APPENDIX A: Goals for Basic and Advanced Tutorials in Current Version of NIA2

BASIC Level Tutorials

Patch: Stationary Signals

Introduction to Neurons in Action
Exploring the Ionic Dependence of the Action Potential

- To become familiar with the overall layout of the panels and graphing windows
- To understand how to stimulate your preparation and record the results
- To become adept at performing an experiment in by changing parameters
- To reproduce the first key observations of the action potential: its dependence on NA and K ions

The Membrane Tutorial
How Currents Depolarize Excitable Membranes

- To understand capacitance and capacitive currents and why they are important for understanding neuronal signaling

Equilibrium Potentials
Determinants of Signals and the Resting Potential

- To understand equilibrium potentials by experimenting with ion concentrations and calculating them using the Nernst equation
- To understand how the resting potential depends on the relative permeabilities (conductances) of Na and K
- To understand how signals can be generated by changing the ratio of the conductances to Na (gNa) and K (gK).

The Na Action Potential
Target of Anesthetics and Toxins

- To observe the action potential and its underlying currents and conductance changes in a uniform patch of membrane
- To experiment with temperature, anesthetic agents, and toxins, and observe their effects on the action potential
- To examine the refractory period following the action potential and its consequences for membrane excitability

Threshold: To Fire or Not to Fire
Treating Myasthenia Gravis

- To investigate whether threshold is at a fixed voltage in neurons
- To understand how threshold depends on the duration of the stimulus and the importance of this relation for synaptic transmission
- To explore the determinants of firing frequency in a train of action potentials generated by a sensory receptor potential

Voltage Clamping a Patch
Measuring Macroscopic Currents Through Channel Populations

- To plot currents in response to individual depolarizing voltage steps and to plot families of voltage steps.
- To plot the conductance increases (due to the opening, closing, and inactivation of the channels) in response to these voltage steps
- To experiment with "tail currents" (which give information on the time course of the closing of the channels when the voltage is returned to rest)
- To use the voltage clamp to demonstrate that a portion of the Na channels are inactivated at rest.
- To experiment with the effect of temperature on the kinetics of the conductance changes

Chattering Ion Channels
Patching Single Na and K Channels

- To observe, measure, and understand the features of the currents flowing through single, voltage-gated Na and K channels
- To appreciate the stochastic nature of the gating of single channels by voltage
- To understand, through experimentation, how depolarization favors the open state of the channel
- To conceptualize how macroscopic currents, in populations of many channels, arise from the aggregate behavior of the microscopic currents through individual Na and K channels

The Ca Action Potential
How to Make a Long, Cardiac-like Action Potential

- To generate a Ca-dependent action potential and observe its special characteristics, particularly how it differs from a Na-dependent action potential
- To generate a hybrid action potential and observe the contributions of the voltage-gated Na and Ca channels to its various phases
- To mimic the basic features of the cardiac action potential

The Neuromuscular Junction
The Classic Model of Synaptic Excitation

- To observe the relationships between the ACh-gated conductance, the resulting current (the EPC), and the voltage change in the muscle fiber (the EPP)
- To experiment with the reversal potential of the ACh-gated EPC and EPP
- To discover the effect on the EPP of adding voltage-gated channels to the muscle fiber

Postsynaptic Inhibition
The GABAergic Synapse: Target of Psychoactive Drugs

- To understand how an IPSP "clamps" the membrane voltage
- To understand disinhibition
- To probe what happens to membrane excitability following an IPSP

Interactions of Synaptic Potentials
Na Channel Kinetics Complicate Synaptic Interactions

- To observe how EPSPs sum in a passive membrane
- To experiment with summation of EPSPs in an active soma membrane (membrane containing voltage-gated Na and K channels)
- To discover how both EPSPs and IPSPs can affect subsequent membrane excitability
- To realize that EPSPs can be inhibitory and IPSPs can be excitatory, contrary to accepted nomenclature

Axons: Signals That Move

The Passive Axon
How Voltages Spread in Axons Without Voltage-Sensitive Channels

- To observe the passive spread of a voltage change along an axon in response to injected current
- To measure the "length constant" of the axon
- To experiment with how membrane resistance and axon diameter affect the passive spread of a voltage
- To investigate whether a change in membrane capacitance affects passive spread
- To observe passive spread when the electrode is located at different positions along this "closed-ends" axon

The Unmyelinated Axon
Experiment with the Impulse Traveling in the Classic Giant Axon of the Squid

- To understand the mechanisms that underlie propagation of the action potential along the axon
- To relate the shape of the action potential as a function of time to its shape as a function of space
- To observe the effects of changing diameter and temperature on the shape and velocity of the propagating action potential

The Myelinated Axon
How Impulses Can Travel at 50 Miles per Hour!

- To observe that the impulse does not "jump" from node to node but spreads out, covering many nodes at once
- To measure the velocity of impulse propagation in myelinated nerve and compare it to unmyelinated nerve
- To change the degree of myelination and the temperature -- two clinically important factors -- and observe the effect on conduction velocity

Partial Demyelination
The Problem in Multiple Sclerosis

- To observe the shape of the action potential as it travels from the demyelinated region of the axon into the myelinated region
- To observe the features of the action potential as it tries to invade the bare axon from the myelinated region
- To observe how changes in the ion conductances in the bare axon and also in temperature affect the ability of the action potential to invade the bare axon from the myelinated portion

APPENDIX B: Goals for Basic and Advanced Tutorials in Current Version of NIA2

ADVANCED Level Tutorials

Patch: Stationary Signals

Extracellular Ca Sensitivity of the Na Channel

Abnormal Serum [Ca] Can Cause Clinical Hypo- and Hyperexcitability

- *To understand how the [Ca]o can influence excitability by affecting the Na channel*
- *To explore the basis of spontaneous firing in conditions of low [Ca]o*

A Dynamical View of Threshold

The Rate of Change of the Voltage Determines Firing

- *To refine the definition of threshold by observing the rate of depolarization (dV/dt) of the membrane*
- *To become acquainted with an informative way of plotting the dynamics of the action potential: as dV/dt versus the voltage (Vm) (known in physics and engineering as a "phase plane" plot)*
- *To explore the factors that can affect the value of the threshold dV/dt*

Na and K Channel Kinetics

Channel Subtypes, Channel Toxins, Membrane Excitability, and Pain

- *To appreciate how changes in the activation and inactivation kinetics of the Na and K channels affect membrane excitability*
- *To understand how , , and change during the action potential and how changes in these variables affect its shape*
- *To explore how Na channel subtypes affect excitability through differing channel kinetics*
- *To observe how Na channel toxins decrease excitability or cause spontaneous firing by altering channel kinetics*

Axons: Signals That Navigate

Axon Diameter Change

How Does an Axon's Impulse Invade a Soma?

- *To observe impulses in the smaller axon struggling to invade the larger axon and understand the curious current patterns that result from the struggle*
- *To explore how the ratio of the two diameters affects the ability of the action potential to invade the larger-diameter axon*
- *To experiment with how temperature changes affect invasion of the larger-diameter axon*
- *To understand why impulses move with no difficulty from the larger to the smaller axon*

Non-uniform Channel Density

Altering Impulse Conduction with Local Anesthetics

- *To test how local application of tetrodotoxin, a Na channel blocker, affects propagation*
- *To test how local application of 4-aminopyridine (4-AP), a K channel blocker, affects propagation*
- *To test how application of the anesthetic lidocaine, which affects both conductances, alters impulse propagation through the anesthetized region*
- *To test how local trauma affects propagation*

Cells

Site of Impulse Initiation

How Geometry Affects Where the Impulse Initiates

- *To interpret movies of impulse initiation and voltage distribution along the cell in response to synaptic input onto its dendrite*
- *To observe the effects of changes in geometry and synaptic parameters on the voltage distribution*

Synaptic Integration

Explore Integration of Excitatory and Inhibitory Inputs on Multiple Dendrites

- *To compare the site of impulse initiation in this neuron, whose axon is myelinated, with that in the unmyelinated axon in the Site of Impulse Initiation tutorial*
- *To explore how impulse initiation depends on the timing, location, strength and reversal potentials of the synaptic inputs*

Impulse Invasion of the Presynaptic Terminal

Using Simulations to Understand Experimental Observations

- *To observe the action potential as recorded in the three major portions of the presynaptic terminal: in the myelinated axon, at the termination of the myelin (the heminode), and along one branch of the presynaptic arbor*
- *To compare simulated currents in the arbor with experimental observations*
- *To experiment with changes in arbor geometry and conductances, observing how they change the currents*
- *To observe how changes in channel density and internode length affect invasion of the arbor*

Coincidence Detection

Achieving Temporal Precision in Integrating Synaptic Inputs

- *To investigate how the insertion of special channels influences the CDW of an auditory neuron*
- *To explore whether faster synapses or higher temperatures significantly narrow the CDW in these neurons*

"Voltage Clamping" Intact Cells

Looking at the Action Behind the Scenes

- *To observe how the capacitance of the (passive) dendritic tree affects currents and voltage steps in a "voltage clamped" soma*
- *To observe how longitudinal currents flowing from the axon or dendrites distort currents one thinks are flowing across the soma's membrane in a "clamped" soma*
- *To observe how the resistance of the electrode can degrade the control of the voltage by the clamp*

APPENDIX C: NIA2 Minimovie Titles by Topic

Movies: Action Potential


Increasing Stimulus Amplitude


Very Near Threshold Stimuli


Changing External Na Concentration


Blocking the K Channels


Ion Conductances Underlying the Action Potential


Blocking the K Channels: View the Conductances


Effects of Cooling

Movies: Axons


Passive Spread of a Depolarizing Stimulus


AP Traveling in a 500 µm diam Unmyelinated Axon


AP Traveling in a 50 µm diam Unmyelinated Axon


AP Traveling in a Myelinated Axon


AP Traveling in an Axon with 5 Myelin Wraps


AP Traveling in a Partially Demyelinated Axon


AP Initiation in a Motoneuron


Synaptic Integration

Movies: Synapses


Postsynaptic Responses to Ach


Saturation of Ach Response


Reversal of Ach Response


IPSP Reversing at Resting Potential

APPENDIX D: Cornell University BioNB 491/BMEP491 Course Syllabus and Calendar

Spring 2007
Principles of Neurophysiology (BioNB 491/BMEP491)
Lecture: Room 213 Kennedy Hall; Lab: B150 Comstock Hall
Web Site: http://instruct1.cit.cornell.edu/courses/bionb491/index.html

Instructors: Dr. Bruce Johnson (brj1@cornell.edu)
Gus Lott (GKL6@cornell.edu)
Rob Bonow (rhb23@cornell.edu)

Week	Lecture-**Lab**	
	Mon	**Tues**-Weds
1 (January 22-24)	Course Objectives, Model Neurons	Electrophysiological Recording Techniques I
	Introduction to Instrumentation and Recording	
2 (January 29-31)	Innervation of a Crayfish Postural Muscle	Electrophysiological Recording Techniques II
	Motor Neuron Physiology	
3 (February 5-7)	The Crayfish Stretch Receptor	Nanobiotechnology and Micro-Electrode Arrays
	Response Properties of Sensory Neurons	
4 (February 12-14)	Generation of the Membrane Battery	Statistics and the Meaning of Life
	Ionic Basis of the Resting Potential	
5 (February 19-21)	Excitability of Snail Brain Neurons	Experimental Animal Welfare
	Excitable Properties of Snail Brain Neurons	
6 (Feb. 26-28)	Action Potential Generation and Conduction	Electrophysiological Recording Techniques III
	Ionic Basis of Action Potentials	
7 (March 5-7)	Electrical Excitability in Plants	Ionotropic Receptors
	Electrical Excitability in a Plant Cell	
8	Metabotropic	Mechanisms of

(March 12-14) Receptors Synaptic Plasticity I

Synaptic Transmission I. Nerve-Target Matching and Synaptic Integration

9 *Spring Break*
(March 19-21)

10 Mechanisms of Synaptic Transmission
(March 26-28) Synaptic Plasticity II and Disease

Synaptic Transmission II. Short Term Synaptic Plasticity

11 Introduction to Central Brain Recording in
(April 2-4) Pattern Generation Behaving Rats

Rhythmic Motor Patterns in Housefly Larvae

12 Hodgkin & Huxley Neurons as
(April 9-11) Experiments I Channel Ecosystems

Voltage Clamp I: Total Membrane Current

13 Hodgkin & Huxley Applied Neurobiology:
(April 16-18) Experiments II Insecticides

Voltage Clamp II: Isolated Membrane Currents

14
(April 23-25) Ion Channels Neuroethology: Seeing the
and Disease Forest Again

Special Projects

15
(April 30-May 2) Nervous System Evolution I. Nervous System Evolution II:
Excitability Transmitter Systems

Special Projects

Required "Texts":

Moore, J.W. and A.E. Stuart (2000) **Neurons in Action 1.5**, Sinauer Associates, Inc. Sunderland, MA.

Wyttenbach, R.A., Johnson, B.R. and R.R. Hoy (1999) **Crawdad: A CD-ROM Lab Manual for Neurophysiology**. Sinauer Associates, Inc. Sunderland, MA.

BioNB/BMEP 491 Neuronal Simulation Assignments[1], Spring 2007

Tutorial Assignment	Page	Due Date
Week 1: Set up	1	January 31
Membrane	5	
Passive Axon	47	
Week 2: Unmyelinated Axon	53	February 7
Week 3: Myelinated Axon	58	February 14
Partial demyelination	62	
Week 4: Patch Resting Potential	13	February 21
Week 5: Patch Action Potential	17	February 28
Week 6: Patch Threshold	29	March 7
Patch Refractoriness	33	
Week 7: Axon Diameter Change	67	March 14
Week 8: Patch Postsynaptic Potential	36	March 28
Patch Postsynaptic Interactions	42	
Week 9:	**Spring Break**	
Week 10: Synaptic Integration	79	April 4
Week 11: Presynaptic Terminal	85	April 11
Week 12: Patch Voltage Clamp	22	April 18
Week 13: Non-uniform Channel Density	71	April 25
Week 14: Site of Impulse Initiation	75	May 2
Week 15: None!		

[1]Moore, J.W. and A.E. Stuart (2000) Neurons in Action 1.5. Sinauer Associates, Inc. Sunderland, MA

Appendix E: Institutions That Have Purchased NIA2 as of November 2008

Institution	Site License	5+ Individual Copies	Had NIA1 Site License*
Agnes Scott College		X	
Amherst College	X		
Arizona State University	X		
Assumption College	X		
Bowdoin College	X		X
Brandeis University		X	
Bridgewater State College		X	
California State University–Fullerton		X	
California State University–Northridge		X	
Carthage College		X	
Centenary College of Louisiana		X	
Clayton State University		X	
Clemson University		X	
Colgate University	X		
Cornell University		X	
CUNY–York College	X		
Delaware State University		X	
Emory University	X	X	X
Grinnell College		X	
Guilford College	X		
Gustavus Adolphus College		X	
Hobart & William Smith Colleges		X	
Keio University (Japan)		X	
Kyoritsu University of Pharmacy (Japan)	X		X
Lycoming College	X		
Mount Holyoke College		X	
Muhlenberg College		X	
Northwestern College	X		X
Northwestern University	X		
Palmer College of Chiropractic		X	
Pomona College	X		X
Purdue University	X	X	X
Queen's University at Kingston (Canada)	X		
Regis University		X	
Rosalind Franklin University	X		
Shepherd College		X	
Sierra College	X	X	
Simmons College	X		X
Southern Wesleyan University		X	
St. Joseph's University		X	
St. Lawrence University	X		

St. Olaf College		X	
The Australian National University (Australia)	X		X
Thiel College	X		
Universidad de Sevilla (Spain)	X		
Universidad Miguel Hernandez (Spain)	X		
University of Adelaide (Australia)	X		
University of Alabama at Birmingham	X		
University of Alberta (Canada)	X		
University of Birmingham (United Kingdom)	X	X	
University of California–Davis	X	X	
University of California–Riverside		X	
University of Connecticut		X	
University of Connecticut Health Center	X		X
University of Illinois at Chicago	X		
University of London	X		X
University of Maine at Orono		X	
University of Maryland		X	
University of Nevada–Reno	X		X
University of North Carolina at Chapel Hill		X	
University of Northern Colorado	X		
University of Nottingham (United Kingdom)	X	X	
University of Pennsylvania	X	X	
University of South Carolina–Beaufort	X		
University of Toronto		X	
University of Toronto at Toronto	X		
University of Waterloo (Canada)	X		
Wesleyan University	X		
Westminster College–Salt Lake City	X		
Whitman College		X	
TOTAL	*40*	*37*	*11*

* Fifty-one institutions purchased a site license for NIA1 and have not yet upgraded to NIA2.

