AC 2010-1661: A TEAM-BASED NERVE CUFF SIMULATION PROJECT IN A THIRD YEAR FOUNDATIONS OF BIOMEDICAL ENGINEERING COURSE

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A Team-Based Nerve Cuff Simulation Project in a Third Year Foundations of Biomedical Engineering Course

Abstract

A nerve cuff simulation group project was used to introduce first semester juniors to bioelectric phenomena. The students are enrolled in the biomedical engineering concentration within the newly accredited general engineering program at East Carolina University. Bioelectric phenomena were introduced through a group project so that, in addition to learning new subject matter, they would (A) integrate knowledge developed in prerequisite and co-requisite coursework in a new setting, (B) develop their independent research skills, (C) gain experience working in teams, and (D) develop facility to apply their new knowledge, not just recite it. These traits are considered to be important aspects of the program goal to producing work-ready engineers.

Teams of 3-4 students were given a model of an axon, surrounding tissue and a stimulating nerve cuff, written in LT-Spice®. Students also received a background reading assignment including a chapter from a textbook, a short research article, and a patent application relevant to the project. Three assignments with progressively decreasing levels of difficulty were given to the students in succession. The original assignment asked them to design and simulate a nerve cuff which would generate a unidirectionally propagating action potential. The second assignment asked them to design stimulation parameters so that a modified model with two identical nerve cuffs could generate a unidirectionally propagating action potential. The third assignment supplied a modified model with two nerve cuffs which generated a unidirectionally propagating action potential. Students were asked to increase the temperature so that it no longer generated a unidirectionally propagating action potential, and then adjust stimulating currents so that the model again generated a unidirectional action potential.

Assessment activities for this course were chosen to both foster to continuous improvement of the course and to support the relevant ABET outcomes of the general engineering program. Assessment methods included an inventory of factual questions, a set of questions related to self-perception of factual learning, and a report of time spent on activities related to the project. Performance was fair on factual questions related to definitions, action potential initiation and parameter scaling, but less well on questions related to applications of defined terms, typical values of physiological parameters and action potential termination. Self perceptions were somewhat better than the factual scored indicated. More time was spent organizing their team effort than on any other single activity. Based on this experience, the course is being re-structured to better support problem-based pedagogy.

Introduction

A nerve cuff simulation group project was used to introduce first semester juniors to bioelectric phenomena. The project involved working with a simulation of an axon and surrounding tissue, with two nerve cuffs. A pulse current generator connected to one nerve cuff generated an action potential which propagated in both directions along the axon. Another current source delivered a blocking current to the other cuff so that the action potential could not propagate through it. Students were asked to modify the model so that the action potential block worked at a higher temperature. Successful completion of the project requires an integrated understanding of the velocity of action potential propagation, the dynamics the Hodgkin-Huxley model of action potential generation, and the role of temperature in modeling biophysical processes. This framework treats action potential generation both as an objective to be attained, and a constraining obstacle to be avoided. Sodium channel inactivation is observed in a design context, in addition to controlling the refractory period after action potential generation.

Before entering this course, they have completed physics, general chemistry, calculus, engineering math, one semester of statistics, and statics. Students and were concurrently enrolled in courses in mechanical dynamics, and beginning electrical circuits. A basic background in axon biophysics was supplied by a reading from standard introductory biomedical engineering textbook [1]. To develop research skills, students were supplied with an influential research article [2] relevant to the project, and instructed on how to use PubMed and ISI Web of Science to find articles related to a key publication. They also received a patent application [3] in the field of their project so that they could learn about the structure of a patent, and what information can be found in patent publications. The particular patent application was chosen because it had a comprehensive and readable disclosure, and figures illustrating a range of applications

Students were supplied with a working design, and asked to modify the design. Grades were based on a poster describing the design and their modifications. Assessment was based on answers to factual questions and a self-assessment questionnaire.

The students were enrolled in the biomedical engineering concentration within the newly accredited general engineering program at East Carolina University (ECU). This program, founded in 2003, capitalizes on institutional strengths to prepare regional students serve regional industry. The core curriculum trains students broadly not only in science, math and the foundations of mechanical and electrical engineering, but also in systems and industrial process engineering. Four concentrations provide advanced training in a specific discipline. These are biomedical engineering, bioprocess engineering, mechanical engineering, and systems and industrial engineering. The program produced its first graduates in Spring 2008, and was awarded ABET accreditation in 2009 - the earliest point of eligibility.

The motivation for attempting this project was so that, in addition to teaching new subject matter, students could develop independent research skills and gain experience working in teams, in a framework that emphasized the application of knowledge, over recitation. Many ECU students share characteristics historically common to the experience of minorities and women in engineering. Such characteristics include being the first in their families to attend college or enter a profession, with the resulting insufficient personal support network; a necessity to hold a job in

order to support their education; and over-reliance on individual study in lieu of group study [9]. The simplest statistic related to these characteristics is that 85% of incoming engineering freshmen qualify for need-based financial aid, based on the federal FASFA financial aid form.

Assignment

The original problem statement for the exercise is included as appendix A. Students were supplied with a SPICE model of a small-diameter squid axon, and a stimulating nerve cuff, and asked to modify the nerve cuff and the applied stimulation so that an action potential was still generated, but its propagation was blocked along one branch of the axon. Students had considerable design freedom, with the option to design many facets of the nerve cuff. This included geometrical properties, such as the number of electrodes and their surface area, the spacing between the electrodes, as well as electrical properties, such as the magnitude and duration of current pulses and ramps. This was sufficiently difficult to intimidate the students, and they did not attempt begin the project.

In order to encourage students to interact with the model, the assignment was simplified. A model with two identical nerve cuffs was supplied. Students were asked to modify the current sources so that one nerve cuff generated bi-directionally propagating action potentials, while the other nerve cuff blocked action propagation, without itself generating a propagating action potential. The revised assignment also allowed students to consider action potential generation and blocking more independently, and did not require them to make any changes to the connections between elements of the model. It did require them to formulate a strategy for manipulating the processes underlying membrane resistance and refractoriness. It also required them to manipulate the model through extracellular stimulation, rather than directly applying a voltage or current across a modeled patch of membrane. This revised assignment also proved to be unreasonably difficult.

In a third revision, students were asked to increase the model temperature so that it no longer generated a unidirectionally propagating action potential, and then to modify the stimulus and blocking currents, so that the revised again generated a unidirectionally propagating action potential at the warmer temperature. This revised assignment required students to understand the mechanisms of the action potential blockade, and how changing temperature affects the Hodgkin-Huxley model. The students failed to achieve this revised objective, and the course moved on without devoting further time to this project.

An overview of the revised model is shown in figure 1. Briefly summarized here, it is described in detail in Appendix D. The model is comprised of 58 segments, with each segment comprised of models for the Hodgkin-Huxley model for the squid axon, and volume conductor models representing the nerve tissue near the axon, and a nonspecific physiological medium surrounding the nerve. In the figure, labeled nets connect the right end of one row of segments to the left end of the succeeding row. Two nerve cuffs are provided, one to initiate bidirectional action potential propagation, and the other to block action potential propagation along one branch of the model.

