

Neural Integration

Once you understand how individual nerve cells (neurons) work, the next level of complexity is to study how a neuron interacts with another neuron, or other types of cells. This is the beginning of understanding the *nervous system* and its functions. In this lab exercise, you will investigate: 1) how more than one neuron work together to produce a spinal reflex; 2) how somatic (=motor) neurons communicate with skeletal muscle cells; and 3) and how neural responses can become more sophisticated with integration by the brain.

Exercise 1: Frog Spinal Reflexes

Aim: Determine (by the process of elimination) which frog activities are controlled by the spinal cord, which are controlled by the brain, and which are controlled by neither.

Background

During our day-to-day lives we detect changes in the environment and react appropriately. An external stimulus is detected by one or more neurons, which send the sensory information to the central nervous system, where it is processed. If a motor response is initiated, it usually involves a series of action potentials that produce a muscle contraction and a movement of one or more parts of the body. A simple reflex is perhaps the easiest of this type of stimulus-response reaction. A loud sound or something flying at your eye makes you blink, while a tap on the tendon under the knee cap produces the knee-jerk reflex.

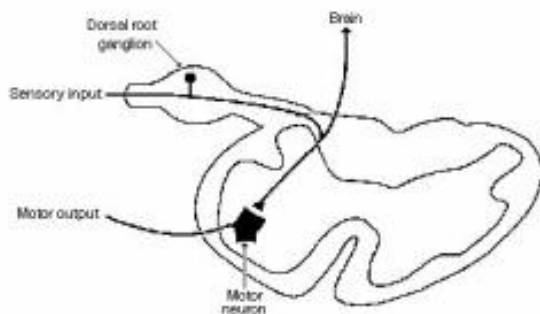


Figure 1: A cross section of the spinal cord showing the single synapse between the sensory and the motor neurons involved in a monosynaptic spinal reflex.

A simple reflex like the knee-jerk reflex is produced via single synapses between sensory axons and motor neurons (a **monosynaptic reflex**). The required circuitry for this reflex is confined to the spinal cord, as shown in Figure 1. Sensory information also ascends to higher centers, but the brain is not necessary or required to perform the reflex. More complex reflexes usually involve additional (inter-) neurons (a **polysynaptic reflex**) and more than one population of motor neurons. Thus, more neurons and synapses are involved, which usually results in a longer delay between stimulus and response, and often a more complex response. One example of such a complex response is the flexion withdrawal reflex, where a noxious stimulus to one leg causes withdrawal of the stimulated leg and extension of the other. In addition to monosynaptic and polysynaptic reflexes integrated in the spinal cord (**spinal reflexes**), some reflexes are integrated in the brain, but these are still “hard-wired” responses to a stimulus. The latter obviously can have more complicated integration.

Procedure

- 1 Observe a live frog for spontaneous activity. Is it coordinated (limbs working together)? When the frog is calm in the dissecting tray, rotate the tray slowly back & forth (45°), and tilt the board so that the frog is head-up or head-down by 4-6 cm. You can also try tilting it side-to-side. Finally, try putting it on its back. Record all your observations.
- 2 Soak a small square of filter paper in 10% acetic acid (about twice the strength of vinegar). Put this on the frog's back or leg for 60 seconds or until you get a response. Then rinse the area with distilled water. Repeat 2-3 times at different places on the frog's back, sides, or legs.
- 3 When the frog is calm, gently touch the two prongs of the stimulating electrode to its toes, and stimulate with 10-20 volts at 5 pulses per second (pps). You can do this by using the iworxs software. Open the LabScribe software, and under settings, open the **Neuromuscular studies**. To change the amplitude of the stimulus, under the **Edit** menu, select **preferences** and under the **stimulator tab**, change the **amplitude** of the voltage to **10-20V**.
- 4 Give the frog to your instructor to be single-pithed. This means that the frog's brain is destroyed (so it is now “brain-dead”), but the spinal cord is still functional. This is sometimes called a “spinal frog”. When the pithing is done, start a timer.
- 5 With the frog on its ventral side in the dissecting tray, pinch the toes firmly about every 20 seconds until you get a response. The time from pithing to the first response is the period of **spinal shock**, which occurs because the pithing overstimulates neurons in the spinal cord. This must wear off before normal spinal reflexes can occur.
- 6 Repeat all the tests that were done with the live frog as stated in #1.
- 7 Poke a small hole in the skin under the frog's chin, and pass the bent hook through this. Connect the handle of the hook to the ring stand such that the frog is dangling and its hind legs are over the dissecting tray.
- 8 Stimulate the toes of one foot with a weak titanic stimulation to obtain a **flexion reflex**. Let the leg return to its relaxed position and give the muscle 30 seconds of rest. Repeat this procedure gradually increasing the voltage, and watch the opposite leg for a **contralateral extention** when the stimulated leg goes up.
- 9 Use a square of filter paper to apply the acetic acid to the side/back of the frog to obtain a **scratch reflex**. Rinse with distilled water and repeat 2-3 times at different locations. Does it appear that the frog “knows” where the acid is?
- 10 Once more, use the acid on the frog's side to get a scratch reflex, and while that leg is up, stimulate the other leg with electricity as you did in step 5 above. Watch both legs, and record what happens.
- 11 Give the frog to your instructor to be double-pithed. When you get it back, both the brain and spinal cord will be destroyed. Quickly repeat all the observations/tests done on the live and spinal frogs. Which activities that a spinal frog can do have now ceased? Which remain?