APPENDIX F: University of Texas at Austin Bio 365L Lab Manual Section 3 Excerpt

Section 3: Mechanisms of Action Potential Generation

Theory: Synaptic integration in central neurons

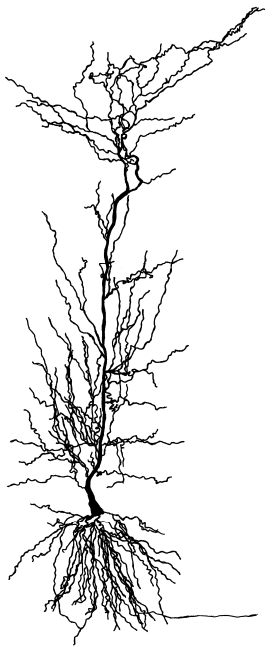


Fig. 1. Dendritic morphology of a CA1 pyramidal neuron of the hippocampus.

All neurons in the brain share a common overall structure, possessing dendrites, a cell body (soma), and an axon (see Figs. 1 and 2). The majority of synaptic input impinges on their highly branched dendrites, and the electrical activity from these events sum together in time and space. Synaptic activity is conveyed ultimately to the soma and axon, the latter of which is the final site of synaptic integration in the neuron. Should the summed activity in the axon exceed a certain voltage threshold, the cell initiates an action potential, the regenerative electrical event that propagates down the axon to the synaptic terminals, triggering calcium influx and neurotransmitter release onto the neuron's network targets.

Temporal summation

If presynaptic inputs to a cell are active within a narrow enough time window, EPSPs and IPSPs in the postsynaptic cell may sum together to form a composite EPSP. The membrane time constant (τ_m) is critical here! It dictates the speed at which the membrane can repolarize, and thus the time window for temporal summation.

Spatial summation

Spatial summation refers to the process of combining EPSPs and IPSPs occurring in different dendritic branches of the neuron. These PSPs sum together and propagate toward the soma and axon.

Cable properties of neurons: Excitatory and inhibitory inputs impinging on the dendrites must propagate to the soma and axon like a wave. In their progress, they undergo a substantial degree of attenuation, analogous to a drop in water pressure in a garden soaker hose as the water propages further and further from its site of "injection". The analogy to the hose goes further, the amount of attenuation is greater the more holes (or open ion channels) that are present. You will recall that according to Ohm's Law, V=IR, more open ion channels would result in a lower resistance and thus a smaller voltage change for a given amount of current. The cable properties of neurons are not disruptive to neurons, but rather give neurons computational power, allowing them to modify the time course and amplitude of synaptic inputs in interesting ways.

Action potential encoding strategies: dynamic range

How is "information" in the nervous system encoded? Put differently, what is it about the pattern of action potential firing that their downstream targets are paying

attention to? Some sensory receptors and central neurons encode information in the frequency of action potential firing (pacinian corpuscles are a good example). As the stimulus increases, so too does the frequency and number of action potentials that are fired. Dynamic range refers to the range of firing frequencies that the neuron is capable of sustaining in response to a stimulus. When a neuron is firing at its maximum frequency (it is at the upper limit of its dynamic range), further increases in the stimulus are no longer reflected in the neuron's action potential output, and ambiguity about the nature of the stimulus will result. Neurons employ many mechanisms by which to keep their firing rates below saturation, including voltage-gated potassium channels, inhibition, and neuromodulation of the properties of intrinsic ion channels.

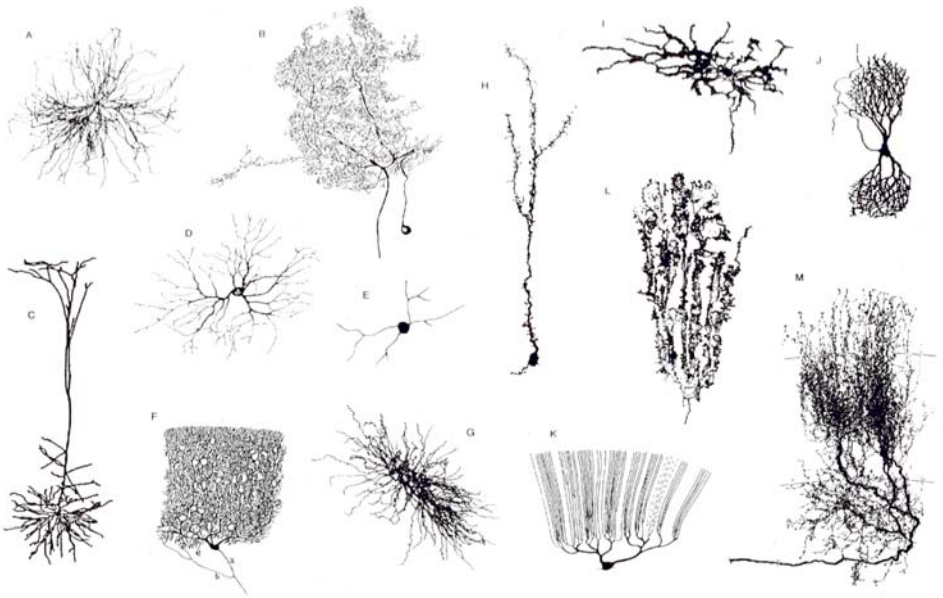


Fig. 2: Diversity of dendritic structure in central neurons. The degree of branching and overall dendritic shape is distinctive for neurons in different functional areas of the brain**.** From Mel, 1993.

Commonly used terminology.

Depolarization. A movement of the membrane potential in a relative positive direction.
Hyperpolarization. A movement of the membrane potential in a negative direction.
Afterhyperpolarization. The silent period of time immediately following the action potential, when the membrane potential undershoots threshold for action potential generation.
Interspike interval. The period between action potentials; the inverse of frequency.
Refractory period. The period of time following the action potential where the membane is relatively inexcitable, due to inactivation of sodium channels. This period typically overlaps the afterhyperpolarization.
Reversal potential. The membrane potential at which currents change polarity (e.g. from inward to outward, and vise versa). For channels that are permeable to primarily a

single ion type, the reversal potential is equivalent to the equilibrium potential for that ion. However, the reversal potential may apply to channels selective for multiple ion types (e.g. H-type channels are permeable to both sodium and potassium), in which case the reversal potential for currents through the channel is intermediate between the equilibrium potentials of the permeant ions.

Voltage-gated channels vs. voltage-insensitive leak channels.

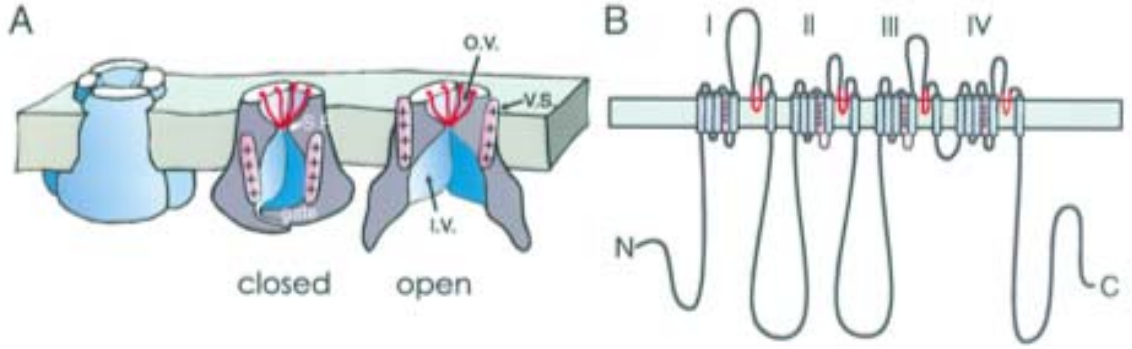
In the previous laboratory module, you learned how the resting potential is the summation of the currents whose individual magnitude and direction are determined by a) the number of channels present, and b) the electrochemical gradient governing the ions that permeate the channel. The current through such a channel was represented by the equation:

$$I_{ion} = G_{ion} * (V_m - E_{ion}) \qquad \textbf{Equation 1}$$

and… $G_{ion} = n * g_{ion}$ **Equation 2**

G_{ion} represents the total ionic conductance, g_{ion} is the unitary conductance through a single channel, n is the number of open ion channels, and $V_m - E_{ion}$ represents the driving force.

Structural features of voltage-gated ion channels



1. Selectivity filter:
   Part of the basis for the ability of both voltage and ligand-gated ion channels to conduct ions of is simply due to the nature of the pore forming part of the channel, which can select for ions on the basis of size and charge.

2. Voltage sensor:
   In order for ion channel proteins to be voltage gated, the protein must have residues that sense voltage. The voltage-sensing region of most voltage-gated ion channels is thought to be on the 4th transmembrane segment, consisting of a series of positively charged amino acids. These residues would thus move during a voltage change, and provide the necessary energy for a conformational change in the protein.

3. Activation ‘gate’
   Channels must activate before they inactivate
4. Inactivation ‘gate’
   There is a good body of evidence supporting that the inactivation gate is located on the C-terminus on the intracellular face of the channel. Channel inactivation is thought to occur due to binding of this intracellular, tethered part of the protein to binding sites on the inside of the channel pore.

Biophysical steps underlying action potential generation.

The action potential is generated through the coordinated activation of both voltage-gated sodium and potassium channels. Should depolarization of the membrane during synaptic activity be sufficient to activate a significant local population of sodium channels, the flow of events leading to action potential generation is as follows:

1. Action potential upstroke: sodium channel activation.
Remember that the equilibrium potential for sodium in neurons is approximately +40-50 mV. When voltage-gated sodium channels open, the electrochemical driving force on sodium is to flow inside the cell, producing a regenerative depolarization that makes up the upstroke of the action potential. During the upstroke, more and more sodium channels are recruited.

2. Action potential repolarization. Without a mechanism to oppose sodium channel activation, the membrane potential would be locked permanently at the equilibrium potential for sodium, at about +40-50 mV. However, two mechanisms terminate membrane depolarization.

2A. Sodium channel inactivation. Following activation, sodium channels rapidly enter an inactivated state. You should remember from your coursework that inactivation is biophysically distinct from channel deactivation. During inactivation, a charged, intracellular portion of the channel protein becomes attracted to charges now exposed in the open pore, and plugs the channel (the “ball and chain” hypothesis). By contrast, deactivation requires repolarization of the membrane, whereupon the transmembrane portion of the channel changes conformation and no longer permits the flow of sodium ions.

2B. Potassium channel activation. During the upstroke of the action potential, voltage-gated potassium channels are activated in addition to sodium channels. The equilibrium potential for potassium is about –90 mV, far away from the peak of the action potential (around +40 mV). Thus the electrochemical driving force strongly drives potassium out of the cell, leaving net negative charges and hyperpolarizing the cell membrane. Fortunately for all living things possessing nervous systems, the speed at which potassium channels activate is slightly slower than that of sodium channels. This allows sodium channels a brief window of time in which to generate

the upstroke of the action potential. Without this mismatch in activation timing, the sodium and potassium currents would cancel out, and the neuron would be inexcitable!

Voltage-gated ion channels and passive membrane properties

It is extremely important to realize that the action potential is an "active" process. The voltage changes during both the upstroke and repolarization of the action potential occur at rates faster than that of passive voltage changes. Action potentials repolarize in less than a millisecond, whereas passive voltage changes, reflected by tau, the membrane time constant, are on the order of many milliseconds. This is possible because the action potential is dominated by the kinetics of the underlying voltage-gated channels (the speed at which they activate, inactivate, and deactivate). *In general, below action potential threshold, changes in membrane voltage are dominated by the passive resistive and capacitive properties of the membrane. Above action potential voltage threshold, voltage-gated channels dominate voltage changes with (in general) faster kinetics.*

Diversity of ion channels in central neurons

To this point, we have portrayed synaptic integration as a rather simple process. Neurons would appear to algebraically sum subthreshold synaptic inputs that occur within a window of time dictated by the passive membrane time constant. Should action potential threshold be exceeded, voltage-gated sodium and potassium channels dominate, producing transient, "all-or-none" electrical events that are propagated down the axon.

However, all neurons in the brain possess many more types of voltage-gated channels which in turn display a staggering variety of properties. This diversity confers different signaling characteristics (threshold for action potential initiation, firing frequency, etc…). In some sense one can regard each neuron type as having an electrical "signature". In this laboratory, we will not consider all types of voltage-gated channels. Below is a description of only some of the ion channel types that are expressed by neurons. However, we can only focus on a few specific channel types in the laboratory exercises.

Voltage-gated potassium channels

1. **Delayed rectifier ($I_{K(D)}$).** A high voltage-activated (HVA) potassium channel. This channel type is a major component of the repolarization of the action potential. This channel type shows little inactivation. At the molecular level, subunits from many different potassium channel families may give rise to this electrophysiological phenotype.

2. **D-type potassium channel (I_D)**. A low voltage-activated (LVA) potassium channel. This channel is fast activating and slowly inactivating. It was first named for its tendency to *delay* the time to action potential firing during membrane depolarization. This channel type is blocked by 50 µM 4-aminopyridine. This channel typically consists of members

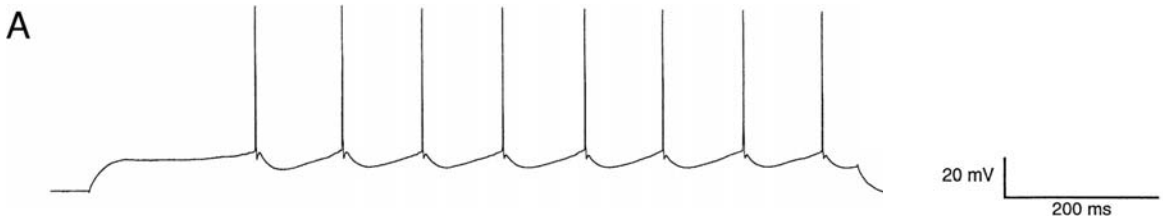


Fig. 6. Delayed firing of a pyramidal neuron in the hippocampus. D-type potassium channel contribute to the initial suppression of firing. Note the "shoulder " of the initial depolarization, caused by the activation of potassium channels.

of the Kv1 family of potassium channels in mammals, or the *Shaker* potassium channel family in drosophila.

3. **A-type K^+ channel (I_A)**. Another LVA K^+ channel. This channel displays strong inactivation. It is blocked by millimolar concentrations of 4-aminopyridine, although the blocker is not specific to A-type channels at this concentration. This channel typically consists of members of the Kv4 family of K^+ channels in mammals, or the*Shal* family in drosophila. Like D-type channels, A-type channel activation can result in a transient suppression of action potential firing, but its inactivating properties renders the channel's influence sensitive to the cell's prior level of depolarization (e.g. see Fig. 7). Both D-type and A-type K^+ channels are present in CA1 pyramidal neurons.

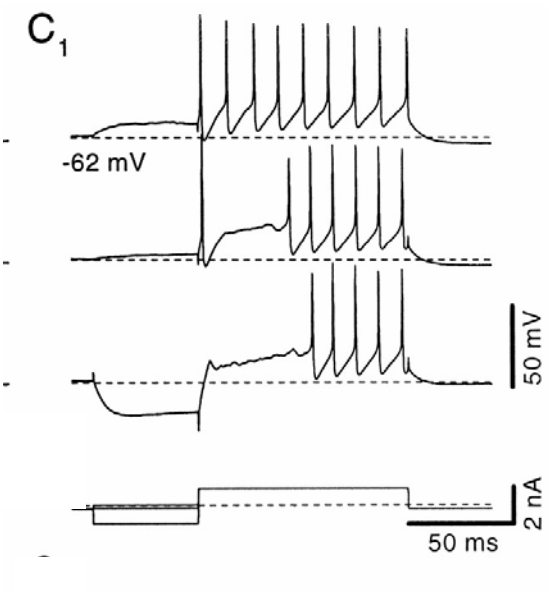


Fig. 7. Effect of A-type channels on the firing pattern of a central neuron. With prior membrane depolarization (via a small somatic current pulse), the cell fires a regular train of action potentials in response to a larger depolarization (resting potential=dotted line). However, a pause in action potential firing is observed in response to the same depolarization when inactivation of A-type potassium channels is removed by prior hyperpolarization (lower trace).

Adapted from Kanold and Manis, 1999.

Final Perspective: Role of voltage-gated channels in behavior and disease.

The critical balance of inward and outward currents is of critical importance. Quite literally, we could not function without this balance, as all behavior and cognition requires the proper coordination not just of single neurons, but entire networks. When this delicate balance is disrupted, the consequences are dire. Epileptic seizures are perhaps the best example of the functional manifestation of an imbalance between inward and outward currents. Indeed, all of the major treatments for epilepsy are drugs that modulate ligand and/or voltage-gated ion channels (e.g. carbamazepine and phenytoin). An understanding of ion channel physiology is thus of critical scientific and medical importance, and has implications for our everyday lives.

APPENDIX G: University of Texas at Austin's BIO365L
NIA2 Na Action Potential Lab Handout (3/26/08)

Page 6.

Neurons in Action: The action potential

Experiments and observations

Generate action potentials.

1. Press R&R to generate an action potential
   You will see an action potential displayed on the Voltage-vs-Time graph in red.
   The brief stimulating current pulse is shown in green.
   INa is shown as a blue line.
   IK is shown as a brown line.

