# **Reflex Functions and Other Physiology Principles: Active Learning and Real Time Analysis**

# Elizabeth McCarthy, Parthena Sanxaridis Mantis and Angela Seliga

Boston University, Department of Biology, 5 Cummington Mall, Boston MA 02215 USA (emccart2@bu.edu; psmantis@bu.edu; amseliga@bu.edu)

At Boston University, we have two large enrollment physiology courses; one for health and rehabilitation and one for pre-medical students. Both courses use equipment from iWorx, which allows students to record and analyze human and animal data in real time. This laboratory module was designed to address misconceptions about reflexes and learned responses, by comparing patellar tendon reflexes to a tactile learned response. ABLE workshop participants also explored our other laboratory modules to demonstrate the breadth of physiology concepts we explore with this equipment, as well as to facilitate discussions on how to develop or adapt these modules.

Keywords: Physiology, Reflexes, Skeletal Muscle, iWorx

## Introduction

The physiology courses at Boston University are large (350-450 students each semester), with a weekly 3hour laboratory component, divided into laboratory sections with up to 24 students in each section, taught by a graduate student teaching assistant (GTA) and an undergraduate assistant (UA). The 200-level course is the third and last core course for students pursuing health and rehabilitation sciences, preceded by introductory biology and anatomy. The 300-level course is for pre-medical students, from majors in neuroscience, human physiology, and biomedical engineering, and is required for most of the specializations for biology majors. Despite the differences in prior knowledge, the laboratory components are very similar. Both courses have nine weeks of laboratory modules that cover key physiology principles with detailed laboratory procedures, and most consist of products from iWorx, which includes the hardware and attachments, and software (LabScribe 2, LS2). Our students collect and analyze physiology data mostly from themselves, while we also have one animal laboratory module. Currently, iWorx products are in six of our nine modules, but in the ten years we have been teaching with this equipment, we have heavily modified the modules by developing tutorial videos, pre-laboratory assignments and post-laboratory questions as well as developed different topics to explore using existing equipment.

Our typical module design is that students read the module prior to class, which includes background, procedures and post-laboratory questions to be completed during class. Students complete a short pre-laboratory assignment and an in-class pre-laboratory quiz and hear an introductory lecture given by a GTA or UA. Some of our modules include pre-laboratory videos that we have created based on the common issues our students have had. For example, the first week consists of 5 short tutorial videos about how to use LS2 software to analyze experimental data. Our Reflexes and Somatosensory module is the fourth week of laboratory modules, typically presented after the first lecture exam covering membrane transport and neurons. Many of the introductory lectures incorporate active learning where students work in groups on a white board activity to assess their level of preparation and to identify and address misconceptions. The pre-laboratory assignment in this module is related to the in-class white board activity asking students to compare the neural pathways of the patellar reflex arc to other reflexes and learned responses. In this 3-hour laboratory module, students work in groups of two or three and act as both the subject and experimenter to test the functions of sensory units, including adaptation and stimulus localization, in different areas of the body, and to test reflexes, including eve, vestibular and tendon reflexes. At our ABLE workshop, only the patellar tendon reflex and learned response activities from this module were explored. This left sufficient time to explore a few of our other iWorx

modules, i.e. cardiac muscle, skeletal muscles, resting metabolic rate (RMR) and lung capacity to demonstrate the breadth of physiology concepts we explore with this equipment.

In the first 90-minutes of the workshop (Part 1), participants completed an abbreviated tutorial of the LS2 software (Activity 1.1), the active learning white board

activity for our Reflexes and Somatosensory module (Activity 1.2), and performed the tendon reflex and tactile learned response activity (Activity 1.3). After the break (Part 2), participants explored our other iWorx modules and discussed the design and adaptation of the modules.

<b>Table 1.</b> Format of Part 1- Kenex Function Laboratory wide
--

#	Activity	Description		
1.1	LS2 Tutorial	Video tutorial to become familiar with the LS2 icons and		
		functions that will be used during the module.		
1.2	Active Learning Activity: Reflex Arcs	Interactive board work activity to establish background on		
		reflexes.		
1.3	Patellar Reflex vs Tactile Learned Responses	Test patellar reflex and tactile learned response using iWorx		
		units and LS2 to visualize responses.		

-			
#	Activity	Description	
2.1	Physiology of Circulation	Compare electrical activity of cardiac muscle to contractio	
		measured by listening to heart sounds.	
2.2	Skeletal Muscle Physiology	Analyze electrical activity in skeletal muscle by comparing	
		the dominant and non-dominant arm.	
2.3	Resting Metabolic Rate (RMR)	Determine the resting metabolic rate.	
2.4	Respiratory Volumes and Capacities	Measure lung volumes and capacities.	

