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PLAB 1: Voltage Clamp

Abstract:

A circuit was constructed to simulate the action of a voltage clamp. The circuit low pass filtered the membrane potential, compared it to the control voltage, amplified the difference by the gain and by that feedback, injected current to compensate. The response of the circuit to various experimental conditions facilitated an understanding of the contributions of individual components, as well as the physiological significance of each. By controlling the value of a variable resistor (Rg), the circuit's steady state response was analyzed for varying gain values and it was determined that larger gain produced smaller deviations between Vm and Vc. Similar studies for Rm showed that small Rm values produced large deviations between Vm and Vc. Comparing rising time constants, for the response and the control, demonstrated the effect on dynamic response resulting from changing parameters. An analysis of bode magnitude plots for various gain values visually demonstrated the low pass filter behavior of the voltage clamp. Sources of error resulted in a few outlying data points, making the experimental results slightly mixed. A hypothetical analysis of each experiment made clearer the trend that was still evident, but clouded, in the experimental data.

Question 2:

Rm and Cm are the membrane resistance and capacitance. In parallel they provide a representation for the physiological state and combination of different membrane channels. Membrane resistance is a function of the number of channels open, whereas membrane capacitance is a function of the thickness of the membrane. A larger cell capacitance results in a slow time constant (slower response to a change in voltage). Rm affects the time constant similarly, but not as much. Re1 and Re2 are the electrode resistances. Re1 can be thought of as a voltage sensing resistor that records Vm, and Re2 a current

inducing resistor that passes current into the cell. Re1 doesn't have much of an effect on membrane response to changes in control voltage. A small Re2 compared to Rm will get K close to 1. Increasing Re2 will make a slower time constant and will also create a larger deviation between membrane voltage (Vm) and control voltage (Vc).

We can control Re1 and Re2 to some degree, and we can also affect Rm somewhat by tinkering with ligand gated ion channels or by affecting voltage regulated ion channels.

Question 3:

An ammeter measures current. An ammeter is important when trying to measure the activity of the ion channels in response to changes in voltage. This includes keeping track of ion movement/behavior (magnitude and direction) and studying/calculating ion channel conductance.

Question 4:

Varying the amplifier gain has an effect on steady state response. Other than overcoming the limitations of the INA118 amplifier (in regards to using too high of a gain), the larger we can make the gain the better. With the use of an ideal amplifier (which isn't possible) an infinite gain would produce a situation where Vc equals Vm. In reality gain values between 100-10000 get pretty close (this is a generalization formed from the experimental results as well as INA 118 performance curves spec sheet and the PLAB lab manual). The results of the steady state experiment are shown in Table 1 and Figure 1. There was a difference in the steady state response for the negative step and the positive step (to -80mV and +100mV for Vc respectively). Because of this, I provided analysis of both. Ideally their % errors should have been identical, but that was not the case. As for overall trend of the experiment, there is a large jump in the theoretical ratio between two values of Rg used in testing (390Ω, and 3330Ω). (See Table 1) Within the range of values between those two, there exists a curve. However,

since we only tested 4 values, it cannot be seen in the experimental data. (See Figure 1) Because of this I graphed a secondary set of theoretical ratios (using eqn 1.9) over an enormously dense range of gain values. (See Figure 2) The trend is shown much clearer there. Essentially, below gain values of ~100, drop off begins quickly. On the increasing side, the ideal behavior is asymptotically approaching 1.

In some instrumentation amplifier data sheets, a higher gain is shown to produce a higher CMRR… and very low gains produce low CMRR. This could be the large source of error in low gain value trials. There are a few other possible sources of error for both the Rg and Rm experiments (ie: questions 4 and 5). Series resistance of Re2 could cause a voltage drop (ie: a large Re2 compared to Rm could cause problem). Additionally there could have been flaws in circuit construction, the recording could have produced noisy data and the hardware/componentry used could have been faulty or untrue to their posted values.