2. Observe the currents underlying the action potential.
To do this, press Membrane Current Plots (in the PkG Manager) to bring up the appropriate graph and then run the simulation (press R&R). You will see graphs of the Na and K currents (patch INa and patch IK) that cause the rise and fall of the action potential.

If you are familiar with voltage clamp experiments, you will note that these action potential current patterns are not the same as those observed with a voltage clamp. The voltage clamp measures currents in response to the relatively simple stimulus of the voltage step (see Patch Voltage Clamp Tutorial); in contrast, the currents flowing during the complicated voltage change of an action potential cannot be measured experimentally but can only be calculated, as NEURON is doing here.

3. Now observe the conductances changing during the action potential.
Press Membrane Conductance Plots (in the PkG Manager) to bring up a graph for plotting the Na and K conductances (Patch gNa and Patch gK) as a function of time. Run the simulation. Of course, you could not make these observations of conductance with a current clamp or even with a voltage clamp in a real experiment. They may only be calculated or seen using a simulator.

4. ***Question:***
What underlies the depolarizing ramp at the beginning of the action potential?

The shape of INa

Why is INa so "kinky" with two plateaus? Why does it not have a smooth time course since the voltage and gNa and even IK all have smooth time courses? Here are two experiments to assist your reasoning.

1. Plot the driving force on the Na ions as a function of time.
Press the "Plot Driving Force for INa" button (in the PkG Manager). An appropriate graph will come up, overlying the Voltage-vs-Time graph. Run the simulation by pressing R&R.
The red line is the action potential
The black line is the driving force on Na (Membrane Voltage minus ENa).

2. Find the time at which the minimum (the notch) in INa occurs.
Use the Crosshairs by clicking the left mouse button on the current trace; read off the time (the x value) in the blue bar at the top of the graph. Find the y-values at that time on the curve that plots the INa driving force and on the plot of gNa. Can you now explain the kink?

3. Measure the size of the peak INa.

Keep a note of this value for comparison with Na currents in later exercises.

4.Close the Driving force for INa-vs-Time graph.
Close this panel to expose the Voltage-vs-Time graph beneath for the remainder of the experiments in this tutorial.

More questions
Does changing either the length or diameter of the patch alter the action potential in any respect? Should it?

Does the location of the stimulating electrode in the patch matter? Should it?

Suppose the lipids in the membranes were not such strong insulators and the membrane's thickness had to be doubled. What would this do to the membrane's capacitance per unit area? Call up the Patch Parameters panel (in the Hoc Manager) and change the value of the capacitance to see how your change would affect the shape of the action potential. When you change the capacitance, there will be changes in the rate of rise and in the final amplitude of the depolarization in response to the current pulse. Are these changes compatible with the explanation of capacity charging in the Membrane Tutorial?

Can you find parameters that would permit you to elicit more than one action potential in this stimulation?

Study the effect of temperature on the action potential and underlying conductances.

1.Change the temperature (in Run Control).
You can warm or cool the patch by using the UP or DOWN arrows (to the right of the white value field) or by typing a new value into the field using the field editor.

2.Compare traces at different temperatures.
Use the "Keep Lines" option (right mouse button menu for comparing traces. You must have your cursor positioned on the graph when you select "Keep Lines."
Note that your experiments so far have been carried out at 6.3 °C, the standard reference temperature for squid, as invertebrate, used by Hodgkin and Huxley. Although the lack of heating in labs in post World War II England is the most likely reason for this low temperature in their experiments, the effect was to slow the changes in the ionic currents to the point where the electronic circuits could control the potential more accurately.

3.Draw conclusions from this important experiment.
What happens to the duration of the action potential if you change the temperature? Can you explain your observations by studying the effect of temperature on the underlying conductances? The results of this experiment are crucial for understanding why temperature affects the ability of an action potential to invade demyelinated regions of myelinated nerve (Partial Demyelination Tutorial).

Probe the axon in unrealistic ways.

1.Open the Patch Parameters panel so that you can do more experiments.

Do this by clicking on the "Patch Parameters" button (in the PAC Manager) if this panel is not already open.

2.Partially block both the Na and K conductances.

The anesthetics procaine and lidocaine reduce both the Na and K conductances by almost equal factors. Reduce the values of the conductances (the Na channel density and the K channel density) by a factor of 2

3.Compare the normal and partially blocked action potentials.

Using Keep Lines, compare action potentials generated at different "concentrations" of anesthetic by continuing to divide by a factor of 2. By how much must you reduce the two conductances to block the generation of the action potential? Make a note of this value for comparison with experiments below using selective poisons of each channel type.

Block the Na channels with the poison tetrodotoxin or saxitoxin.

1.Block the Na channels by reducing the Na conductance.

Restore the value of only the Na channel density by dividing by a factor of 2 as you did above until the regenerative response disappears. Use Keep Lines to compare the action potentials in normal saline and at different degrees of block of the Na channels.

3.*Question:*

Which is more effective at blocking action potentials, a toxin that selectively blocks Na channels or the anesthetics (investigated above) that block both Na and K channels? Why?

Change the external and internal Na concentrations.

You can reproduce the experiments of Hodgkin and Katz (1949) on squid giant axons. They altered the external Na concentration and found that the amplitude and rate of rise of the spike were tightly coupled to the changes in $[Na]_o$. Furthermore, you can also do an experiment for which the techniques were not available at the time: you can change the internal Na concentration.

1.Change the $[Na]_{out}$.

Make your changes over a wide range (e.g., by 2 fold steps) from double the default value down to a value equal to the internal concentration. Plot the peak of the action potential and I_{Na} as a function of $[Na]_{out}$. How do your results compare with those of Hodgkin and Katz? You can check your plot of I_{Na} versus $[Na]_{out}$ against ours.

2.Change the $[Na]_{in}$.

Go beyond what Hodgkin and Katz were able to do and perfuse the axon internally with a solution of your choice. You can simulate a "very thick nerve" experiment by doubling the $[Na]_{in}$ and then repeating changes in the $[Na]_{out}$. How should the plots change if the internal Na concentration is doubled?

APPENDIX H: University of Texas at Austin Bio 365L Lab Manual Laboratory 4 Excerpt

Laboratory 4: Action potential signaling in hippocampal neurons

Experiment 1: Voltage threshold for action potential initiation

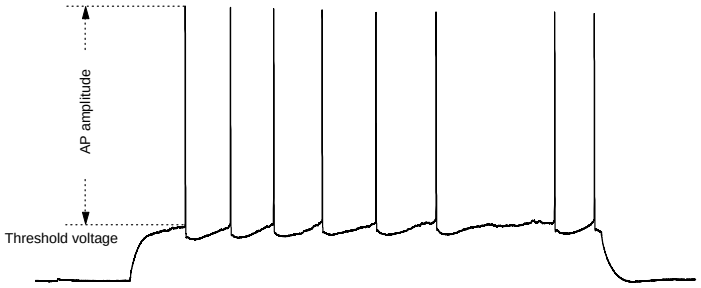
Rationale

Action potentials are initiated when inward sodium currents exceed outward potassium currents, and become regenerative. You will investigate whether this initiation point occurs at a specific voltage threshold in response to stimuli of different strengths as well as during repetitive firing.

Experiment

A. Deliver 1000 ms step pulses at a threshold current intensity. Now deliver the same duration pulse at 5 larger intensities (but below 100 pA). The action potential voltage threshold is the voltage at which action potentials are first initiated (see diagram).

B. Change the resting potential (<10 mV either direction) with constant current injection through the amplifier and deliver your current pulses.



For your results section

1. Plot threshold voltage vs. current intensity for the first action potential initiated in each train
2. Superimpose 2-3 select traces in a graph to illustrate your findings in the above plot.

For your discussion section

1. Measure the voltage at which the first action potential is initiated. Is it roughly the same (i.e. within 5 mV of one other) or different for the different step amplitudes? Plot threshold voltage vs. step amplitude.
2. Do later action potentials appear to be initiated at the same voltage that the first one does? Why or why not? What does this result say about the relative balance between sodium and potassium currents during repetitive firing?
3. Does the value of the resting potential from which the action potential is initiated matter, in terms of the threshold voltage? Explain fully.

Experiment 2. Dynamic range of frequency encoding with action potentials.

Rationale:

Hippocampal neurons encode information about the strength of their excitatory inputs in the rate of their action potential firing. Here you will determine over what range of firing frequencies this encoding can occur.

Experiment:

Use the steppulse command module to deliver 1 second duration depolarizations. Start from threshold stimulation, and increase the amplitude by 2-5 pA steps, until you detect the firing frequency (or number of action potentials during the step) no longer changes significantly. You should adjust your amplitude of your step size according to the sensitivity of the cell's firing rate. Use smaller step increments at lower stimulus intensities when firing rate is highly sensitive to step size. Use larger step increments when you approach the upper end of the cell's dynamic range (firing rate begins to saturate).

For your results section.

1. In the same graph, show 4-5 selected traces showing a range of different response frequencies. You can offset the traces so that they do not overlap. Simply click on a given trace, and hold the mouse button down for about a second. You can then move the trace with the mouse.
2. Plot AP firing frequency (Hz) vs. current strength (pA).

For your discussion section.

1. What does dynamic range mean?
2. What does your plot of firing frequency vs. current strength say about the dynamic range of the cell?

Experiment 3: Action potential refractory period

Rationale

You have learned in your coursework that a refractory period is the time after an action potential where a neuron is transiently rendered less excitable, or entirely inexcitable. In this experiment, you will probe this phenomenon directly using pairs of brief depolarizations. The refractory period is not only extremely important in regulating repetitive firing in neurons, it also serves as an easy experimental assay with which to eavesdrop on the complicated interactions of voltage-gated sodium and potassium channels.

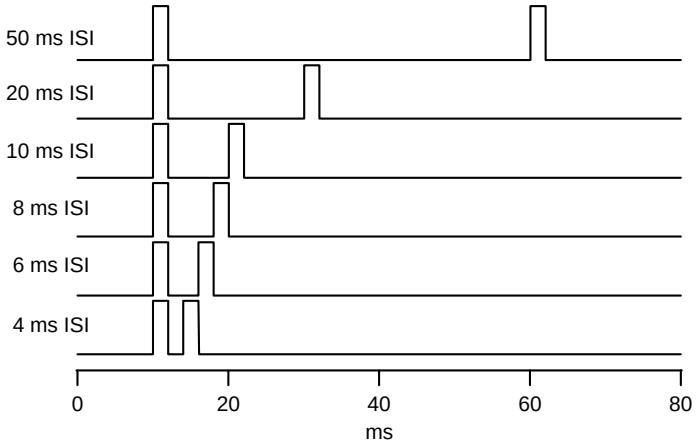
Experiment

Select a paired pulse stimulus protocol, and find threshold by increasing the value in the multiplier window next to the stimulus protocol (the waveform is set to 1 pA amplitude, so the multiplier indicates the amplitude of the stimulus entirely). Each pulse is short, only 1 ms long. Deliver twin pulses of differing InterStimulus Intervals (e.g. pair50ms_DAC, pair10ms_DAC). At shorter ISIs the action potential will either change shape and/or amplitude, or fall subthreshold entirely. Now increase the stimulus

amplitude by increments, up to 2000 pA. Can you trigger the second action potential? If you can, does it look the same as the first action potential? If you are able to initiate a second action potential, record the stimulus current. You are within a window of time referred to as the *relative refractory period*.

At very short ISIs, you will be unable to elicit an action potential with the second stimulus even with stimulus amplitudes of 2000 pA. This is the *absolute refractory period.* Make a note of the value of this ISI.

The stimuli:



For your results section.

1. Make a plot of threshold current (for the second action potential) vs. interstimulus interval (ISI). As in all of your graphs, be sure to label both axes clearly, including the units in parentheses. For example, the Y-axis will be labeled Threshold current (pA).
2. Make a graph with responses to just suprathreshold stimulation. Superimpose responses to different ISIs.
3. Plot action potential amplitude (relative to rest) as a function of interstimulus interval.

For your discussion section

1. Within the relative refractory period, why does the generation of the second action potential require a larger stimulus strength compared to the first? In regard to sodium channels, what are you actually changing as you increase the size of the current step (and thus the magnitude of the depolarization)? Discuss the mechanisms underlying both the absolute and relative refractory periods.
2. As you decreased the interstimulus interval, you likely noticed that the amplitude of the action potential decreased in a graded manner. Why? Be sure to discuss in your answer both sodium channel inactivation and potassium channel deactivation, and how these processes might contribute to this phenomenon.

Experiment 4: Variations on the theme

Group 1:

Measure the action potential firing characteristics of your choice (as many as you can manage) in various interneurons in either the stratum radiatum or oriens. Compare and contrast these properties with pyramidal neurons.

Group 2:

Bath apply 250 µM TEA-Cl. At this low concentration this substance blocks primarily (though not exclusively) a type of potassium channel called a calcium-activated potassium channel, or "BK". Design an experiment to observe whether action potential repolarization is compromised. Does this manipulation affect refractory period?

Group 3:

Test the effects of temperature on the electrophysiological properties of your choosing. Your instructor can provide your with a small temperature probe to measure ACSF temperature near your slice. You can raise and/or lower temperature by changing to a solution kept in either a cooled or heated water bath.

Group 4:

Partially block voltage-gated sodium channels with a low concentration of tetrodotoxin (10-20 nM). How does this affect action potential firing threshold and frequency? Action potential repolarization? How is the refractory period affected?

Group 5:

To be announced.

APPENDIX I: Amherst College Biology 35 Lab Instructions

Biology 35 Computer simulation of synaptic transmission Fall, 2008

The "Neurons in Action" computer program we used to run the Hodgkin-Huxley equations also contains a simulation of synaptic transmission. This isn't a computation from first principles as for the HH equations; rather, the synapse simulation uses a plausible function to estimate the time course of conductance change at chemical synapses, and then calculates currents and membrane potentials from there.

As in our previous HH computer lab, go to the Biology/Psychology computer room (Life Sciences 425), log on to a computer, then Start | All programs | Course-related | NIA2PC.

Select Tutorials | The Neuromuscular Junction. Read the introductory paragraphs, then click on Start the Simulation. You will see several popup windows as before. Follow the instructions in this handout; after finishing the neuromuscular junction tutorial, close all windows and move on to the Postsynaptic Inhibition tutorial. Follow this tutorial through AReveal the IPSP@ on p. 65. Then do "Interactions of Synaptic Potentials."

After you have completed the attached instructions, do one additional exercise that isn't in the printed protocols: observe the inhibitory effect of a depolarizing inhibitory synapse. To do this, keep the windows open from the "Interactions of Synaptic Potentials" tutorial.

In Alpha synapse (1), set onset to 1 msec, $g_{max} = 0$ (initially, to observe the effect of the other, excitatory synapse), and reversal potential e = -63 mV. Since the resting potential here is -65 mV, this synapse will cause a small depolarization of the postsynaptic membrane when active.

Alpha synapse (2) is a normal excitatory synapse: onset = 4 msec; $g_{max} = .505$ conductance units (just above threshold); reversal potential e = 0 mV.

When you Reset and Run, you should see an impulse generated by alpha synapse(2). Now add the depolarizing inhibitory synapse (1), by changing its g_{max} to 3. When you Reset and Run, the combined synapse should fail to evoke an impulse.

Explain why adding the depolarizing synapse (2) is inhibitory, based on your knowledge of the relation between reversal potentials and excitation vs. Inhibition.

APPENDIX J: Amherst College Biology 35 Exam 2

Biology 35 Exam 2 October 16, 2008

This is an open-book exam. You may consult any written, printed or web sources you wish. However, you may not communicate about the exam with any other person in any way while you're taking it.

Although no question requires it, and I don't think it will be needed during the exam, you are permitted to use the "Neurons in Action" program in the computer room on the 4th floor in Life Sciences. I can't guarantee hall door access to the 4th floor, however, after around 10 PM.

Put the completed exam in Prof. George's mailbox on the 3rd floor of the Life Sciences building in the evening (the front door of Merrill is open until 1 AM and you can reach the Bio. office area from there), or Friday morning at Prof. George's office no later than 10 AM.

If you wish, you may prepare answers using a word processing program and e-mail your answers in an attached file to sageorge@amherst.edu. However, if you include graphs or equations in some of your answers, it may be easier to write them out by hand. Please write legibly using a pen or a reasonably dark pencil.

Answer question 1 (60 points), and <u>either</u> question 2 <u>or</u> question 3 (40 points).

---

1. (A) Refer to figure 7.7A on p. 129 in our text, showing impulses in dendrites of Purkinje cells. Accept that these are indeed calcium impulses in the distal dendrites and sodium impulses in the cell body (soma), as the figure legend claims.
(i) Note that continuous depolarization of the dendrite leads to cycles of only two Ca spikes and then a pause, but when the soma is depolarized, there are many Na spikes, then a single Ca spike (indicated presumably by the slightly different shape of the Ca spike) and then a pause. Suggest a plausible physiological mechanism that would cause Ca spikes, but not Na spikes, to be followed by a pause in firing.
(ii) Assume the electrode positions in Fig. 7.7c and 7.7d are 200 μm apart. Use a ruler to measure the approximate amplitude (i.e. size) of the Na spikes at the two points (or if you don't own anything as low-tech as a ruler, estimate by eye and say that's what you did), and from that measurement estimate the space constant λ in this dendrite. Show your calculation.
(iii) Using the size of spikes to find λ gives only an approximate value; what assumption about the definition of λ are we violating by using these responses to calculate λ?