The Student Outline contains customized and abbreviated versions of the Patellar Reflex vs Tactile Learned Response activities using iWorx. Our students receive these protocols in our published laboratory manuals (Rycroft *et al.* 2013, Seliga and Narain 2013.) Details for the activities presented in Part 2 of the workshop can be found in the Appendices.

In Materials, all of the supplies and equipment necessary to run all of the iWorx activities we will

demonstrate are listed, based on 12 student stations with 2-3 students/station.

In Notes for the Instructor, we included the learning objectives and background of the Patellar Reflex vs Tactile Learned Response activities. This section also contains pre-laboratory setup details, in-lab instructions, troubleshooting guides, and discussion and extensions of the activities. The details for the activities presented in Part 2 can also be found in the Appendices.

# **Student Outline**

#### Part 1: Activities for Reflex Functions Lab

In Part 1, participants will act as students for the reflex activities that are part of our larger Reflexes and Somatosensory module. In Part 2, during the second portion of this module, participants can choose at least one of our other iWorx activities that will be set up to explore other physiology concepts our students learn. Some of the stations are relatively easy and multiple stations could be visited, but RMR in particular is a lengthy procedure.

## Activity 1.1: LS2 Tutorial

#### 1.1A Activity Objective

LS2 will be used to collect data in this module and this tutorial will help you learn the key icons and functions need to gather and analyze your data.

#### 1.1B Results

After watching the tutorial video, describe the LS2 icons and functions that you will be using in today's activities in Table 3.

Icon or Feature	Name	Function (What happens when you use it? When should you use it? )
N	Main	
2	Analysis	
Mark 💌	Mark box	
4	Double Display Time	
***	Double Cursor	
<b>⊕</b>	Zoom between cursors	
Record	Record	
AutoScale	AutoScale	
$\nabla$	Invert	
T2-T1	T2-T1	

Table 3. Common LS2 icons and functions used to collect and analyze data

Indicate on the example screenshot in Figure 1:

- Which channel represents the EMG recording of the reflex response? Learned response?
- Which segment contains noisy data?
- Where you would put your cursors to determine the latency of your learned response?



Figure 1. Example screenshot of data collected during Patellar Reflex vs Tactile Learned Response activity.

# Activity 1.2: Active Learning: Reflex Arcs

1.2A Activity Objective

The general components of a reflex arc are the stimulus, receptor, integrating center, effector, and response. Identify the specific components of a patellar tendon reflex.

## 1.2B Results

Fill in the general components of the reflex arc in Figure 2.





#### 1.2C Discussion Questions

Compare this reflex arc to other reflexes and learned responses.

#### Activity 1.3: Patellar Reflex vs Tactile Learned Response

#### 1.3A Activity Objective

Reflexes are automatic responses caused by the stimulation of a sensory receptor that generates the appropriate rapid response. Reflexes do not require action from higher brain centers; tendon reflexes function through integration in the spinal

cord. On the other hand, tactile learned responses are reactions to stimuli that require higher brain centers to integrate those stimuli before producing a response. These actions can be trained over time to become faster, but they are not reflexive. In this activity, you will test both your patellar reflex and tactile learned response, while recording the total electrical activity (or electromyogram; EMG) generated to contract your quadriceps femoris muscle using LS2. You will be able to visually see the stimulus (tendon tap), the reflex response (EMG) and the event marker (learned response), and determine the latency between the stimulus and the responses. See Tables 5 and 6 in Materials for the equipment for this activity.

#### 1.3B Methods

Setup Student Station

- 1. Check that the EMG cable is plugged into "Channels 1 & 2", the reflex hammer into CH2 and the event marker into CH3 of the iWorx unit.
- 2. Turn on the iWorx unit by flipping the switch on the back right of the black box.
- 3. Click on the LS2 icon (white with a red i) from the dock on the computer screen.
- 4. Click on the Settings pull down menu and select "Load Group" and do the following
  - Select the "Settings" folder then click on ABLE.iwxgrp for our customized settings.
    - Click on the Settings pull down menu again and select "Learned Responses" and "TactileReactionLS2" (see Figure 3). These settings have three recording channels.



Figure 3. Screenshot to load correct settings for Activity 1.3.

#### Test Patellar Reflex

- 1. Expose your quadriceps femoris muscles; change clothes if necessary.
- 2. Test each other's patellar tendons to determine who is the easiest to elicit a reflex response from by performing the following:
  - The subject should sit on the bench such that the hamstring muscle (muscle above the back of the knee) is FULLY supported by the bench, sitting as far back as he/she can with one leg hanging in a relaxed manner (foot should be off the ground and not resting on a chair leg/foot support).
  - Find the subject's tendon, which is the springy band of tissue below the hard patella (knee cap) and above the hard tibial bone.
  - While the subject's eyes are closed, strike the tendon with the blunt side (black) of the rubber mallet. The normal response is contraction of the quadriceps femoris muscle, which causes an extension of the leg, i.e. the knee-jerk response.
  - Repeat two more times. If the subject has three responses, this is the knee to use! If not, try the other knee. If neither knee works AND you are hitting the target, switch to test the other partner's knees.
  - Proceed with the subject's knee that had the most number of responses to three hits.