Table 1: Steady State Response: Ratios of Vc/Vm compared for varying experimental gains.

Rg (Ω)	Gain	Theoretical Ratio for Vc = 97.0mV or -79.9mV	Observed Ratio for Vc = 97.0mV	% Error	Observed Ratio for Vc = -79.9mV	% Error
100	501	0.9979	0.9800	1.8	1.0173	1.9
390	129.2	0.9920	0.9705	2.2	1.0060	1.4
3300	16.15	0.9391	0.8997	4.2	0.9343	0.5
Infinite	1	0.4885	0.3913	20.0	0.4061	16.9

Figure 1: Theoretical Response compared to observed response for experimental gain values set by Rg.

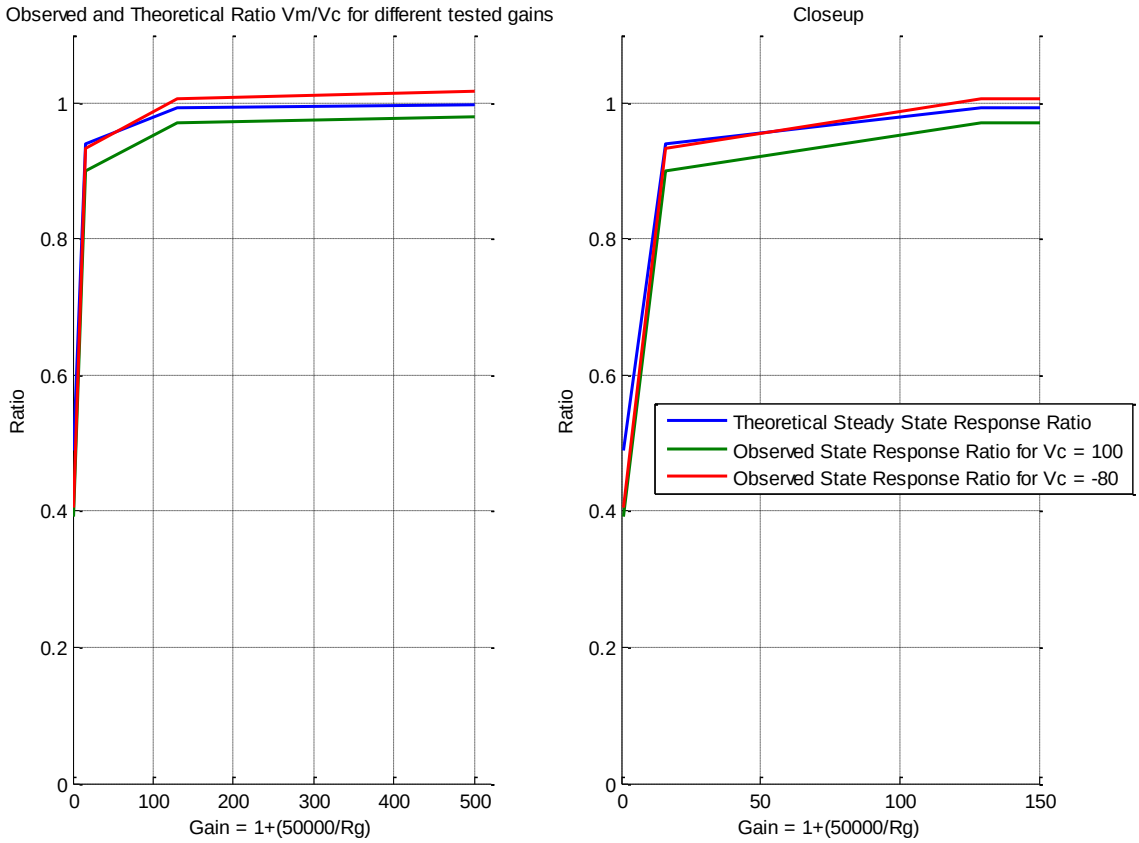
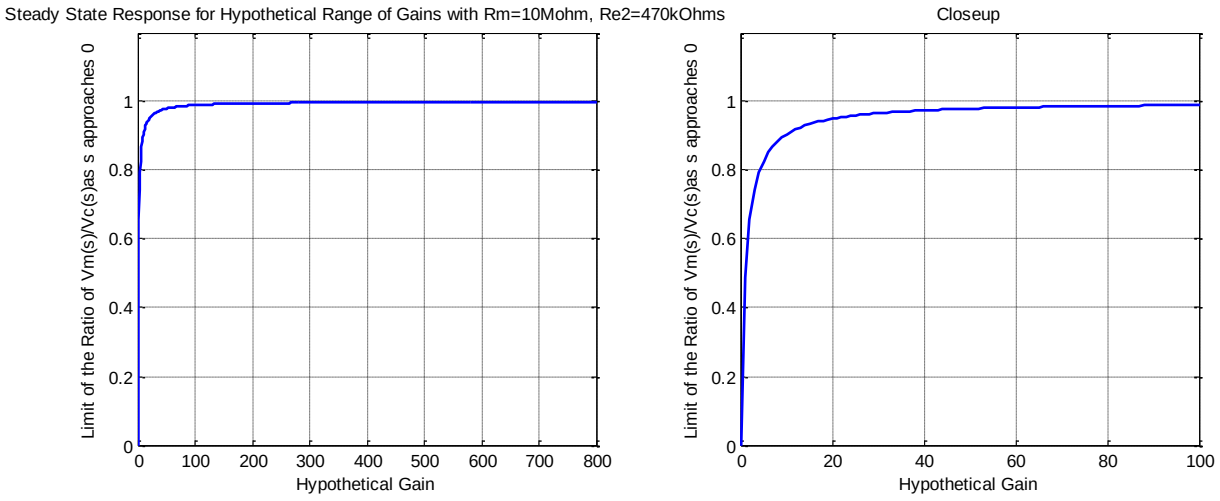


