Bio 2424 - Human Physiology Lab
Frog Spinal Reflex Experiments Protocol

Laboratory animals have been used in secondary schools and universities for many years to help students to see physiological processes in vivo. The instructors in this course seek to provide you with the most meaningful demonstrations in the laboratory as possible. The lab instructors in this course ask you to remember some important points:

1. The animals we use in this laboratory will be treated by the staff members and students with respect and as humanely as possible.
2. No animal will be used needlessly, therefore you must be prepared to perform the experiments only after you have read through them and follow the steps outlined.
3. Because various bacteria are carried by frogs, refrain from putting your hands, fingers, or any objects in your mouth while working with the frog. When you have completed your experiments be sure to wash all glassware and supplies for the next lab.
4. All frogs will be discarded in double bags by your instructor. DO NOT DISCARD THEM IN THE LAB'S WASTE BASKETS.
5. Wash your hands with soap and water before you leave the lab today.

The Reflex Arc

The basic unit for behavior is the reflex. Reflexes generally occur quickly and are the result of the excitation of 1. sensory receptors 2. conduction over afferent (sensory) nerve fibers 3. integration by the central nervous system 4. transmission of motor impulses over efferent (motor) nerve fibers 5. excitation of an effecter organ / tissue. The spinal cord may act as the primary integrator and source of a motor nerve response (spinal reflex) or as a modulator of motor responses when the brain or higher regions of the spinal cord are involved. The integrating and modulating activity or reflex activity is observed best when the controlling influences of the brain are removed. Removal is accomplished by ablating the neural structures anterior to the spinal cord i.e. the brain.

Ablation of the brain is achieved by performing a procedure known as single pithing. Two techniques for single pithing may be used. In one technique the lower blade of pair of scissors is inserted transversely between the upper and lower jaws of the frog such that the top blade is superior to the head. Care is taken too insure that when the blades move toward each other that they meet to section the head behind the tympani (the two large circles of the lateral sides of the head). It is generally best to insert the scissors so they are back against the joint between the upper and lower jaws before cutting. We will use this technique since it is more humane and accomplishes the task more quickly than other methods. Generally the amount of blood loss is minimal. The frog at this point is said to be single pithed. Single pithing then, effectively breaks all connections between the brain and the spinal cord, it therefore eliminates any influence they brain could have on reflex activity. The frog will regain some activity based upon spinal reflexes it is therefore known as a "spinal frog". If we wanted to eliminate all control by the central nervous system in this procedure the spinal cord is destroyed by the insertion of a metal dissection probe/needle into the open vertebral canal by the single pith. Because both the brain and spinal cord have been ablated the frog is said to be double pithed.
In considering reflexes one must remember that many of the responses that include the structure anterior to the spinal cord are frequently too labile or complex to be considered reflexes; rather, these responses should be labeled "reactions". Two points that must be considered in understanding reflex actions are:

1. The spinal cord is not strictly organized for function in terms of its segments; but, rather, it is organized with regard to the muscles that are innervated by specific spinal nerves.

2. Two major reflex patterns operate in the spinal cord: a. circumscribed b. diffuse

In circumscribed reflexes some of the fibers of a given dorsal root (sensory) make direct connections with motor neurons within a few segments. These are then referred to as monosynaptic reflexes.

In diffuse reflex arcs sensory neurons enter the spinal cord in from the dorsal root but then synapse with interneurons within the gray matter which in turn synapse with the bodies of motor neurons in the ventral gray horn. The axons of these neurons then exit via the ventral root and travel to effector organs, especially skeletal muscle.

Spinal reflexes vary in complexity depending on the involvement of spinal segments above or below the entry point for sensory action potentials. Examples of these are: The Myotatic / Patellar Tendon Stretch Reflex (seen in your previous lab exercises), The Flexion / Withdrawal Reflex, Crossed Extensor Reflex.

EXPERIMENTAL PROCEDURE

1. Reflex Reactions of Normal Frog

Place a normal frog on a frog board as demonstrated by your lab instructor and determine the frog's normal responses to each of the following conditions: ability to right itself after placement on its back (time), voluntary activity (escape behaviors), swimming in a water filled pan, coordination of movements and equilibrium, response to 1% acetic acid (rinse with finger's solution). Immediately after response is observed and other noxious stimuli such as pinching of the toes with forceps.

Note reaction times on a separate piece of paper along with any other observations made concerning its behavior.