#### Electrode Placement

1. The selected subject should remove all jewelry or other metal from his/her legs and feet, and move all nonlaboratory electronics, such as a cell phone, as far away as possible.

- 2. The subject should sit on the bench and extend the lower leg such that the lower leg is parallel to the floor and maintain this position. Feel and observe the quadriceps femoris muscle contract. These are the muscle fibers you will record EMG activity from.
- 3. Use an alcohol swab to scrub the quadriceps femoris muscle where the electrodes will be placed.
- 4. After the areas dry, attach the disposable electrodes in the following way: one is about 12cm above the knee (on the "meat" of the muscle), the second is about 10cm above the first (closer to groin), and the third is outside of the kneecap.
- 5. Snap the lead wires onto the electrodes on your leg so that:
  - "+1" lead attached to the electrode closer to the groin, red
  - "-1" lead attached to the electrode closer to the knee, black
  - "C" lead (the ground) is attached to the electrode on the knee, green

#### Testing Equipment

- 1. Click Record on LS2.
- 2. Place a "test EMG" mark in the marks box on LS2 and hit enter.
- 3. Refer to the LS2 icons found in the upper left corner of the recording screen (see Figure 4) to test the EMG signal by performing the quadriceps extension (contract and hold, then relax). After AutoScaling the top channel, if an obvious signal is not observed, the red and black electrodes need to be moved to a different position on the muscle. Repeat as necessary.



Figure 4. Common icons used in LS2.

- 4. Place a "test hammer" mark in the marks box on LS2 and hit enter.
- 5. Test the reflex hammer signal by tapping the bench and AutoScale the second channel.
- 6. Place a "test marker" mark in the marks box on LS2 and hit enter.
- 7. Test the event marker by pressing and releasing several times and AutoScale the third channel.
- 8. You can stop the recording or proceed directly to the next section.

#### Data Collection

- 1. Place "patellar tendon" mark in the marks box on LS2 and hit enter.
- 2. While the subject's eyes are closed, strike the tendon with the blunt side (black) of the rubber mallet to elicit the patellar reflex response.
- 3. Repeat step 2 with the following modifications:
  - The subject should hold the event marker and close his/her eyes.
  - When the subject feels you hit the tendon, the subject should press and release the event marker.
- 4. Repeat step 3 for a total of 3 stimulus-response sets.
- 5. Click Stop.

### 1.3. C Results

- 1. Go to the Mark labeled "patellar tendon" and click the AutoScale buttons for all three channels.
- 2. Use the Double Cursors icon and Zoom Between Cursors icon (see Table 1 and Figure 5) to show all three stimulus-response sets. AutoScale all channels for an optimized view.
- 3. Click on the Single Cursors icon and drag to the baseline just before the visually strongest EMG of the three stimulus-response sets.
- 4. Copy a screenshot of all channels by clicking on the Edit pull down menu and select "Copy" and "Paste" into a MS Word file.

5. Click the Double Cursors icon so that two blue vertical lines appear over the EMG channel on the Main window. Click the Analysis icon, do the following measurements and record in Table 4:

EMG Latency (delay of the reflex response): Drag the right cursor at the peak of the largest EMG and the left cursor at the peak of the tendon strike that caused that EMG. T2-T1 is already displayed in the toolbar.

Tactile Latency (delay of the learned response): Leave the left cursor at the peak of the tendon strike and drag the right cursor at the start of the Event Marker. T2-T1 is already displayed in the toolbar.

Table 4. Fatellal Kellex vs	Tacthe Learned Responses Data.
Subject:	
•	
	Strongest Patellar Tendon EMG
EMG Latency ( )	
Tactile Latency ( )	

Table 4. Patellar Reflex vs	Tactile Learned Resp	onses Data
-----------------------------	----------------------	------------

#### 1.3.D Discussion Ouestions

Compare the latencies during the patellar reflex test and tactile learned responses. Is this what you would expect based on the neural pathway for this reflex? Explain based on neural pathways.

