

Experiment AN-1: Membrane Potentials

Exercise 1: Impaling Muscle Fibers

Aim: To measure the membrane potentials in different muscle fibers.

Procedure

1. Look through the microscope; you should see the tip of the microelectrode in the bath solution. Use the controls of the micromanipulator to move the tip of the microelectrode over a bundle of muscle fibers.
2. Type Muscle Fiber 1 in the Mark box to the right of the Mark button.
3. Click Record to begin recording.
4. Use the micromanipulator's controls to move the tip of the microelectrode toward the muscle fibers, gradually. Once the tip of the microelectrode is close to the membrane of any of the muscle fibers in view, begin to watch the recording of the membrane potential on the computer screen
5. When the microelectrode tip touches a muscle fiber membrane, you will see a small deflection of the recording, either up or down.
6. At this stage, penetrate the membrane of the muscle fiber by either:
 - Pushing the microelectrode tip through the membrane using the controls of the micromanipulator; or,
 - Gently tapping the base of the micromanipulator to create a small amount of vibration in the microelectrode tip, which will aid in the penetration of the membrane.
7. As the tip of the microelectrode penetrates the membrane, the recording displayed on the computer screen deflects downward rapidly ([Figure AN-1-L1](#)). When this happens, do not touch the manipulator! The tip of the microelectrode is inside the muscle fiber. Click AutoScale to view the voltage levels before and after the microelectrode penetrated.
8. Click Stop to halt recording.
9. Select Save in the File menu.

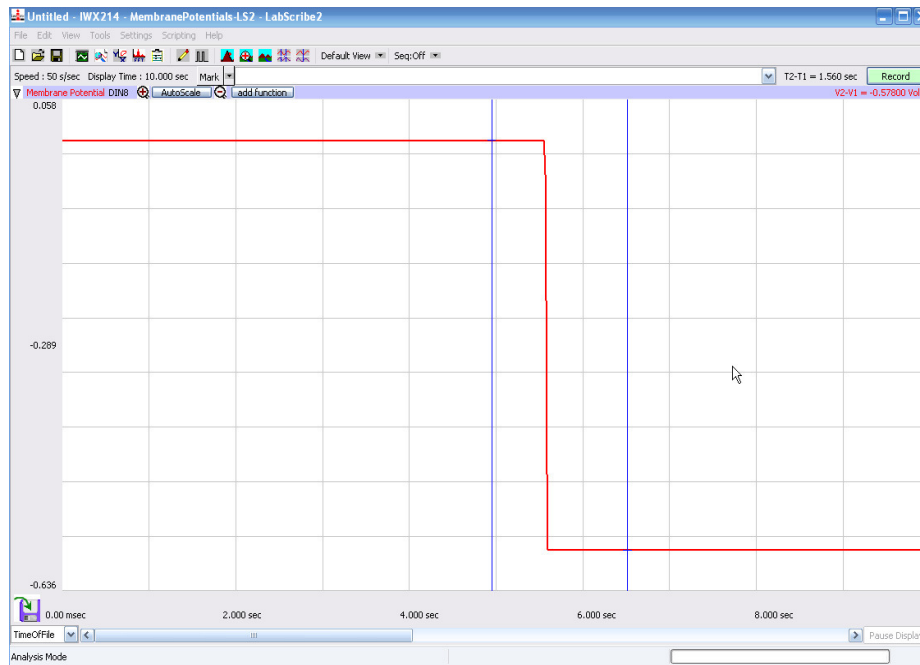
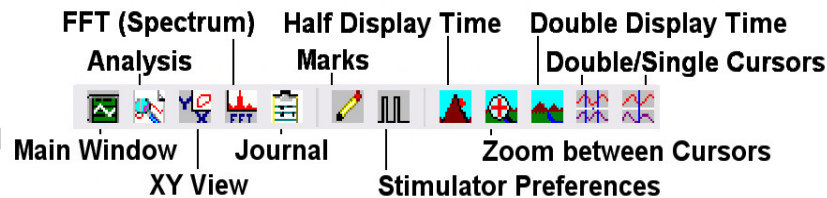


Figure AN-1-L1: Recording of the downward deflection when a muscle fiber is impaled. The cursors are used to measure a voltage difference, (V_2-V_1) of 0.578 Volts or 578 millivolts.

