

Experiment AN-2: Compound Action Potentials

Equipment Required

PC Computer

IXTA, USB cable, IXTA power supply

NBC-401 or 402 Nerve Chamber

iWire-B3G input cable

C-ISO-P5 pin connector-pinjack lead wires (3)

Glass hooks

Pasteur pipettes and bulbs

C-BNC-P2 BNC-dual pinjack stimulator cable

Pinjack-male banana ground cable

Room Temp & Chilled amphibian Ringer's solution

Note – the iWire-B3G needs to be plugged into the IXTA prior to turning it on.

NBC-401 or 402 Nerve Bath Chamber Setup

1. Locate the following items: NBC-401 or 402 nerve bath chamber and the C-BNC-P2 stimulator cable.
2. Also, locate the iWire-B3G recording cable and C-ISO-P5 nerve chamber lead wires in the iWorx kit.

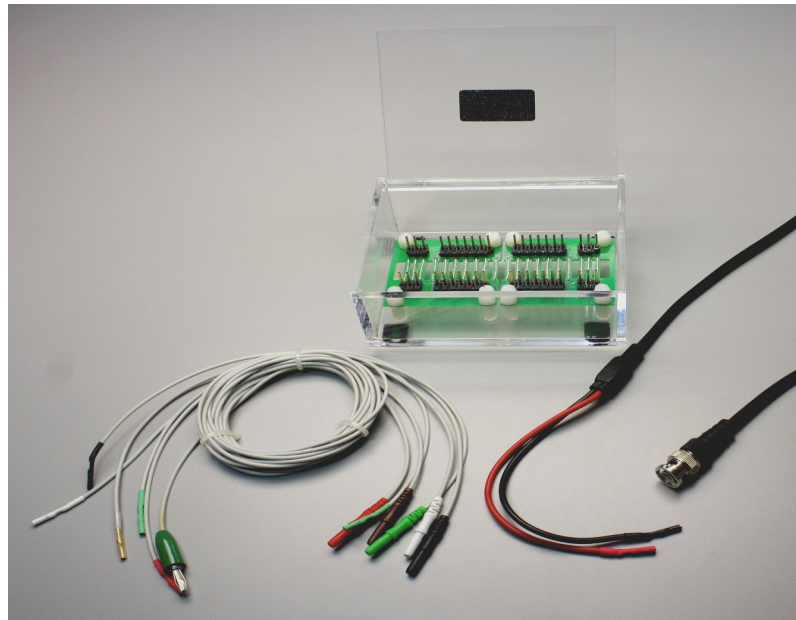


Figure AN-2-S1: The NBC-402 nerve bath chamber. Note that the NBC-401 is approximately half the size.



Figure AN-2-S2: The C-BNC-P2 stimulator cable.



Figure AN-2-S4: The iWire-B3G recording cable.

3. Attach the BNC connector of the C-BNC-P2 stimulator cable to the IXTA Stimulator 1. Place the sockets, at the other end of the stimulator cable, on the closely-spaced electrodes at one end of the NBC-401 or 402 nerve bath chamber. The red socket goes on the positive stimulating electrode (+S), which is the electrode closest to the end of the chamber. The black socket goes on the negative stimulating electrode (-S), on the opposite side of the chamber to avoid the possibility of the short circuit between the two stimulator cables.
4. Insert the connector on the end of the iWire-B3G cable into the iWire 1 input on the front of the IXTA.
5. Attach the red, black, and green C-ISO-P5 nerve chamber lead wires to the corresponding sockets on the iWire-B3G cable. Place the sockets on the ends of the lead wires, on the appropriate electrodes of the nerve bath:
 - The green (C) lead is attached to the electrode (G) between the negative stimulating electrode (-S) and proximal recording electrode (-R).
 - The black (-1) lead is attached to the proximal recording electrode (-R), the recording electrode closest to the ground electrode (G).
 - The red (+1) lead is attached to the distal recording electrode (+R) on either the distal end of the nerve or the thread holding the distal end of the nerve in place.



Figure AN-2-S5: The stimulator cable and iWire-B3G cable with three lead wires connected to the IXTA.

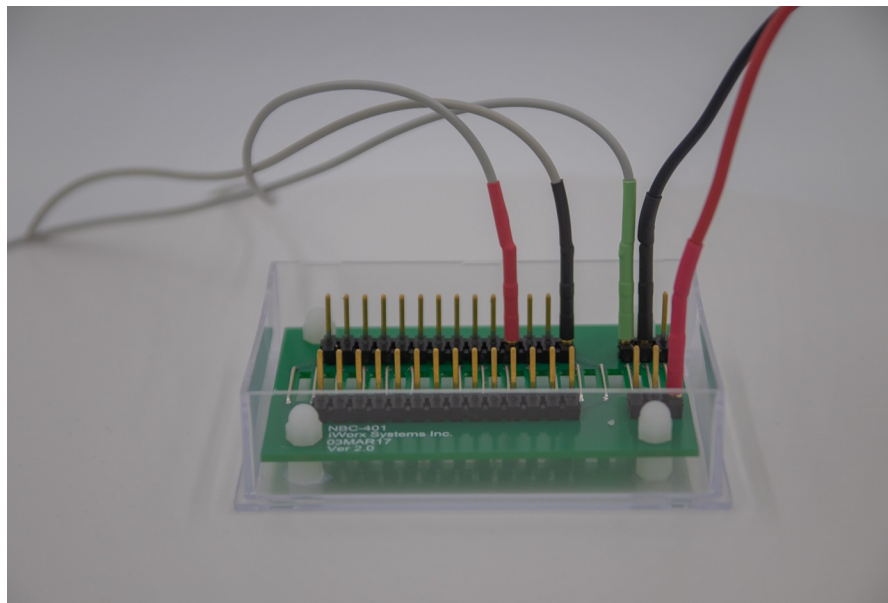


Figure AN-2-S6: The NBC-401 nerve bath chamber with stimulator and recording lead wires attached. Notice that the stimulator leads are attached to the electrodes that are spaced closer together.

