

# SYLLABUS

NSC 4353 Fall 2009

## NEUROBIOLOGY LABORATORY METHODS

Room GR4.708

Wednesdays 2:30 to 5:15

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This course will provide students with hands-on experience working with living biological preparations and training in the basic skills of neurobiology research including: electrophysiological signal acquisition and analysis; surgical and stereotaxic techniques; tissue dissection and histological preparation for anatomical analysis.

- Initial experiments will involve the use of electrical stimulators, amplifiers and oscilloscopes for the analysis of electronic models of neurons.
- These skills will then be applied in experiments employing the *in vitro* neuromuscular preparation obtained from frogs. This excitable preparation will demonstrate the analysis of bioelectric signals (action potentials and synaptic potentials) and has been used as an experimental model for human nerve-muscle disorders such as multiple sclerosis and myasthenia gravis.
- Further experiments will involve deeply anesthetized rats which provide the simplest mammalian model of human brain structure and function. Experiments will demonstrate the analysis of the brain's response to sensory inputs.
- The final advanced experiments will examine putative physiological mechanisms involved in learning, memory and epilepsy.
- Grading will be based upon lab participation and four publication-style lab reports (best four of five).
- Class size will be limited to 16 students and will require a small laboratory fee.
- This course satisfies the Neuroscience writing requirement and can be used for science credit by pre-med students.
- There are no prerequisites for this course except maturity.

**A complete set of experimental protocols may be downloaded from my internet site:**

**<http://utdallas.edu/~lcauller>**

CLASS SCHEDULE:

Week 2

Introduction to Electronics and Electrophysiology Methods  
Publication-Style Format I  
Electronic Model of a Neuron

Week 3

EXP #1: Measurement of Model Membrane Constants

Week 4

Introduction to *In Vitro* Frog Sciatic Nerve Preparation (EXP #1 Report Due)

Week 5

EXP #2: Compound Action Potential: Conduction Velocity

Week 6

Review of Exp #1 paper and Demonstration of Exp #3

Week 7

EXP #3: EndPlate Potential

Week 8

Introduction to *In Vivo* Electrophysiology (EXP #2 Report Due)

Week 9

Introduction to Rat Surgical and Stereotaxic Techniques

Week 10

Demonstration of Surgery and electrophysiology (EXP #3 Report Due)

Week 11

EXP #4: SomatoSensory-Evoked Cortical Responses

Week 12

Hippocampal Anatomy and Physiology

Week 13

EXP #5: Hippocampal Plasticity: Long-Term Potentiation (EXP #4 Report Due)

Week 14

**FINALS WEEK – All papers due**

## Basic elements of a publication-style research report.

### Sections:

**Title** - one sentence that says it all.

**Abstract** - paragraph of sentences summarizing each section.

**Introduction** - Why we did this experiment.

**Methods** - How we did this experiment.

**Drawing of experimental configuration**

**Results** - What we found.

**Figures and Tables**

**Discussion** - What the findings mean.

**Bibliography** - references to other literature

### Order of writing:

### Value for this class:

<b>Methods</b>	<b>40%</b>
<b>Results</b>	<b>40%</b>
<b>Text</b>	<b>20%</b>
<b>Figures and Tables</b>	<b>20%</b>
<b>Introduction/Discussion</b>	<b>5%+5%</b>
<b>Abstract</b>	<b>5%</b>
<b>Title</b>	<b>5%</b>

### Key points about each section:

#### Methods:

The Methods section should give enough information for another researcher to exactly replicate your experiment.

- Subjects - species, sex, weight/age
- Preparation - anesthesia type and dosage (mg/kg weight), special handling (eg. cooled in icebath), dissection/surgical technique, stereotaxic.
- recording/stimulating configuration
- data analysis methods - statistics or computer programs
- Histological tissue preparation - marking lesions, perfusion, sectioning and staining.

#### Results:

The results consists of two parts, TEXT and FIGURES.

