Measuring and Manipulating Passive Membrane Properties

Abstract

Biological membranes exhibit the properties of capacitance and resistance, which allow them to respond to stimuli with varying speeds and sensitivities. Membrane capacitance is created by the phospholipid bilayer that effectively separates charges on either side of the membrane. Resistance is created by ion channels in the membrane that selectively allow certain ions to pass through. Biological membranes act in accordance with Ohm's law, which outlines the relationship between current, voltage, and resistance. In order to investigate and observe the passive properties of cell membranes, a series of step pulses and excitatory potentials were delivered to hippocampal neurons under varying saline bath conditions. Cell responses were tracked and compared to identify the membrane properties responsible for the elicited responses. Here we find that the opening and closing of ion channels in the cell membrane alters membrane resistance and that membrane resistance varies directly with the time constant of the cell.

Introduction

Experiment 1: Measuring Passive Membrane Properties

Biological membranes possess the properties of resistance (R) and capacitance (C), which determine the rate at which passive voltage changes can occur. By separating the intracellular and extracellular environment, the phospholipid bilayer allows charge buildup on both sides of the membrane, thus acting as a capacitor. Ion channels contain transmembrane pores that allow ions to traverse the membrane. The presence of these ion channels introduces the property of resistance. Responses of membranes to external stimuli are governed by their resistance and capacitance. Together these properties determine the membrane time constant (τ), the product of resistance and capacitance, $\tau = RC$. Thus, the amount of charge stored on the phospholipid bilayer and presence of ion channels in the membrane determine the membrane time constant. When recordings from the same cell are compared, the capacitance is generally assumed to remain constant. As a result, any changes in the time constant can be attributed to a change in resistance.

Biological membranes act in accordance with Ohm's law, which states that the voltage is equal to the product of the current and the resistance, $V = IR$. When current is held constant, the voltage varies directly with the resistance. An increase in resistance yields an increase and voltage and a decrease in resistance yields a decrease in voltage. The purpose of this experiment is to understand how passive membranes limit the speed of voltage changes induced by synaptic input. In order to do so, a "simulated postsynaptic excitatory synaptic current" (EPSC) will be delivered to a cell and compared to the time course of the induced voltage change to that of the EPSC delivered to the cell. It is hypothesized that there will be a relatively longer time course in cells with the injected EPSC compared to cells with the recorded voltage change, possibly due to the resistance in the membrane.

Experiment 2: Manipulating Passive Membrane Properties

Neurons respond to varying stimuli at different speeds and sensitivities. The passive membrane properties of a cell, resistance and capacitance, determine its response to an excitatory stimulus. Changing the passive membrane properties of a neuron alters its response to a stimulus. The time constant of the cell determines the speed with which it reacts to a current stimulus. As the time constant varies directly with resistance, a decrease in the resistance will elicit a decrease in the time constant. Additionally, a decrease in resistance will decrease the magnitude of a cell's voltage response to a given current. The purpose of this experiment is to examine how the addition of GABA, an inhibitory neurotransmitter that opens up chloride channels, affects the passive membrane properties of the cell. In order to do this, GABA will be added to the saline bath and the cellular response to simulated EPSCs in normal and GABA containing saline baths will be compared. It is hypothesized that cells bathed in saline containing GABA will exhibit greater temporal sensitivity and increased speed in response to EPSCs. The addition of GABA to the saline bath will open up chloride channels in the membrane thereby reducing its resistance. As a result, there will be a greater flux of ions across the membrane which will allow greater sensitivity and increased speed.

Experiment 3: Variations on the Theme, Rig # 3

HCN channels are voltage-sensitive channels that are activated by hyperpolarization. At resting potential, a small number of HCN channels are open. CsCl is a known blocker of HCN channels and K^+ leak channels. The purpose of this experiment is to observe and understand the effect of CsCl on the neuron. In order to study the effect of CsCl, neurons will be bathed in a solution containing CsCl. The resting conduction of the neuron will then be measured and compared to the resting conduction of neurons in a saline bath lacking CsCl. Conductance is a measure of the ease by which ions can move through a medium or membrane. In addition, conductance is the inverse of resistance. Addition of CsCl to the saline bath will block HCN and K^+ leak channels, thereby increasing the resistance and thus decreasing the resting conduction. Thus, when comparing the conductance of neurons in the baths containing and lacking CsCl, there should be lower resting conductance in neurons bathed in CsCl.

