak conductance was constant (50 nS) and had a reversal potential of -70 mV.

Students generated different model neurons by varying \( g_{\text{max}} \) for each conductance such that each model neuron had a unique combination of maximum conductances for the persistent (non-inactivating) sodium current (\( h_{\text{NaP}} \)), a non-delayed rectifier voltage-gated potassium current (\( i_k \)), an A-type potassium current (\( i_A \)), an inwardly-rectifying potassium current (\( i_n \)) and a hyperpolarization-activated inward current (\( i_{Na} \)). Random conductance magnitudes (from 0 to 300 nS) were selected using a random number generator (http://www.random.org/integers/). Other model parameters were kept constant at their default levels according to the Neuron impulse_frequency.ndl lesson module. A unique combination of maximum conductances is hereafter referred to as “a model.”

Students manually entered each combination of conductances (representing one model) into the appropriate parameter boxes in the Neurodynamix II simulator. They observed the membrane potential for one second in the absence of electrical stimulation, which was long enough for membrane potential to stabilize. Then, they stimulated the cell for 400 ms with 1 nA depolarizing current and recorded the firing rate in Hz as a measure of the model’s excitability.

**Learning Assessment:** Students completed three short writing projects: a one-page paper answering the question “What role do ion channels play in nerve electrical activity?” (due week 4); a two-page research proposal for the class research project including expected rationale and expected results (due week 7); and a three-page response to the statement “Neuronal excitability is a property that emerges from the interactions between ion currents.” Using specific examples from the class research project and at least three cited journal articles (due week 15). Students were also expected to take part in a poster presentation on the results from the class research project at the Midbrains Consortium Conference at St. Olaf College.

The approval of the St. Olaf College Institutional Review Board was obtained for all matters of data collection and analysis concerning student learning in this study.

**RESULTS**

**Student Findings from the Structured-Inquiry Project**

Students then analyzed data from 711 model neurons generated with random combinations of \( g_{\text{max}} \) values (see Methods). Models were classified as spontaneously active if no current injection was required for them to fire impulses, low threshold if 1 nA was sufficient to cause them to fire, and high threshold if more than 1 nA was required to provoke impulse activity. Seventy-five percent of models examined were observed to be spontaneously active, 8% were classified as low threshold and 13% were classified as high threshold. The remaining fraction did not fit these three categories; for example, some of these showed an increased firing frequency during current injection that outlasted stimulus duration, suggesting a possible biphasic firing property or model instability.

Figure 1 shows the mean \( g_{\text{max}} \) values for the three classes of model neurons defined above. They found some of these results to be quite surprising. For example, prior to the research project, students anticipated that a higher \( g_{\text{NaP}} \) would be a factor “enabling a high frequency of impulses to occur,” as one student wrote in the anticipated results section of the research proposal assignment. Similarly, another student predicted that “The persistent non-[in]activating sodium current has been shown to contribute to steady-state firing. Presumably, a higher conductance would lead to an increased rate of fire.” True to their predictions, they found that if they set all other conductances to zero and varied \( g_{\text{NaP}} \), the effect of \( g_{\text{NaP}} \) was linearly related to firing frequency. However, when they plotted the relationship between \( g_{\text{NaP}} \) and firing frequency in the randomly generated, 711 model data set, \( g_{\text{NaP}} \) was not a good predictor of firing frequency (Fig. 1). When they varied \( g_{\text{NaP}} \) in a model in which other \( g_{\text{max}} \) values equaled their respective means in 711 model data set, \( g_{\text{NaP}} \) exerted little influence on firing rate (Fig. 2).

To quantify the contributions of each individual conductance to firing frequency, the students fit the model data set with a multiple regression of the form:

\[
f = a \cdot g_{\text{NaP}} + b \cdot g_{\text{K}} + c \cdot g_A + d \cdot g_H + e \cdot g_{IR}
\]  

(4)

Where \( f \) is firing frequency in Hz and \( a-e \) are regression coefficients (betas). The data were well-fit by this simple, linear model (\( F_{5,707} = 445.38; \) adjusted \( R^2 = 0.76; \) \( p < 0.001 \)). The students compared the coefficients as weighted contributions of each conductance to excitability, and concluded that although four out of five coefficients were statistically significant (\( p < 0.05 \)), the relative influence of
Figure 1. Mean $g_{\text{max}}$ of model neurons according to classification of spiking behavior. Note that the three classes of models do not differ in their average $I_{\text{NaP}}, I_{\text{K}}$ or $I_{\text{A}}$, but show interesting differences with changes in mean $I_{\text{H}}$ and $I_{\text{IR}}$. Note that low $g_{\text{H}}$ and high $g_{\text{IR}}$ increased spike threshold, while high $g_{\text{H}}$ and low $g_{\text{IR}}$ decreased spike threshold.