You MUST describe the figures using words. It is not enough to simply show the data, you must describe it. (eg. The maximum slope of the stimulus response curve occurred for stimuli between 5-7 V.)

#### Introduction:

This section should state why we did the experiment and what we expect to find (if applicable). This section normally contains a brief literature review which is not necessary for this course.

Discussion:

This section should begin with a restatement of the findings reported in the Results section in general terms (e.g. We observed an increase in muscle excitability when we added physostigmine.) Then explain your interpretation of these findings (e.g. This is consistent with an increase in the availability of synaptic acetylcholine.) Normally this section involves hypothesis building and a critical review of potential pitfalls of the experiment.

Abstract:

The abstract is the face of your report. It should have at least one sentence describing each section (Intro, Method, Results, Discussion).

Title:

Writing a good title is an artform. In one sentence the title should specify the methods, independent and dependent experimental variables and (if lucky) the findings or conclusion.

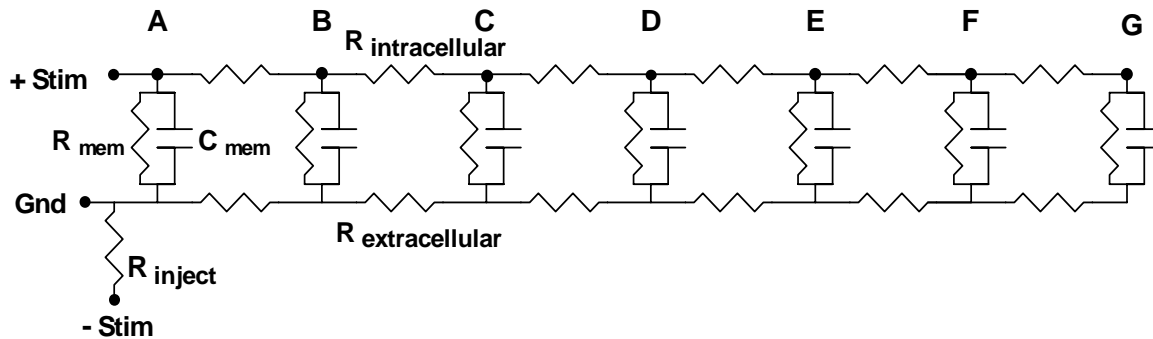
**A long report is not necessarily a good report. You will get bonus points for brevity. Concentrate on making good figures because this conveys the most information.**

## NEUROBIOLOGY LAB Fall 2009

### Experiment #1: Measurement of Model Membrane Constants

At each lab station, you will find an electronics breadboard prepared with a network of resistors and capacitors to simulate the passive cable properties of a neuronal dendrite or axon. You will use the Grass SD9 stimulator to inject current into this model neuron and you will use the Igor Acquisition Application to record its response. A schematic of the model membrane and points of connection is shown below:

FIGURE 1. The model membrane network.



At each lab station, the value of each component of the membrane model will be written on a card. These values should be included in your METHODS section description of the model you used. For the first part of this experiment, the membrane capacitors (C<sub>mem</sub>) will be omitted while you measure the length constant. In the METHODS section of your lab report, you should draw the experimental configuration including the stimulator and the model membrane network. In this drawing you should indicate where you measured the responses.

Connect the oscilloscope channel 1 input to measure the voltage at point A of the model membrane with the ground shield attached as shown in the schematic. Connect the positive electrode of your stimulator to the intracellular point A and the negative electrode to the extracellular point Gnd as shown above. Adjust the stimulator controls to deliver an electrical pulse (6 volts, 100 ms duration, 5 ms delay) at a repeat frequency of about 1 pulse per second. Adjust the oscilloscope controls to fill the screen with the response of the model membrane to optimize time and voltage measurements. Ask a laboratory assistant to check your setup before proceeding.