Exercise 2: Neuromuscular Junctions

Aim: Determine how and where curare works, to better understand the operation of neuromuscular junctions.

Background

Neuromuscular junctions are analogous to synapses in many ways, but are the spaces between somatic nerves and skeletal muscles. They are involved in spinal reflexes which result in the movement of a skeletal muscle, but are also used for normal muscle movements.

The neuromuscular junction of vertebrates has been intensely studied as a model of general synaptic function because its size and accessibility are greater than synapses within the central nervous system (Figure 2). This "synapse" is a critical point in communication between the neural and muscular systems.

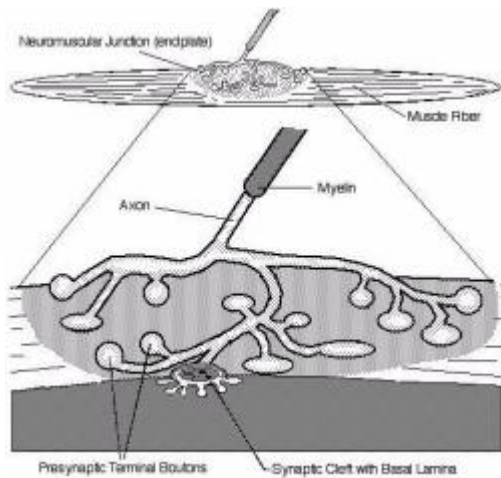


Figure 2; The Neuromuscular Junction.

The distal end of the axon branches into many presynaptic terminals to reach many muscle fibers with one somatic (=motor) neuron. The axon terminals innervate the muscle at a specialized region of the muscle membrane known as the **motor endplate**. The space between the terminal and the end-plate is 20 to 30 nanometers wide.

Somatic neurons use **acetylcholine (ACh)** to transmit nerve impulses across the neuromuscular junction to the muscle fibers. The presynaptic terminals have a high concentration of membrane-bound synaptic vesicles containing ACh. These vesicles fuse with the presynaptic membrane to release ACh into the synaptic cleft (Figure 3). Although the mechanism is not completely understood, calcium (Ca^{++}) is known to be necessary in the process. When a nerve action potential depolarizes the presynaptic terminal, voltage-dependent Ca^{++} channels are opened causing an influx of Ca^{++} into the terminal. Once inside the terminal, Ca^{++} aids with fusion of synaptic vesicles to the terminal membrane. The time consumed during the processes which occur after the arrival of the presynaptic action potential and before the elicitation of the muscle action potential is known as the synaptic delay. The fusion of vesicles to the membrane and the subsequent release of ACh occur about 0.3 msec after the arrival of the nerve action potential, which accounts for most of the synaptic delay. Once released, the acetylcholine rapidly diffuses (in 0.01 msec) across the neuromuscular junction and binds with specific protein receptors on the muscle end-plate. This process greatly increases the

permeability of the muscle membrane to positive ions (via messenger-dependent Na^+ channels). Na^+ enters the muscle fiber, hypolarizing the membrane. This local hypolarization is called an **end-plate potential**. When the end-plate potential reaches threshold value, a muscle action potential is elicited (using voltage-dependent Na^+ & K^+ channels).

