

Experiment AN-3: Neuromuscular Studies

Equipment Required

PC or Mac Computer

IXTA, USB cable, IXTA power supply

NBC-401 or 402 Nerve Chamber

iWire-B3G input cable

C-ISO-P5 pin connector-pin jack lead wires (5)

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

Small amounts of reagent solutions

NOTE – plug the iWire-B3G into the IXTA prior to turning it on.

NBC-401 or 402 Nerve/Muscle Bath Chamber Setup

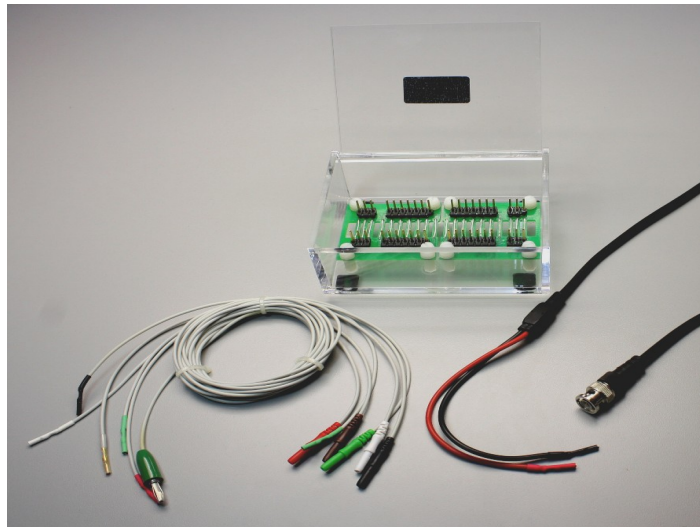


Figure AN-3-S1: The NBC-402 nerve bath chamber. Note that the NBC-401 is approximately half this size.

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



Figure AN-3-S2: The C-BNC-P2 stimulator cable.



Figure AN-3-S3: The iWire-B3G recording cable.

3. Attach the BNC connector of the C-BNC-P2 stimulator cable to the Stimulator 1 input. 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. Attach the red, black, white, brown, and green C-ISO-P5 nerve chamber lead wires to the corresponding sockets on the lead pedestal of the iWire-B3G biopotential cable. Place the sockets, at the other end of the lead wires, on the appropriate electrodes of the nerve bath:
 - The green (C) lead is attached to the electrode (G1) between the negative stimulating electrode (-S) and the proximal recording electrode for the nerve (-N).
 - The black (-1) lead is attached to the proximal recording electrode for the nerve (-N), the one closest to the ground electrode (G1).
 - The red (+1) lead is attached to the distal recording electrode for the nerve (+N), the one between the proximal recording electrode for the nerve (-N) and the ground electrode on the muscle (G2).

- The brown, or clear, (-2) lead is attached to the negative recording electrode for the muscle (-M), which is between the second ground electrode (G2) and the proximal recording electrode for the muscle (-M).
- The white (+2) lead is attached to the positive recording electrode for the muscle (+M), which is next to the negative muscle recording electrode (-M).
- A jumper wire with sockets or insulated alligator clips is attached between the ground electrode on the head of the muscle (G2) and the other side of ground electrode on the nerve (G1). This ground is needed to prevent the artifact from the compound action potential of the nerve from being recorded through the recording electrodes on the muscle.