In this experiment you will:

1. characterize the decay of the transmembrane voltage as you move away for the current injection site and measure the length constant ( $\lambda$ ) of the model membrane using two values of the membrane resistance;
2. add the membrane capacitors, record the charging curve and measure the time constant ( $\tau$ ) for each value of membrane resistance;
3. characterize the spread of the response down the complete membrane model.

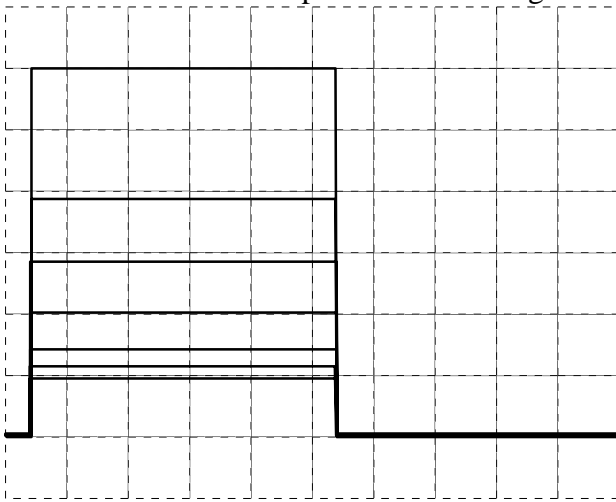
The *transmembrane voltage* ( $V_m$ ) is recorded intracellularly (e.g. point A) relative to an extracellular reference or ground (Gnd). The response you are starting with is the  $V_m$  at site A.

**Record the DECAY of  $V_m$  with distance and measure the LENGTH CONSTANT:**

Not surprisingly, the  $V_m$  decreases as you move away from the current injection site. For this experiment, we will treat each resistor segment as one distance unit, such that the total length of the model dendrite is six segments. To measure the decay of the response, you will apply constant input stimuli and record the responses at each intracellular site along the model membrane (points A-G in Figure 1).

**FOR YOUR LAB REPORT:** Make a plot superimposing the responses recorded at each site along the model membrane. This should look something like:

FIGURE 2. Oscilloscope record of voltage decay along the model membrane.



(Indicate the site where each response was recorded. REMEMBER to always write the V/DIV and ms/DIV on your plots!)

Now you should replace the seven 100 k $\Omega$  membrane resistors ( $R_{mem}$  in Fig. 1) with the seven 10 k $\Omega$  resistors you will find at each lab station. Notice that you must increase the stimulus intensity to get the same response at site A that you got with  $R_{mem} = 100$  k $\Omega$ . (Extra credit if you can think why this is so. HINT: Ohm's)

**FOR YOUR LAB REPORT:** Make another plot superimposing the responses recorded at each site along the model membrane with  $R_{mem} = 10$  k $\Omega$ .

**LENGTH CONSTANT ( $\lambda$ ):** As we discussed in our lab meeting last week, the  $V_m$  decays *exponentially* as you move away from the site of current injection. The equation describing this decay is:

Equation (1)  $V(x) = V_{origin} * e^{-x/\lambda}$

[ You may be able to show:  $d^2V/dx^2 = 1/\lambda^2 * V$  . ]

