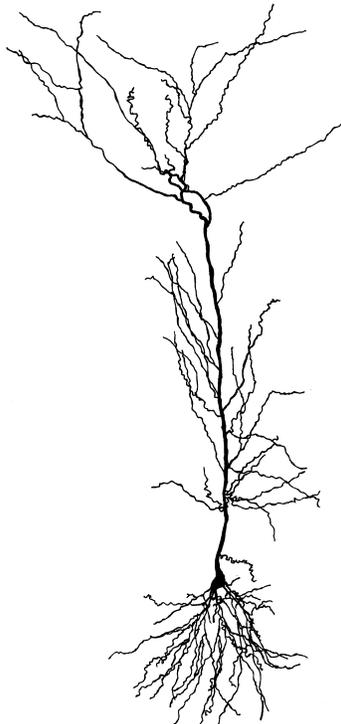


# **Neurobiology Laboratory**

**(BIO 365L)**

Spring Semester 2011  
Friday 1-6 pm, PAI 1.04



## **Lab Manual: Module 1**

### **Passive Membrane Properties**

## Module 1: Passive Membrane Properties

### Introduction to Membrane Biophysics and Electrophysiology Equipment

#### **Goals:**

1. Overview of the course syllabus, attendance and grading policy;
2. To review basic membrane biophysics and terminology;
3. To introduce the physiology rig, computer software, and their applications in electrophysiology;
4. To review RC circuit and measure  $\tau$ ,  $R$ , and  $C$  using a model cell.

**Additional Reading:** Chapter 1 of the Axon CNS guide is **strongly recommended**

### **Part I: Summary of Neurophysiology Terminology**

An important step toward becoming an electrophysiologist is to understand the biological membrane from the point of view of a physicist. To this end, we will first review the basic concepts in physics that you may have learned sometime ago.

#### **1.1. Charge (Q)**

All matter is made up of charged particles, and can exist as positive protons (+) or negative electrons (-), in more or less equal numbers. Like charges repel one another, and dissimilar charges attract. The unit of charge that we will commonly deal with is the coulomb (C). How much is a coulomb? The charge on a gram of a monovalent ion such as  $\text{Na}^+$  would be 96,500 C.

#### **1.2. Current (I)**

Current is the flow of charge past a location per unit time ( $dQ/dt$ ). The unit of current is the **Ampere**, which equals **1 coulomb/sec**. Currents in neurons and muscles are usually on the order of picoamps (pA;  $10^{-12}$  A) or nanoamps (nA;  $10^{-9}$  A). By convention, current moves from positive to negative. Different from electric current used to power light bulbs or computers, biological current is carried by the movement of charged salt ions (e.g.  $\text{Na}^+$ ,  $\text{Cl}^-$ ).

#### **1.3. Potential difference, or Voltage (V)**

A potential difference, or voltage, is the work that must be done to separate two charges (negative vs. positive). Voltage is expressed in volts (**V**) or millivolts (**mV**, 1 V = 1,000 mV).

#### **1.4. Resistance (R)**

Charge flowing in a medium encounters atoms, which impede their flow, generating heat in the process (that's why computers and electronics get warm as they

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run). This is resistance. Similarly, ions flowing across ion channels on the membrane are also met with resistance, as they will have to go through the water-filled pore of the protein and interact with charged residues in its lining. The resistance of cells is usually expressed as megaohms ( $M\Omega$ ).

One concept used often by electrophysiologists is conductance, which is the inverse of resistance, or  $G=1/R$ , and refers to the ease of which ions can move through a medium or membrane. The unit for conductance is siemens; most single channel conductance is in picosiemens (pS).

### 1.5. Capacitance (C)

When two conductors (e.g. two metal plates) are in very close proximity to one another but are separated by a nonconductive medium (e.g. air), charges on each side attract one another and impede one another's movement. The conductors thus can be said to store charges. The amount of this charge storage, or capacitance, is proportional to the area of the conductor, and inversely proportional to the distance separating the conductors. The unit capacitance is the farads (F), which equals 1 coulomb of charge a capacitor can store for each volt applied to it. Hence,  $C=Q/V$ .