Figure 2: Theoretical Response for a Hypothetical Range of Gains... Shows asymptotic behavior.



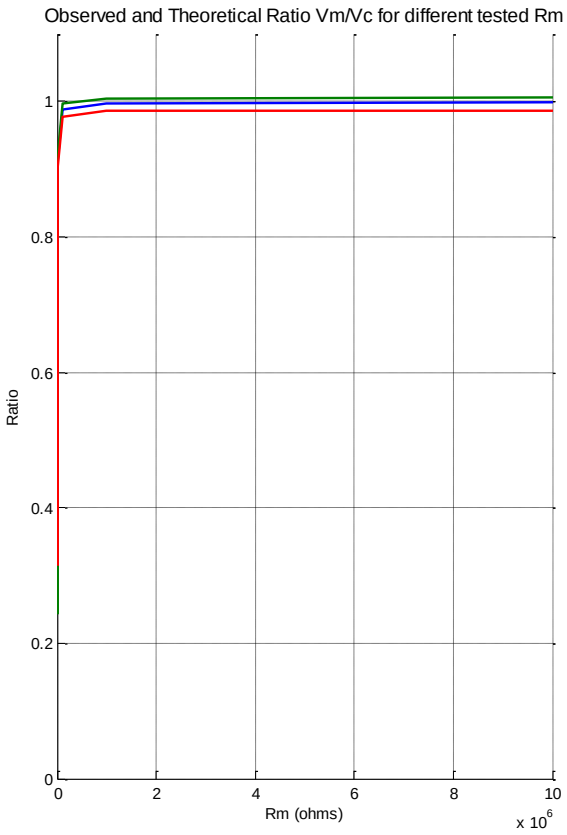
Question 5:

By purely theoretical analysis, from a hypothetical range of Rm (See Figure 4), Rm values above ~7000 produced resultant ratios greater than 0.99, ie: extremely close to 1. But by experimental testing (see Figure 3), it was Rm values above 100,000 ohms that produced results that close. Again the positive and negative steps in Vc produced different ratios when Vc was compared to the membrane response. I cannot account for this. But I have shown both in Figure 3. Again, sources of error could include series resistance of Re2 causing a voltage drop (ie: a large Re2 compared to Rm), flaws in circuit construction, noisy data, and faulty/ inaccurate hardware or circuitry components. As an overall trend, small values of Rm greatly affect the steady state response, however in practice larger values (>100kΩ) have little difference between one another.