Within 1 msec of its release from the nerve terminal, much of the excessive amount of acetylcholine has already diffused out of the neuromuscular junction, and no longer has a chance of acting on the muscle cell. The remaining molecules are hydrolyzed to choline and acetate by the enzyme, **acetylcholinesterase**, which is in the neuromuscular junction. The hydrolysis can occur before or after acetylcholine binds to the receptor sites since the binding is freely reversible. The short period of time that some acetylcholine molecules remain in contact with the muscle fiber membrane is almost always sufficient to excite a muscle action potential. After the hydrolysis of acetylcholine, choline is actively transported back into the axon terminal by a pump in the presynaptic membrane. Inside the neuron, choline is acetylated by choline acetyl transferase, and the resynthesized acetylcholine is stored in presynaptic vesicles.

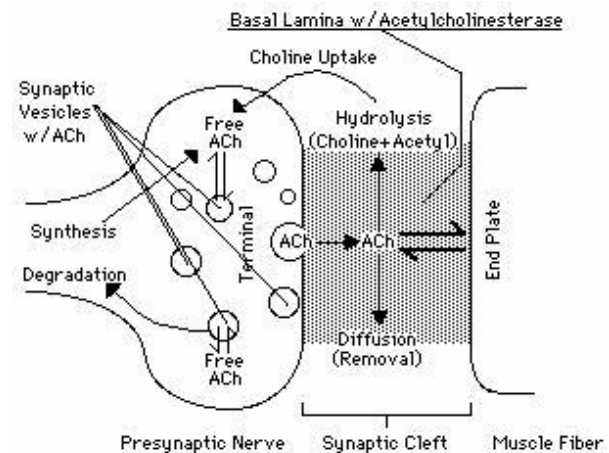


Figure 3: Disposition of Acetylcholine at the neuromuscular junction.

Equipment Required

Stimulator and BNC-P2 Stimulator cable
Force transducer
Glass hooks, Pasteur pipettes
Dissecting instruments and pins
(2) Grounding leads (green)

Equipment Setup

- 1 Attach the force transducer to the channel 3 input on the iWorx unit.
- 2 Plug the BNC-double banana adapter into the positive (red) and negative (black) sockets of the iWorx 214 stimulator. To insure the correct polarity of stimulation, check the side of the double banana adapter for a tab. This is the side of adapter that goes into the negative (black) socket of the stimulator.
- 3 Attach the BNC connector of the stimulator cable to the adapter already on the stimulator.

The Dissection

- 1 On your double-pithed frog, cut the skin all the way around each knee and then pull down to remove the skin from the lower legs.
- 2 Find the gastrocnemius muscle on each side. Use scissors to free the Achilles tendons from the connective tissue around the heel of the feet. Then firmly tie a thread around each Achilles tendon as close to the toes as possible, leaving the ends of the thread long enough (at least 4") to secure to the muscle force transducer.
- 3 Cut the Achilles tendons below each thread, so the thread is still attached to the gastrocnemius muscle.
- 4 Use a glass hook to separate the gastrocnemius muscle from the bone and other muscles of the lower leg. The muscles should be free all the way up to the knees. Moisten the exposed muscles with Ringer's solution every five minutes or so.
- 5 Laying the frog on the tray with dorsal side up, slit the skin on the thighs and separate the muscles of the upper leg to expose both sciatic nerves.
- 6 Use a glass hook to separate the nerve from the fascia and the vessels for each leg. If possible, avoid cutting the blood vessels. If bleeding does occur, rinse away the blood with lots of Ringer's solution.
- 7 Free each nerve from the knee joint to the pelvis. Use the glass hook to place a thread under each nerve, but do not tie them. Bring the ends of each thread together to make a "handle" for gently lifting that nerve for stimulation. Keep the exposed nerve moist at all times with Ringer's solution.
- 8 For one leg, pass a second thread under the sciatic nerve. Bring the ends of this thread around to the front of the leg and tie as tightly as possible, forming a tourniquet that includes everything in the upper leg except the sciatic nerve. If one leg bled during dissection, use this leg, so that you have one leg with good circulation and the other with no circulation.
- 9 Using 4 dissecting pins for each leg, pin the knees down to the tray, making an "X" above and below each knee. Do not interrupt blood flow to the gastrocnemius muscles.
- 10 Tie the two threads attached to the Achilles tendons together after passing one of them through the loop on the force transducer, so that contracting either muscle will pull down on the transducer. Raise the force transducer so that the threads are taut.