### 1.6. Ohm's Law ( $V = IR$ )

This relationship between voltage, current and resistance is critical in understanding the electrical behavior of neurons and muscle.  $1\text{ V} = 1\text{ A} * 1\ \Omega$  or  $1\text{ mV} = 1\text{ nA} * 1\ M\Omega$

This analogy might help you develop an intuitive understanding of the relationship between voltage, current, and resistance: *Imagine you turn on a faucet, forcing water through a garden hose. In this case, pressure is analogous to voltage (V). Water current and the resistance it encounters going through the hose is analogous to current (I) and resistance (R) in electricity. For a given hose (i.e. R remains the same), water flow (I) increases if one turns up the faucet to increase the water pressure (V). Similarly, for a given water pressure (V), the amount of water coming out of the hose will decrease if the hose diameter is too small (i.e. R is high) or vice versa.*

## Part II. Principles of Membrane Biophysics

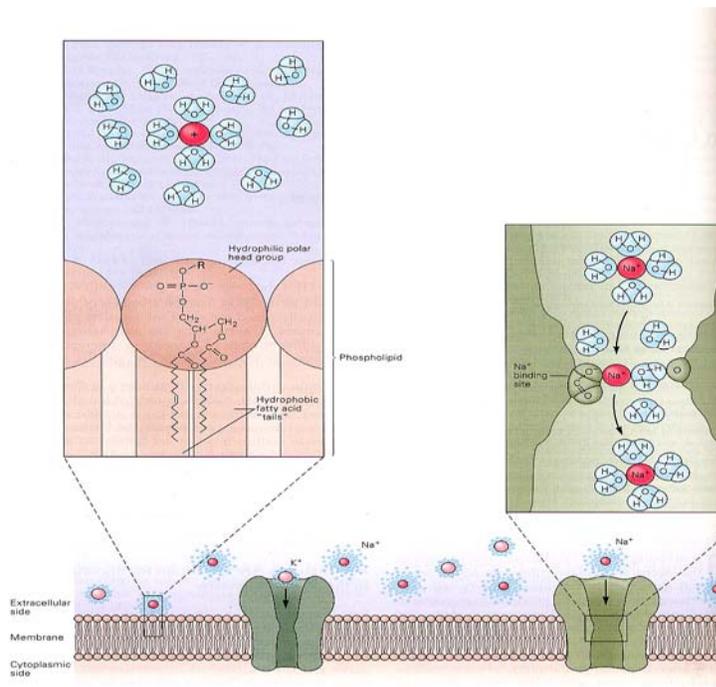
### 2.1. The Physics of Biological Membranes

Biological membranes consist of a phospholipid bilayer, with the charged polar heads of the phospholipids facing the internal and external ionic environments, and the hydrophobic fatty acids facing the interior. In electrophysiologists' eyes, biological membranes have many similarities to the physical property described earlier. In other words, a membrane contains resistors, capacitors, batteries, and pathways for current to flow.

### Membrane Resistance

Membranes possess ion channel proteins, most of which selectively allow charged ions to pass through their water-filled pores.

Ions exist in solution surrounded by water. This is called a sphere of hydration. Charged ions exchange water molecules with charged residues in the channel pore, allowing them to pass through. Membrane resistance is usually determined by the number of ion channels and the state (open or closed) of these channels.



The flow of charged ions through membrane channels represents transmembrane current flow. The resistance of this current flow across the membrane results in a transmembrane voltage change. The relationship between current, resistance, and voltage is reflected by **Ohm's Law**:

$$\text{Ohm's Law: } V = I * R$$

Equation 1

### Membrane Capacitance

Membranes also act as capacitors, as they are able to store charge on their surfaces. This is because there are two conductors (the intracellular and extracellular aqueous environments) that are separated by an extremely thin insulator (the fatty acids of the bilayer). The thickness of the membrane is only about 6-7 nm. The capacitance of the membrane is described by:

$$\text{Membrane capacitance: } C = Q/V$$

Equation 2

This equation states that the membrane capacitance is equal to the amount of charge that can be stored on a membrane for a given transmembrane voltage change.

Rearranging,

$$Q = CV$$

Equation 3

Now differentiate the equation:

$$dQ/dt = C * dV/dt$$

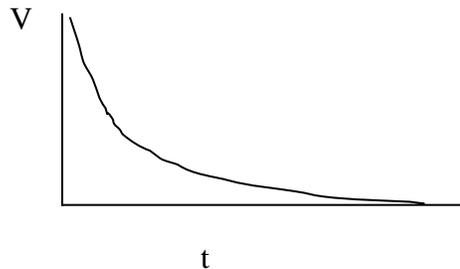
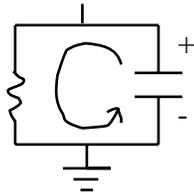
Equation 4

Since  $dQ/dt$  is current ( $I$ , the flow of charge past a point per unit time), this equation states that current onto and off the membrane (for storage) is proportional to the **change** in membrane voltage. In other words, there is no capacitive current when the voltage is stable (e.g. at the resting potential).