#### Part 2: Additional Physiology iWorx Laboratory Modules Activity 2.1: Physiology of Circulation

#### 2.1 Activity Objective

Electrocardiograms (ECGs) record the electrical activity of the heart that cause contraction and relaxation of the heart muscle. Different peaks in the ECG recording correspond to different parts of the cardiac cycle and this electrical activity can be recorded and visualized using the same cables and electrodes as Activity 1.3. In this activity you will be able to visualize the electrical waves as they move through the heart. The addition of the event marker (also used in Activity 1.3) and a stethoscope allow you to compare the mechanical closure of the heart valves during ventricular contraction to the ECG. See Tables 5 and 7 in Materials for the equipment and Appendix A for methods for this activity.

#### Activity 2.2: Skeletal Muscle Physiology

#### 2.2 Activity Objective

In this activity students will record EMGs generated by the contraction of forearm muscles to explore the relationship between electrical activity and muscle contraction strength. The EMG can be recorded using the same cables and electrodes used in Activity 1.3, while muscle strength can be recorded using a hand dynamometer attached to the iWorx unit. By squeezing the hand dynamometer, you can compare EMG intensity with increased muscle strength. This activity compares the dominant and non-dominant arms. See Tables 5 and 8 in Materials for the equipment and Appendix B for methods for this activity.

#### Activity 2.3: Resting Metabolic Rate (RMR)

#### 2.3 Activity Objective

Metabolic rate refers to all the chemical reactions that are occurring in the body at a given point in time. This measurement provides information about how the body is using energy. The RMR of a subject can be determined by measuring the rate of oxygen utilization with indirect calorimetry. In this activity you will determine your RMR using a  $CO_2/O_2$  gas analyzer and reusable mask attached to an iWorx unit. See Tables 5 and 9 in Materials for the equipment and Appendix C for methods for this activity.

#### **Activity 2.4: Respiratory Volumes and Capacities**

#### 2.4 Activity Objective

Measuring lung capacities and volumes provides information about the respiratory efficiency of a patient and can indicate their health and fitness levels. Vital Capacity (VC) is the total volume of air that can be exchanged in one breath and is one of the factors measured when while diagnosing lung disease. In this activity, you will measure your vital capacity (Functional Vital Capacity) using a spirometer and a flow head, and compare that measured value to your predicted VC. A healthy VC is considered to be measured within +/- 10% the the predicted VC for your age, height and weight. See Tables 5 and 10 in Materials for the equipment and Appendix D for methods for this activity.

# Materials

#### **Module Part 1**

The following six tables list the materials needed to conduct this workshop. Table 5 provides a general list of equipment required at each student station to conduct all of the activities in this workshop that use iWorx and LS2. The kits that can be purchased from iWorx include several customizable components depending on the types of laboratories instructors want to run. The specific iWorx attachments and consumable supplies for each activity are listed in Tables 6-10 and are listed by unit price. For a class of 24 students, resulting in 12 student groups, you will need 12 student stations. Each computer at a student station will need the custom LS2 settings for the workshop loaded on them. Although not listed in any of the following tables, you could also use a chalk or white board in order to conduct learning activities at the beginning of the laboratory session, in addition to a projector to showcase your in-class presentation.

Table 5. General Equipment for Each Student Station

ITEM	CATALOG #	PRICE		
Computer (MAC)	iMac (Apple)	\$1000.00		
iWorx Unit	AHK-214 (iWorx)	\$5000.00		
LabScribe 2 (LS2)	(download from iWorx)	Included w/AHK-214		
USB Cable	C-USB (iWorx)	Included w/AHK-214		

Table 6. Activity 1.3: Patellar Reflex vs Tactile Learned Responses.

ITEM	CATALOG #	PRICE
C-AAMI-504 Cable & Electrode Lead wires (red,	C-AAMI-504 (iWorx)	Included w/AHK-214
black, green)		
PRH-100 Reflex Hammer	PRH-100 (iWorx)	\$85.00 each
EM-100 Event Marker	EM-100 (iWorx)	Included w/AHK-214
Disposable EMG Electrodes (3/subject)	FS-TB1 (Skintact)	\$6.00/30 pcs.
Alcohol Wipes	06-669-62 (Fisher Sci)	\$83.00/4000 pcs.

#### Module Part 2

**Table 7.** Activity 2.1: Physiology of Circulation.

	· · · · · · · · · · · · · · · · · · ·	
ITEM	CATALOG #	PRICE
C-AAMI-504 Cable & Electrode Lead wires with 5	C-AAMI-504 (iWorx)	Included w/AHK-214
ECG Leads		
EM-100 Event Marker	EM-100 (iWorx)	Included w/AHK-214
Stethoscope	71-872 (Fisher Sci)	\$12.00 each
Disposable ECG Electrodes (5/subject)	FS-TB1 (Skintact)	\$6.00/30 pcs.
Alcohol Wipes	06-669-62 (Fisher Sci)	\$83.00/4000 pcs.