Start the Software

- 1 Open the LabScribe folder on the desktop, click on Settings at the top, and open the file called **Skeletal Muscle Weight-Work**. Then close the pdf file that opens with it.

Procedure

- 1 Using the "handle", gently lift up on a nerve just enough to slide the two prongs of the electrode under it. Stimulate at a very low voltage, gradually increasing it until you get a

very small muscle twitch. Record this "threshold voltage", and then repeat with the sciatic nerve on the other leg.

- 2 Have the lab instructor inject the frog with curare (1 ml)
- 3 Every 5 minutes for at least 30 minutes, repeat your measurement of the threshold voltage for each leg.
- 4 At the end of the exercise, directly stimulate the two gastrocnemius muscles, and determine their thresholds. Record this data.

Exercise 3: Human Responses to Stimuli

The next two exercises can be done while waiting for the curare effect to be seen, during the 5-min. intervals between determining the frog muscle thresholds. These involve measuring the time which occurs between a stimulus and the response, which is an indicator of the amount of integration that occurs.

Aim: Observe and compare the time reaction time of your lab partner to auditory vs. visual stimuli.

Background

The integration in a spinal reflex is fast, but not very sophisticated. More complicated integration required the brain, and more time elapsed between the stimulus and the response.

Equipment Required

EM-100 Event marker
PT-104 Pulse plethysmograph

Equipment Setup

- 1 Connect the iWorx unit to the computer.
- 2 Plug the DIN connector of the event marker in Channel 3.
- 3 Plug the DIN connector of the plethysmograph into Channel 4. The equipment should look like Figure 4.

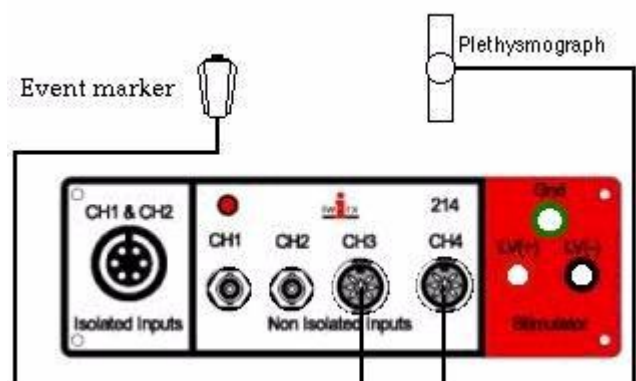


Figure 4: The equipment used to measure reaction times from a volunteer.

Start the Software

- 1 Click on the **Settings** menu and select the **Auditory-VisualReflexes** settings file.
- 2 After a short time, LabScribe will appear on the computer screen as configured by the **Auditory-VisualReflexes** settings.

3 Close the pdf file that opens with it.

3A: Reaction Time and Sound

Procedure

1 Have the subject relax in a chair placed so that the subject's back is to the computer screen and the keyboard.

2 Have the subject listen as another student taps the white surface of the plethysmograph with a pencil. Make sure the subject can hear the tapping sound.

3 Ask the subject to click the event marker as soon as he/she hears the tap.

4 Click **Start**.

5 Present the subject with a total of 10 taps, but deliver the taps in a pattern that is difficult for the subject to predict.

6 Click **Stop** to halt recording.

7 Select **Save As** in the **File** menu, type a name for the file. Choose a destination on the computer in which to save the file (e.g. the **iWorx** or class folder). Click the **Save** button to save the file (as an *.iwd file).