Table 2: Steady State Response: Ratios of Vc/Vm compared for varying experimental Rm values.

Rm	Theoretical Ratio for Vc = 97.0mV or -79.9mV	Observed Ratio for Vc = 97.0mV	% Error	Observed Ratio for Vc = -79.9mV	% Error
10MΩ	0.9979	1.0053	0.7	0.9864	1.2
1MΩ	0.9971	1.0045	0.7	0.9859	1.1
100kΩ	0.9888	0.9965	0.8	0.9775	1.1
10kΩ	0.9126	0.9213	1.0	0.9033	1.0
1kΩ	0.5154	0.2430	53.0	0.3148	39.0

Figure 3: Theoretical Response compared to observed response for experimental values of Rm



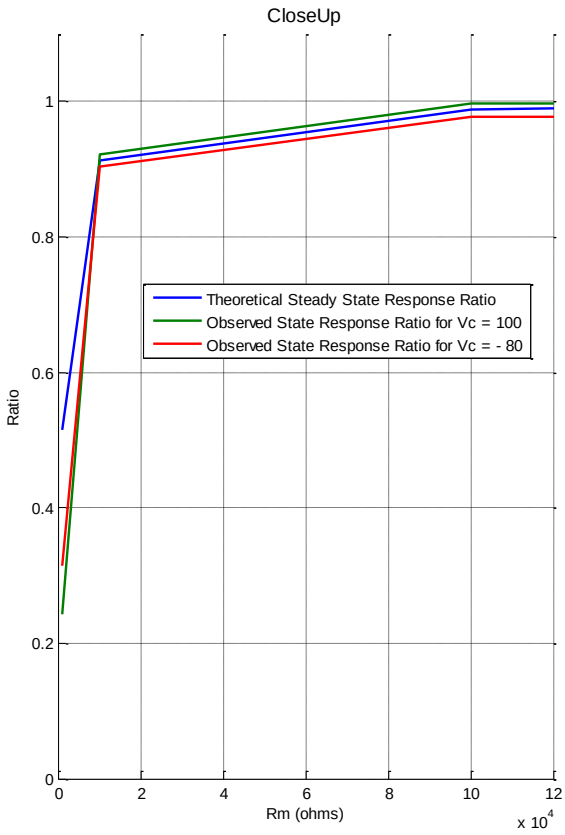
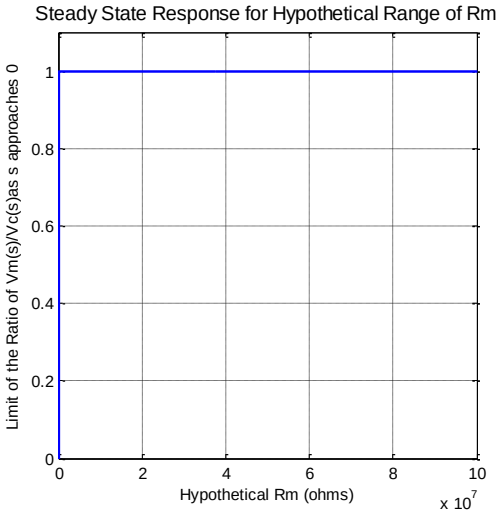
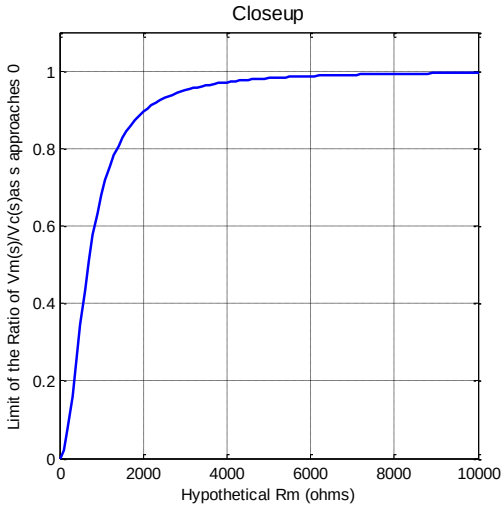