Methods

For all of the experiments, brain sections of rat hippocampal regions were made and supplied with a continual flow of oxygenated artificial cerebral spinal fluid (ACSF) to mimic conditions *in vivo*. After successfully patching onto the cells, various types of current injections were delivered to cells in normal saline, saline containing GABA, and saline containing CsCl. The changes in potential difference were measured, recorded, and compared.

Results

The overall findings from this series of experiments are that the opening and closing of ion channels in the cell membrane affect resistance and that membrane resistance varies directly with the time constant of the cell.

Experiment 1: Measuring Passive Membrane Properties

Cell bodies in the pyramidal cell layer in the CA1 region of the hippocampus were patch clamped and given a positive step pulse of 250 ms to elicit a voltage response of approximately 3 mV in amplitude. 10 sweeps of the 250 ms step pulse were taken. The membrane time constant was determined by using the average of the 10 sweeps and adding the best fit overlay shown in blue for the 250 ms square pulse shown in red. As the current injection was 60 pA, the input resistance was calculated to be 5 x 10^7 Ω. The membrane time constant was determined to be 76.65 ms. Error in the resting potential is due to inaccurate setting of the offset balance.

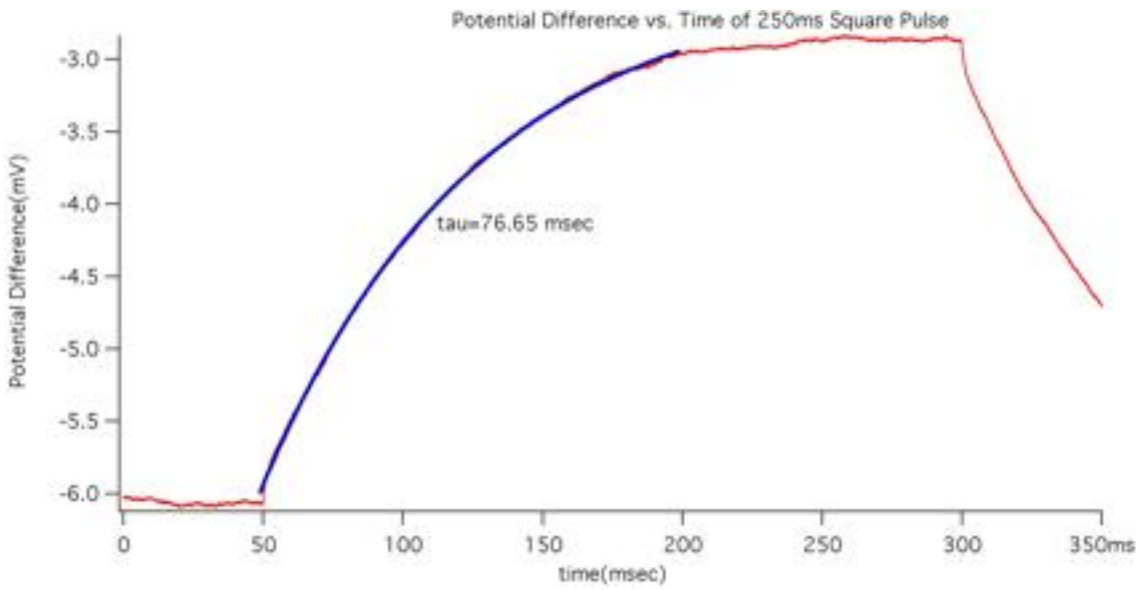

Figure 1: Measuring the membrane time constant from a 250 ms square pulse response. The time constant was determined 76.65 ms. The red line depicts the 250 ms square pulse. The blue line is the best fit overlay used to determine the time constant.

Cell bodies in the pyramidal cell layer in the CA1 region of the hippocampus were patch clamped and given a postsynaptic excitatory synaptic current (EPSC). The EPSC that was delivered to the cell is depicted by the green line on the graph. The membrane response is shown in red. The time constant was determined once again by using the average of 10 sweeps and adding a best fit overlay shown in blue. The time constant was determined to be 90.398 ms. When comparing the current input to the membrane output, it is clear that there is a delay in the membrane output. The membrane response rises more slowly and has a longer duration than the simulated EPSP. Error in the resting potential is due to inaccurate setting of the offset balance.