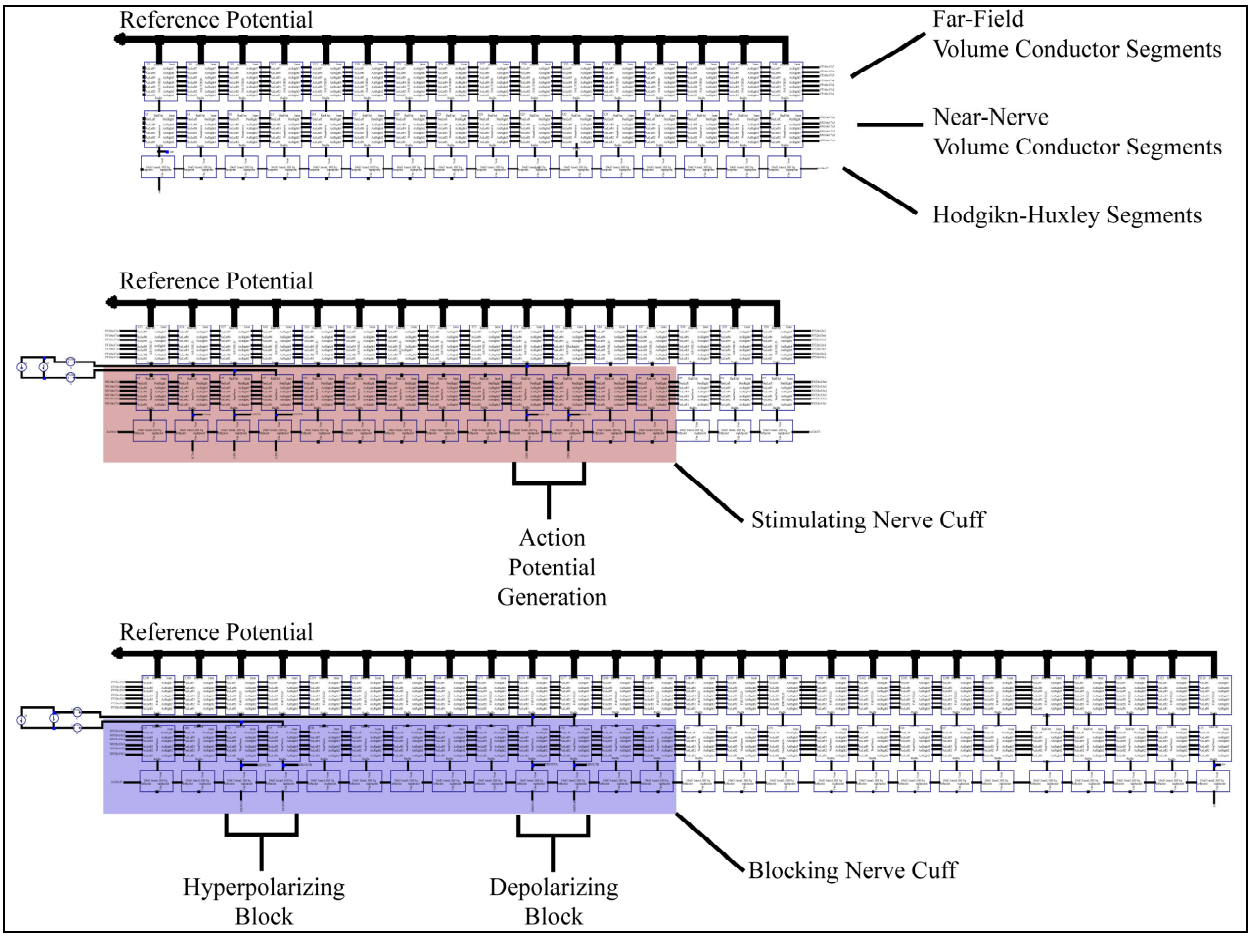

Figure 1. Axon with stimulating and blocking nerve cuffs. The red and blue background shading simply highlights the position of the stimulating and blocking nerve cuffs, with no computational significance.

Assessment

The assessment was designed to support continuous course improvement, and to document the achievement of program outcomes. The goal of the assignment was for students to learn the basics of electrobiology, and to be introduced to materials and practices relevant to working as a biomedical engineer. Three assessment instruments evaluated the achievement of those objectives. First, responses to factual questions, found in appendix B, evaluated learning of the subject matter. Table 1 reports the instructor evaluation of student responses to these questions. Second, students rated their self perceptions of learning on a five-point scale from strongly agree (score=1) to strongly disagree (score=5). The self-perception inventory can be found in appendix C, and student responses are summarized in table 2. Third, students reported how much time they spent on several activities related to their project. Time is reported in table 3 as the average hours per person, for teams of three or four students. Assessment was obtained after all

revisions of the project were completed. Responses were received from 6 students out of a class of 11 students.

Objective and self-perception assessments complement each other. Answers to objective questions are a good indicator of whether students achieved an ability to function independently, and provide definitive answers to specific questions. Even when self-perception measures differ from objective measures, they provide valuable information that can inform the continual course improvement process. A finding of perceived learning may indicate that students obtained a foundation for greater learning through a second exposure to the subject matter in a subsequent course, or that it may be possible to construct an inventory questions that objectively demonstrates some level of competency. Also, students perceive learning compared to their initial knowledge, whereas factual assessment compares knowledge to a desired performance standard.

Learning within the problem domain did not meet the performance objective. Interestingly, topics most strongly related to the structure showed among the lowest levels of achievement (action potential termination, 30% correct) and confidence in learning (know how to block an action potential, score=3 – neither agree nor disagree).

This project was the main deliverable for the first 5 weeks of a 3 credit hour course. Self-reported effort was commensurate with the course load. More time was spent organizing team efforts than any other single activity - almost as much as performing calculations and running and interpreting simulations combined. This was about 23% of the total time spent on the project. Students had a good appreciation of the need for lifelong learning.

Factual Learning Topic	**Percent Correct**
chronaxie definition	60
rheobase definition	100
use of chronaxie and reheobase	40
kcl and membrane potential	50
space constant	60
time constant	60
action potential initiation	80
action potential termination	30
physiogical potential values	30
scaling propagation velocity	60
scaling space constant	0
scaling time constant	0
awareness of applications	53

Table 1. Instructor evaluation of student responses to factual questions.

Self-Perception Topic	Average Score
resting potential	2.8
ion channels	2.6
temperature scaling	2
intracellular stimulation	2.6
extracellular stimulation	2.8
block action potential	3
learned new sources for technical information	2.6
need for lifelong learning	1.4
information in patent	2.2
information in articles	2

Table 2. Self perception of student learning.

Activity	Time Spent (hours)
working tutorials	3.8
reading posted articles	1.5
library research	3.5
performing calculations	3.3
running simulations and interpreting results	4.4
organizing team efforts	7.1
discussion results with team	5.5
learning to use LT-Spice®	4.7
making poster	4.6
figuring out how to make a poster	2.1
total time	31

Table 3. Student accounting of time spent on the project.

Discussion: General

This report describes a SPICE model of an axon, with two nerve cuffs attached to current sources. The model is organized so that an action potential propagates along one branch o the axon, but its propagation along the other branch is blocked. It describes assignments related to the model at three levels of difficulty. All three levels require students to interact with the

Hodgkin-Huxley model through a simulation of extracellular stimulation, without directly controlling membrane voltage or current. All three levels present the Hodgkin-Huxley model in a problem-solving context, rather than as a mechanism that generates a sequence of ion channel conductance states. The first two levels of difficulty require significant design effort, with the necessity to choose a strategy, and the possibility of evaluating alternative strategies. The third level of difficulty requires manipulating a given strategy.

The rationale for this group project was that students would (1) learn new subject matter, (2) integrate knowledge developed in prerequisite and co-requisite coursework in a new setting, (3) develop their independent research skills, (4) gain experience working in teams, and (5) develop facility to apply their new knowledge, not just recite it. The project builds upon student experience from other classes. Pre-requisite courses in chemistry support an understanding of the underlying dynamics of the Hodgkin-Huxley model of the squid axon, and experience with voltage dividers supports an understanding of how extracellular stimulation can influence and manipulate membrane currents, which control the model dynamics. Pre-requisite courses in differential equations and mechanical dynamics provide a further foundation for appreciating a general dynamical system. This objective focuses attention on the inactivation particle of the Hodgkin-Huxley model, which is easy to interpret as the fraction of sodium channels that are available for voltage-controlled opening.

Assessment data indicates that not all of the anticipated benefits were obtained in this implementation of the exercise. Students did feel that they learned something through their participation in the exercise. The course is being adjusted to provide more support within the course for the use of the model, and to take better advantage of a co-requisite circuit analysis course.

Discussion: Assessment Outcomes

Although learning was fair within the problem domain, a key motivation for using the problem-based approach was to attain excellent learning of basic concepts and high confidence in learning. Successful posters could provide evidence of skill in design and use of advanced engineering tools. Several factors contributed to this outcome.

First, the initial project assignment was too challenging, and the assignment updated after the project was assigned. The original assignment provided students with the stimulating nerve cuff, and asked them to design a blocking nerve cuff. The initial project intimidated students. They delayed starting the project, and then did not direct their initial efforts productively. Students may have benefited more by working simple exercises, or completing modeling exercises that incrementally developed the model up to the level of the blocking nerve cuff.

Second, this was the first time that students encounter SPICE modeling. The exposure occurred concurrently with the beginning of an introduction to circuits class. The initial project required students to design a nerve cuff, and perform simple modifications to the model connections in order to implement it. The second-level project did not require any connectivity changes, but only required changes to parameters controlling current sources. These students needed

significant support to gain confidence manipulating the LT-Spice® program, even to view graphs representing dynamical processes in the model, without changing it at all. Introducing the model before they had mastered transient analysis of simple RC circuits may have exacerbated their need for support.

