

## **BSL Frog Lesson**

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# Frog Pith & Preparation



## **Overview**

The following includes detailed instructions for the frog preparation procedures listed below:

- Pithing the frog's brain (and sever the spinal cord if necessary).
- Removing the skin.
- Isolating the sciatic nerve.
- Attaching the nerve to the transducer.
- Isolating the gastrocnemius muscle.
- Attaching the muscle to the transducer.

## **Objectives**

To become familiar with the procedure for preparing the frog gastrocnemius muscle and its sciatic nerve (pithing, skin removal, muscle and nerve isolation) in order to complete subsequent lessons with the following objectives.

## **BSLPRO-A02 Neural Control of Skeletal Muscle**

- To determine and compare the twitch subthreshold and the threshold response for skeletal muscle and its motor nerve.
- To determine the maximal twitch response of the muscle.
- To record a skeletal muscle twitch and determine the latent, contraction, and relaxation periods.
- To record and measure the effect of motor unit summation on the amount of skeletal muscle contraction.
- To determine the point of complete tetanus in the skeletal muscle.
- To observe and measure the beginning and end of skeletal muscle fatigue.
- To record and measure the mechanical work achieved by an isotonically contracting skeletal muscle.
- To measure the effects of initial length and load on the ability of skeletal muscle to do work.

## **BSLPRO-A03 Compound Action Potential and Conduction of the Nerve Impulse**

- To record the compound action potential of the frog sciatic nerve and measure the latent period.
- To record the effect, on the amplitude of the compound action potential, of subthreshold, threshold, submaximal, maximal, and supramaximal stimulation of the sciatic nerve.
- To observe and record nerve refractoriness by stimulating the nerve using maximal stimuli of increasing frequency.
- To record the effects of temperature on the velocity of nerve impulse conduction.
- To record the effect on the ability of the nerve to conduct impulses before and after the application of a local anaesthetic.

## **BSLPRO-A04 Physiology of the Heart: the Cardiac Cycle & Properties of Cardiac Muscle**

- To observe and record atrial and ventricular systole and diastole in the frog heart.
- To observe and record the cardiac phenomena of refraction, ventricular extrasystole, and the compensatory pause in the frog heart.
- To observe and record the effects of atrioventricular block induced by Stannius ligature in the frog heart.
- To observe the property of myogenicity in the excised frog heart.
- To observe and record the effects of epinephrine, acetylcholine, atropine, and pilocarpine on the frequency and amplitude of cardiac muscle contraction in the frog.
- To observe and record the effects of vagal stimulation on the frog cardiac cycle.
- To observe and record the effect of increasing and decreasing the temperature of cardiac muscle on cardiac rate and contractility.
- To observe and record the positive inotropic effect of digitalis on the frog heart.