### Time Constant ( $\tau$ )

The change in membrane voltage would occur instantaneously if resistance to current flow did not exist. This can be appreciated using Ohm's law. Using Ohm's Law, the maximum current ( $I$ ) equals  $V/R$ . When  $R$  is 0, the current is infinitely large such that a capacitor can be charged or discharged with a rapid rate. When  $R$  is large, the current flow reduces, taking a long time to change the voltage across the capacitor.

The rate of discharge at a given time is defined as  $-dQ/dt$  (negative because the charge is decreasing with time), which is essentially the same as the magnitude of the current. Hence, equation 4 becomes



$$dV/dt = -(1/C) * dQ/(dt)$$

$$dV/dt = -V/ (R*C)$$

Equation 5

Where  $V$  is the initial voltage and decreases as the capacitor is discharged. This equation states that the speed of the voltage change from the capacitor is proportional to the voltage remaining. Thus, as voltage decreases, the rate also decreases. **RC is the time constant or tau.**

*This effect is analogous to water draining from a bathtub.* The amount of water the bathtub "stores" is analogous to capacitance. The pressure on the water to flow through the drain pipe is analogous to voltage, and the size of the drain pipe determines the resistance to water flow. When the bathtub is full, there is a lot of driving force on the water to flow through the pipe, and so the flow of water out of the bathtub is initially fast. However, as the water nears the bottom, there is not much driving force pushing the water out, and so this water drains out more slowly. Of course, the pipe size also influences the draining rate. A larger pipe (i.e. smaller  $R$ ) will drain faster than a smaller pipe.

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This example shows how membrane resistance and capacitance are BOTH involved in regulating how fast membrane voltage changes can occur. If resistance is high (a narrow pipe in the bathtub example), a given amount of current takes longer to pass through. If membrane capacitance is high (there is a lot of water stored in the bathtub), it also takes longer for the larger amount of current to “drain” away, so to speak.

Functionally, the membrane time constant reflects how fast a neuron or muscle can respond to a synaptic signal. The more open channels (low R), the faster the response.

The equation 5 is a first-order differential equation whose solution is:

$$V(t) = V e^{(-t/RC)}$$

The membrane time constant, or tau is the time it takes for voltage to fall to 1/e, or 36% of the initial voltage (or ~63% of the final voltage).

$$\tau = RC$$

When a capacitor is charged through a resistor (e.g. during a step depolarization), the equation is:

$$V(t) = I R + C \cdot dV/dt R$$

the solution:  $V(t) = I * R (1 - e^{(-t/RC)})$

Here, tau refers to the time required to reach 63% of the final voltage.

A time constant is the time it takes for any exponential process to reach 63% of its final value. In the case of biological membranes, the exponential process is a change in membrane voltage. The membrane time constant, or tau, is directly proportional to the membrane resistance and the membrane capacitance (per unit area). That is,  $\tau = R_m C_m$ . *Since  $C_m$  is largely the same in different membranes ( $1 \mu F/cm^2$ ), the membrane time constant thus reflects the number and type of ion channels expressed in the neuron (which determines  $R_m$ ).*

### **The Pathway for Current Flow Across the Membrane**

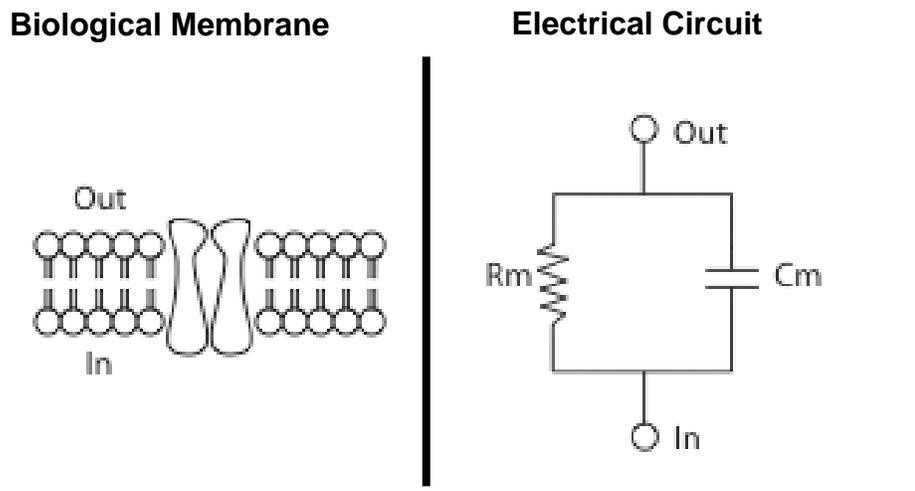
Movement of charged ions across the membrane carries the membrane current. Ions are charged particles that will not flow easily across the membrane lipid bilayer. Rather, ions pass through the membrane through specific ion channels. Therefore, ion channels are the ‘metal wire’ of a circuit so to speak.