Third, students needed to develop their teamwork and independent thinking skills. The general engineering program includes a team project in a freshman introduction to engineering class, but no teamwork or project activities in the sophomore year. Developing teamwork and independent thinking is a key goal of the project-based approach. The initial experience with teamwork did appear to have benefits for another team project later in the class, although that project was not assessed as thoroughly as the project described here. One interesting outcome of the class is that four students who identified each other as working well in teams took the initiative to register for a research class together in the spring semester. The students are pursuing background work related to a potential senior design project with a sponsor at NASA.

Discussion: Model Implementation

A system for implementing a physiological system simulation for teaching engineers should efficiently support student learning. It should facilitate understanding by allowing the mathematical expression of the model to be clearly parallel with the model implementation expression of concepts, thereby supporting understanding. It should be usable, supporting an efficient student workflow. It should develop skills that are generally useful in other problem domains. Implementing the axon model in LT-Spice® delivers good understanding, usability and generality:

(1) It supports an a highly interactive style for exploring the model (understanding),
(2) Behavioral sources allow clear exposition of complicated formulas (understanding),
(3) Complexity can be encapsulated through use of a model hierarchy, with straightforward definition of new symbols (understanding),
(4) Graphic representations of models and simulation results can be exported as windows metafiles, which can be directly incorporated into student posters and presentations (usability),
(5) It has reasonable performance on student computers (usability),
(6) it has good facilities for exchanging time-domain data with other systems (generality),
(7) It has broader utility for simulating other circuits, and such circuits could be combined with the axon model (generality),
(8) It runs on several versions of Microsoft Windows operating systems, as well as on Linux and Macintosh systems via the Wine emulation system (usability).

LT-Spice® facilitates student understanding by supporting a highly interactive style for exploring the model, as well as for modifying the model. Checkbox options can request that all voltages and currents in a hierarchical model be saved. Students can then interactively test their hypotheses about what happened in a simulation, generating a time-domain plot of a variable with a mouse click. If a text file with key equations is kept open alongside the simulator, functions of simulation variables, like the membrane resistance plot shown in figure 4-C, can be generated quickly with a cut-paste-edit sequence. SPICE nets that are understood to be interesting *a-priori* can be labeled, to improve the efficiency of reference, and communication to

others. Beginning users appreciate being able to right click on a pictorial representation of a circuit element in order to interact with a popup parameter editor. More advanced users can easily loop simulations over a range of parameter values.

LT-Spice® supports understanding of the model through provision of labeled nets, direct entry of nonlinear algebraic expressions, and direct expression of numerical integration and initial conditions. This is simpler than approaches required by some other SICE systems, such as using a piece-wise-linear (PWL) block to express nonlinear functions [6], or using a capacitor as an integrator block [6,7]. Variables must still be linked to node voltages and component currents, however, which adds some confusion over the more flexible naming provided by other general systems such as Matlab Simulink®. It is also more indirect than the representations provided by special purpose neuronal simulation packages such as NEURON [8]. On the other hand, the stimulation presented here straightforwardly incorporates a simplified volume conductor model, a task that is not straightforward in NEURON.

LT-Spice® has a suitable workflow for student projects. Data can be exchanged with other systems in batch mode, though not dynamically during the simulation. Current and voltage sources can depend on data files in the Microsoft WAV format. Selected node voltages and component currents can be written to WAV files, for audio playback or use by other systems. LT-Spice® models and simulation results can be written to windows metafiles (.WMF), and imported directly into Microsoft Office programs such as Word and PowerPoint, adequately supporting student posters and reports. More advanced tools can import windows metafiles exported by LT-Spice®, and apply any requested formatting to yield publication quality figures. The figures in this paper were prepared by reading windows metafiles into Adobe Illustrator. Illustrator was used to change the font to Times New Roman 12 pt., to control the color and stroke weight of graphical curves, and to align, and in some cases reshape and document features within the figure.

The simulation runs efficiently enough to perform well on student computers. To achieve good performance, it is important to specify initial conditions for the integrators, and specify the “uic” option to request that supplied initial conditions are used. The single axon segment runs very quickly. One run of the full axon simulation on a VM Ware virtual workstation, identifying itself as running on a Xeon® Anecdotally, students report simulation times of about 5 minutes. 3.4 GHz processor, incremented CPU time used by the process by 7 minutes and 35 seconds. Much of this time is at the beginning of the run, as the simulation converges towards an additional operating point, with the dynamical portion of the simulation proceeding relatively quickly. Simulation time scales linearly with the length of the axon. Tighter coupling between segments can increase run time quite noticeably. Example manipulations which lead to much longer run times are increasing the space constant, or changes which increase the speed of action potential propagation (in segments per second).

One issue that requires care when implementing hierarchical models is parameter scope. No conflict occurs when the same name is set in both higher and deeper levels of the hierarchy, but the value in the higher context does not override the value set in the deeper context. On the other hand, deeper contexts will make use of parameter values set in higher contexts. Such behavior can be confusing when stepping through parameter values at the top level context. Parameters

can be passed up or down the hierarchy by letting them specify the value of voltage or current in a DC source, with the advantage that connecting two sources to the same node will cause the simulation to fail. The error messages produced in this case can be difficult to interpret, however, especially for beginners.

Discussion: Curriculum changes

The experience with this first offering of the course suggests a number of changes, which can be assessed for improved outcomes. In order to support this and other problem-based exercises, the course schedule has been updated, from 3 hours lecture per week, to 2 hours lecture and 2 hours laboratory per week. The laboratory sessions will allow for more in-class, hands on instruction with the advanced engineering tools used in projects. In order to better prepare students to use this model, the nerve simulation project will be delayed until students obtain more experience with circuits in a co-requisite class. Because students reported spending as much time on teamwork issues as on calculation and simulation, new material on project management and working in teams will be added to the course.

Conclusions

This report describes a SPICE implementation of the Hodgkin-Huxley model of the squid axon, along with models of nerve cuffs and current sources to support generation of a unidirectionally propagating action potential. It describes three assignments that require manipulating the model at three different levels of complexity. Two of the assignments required significant design effort. All three assignments require more of an engineering, problem-solving understanding of the Hodgkin-Huxley model, compared to traditional introductions to nerve physiology. This approach was undertaken in order to achieve superior student achievement. Although students felt that they learned something, responses to factual questions did not achieve the desired performance standard. The course is being reorganized to retain the problem based approach, and to provide more supervised practice with the model and the advanced engineering tools on which it depends.

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Appendix A: Original Problem Statement

Design and simulate a nerve cuff and stimulation protocol which will generate a unidirectional action potential in a simulated axon at 6.3 °C and at 16.3 °C.
The design must specify:

- The length of the nerve cuff
- The size and placement of the electrodes
- The magnitude and duration of any current or voltage applied to the electrodes

A great design will keep applied current within a current density constraint of 65 μCoulombs/phase/cm^2.
Bragging rights go to the design that can support the fastest rate of stimulation, while still only generating action potentials in one direction.
Report your design in poster format. The poster must be less than 40" wide, and less than 3' tall. The poster must have a title and identify the group members at the top.
The poster must include a problem statement including constraints that the design satisfies and how well it satisfies them, background material which may optionally address standards and safety concerns, an explanation of the solution strategy including key reference dimensions and time constants and how they were estimated, a diagram of the mechanical and electrical features of the design, example stimulation results, a report of the maximum stimulation rate that the design can generate, and references.

Appendix B: Factual Questionnaire

1. Define chronaxie.

2. Define rheobase.

3. Briefly explain why an engineer might report or look up values of chronaxie and rheobase

4. Explain what would happen to action potential generation and transmission if the extracellular potassium concentration suddenly increased to be the same as the extracellular sodium concentration – for example if KCl solution was accidentally injected into a patient instead of a NaCl solution.

5. What is the "space constant" or "membrane length constant" ? Please explain and write a formula for membrane length constant.

6. What is the membrane time constant ? Please identify the biophysical parameters which determine the time constant and give a formula.

7. An action potential is initiated when a nerve cell membrane is depolarized, opening channels in the membrane which permit only _____ ions to pass.

8. What two ion channel mechanisms contribute to ending an action potential and repolarizing a nerve cell membrane ?

9. Please write down in order of increasing voltage the resting potential of a nerve cell membrane, zero volts, and the Nernst potentials for sodium and potassium.