Figure 2. Students observed different effects of $g_{\text{NaP}}$ on excitability depending on the magnitudes of the other conductances. When $g_{\text{K}}, g_{\text{A}}, g_{\text{H}}$ and $g_{\text{IR}}$ were each zero, firing frequency of the models increased as a function of $g_{\text{NaP}}$. However, when $g_{\text{K}}, g_{\text{A}}, g_{\text{H}}$ and $g_{\text{IR}}$ were set to their respective mean magnitudes as represented in the model database, $g_{\text{NaP}}$ exerted little influence on firing frequency over the magnitude range the students examined.

Figure 3. Relative contribution of each conductance to excitability. Firing frequency was fit with a multiple regression of the form $f = a \cdot g_{\text{NaP}} + b \cdot g_{\text{K}} + c \cdot g_{\text{A}} + d \cdot g_{\text{H}} + e \cdot g_{\text{IR}}$, where $a$-$e$ are coefficients plotted on the y-axis above. Coefficient magnitude indicates the relative strength of the contribution of the associated conductance to excitability, while the sign of the coefficient indicates whether the conductance enhances or diminishes repetitive firing. Asterisks indicate statistical significance (p < 0.05).

What Students Learned about Neuronal Excitability

Written responses to the question “How do ion channels influence neuronal firing?” were analyzed from eight students on week 6 of the semester and six students on week 15. Although there was no difference in the number of words written during the 10-min writing exercises between week 6 (249 ± 31 words; n = 8) and week 15 (243 ± 14 words; n = 6), the mean Flesch-Kinkaid grade level increased from 12.4 ± 0.7 (week 6) to 14.4 ± 0.9 (week 15), indicating an increase in the complexity of words and sentences used to convey ideas in students’ answers. Week 15 answers included a greater frequency of terms pertinent to the research project. For example, use of “current(s)” increased from 5 to 28 mentions, “fire/firing” increased from 35 to 45 mentions and “frequency” increased from 1 to 15 mentions. These changes are particularly striking because week 6 analysis included a total of 1988 words, which decreased to only 1437 words by week 15, due to class size attrition.

Qualitatively, the students’ answers to these questions on week 6 principally conveyed the idea that nerve impulses reflect time-dependent changes in sodium and potassium conductances. Half (4 out of 8) of the answers contained textbook-style descriptions of the ionic basis of the action potential while the remaining four wrote in more general terms about currents and equilibria. Overall, the week 6 answers reflected that multiple players contributed...
to neuronal excitability but did not highlight interactions between these mechanisms (see Table 2, week 6).

In contrast, week 15 answers reflected a deeper understanding that interactions between ion currents underlie the emergent property of excitability. All six students wrote about interactions in their week 15 answers (see Table 2, week 15). Five of these six gave specific examples of interactions that contribute to excitability. For example, one student wrote “No single ion channel or class of ion channels controls cell firing... All the different types of channels influence each other and interact.”

What Students Learned about Neuronal Modeling

In their written assignments (research proposal and short research paper), many students expressed an appreciation for the potential of computational methods in modern neurobiology research. For example, one student wrote optimistically that through “the use of computer simulation it is possible to understand the functioning of the cell.” Another student suggested that this methodology may have important potential for drug discovery, mainly because of the ability to generate a large data set quickly and easily with a small financial input.

At the same time, the students often wrote of the limitations of computational methods. One student wrote that “neuronal modeling is far from perfect, and it will take many advances before computational methods can truly reflect all the ion current interactions in a neuron.” Not surprisingly, given the nature of the project, students wrote of the importance of developing a range of models representing different possible combinations of parameters. One wrote:

“Rather than developing a single model to encompass actual biological reality, with today’s computing power it is more practical to create a population of models that represent... different biological conditions. This approach is more appreciative of true biological complexity and will most likely provide scientists with a set of models that are more robust to the natural fluctuations observed in actual biological data.”

Another wrote that “computer models are able to accurately represent actual neurons as long as the currents are analyzed in context with other currents.”

Students also wrote about the interplay between computer simulation research and in vivo research. For example, a student wrote of the class project results that “this experiment also opens up the door for possible in vivo experiments in the future for biological confirmation of these modeled findings.”

DISCUSSION

The structured-inquiry approach to teaching neurophysiology using computer simulation covered substantially less course material (in terms of facts, definitions and relationships) than the students would typically get by working through the MetaNeuron manual. For example, synaptic integration and voltage-clamping methods were hardly touched upon during the project. The students learned a great deal of content from working through the Neurodynamix II exercises in parallel with the research project. However, the students seemed qualitatively more engaged in this project than in the exercises, which one student called “dry.”