## **Equipment**

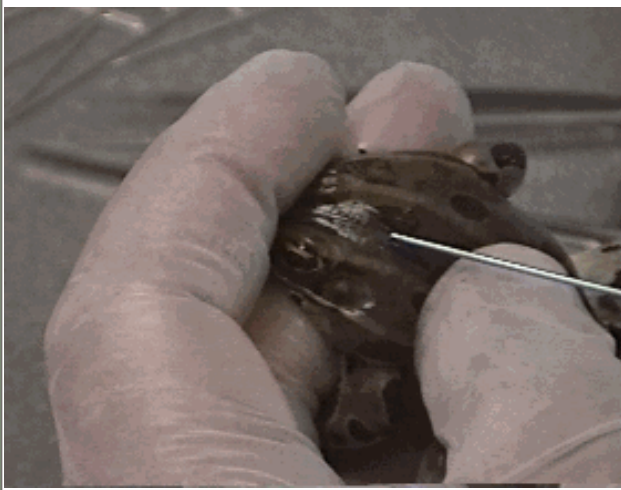

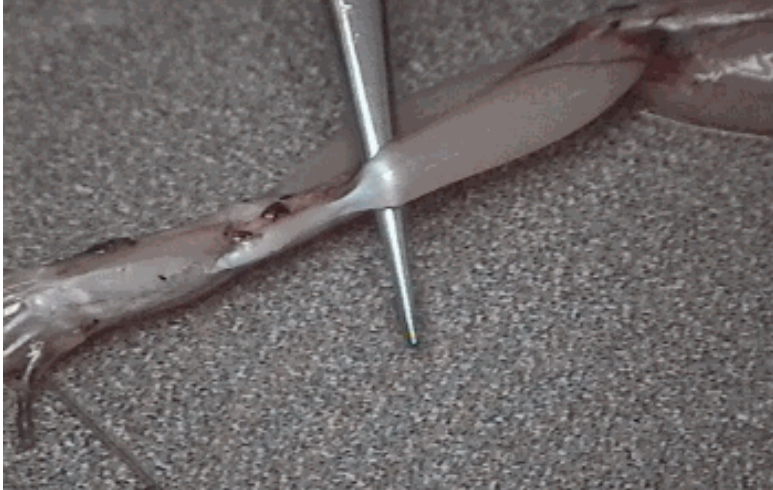
- BSLSTM Stimulator
- Force Transducer Assembly
- Bipolar Electrode (for sciatic nerve)
- Pin Electrodes (for gastrocnemius muscle)
- Thread (nylon preferred vs. cotton)

- Live Frog
- Acrylic Board
- Goggles
- Rubber Gloves
- Amphibian Ringer's solution
- Dissection Pan
- Dissection Kit: Scalpel, Forceps, Scissors, Dissection Pins, Probes
- Glass probes (you can make your own by heating a glass rod under a Bunsen burner and pulling it to a fine point.)

## Storing Live Frogs

- Live frogs should be stored in the refrigerator at about 35°F to keep their metabolism down.
- Frogs can be kept in a covered cake pan and should be kept moist with amphibian Ringer's solution.
- Clean the frogs before beginning preparation for any experiment.

## Frog Anatomy Reference

Cranial cavity (brain)	Spinal cord	Achilles tendon
Between the eyes, soft spot at the base of the (hard cartilage of the) skull	Extends from cranial cavity down to about the middle of the back, severed via the cranial cavity.	There is a tendon that wraps around the posterior part of the ankle and is inserted to the foot. This is called the Achilles tendon (it's the same in humans).
		

<b>Gastrocnemius muscle</b>	<b>Sciatic nerve</b>
Posterior muscle of leg. Anatomically the same as the calf muscle in humans.	Runs deep beneath the muscles of the thigh, supplies the gastrocnemius muscle.



Spinal cord	Leg
Extends from cranial cavity down to about the middle of the back, severed via the cranial cavity.	Knee to ankle

## Pithing Procedure

Before the experiment, you need to render the frog insensitive to pain. Pithing is one procedure to accomplish this. Pithing will destroy the brain, which is located between the eyes. (For some experiments, pithing will include severing the spinal cord.) Pithing is relatively painless to the frog. Read the entire procedure before beginning so you can perform the procedure quickly.

1. Hold the frog facing away from your body, with the lower extremities extended.
2. Grasp the frog with your first two fingers: first finger on the nose, second finger under the jaw. Flex the head forward (away from your body).
3. Move probe down midline until you come to the soft spot.

Do not use undo pressure on the frog skin.  
Frog skull is fairly hard cartilage; soft spot is at the end of the skull.



4. Insert the probe quickly into the cranial vault and sever the brain and spinal cord.
5. Move the probe into the cranial vault and move it from side to side to destroy the brain.

You can feel inside the cranial vault, but the frog can't feel anything.  
Without the brain, perception of any sensory phenomena is impossible.





6. Test for reflexes to confirm that sensory perception has been destroyed and ensure that the spinal cord is still intact, although it is now in shock.

<b>Corneal Reflex</b> (sensory perception check)	<b>Reflex Withdrawal Response</b> (spinal cord check)
In a normal frog, as soon as you touch its eyelid, it will blink. With no brain, there is no response.	Pinch the frog's leg and it should withdraw as shown below.
	

**NOTICE:** The frog may crouch, jump, or even make noise, but it is not in pain. These actions are neural reflexes controlled via motor senses in the spinal cord, not brain functions.

7. Keep the frog moist. The frog will still be breathing because frogs breathe primarily through the skin, not the lungs, so it is important to keep the frog moist.