10. Please circle: All other things being equal, action potential travels (faster at the same speed slower) along an axon with larger diameter, compared to an axon of smaller diameter.

11. True or False: All other things being equal, the "membrane length constant" is greater for a larger diameter axon compared to a smaller diameter axon.

12. Please circle: The time constant for a nerve cell membrane is (greater about the same briefer) when the temperature increases by 10 degrees, all other things being equal.

13. Please list 3 applications of electrical stimulation to produce an action potential in biomedical engineering (the action potential could be generated in a nerve cell or a muscle cell):

Appendix C: Self Perception of Learning Questionnaire

1 – strongly agree, 2 - somewhat agree, 3 - neither agree nor disagree, 4 – somewhat disagree, 5 – strongly disagree.

1. 1 - 2 - 3 - 4 - 5 : I understand what determines the nerve cell membrane resting potential.
2. 1 - 2 - 3 - 4 - 5 : I understand the role of the relative numbers of sodium and potassium channels in a nerve cell membrane in determining the action potential threshold
3. 1 - 2 - 3 - 4 - 5 : I understand how temperature affects action potential propagation
4. 1 - 2 - 3 - 4 - 5 : I understand how to stimulate an axon intracellularly so that an action potential is generated
5. 1 - 2 - 3 - 4 - 5 : I understand how to stimulate an axon extracellularly so that an action potential is generated
6. 1 - 2 - 3 - 4 - 5 : I understand how to use electrical stimulation to block action potential generation
7. 1 - 2 - 3 - 4 - 5 : I learned some new places to look for technical information related to biomedical engineering
8. 1 - 2 - 3 - 4 - 5 : After I graduate, success as a biomedical engineer will require me to regularly learn new things
9. 1 - 2 - 3 - 4 - 5 : I understand the sorts of information that I might obtain from reading a patent.
10. 1 - 2 - 3 - 4 - 5 : I understand the sorts of information that I might obtain from reading a research or review article.

Appendix D: The Model

The model is implemented in LT-Spice®, available as a free download from Linear Technology[4]. LT-Spice® integrates schematic capture and circuit simulation. It provides extensions which simplify expression of nonlinear models, such as the Hodgkin-Huxley model of action potential generation. As in any SPICE, state variables are represented as node voltages or current through components. LT-Spice® provides behavioral voltage or current sources, which can be governed directly by equations. Many special functions are provided, as well as an integration operator, which facilitates direct expression of integration of a first order differential equation. Values which are parameterized may be defined as explicit constants, algebraic formulas which are evaluated once, or can be systematically varied across a sequence of simulation runs using the .STEP function.

The figures below include the equations governing the model, in LT-Spice® notation. The circuit diagrams therefore serve both to describe the model architecture, and as a program listing for this graphical programming language. Constants are expressed a numerals, with an optional suffix indicating the magnitude. For example, 1m corresponds to 1×10^{-3}, and 1u corresponds to $1\times10^{=6}$. Connections or wires are referred to as "nets". A net can be referenced by a numerical index, or optionally by a name. Parameterized values for resistance and capacitance are enclosed within braces.

The model is hierarchically organized, with the axon model incorporating sub-models for an axon segment, and the volume conductor surrounding the axon. The axon segment model in turn incorporates a sub-model for Nernst potentials. A total of 58 segments comprise the axon model, with each segment in turn comprised of an axon segment, a near field volume conductor segment representing nerve tissue surrounding the modeled axon, and a far field volume conductor segment representing a nonspecific physiological medium. The axon segment sub-model makes extensive use of behavioral sources. The model includes two nerve cuffs, one for generating a bidirectional action potential, and one for blocking action potential propagation along one branch of the axon. Model parameters are drawn from an introductory biomedical engineering textbook [1], and a membrane biophysics textbook [5].

The axon segment sub-model itself is a suitable foundation for simple exercises for beginning electrobiology students. The effects of changing ion concentrations and changing temperature can be quickly demonstrated across a sequence of simulation runs. The model facilitates detailed examination of action potential generation, the refractory period, and membrane resistance.

A Nernst potential sub-model is the foundation of the axon model, diagrammed in figure 1. It makes use of three behavioral voltage sources, governed by the Nernst equation for potassium, sodium and chloride ions. Ion concentrations are expressed in units of molarity, scaled by the "m" suffix. Concentrations are parameterized. Values appropriate to the squid axon are defined as literal constants. If it is desired to automatically step through values of an ion concentration in a higher level of a hierarchical simulation, the parameter must be defined at the higher level, and commented out or deleted from the lower level.

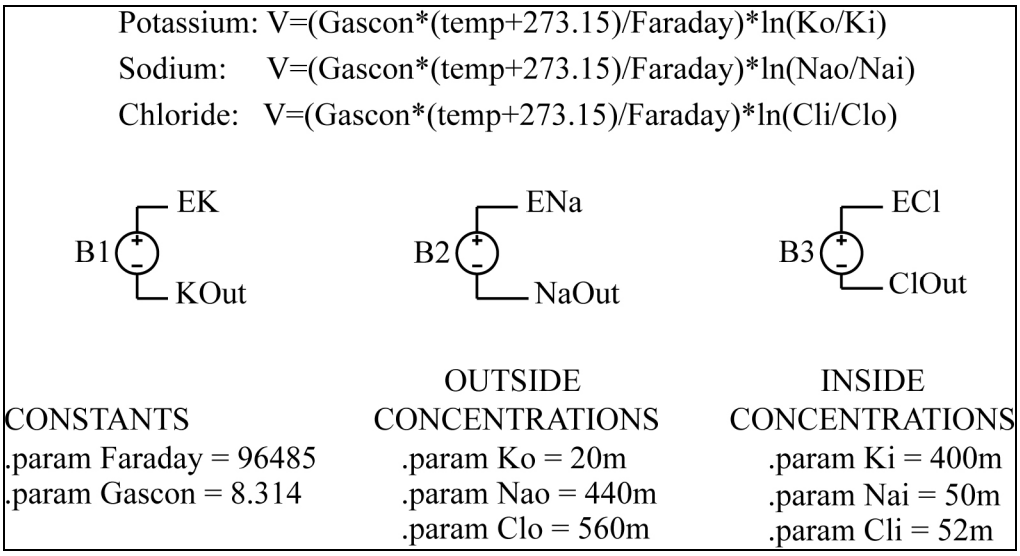

Figure D1. Nernst Potential Sub-Model. Labled nets EK, ENa and ECl are governed by the equations for Potassium, Sodium and Chloride, respectively, above the diagrams. Pins in this model's symbol reference these nets, and the labeled nets KOut, NaOut and ClOut. Values are appropriate to the squid giant axon.

Figure D2 illustrates the axon segment model. It simulates a segment 1mm long and 10μ in diameter. It incorporates the Nernst potential model, in the block titled "SQUID". Three behavioral current sources simulate current through the membrane sodium, potassium and chloride channels. The current in each sodium and potassium sources depends upon (1) the voltage across the membrane, Vout – Vin, (2) a maximum value of conductance per cm^2 membrane area, (3) temperature, via QTenConductance, (4) the integrated particle state computed through the Hodgkin-Huxley formalism, and (5) the Nernst potential for the corresponding ion. The chloride source is similar, but lacks the Hodgkin-Huxley voltage dependence. Axial resistance is modeled as proportional to cross sectional area. The model as depicted defines the potential Vout as ground, SPICE node 0. It includes a pulse current source for testing. When the segment model is incorporated into the model of the full axon, the ground and current source are deleted. The model symbol references labeled nets referring to the potential inside and outside of the axon, and for the left and right ends of the segment.

Figure D3 illustrates the implementation of the Hodgkin-Huxley voltage dependence of axon segment model. Each particle makes use of three behavioral sources. Two sources are governed by nonlinear voltage-dependent algebraic expressions for the rate constants. The third source explicitly integrates the differential equation describing the state of the controlling particle. Potassium channel opening depends on the fourth power of the particle n state variable, computed by behavioral source Bn. Sodium channel opening depends on the product of the third power of the particle m state variable, computed by source Bm, and the first power of the particle h state variable, computed by source Bh. The function idt(A, B) performs the integration, where A is the expression to be integrated, and B is the initial condition.