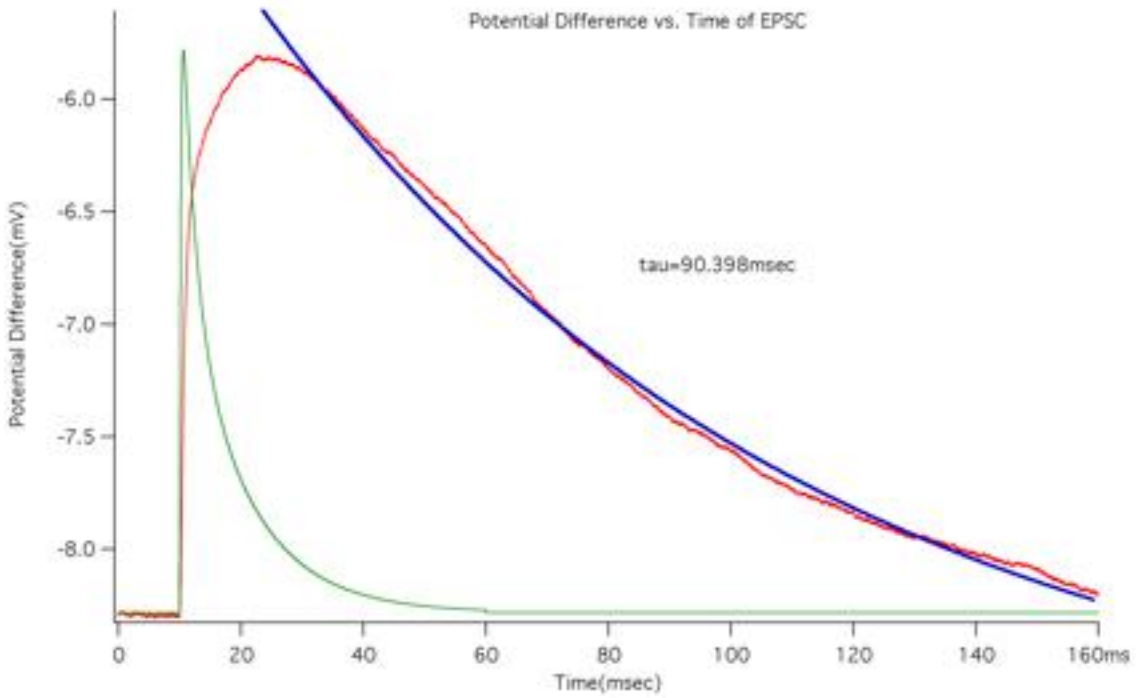

Figure 2: Measuring the membrane time constant from a postsynaptic excitatory synaptic current (EPSC). The time constant was determined 90.398 ms. The green line represents the simulated EPSC. The red line depicts the membrane response to the input. The blue line is the best fit overlay that was used to determine the time constant.

Experiment 2: Manipulating Passive Membrane Properties

A 250 ms, 50 Hz train of excitatory postsynaptic potentials (EPSPs) was delivered to a hippocampal neuron in a normal saline bath in order to elicit an amplitude of approximately 5 mV. The response of the cell in normal saline is indicated by the red waveform in Figure 3. With each pulse, the potential difference increased, but the cell did not hyperpolarize back to its resting potential in between pulses. Subsequently, the saline bath was changed to a solution containing 100 µM of GABA. A 50 Hz train of EPSPs was delivered to the neuron in the GABA containing bath. The response of the cell is depicted by the blue waveform. Unlike the cells in normal saline, the GABA treated cells depolarized to a potential of approximately 2.2 mV and hyperpolarized back to resting potential with each pulse.

