

Experiment AN-3: Neuromuscular Studies

Appendix I:

Recipe for Amphibian Ringer's Solution.

Concentration (mMolar)	Salt	Grams/Liter DI H ₂ O
111.0	Sodium Chloride	6.49
1.9	Potassium Chloride	0.142
1.06	Calcium Chloride*2H ₂ O	0.154
1.0	Tris	0.121
5.55	Glucose	1.00
Adjust the pH of the solution to 7.6 with 6N HCl		

Concentrations of Reagents in Ringer's That Alter Nerve and Muscle Activity.

Concentration (mMolar)	Reagent	mg/ml in Ringer's
0.036	Eserine	0.01
0.016	D-Tubocurarine Chloride	0.01
0.035	Atropine Sulfate	0.01
0.061	Acetylcholine Chloride	0.01
0.055	Nicotine Sulfate	0.01
10	Dantrolene	3.14
20	Magnesium Chloride*6H ₂ O	4.06
20	Calcium Chloride*2H ₂ O	2.94

Appendix II: Electrical Noise

Electrical noise is the most common problem associated with the recording of bioelectric signals. It radiates through the air and comes from electrical devices in the lab room or building: lights, power outlets, computers, monitors, and the power supplies. Since the source of power for these devices is 60Hz alternating current (AC), this electrical noise appears as a distorted sine wave with a repeating period of 16.7 milliseconds (msec).

There are two major sources of electrical noise: pickup and ground loops.

Pickup

Pickup is caused by electrical radiation that produces currents in the electrodes and wires leading to the amplifiers in the recording system. Because the resistance in the electrodes is high, small currents produce large voltages that may be greater than the biopotential being recorded. The major ways to reduce pickup are:

- Faraday Cage: Put a grounded, screened enclosure, known as a Faraday cage, around the preparation and the electrodes. The enclosure separates the source of the radiation from the electrodes. The person operating the equipment might also be a source of noise, and he or she may need to be grounded.
- Shielded Cables: Use shielded cables to carry the signals from the electrodes to the amplifier and the recorder; this puts a protective ground around the wires carrying the bioelectric signal.
- Differential Recording: Record using both a positive and a negative recording electrode placed on the nerve. The noise signals that are equal in magnitude, but opposite in polarity, will cancel each other out and leave a flat baseline.
- Short Cables: Use the shortest cables available to reduce the length of wiring exposed to electrical noise.
- Direct Current Equipment: Use equipment, like preamplifiers and illuminators, that are powered by batteries or direct current (DC) transformers.
- Equipment Removal: Unplug or remove unused alternating current (AC) equipment from the area.

Ground Loops

Ground loops are a troublesome source of electrical noise caused by the ground cable itself serving as an antenna for the noise radiating in the room. Using a Faraday cage to shield the preparation and the recording electrodes does not remove the electrical noise caused by ground loops. To avoid ground loops, use the following techniques:

- Ground Hub: Ground all the equipment around the preparation to a common grounding point (hub). This includes all the items that are electrically powered or are made of metal, like illuminators or microscopes. Use simple cables, like banana cords equipped with alligator clips, to connect each device directly to the common grounding point. The common grounding point is connected to the ground of the recording device with a single cable. The recording device is

connected to the building ground.

- **Simple Chain:** Ground the devices to the common grounding point using the simplest route that links the first device to the second device, the second device to the third device, and so on. Start the chain at the device that is the farthest from the common grounding point. End the chain by connecting the last device to the common grounding point, which is connected to the ground of the recording device.
- **Free-Floating:** In addition to using one of the grounding techniques described earlier, plug all devices powered by alternating current (AC), like illuminators, amplifiers, and recording units to power outlets using three-prong adapters.

High-Frequency Noise

High-frequency noise can also be a problem when recording bioelectric potentials. This type of noise is seen as the thickening of the recorded line. This noise contains many frequencies, and the amplitude of the noise is proportional to the resistance of the electrode. Therefore, intracellular electrodes, with high resistances, pick up a greater amplitude of high-frequency noise than extracellular electrodes, with low resistances.

Mechanical Noise

Mechanical noise, like vibrations from the ventilation system in the room, can cause the electrodes to vibrate and produce voltage changes with each vibration cycle. To alleviate this problem, isolate the platform holding the preparation with foam pads or bicycle inner tubes. Also, avoid bumping the table when the recording electrodes are in place.

Grounding

The iWorx unit has two different connections for grounding:

One method uses the ground (C) input on the C-AAMI-504 recording cable as described in the section showing the setup of the NBC-401 or 402 nerve bath chamber.

The other method uses either of the green (Ground or GND) banana jacks. One jack is located on the front panel in the stimulator section; the other jack is located on the back panel.

1. Try recording when the nerve/muscle preparation in the NBC-401 or 402 nerve/muscle bath chamber is grounded to the ground (C) input on the C-AAMI-504 recording cable, that is connected to the isolated inputs of the IWX/214, or the ground on the iWire-B3G. In this experiment, the muscle is also grounded through the electrode near the knee joint. Grounding the head of the muscle prevents the nerve CAP from being recorded by the muscle recording electrodes, and the muscle CAP from being recorded by the nerve recording electrodes. Attach the green C-ISO-P5 electrode lead wire to the ground (C) socket of the lead pedestal of the C-AAMI-504 recording cable or on the iWire of the IXTA.
 - Attach the pin jack on the other end of the green lead wire to the electrode between the negative stimulating electrode and the negative recording electrode. The ground electrode separates the stimulating electrodes from the recording electrodes to reduce the

amplitude of the stimulus artifact picked up by the recording electrodes.

- Connect the ground electrode on the muscle to the ground electrode on the nerve with a simple alligator-alligator cable. Make sure the electrode used for grounding the muscle is between the positive recording electrode on the nerve and the negative recording electrode on the muscle.
2. If the recordings of the CAP have a lot of 60Hz noise while using the ground of the C-AAMI-504 cable or the iWire, connect the ground electrode of the NBC-401 or 402 nerve bath chamber to one of the green banana jack connectors on the IWX/214 or the IXTA.
 - Attach an optional pin jack-male banana electrode lead wire.
 - Attach the pin jack on one end of this electrode lead wire to the electrode between the negative stimulating electrode and the negative recording electrode.
 - Attach the male banana plug on the other end of this lead wire to the green banana jack on either the front or back panel of the iWorx unit.

Signal Improvement

1. To improve recordings of the compound action potential, move the nerve/muscle chamber away from sources of 60Hz noise. These sources include outlets, computers, monitors, lights, refrigerators, water baths, and other AC-powered devices.
2. If the recording still contains a great deal of electrical noise, apply the digital filtering function to the data. Click on the Add Function button in the upper margin of the Nerve or Muscle Compound Action Potential channel. Select Filter from the menu of computed functions.
3. In the Filter Setup Dialog window, the Filter Mode is set to the Hamming Window and the Filter Order is set to 51; these are the default settings that should be used.
4. Set the Low Cutoff filter value to 65 and the High Cutoff filter value to 8000. The values for the filter cutoffs can be set by:
 - Typing the values for the filter cutoffs in the boxes to the right of the names of the filter cutoffs.
 - Clicking on the up or down arrows to the right of the boxes displaying the values of the filter cutoffs.
 - Clicking on the margins of the colored area in the graphic display of the filter and dragging the margins to the values required.