Squirt the frog with amphibian Ringers (a special saline solution for frog electrolyte concentration).

OR

When you no longer require tissue experimentation, place a moist napkin (soaked in Ringers) over the frog.

8. Sever the spinal cord: Keep the probe in the cranial canal and turn the probe around into the vertebral canal.



When the spinal cord is severed, the frog legs become completely limp (due to flacid paralysis of skeletal muscle). The frog will never assume a crouching position again, and if you pinch the frog after the spinal cord is severed, which is difficult because the frog may be slick, the frog will not feel anything and there will not be a reflex withdrawal. In other words, spinal severing makes muscle work easier because the muscle no longer reflexes. Local muscle twitching resulting from spinal nerve ending stimulation may still occur.

Continue to keep the frog moist so it can continue to respire through the skin (tissue remains vital) and the heart can beat.

## **Skin Removal**

1. Sever the skin at the hip.
2. Lift the skin away from the underlying fascia
3. Slip one jaw of the scissors beneath the skin
4. Cut the skin all the way around, being careful not to damage the underlying muscle.
5. Grasp the skin with the forceps, hold the frog, and pull the skin down.
6. Remove the skin from the hip down, over the knee, over the ankle, off the toes to expose the musculature of the nerve.



## Isolating the Sciatic Nerve

The sciatic nerve runs deep beneath the muscle in the thigh and supplies the gastrocnemius muscle.

1. To expose the sciatic nerve, penetrate the overlying muscle fascia to separate the muscles of the thigh.

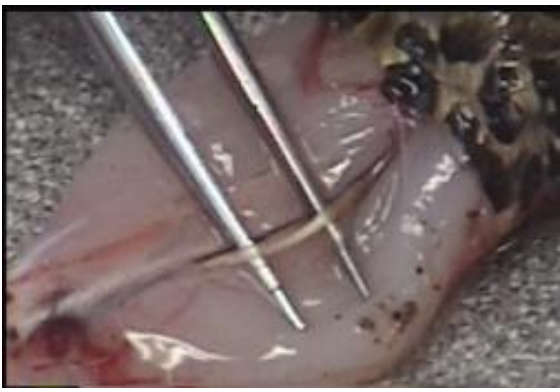
### *Hints for locating the sciatic nerve...*

With the thigh spread, you can see the femur and a blood vessel running along it. Next to that is the sciatic nerve.

From the posterior aspect of the thigh, the nerve lies in a groove on either side of the muscle under the blood vessel (which is a thin, dark line). The nerve is a white, tendonous strip.



2. Use a blunt dissection to free up the nerve.
3. Separate the nerve from the blood vessel.



4. Loop a thread under the nerve so you can pull up on the thread and lift the nerve.

When you release the thread, the nerve will drop back between the muscle,  
This helps prevent the nerve from drying out.

**TIP:** Nylon thread is preferred because it is stronger than cotton thread and not as distensible.

### **Attaching the Sciatic Nerve to the Transducer**

Use a bipolar (two pronged) electrode. Insert the electrode prongs underneath the nerve to separate it from the muscle.

### **Isolating the Gastrocnemius Muscle**

You need to attach the muscle to the transducer so you can stimulate the muscle using pin electrodes that will be inserted directly into the muscle belly (one at each end). A current flows between the two, and if its voltage is adequate the muscle will respond by contracting.

1. Take the blunt end of the forceps and place it under the belly of the gastrocnemius muscle to free it from the surrounding fascia.





2. Place a probe under the Achilles tendon and exit from the other side.



The Achilles tendon, located at the distal end of the gastrocnemius muscle, wraps around the posterior part of the ankle and is inserted into the foot.

3. Leaving the probe between the muscle and surrounding fascia of the leg, loop some thread around the Achilles tendon and tie a knot at the distal end of the muscle which will be cut and attached to the transducer. The proximal end of the muscle (attached at the knee) will remain attached to the frog.



- Cut the gastrocnemius muscle at the distal end (distal to the knot on the Achilles tendon) so it can be attached to the transducer and elicit a response.



TIP: Try to get as much tissue distal to the knot as possible in case the knot slips.

- Moisten the leg with Ringers so you don't lose function from drying out.