2. Reflex Reactions of Spinal Frog: Spinal Shock

Carefully pinch the frog as directed on the previous page. BE SURE TO NOTE THE PRECISE TIME AT WHICH THIS IS DONE. Immediately after the procedure is complete the frog should remain motionless and lose muscle tone. This is a sign that the frog is in spinal shock because the spinal cord is still in tact the frog is called a spinal frog. A spinal frog cannot perceive pain.

After a recovery period, however it will be able to resume its ability to carry on spinal reflex activity which makes it appear as if it is consciously responding to sensory perceptions, i.e. painful stimuli. The lack of usual muscle tone is an indication that the frog is in spinal shock.

After pinching the frog, test for spinal reflexes pinching the toes periodically with a forceps. Continue to do so approximately every 15 seconds until reflex muscle contractions in response to the stimulus are observed. Note the time, which represents recovery from spinal shock.
Spinal shock in the frog usually lasts from 2-3 minutes. In humans suffering from spinal injuries it spinal shock could last for several months. When spinal reflexes have returned, repeat the same conditions as in #1 above when the frog had a functional brain & spinal cord. Note your observations and particularly the following: Can the spinal frog right itself when placed on its back? To what stimuli can it respond? How much and what type of "voluntary activity" does it perform? Will it swim as it did before pithing?

3. Flexion Reflex and irradiation of Reflex Activity
Suspend the frog by its bottom jaw from a femur clamp on the ring stand. Use the electronic stimulator and a stimulating electrode to apply a short burst of weak tetanizing stimulus. (ASK YOUR INSTRUCTOR TO HELP YOU WITH THIS) If the stimulus is adequate a flexion reflex will be elicited in the leg and foot being stimulated. Again with your instructor's assistance increase the stimulation strength. Pay particular attention to generation of response in the contralateral (the other side) leg. Stimuli of sufficient intensity can induce radiating synaptic activity in the spinal cord causing stimulation of motor neurons on the other side of the spinal cord and subsequent contraction of the extensors of the contralateral leg even though the stimulus is entering from the opposite side. This demonstrates the crossed extensor reflex.

4. Coordinated Nature of Reflexes
Saturate a 1-cm² piece of filter paper in 10% acetic acid and place it on the lower part of the frog's back. Record the time that elapses before the foot is raised as if to scratch the paper away. Rinse as soon as a response is observed with Amphibian Ringer's solution. Record the average of three trials. Have you ever elicited a scratch reflex in a pet dog or cat? Does a scratch reflex require voluntary cerebral cortex activity?

5. Inhibition of Reflexes
Place a small piece of 10% acetic acid paper on the right side of the frog's back as in exercise #4, to elicit a withdrawal (flexor) reflex. Then pinch (or electronically stimulate) the toes of the left foot. What happens to the right leg? You should be able to explain the cerebral inhibition that causes this response.

6. Convulsive Reflexes: Strychnine Activity
Two groups will combine to perform this activity. Lab groups at the front tables will join with the groups working directly behind them at the back of the lab. If you are at one of the front tables take your frog to the back table in one of the larger beakers on your table. Pour enough Amphibian Ringer's solution into one beaker to fill it to a depth of about 2 cm. Place one of the frogs in the beaker and cover it by keeping one hand over the opening. Pour 1% Procaine to a depth of approximately 2 cm. Place the other frog in the beaker and cover it by keeping one hand over the opening. Label the beaker so you know which frog is in which solution. Allow both frogs to soak in the solutions for 10 minutes. The ask your instructor to inject 1.0 ml 0.5% strychnine solution into each frog's peritoneal cavity. (CAUTION! This is a very potent poison and can cause respiratory failure if handled improperly) Return the frog to its beaker.

After another 10 minutes, stimulate both frogs by banging on the table. Note any developments of excitability or muscle contractions in the frogs. If convulsions do not appear in at least one of the frogs ask your instructor to inject an additional 1 ml of strychnine into the frogs.

If the spinal synapses are strongly activated (i.e not inhibited as in normal conditions) the resulting muscle contractions will cause the frog to assume an upright posture in the beaker in
a rigid fashion. At the height of its effect strychnine will cause total contracture of all muscle in
response to a minimal stimulus, such as a puff of air. You should note arching of the back. With
all muscles contracting simultaneously the flexors cause arching of the back since the flexors
represent the stronger muscles.
Eventually the frog will suffocate as the intercostal muscles go into contracture and fail to relax
to allow air to enter and leave the lungs.

Which frog is easier to stimulate and produce convulsive activity in? Why? Explain this
phenomenon.

When the convulsions are well developed, double pith the frog showing the signs described
above and note whether the convulsions persist. What does your observation tell you about the
location where strychnine has its effects? Why? Explain this phenomenon.