(B) Refer to Figure 2.6 on p. 33 in our text, showing currents recorded from a single K channel.
(i) What feature of the graph in fig. 2.6E indicates that this K channel doesn't have the same properties as the K channels that are active during nerve impulse repolarization in axons?

(ii) Sketch the shape of the comparable graph for axon K channels. (Axis scales or numbers other than the (0,0) point are not needed – just the shape of the graph.) Explain why the graph for axon K channels would have the shape you are saying it has.

(iii) For purposes of this part of the question, assume that the single-channel recordings in fig 2.6 B,C,D came from a squid axon K channel, even though they actually didn't. Make a rough estimate the value of ***n***, the potassium activation parameter for a squid axon K channel, from the single channel data and the relation between squid axon K channel subunit activation and K channel opening. Explain the thinking behind your answer.

Answer **either** question 2 or question 3.

2. Normally impulses travel in one direction: from the site of initiation to the terminal of the axon. However, in the lab we can stimulate axons at any point, and we find that an impulse can be initiated at the "wrong" end of the axon and travel "backwards" to the normal initiation site. Question: Reason out what would happen if two impulses collided in an axon. In other words, suppose impulses are simultaneously initiated at the normal initiation site and also at the far end of the axon, so the impulses travel towards each other and collide somewhere along the axon. For example, would the impulses pass through each other and continue on, like two approaching waves in water? If not, what would happen, and why?

3. Figure 6.1 in our text (p. 92) shows how extracellular sodium concentration affects the action potential in a squid axon. The peak of the impulse declined from +40 mV in normal sea water (i.e. normal bathing solution for the axon), to +15 mV in sea water containing only 50% as much $[Na^+]_o$

(A) In the case of normal $[Na^+]_o$ and the +40 mV impulse peak, assume that the membrane is permeable only to sodium at the peak of the impulse. Question: how many times greater is the concentration of sodium in the sea water compared to inside the axon? Show your calculation.

(B) Suggest an explanation for the slower time course of the action potentials in low extracellular sodium.

Extra credit question(s) (1 point)
Answer any one (or, if you're procrastinating, more than one) of these; all answers accepted! (Thanks to our Tuesday TA, [name removed])

Why did the action potential cross the optic chiasm?
Why did the neuron like to sleep (or, let's say, 'rest') in the top bunk bed?
Why are postmitotic neurons bad at math?
What did the stimulus do to the neuron after they got married?

Appendix K: Williams College NEURO 201 NIA2 Lab

Neurons in Action

Adapted from John W. Moore and Ann F. Stuart. 2007 *Neurons in Action 2.0, Tutorials and Simulations using MATLAB*. Sinauer Associates, Inc, Sunderland MA.

Today you will use a tutorial based approach to review some concepts presented in lectures. Neurons in Action has been loaded onto the laboratory computers. You will focus on two tutorials during this laboratory period. The first focuses on Equilibrium Potentials, while the second, Introduction to NIA, allows you study the ionic dependence of the action potential. Please feel free to browse others and you are welcome to come back to the laboratory to work more on any of the tutorials.

The goals of these tutorials are

1. Review of concepts presented in the lectures
2. To understand how to stimulate your preparation and record the results
3. To perform experiments in NIA by changing parameters
4. To reproduce the four key observations of the action potential by depending on V_m and K ions

Prior to starting, the instructor will go over some general features of NIA, to facilitate your experience. A quick guide to these features is presented in the next section.

Navigating NIA

Navigating NiA

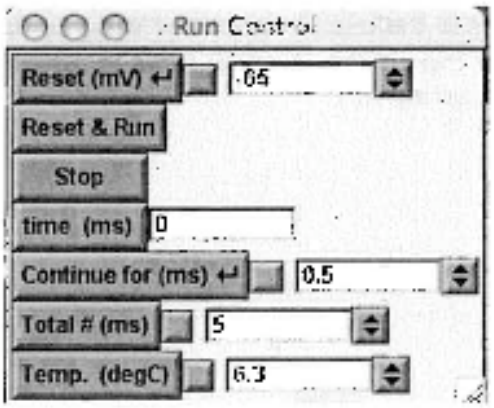
Neurons in Action has been loaded onto your computer. Open NIA2 on the desktop, which will open a Firefox browser window. From the home page, click Tutorials, then choose the tutorial described in this manual. Follow the instructions in this manual, which we have condensed from the instructions in the program.

We will go over the first part of the Introduction to Neurons in Action tutorial together at the start of class. You can toggle between the Firefox browser with the documentation and the simulation windows (Alt-Tab)

Click the "Start the Simulation" button (green). 

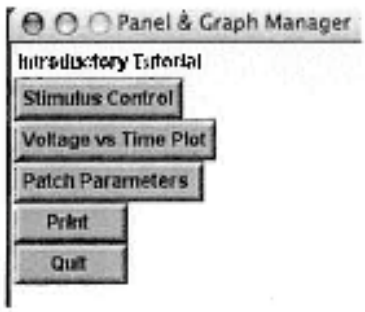
Two panels will open: *Run Control* and *Panel and Graph* manager.

Run Control triggers and controls the simulation runs, and is where you can set the membrane potential, the duration and the temperature at the start of the simulation. You can pause or advance a simulation along a time axis.

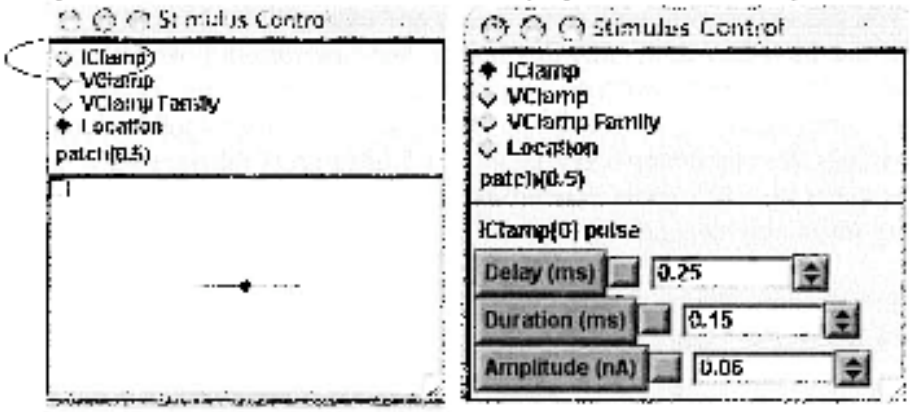


- Reset: the value is the initial membrane potential at the start of the simulation, the resting potential. If you change it, NiA will calculate new conductances. (for this lab we will assume conductance =permeability).
- Reset and Run (R&R): Resets to initial voltage and conductances, and runs a new simulation.
- Stop: stops the simulation.
- Time (ms): shows physiological time as simulation progresses.
- Continue for (ms): Allows you to run simulation in pause-advance mode. Each click steps forward through the run by the time shown in the box.
- Total # (ms): Sets the duration of the run and the time scale of the graphs.
- Temp (degC): Sets the temperature for the simulation.

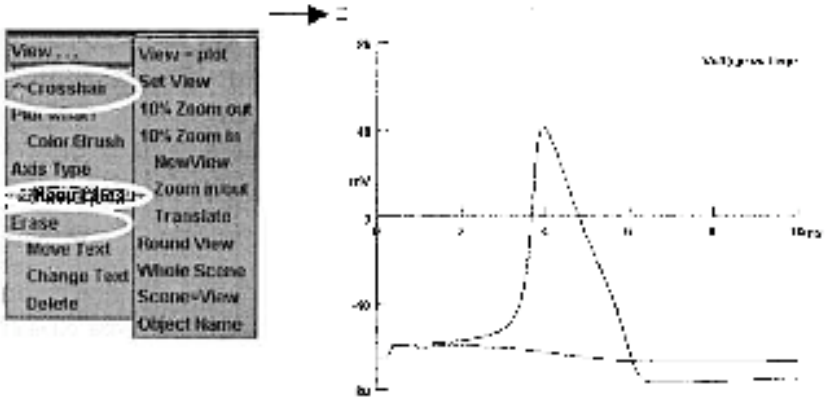
The **Panel and Graph Manager** allows you to open other panels and graphs, print, or quit the tutorial.



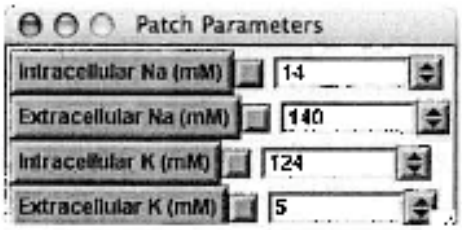
- **Stimulus Control** opens a panel to control the stimulus parameters. It is like inserting an electrode into the cell. If you select iClamp (current clamp) the parameters of the stimulus will be displayed in the control panel as on the right.



- **Voltage vs. Time Plot** launches a graph to show the voltage response to a stimulus. A right click over this plot (or a click on the box in the upper left corner) brings up the plot options menu. Crosshair allows you to measure voltage and time precisely. Keep lines preserves previous traces. Erase gets rid of previous traces.



- **Patch Parameters** opens a panel for ion concentration (and channel density in the second tutorial).



Warning: do not double-click the buttons. If you do, it will open 2 windows. If you have 2 stimulus control windows, you will end up inserting 2 electrodes into the patch, so your results will be puzzling. If you open 2 windows, quit the simulation and re-launch it.

Equilibrium Potential Tutorial
Open Neurons in Action using the green icon on the Desktop for NIA2. Select the Equilibrium Potentials tutorial (Tutorials>Equilibrium Potential).

In this tutorial, you can experiment with intracellular and extracellular Na and K concentrations to see how they determine E_{Na} and E_K, the equilibrium potentials for these ions. You can then vary the ratio between the cell's conductances (for this exercise we will assume that conductance = permeability) to K and Na to understand how ion selectivity determines the membrane potential (V_m). V_m controls the opening of ion channels and the generation of signals in neurons, skeletal muscle, and heart muscle. Thus, excitability ultimately depends on precisely regulated ionic concentrations.

Equilibrium potentials are calculated with the Nernst equation. Please use the NIA manual to review the Nernst equation.

$$E_{ion} = \frac{RT}{zF} \ln \frac{[ion]_o}{[ion]_i}$$

Click on *Start Simulation*. *Run Control* is as before. *Patch Parameters* allows you to change ionic concentrations and conductances. *Voltage Plots* on *Panels and Graphs* allows you to choose what you want to graph.

1. Glial cell, permeable solely to K ions.
   a. Click on the *K conductances only* button in the *Voltage Plots* panel.
   b. Click on *Run* in the *Run Control* panel and an equilibrium potential will appear on the voltage vs time plot, based on default values.
   c. Measure its value using the crosshair function (left-click on the blue line) and compare to E_K in *Patch Parameters*. Because the cell is permeable only to K^+, $V_m = E_K$ in this cell.
   d. Select *Keep Lines* from the right click menu.
   e. Change $[K]_o$ up and down.
   f. Click the *red button* next to $[K]_o$ to revert to the default value.

Q1 What value of $[K]_o$, in mV, causes E_K to be exactly zero, and why?
When $[K]_o$ is equal to $[K]_i$ then $E_K=0$. This is because $K_i/K_o = 1$; $\ln_{ion} 1 = 0$.
Q2 If the $[K]$ values were reversed such that $[K]_o$ = 131 mM and $[K]_i$ = 5mM, what would be the value of E_K?
The sign of E_K would change. E_K would be +75mV.
Q3. For the same values of $[K]_o$ and $[K]_i$, would E_K be different in a mammalian vs a squid nerve? Why or why not?
E_K is determined by a ratio of $[K]_i/[K]_o$. However, temperature is a factor in the Nernst equation, so different nerve tissues would affect E_K. Temperature in the Nernst equation is in Kelvin (absolute temperature), so the change would be small.

2. Cell permeable only to Na ions.
   a. Click on *Na conductances only* in the *Voltage Plots* panel.
   b. Run the simulation.

Q4. What happens to Vm if the membrane is permeable only to Na? Why is the value positive?

If a membrane is permeable only to Na, and ratio of [Na]o/[Na]i=10, the log(ratio) of 10 is 1, so Vm is a positive value.

3. What determines the resting membrane potential (Vm), and how does it depend on ion concentrations?

A typical nerve is permeable to both K^+ and Na^+ ions.

a. Return [K] and [Na] to their default values.
b. In *Voltage Plots*, click *Na and K conductances*. Note that the conductances, gK and gNa (which we here assume are equal to permeability) are here expressed as a ratio of channel densities (default 1:1).
c. Select *Keep Lines* from the right-click menu and run the simulation.
d. Measure Vm, the black line, with the cross hairs. Why is Vm resting at this value?
e. Set the K conductance, gK, to 10 and then 50 times greater than the Na conductance, gNa. If you could observe K and Na currents across the membrane at a gK:gNa ratio of 50:1, what would you observe?

Q5. What determines the value of Vm at which the neuron rests. Vrest?

Ratio of gK:gNa

f. Keeping gK:gNa at 50:1, change [K]o to several values between 5 and 500mM. Notice how Vm (=Vrest) tracks EK. From this experiment you can see how a change in extracellular [K]o would affect Vrest of cells.
g. Now reset [K]o to default, but keep the gK:gNa at 50:1. Decrease [Na]o stepwise by factors of two.

Q6. Why is the resting potential so insensitive to [Na]o?

Because gK is much higher than gNa.

4. Changing conductance ratios.

You can now imagine what will happen to Vm if you reverse the conductance ratio and make the membrane far more permeable to Na than K.

a. Set the conductance ratio so that gK:gNa is 1:100. Now you should expect to see Vm come close to ENa.
b. If you now reverse the ratio, such that gK:gNa is 100:1, Vm will return to very close to EK.

Q7. From this experiment, what do you conclude happens to ratio of gK:gNa over time when a neuron is stimulated and and action potential is generated?

gK:gNa becomes small during action potential, then becomes large as Na channels inactivate and K^+ is the primary permeable ion.

When you have answered these questions, choose Quit at the bottom of the Panels and Graphs manager.

Introduction to Neurons in Action—Exploring the ionic mechanisms of the action potential

This tutorial will allow you to observe the action potential and study effects of stimulation parameters, temperature and intracellular and extracellular concentrations of ions on the action potential.

1. Action potential
   A. Response to stimulus current.
      i. Set *Stimulus Control* to *Keep* (second choice)
      ii. Choose *Keep lines* (right click menu on *Voltage* or *Time*).
      iii. Click *Reset & Run*.
      iv. Increase the amplitude of the stimulating current in five steps, using either the arrows or typing into the field (*Stimulus Control> Amp> amplitude*). (Click the red box to restore the default value).
      v. Measure the peak amplitude using the Coordinate function (left click) on the *Voltage vs Time* graph.
      vi. Erase the lines after annotating the question below (right-click menu). Do this each time you move to a new section.

Observe the changes in response to the different stimulus amplitudes.

Q1. What is the reason for this set of responses to an increase in the stimulating current?

Larger stimulating currents activate more Na channels/cell. This resulting in more rapid depolarization such that the membrane reaches threshold more quickly.

   B. Observe the duration of the undershoot and return of the voltage to rest.
      i. Increase *total t (ms)* of the simulation to 25 ms (*Run Control* panel).
      ii. Keep the amplitude set to a value that gives an action potential.
      iii. Click *Reset & Run*.

Observe how long it takes for the membrane to return to resting potential.

Q2. Using what you know about channel properties, why is the return to the resting potential so slow?

The undershoot is the result of voltage sensitive K^+ channels opening. The long return to rest results from the slow closing of these channels.

      iv. Reset *total t (ms)* of the simulation to 5 ms (red button resets to default).

2. Change $[Na]_o$, $[Na]_i$, and $[K]_o$.

This section reproduces the classic squid giant axon experiments of Hodgkin and Katz (1949). Open the *Panels Parameters* window with the button in *Panels and Graphs*.

   A. Change the extracellular sodium concentration, $[Na]_o$.
      i. Set the amplitude of the stimulus to 0.1 nA (*Stimulus control> IClamp*).

i. Increase the extracellular Na^+ concentration, $[Na]_o$, in two-fold steps *(Point Parameter)* from the default value of 140mM
ii. Decrease $[Na]_o$ from 140mM in two-fold steps.
iv. Return $[Na]_o$ to its default value.

Q12. How is the effect in decreasing $[Na]_o$ on the action potential? Why?
$V_{m,rest}$, E_{Na} during an action potential. If E_{Na} is reduced so will the height of the AP.

B. Change the intracellular Na^+ concentration, $[Na]_i$.
Changing $[Na]_i$ was not technically possible when Hodgkin and Katz did their classic study. It was eventually accomplished by squeezing out the cytoplasm of the squid giant axon and replacing it with saline.
i. Now perform the same calculations of your choice.
ii. You mimic a very thin nerve by doubling the $[Na]_i$ and repeating changes in the $[Na]_i$.