### Attaching the Muscle to the Transducer

- Place the frog knee just beneath the transducer hook.
- Pin the knee in place to stabilize its position.
- Use muscle of the anterior thigh.
- Use muscles of the anterior leg on the other side of the knee.
- Lay the thread from the muscle over the transducer hook.
- Pull the thread so there is a little tension on the thread.
- Position the muscle so the thread is vertical, so the muscle is not pulling at an angle (you want an accurate reflection of the muscle's contractile force).
- Use a double square knot to ensure a firm hold when you start stimulating the muscle.
- Remove any additional thread.
- Moisten the frog with Ringers Solution.
- Adjust the ring stand height so the connection between the muscle and the transducer is relatively taut.
- Use the fine adjuster knob (at the top of the stand) to remove any remaining slack without stretching the muscle.



### Dissecting the Sciatic Nerve

The following procedure assumes the frog has been double-pithed (brain and spinal cord). The pictures are available as a quick reference, and should not be used alone to complete dissection. Follow the written directions closely..

## 1. Remove the skin of the leg.

Use dissecting scissors to cut and loosen skin around the leg.

Cut all the way around the leg. Use tooth forceps if slippery.

Don't get down into muscle but otherwise doesn't have to be too delicate.

Cut all the way around at the hip, pull down and off the foot.

Use paper towel if slippery and pull over and off the foot.

## 2. Secure the frog with T-pins.

Use t-pins to secure the leg so you don't get a lot of sliding.

Be careful not to put pin at nerve since you will dissect both nerves.

## 3. Separate the muscle bundles.

Notice two muscle bundles on back of thigh.

Incise fascia so you can separate the two muscle bundles.

Use sharp point of scissors to get in between bundles.

Sometimes you'll see the muscle twitch which is normal since the frog is still functional.

Separate between the muscle bundles. You may see a showing of purple from the veins and artery; this is also where the nerve is.

Be gentler; still incise fascia just to be able to separate it a little better. Don't touch the nerve with your hands or anything metallic, as both are conductors. Use glass probes.

Separate and get down into nerve area using gentle separation.

## 4. Cut the fascia.

When you can see the nerve, you can use scissors to cut fascia.

Continue to separate down to the knee and up to the spinal cord.

Cut tissue around and later artery and vein off.

Don't pull on the nerve because that will discharge action potentials and perhaps cause more permanent trauma.





## 5. Dissect the nerve.

Identify nerve and use probe to help you hold it aside and continue the dissection.

## 6. Expose spinal cord.

Cut skin up the back to expose spinal cord.

Nerves come around corner of bone ridge; nerve runs deep.

Gently open corner area at the top and separate.

Nerve is under muscle.

Corner area is tricky—identify nerve and know how far to go. Just go slightly to the side.

Use glass probes to lift nerve aside as you dissect.

## 7. Identify nerve and perform a blunt dissection.

Gently loosen and pull apart tissue.

Open up the spinal section, being aware of nerve.

Notice spinal root coming out (Medial aspect = other nerve).

See fascia again and blood vessels.

Know that nerve is under that.