Data Analysis

1. Scroll through the data file and locate the section of the recording made when the microelectrode penetrated the muscle fiber membrane.
2. Use the Display Time icons to adjust the Display Time of the Main window to display a seventy-second section of recording with a consistent slope on the Main window. This section of data can also be selected by:
 - Placing the cursors on either side of the seventy-second section of the recording, and
 - Clicking the Zoom between Cursors button on the LabScribe toolbar (Figure AN-1-L2) to expand or contract the seventy-second recording to the width of the Main window.



3.

Figure AN-1-L2: LabScribe toolbar.

4. Click on the Analysis window icon in the toolbar or select Analysis from the Windows menu to transfer the data displayed in the Main window to the Analysis window.
5. Look at the Function Table that is above the [Oxygen] channel in the Analysis window. The mathematical function, V_2-V_1 , should appear in this table. The value for V_2-V_1 are displayed in the table across the top margin of the Membrane Potential channel.

6. Once the cursors are placed in the correct positions for determining the change in voltage ($V_2 - V_1$) as the tip of the microelectrode enters the muscle fiber, the value for $V_2 - V_1$ can be recorded in the on-line notebook of LabScribe by typing the names and values of the parameters directly into the Journal.
7. The functions in the channel menu of the Analysis window can also be used to enter the name and value of the voltage change from the recording to the Journal. To use these functions:
 - Place the cursors at the locations used to measure the oxygen concentration in a minute.
 - Transfer the names of the parameters to the Journal using the Add Title to Journal function in the Membrane Potential channel menu.
 - Transfer the values for the parameters to the Journal using the Add Ch. Data to Journal function in the Membrane Potential channel menu.
7. On the Membrane Potential channel displayed in the Analysis window, use the mouse to click on and drag a cursor to the baseline recorded before the electrode test button was pushed. Drag the other cursor to the voltage level that results from the electrode test button being pushed.
8. Record the value for the voltage change ($V_2 - V_1$) in the Journal using the one of the techniques described in Steps 5 or 6, and in [Table AN-1-L1](#).
9. Divide the value for $V_2 - V_1$ by the gain of the IC-200 intracellular probe to obtain the membrane potential. For example, a voltage difference ($V_2 - V_1$) of 620 millivolts recorded by an IC-200 with a gain of 10X is equal to a membrane potential of 62 millivolts:

$$620 \text{ millivolts} / 10X \text{ Gain} = 62 \text{ millivolts}$$

Exercise 2: Membrane Potentials from Different Fibers

Aim: To measure any variations in the membrane potentials between muscle fibers.

Procedure

1. Repeat Exercise 1 on two additional muscle fibers in the same segment of the crayfish tail (Location A). Label the recording from each fiber
2. Record from fibers on the contralateral side of the same segment (Location B) and from fibers in other segments (Locations C and D).

Analysis

1. Use the same procedures used in Exercise 1 to measure the voltage changes recorded when the microelectrode penetrated the other fibers.
2. Convert the voltage changes into membrane potentials as performed in Exercise 1 by dividing the voltage change by the gain programmed into the IC-200.
3. Enter the data into the Journal using either of the two techniques described in Exercise 1. Enter the membrane potentials into [Table AN-1-L1](#).

Questions

1. Were the resting membrane potentials of fibers in the same muscle bundle the same?
2. Was the average resting membrane potential for fibers from one bundle the same as the average potential for fibers from the bundle on the opposite side of the segment?
3. Was the average resting membrane potential for fibers from one segment the same as the average potential for fibers from the same side of another segment? The same as fibers from the opposite side of different segments?
4. Why do resting membrane potentials recorded from different fibers vary?

Table AN-1-L1: Resting Membrane Potentials (E_m) of Muscle Fibers Bathed in Crayfish Saline.

	Fiber Location-Segment	E_m in Normal Crayfish Saline
Fiber 1		
Fiber 2		
Fiber 3		
Mean	A _____	
Fiber 4		
Fiber 5		
Fiber 6		
Mean	B _____	
Fiber 7		
Fiber 8		
Fiber 9		
Mean	C _____	
Fiber 10		
Fiber 11		
Fiber 12		
Mean	D _____	

Exercise 3: Membrane Potentials and Extracellular Potassium

Aim: To measure changes in membrane potential in response to changes in the extracellular concentration of potassium.