Table 8. A	Activity	2.2:	Skeletal	Muscle	Physiol	logy.
------------	----------	------	----------	--------	---------	-------

ITEM	CATALOG #	PRICE		
C-AAMI-504 Cable & 3 Electrode Lead wires (red,	C-AAMI-504 (iWorx)	Included w/AHK-214		
black, green)				
Disposable EMG Electrodes (6/subject)	FS-TB1 (Skintact)	\$6.00/30 pcs.		
Hand Dynamometer	FT-325 (iWorx)	Included w/AHK-214		
20lbs (9.07kg) weight	BR-020 (Troy)	\$22.00 each		
Alcohol Wipes	06-669-62 (Fisher Sci)	\$83.00/4000 pcs.		

ITEM	CATALOG #	PRICE
GA-300 CO <sub>2</sub> /O <sub>2</sub> Gas Analyzer with Filter	ETS-300 (iWorx)	\$4000.00
A-FH-1000 Flow Head	A-FH-1000 (iWorx)	Included w/ETS-300
Reusable Mask (small) with straps and Non-	113641 (Hans Rudolph)	Included w/ETS-300
Breathing Valve		
Reusable Mask (small), extra	669197 (Hans Rudolph)	\$71.04 each
Mask adapter to pulmoguard filter	CC1842 (Hans Rudolph)	\$467.94 each
Filter to valve adapter	171099 (Hans Rudolph)	\$33.76 each
Pulmogard filter	29-7950-050 (SDI diagnostics)	\$100.00/50 pcs.
SP-304 Spirometer with Flowhead and Tubing	SP-304 (iWorx)	Included w/AHK-214

|--|

Table 10. Activi	ty 2.4: Respiratory	Volumes and	Capacities.
------------------	---------------------	-------------	-------------

ITEM	CATALOG #	PRICE
SP-304 Spirometer (small black box) with flow	SP-304 (iWorx)	Included w/AHK-214
head tubing (white)		
FH-300 Flow Head	A-FH-300 (iWorx)	\$85.00 each
Disposable cardboard mouthpiece	162702 (A-M Systems)	\$19.32/100 pcs.

# Notes for the Instructor

Part 1 of this workshop is part of a larger 3-hour laboratory module that focuses on reflexes and somatosensory function. Our laboratory courses are set up to complement lecture material from two different associated physiology classes. The lecture professors in the different courses selectively cover reflexes and somatosensation with different levels of detail and emphasis, which means that the background knowledge that the students in the different courses come to class with varies. Even so, the activities are flexible enough to be easily modified for effective presentation to students enrolled in both courses. In previous modules, students will also have covered general neuron structure and function concepts, including resting membrane potential, threshold and summations, as well as the difference between action potentials and graded potentials. This module occurs a third into the semester after the students have already had an extended version of the LS2 tutorial (Activity 1.1) and have been working in the same groups of 2-3 students. This is important because in order to be successful, this module depends heavily on familiarity with the LS2 program, which can be challenging for students. This module also occurs at a point in the semester when the teaching staff has received multiple, weekly training sessions so that they are familiar with the program and procedures and are better able to help the students. Students have the background and procedures, as well as the guidelines for the pre- and post-laboratory assignments, written out in their laboratory manuals (Rycroft et al. 2013, Seliga and Narain 2013.) We have generated specific tutorial videos on using LS2 with the reflex module that students can view before coming to class.

#### Part 1: Notes for Reflex Functions Lab

## Activity 1.1: LS2 Tutorial (15-20 min)

1.1 Activity Objective/Learning Goals

Familiarity with LS2 is essential to many of the laboratory modules taught in our physiology courses. Students use LS2 to collect and analyze data in six out of nine modules over the course of the semester. The first module of the semester is dedicated to learning how to use this program so that by the time students reach the Reflex and Somatosensation module presented in the first part of this workshop, they are comfortable with the program. Students are given access to tutorial videos that explain the functions and power of LS2 for collecting physiology data and also have a pre-laboratory assignment to complete before the first module. Activity 1.1 presented in this workshop is a modified version of this assignment given to our students so that participants in the workshop can become familiar with LS2 before starting the rest of the activities.

# Activity 1.2: Active Learning Activity: Reflex Arcs (10-15 min)

#### 1.2A Activity Objective/Learning Goals

In this activity students are asked to work in groups to fill in the components of a general reflex arc. The goal of this activity is to create discussion about reflex arcs and to draw connections to the reflex tests performed in lab. This diagram is very flexible and can be adapted to describe multiple different reflexes at different levels of detail.

#### 1.2B Pre-laboratory Set-up

We pre-draw four sets of the diagram shown in Figure 2 of the Student Outline on the white boards in the classroom and have dry erase markers available to the students. The activity could also be turned into a worksheet that students could complete at their stations.