### **2.2. Biological Membrane as Electrical Equivalent Circuits**

One can view the membrane as the following electrical equivalent circuit.

**Features:**

- Ion channel proteins possess transmembrane pores that conduct ions across the membrane. This introduces membrane resistance ( $R_m$ ), which is the inverse of conductance. The value of the resistor is thus proportional to the number of channels that are open.
- The phospholipids bilayer stores electrical charge, giving rise to the property of membrane capacitance ( $C_m$ ).
- The behavior of a biological membrane may be simulated closely by the behavior of a simple RC electrical circuit with solid-state components.



**2.3. Electrical Noise**

In electrophysiological recordings, the goal is to record biologically relevant signals from muscles and neurons. However, in practice, these recordings are subject to other contaminating signals (termed “noise”) that arise from other, non-biologically relevant sources, such as electronic equipment, power supplies, electrical outlets, and even (sometimes) fluorescent lights. Noise contamination can be minimized, but it can be a non-intuitive process.

***The take-home message***

- Biological membranes possess 2 primary “passive” electrical properties: resistance and capacitance, which, together, determine the rate at which passive voltage changes can occur.
- It follows that membrane resistance and capacitance determine the window over which synaptic potentials can sum with one another in neurons and muscles. Passive membrane properties help determine the speed at which neurons signal within their networks.

## **Laboratory 1: Passive Membrane Properties in Hippocampal Pyramidal Neurons**

### **Introduction: Patch Clamping in Brain Slices**

#### **The overall approach**

For the next semester you will be recording the electrical activity of different classes of neurons contained in living brain slices taken from rodents (Long-Evans rats). You will visualize the neurons at 40x magnification with a contrast-enhancing camera system, and then use patch recording technology to study the electrical excitability of neurons. You will observe how cells initiate action potentials and integrate synaptic activity, all the cellular building blocks that contribute to sensory perception, learning and cognition, and the writing of laboratory reports.

#### **The use of animals**

For practical reasons, you will not be involved in the process of preparing brain slices. In fact, you will never see the animals that are sacrificed for this laboratory. Nevertheless, it is important that you understand what is involved in preparing brain tissue, and also that you are aware of the steps that are taken to ensure that animals experience no pain or suffering. You will be recording from brain circuits that are common to all mammals, including humans, so we hope to impress upon you in the course of the laboratory the relevance of these experimental approaches in understanding both normal neural function as well as the underlying mechanisms of brain disorders such as epilepsy and Parkinson's Disease.

#### **Care and maintenance of brain tissue *in vitro***

Animals are fully anesthetized and decapitated, using guidelines approved by the University of Texas Animal Care and Use Committee. The brain is rapidly removed and submerged in a saline solution that mimics the cerebrospinal fluid that normally bathes the brain (We will refer to this solution as artificial cerebrospinal fluid, or ACSF). A block of the brain area of interest is glued to a stage of an oscillating tissue slicer, and brain slices are cut at a thickness of 300  $\mu\text{m}$ . They are allowed to recover from the slicing procedure submerged in ACSF at 35°C for at least 30 minutes. Finally, the holding chamber containing the slices is transported to the teaching laboratory and held subsequently at room temperature. In all conditions, ACSF is constantly bubbled with a mixture of 95% oxygen and 5% carbon dioxide, a routine that provides badly needed oxygen to the brain tissue via diffusion (the circulatory system is disrupted immediately upon slicing).

Brain tissue is sensitive to anoxia (lack of oxygen), so during recordings, you will be constantly bathing the brain slice with fresh, oxygenated ACSF. In addition, maintaining the tissue at room temperature (rather than at body temperature) improves the viability of slices, presumably because the lower temperature reduces the metabolic demands of the neurons.