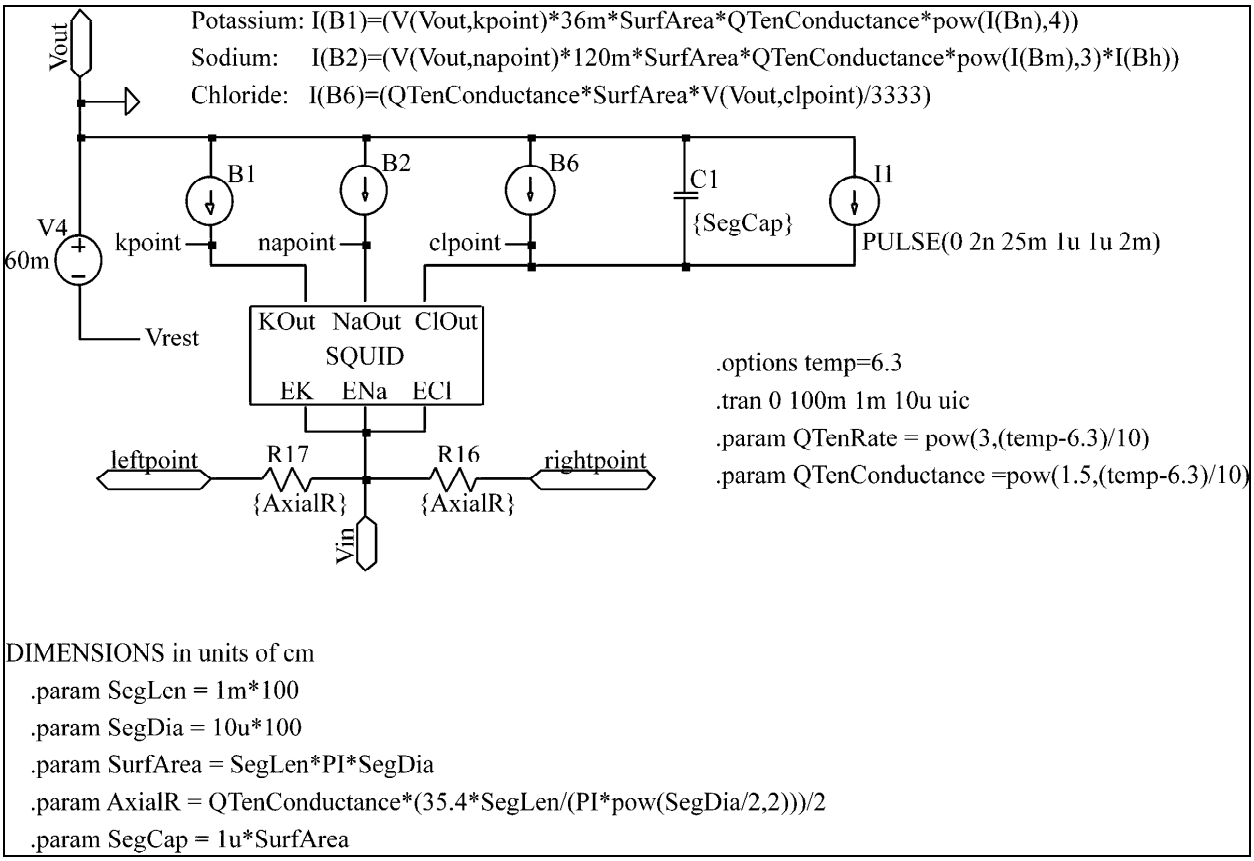

Figure D2. Axon Segment Sub-Model. The Nernst potential sub-model is incorporated by the block labeled “Squid”, that the concentrations are a appropriate to the Squid. Current sources B1, B2 and B6 represent the transmembrane potassium, sodium and chloride currents, respectively, governed by the equations above the model. The Hodgkin-Huxley particle state is represented by the current in behavioral current sources Bn, Bm and Bh, computed as diagramed in Figure D3. This diagram shows the model instrumented for testing, with the inclusion of a current pulse generator, and a defined ground point (SPICE node 0) at the outside of the membrane. These components are omitted from sub-models incorporated into the full axon model.

Figure D4 illustrates an example simulation of the axon segment sub-model depicted in figures D2 and D3. With the initial conditions shown in figure D3, it takes about 10 msec for the membrane potential to reach steady state. A pulse current stimulus of 2 nA magnitude and 2 msec duration was applied across the membrane, beginning at a stimulation time of 25 msec. This stimulus is near threshold. Figure D4-A shows a biphasic action potential, with a depolarizing phase about 2.5 msec in duration.

Figure D4-B shows the state of the sodium channel inactivation particle. The minimum value attained is 0.0799 at 29.3 msec. Since sodium conductance is linearly proportional to this value, it can be interpreted as the fraction of sodium channels that are available to contribute to action potential generation at any time during the simulation. The absolute and relatively refractory

period correspond to the time course of the recovery of sodium channel inactivation. A pedagogically interesting exercise to demonstrate the relatively and absolutely refractory periods is to add a second pulse current source in parallel with the source diagramed in figure D2, with pulse magnitude somewhat larger, for example 5 nA, and pulse onset specified as a parameter. If the parameter value is sequenced with a SPICE .STEP directive (for example .step param T2 27 40 1), the successive traces appear in the plot window. Plots generated by pulses with brief inter-stimulus intervals fail to generate an action potential, while plots generated with longer inter-stimulus intervals do generate action potentials.

Figure D4-C shows the Thevenin equivalent membrane resistance, based on the conductivities of the potassium, sodium and chloride conductances. The calculation was implemented by adding a computed trace to an LT-Spice® graphics window, and entering the expression plotted adjacent to the curve interactively to define the trace. The Thevenin resistance R_{Th} is useful for computing the membrane space constant, $\lambda = \sqrt{R_{Th} / R_{Axial}}$, where R_{Axial} is the segment axial resistance [1]. Using $R_{Th} = 6.06 M\Omega$ and $R_{Axial} = 4.46 M\Omega$, the space constant is 1.17 segments, slightly shorter than the values of 1.33 obtained by subjecting a segment of the full model to a small hyperpolarizing current pulse.