Data Analysis

1 Click the **2-Cursor** icon (Figure 5), so that two blue vertical lines appear over the recording window.

2 Drag the cursors left and right so that the large spike on the plethysmograph channel and the signal from the event marker are located between the two blue lines.

3 Click the **Analysis** icon (Figure 5) to open the **Analysis** window.

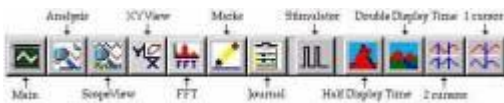


Figure 5: The LabScribe toolbar.

4 Display Channels 3 (**Reaction**) and 4 (**Stimulus**) by deselecting Channels 1 and 2 in the **Display Channel** list, on the left side of the **Analysis** window. Select **Title** and **T2-T1** from the **Table Functions** list.

5 Use the mouse to click and drag one cursor to the beginning of the spike on the Stimulus channel and the second cursor to the onset of the signal from the event marker on the **Reaction** channel (Figure 6).

6 Data can be entered into the **Journal** by either typing the titles and values directly or by using the **right-click** menu. Place the cursors to take measurements; then, select **Add Title to Journal** or **Add Data to Journal** from the **right-click** menu to add the measurements to the **Journal**.

7 While in the **Analysis** window, use the scroll bars to move through your data and repeat the measurements for all 10 trials.

8 Drop the longest and shortest times from the data set, and average the remaining eight values to determine the **mean reaction time**.

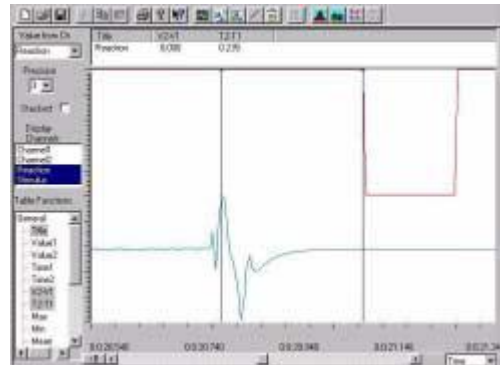


Figure 6: Data produced by tapping the plethysmograph, which entered a large spike on the lower trace and produced a sound, which the volunteer used as a cue to press the event marker (upper trace). The data are displayed in the Analysis window and the two cursors are positioned to measure the reaction time ($T2-T1$).

3B: Reaction Time and Visual Cues

Procedure

1 Have the volunteer sit in a chair and face the computer screen.

2 Have the subject watch the screen and quickly press the button of the event marker as soon as they see a deflection in the stimulus trace.

3 Out of sight of the subject, a second student should prepare to gently tap the plethysmograph. They should avoid giving any auditory cues.

4 Click **Start**. Present the subject with a total of 10 trials, delivered in a pattern that is unpredictable.

5 Click **Stop** to halt recording.

6 Select **Save** from the **File** menu.

Data Analysis

Your result may look like Figure 7.

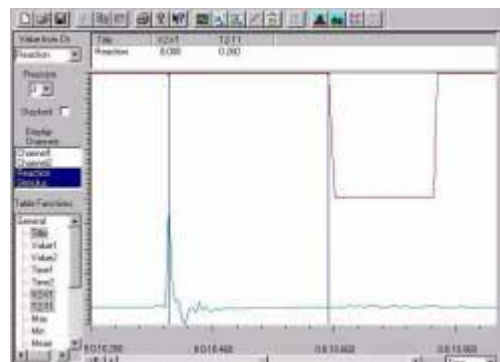


Figure 7: Data produced using a visual cue from the plethysmograph. The volunteer used this visual cue on the screen (lower trace) to press the event marker (upper trace). The data are displayed in the Analysis window and the two cursors are positioned to measure the reaction time ($T2-T1$).

1 Use the cursors to measure the time delay between visual stimulus (the onset of the tap) and response (the onset of the mark).

2 Repeat the measurements for all 10 trials.

3 Drop the longest and shortest times from the data set, and average the remaining eight values to determine the mean reaction time.

Questions

How does the average reaction time from Exercise 3A compare to the data from Exercise 3B? What would cause reactions times to oral and visual cues to differ?