### **Patch recordings**

Patch electrodes are comparatively blunt, and exhibit tip diameters of about 1  $\mu\text{m}$  (vs. about 0.1  $\mu\text{m}$  for sharp microelectrodes). Instead of penetrating the cell membrane with the electrode, you will form a high-resistance seal (several GigaOhms ( $G\Omega$ )...that's 1000M $\Omega$ !) between the blunt glass lip of the patch electrode and the cell membrane. In order to get this seal, you will quite literally cram the pipette against the side of the cell membrane (which you will visualize directly with the aid of your microscope). You then rupture the small area of membrane under the electrode tip with brief pulses of suction, thereby establishing an intracellular recording.

### **Pipette solutions**

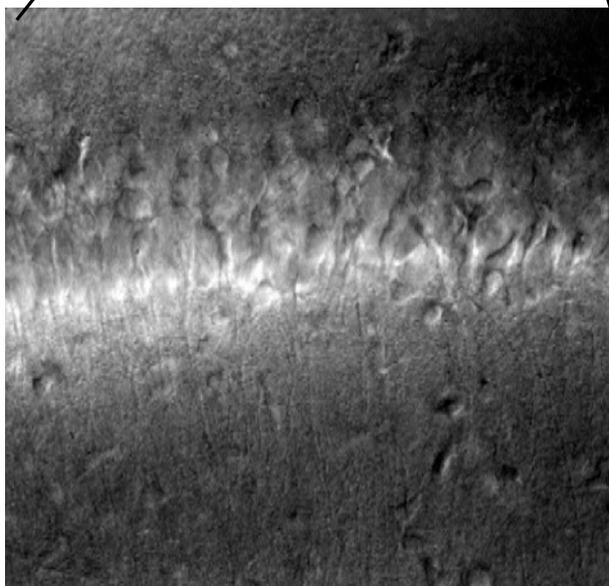
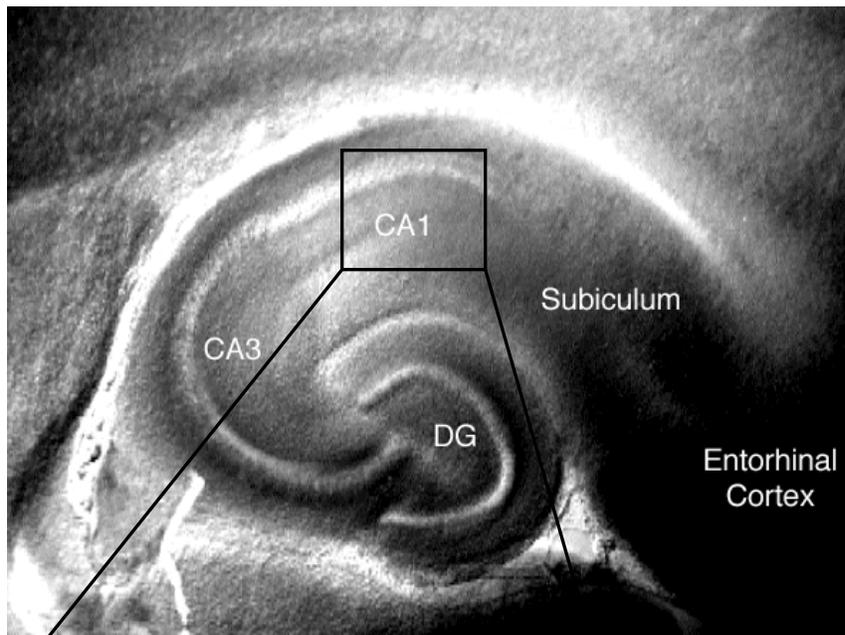
The salt solution used inside patch pipettes is rather complicated. Upon “breaking in” to the cell, there is considerable exchange between the pipette solution and the cytoplasm inside the cell. Thus the pipette solution must closely mimic the ionic conditions inside the cell (high potassium, low sodium, low calcium, etc...). Intracellular dialysis with patch pipettes has a disadvantage, in that critical cytoplasmic constituents (e.g. organelles) can be lost into the pipette. To compensate for the partial loss of soluble cellular machinery, ATP and GTP are included in pipette solutions.

A critical consideration is that *pipette solutions must be extremely clean*. Your pipette solutions have been sterile-filtered through a 0.2  $\mu\text{m}$  filter. To keep the solution clean you must take care not to touch the solution and pipette fillers with anything. Be especially careful about touching solutions with your fingers. The silver chloride wire that comes in contact with the pipette solution must also be clean. It is quite easy to accidentally brush the wire with your hand or finger. In doing so you risk transferring cellular detritus to your pipette solution. This detritus is remarkably good at plugging up the tip of your pipette (this material is sometimes informally referred to as “tip sh--”). Its presence will require you to discard the electrode.