Q13. How should the action potential change if the $[Na]_i$ is doubled? Does this differ from halving $[Na]_i$?
E_{Na} will be lower and so will the height of the AP. There is no difference in E_{Na} between doubling $[Na]_i$ and halving $[Na]_i$, because the Nernst equation depends on the ratio of the concentrations between inside and outside of cells, which is unchanged.

iii. What happens if you set the $[Na]_o$ to the same value as the $[Na]_i$?
iv. Is this the same result if you set the $[Na]_i$ to the default value of the $[Na]_o$?

C. Increase $[K]_o$.
i. Return $[Na]_o$ and $[Na]_i$ to their default values.
ii. Increase $[K]_o$ in several steps
iii. What if you increase $[K]_o$ to absurdly high levels like 2000mM?

Observe the changes in the action potential when you change $[K]_o$.
Q14. What part of the action potential depends on $[K]_o$? Why?
K_+ ion leaks out the cell after action potential when the voltage sensitive K^+ channels open. Halving $[K]_o$ results in a smaller (less negative) E_K. The action potential will reach this potential after Na^+ channels inactivate.

When you have answered the questions, choose *Quit* on *Panels and Graphs*.

We will re-convene to discuss the answers to the questions in the tutorials, which will be our discussion questions for the week.

APPENDIX L: Emory University's BIO 360L/NBB 301L Course Syllabus

BIO 360L / NBB 301L: Neurobiology Simulation Lab

to accompany BIO 360 / NBB 301: Introduction to Neurobiology

Fall Semester 2008

Instructors and contact information:

Astrid Prinz
Assistant Professor of Biology
astrid.prinz@emory.edu
Office: Rollins Research Center, Room 2105
Tel.: 404-727-5191
Office hours: by appointment

Dieter Jaeger
Associate Professor of Biology
djaeger@emory.edu
Office: Rollins Research Center, Room 2129
Tel.: 404-727-8139
Office hours: by appointment

Michael Wright
Neuroscience graduate student, Teaching Assistant
terrence.m.wright@emory.edu

Class schedule and location:
Tuesday 4:00pm – 6:00pm
Room 109 in 1462 Clifton Road
Course conference on Blackboard: classes.emory.edu

Required materials:
Neurons in Action 2: Tutorials and Simulations using NEURON, John W. Moore, and Ann E. Stuart, 2007 CD and Text
The course will loosely follow the content covered in BIO 360 / NBB 301, which uses Kandel, Schwartz, Jessell "Principles of Neural Science" (McGrawHill). Other course materials will be provided in class or on the Blackboard site.

Course description and goals:
In this lab course, students will explore topics in cellular and small network neuroscience by performing virtual electrophysiology experiments on the computer. The content of the course matches material covered in the Introductory Neurobiology course, BIO 360 / NBB 301, and will help students understand neurons and neuronal networks in greater depth.

Course structure:
Each session will begin with an introduction of the topic covered by and the software used for the virtual experiments in that session. Students will then perform the experiments and work on the assignment for that class in the computer lab. Assignments will be handed in the following week for grading.

Attendance:
Attendance in class is required, and excused absences due to medical or other valid reasons must be arranged with the instructors ahead of time. More than three unexcused absences will lead to a failing grade in the course.

Grading:
There will be 13 weekly class assignments. All 13 must be handed in electronically as Word documents or pdf files for grading, but only the 10 best will count for the class grade. There will be no exams. As always, the Emory College honor code applies to all components of the class.

Course schedule

date	topic	instructor
09/02	Electronics and lab familiarization	AP
09/09	Passive membrane properties	AP
09/16	Resting membrane potential	AP
09/23	Action potential – membrane patch and voltage clamp	DJ
09/30	Ion channels	DJ
10/07	Passive cable	DJ
10/14	**Fall Break – no class**	
10/21	Active cable, unmyelinated and myelinated	DJ
10/28	Postsynaptic potential	DJ
11/04	Synaptic integration	DJ
11/11	Coincidence Detection	DJ

11/18	Simple circuits and neuromodulation	AP
11/25	Homeostatic regulation	AP
12/02	Second messengers	AP
12/09	Sensory receptive field	AP

APPENDIX M: Emory University BIO 360L/NBB 301L
Coincidence Detection Lab 10 Notes (Given out in class.)

BIO 360L / NBB 301L: Neurobiology Simulation Lab, Fall 2008

Session 10: Coincidence Detection

This class and homework use the Site of Impulse Initiation and Synaptic Integration tutorials

Reading:
Chapters 12, 30 of Kandel, Schwartz, Jessell.

Near Key Terms: coincidence detection window (CDW), axonal localization, interaural time delay, Ih (HCN) and Kv1.1 (Kvs) conductances, shunting.

BIOLOGY ASSIGNMENTS

Experiment: What is the value of CDW for different synaptic properties and different conductances in the auditory neuron ?

Familiarize yourself with the Coincidence Detection tutorial.

Change the temperature to 22 degrees C, typical for mammalian slice experiments.

Double the densities of Ih and K when conductances – then proceed

Group 1: Perform experiment with default synapses and no IH/ Kvs
Group 2: Perform experiment with fast synapses and no IH / Kvs
Group 3: Perform experiment with default synapses and IH / Kvs
Group 4: Perform experiment with fast synapses and IH/ Kvs

Find the duration of the CDW for a cell with two antidromic excitatory inputs.

1. **First, find the membrane current that is just at threshold for triggering the action potential.**
   - **Click the "Find Threshold I" button in the PKO Manager.**

     When you click this button, NEURON will search for the threshold current and display its value in the "AlphaSynapse[1]" panel. Think of the stimulator delivering pulses of neurotransmitter from the virtual presynaptic terminal at AlphaSynapse[1] onto the postsynaptic cell. The stimulus pulses will be of constant duration given in ms below the threshold value until a contraction is found that is just at threshold

   - **If you Keep Lines in the Voltage-vs-Time graph, you can more easily mark the current of Ending for threshold.**

You may find it interesting to repeat this process several times as sometimes NEURON approaches threshold from above and sometimes from below.

- Where does the impulse initiate?

  Does it initiate in the soma (or in the axon at the Site of Impulse Initiation marker)?

2. **Specify a conductance value for each subthreshold EPSP.**

   To make a narrow CDW, you want the sum of the EPSPs to be *reliably above, but very near,* threshold. How large should their amplitudes be?

   - Choose the amplitude for each synapse input.
     - If each synaptic conductance were exactly half the threshold value, then their sum might—or might not—reach threshold and the postsynaptic soma would fire unreliably.
     - Should you set each synaptic conductance at 1% above the half-threshold value? In a simulation, this small increase above the half-threshold value should lead the EPSPs to sum reliably above threshold, but in reality, 1% would probably be too small to cover variability.
     - Here we arbitrarily choose 10%.
     - **What is the value of your threshold + 10% conductance ?**
3. **Now determine the CDW.**
   - Enter the conductance (gmax) value of 50% of your determined value (threshold + 10%) in each AlphaSynapse panel.

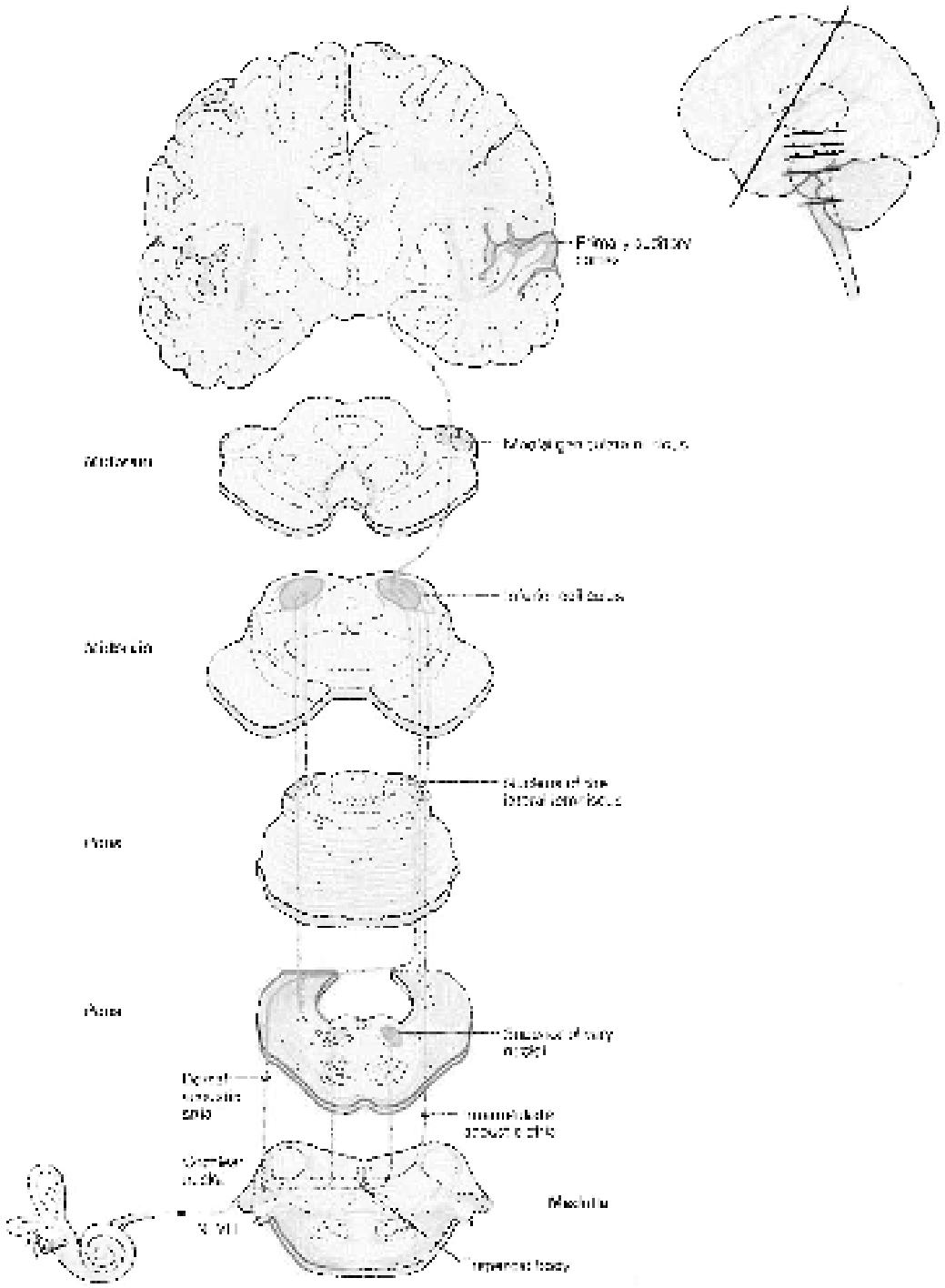
     This is the value determined in the last step.

   - Delay the onset of AlphaSynapse[2] until this EPSP fails to sum with the first to evoke an action potential.
   - **What is the CDW ?**

Extra Credit: If the difference in distance the sound has to travel to the right and left ear is 5 cm, what is the time difference for input to the two ears? Is your CDW good enough to detect this? How many cm of distance is your CDW good for ?

BIO 360L / NBB 301L: Neurobiology Simulation Lab, Fall 2008

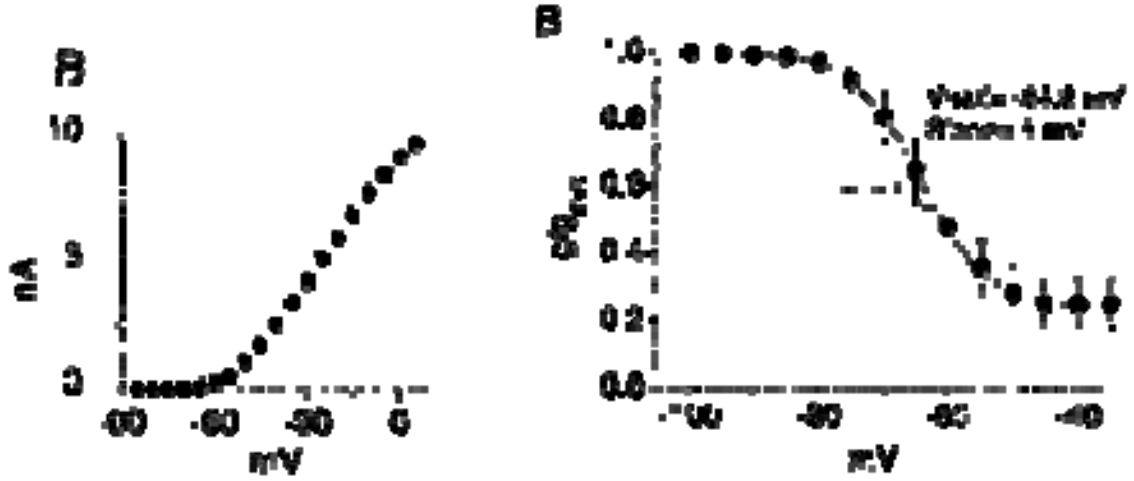
Auditory Pathway



BIO 3601 / NBB 3011: Neurobiology Simulation Lab, Fall 2008

Coincidence Detection in Medial Superior Olive

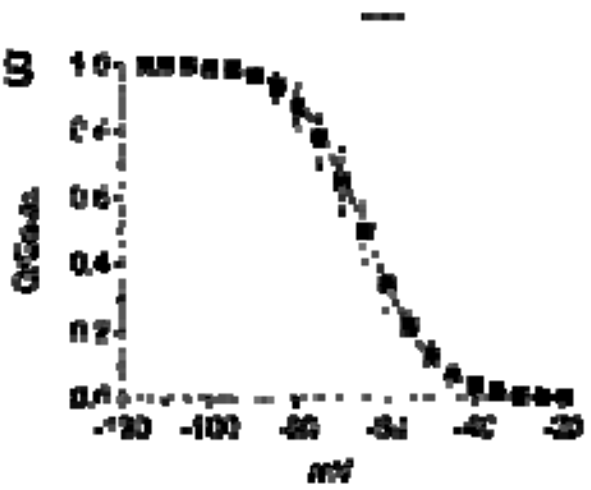
Kv1.1 activation **Inactivation**



Ivanov Bal R and Oertel D. (2001).
Potassium currents in octopus cells of the mammalian cochlear nucleus.
J Neurophysiol 86:2299-311.

BIO 360L / NBB 301L: Neurobiology Simulation Lab, Fall 2008

H Activation



From: Bal T. and Oertel D. (2000).
Hyperpolarization-activated, mixed-cation current (Ih) in octopus cells of the mammalian cochlear nucleus.
J Neurophysiol. 84:806-17.

Spec. of remark: 383 mb (Whipple)

APPENDIX N: Emory University BIO 360L/NBB 301L
Coincidence Detection Homework Set

BIO 360L / NBB 301L: Neurobiology Simulation Lab, Fall 2008

HOMEWORK ASSIGNMENT #9 (Answer) : Total Points: 100 = 100%

This homework is due on Friday, November 21th, 2008, and can be e-mailed to [redacted] or delivered to my mail box in room 2008 Rollins. For any late submissions we subtract 2% from the grade per day. Special arrangements in case of hardship can be arranged BEFORE-HAND only.

You can get partial credit for incorrect answers if you show all your work leading to the answer. Thus it is important to show your approach to the solution, not just the solution!

The homework sets are open notes, open book, and open web. HOWEVER, you have to do the set completely on your own without help from others and without exchanging information with other students in the class. Follow the Honor Code System.

Total points: 100 = 100%

For all problems: As during class, set the temperature to 36 degree Celsius, and double both Na and K chan densities.

Note: For all problems: The definition of triggering an action potential is when a definite spike is seen in the axone.v(0.9) trace (green). Small spikelets in the soma that do not trigger an axonal spike do not count.

Problem 1 (50 points) Run the Coincidence Detection Tutorial

Let's determine the influence of synaptic conductance amplitude on the CDW. Use both "Add Ih and Kv4" and "Faster Synapses". Then proceed to find your threshold conductance for 1 synapse to trigger an AP, and add 10% to this value. What is this value of threshold + 10% conductance? Determine the CDW for this value, i.e. divide this conductance evenly between both synapses and determine at which minimal delay the 2 synapses fail to trigger an AP. What is the CDW? (Be precise to the closest 10 microsecond (0.01 ms) level). Now determine the CDW if you augment 6 %, or add 10, 20, or 30 % more conductance to both synapses. You now have a total of 5 CDW values. Draw a graph of CDW (y-axis) vs. the total conductance of both synapses. Describe and Explain the relationship you find. For each CDW, how many ms of difference in the distance between 2 cells and a sound source could the neuron discriminate? (Make a 2nd graph or a table). What value of conductance (among your 5) should the nervous system use to get the best possible discrimination of interaural time delays? Is there any disadvantage to using this value?