Figure 4: Theoretical Response for a Hypothetical Range of Rm Values... Shows asymptotic behavior.





Question 6:

Theoretically, the time constants for the control voltage should have all been identical. However in the experimental data recorded, this was not the case. The time constants varied slightly even within the control. The source of error here could be inaccurate or imprecise method coded to calculate Tau_c. Table 3 shows the parameters given in lab that were used in each trial. Table 4 shows the time constants for the control and response as well as the ideal or expected time constant Tau_o, the time delay and the % error comparing Tau_o to the delay. Some of these values are close, but the highest was 274% error off. These errors most likely result from the method used to calculate the time constant, paired with the format of the data input to the method. Figure 5 shows each trial's dynamic response.

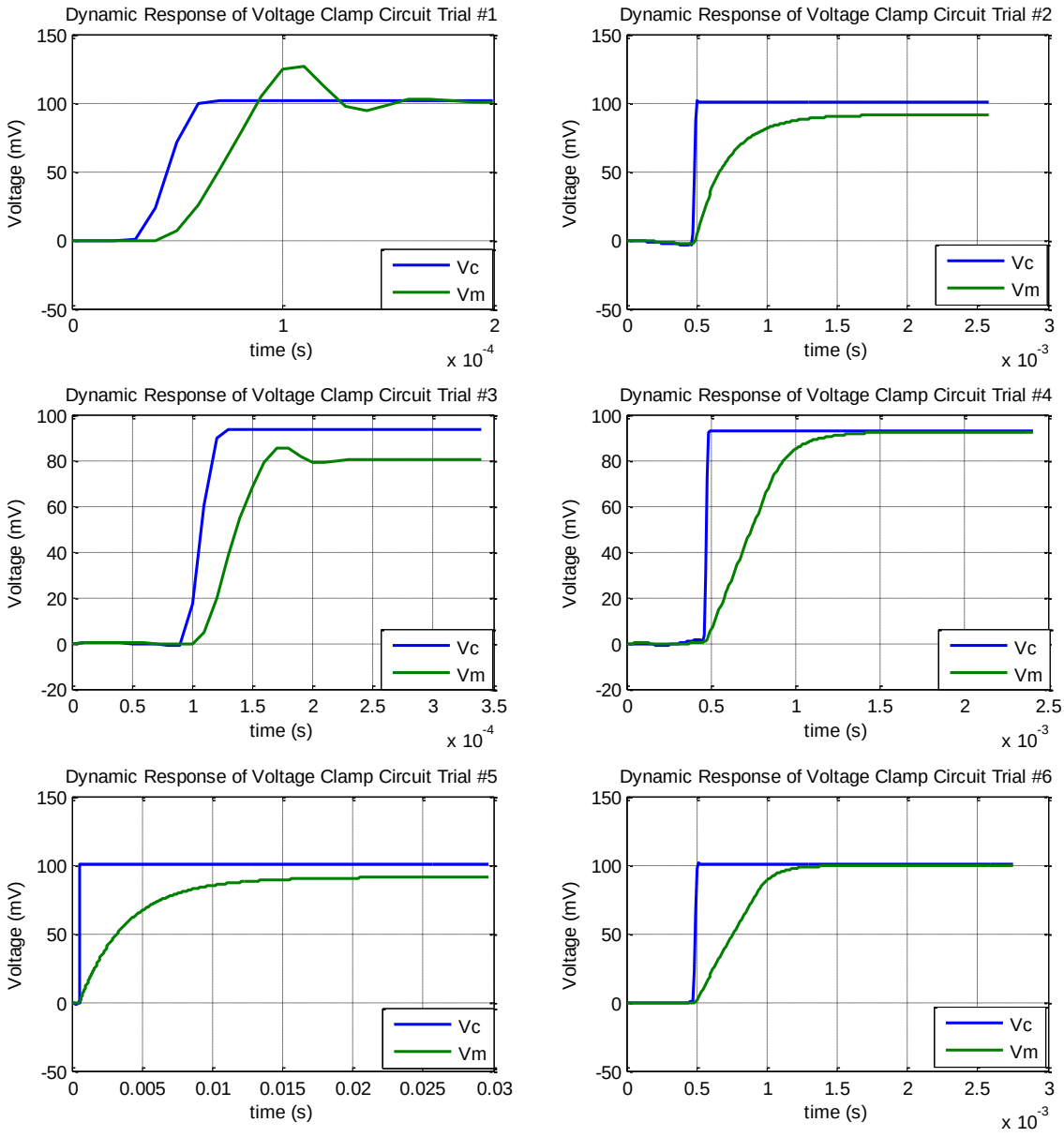
Table 3: Parameter Values for Dynamic Response Trials