At  $x = \lambda$ ,

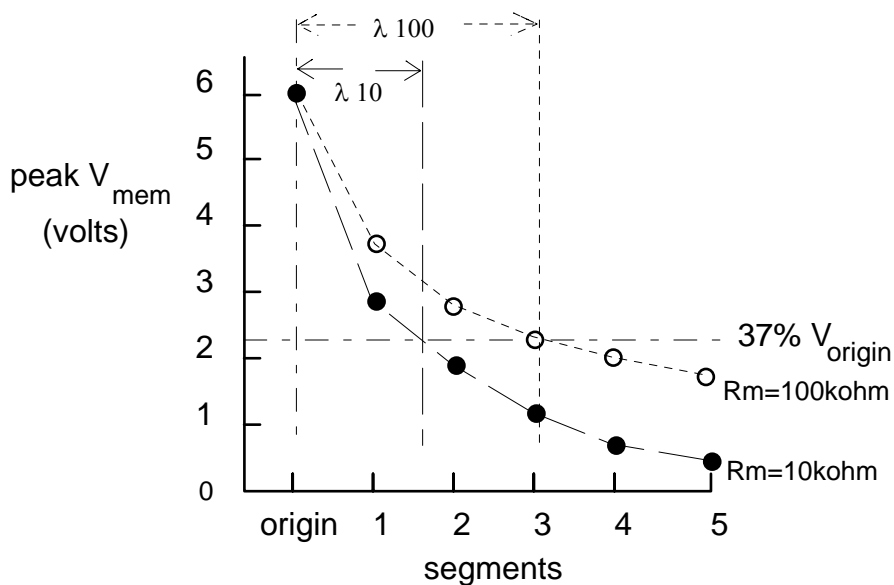
$$V(\lambda) = V_{\text{origin}} * e^{-\lambda/\lambda} = V_{\text{origin}} * e^{-1} = V_{\text{origin}} * 1/e = 37\% V_{\text{origin}}$$

[ The value of  $e \cong 2.718\dots$ , such that  $1/e \cong 37\%$ . ]

The length constant is simply the distance from the origin to the point where the voltage falls to 37% of the voltage at the origin.

**FOR YOUR LAB REPORT:** Prepare a graph which plots  $V_m$  versus distance for both values of  $R_{\text{mem}}$  and indicate the length constants. This graph should look something like:

FIGURE 3. Decay of  $V_m$  along a model membrane .



**EXTRA CREDIT:** By simply changing the vertical scale on your graph, you should be able to indicate the transmembrane current at each site along the membrane. **HINT:** Ohm's law.

**Record the CHARGING CURVES and measure the TIME CONSTANT:**

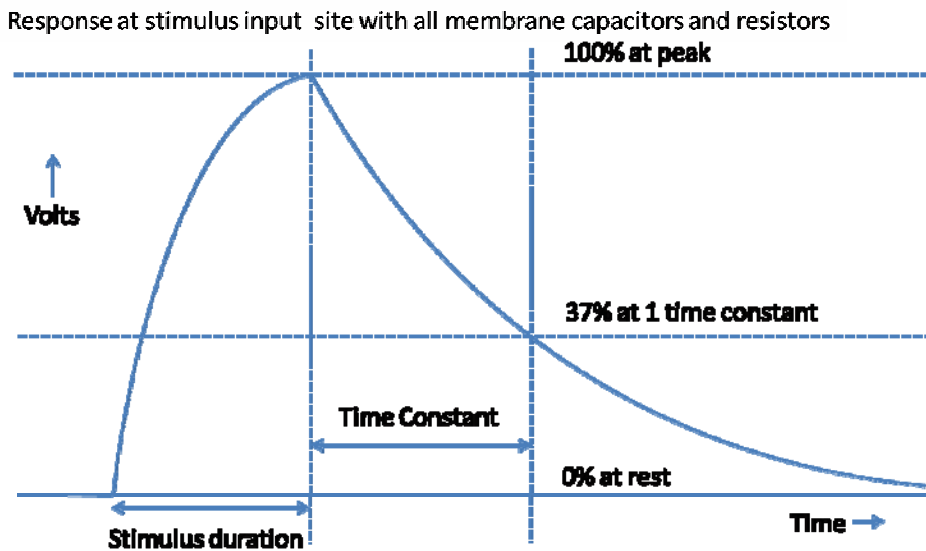
Now install a membrane capacitor ( $C_m$ ) at each segment as in Fig. 1. **MOVE THE NEGATIVE STIMULATOR INPUT** to the other end of the injection resistor ( $R_{inject}$  in Fig. 1). The charging curve and time constant should be recorded at the same site where the current is injected (i.e. site A).