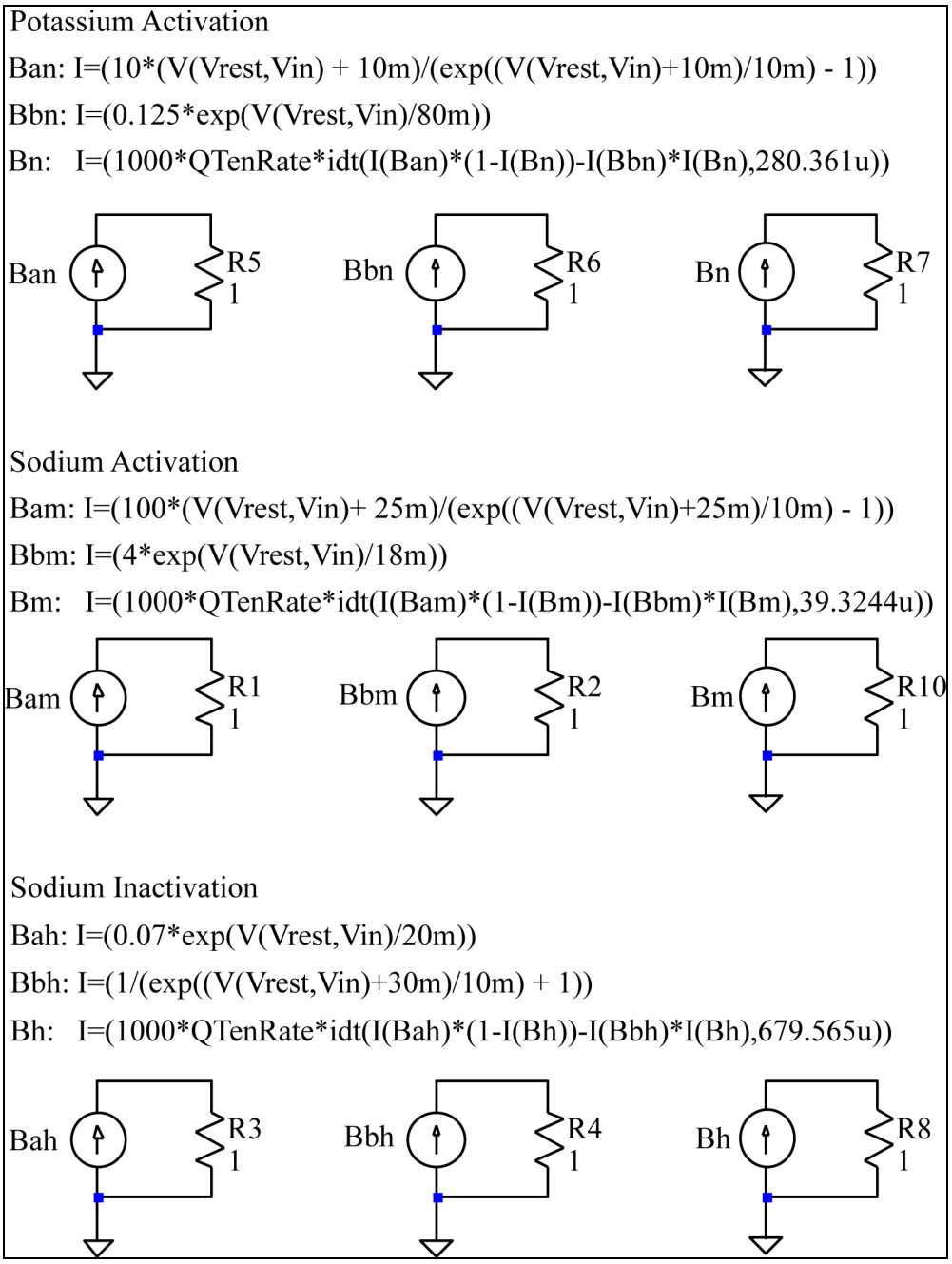

Figure D3. Computation of the Hodgkin-Huxley Particle State. The behavioral current sources make use of LT-Spice® extensions to compute the algebraic parameters and integrate the differential equations explicitly, without introducing a capacitor as a charge integrator, or piece-wise linear approximations to nonlinear functions. State variables still must correspond to voltages or currents, however.

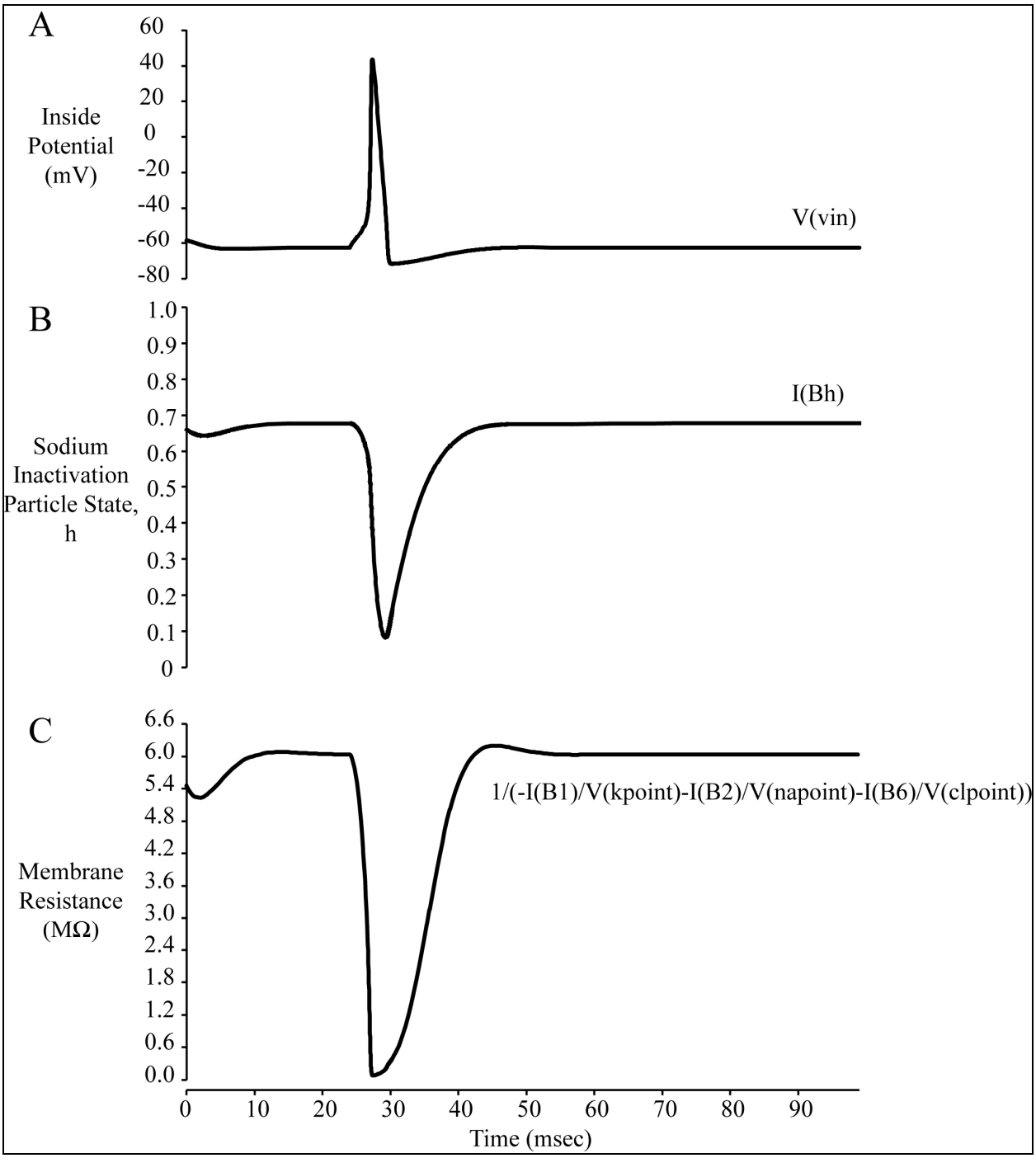

Figure D4. Example Simulation of the Axon Segment Sub-Model. The model diagramed in figures D2 and D3 was simulated. A. The potential inside the axon. B. The sodium inactivation particle state, h. C. The Thevenin resistance of the segment membrane. This plot was obtained by interactively entering the expression shown into an LT-Spice® plot window. The model takes about 10 msec to approach steady state.

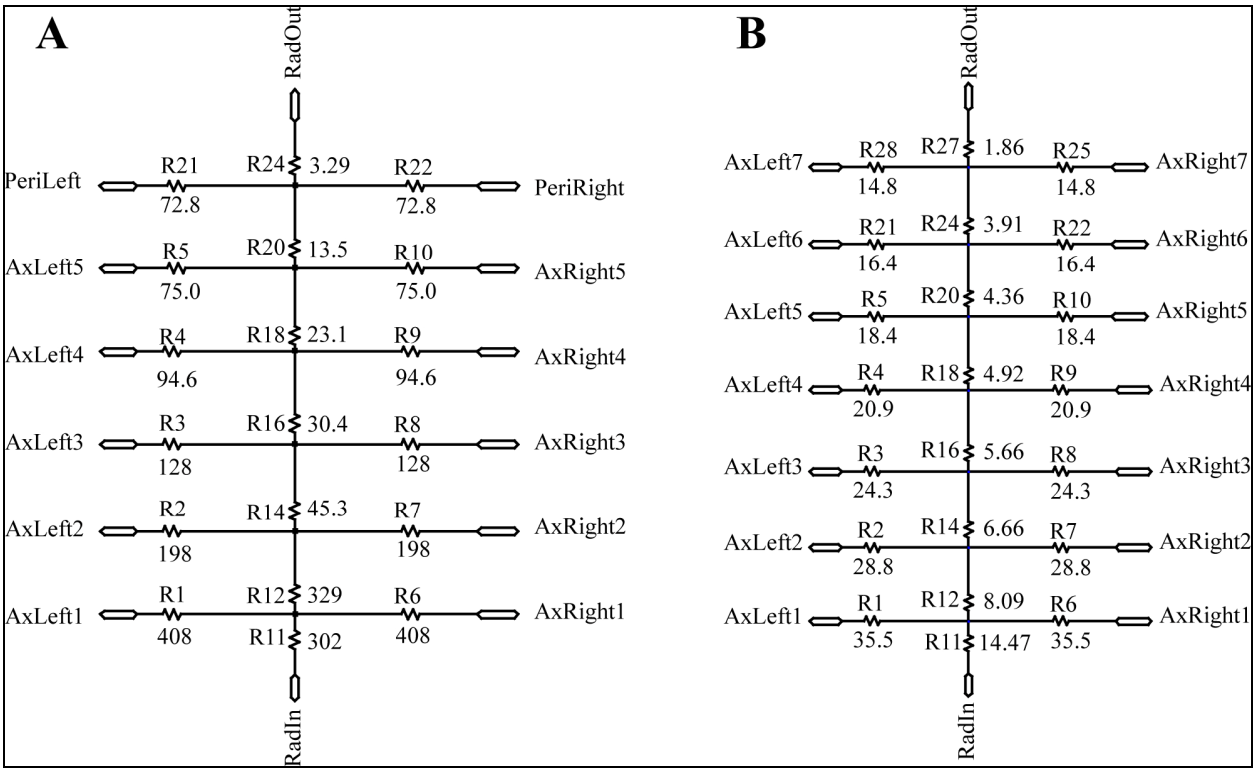

Figure D5. Volume Conductor Sub-Models. The volume conductor modeled by two resistor networks, representing the axial and radial resistance of tissue annuli coaxial with the modeled axon. A. Resistor network representing anisotropic conductance of nerve tissue adjacent to the modeled axon. B. Resistor network representing isotropic conductance in a nonspecific physiological medium surrounding the nerve.