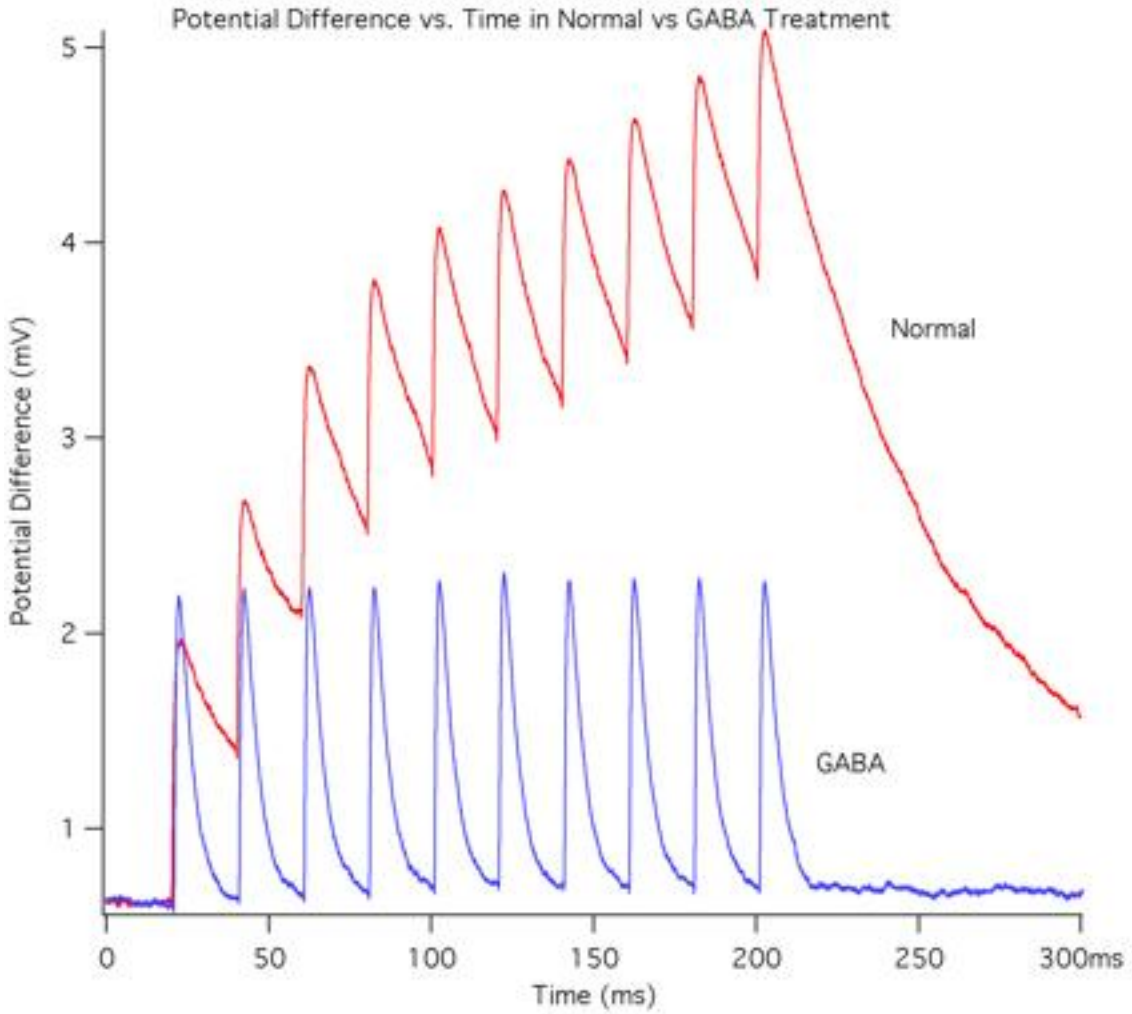

Figure 3: 50 Hz train of EPSPs in a neuronal cell in normal and GABA treated saline. The red waveform illustrates the response of the cell in normal saline. The blue waveform depicts the response on the cell in saline containing GABA.

A 250 ms step pulse was delivered to a hippocampal neuron in a normal saline bath. The response of the cell is indicated by the blue waveform in Figure 4. The cells in normal saline show a large change in potential difference when stimulated. Subsequently, a 250 ms step pulse was delivered to the neuron in a saline solution containing 100 µM of GABA. The cellular response is depicted by the red waveform in Figure 2. The amplitude of depolarization of cells treated with GABA is significantly lower than that of cells in normal saline.