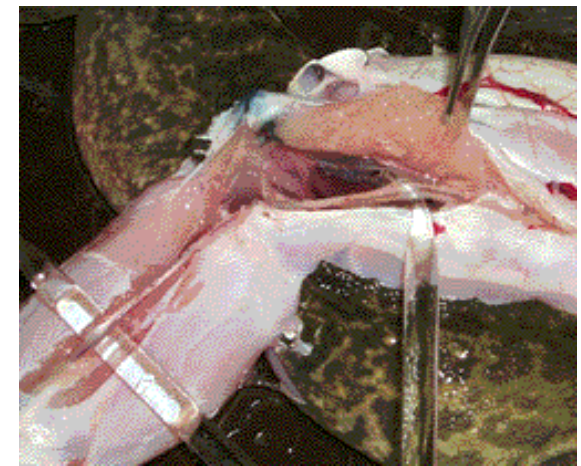
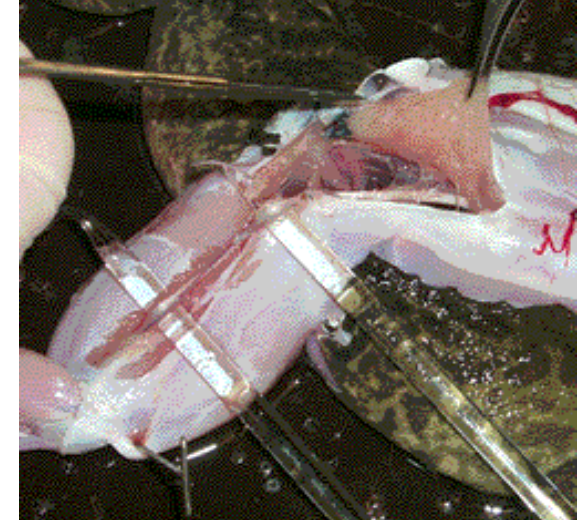
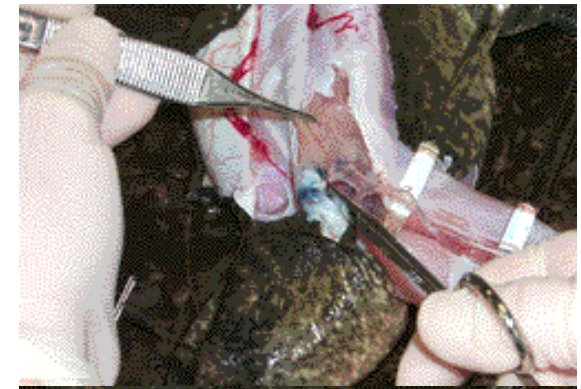
Gently pull on fascia, cut away muscle.

Get rid of connective tissue.

See roots of spinal nerves (all conductive tissue).

Can trim backbone to get more nerve.

Detach completely from surrounding tissue.





### 8. Tie thread around nerve ends.

Loosen nerve before you ligate it, this gives you some ability to lightly pull on the nerve as you loosen it.

Pull gently on nerve

Hook thread and pull underneath, pull as far down the nerve as you can get (edge it down around the roots, must be freed up)

Cinch nerve down – will cause action potential and twitches

### 9. Cut nerve at both ends.

Tie thread and cut nerve beyond the knot (proximal to knot, where nerve is still attached)

Pin tissues apart if necessary.

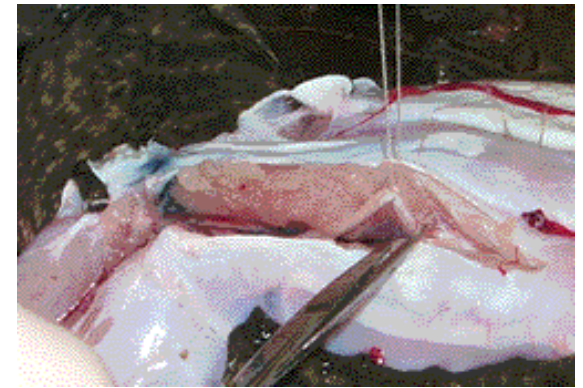
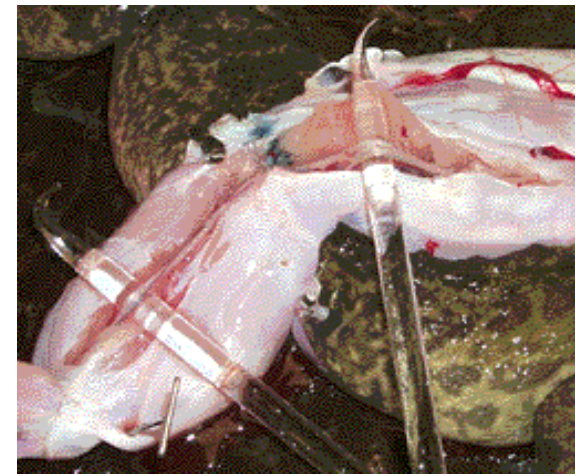
Cut fascia to free up

Use scalpel to cut spinal twigs

Do not over-pull the nerve...OK to pull on blood vessels and to break them off

\*You may think the nerve looks shorter once it's cut.

### 10. Place dissected nerve in Ringers.



11. Repeat procedure to dissect other sciatic nerve.

12. Clean up before beginning a new lesson.