Figure D5 illustrates resistor networks simulating the volume conductor around the axon segment. It is a very simple model, independently modeling axial conductance, and axisymetric radial conductance around the axon. This is a reasonable approach for this model because for the purpose of stimulating a nerve with a nerve cuff, the most important currents are in the radial and axial directions. The axon is modeled as surrounded by coaxial annular shells. The shells are organized into an inner group with anisotropic resistivity, representing nerve tissue, and an outer group with isotropic resistivity, representing a nonspecific physiological medium. For the resistance calculation, panels are modeled as simple boxes, with axial length equal to the segment length (1 mm), thickness equal to the annular thickness, and width equal to the circumference of the annulus at radius equal to the geometric mean of the inner and outer radii. Panel A diagrams the model circuit for the inner group of annuli. Resitivity in the axial and radial directions are set to 10.2 Ω-cm and 30.7 Ω-cm, respectively. The innermost annulus is bounded by the axon, 10μ in diameter, and extends radially to 400μ in diameter, for a shell thickness of 195μ. The surrounding annuli are each 150μ thick, extending to an outer diameter of 1900μ. Panel B diagrams the model circuit for the outer group of annuli. Isotropic resistivity is set to12 Ω-cm. Shell thickness is 250μ for each shell, with the outermost shell extending to 5400μ in diameter.

Figure 1 illustrates the full model including the axon, volume conductor, and two nerve cuffs. The nerve cuffs are conceived to be insulators surrounding a nerve, with two perfectly conductive bands on their interior surface serving as electrodes. The model is comprised of 58 axially coupled segments, each comprised of an axon segment radially coupled to a nerve tissue segment, in turn radially coupled to a surrounding tissue segment. Labeled nets connect the right end of one row of segments to the left end of the succeeding row. The upper left and lower right segments are simply left unconnected at their outward terminals, with no special termination impedance. The presence of a nerve cuff is modeled by breaking the radial connections between the nerve tissue segments and the surrounding tissue segments over a span of 13 segments. Stimulating electrodes are modeled by short-circuiting the outer radial connection of two adjacent nerve tissue segments to each other, and to a current source. The outward connections of the surrounding tissue segments are all coupled to the reference potential (ground, SPICE node 0). Each phase of the stimulus is controlled by a separate current source, with two current sources connected in parallel for each nerve cuff. Zero-volts DC voltage sources are inserted in series with the current source lead wires, for convenience of displaying the net current from the parallel combination of sources. For example, the current in a voltage source named Vprobe1 is referenced as I(Vprobe1) and represents the total current through the electrodes, generated by any number of parallel sources. Current flows through the volume conductor within the nerve tissue around the cuff and around the cuff in roughly equal proportions.

Figure D6 illustrates an expanded view of a portion of the model. It expands the view of the right end of the stimulating nerve cuff, which is the site of action potential initiation. A normal segment of the model can be seen at the right of the figure. It is comprised of an axon segment and its adjacent volume conductor blocks, representing coaxial resistive shells. The remaining segments are similar, but omit the radial connection between the near nerve and far field volume conductor blocks. One of the cuff's two stimulating electrodes is shown as a short-circuit between the outer terminals of two adjacent segments of the near nerve volume conductor. The

other end of the nerve cuff is a mirror image of the section shown. The blocking nerve cuff geometry is identical to the stimulating nerve cuff geometry.

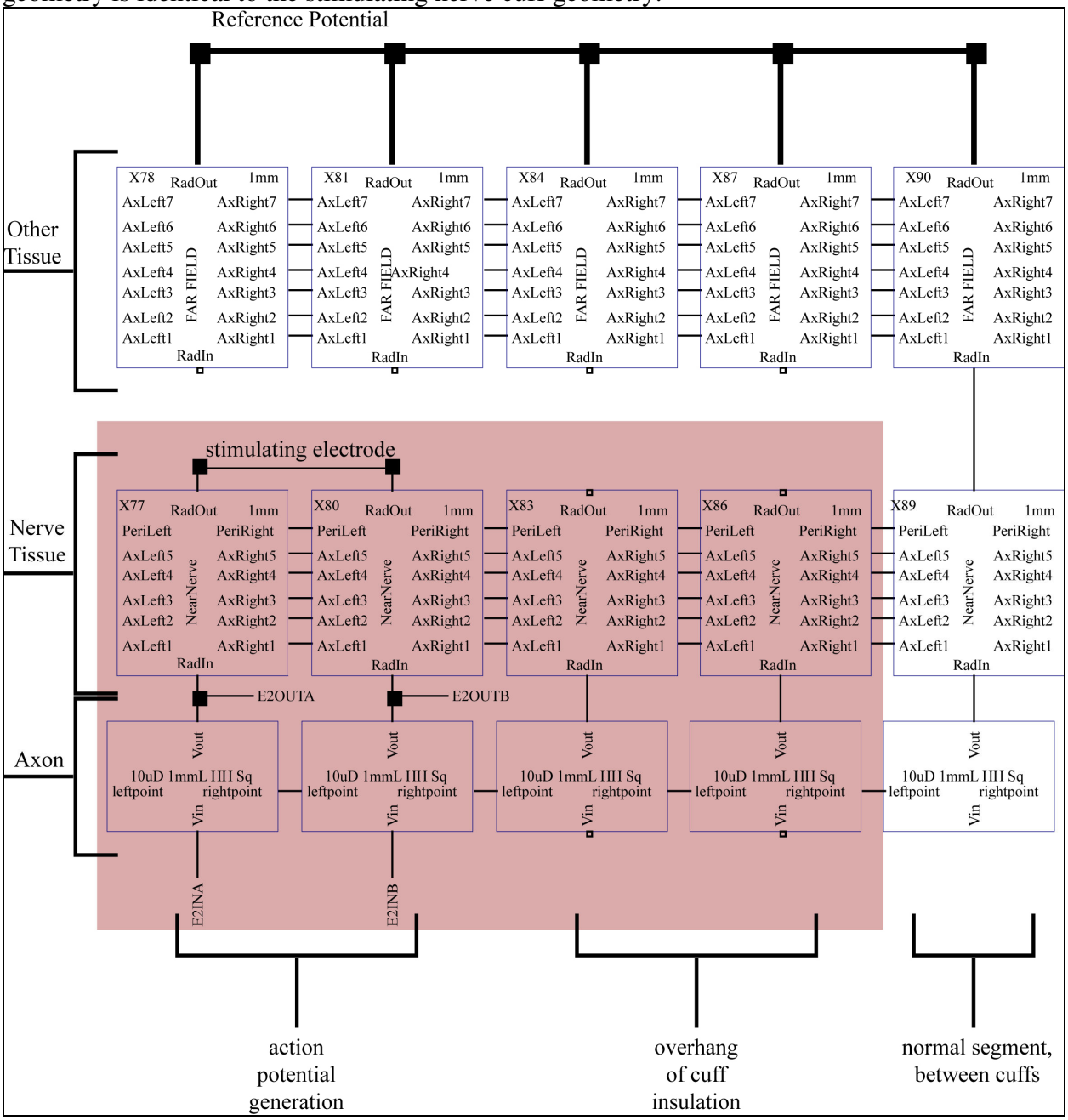

Figure D6. Detailed view of the nerve cuff in the vicinity of the action potential generating electrode. An expanded view of the right end of the nerve cuff on the second row of segments, as shown in full in figure 1. Electrodes are placed two segments in from the end of the nerve cuff, to control shunting of current and keep simulated electrode current density to a reasonable level. Current within the cuff is of the same order of magnitude as the shunt current around the cuff.