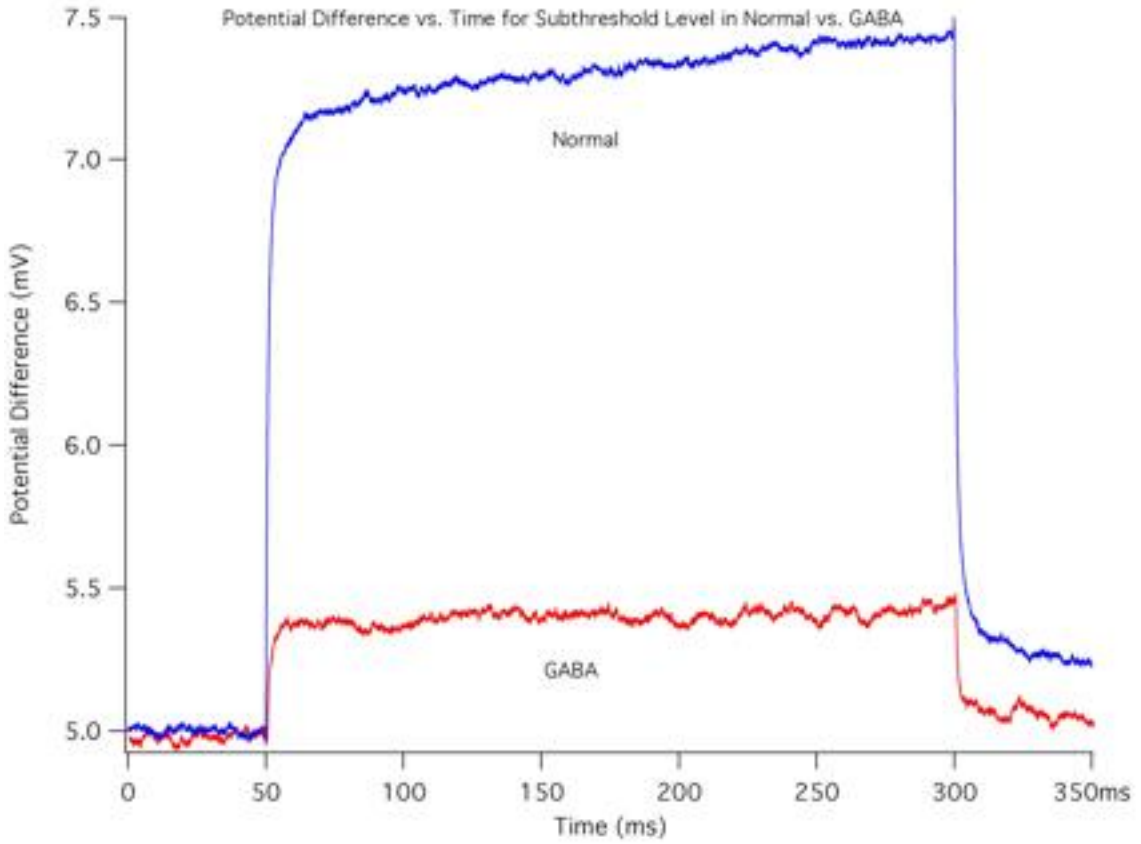

Figure 4: 250 ms step pulse in a neuronal cell in normal and GABA- treated saline. The blue waveform represents the response of a neuronal cell in normal saline to a 250 ms step pulse. The red waveform represents the response of a neuronal cell in saline containing 100 µM GABA to a 250 ms step pulse.

Experiment 3: Variations on the Theme

Current injections of -5 pA, -10 pA, and -15 pA were delivered to a hippocampal neuron in a normal saline bath for 1000 ms. The potential difference of the neuron was measured for each of the current injections. The potential difference for the -5 pA current injection is indicated by the green waveform in Figure 5. The potential difference for the -10 pA current injection is represented by the blue waveform in Figure 5. The potential difference for the -15 pA current injection is depicted by the pink waveform in Figure 5. In each of the waveforms, a sag that was proportional to the magnitude of the respective current was observed.