#### 1.2C In-lab Instruction

Students are split into groups and told to fill in the components of the reflex arc. The pre-laboratory assignment for this module contains questions about reflex arcs and learned responses. With multiple white boards, the GTA leads discussion at one board (containing 2 diagrams and two student groups of three students each) and the UA leads at the other board with two other groups. It can also be helpful to have a list of possible categories written on the board (receptor, integrating center, etc.) to help guide the students through the activity and to give them a frame of reference, or the diagram can be completed entirely through discussion with the students. A completed reflex arc for the patellar reflex is shown in Figure 5.



Figure 5. A sample of a completed general reflex arc diagram.

#### 1.2D Troubleshooting

If students appear to have difficulties with this task you could assist by providing some terms and have them place the terms in the diagram. You could promote discussion by giving them examples and walking them through the arc. You could also ask questions that will lead them to the correct diagram terms without actually giving the students the answers directly.

#### 1.2E Discussions and Extensions

The general diagram can be modified to include specific components of the reflex arcs studied in this module. For example, the diagram above could be completed for the Achilles reflex (stimulus  $\rightarrow$  stretch receptor  $\rightarrow$  spinal cord  $\rightarrow$  gastrocnemius  $\rightarrow$  response). In this module, we also discuss the Jendrassik's maneuver and its impact on the reflex response, which can be added to the diagram during discussion. This diagram can be modified to include the reflexes of the cranial nerves as well as the role of the brain in tactile learned responses to link together multiple activities in this module.

#### Activity 1.3: Patellar Reflex vs. Tactile Learned Responses (40 min)

#### 1.3A Activity Objective/Learning Goals

In this activity, students investigate patellar tendon reflexes and tactile learned responses (pressing the event marker when they feel the tendon strike) using iWorx and LS2. Testing the patellar tendon reflex is a common clinical test done to determine the integrity of a patient's reflex arc. The normal response to a patellar reflex test is an extension of the knee and contraction of the quadriceps femoris muscle. Students will be able to visualize the responses to the stimulus in real time and then analyze different aspects of those responses, such as response latency. From this activity, students will be able to visually see and measure that learned responses, which require integration in the brain, take much longer to carry out than reflexes, which only require processing in the

spinal cord. Students should also be able to relate the stimulus-response sets they are seeing to the reflex arcs we presented at the start of class.

#### 1.3B Pre-laboratory Set-up

Before each lab, the student laboratory stations need to be set up (see Tables 5 and 6 in Materials section). Each station consists of a computer that has the LS2 software loaded and is attached to an iWorx unit. Each station also needs an EMG cable with three leads attached (green, red and black) and a reflex hammer. The EMG cable plugs into Channels 1 and 2 of the iWorx unit, the reflex hammer plugs into Channel 2 and the event marker plugs into CH3 (Figure 6.) Each station also needs three disposable EMG electrodes to attach the subjects to the EMG cables and alcohol swabs to clean areas of the leg prior to attaching the electrodes. All of the iWorx equipment can be set up once for the entire week of laboratory sections, and only the disposable components (electrodes and alcohol wipes) would need to be replaced at each station before each section.



**Figure 6.** Student station set up with a computer and an iWorx unit. The unit has the EMG cable with three leads attached to Channels 1 & 2 and the reflex hammer attached to Channel 2 (CH2). The event marker is also shown in this diagram attached to CH3.

For this activity, students need to be able to expose their quadriceps femoris so we tell them the previous week that they should bring shorts for this module or bottoms that can lift high enough. We also tell students the electrodes are very sticky and can be difficult to remove from subjects with a lot of leg hair. Students watch the pre-laboratory video we created prior to class that describes correct electrode placement on the quadriceps muscle as well as data collection and analysis.

#### 1.3C In-lab Instruction

Have students load the custom settings, after opening LS2, to set up the program and have the students do the protocol detailed in the Student Outline (Activity 1.3B). It is important to remind students to perform this test with their eyes closed so that they cannot anticipate the tendon strike and for the partner to hit the tendons accurately. Students really enjoy this activity, which allows them to

see reflexes in action both in the actual reflex response observed in the subject and in the data recording.

Data analysis for this activity requires familiarity with LS2 and can be frustrating to students. We run this module a few weeks after our introductory tutorial module with our pre-laboratory videos. We recommend that students re-watch those videos before starting this module. During the pre-laboratory lecture, our GTAs draw a mock screenshot on the board and walk through what data the students can expect to see. We also give them an approximate time frame they can expect to have the data collection completed in. We recommend that the students collect all of the reflex and learned response data before they start any data analysis to streamline analysis. While students are doing these activities, instructors walk through the room to make sure that students are collecting data correctly and to answer any questions that may come up. An example screenshot of data students will collect is shown below in Figure 7.