## The Hippocampal Slice Preparation and Circuitry

### The “trisynaptic circuit”

The hippocampus processes information about spatial location, and accordingly receives synaptic input from multiple sensory modalities. The majority of inputs arise from the entorhinal cortex and synapse on granule cells in the dentate gyrus (DG). The axons of granule cells then project to pyramidal neurons in CA3, which in turn project to the pyramidal neurons in CA1. Finally, the axons of the CA1 pyramidal neurons exit the hippocampus and contact neurons in the subiculum and entorhinal cortex.

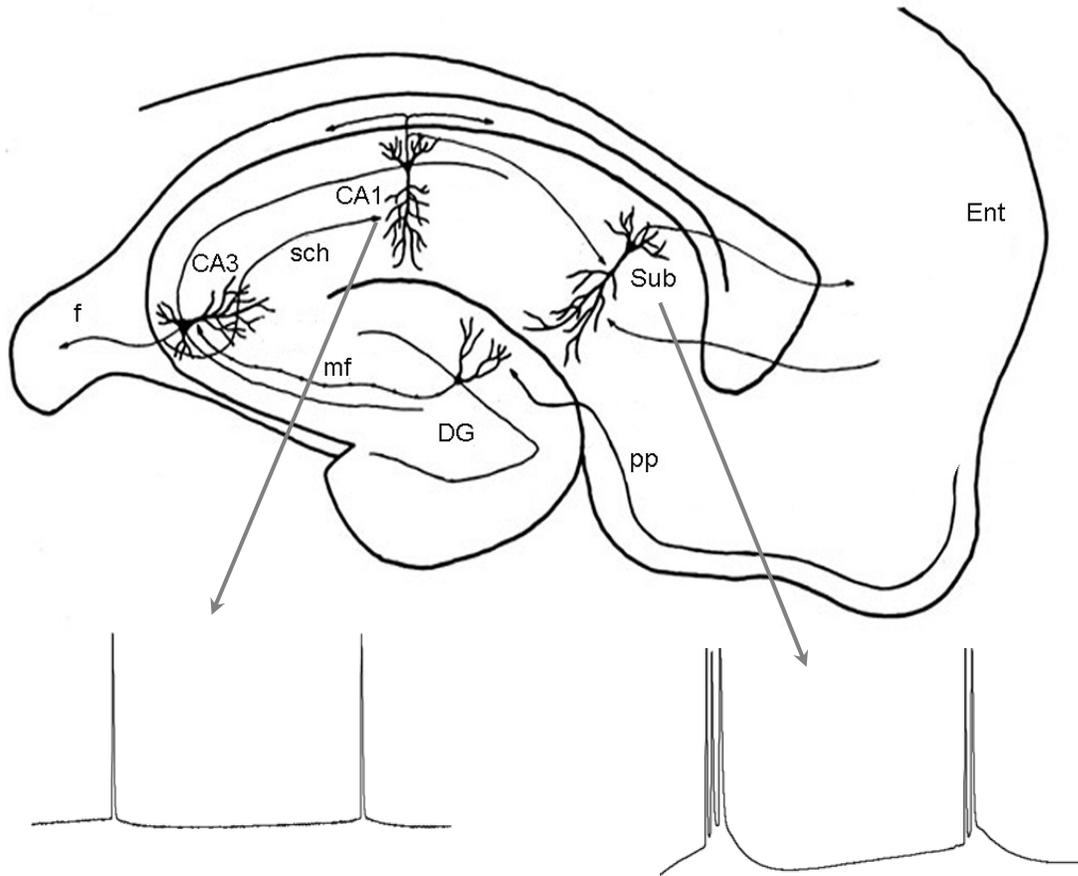


Stratum Oriens

Stratum pyramidale (layer containing cell bodies)

Stratum radiatum (layer containing Schaffer collaterals)

Stratum lacunosum-moleculare (out of view)



### Connectivity of the Hippocampus

Abbreviations:

**Ent**= entorhinal cortex; the origin of excitatory inputs to the hippocampal circuitry

**pp**=perforant path; the axons of entorhinal cortical neurons enter the hippocampus here

**DG**=dentate gyrus; neurons in this area are called granule cells

**CA3**; cornu ammonis (latin for Ammon's Horn) area 3; pyramidal neurons here receive granule cell input (via mossy fiber axons, abbreviated "mf")

**CA1**= cornu ammonis (latin for Ammon's Horn) area 1; pyramidal neurons receive excitatory input from CA3 pyramidal neurons via schaffer collaterals (abbreviated sch).