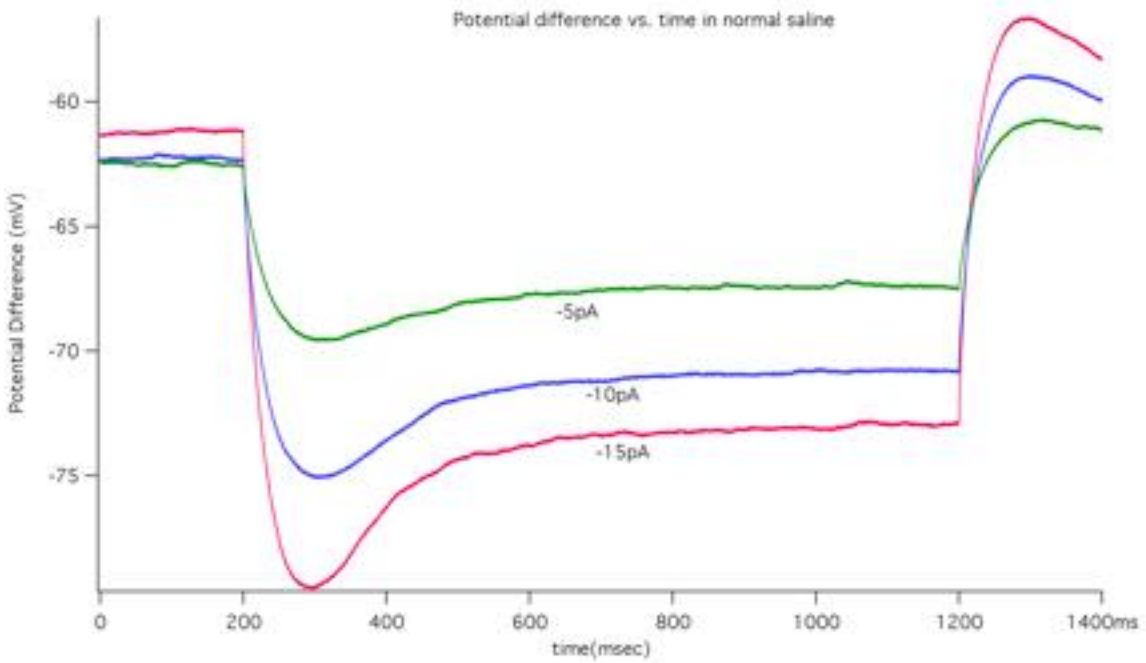

Figure 5: 1000 ms current injections of -5 pA, -10 pA, and -15 pA in a hippocampal neuron in normal saline. The waveforms represent the response of the neuron to the current injection. -5 pA is shown in green, -10 pA is shown in blue, and -15 pA is shown in pink.

Current injections of -5 pA, -10 pA, and -15 pA were delivered to a hippocampal neuron in a 5 mM CsCl bath for 1000 ms. The potential difference of the neuron was measured for each of the current injections. The potential difference for the -5 pA current injection is indicated by the green waveform in Figure 6. The potential difference for the -10 pA current injection is represented by the blue waveform in Figure 6. The potential difference for the -15 pA current injection is depicted by the pink waveform in Figure 6. No sag was observed in cells treated with CsCl.