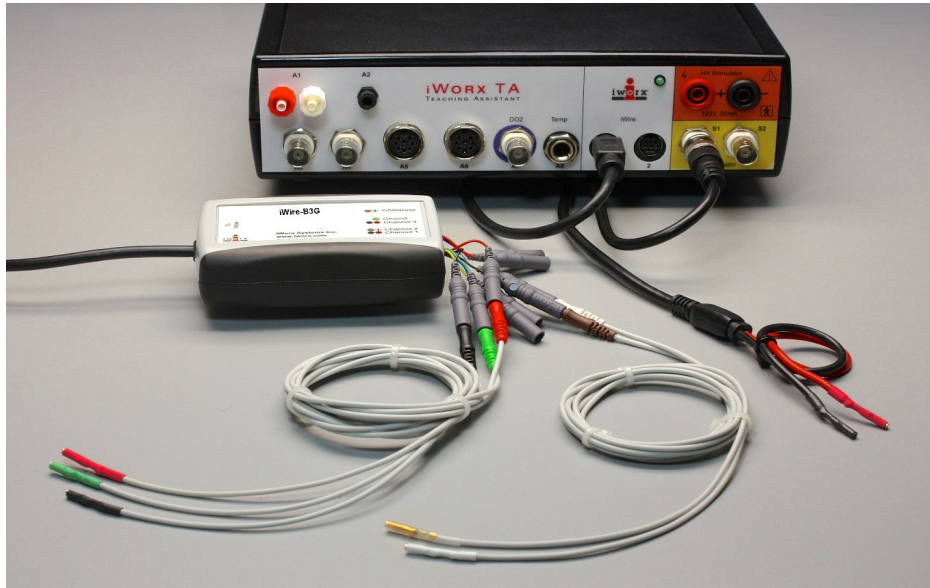


Figure AN-3-S5: The female BNC to Dual Banana Adapter; stimulator cable, and iWire with five leads.

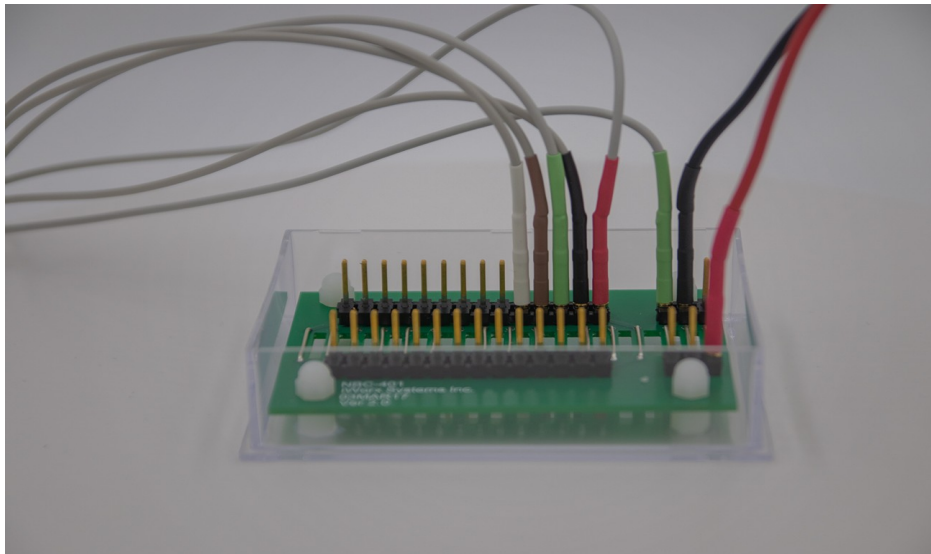


Figure AN-3-S6: The NBC-401 nerve bath chamber with stimulator and recording lead wires attached.

Notice that the stimulator leads are attached to the electrodes that are spaced more closely.

Stimulator Settings for Experiment AN-3.

Parameter	Units/Title	Setting
Stimulus Mode		Pulse
Stimulator Start		With Recording
Time Resolution	msec	0.01
Toolbar Step Frequency	Hz	1
Toolbar Step Amplitude	Volts	0.01
Toolbar Step Time	Sec	0.0001
Delay	Sec	0.005
Amplitude (Amp)	Volt	0.250
Pulses (#pulses)	Number	1
Pulse Width (W)	msec	0.1
Time Off (T Off)	msec	0.9
Time Off Amplitude	Volts	0
Holding Potential (HP)	Volts	0