BIO 360L / NBB 301L: Neurobiology Simulation Lab, Fall 2005

Problem 2 [50 points total] Run the Coincidence Detection Tutorial

Let's determine the influence of the IH (HCN) conductance on the CDW. To examine the amplitude and time course of IH we need one additional plot. Go to 'Neuron Main Menu' -> 'Graph' -> 'Current Axis'. Open the command menu on the empty plot that pops up and select 'plot what?' -> 'soma' -> 'ihcn(0.5)'. This creates a plot of the ih current flowing in/out of the soma.

A [25 points]. Move the onset of both synapses to 5 ms. Use both "Add Ih & Kiva" and 'Faster Synapses'. We are now going to manually set the Ih conductance to different values (0.0001, 0.0005, 0.0025, 0.005, and 0.01) and for each setting of Ih determine the following values: 1) amount of Ih current at t = 4.9 ms, 2) level of Vm at t = 4.9 ms, 3) threshold of synapse 1 to trigger an AP in its own 4) CDW (i.e. and "50% of membrane to threshold, divide evenly between both synapses, and look for largest delay between activating synapses (keep synapse 1 at 5 ms) with 10 microsecond precision that still triggers an AP. **Hand in a table of your values for 1-4, and a graph of the CDW (yaxis) vs level of IH.**

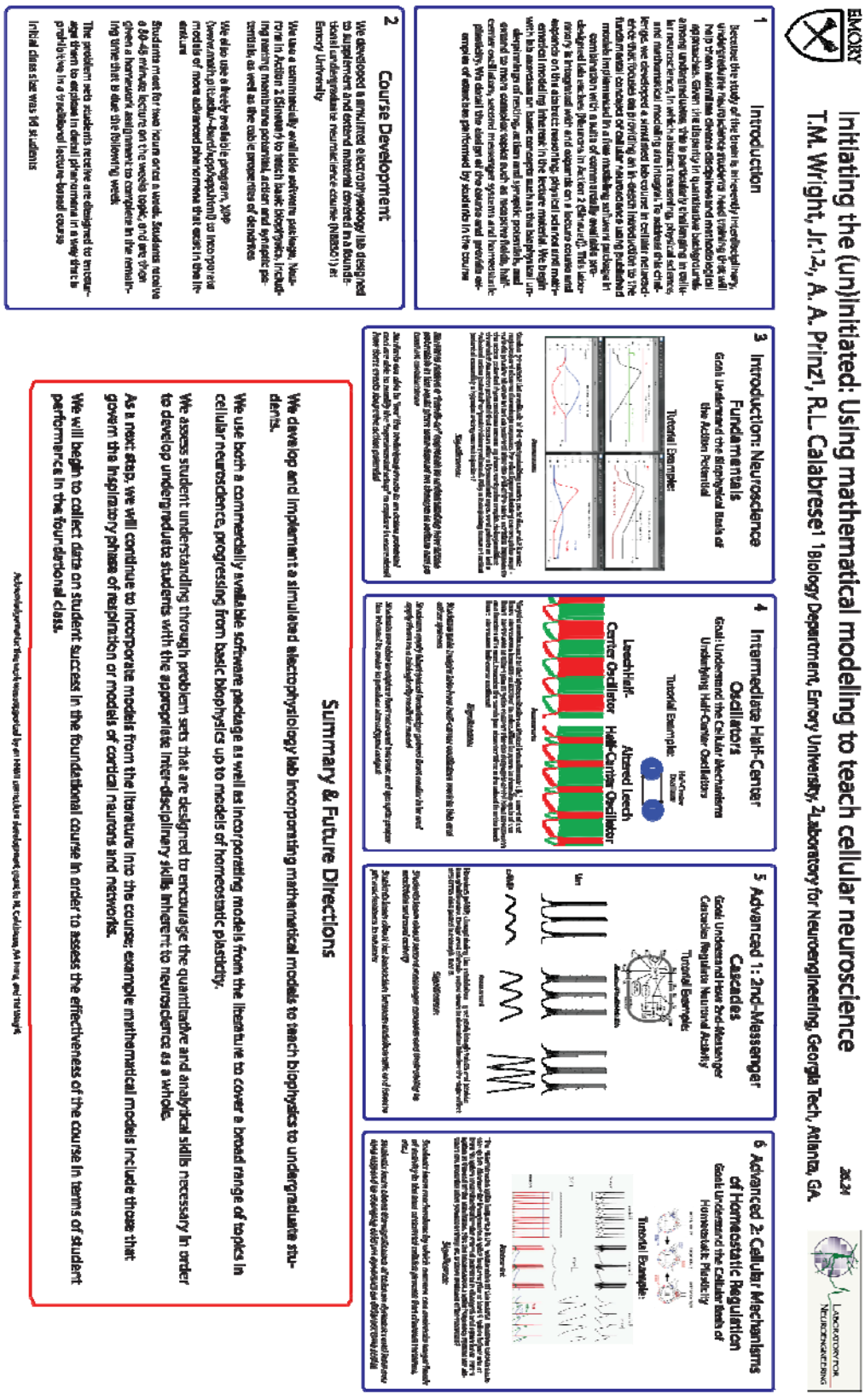
B [5 points]. Explain the relationship between the levels of IH you set in (A) and the value of Vm at 4.9 ms. Does increasing IH get you closer or further away from spike threshold? Why?

C [5 points]. Describe the relationship between the level of IH and the value of threshold synaptic conductance needed to trigger an AP. Does this relationship surprise you given your finding in part B above? Explain how it may come about.

D [10 points]. Run an additional simulation with IH = 100 and gmax of synapse 1 of 5000 (onset of 5 ms). Show the voltage and IH current plot of this simulation. **Explain these data. You should be able to tell me a pretty accurate value for the reversal potential of Ih now.** Hint: It is possible that this simulation sheds additional light on what a good answer to problems B and C above is, and maybe you could improve your answers to these parts now. Note: These levels of IH and synaptic conductance are unbiologically high – but in a simulation they can help us to better understand the mechanisms behind our data at lower levels of conductance.

E [5 points]. Describe the relationship between the level of IH and the CDW (from your graph in part (A)) and explain as best as you can.

APPENDIX O: Emory University BIO 360L Teaching Assistant's Poster Presentation on Teaching Neuroscience with NIA2 at Society for Neuroscience Conference



APPENDIX P: Agnes Scott College Biology/Psychology 250 Foundations of Neuroscience 1: Excitable Cells and Synapses Course Syllabus

Foundations of Neuroscience I: Excitable Cells and Synapses
Biology/Psychology 250 Fall 2008

Karen J. Thompson, Department of Biology, Room 202E, Bullock Science Center

Date		Topic	Chapter*	Laboratory (W)
Aug	28	Intro to Neuroscience	1B	
Sept	2	Neurons, Neuroglia	2B, Int G	Neurons in Action
	4	cont.	2B	Membrane **(10 pts)**
	9	Resting Neuronal Membrane	3B	Equilibrium Potentials
	11	cont.	3B	**(10 pts)**
	16	Action Potentials	4B 1G	Na^+ Action Potential
	18	cont.	4B 1G	Threshold **(10 pts)**
	23	cont.	4B	**Test #1 (70 pts)**
	25	Chemical Signals Discussion	2G	
	30	Synaptic Transmission	5B	Neuromuscular Junction
Oct	2	cont.	5B	Voltage Clamp **(10 pts)**
	7	Modulation Discussion **(5 pts)**	4G	Action Projects (w/NIA2)
	9	Neurotransmitter Systems	6B	
	14	cont.	6B	3G Discussion **(10 pts)**
	16	*Fall Break*		
	21	Nervous System Structure	7B-p167-	**Test #2 (70 pts)**
	23	cont.	p192	
	28	Somatic Sensory System	12B	Outline Exchange **(10 pts)**
	30	Somatic Sensory System cont, Pain	12B	
Nov	4	Love on the Fly Discussion **(5pts)**	7G	Action Projects
	6	Spinal Control of Movement	13B	
	11	Spinal Control of Movement	13B	Presentations and
	13	Brain Control of Movement	14B	Discussion **(20 pts)**
	18	No class		**Test #3 (70 pts)**
	20	Brain Control of Movement	14B	
	25	Neural Networks Discussion	6G	N/A
	27	*Thanksgiving*		
Dec	2	Invertebrate Neuroethology	PowerPoint	Motor Control Lab

4	Circadian Rhythms Discussion	5G	worksheet **(10 pts)**

* B = Bear et al; G = Greenspan

Dec 11 – 16 Self Scheduled Final Exam (100 pts)

Text Books:

Bear, MF, BW Connors, MA Paradiso (2007) Neuroscience Exploring the Brain, 3rd Edition. Lippincott Williams and Wilkins. Philadelphia, Pennsylvania.

Greenspan, RJ (2007) An Introduction to Nervous Systems. Cold Spring Harbor Laboratory Press. Cold Spring Harbor, New York.

Lab Manual:

Moore, JW and AE Stuart. (2007) Neurons in Action Tutorials and Simulations Using NEURON, Version 2. Sinauer Associates, Inc., Sunderland, Massachusetts.

Course Resources:

A copy of the syllabus and other course materials will be posted on Blackboard. You can also view your grades at any time in the grade book on Blackboard. PowerPoint files from lectures will be stored in Blackboard and on the W:drive under Dept Dirs, Biology, KThompson.

Course Assistant:

Ms. Virginia Vachon of the biology department will provide assistance for the laboratory of this course. Her office is located off the Bullock Science Center atrium in Rm 202.

Grading:

Exams (3 X 70)	210 pts
Discussion Qs	20 pts
Laboratory	70 pts
Final Exam	100 pts
Total Points:	400 pts

Grading Scale: 90-100 = A, 80-89 = B, 70-79 = C, 60-69 = D, <59 = F

Course Goals:

This course is the first of a two-semester sequence in basic neuroscience, a required course for the Neuroscience major. Because you have taken the prerequisite introductory biology and psychology courses, you are now well prepared to investigate the operation of the nervous system and to explore its complexity. This course focuses on the cellular elements of the nervous system, the importance of passive and excitable properties of cells, the functional connections between neurons, neural circuits, and nervous system development. Motor systems are also examined, revealing how neural circuits are capable of generating purposeful animal movement. In the lab, you will gain experience with computer simulations of nerve cell membranes and synapses, you will conduct an original project based on NEURON software, present your findings to the class, and you will explore descending neural control of movement in a live animal.

Attendance:

Class and laboratory attendance are expected. No make up labs are offered, nor are tests delayed for individuals unless there is written *and convincing* documentation. Attendance Policy is posted on Blackboard.

APPENDIX Q: Agnes Scott College Biology/Psychology 250 Foundations of Neuroscience 1: Action Project Assignment

Action Project Laboratories

Individual oral presentation.

The objectives of this lab project are for you to develop an independent, creative project, and to share your accomplishment with the rest of us. You will use new NIA2 exercises as the starting points for your talks, but you will be expected to go beyond the lab exercises and present your own protocols and information from linked publications or others that you find. At some level these presentations will be exercises in teaching for you because the rest of the class will not have tried your tutorial and you will need to make the simulations, experiments, and the neurobiology clearly understandable to the class. Use the evaluation sheet attached to reflect on your presentation and to help judge how to make it strong.

Oct 8 Try out various project options, and give Dr. Thompson your top two choices.
Areas to be covered for each project
Basic science covered in the tutorial
Running important components of the tutorial
Presenting the classic paper and/or other references to expand on the basic neurobiology in the tutorial
Relating the neurobiology to a medical condition or experimental situation and presenting the tutorial in a creative mode, beyond following the set steps.

Oct 15 (3G Discussion 10 pts)
Fall Break
Oct 22 (Test #2 70 pts)

On your own time: Work out more details about the project and how you will present to the class. Try out the simulations so that you understand the NIA program that is related to your project. Develop a plan for the presentation.

Oct 29 Action Projects Outline and Plan Exchange
Bring <u>two copies</u> of an outline of your presentation to lab (2 pgs). You will turn one copy in to Dr. Thompson and exchange one copy with someone in the lab for feedback. The outline needs to be specific as to the content in each section. You will provide comments on the other person's outline and you will both receive 10 points (5 pts for your outline and 5 pts for your critical review of the other person's outline).

Nov 5 A day to independently work on projects in the lab. This would be a good time to finalize the power point images and the tutorial demonstrations that you will do during your talk.

Nov 15 Presentations. This lab will be a series of presentations. Ginny Vachon and I will both evaluate your presentations with respect to the guidlines we gave you earlier. Each of you will speak for 15 to 20 minutes and there will be time afterwards for questions. Please be sure to have your presentation well prepared and ready to go so the class doesn't have to wait for you to get yourself organized for your talk. We have NIA2 loaded on the computer in the lecture room. (20 pts for the presentation).

Action Project Options

1. Chattering ion channels (p 43).

2. GABAergic synapses (p 67) target for psychoactive drugs, search for drugs and inhibition.

3. Partial demyelination, the problem in MS (p 89).

4. Extracellular Ca sensitivity of the Na channel (p 95), and dynamic view of threshold (p 99).

5. Na and K channel kinetics, drugs (105).

6. Local anaesthetics, trauma and AP conduction (p 119).

7. Simplified multiple dendrite cat motor neuron and integration (127).

8. Simulation vs real experiments APs invading terminals (133).

9. Focus on Hodgkin and Huxley classic 1952 papers (choose one).

APPENDIX R: Agnes Scott College Student's Components of Na and K Channel Excitability Project Presentation Slides

Components of Na and K Channel Excitability

[name deleted]

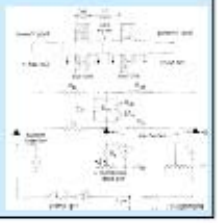
More than 1channel type exists

- Different channels in nerve and cardiac muscle.
- Resistant to different types of drugs.
- Vary in conformational changes elicited.
- Discovered in TTX experiments

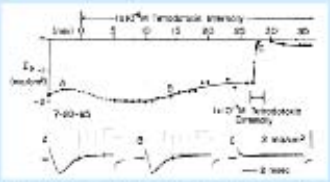
(Merre, J.W., and Narahashi, T.(1967))

TTX Experiment (Moore & Narahashi)

- Voltage Clamping of Lobster Nerves
- Sucrose gap method
- "artificial node"



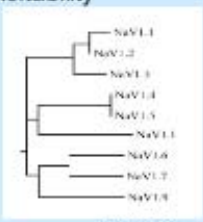
TTX Experiment (Moore & Narahashi)



Ineffective on cardiac and smooth muscle; powerful blocking agent for axon and skeletal muscle action potentials.

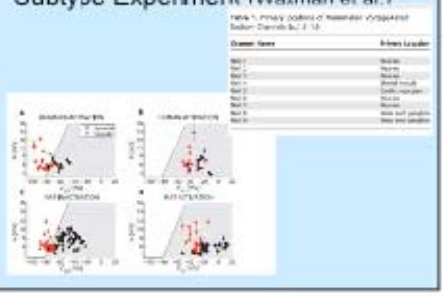
Channel subtype determines neuronal excitability

- Based on alpha and beta subunits
- K+(80 genes)
- Na+(9 genes)
- Suited for a paticular job



En.wikipedia.org

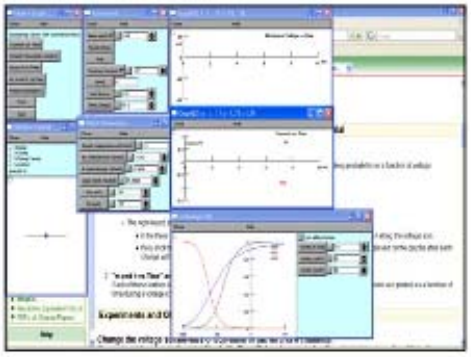
Subtype Experiment (Waxman et al.)