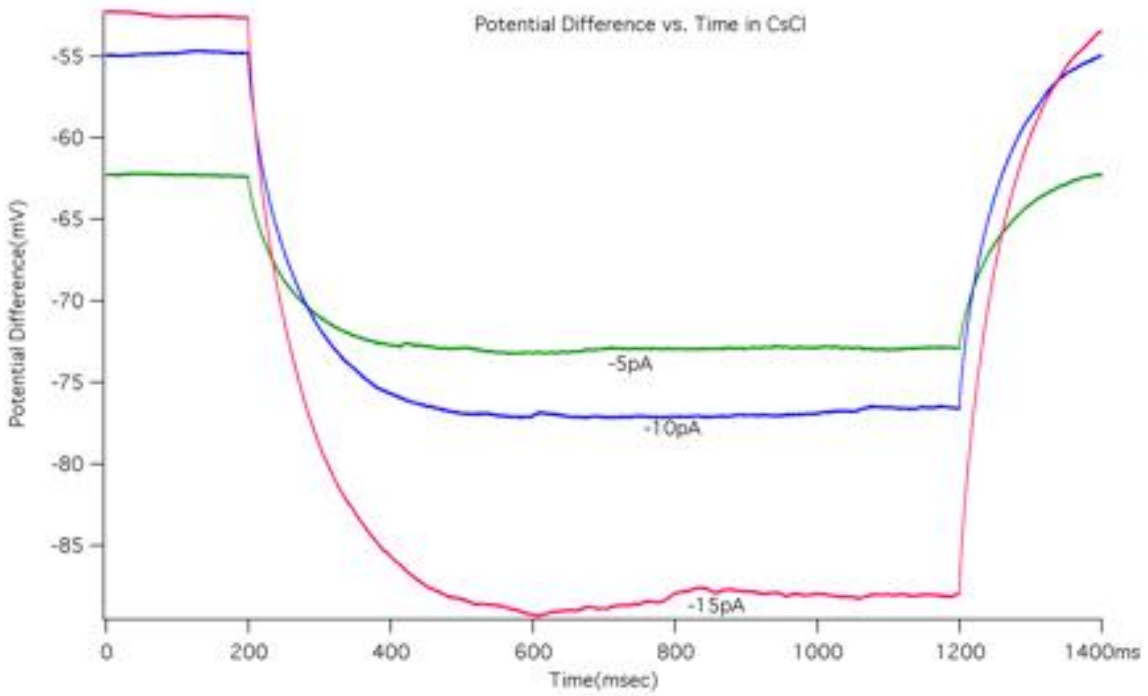

Figure 6: 1000 ms current injections of -5 pA, -10 pA, and -15 pA in a hippocampal neuron in a 5 mM CsCl saline bath. The waveforms represent the response of the neuron to the current injection. The -5 pA current is shown in green, the -10 pA current is shown in blue, and the -15 pA current is shown in pink.

The response of the hippocampal neuron in normal saline was compared to that of the neuron in the 5 mM CsCl bath. The cells in normal saline exhibit a sag in the potential. This sag is not present in the cells bathed in a solution containing CsCl. The contribution of the voltage-sensitive channels to the overall resting conduction is 7 mV. This was determined by comparing the potential difference of cells in normal saline with cells in the CsCl solution.