**Figure 7.** Sample recording from LS2 showing all three channels (from top to bottom: reflex EMG, tendon strike and the learned response). Cursors are in position to determine the latency of the learned response for the second stimulus-response sets.

#### 1.3D Troubleshooting

Finding the patellar reflex can sometimes be difficult. Students often strike the wrong part of the leg (the knee cap) with the reflex hammer and fail to elicit the reflex, or they will not strike the tendon hard enough to produce a reflex response. Also if students have any tendon damage from an injury they will not make a good subject for this experiment. Attaching the electrodes correctly (tightly and in the correct orientation) is also critical to getting a good signal. It is also important for the students to test the event marker and make sure that it is being registered in LS2.

During data collection, students can have difficulty getting clean recordings. Noise in the recording can be eliminated or reduced by removing metal jewelry and cell phones and placing them away from the iWorx unit and by making sure that all wires are securely connected. It is also possible that the electrodes are not placed in the best orientation. Adjusting the positioning of the red and black electrode (the two on the quadriceps femoris muscle) to make sure that they are placed on the large part of the muscle and not placed either too far or too close together can also help produce a cleaner recording. If students are able to distinguish between the baseline recording and the peak of the EMG after the tendon strike, they will be able to analyze their data. Remembering to AutoScale the data is also important to make sure students are getting a strong signal.

#### 1.3E Discussions and Extensions

Discussions include relating the learned response to the reflex arc discussed during the introductory background and comparing the different lengths of the neural pathways for a reflex and a response with the latencies observed in the experiment. This activity can be expanded to include additional tendon reflexes; we have students test the Achilles reflex, which has a longer latency and smaller EMG amplitude than the Patellar reflex. We also have students measure the amplitude of the EMG response for each reflex with LS2. Measuring the EMG amplitude of each response becomes important when looking at the role of central inhibition on reflex response with the Jendrassik's maneuver. This is an isometric contraction of the arm and shoulder muscles carried out while performing a tendon reflex test to allow students to examine the role of inhibitory signals from the brain in the strength of that reflex response. Additionally, while the activities presented here use a special reflex hammer that can be attached to an iWorx unit to record the stimulus tendon tap, these activities could be performed with a regular reflex hammer. Students would be able to record their reflex response and determine

EMG amplitude using LS2. Students have post-laboratory questions to answer based on these activities that are collected at the end of the 3-hour class; some have been inserted into the Student Outline. Students also graph the results comparing the EMG strength of the different tendon reflexes alone and with the Jendrassik's maneuver. In our 300-level course, students are given minimal guidance about how to create graphs and only analyze group data. In our 200-level course, we incorporate an additional topic in almost every module that details exactly how to create graphs in Excel and how to work with data tables. Students in this course learn to calculate averages, standard errors, and Student ttests in Excel to describe class data.

# Part 2: Additional Physiology iWorx Laboratory Modules

The additional physiology modules using LS2 that participants explored in this workshop follow a similar format to the reflex and learned responses module presented in Part 1. The different modules occur at times in the semester that correspond to the material of the lectures associated with the course to help reinforce these concepts with the students. The modules all follow the same format of a pre-laboratory assignment and background reading for the students before they come to class, a pre-laboratory quiz, an introductory lecture with an active learning component, and then the physiology activities and post-laboratory questions. Specific details for each of the additional activities can either be found in the Appendices or in our laboratory manuals (Rycroft *et al.* 2013, Seliga and Narain 2013.)

#### **Literature Cited**

- iWorx Corporation. 2014. Complete Standard Teaching IPLMv4 Lab Manual. <u>www.iworx.com</u>. November 7, 2014.
- Rycroft, N., A. M. Seliga and P. Sanxaridis Mantis. 2013. B1315 Systems Physiology Laboratory Manual.

McGraw-Hill Education, New York, New York, 133 pages.

Seliga, A. M. and A. Narain. 2013. *BI 211 Human Physiology Laboratory Manual*. McGraw- Hill Education, New York, New York, 121 pages.

#### Acknowledgments

The authors of this workshop would like to thank all the GTAs and UAs at Boston University who have contributed to the development of the presented activities. We would specifically like to thank Nathan Rycroft for his contribution to the design and refinement of these laboratory modules. We would also like to thank Archana Narain, Rajendranath R. Selagamsetty, Kathryn Millen, Brittany Silva, Mariah Burch, Conor Fowler and Brian Gambardella for their contributions to the laboratory activates and pre-laboratory videos. Also thanks to Fred Earthrowl for preparing the classrooms for our modules.

#### About the Authors

Elizabeth McCarthy is a Ph.D. student in the Biology Department at Boston University. She has been a Graduate Student Teaching Assistant for numerous laboratory and discussion based courses including Physiology, Neuroendocrinology and Introductory Biology. She is also currently a co-author on the Physiology Laboratory Manual at BU where she has helped to create and adapt laboratory exercises for different physiology courses.