**S**=subiculum; pyramidal neurons here receive CA1 excitation. Some but not all of these neurons fire action potentials in a bursting pattern.

**alv**=alveus (an output layer of the hippocampus that consists of the axons of CA1 pyramidal neurons)

\* All excitatory synaptic transmission from pyramidal neurons in the hippocampus is glutamatergic, consisting of both AMPA and NMDA receptor components.

\*\* Inhibition in the hippocampus is GABAergic

## Module 1 Experiments

### Experiment 1: Measuring passive membrane properties

#### PreLab

The point of this experiment is to examine the limiting effect of passive membrane properties on the speed of voltage changes induced by synaptic input. To do so, you will deliver a "simulated EPSC (postsynaptic excitatory synaptic current)". That is, a waveform shaped like a glutamatergic excitatory current (AMPA receptor current to be precise) found in CA1 pyramidal neurons. You will adjust the amplitude of this current to produce a voltage change of a few millivolts. The precise amplitude, as long as it is relatively low, is irrelevant for your purposes. You will simply compare the time course of the recorded voltage change with that of the EPSC you injected.

#### InLab

1. Set the capacitance compensation and bridge balance controls. Use the test pulse. Duration 10 ms, amplitude 5 pA
2. Deliver a positive step pulse 1000 ms long. Adjust the current injection amplitude to elicit a voltage response between 2-5 mV in amplitude. Once you have set your current amplitude, get 10 sweeps of data, waiting a few seconds between each sweep.

Record the sweep numbers here: \_\_\_\_\_ to \_\_\_\_\_

3. Elicit a simulated EPSP. In the ADC/DAC control panel at the bottom of your screen, replace StepPulse\_DAC with EPSC\_DAC. The amplitude of this waveform is set to 1 pA, so you must multiply this value to adjust the voltage response to approximately 3 mV (see an instructor about how to do this). Once you have set your current amplitude, get 10 sweeps of data, waiting a few seconds between each sweep.

Record the sweep numbers here: \_\_\_\_\_ to \_\_\_\_\_

#### PostLab

*For your results*

1. Average your 10 waves for the 1000 ms square pulse and save the averaged wave with a new name. Make a graph containing your averaged 1000 ms square pulse response. Measure the membrane time constant of the averaged response from the *onset* of the current pulse to where the voltage reaches steady-state. Do this by fitting an exponential to this segment of the response. Overlay the fit that you made to calculate the membrane time constant on your graph. Increase the thickness of the fit to ensure it stands out from the voltage response.. Calculate membrane resistance as usual, using Ohm's law.

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2. Average your 10 waves for the simulated EPSP response and save the averaged wave with a new name. Superimpose on one graph: EPSC\_DAC (this is your preformulated stimulus waveform, and it can be found in your list of waves in addition to the traces you recorded ) and the averaged EPSP response.

### Graphing notes:

You can superimpose your artificial EPSC and your resulting voltage response...simply make a graph and put EPSC\_DAC and your recorded voltage waveform ad1\_something in the same graph (you will have to offset one of the waveforms by holding your cursor on the trace for a second, and then moving it with your mouse. The two waveforms won't be the same size, but fear thee not: you can scale the EPSC by multiplying it by a constant factor: in the command line at the bottom of the ADC/DAC control panel (bottom window), type  $EPSC\_DAC=EPSC\_DAC*1.3$ , or whatever factor you need to match the voltage amplitude.

### For your discussion

1. What electrical properties determine the speed and magnitude of membrane voltage changes (you may want to review your first lecture on passive membrane properties)?
2. Why is the simulated EPSP so much more slowly rising and longer in duration than the current waveform that elicited it? To help you with your answer, how does the value of the membrane time constant you measured from the square pulse response compare to the time that it takes the EPSC voltage response to repolarize to rest (from the peak).

## **Experiment 2: Manipulating Passive Membrane Properties – Changing resistance**

### **PreLab**

Different neurons have different passive and active membrane properties, tailored to allow that neuron type to respond to excitatory stimuli at different speeds and sensitivities. You can observe this for yourselves without having to record from a different neuron type in a different brain area. Instead, you will change the passive membrane properties in a hippocampal neuron experimentally, by applying one of two different compounds to the bath. One is the inhibitory neurotransmitter GABA (gamma-aminobutyric acid) which opens chloride-permeable GABA receptors for as long as GABA is present in the bath, thus *drastically* increasing the number of open ion channels in the membrane. The other compound is cesium chloride (CsCl). Cesium ions block potassium-permeable channels that are open near resting potential, thereby reducing the number of open ion channels in the membrane. Your group will not know which of these compounds you are using, so you will need to use your experimental results to determine whether the test compound increased or decreased membrane resistance.