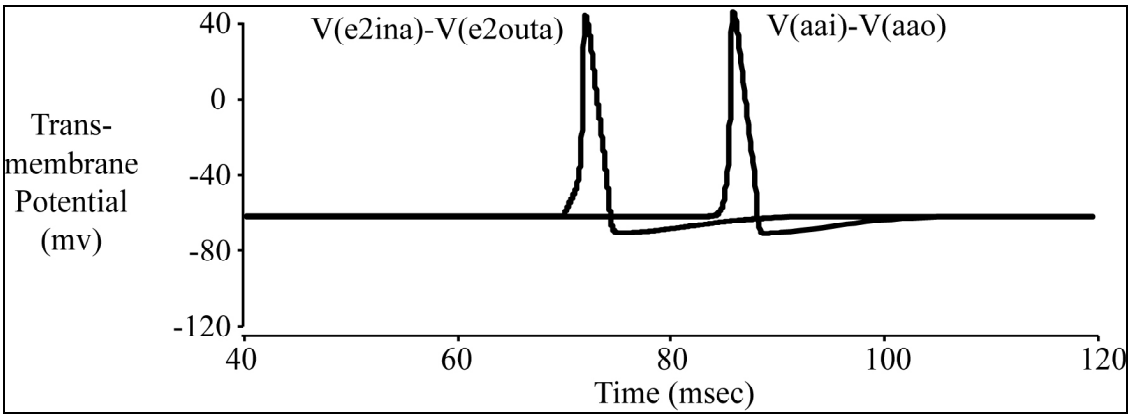

Figure D7. Conduction Velocity. Two action potentials are shown. The left action potential (V(e2ina)-V(e2outa)) is from the site of action potential generation. The right action potential (V(aai)-V(aao)) is from the segment depicted at the upper left of the model depicted in figure 1.

Figure D7 illustrates a propagated action potential in a segment near the electrode depicted in figure D6, and in the segment at the upper left corner of figure 1. The action potential travels 26 segments in 13.9 msec, corresponding to a conduction velocity of nearly 1.9 m/sec. With a depolarizing phase of 2.5 msec, a propagating action potential depolarizes 4-5 segments at any one time. Membrane conductances are near resting values after 14 msec, so that the sight of action potential initiation is nearing its resting state just as the action potential reaches the upper left end of the model.

Figure D8 illustrates some of the events associated with action potential generation, and blocking action potential propagation in one branch of the axon. Figure D8-B shows the stimulating cuff current, and the blocking cuff current. The stimulating cuff current is a brief charge-balanced pulse of duration 2 msec per phase, and magnitude 2 mA. Stimulus onset is at 70 msec into the simulation. The blocking current is comprised of a sawtooth block phase, followed by a brief pause and a triangular recovery phase.

Figure D8-A shows the membrane potential at the site of action potential initiation (V(e2ina)-V(e2outa)), and shows that the action potential does not propagate through the blocking nerve cuff. During the block phase, the left end of the blocking cuff is significantly hyperpolarized, while the right end is slightly depolarized. Hyperpolarization near the time of action potential arrival prevents action potential propagation by. After the action potential is quenched, the blocking current is paused for 11 msec, so that the sodium channel inactivation at the left end of the blocking nerve cuff can recover before a depolarizing current is applied there. If the stimulating cuff current pulse is not applied, so that a propagating action potential is not generated, an releasing the sawtooth current generates an action by anode break. This is because the blocking current removes sodium channel inactivation, increasing the proportion of sodium channels available for action potential initiation, (figure D8-C, I(x151:Bh)), thereby lowering the threshold for action potential generation to below the resting potential. The maximum slope of the sawtooth current is limited by the requirement to not generate an action potential at the right end of the blocking cuff. Although the sawtooth current depolarizes the right end of the blocking

cuff (figure D8-A, V(eb2ina)-V(eb2outa)), it also adds sodium channel inactivation (figure D8-C, I(x172:Bh)), raising the action potential threshold.

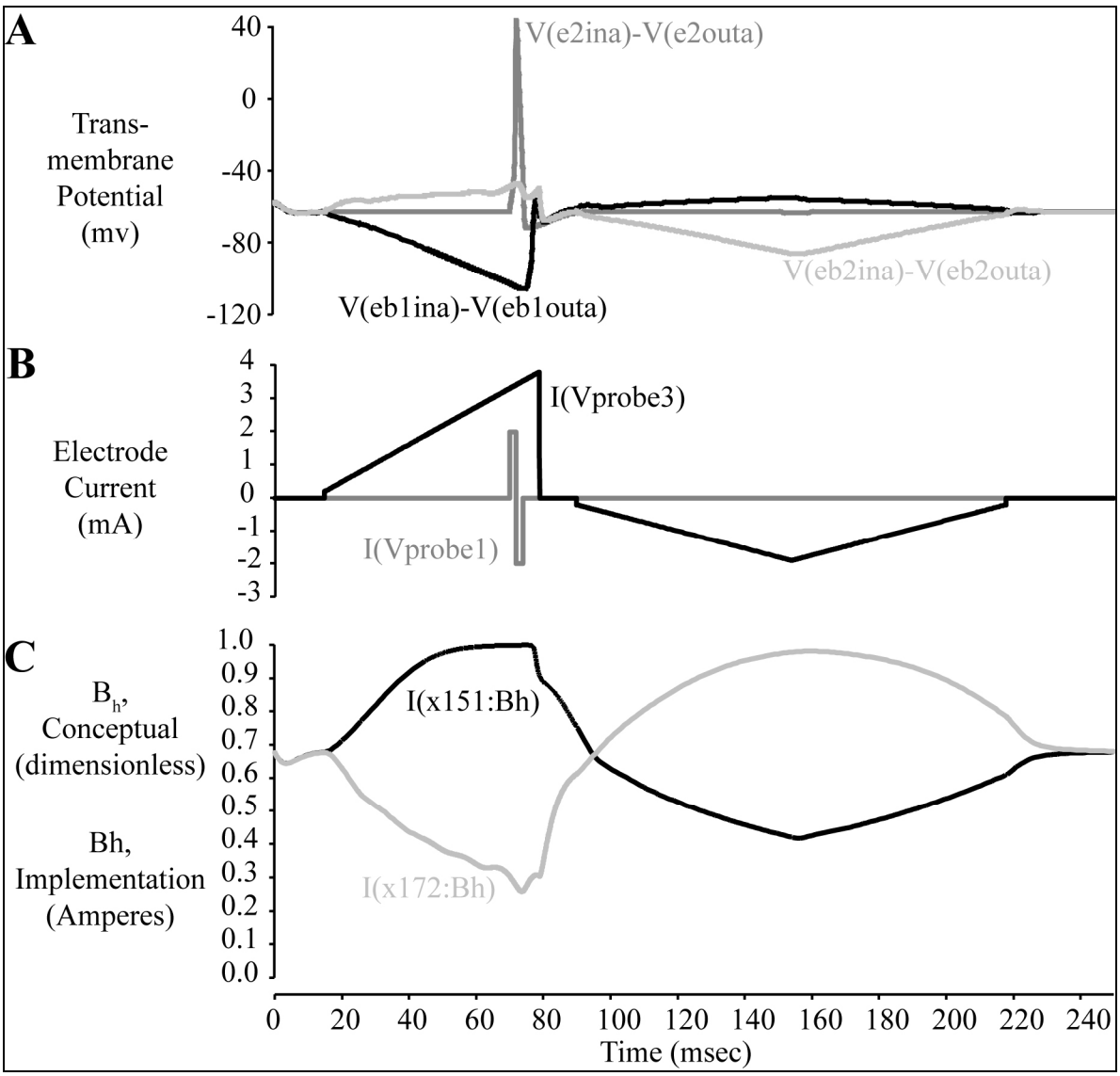

Figure D8. Mechanism of Nerve Block. A. Membrane potential at the site of action potential initiation (V(e2ina) – V(e2outa)), the hyperpolarizing end of the nerve cuff (V(eb1ina) – V(eb1outa)), and the depolarizing end of the nerve cuff (V(eb2ina) – V(ev2outa)). B. Electrode current for the stimulating nerve cuff (I(V(probe1)) and the blocking nerve cuff (I(Vprobe3)). C. The inactivation parameter of a membrane segment model at the hyperpolarizing electrode (I(x151:Bh), and the depolarizing electrode (I(x172:Bh)). Hyperpolarized segments block action potential propagation. The rate at which those segments can be hyperpolarized is constrained by the inactivation parameter at the depolarizing end of the nerve cuff.