Trial #	CM (µF)	RM	RG
1	0.01	10MΩ	100Ω
2	0.01	10MΩ	3.3kΩ
3	0.01	5.6kΩ	100Ω
4	0.1	10MΩ	100Ω
5	0.1	10MΩ	3.3kΩ
6	0.1	100kΩ	100Ω

Table 4: Dynamic Response Time Constant Comparisons

Trial #	Tau_C	Tau_R	Tau_0	Tau_R-Tau_C	%Error
1	30µs	50µs	9µs	20µs	114
2	30µs	280µs	273µs	250µs	9
3	20µs	50µs	8µs	30µs	274
4	300µs	550µs	94µs	250µs	167
5	260µs	3570µs	2733µs	3310µs	21
6	300µs	510µs	93µs	210µs	126

Figure 5: Dynamic Response for each Trial



Question 7:

The variables changed in the time constant experiment were Cm, Rm and Rg. See Table 3 for the parameter values of each trial.

When changing Cm (shown clearly between trials 1 and 4 where Cm is a factor of 10 different), a bigger Cm means no overshoot or oscillation and the time constant of the response should be linearly proportional to Cm. That is as Cm gets bigger, Tau gets bigger. Physiologically this means a longer time is required to change the voltage of the membrane (meaning a longer time to get Vm to equal Vc). In a physiological voltage clamp setup, you want Vm to equal Vc as fast as possible so that you can properly measure any changes in current and ion flow/ channel behavior. This makes sense physiologically and mathematically (eqn 1.11 where Cm is in the numerator).

Greatly reducing Rm (compare trial 1 to 3) decreased overshoot, oscillation and also decreased the attainable steady state value. Rm affected tau as well. Increasing Rm increased tau, decreasing decreased tau. Physiologically increasing Rm is decreasing portion of open ion channels, which reduces current flow and increases the time it takes to change the membrane voltage (ie: tau)… this makes sense both mathematically (eqn 1.11) & physiologically as just described.

Increasing Rg (compare trail 1 to 2) decreased the gain and therefore decreased the ability of the system to respond quickly. Decreased attainable steady state was a result. This factor contributed the most to changes in the response time constant. Rg increasing will decrease tau, and decreasing Rg increases tau. Although this has little physiological analog (it is part of the voltage clamp), it does make mathematical sense given that gain only appears in the denominator of eqn 1.11.