Note: When the positive and negative recording electrodes are on the same healthy section of a nerve, a biphasic compound action potential (CAP) is recorded. The upward deflection of the biphasic CAP occurs when the potentials propagating down the axons in the nerve pass the first (negative) recording electrode, and are recorded in reference to the voltage at the second (positive) recording electrode. The downward deflection of the biphasic CAP occurs when the potentials propagating down the axons pass the second (positive) recording electrode, and are recorded in reference to the voltage at the first (negative) recording electrode. When a biphasic CAP is recorded, the A α fibers are usually the only fiber type seen in the CAP. When a monophasic CAP is recorded, other fiber types (A β , A γ , A δ , C) may appear in the recording in addition to the A α fibers.

To record a monophasic CAP move the positive recording electrode from the healthy section of nerve to the thread holding the nerve in place. B fibers, which are preganglionic autonomic types, are not found in the sciatic nerve.

If there is an issue with electrical noise – please see the appendix.

The Dissection

1. Place a frog in ice water for 15 minutes. Double pith the frog as soon as it is removed from the ice water.
2. Remove the skin from the legs by making an incision through the skin around the entire lower abdomen. Cut the connections between the skin and the body—especially around the base of the pelvic girdle. Use stout forceps to pull the skin off the frog in one piece (like a pair of pants).
3. Place the frog in a dissection tray with its dorsal side up. Moisten the exposed limbs of the frog with Ringer's solution every five minutes or so.
4. Separate the muscles of the upper leg to expose the sciatic nerve. Muscles are surrounded by connective tissue called fascia, and the large medial and lateral muscles on the dorsal side of the upper leg are joined to each other by a fusion of their fascia along a thin "white line". Grab the muscle groups on either side of the "white line" with a forceps, and firmly pull the muscle groups apart. The fascia will tear.
5. Deflect the muscles away from each other to expose the cream-colored sciatic nerve lying deep between the muscles. The sciatic nerve is covered with fascia, which also includes some blood vessels.
6. Use a glass hook, made by flaming the tip of a Pasteur pipet, to separate the nerve from the fascia and the vessels. If possible, avoid cutting the blood vessels. If bleeding does occur, rinse away the blood with lots of Ringer's solution. Free the nerve from the knee joint to the pelvis.
7. Use the glass hook to place a thread under the nerve. Move the thread as close to the knee joint as possible. Ligature the nerve; the foot should jump as the knot is tied tightly.
8. Carefully separate the muscles of the pelvis to expose the sciatic nerve. Remember to rinse any blood away with Ringer's solution. The sciatic nerve enters the abdomen of the frog through an opening at the end of the urostyle, a bone that forms part of the pelvis.
9. Carefully expose the remainder of the nerve through an opening along the lateral side of the urostyle. To avoid cutting the nerve, lift the end of the urostyle with forceps as the muscle is cut away from the urostyle with a blunt scissors. Cut along the urostyle from its tip to the vertebral column.
10. Deflect the muscle away from the urostyle to expose the sciatic nerve. Use a glass hook to separate connective tissue from the nerve and to place a piece of thread under the nerve. Move the thread as close to the vertebral column as possible. Ligature the nerve; the leg should jump as the knot is tied tightly.
11. Prior to cutting the nerve, measure the in situ length of the nerve between the two ligatures.

12. Cut the upper end of the nerve between the knot and the vertebral column. Cut the lower end of the nerve between the knot and the knee joint. Keep the exposed nerve moist with Ringer's solution at all times.
13. Use the thread to lift the proximal end of the nerve from the abdomen of the frog. Do not pinch or stretch the nerve. Remove any connective tissues, blood vessels, or nerve branches that may still keep the nerve attached to the frog. Continue to lift the nerve out of the frog until it is clear of the abdomen, the pelvis, and the thigh.
14. Grasp the threads at either end of the nerve, and carefully place the nerve in the bath chamber: Place the proximal end of the nerve, that was connected to the spinal column, over the stimulating electrodes. The distal end of the nerve, that was near the knee joint, should be over the recording electrodes.
15. To improve contact between the nerve and the electrodes:
 - Place the thread on the proximal end of the nerve under the outermost stimulating (positive) electrode.
 - Place the thread on the distal end of the nerve under the outermost recording electrode at the other end of the chamber.
 - Gently pull the thread on the proximal end of the nerve to position the section of nerve that is just inside the knot under the outermost stimulating electrode. Secure the thread on the wall of the chamber with soft wax or dental wax.
 - Gently pull the thread on the distal end of the nerve until the nerve is at its in situ length. Secure the thread on the wall of the chamber with soft wax.
16. Fill the nerve bath chamber with Ringer's solution to immerse the nerve. Cover the nerve bath chamber to prevent evaporation of the Ringer's solution when the nerve is bathing, or dehydration of the nerve when the Ringer's solution has been removed.

Warning: The nerve preparation used in this experiment is functional for a limited period of time. If the nerve is bathed periodically in Ringer's solution, it will work for about four hours. To conserve time, complete all the exercises in the experiment before analyzing the data.