Procedure

1. Remove the normal crayfish saline solution from the preparation dish.
2. Refill the preparation dish with a modified crayfish saline containing less sodium chloride than the normal saline:
 - Normal saline contains 205mM NaCl, and the modified saline contains 160mM NaCl.
 - The molarity of the low-sodium crayfish saline is adjusted with 45mM choline chloride, or sucrose, to allow for the 45mM NaCl reduction.
 - Neither choline nor sucrose molecules will pass through the membrane or channels.
3. Bathe the preparation in the low-sodium crayfish saline for two minutes. Remove the low-sodium saline from the preparation dish and refill the dish with fresh low-sodium crayfish saline. Bath the preparation in this saline for another three minutes before recording any membrane potentials.
4. Record the resting membrane potentials from five muscle fibers bathed in the low-sodium saline.
5. Remove the low-sodium crayfish saline solution from the preparation dish.
6. Refill the preparation dish with a modified crayfish saline containing more potassium chloride than the normal saline:
 - Normal saline contains 5mM KCl and 205 mM NaCl, and the modified saline contains 50mM KCl and 160mM NaCl.
 - Neither choline chloride nor sucrose have been added to this saline since the higher concentration of KCl will give this solution the same osmolarity as normal saline.
7. Bathe the preparation in the high-potassium crayfish saline for two minutes. Remove the high-potassium saline from the dish and refill the dish with fresh high-potassium crayfish saline. Bath the preparation in this saline for another three minutes before recording any membrane potentials.
8. Record the resting membrane potentials from five muscle fibers bathed in the high-potassium saline.

Data Analysis

1. Use the same procedures used in Exercise 1 to measure the voltage changes recorded when the microelectrode penetrated the fibers.
2. Convert the voltage changes into membrane potentials as performed in Exercise 1 by dividing the voltage change by the gain programmed into the IC-200.
3. Enter the data into the Journal using either of the two techniques described in Exercise 1. Enter the membrane potentials into [Table AN-1-L2](#).

Table AN-1-L2: Resting Membrane Potentials (E_m) of Muscle Fibers Bathed in Modified Crayfish Saline.

	Normal Crayfish Saline	Low $[Na^+]$ Crayfish Saline	High $[K^+]$ Crayfish Saline
Fiber 1			
Fiber 2			
Fiber 3			
Fiber 4			
Fiber 5			
Mean			

Questions

1. What happens to the resting potential if you change the level of sodium in the crayfish saline?
2. What happens to the resting potential if you change the level of potassium in the crayfish saline?
3. Why does an increase in $[K^+]_{outside}$ create the observed changes in resting potential?

Appendix

Recipe for Crayfish Saline Solution with Tris.

Concentration (mMolar)	Salt	Grams/Liter DI H ₂ O
205.34	Sodium Chloride	12.00
5.36	Potassium Chloride	0.40
13.43	Calcium Chloride*2H ₂ O	1.97
2.61	MgCl ₂ .6H ₂ O	0.53
9.99	Tris	1.21
Adjust pH to 7.4 with 6N HCl		

Recipe for Low [Sodium] Crayfish Saline Solution with Tris.

Concentration (mMolar)	Salt	Grams/Liter DI H ₂ O
160	Sodium Chloride	9.35
45	Choline Cl	6.28
5.36	Potassium Chloride	0.40
13.43	Calcium Chloride*2H ₂ O	1.97
2.61	MgCl ₂ .6H ₂ O	0.53
9.99	Tris	1.21
Adjust pH to 7.4 with 6N HCl		

Recipe for High [Potassium] Crayfish Saline Solution with Tris.

Concentration (mMolar)	Salt	Grams/Liter DI H ₂ O
160	Sodium Chloride	9.35
50	Potassium Chloride	3.73
13.43	Calcium Chloride*2H ₂ O	1.97
2.61	MgCl ₂ .6H ₂ O	0.53
9.99	Tris	1.21
Adjust pH to 7.4 with 6N HCl		

Electrode Filling Solution:

3M Potassium Chloride in deionized water.

Chloriding silver wire with 9V battery:

1. Attach an alligator clip to each lead of the connector for a 9V battery.
2. Coil two 16" pieces of silver wire around a large pencil. Clip a coil of silver wire on each lead of the battery connector.
3. Connect the battery lead, with the silver wire coils, to the 9V battery. Submerge the wire coils in a beaker of 3M KCl. Make sure the coils do not touch each other.
4. Let the current flow through the silver wires for 5 minutes, Remove the wires from the 3M KCl and reverse their position on the alligator clips. Return the coils to the beaker of 3M KCl, and let the current flow through this reversed circuit. For example, one coil will be on the positive battery pole for five minutes, followed by five minutes on the negative battery pole.
5. Reverse the position of the coils four more times for a total of 30 minutes.
6. After thirty minutes, the silver wire should be evenly chlorided. The wire should be a dull gray.