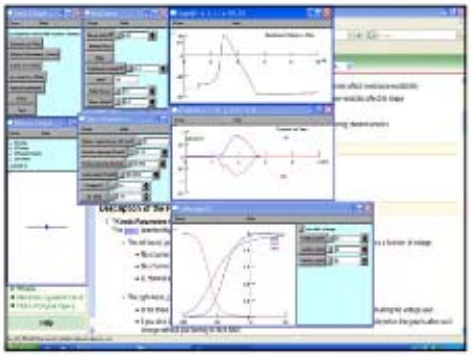


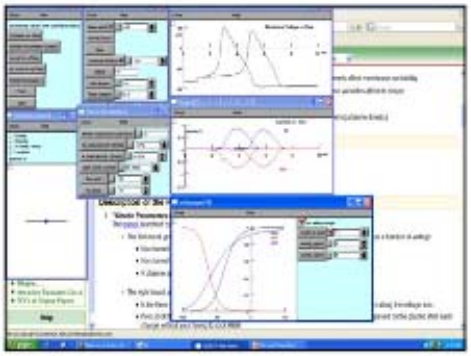
1

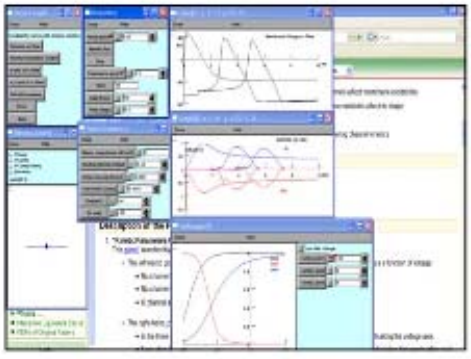
Outline of Experiment

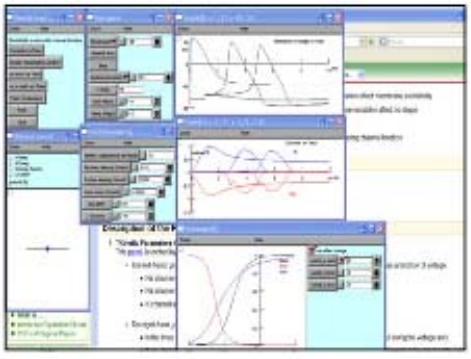
- 1. Changing Voltage Sensitivities
- 2. Inactivating Na channels
- 3. Stimulating Na channel subtypes
- 4. Mimicking effects of toxins











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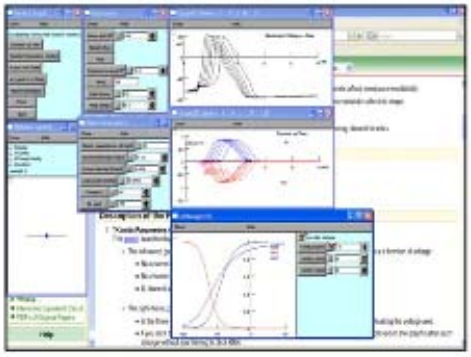
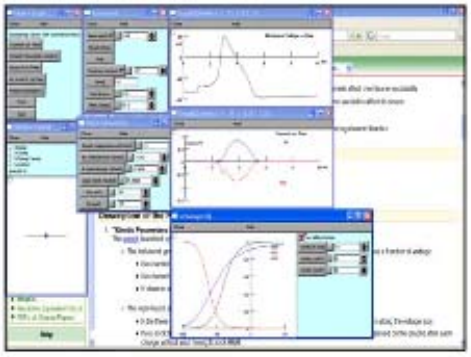
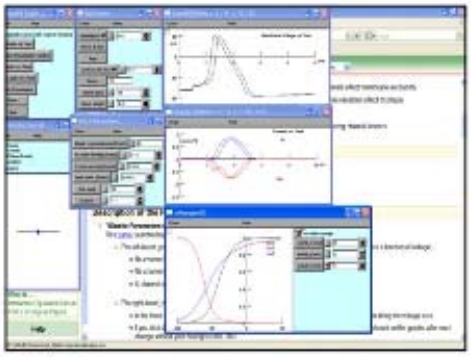
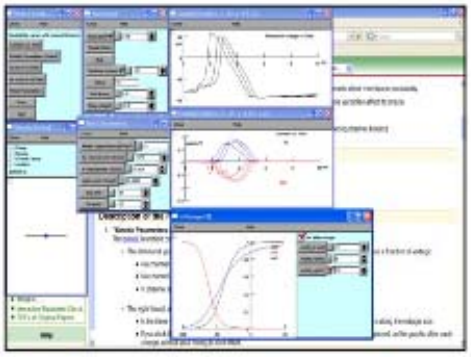
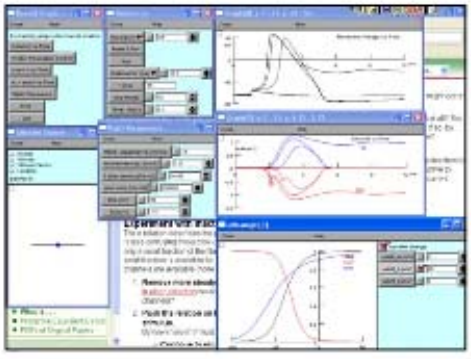
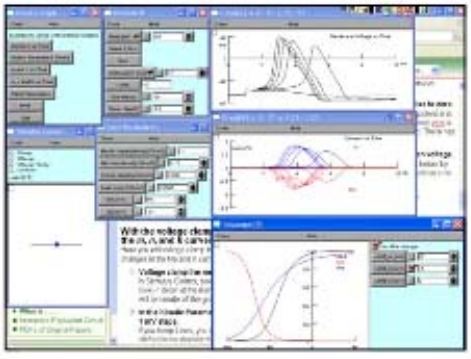

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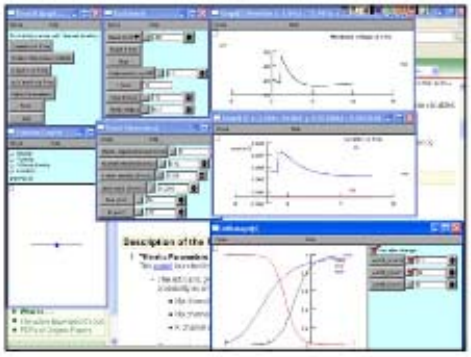
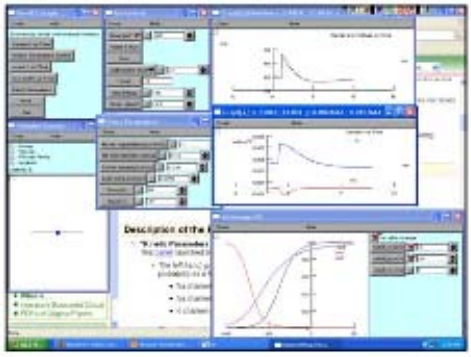
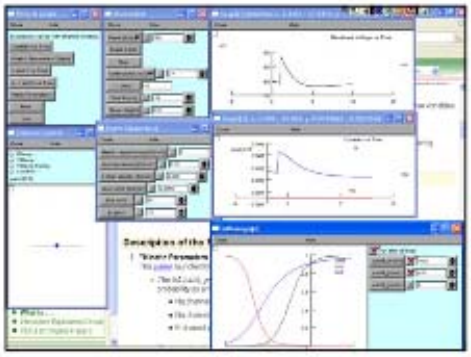
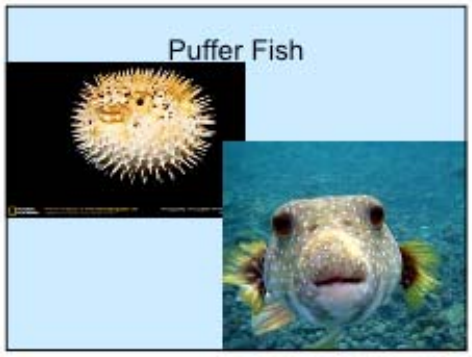
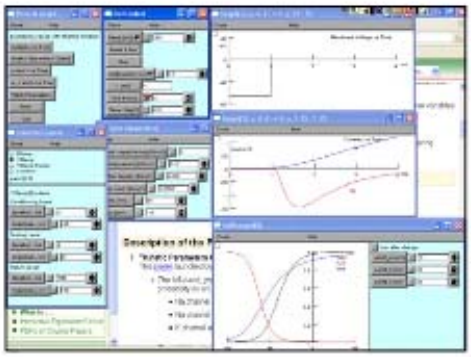
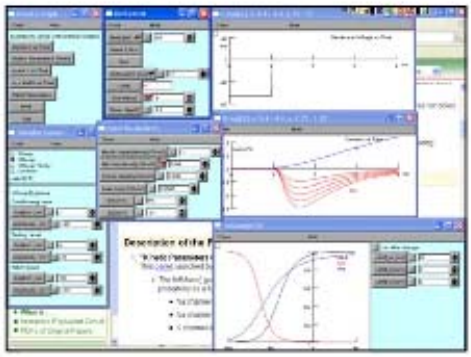






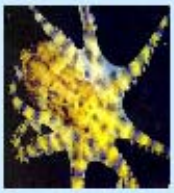




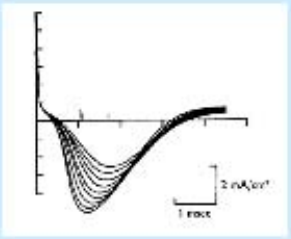


4

Maculotoxin: blue-ringed octopus



Maculotoxin (TTX & 2ND Component)



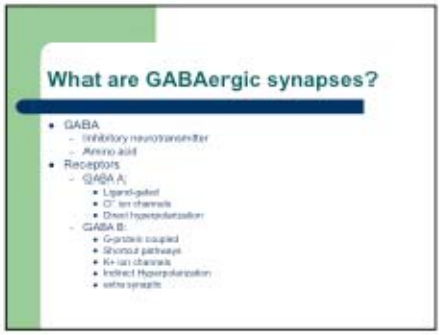
Conclusion

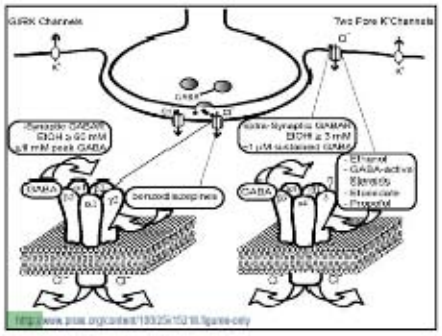
- subtype range= Na excitability.
- sodium channels allow the propagation of action potentials
- Toxins can inhibit or activate
- as the voltage becomes more negative, the probability that the Na channels are *not* inactivated increases
- increasing the amplitude of the stimulus pulse will once again excite the neuron.

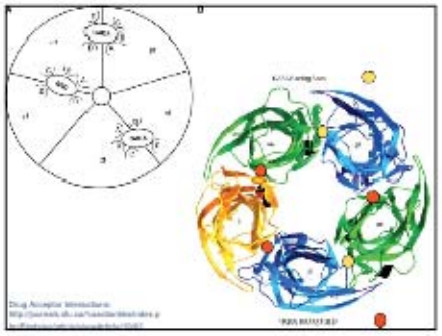
5

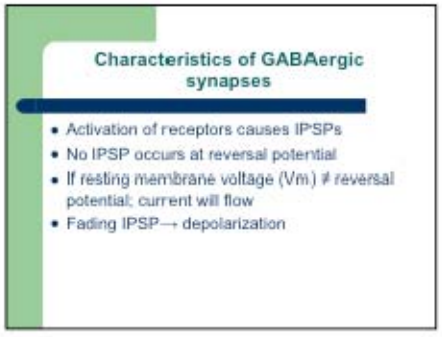
APPENDIX S: Agnes Scott College Agnes Scott College Biology/Psychology 250 Foundations of Neuroscience 1: Action Project Assignment Grading Rubric

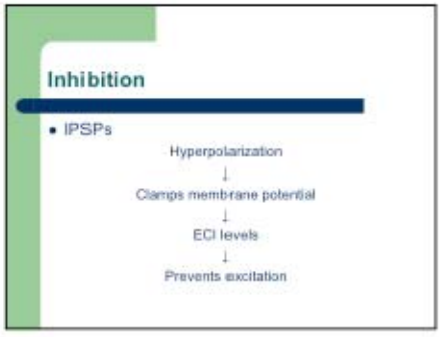
APPENDIX T: Agnes Scott College Student's Gabaergic Synapses: Target for psychoactive drugs, search for drugs and inhibition Project Presentation Slides







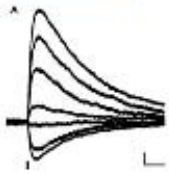




1

Resting Potential = Reversal Potential

- Reversal potential
  - Reversed flow of ions
  - No net current flow
  - No change in membrane voltage (Vm)
- Resting Potential
  - Membrane is at rest
  - No change in current or Vm



Activate GABA A receptors

- Run simulation 1
- Resting potential = reversal potential
- Why is there no current?
  - Ohm's Law (I = V/R)
- If no current flows how is the cell made less excitable?
  - Cl^- is flowing

Changes in membrane potential and IPSPs

Deviations from reversal potential + activated GABA receptors

↓

Cl^- Current flow

↓

Cell hyperpolarized

↓

IPSP

Create an IPSP

- Run Simulation 2
- Vm ≠ reversal potential
- IPSP occurs and current flows
- Why does the IPSP point towards the reversal potential?
  - Cl^- current brings cell to ECl

Reverse the IPSP

- Run Simulation 3
- What happens when the IPSP is reversed?
  - Direction of current reverses
  - Direction of IPSP reverses
    - Toward reversal potential

After the IPSP (Disinhibition)

- Once IPSP fades…
  - Cell is brought back to resting potential
  - Increase in Vm
  - Slight depolarization occurs
    - Causes "off responses"
- "Off responses"
  - Na+ channels have the depolarization they need to open.

2

Create an "Off response"

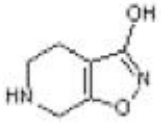
- Run simulation 4
- When does an impulse occur?
- Why does it occur at this time?
  - Na+ channels ready to open
  - HH cycle
- Define an "Off response"
  - Hyperpolarization turned off → response occurs
- Why do larger hyperpolarizations cause greater "off responses?
  - More Na+ has to flow to bring Vm back to resting.

GABAergic synapses and psychoactive drugs

- Antagonists
  - Convulsants
- Agonists
  - Muscimol
    - Hypnotic drug
    - Mushrooms of the Amanita genus
      - Delusional
      - Psychedelic
    - GABA A receptors
    - Inhibits CNS
      - Creates hallucinations

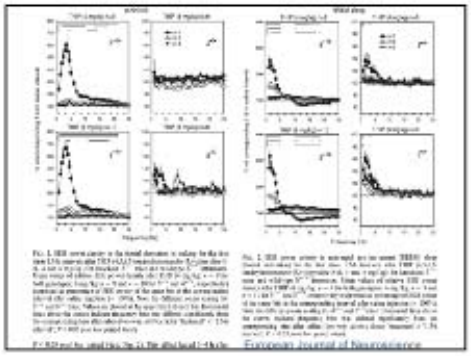
THIP

- Hypnotic agonists
- α4β3δ receptor
  - Regulates sleep
  - Thalamus and neocortex
- Not easily recycled
  - Stays at synapse
  - Long responses
- Uses
  - Sedative, anticonvulsant, anti-anxiety medication, insomnia medication



THIP and sleep

- Where does THIP bind?
  - δ subunits in mice
- Discoveries
  - δ+ mice:
    - Increase in NREM
  - δ- mice:
    - Slight increase in NREM
  - Higher doses = longer/greater effects



European Journal of Neuroscience

References

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3

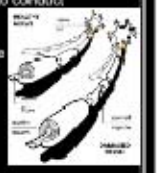
APPENDIX U: Agnes Scott College Student's Partial Demyelination: the problem with MS Project Presentation Slides

Effects of Partial Demyelination
On Action Potentials

[name deleted] 11/12/08

MULTIPLE SCLEROSIS

Axons demyelinated in patches due to inflammation and scarring cause disrupted ability to conduct action potentials



Possible cause- autoimmune disease
Genetic factors and susceptibility
Attacks triggered by a virus?
Gender: Women 50% more likely
To develop MS

Types of MS

Benign - 1 or 2 attacks with complete recovery

Relapsing remitting - partial or total recovery after attacks

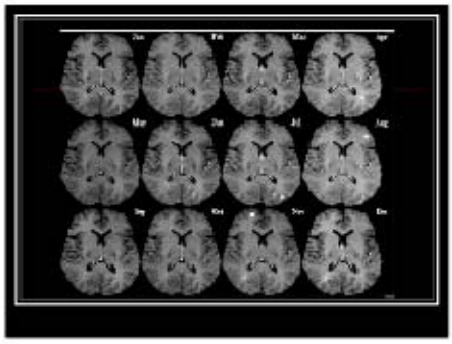
Secondary progressive – relapsing-remitting course which becomes steadily progressive

Primary progressive - progressive from onset

Symptoms

There is no set pattern so they depend on which areas of CNS have been affected

- blurred vision
- involuntary rapid eye movement
- loss of balance
- lack of coordination and weakness
- pins and needles, tingling
- Fatigue
- Sensitivity to heat (causes worsening of symptoms)
- Loss of concentration, judgement, or reasoning



Treatment

Because it is thought to be an autoimmune disease, suppressing the immune system is a goal of treatment.
Steroids: stop inflammation at site of demyelination, allow normal funciton to return more rapidly

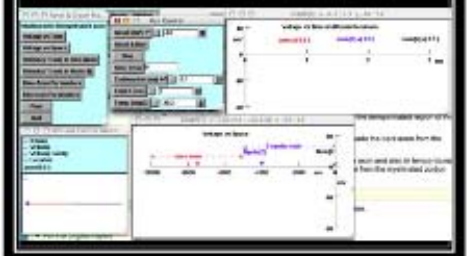
No cure: conventional therapy is only partially effective, and may cause side effects

1

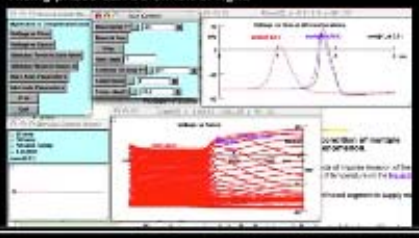
NIA Tutorial

Simulates AP in myelinated nerve as it tries to travel through a bare (unmyelinated) region
Bare axon has normal density of channels in unmyelinated nerve
Goals: Watch change of shape of AP as it travels from myelinated to bare axon & from bare to myelinated
Observe how changes in ion conductances in bare axon and also in temperature affect the ability of the action potential to invade bare axon from myelinated portion

Panels & Windows

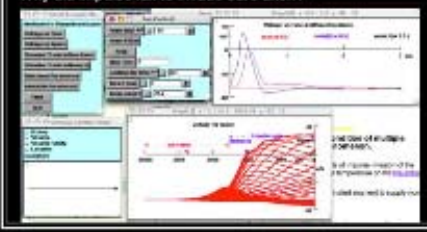


Pressing R&R- impulse travels from left to right
Why are 2nd and 3rd impulses closer than 1st and 2nd?
Rising phase can be on left or right.