Question 8:

Sodium activation is an extremely quick process. It happens immediately upon onset of voltage increase. To precisely measure sodium currents, one would want the time constant for the activating voltage (ie. The control) to be less than the time constant for the activation of voltage gated sodium channels. Therefore, the most suitable trials would be those with the lowest control time constant. Voltage gated sodium channels have an activation time constant less than 200μs (Cui, lecture 2, page 16). Trials 1,2 and 3 had the lowest control time constants, well less than the activation time constant for sodium channels. Trial 2 however, had a response time constant that was larger than the sodium activation time constant. Therefore I would be safe saying the ideal setups (out of the 6 trials tested) are trial 1 and trial 3.

Question 9:

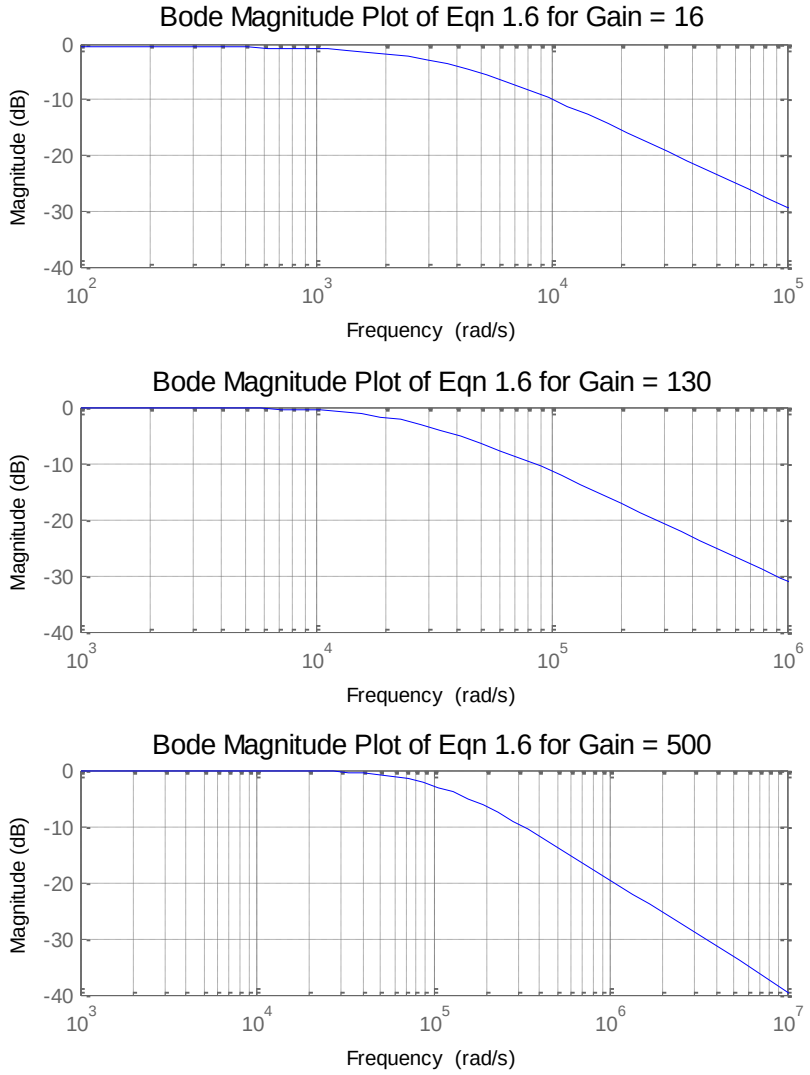
The bandwidth got larger as gain increased. The bode plots resemble a low pass filter. (see Figure 6) This makes sense because the membrane of the hypothetical cell s clamped to the low pass filtered membrane potential. To be more specific, consider the basic principle steps of this voltage clamp: 1. Low pass filter the membrane potential 2. Compare it to the control voltage 3. Amplify the difference by the gain 4. Inject current to compensate. (Source: Low frequency voltage clamp: recording of voltage transients at constant average command voltage by Florian Peters, Arne Gennerich, Dirk Czesnik, Detlev Schild *)

```
%Question #9
close all; clear all; clc;
gain = [ 16, 130, 500];
Rm = 10*10^6;
Re_2 = 470000;
Cm = 0.01*10^-6;
K = Rm/(Rm + Re_2);
tau_p = Rm*Re_2*Cm/(Rm+Re_2);

s = tf('s');
eqn1 = gain(1)*K /(s*tau_p + gain(1)*K + 1);
subplot(3,1,1);  bodemag(eqn1); title('Bode Magnitude Plot of Eqn 1.6 for
Gain = 16','FontSize',11); grid on;
cutoff_freq(1) = bandwidth(eqn1);
s = tf('s');
eqn2 = gain(2)*K /(s*tau_p + gain(2)*K + 1);
subplot(3,1,2);  bodemag(eqn2); title('Bode Magnitude Plot of Eqn 1.6 for
Gain = 130','FontSize',11);grid on;
cutoff_freq(2) = bandwidth(eqn2);
s = tf('s');
eqn3 = gain(3)*K /(s*tau_p + gain(3)*K + 1);
subplot(3,1,3); bodemag(eqn3); title('Bode Magnitude Plot of Eqn 1.6 for Gain
= 500','FontSize',11);grid on;
cutoff_freq(3) = bandwidth(eqn3);

for i= 1:length(cutoff_freq)
    tau(i) = 1/cutoff_freq(i);   %in seconds'
    tau_ms(i) = 10^3/cutoff_freq(i);   %in ms'
end
```