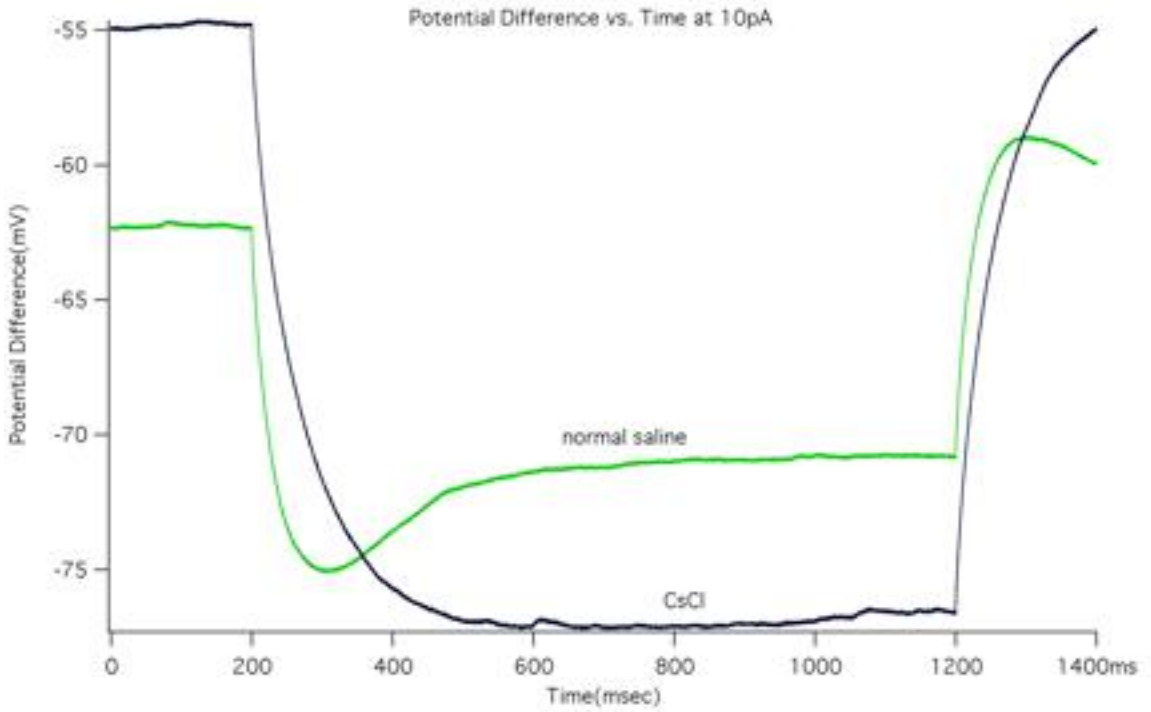

Figure 7: Comparision of a neuron in normal saline to a neuron in a 5 mM CsCl bath when a 10 pA current is delivered for 1000 ms. The green waveform depicts the response of the neuron in normal saline to a 1000 ms long 10 pA current. The blue waveform depicts the response of a neuron in a 5 mM CsCl bath to a 1000 ms long 10 pA current.

Discussion

The membrane time constant is the time it takes for the membrane to reach 63% of its final potential. This measurement is indicative of the speed of membrane potential changes. A large time constant denotes a slow change in membrane potential while a small time constant denotes a rapid change in membrane potential. The time constant varies directly with both resistance and capacitance, as illustrated by the equation $\tau = RC$. In Figure 2 there is an observable delay in the membrane response compared to delivered current stimulus; the simulated EPSP is much more slowly rising and longer in duration than the current waveform that elicited it. This is due to the slow decay in current. The residual current from the injection prolongs the membrane hyperpolarization causing the EPSP to be longer in duration.

The membrane time constant is dependent on two properties: resistance and capacitance. When recording from one cell, capacitance remains roughly constant such that any variation in the time constant can be attributed to a change in the resistance. As the opening and closing of ion channels in the membrane alters the flow of ions in and out of the cell, it affects membrane resistance. Opening ion channels decreases membrane resistance while closing ion channels increases membrane resistance. In Figure 3 we observed a marked difference between the time constant of cells in normal saline and

cells treated with GABA. The membrane resistance and time constant both decreased in the GABA- containing solution. GABA is a neurotransmitter that causes Cl^- ion channels to open. Increasing the number of open ion channels decreases the membrane resistance, the voltage (due to V = IR), and the time constant (τ = RC). Thus we found that cells treated with GABA attained a lower voltage amplitude. Cells in normal saline exhibit more effective temporal summation. In the normal saline bath, fewer ion channels are open, thus the cell has a higher membrane resistance. As a result, the time constant is higher; it takes a longer time for the cell to depolarize and hyperpolarize. A less leaky neuron with high input resistance would be more sensitive to small amplitude synaptic inputs because the higher resistance allows it to integrate signals more effectively. The excitation best reflects the timing of the stimulated synaptic input under the GABA condition. The lower resistance allowed by the GABA condition allows the cell to track the inputs more rapidly, giving it greater temporal accuracy. Varying resistance confers different properties to cells. There is a tradeoff between sensitivity and temporal activity. A cell with high sensitivity or high resistance can integrate input signals but its response cannot accurately indicate temporal frequency of the stimulus. A cell with temporal accuracy or low resistance can accurately encode the frequency of an input, but cannot effectively summate signals temporally.

In assessing the affects of CsCl on cellular response to a current stimulus we find that cell in normal saline show a sag current (Figures 5 and 7). This occurs because more channels are opening when the cell is hyperpolarizing. The opening of more channels decreases the resistance of the cell and thus decreases the voltage, consistent with Ohms' law. The addition of CsCl causes channels decreased conductance through K^+ leak channels, preventing the decrease in resistance, and thus eliminating the sag (Figures 6 and 7).

Conclusion

This series of experiments helps elucidate how altering membrane resistance affects the cellular response to a current stimulus. It was found that the time constant varies directly with resistance. An increase in resistance yields a slower change in membrane potential while a decrease in resistance yields are faster change in membrane potential. Furthermore the opening and closing of ion channels alters membrane resistance. When ion channels are opened, more ions are able to traverse the membrane. Thus, the membrane resistance is decreased. Conversely, when ion channels are closed, there is an increase in membrane resistance. We observe that the neurotransmitter GABA mediates the opening of Cl^- channels, thus decreasing the resistance and time constant of the cell. Similarly, CsCl increases membrane resistance by blocking K^+ leak channels. This eliminates the sag current generated in cells in which K^+ leak channels are open.