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You will first take a set of baseline measurements in normal saline. Start with the usual measures of passive properties (step pulse responses), then you will record the membrane voltage response to brief trains of simulated EPSCs. You will then apply your test compound and repeat the measurements from baseline so that you can determine what functional properties of the neuron have changed and how these changes affect the membranes responsiveness.

### **InLab**

Start by patching a cell.

1. Set capacitance compensation and bridge balance appropriately.
2. Deliver a positive step pulse 1000 ms long. Adjust the current injection amplitude to elicit a voltage response approximately 5 mV in amplitude. Once you have set your current amplitude, get 10 sweeps of data, this time using the macro GetAverage, which will automate the process of running multiple sweeps and creating an averaged wave. Consult with your Instructor or TA for help using this macro.

Record the sweep numbers here: \_\_\_\_\_ to \_\_\_\_\_

Record the current amplitude here: \_\_\_\_\_

3. Deliver a negative step pulse 1000 ms long. Adjust the current injection amplitude to elicit a voltage response approximately 5 mV in amplitude. Once you have set your current amplitude, get 10 sweeps of data, using the macro GetAverage

Record the sweep numbers here: \_\_\_\_\_ to \_\_\_\_\_

Record the current amplitude here: \_\_\_\_\_

4. Deliver a 50 Hz train of simulated EPSPs. To do so, replace the StepPulse\_DAC stimulus file with EPSCtrain50Hz\_DAC. This new file contains EPSC waveforms designed to mimic real glutamatergic EPSCs in hippocampal neurons. The amplitude of the EPSC peaks equals 1, so you must enter the desired amplitude in the multiplier window next to the stimulus file name. Current amplitude should be sufficient to elicit subthreshold responses of between 2-5 mV in amplitude (current injection may be a few hundred pA). Once you have set your current amplitude, get 10 sweeps of data using the macro GetAverage which will automate the process of running multiple sweeps and creating an average of the 10 sweeps. Ask your instructor or TA for assistance with using this macro.

Record the sweep numbers here: \_\_\_\_\_ to \_\_\_\_\_

Record the current amplitude (multiplier) here: \_\_\_\_\_

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5. Change to the saline solution containing your test compound (and wait ~5-10 min. for exchange).

6. Deliver a 50 Hz train of simulated EPSPs. Keeping your current amplitude the same. Get 10 sweeps of data, waiting a few seconds between each sweep.

Record the sweep numbers here: \_\_\_\_\_ to \_\_\_\_\_

7. Deliver a 1000 ms positive step pulse of identical amplitude as you did in normal saline. (Use the current amplitude you recorded in Step 2 above. Get 10 sweeps of data, waiting a few seconds between each sweep.

Record the sweep numbers here: \_\_\_\_\_ to \_\_\_\_\_

8. Deliver a negative step pulse 1000 ms long, using the same amplitude as you did in normal saline. Once you have set your current amplitude, get 10 sweeps of data, using the macro GetAverage

### **PostLab**

*For your results:*

1. Superimpose in a graph, the averaged voltage responses to the positive and negative 1000 ms current pulses in both normal saline and saline containing the test compound.
2. Calculate the membrane resistance and time constant for each of the four averaged voltage responses
3. Superimpose the subthreshold EPSP responses (identical current injection amplitudes) in both normal and experimental conditions. Describe their differences.

*For your discussion*

1. In normal saline, was there a difference in the resistance you measured in response to the positive and negative current steps? Did you observe any differences in the nature of the voltage responses to the positive and negative currents steps. What could account for these observations?
2. In the experimental condition, was there a difference in the resistance you measured in response to the positive and negative current steps? Did you observe any differences in the nature of the voltage responses to the positive and negative currents steps. What could account for these observations?

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3. How did the membrane resistance and time constant change in when you applied your test compound? To answer this question, examine your responses to step pulses in the normal and experimental conditions. Why did this happen?
4. Compare temporal summation of subthreshold EPSP responses in both experimental conditions. In which condition is there more effective temporal summation? What kind of neuron would be more sensitive to small amplitude synaptic inputs: a “leaky” neuron, with low input resistance, or a less leaky neuron with high input resistance?
5. Examine the subthreshold responses in the normal and experimental conditions. In which condition does excitation best reflect the timing of the simulated synaptic input, and why?
6. From these analyses, would you agree or disagree that there is a trade off between sensitivity and temporal accuracy?