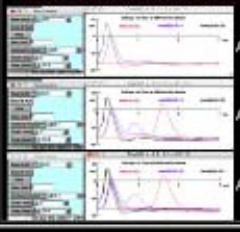
Reversing direction of stimulation

Why did impulse fail to invade bare axon?



Change in temperature and condition of MS patients

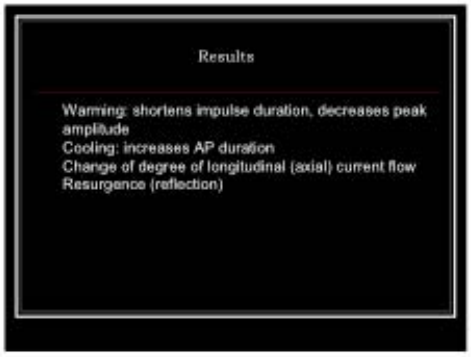
What change will enable AP in myelinated segment to supply more current to the bare axon?

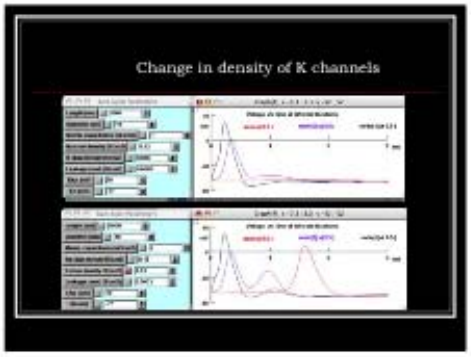


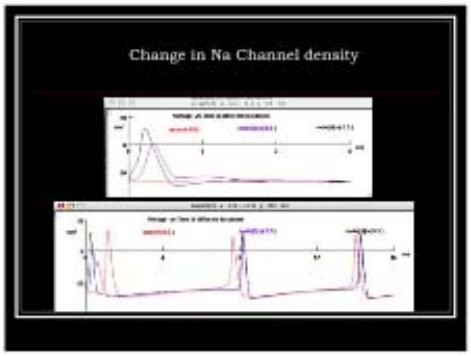
Uhthoff phenomenon

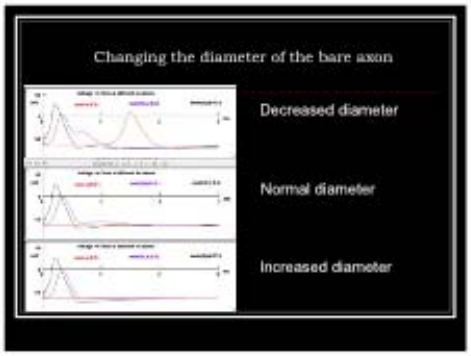
Classic feature of MS. Temporary induction, by heat or exercise, of symptoms such as unilateral visual blurring

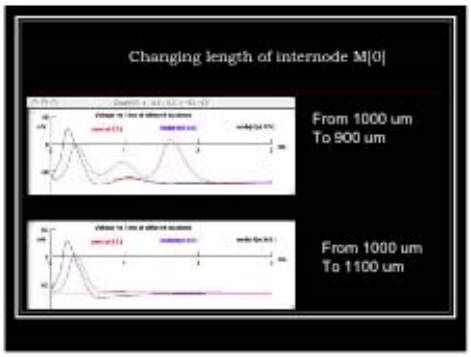
2

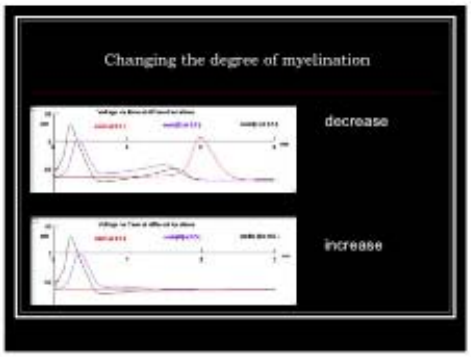

Results
Warming: shortens impulse duration, decreases peak amplitude
Cooling: increases AP duration
Change of degree of longitudinal (axial) current flow
Resurgence (reflection)


Change in density of K channels

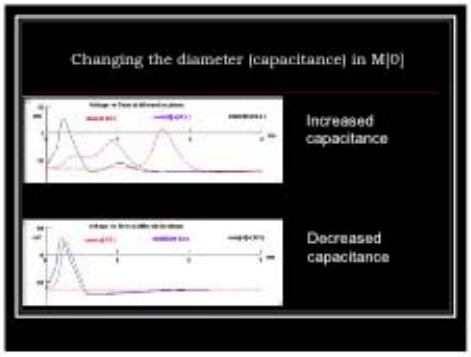

Change in Na Channel density

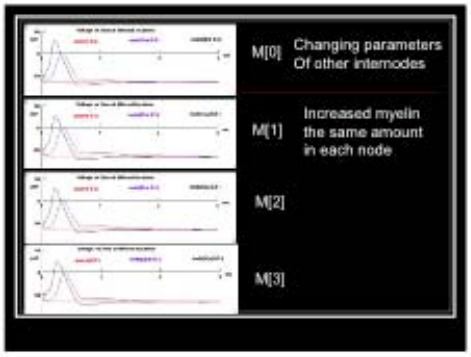

Changing the diameter of the bare axon
Decreased diameter
Normal diameter
Increased diameter


Changing length of internode M|O|
From 1000 um
To 900 um
From 1000 um
To 1100 um


Changing the degree of myelination
decrease
increase

3


Changing the diameter (capacitance) in M[0]
Increased capacitance
Decreased capacitance

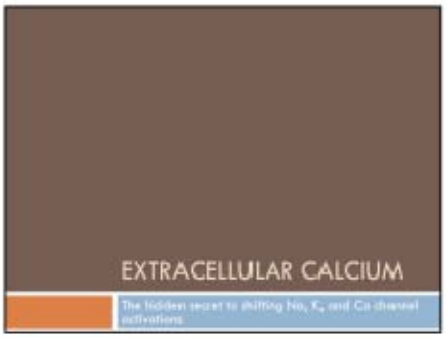

M[0]
Changing parameters Of other internodes
M[1]
Increased myelin the same amount in each node
M[2]
M[3]

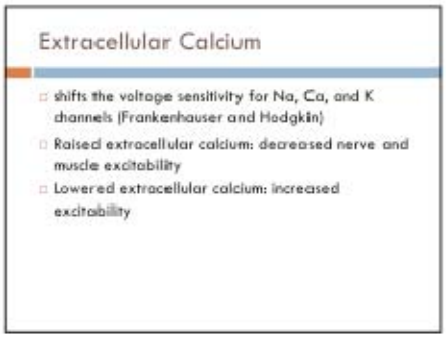

Conclusion

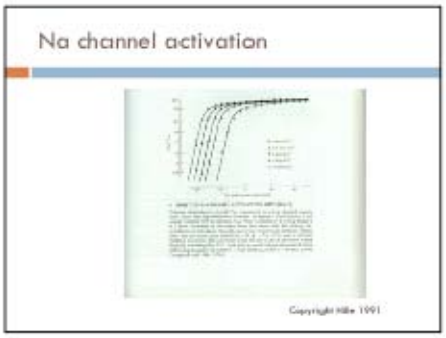

SOURCES
National Library of Medicine / National Institue of Health at http://www.nlm.nih.gov/medlineplus/multiplesclerosis.html
MS International Federation at http://www.msif.org/en/
Neurons In Action book & cd

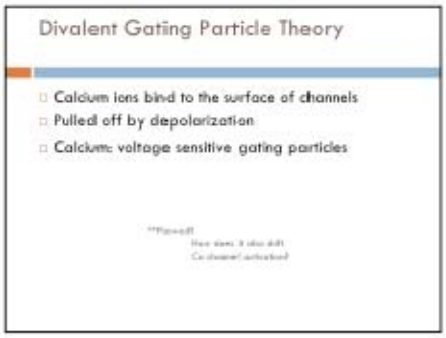
4

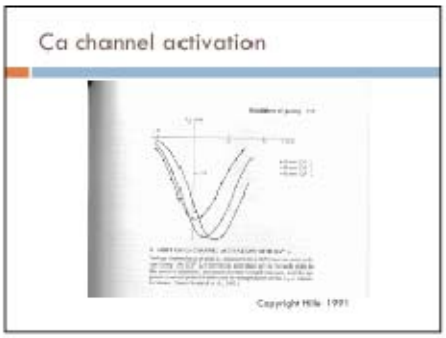
APPENDIX V: Agnes Scott College Student's Extracellular Calcium Sensitivity of the Na Channel and Dynamic Threshold Project Presentation Slides

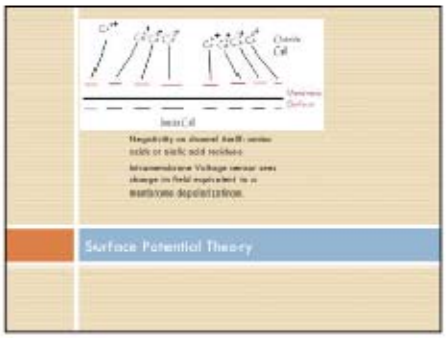










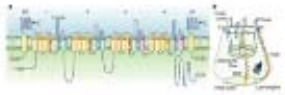


Surface Potential Theory

- Shortcomings:
  - Charge not uniformly spread
  - Na vs. K vs. Ca
  - Ca would have to shift voltage dependence of all gating parameters equally

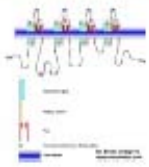
Recent Modifications

- Calcium attaches to the surface acting as a voltage gated sensor
- AND it blocks the pore itself
- S4 conformational changes responsible for Na and K channel openings: Possibly the removal of Ca ion?



Armstrong

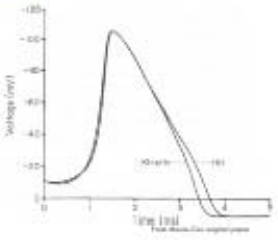
- Can unblocked sodium channels close?
- Saxitoxin: prevents access to binding site
- Ca requires access to the pore
- Other binding sites play a minor role



Current Equations

- Hodgkin and Huxley
  - Does not account for extracellular calcium
  - Reason for "anomalous hump"
- Moore and Cox
  - Developed a model that predicted the Frankenhauser and Hodgkin data
  - Able to better predict experimental results: no hump

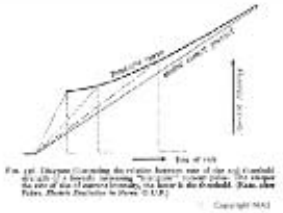
Anomalous Hump



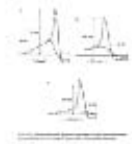
History of Threshold

- Louis Lapicque
  - The duration of the stimulus could be determined by the distance between A & C
  - Started proof: need larger stimulus for shorter stimulues

Katz

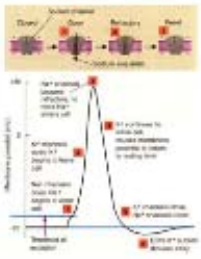


Cole



- Constant charge
- Constant current

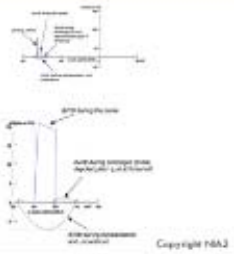
Hodgkin and Huxley



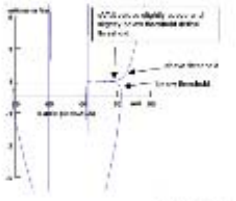
Current or Voltage Threshold?

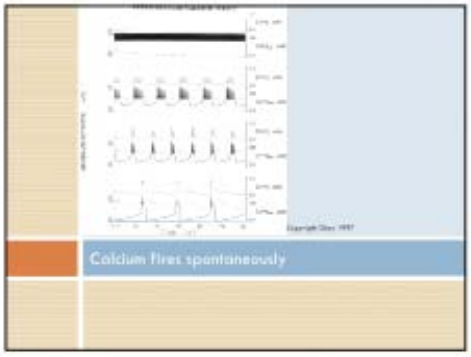
- Cole forgot what he had already done and daimed to find a threshold voltage
- Fitzhugh and Antosiewicz prove once and for all that there is no threshold voltage

Phase Plane Plots



Threshold





Spontaneously Firing Cells: Do they have a threshold?

- Pacemaker cells that display bursting activity as is seen in Aplysia

Spontaneously Firing Cells: Do they have a threshold?

- Look at affect of Increasing extracellular Potassium levels
  - Increased concentration leads to a less negative potassium equilibrium potential
  - We can now more easily go toward sodium equilibrium potential
  - This leads to spontaneous action potentials
  - There is a set value of extracellular potassium that is above and below threshold.

Physical Consequences

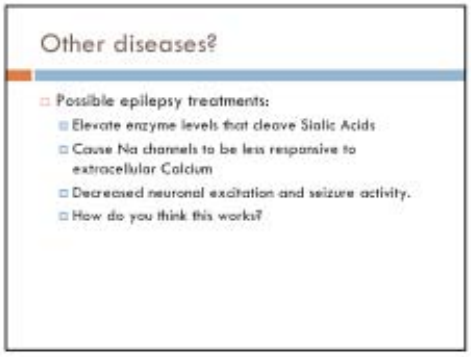
- Hypocalcemia
  - Causes: Parathyroid hormone
  - Symptoms:
    - Headaches, nausea and vomiting
    - muscle spasms, spasms in the hands, facial grimacing and sometimes, spasms of the airways, larynx or bronchial tubes)
    - Confusion, irritability, depression and psychosis
    - Cardiac arrhythmias
    - Seizures
- Hypercalcemia
  - Causes: Parathyroid hormone
  - Symptoms:
    - Nausea, Vomiting, Alterations of mental status, Abdominal or flank pain
    - Constipation, Lethargy, Depression, Weakness
    - Fatigue, confusion, dehydration
    - Cardiac arrhythmias,
    - Coma

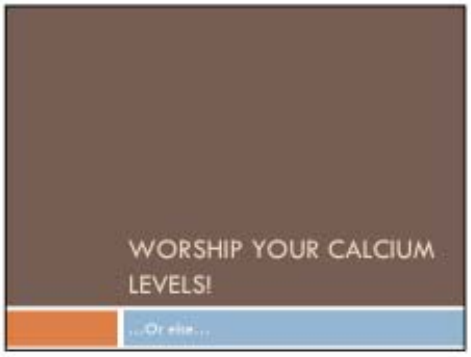
Treatment: Hypercalcemia

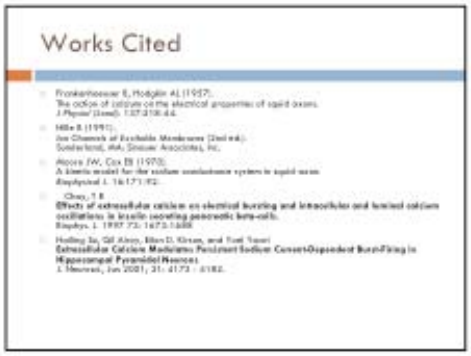
- Initially: HYDRATE!
- Signs of renal or heart failure: dialysis
- Hyperparathyroidism: possible surgery
- Drugs: Biphosphinates, Phosphate salts
- Vitamin D toxicity, Malignancy? : Corticosteroids
- Calcimimetic Agents

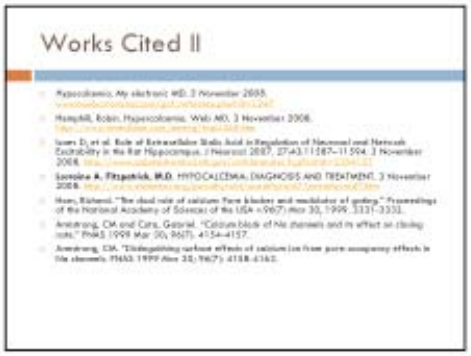
Treatment: Hypocalcemia

- Acute? : intravenous calcium gluconate
- Long term? : oral caldum supplementation and Vitamin D if needed


Other diseases?
Possible epilepsy treatments:
Elevate enzyme levels that cleave Sialic Acids
Cause Na channels to be less responsive to extracellular Calcium
Decreased neuronal excitation and seizure activity.
How do you think this works?


WORSHIP YOUR CALCIUM LEVELS!
...Or else...


Works Cited


Works Cited II