Table 5: Bode Plot Results for various Gains

Gain	-3dB Cutoff Frequency (rad/s)	Associated Tau (µs)
16	$3.62*10^3$	276
130	$2.78*10^4$	36
500	$1.06*10^5$	9

Figure 6: Bode Plots for various Gains



Question 10:

We set the sampling rate to 100k/s for the dynamic response because the responses could be super quick under certain conditions. To measure the time constant of something rising extremely fast, you need to use an extremely high sampling rate to have the resolution required for analysis and calculations.

Question 11:

Common mode rejection ratio CMRR is 90dB for the ina137 vs. 110dB for the ina118… so the ina118 is the better amplifier. Given the two are both so cheap, I would not recommend replacing the ina118 with the ina137. (source: http://www.ti.com/lit/ds/symlink/ina118.pdf vs. http://www.ti.com/lit/ds/symlink/ina137.pdf)

Question 12:

Criterion 1: High fidelity: Vm=Vc

The amount this was accomplished is left to the viewer. Depending on the cutoff for what determines relative equality, the clamp either failed or performed perfectly. Using the right setups, errors below 1% were attainable. If that is within the realm of acceptable, then yes, this clamp met the criteria.

Criterion 2: Fast dynamic response, clamp settles before the onset of current

Given that a few time constants were small enough to measure sodium currents, I would say this was successful.

Criterion 3: Accuracy: the clamp should control Vm alone.

Given that the clamp did not affect other values (they were fixed as circuit components, ie resistors) this is a difficult question to answer for the context of physiology and an easy one to answer for our experiment. The clamp was accurate for this experiment. I do not know how it would have affected a real physiological setup with parameters subject to change.

Unofficial References:

1. PLAB 1. Manual
2. CUI Lecture Slides (Lecture 2, Page 16)
3. Texas instruments INA137 and INA 118 Amplifier Data Sheets.
    a. http://www.ti.com/lit/ds/symlink/ina118.pdf

       http://www.ti.com/lit/ds/symlink/ina137.pdf
4. Low frequency voltage clamp: recording of voltage transients at constant average command voltage (by Florian Peters, Arne Gennerich, Dirk Czesnik, Detlev Schild)

Discussants:

Jordan Nick, Jodi Small, Satish, Akhil Sundar, Lauren Bedell, Paras Vora, Matt Everett