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. Use the glass hook to place a thread under the nerve. Keep the exposed nerve moist at all times with Ringer's solution.
7. 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.
8. 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 you cut the muscle away from the urostyle with a blunt scissors. Cut along the urostyle from its tip to the vertebral column.
9. 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. Ligate the nerve; the leg should jump as the knot is tied tightly.
10. Cut the nerve between the knot and the vertebral column. Keep the exposed nerve moist at all times with Ringer's solution.
11. 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 body of the frog.
12. Continue to lift and release the sciatic nerve from any tissue that still keeps it attached to the pelvis or thigh. The nerve should be separated from connecting tissues to a point just above the knee joint.
13. Lay the nerve over the muscles of the lower leg and bathe the nerve and muscles with Ringer's solution. This nerve and the muscles of the lower leg are the preparation that needs to be preserved for this experiment.
14. Expose the femur bone by dissecting away the muscles of the upper leg as close to the knee joint as possible without damaging the sciatic nerve. Use a stout pair of scissors to cut the femur bone as close to the knee joint as possible. Rinse the preparation with Ringer's solution to moisten the tissue and rinse away any blood.
15. Separate the lower leg and the nerve from the muscles and bone of the upper leg. Cut across the muscles of the thigh and the femur bone as close to the knee joint as possible and without damaging the nerve.

Warning: Moisten the exposed limbs of the frog with Ringer's solution every five minutes or so.

16. Either muscle of the lower leg can be used with the sciatic nerve in this experiment. The gastrocnemius muscle can be used in larger nerve/muscle chambers, but the diffusion of drugs through this muscle takes more time. The tibialis anterior muscle fits into smaller chambers and the diffusion of drugs takes less time through this smaller muscle. In either case, the muscles must be separated from each other.
17. Use a glass hook to separate the gastrocnemius muscle from the bone and other muscles of the lower leg.
18. Use scissors to free the Achilles tendon from the connective tissue around the heel of the foot.

If the gastrocnemius muscle is used:

1. Firmly tie a thread around the Achilles tendon, leaving the ends of the thread long enough to secure the muscle in the nerve/muscle chamber.
2. Cut the Achilles tendon as close to the bottom of the foot as possible, so the thread is still attached to the gastrocnemius muscle.
3. Move the gastrocnemius muscle away from the rest of the lower leg. Cut the tibiofibula bone and tibialis anterior muscle just below the knee to separate the rest of the lower leg from the preparation. Avoid damaging the gastrocnemius muscle or the sciatic nerve. Rinse the preparation with Ringer's solution to moisten the tissue and rinse away any blood.

If the tibialis anterior muscle is used:

1. Firmly tie a thread around the ankle. Leave the ends of the thread long enough to secure the muscle in the nerve/muscle chamber.
2. Cut the Achilles tendon as close to the bottom of the foot as possible. Move the gastrocnemius muscle away from the rest of the lower leg. Cut the across the head of the gastrocnemius muscle as close to the knee joint as possible to separate it from the rest of the lower leg. Avoid damaging the tibialis anterior muscle or the sciatic nerve. Rinse the preparation with Ringer's solution to moisten the tissue and rinse away any blood.

Placement of Preparation in the Chamber

1. Before moving the nerve/muscle preparation from the dissecting tray to the nerve/muscle chamber, read the following directions.
2. Orient the chamber so that the nerve/muscle preparation can be easily transferred to the nerve/muscle bath chamber. The proximal end of the nerve will be at the end of the chamber where the electrodes are closer together, and the distal end of the muscle will be at the end of the chamber where the electrodes are farther apart.

Warning: Do not use the thread on the nerve to move the preparation into the bath chamber. Putting too much tension on the nerve could separate the connections between the nerve and the muscle and prevent signals from being conducted from the nerve to the muscle.

3. Carefully lift the preparation from the dissecting tray and place it in the nerve/muscle chamber using the thread on the end of the muscle and a forceps on the tissue above the knee joint.

Warning: Do not touch the nerve with the forceps.

4. 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.
 - 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.
5. Carefully move the muscle to a position over its three recording electrodes using the same technique described in Step 3. Do not stretch the nerve past its in situ length. Secure the thread on the end of the muscle to the wall of the chamber with soft wax or dental wax.
6. Fill the nerve/muscle bath chamber with Ringer's solution to immerse the preparation. Cover the bath chamber to prevent evaporation of the Ringer's solution when the preparation is bathing, or dehydration of the preparation when the Ringer's solution has been removed.

Warning: The nerve/muscle 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.