In the introductory neuroscience course, our MetaNeuron lab follows a paper problem set in which students work in groups to solve word problems such as “How many sodium ions flow through the membrane during an action potential in a spherical cell with a diameter of 10 microns?” The students complain of these problems as
difficult, but complain more about the computer exercises. The group problem-solving exercise differs from the simulation exercises in several ways. In the simulation environment, students have proscribed choices for how to manipulate the simulator to solve the problem, whereas word problems are fundamentally open-ended. The paper problem set engenders a rich environment of peer teaching and student-faculty interactions, whereas the amount of discussion in the room during the simulation problem set is minimal except among the most naturally inquisitive students. Finally, although each problem takes the students a long time to puzzle through, there are only a dozen problems, each quite distinct, so that the experience is much less repetitive than the simulation problem set.

In some respects, the structured-inquiry project is more closely linked to the paper problem set than the computer exercises, even though it was founded in computer simulation. The structured-inquiry project was executed by a student team, which was held collectively responsible for their work through a shared file data log, assigned workload and the group poster at a multiple-campus research meeting. The question was open-ended, and although the general methodology was proposed to them, it was made clear that this was only a starting point, and results would dictate where they went from there. In fact, a week into data collection, the students identified a potential flaw in the experimental design; they found that their simulation results were different depending on how long they waited between starting a simulation and issuing a stimulus. This called for standardization of methods. At their request, they elected (by a class vote) to throw out all the data collected thus far and begin again with a longer delay to minimize the influence of the initial state on the simulation results. Through in-class discussions, online asynchronous forums and out-of-class meetings, the students maintained a rich environment of peer interactions that helped to make the project a success.

It is not always feasible to incorporate a semester-long research project into a neuroscience lab course. However, the general idea behind the project presented here can be extracted and used to develop diverse structured-inquiry approaches to using computers to teach neurophysiology. Likely, the most critical aspect of this project was that the students took ownership of the research question. To encourage this development, a novel, but answerable research question was selected as the topic of inquiry. Multivariate sensitivity analyses, like the one conducted here, present an endless array of potential research questions. Instead of selecting evoked firing frequency, the author might have selected spontaneous discharge rates, post-inhibitory rebound firing rates, spike frequency adaptation, synaptic fatigue, or membrane time constant, just to name a few examples of potential dependent variables. Instead of varying maximum conductance, the class might have varied the voltage at which each current is half-activated, half-inactivated, or the time constant of activation as independent variables. Furthermore, the students were held accountable for their efforts by the requirement that they generate something presentable at a public forum. Departmental seminars, campus poster presentations and regional intercollegiate meetings represent rich opportunities for public presentations.

While the data generation portion of the project may have some intrinsic special merit, it was certainly a limiting factor with respect to time. The author felt strongly that the students should do the data generation manually so as to truly understand what the numbers in the data set meant. In a larger lab section, it may take much less time to generate a statistically useful set of models and measurements. More recent attempts to implement this project as a three-hour lab activity found that groups of two to three students could test 20-30 models in 50 mins. This left adequate time for a pre-lab lecture that introduced both the research question and software, a student-lead discussion of experimental design, and 50 mins. at the end of lab for data analysis and discussion. In these attempts, results from 150-180 model cells were obtained, analyzed and discussed.

As a time-saving alternative, students might be encouraged to generate a small number of models and measurements, and then add them to a large, pre-existing data set prior to analysis. Often, parallel computing clusters can be programmed to automate the type of data generation that students in my course did manually. Students can get a sense of what the data means and where it comes from, but the bulk of the available lab time can be used for analysis and discussion.

The extent to which structured-inquiry approaches to teaching neurophysiology using computer simulation should replace more traditional computer-based exercises such as those for MetaNeuron depend on the instructor's intended goals for the lab. This class project was conducted throughout the semester in parallel with exercises from the Neurodynamix II textbook. In this way, students learned a broad foundation in neurophysiology from computer-based exercises. The broader goal of the long-term project was to take the students deeper into one small area of neurocomputation, the role of current interactions in shaping cellular excitability. The approach used to accomplish this goal engaged the students in thinking as computational neuroscientists, and encouraged them to develop an appreciation of computational methods that was both critical and inquisitive. With wet laboratory exercises, instructors are willing to compromise the amount of content that will be delivered in a lab session in order to engage the students in thinking and doing neuroscience. Perhaps computer-based neurophysiology exercises should be held to a similar standard.

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