The membrane charging curve is simply the trace you see on the oscilloscope when you inject current across the membrane capacitance. Notice that unlike the stimulus pulse, the charging curve is not square. The beginning of the pulse is rounded off and a tail extends from the end of the pulse. In other words, the voltage approaches any new value *exponentially*. (See section 2 of <http://www.utdallas.edu/~lcauller/Courses/NeuroPhys/NPhysWSderivations.doc> for a detailed derivation of the exponential nature of the time and length constants.)

For this experiment, you should try to measure the time constant as accurately as possible by finding the point along the decaying tail after the end of the stimulus when the voltage falls to 37% of the peak voltage just before the stimulus ended. Try adjusting the stimulus intensity and the vertical position of the charging curve such that the peak voltage aligns with a grid line near the top of the oscilloscope and the prestimulus baseline aligns with a grid line near the bottom. Then you can accurately measure the decay time by adjusting the horizontal axis scale such that the tail extends across the full width of the graph.

**FOR YOUR LAB REPORT:** Make a plot of the full charging curve superimposed upon a fast trace indicating the time constant. This should look something like Fig. 4.

FIGURE 4. Exponential waveform of membrane charging and discharge decay.



Now, replace the 10 k $\Omega$  resistors with the 100 k $\Omega$  resistors and repeat the procedure above to measure the corresponding time constant. REMEMBER: the same membrane resistor values must be installed for all 6 segments.

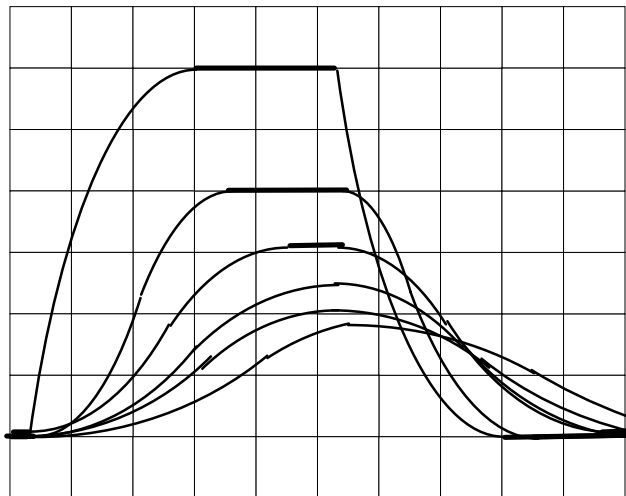
**FOR YOUR LAB REPORT:** Include two separate, or one combined plot showing the exponential charge/discharge responses with each membrane resistance value, indicating how their time constants were measured. REMEMBER: report these measurements in the text of the Results section.

**Characterize the TEMPORAL and SPATIAL DECAY of  $V_m$ .**

To characterize the spread of the response along the complete model membrane, simply record the response at each site (A-G) with both  $R_m$  and  $C_m$  in place.

**FOR YOUR LAB REPORT:** Make a plot superimposing the  $V_m$  responses recorded at each site along the complete model membrane for  $R_m = 100$  k $\Omega$  and another for  $R_m = 10$  k $\Omega$ . Each of these plots should look something like Fig. 5. Adjust the axis scales for the best illustration of these recordings.

FIGURE 5.



You should try to describe how the response spreads along the membrane in your RESULTS section noting outstanding differences (i.e. how would you describe the state of the axon toward of the end of the responses with the high membrane resistance?). Also, in the DISCUSSION section, specifically comment on how you think this might affect the neural functions (i.e. neuromodulated arousal states) described in the INTRODUCTION.

**LAB REPORT REMINDERS:**

- The METHODS section should describe the experimental setup and procedures well enough to let someone repeat your experiment without knowing what you did otherwise. Include specific names of your experimental apparatus (i.e. Grass SD9 stimulator) and the values of your model components (e.g.  $R_{\text{intracellular}} = 100\Omega$ ).
- In addition to figures showing examples of your raw data plots and graphs showing your analysis, the RESULTS section should clearly state your findings. For example: "The length constant of the model membrane was 3.2 segments when the membrane resistance was 10 k  $\Omega$ ."