Parthena Sanxaridis Mantis earned her Ph.D. in biology at Boston University and is currently a co-author on the Physiology Laboratory Manual at BU. She has taught the Physiology laboratory course several times wherein she was involved in several reformations of lab and presentations, and has exercises mentored undergraduate assistants in the lab course interested in gaining teaching experience in science. After her postdoctoral training at BU, her passion for scientific research, scientific education and working with young scientists eager to conduct hands-on research made her excel as the coordinator for the Research in Science and Engineering (RISE) program at BU. For the RISE program, she was involved in reviewing high school student applications, successfully matching students with research faculty, mentoring high school students from across America during an intense 6-week summer program at BU all leading up to a poster symposium. It is extremely gratifying to know that some of these students go on to win awards for their work, some go on to publish with their summer lab mentors and all eagerly go on to bright futures in science.

Angela Seliga earned her Ph.D. in biology from Boston University and is currently the Physiology Laboratory Manager at BU, where she teaches, trains undergraduate and graduate students to teach, and develops curriculum for multiple physiology courses, as well as train educators across the STEM disciplines. As a graduate student at BU, she participated in the NSF funded GK-12 program where she taught biology and chemistry to high school students with special needs. She has designed several laboratory modules for outreach programs and continues to train undergraduate and graduate students to design and teach in these programs.

# Mission, Review Process & Disclaimer

The Association for Biology Laboratory Education (ABLE) was founded in 1979 to promote information exchange among university and college educators actively concerned with teaching biology in a laboratory setting. The focus of ABLE is to improve the undergraduate biology laboratory experience by promoting the development and dissemination of interesting, innovative, and reliable laboratory exercises. For more information about ABLE, please visit http://www.ableweb.org/.

Papers published in *Tested Studies for Laboratory Teaching: Peer-Reviewed Proceedings of the Conference of the Association for Biology Laboratory Education* are evaluated and selected by a committee prior to presentation at the conference, peer-reviewed by participants at the conference, and edited by members of the ABLE Editorial Board.

#### **Citing This Article**

McCarthy, E. A., P. Sanxaridis Mantis, and A. M. Seliga. 2016. Reflex functions and other physiology prinsciples: Active learning and real time analysis. Article 12 in *Tested Studies for Laboratory Teaching*, Volume 37 (K. McMahon, Editor). Proceedings of the 37th Conference of the Association for Biology Laboratory Education (ABLE), http://www.ableweb.org/volumes/vol-37/?art=12

Compilation © 2016 by the Association for Biology Laboratory Education, ISBN 1-890444-17-0. All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording, or otherwise, without the prior written permission of the copyright owner. ABLE strongly encourages individuals to use the exercises in this proceedings volume in their teaching program. If this exercise is used solely at one's own institution with no intent for profit, it is excluded from the preceding copyright restriction, unless otherwise noted on the copyright notice of the individual chapter in this volume. Proper credit to this publication must be included in your laboratory outline for each use; a sample citation is given above.

# Appendix A Physiology of Circulation

#### A.1 Student Outline

Methods

#### Set-up Student Station

The student station should be set-up by the laboratory instructor. The EMG cable should be connected to Channel 1 & 2 of the iWorx unit and the connector of the event marker CH3 of iWorx. The Physiology of Circulation laboratory custom settings ("ECGHeartSounds") should already be open in LS2.

#### Electrode Placement

- 1. The subject should remove all jewelry or other metal from their arms, wrists and hands, and all non-lab electronics as far away as possible.
- 2. Use an alcohol swab to scrub an inside region of each wrist and ankle that has little or no hair.
- 3. After the areas dry, attach the disposable electrodes. On the right wrist, place a fifth electrode adjacent to the other, or underneath if your wrists are small. Snap the other ends onto the disposable electrodes, so that:
  - "+1" electrode is attached to the left wrist, red
  - "-1" electrode is attached to the right wrist, black
  - "+2" electrode is also to the right wrist, white
  - "-2" electrode is attached to the left ankle, brown
  - "C" electrode (the ground) is connected to the right ankle, green

#### Data Collection

- 1. Subject should sit quietly with their hands in their lap and not move the ECG cords.
- 2. Click Record, then click AutoScale on both channels to observe the recordings. You should see the ECG in the top channel.
- 3. Record at least four ECG cycles and keep recording.
- 4. Subject should press the bell of the stethoscope over the clothing but against bare skin over the AV valves (tricuspid region), located on the left sternal border between the 3<sup>rd</sup>-6<sup>th</sup> intercostal spaces. His or her partner should listen for the lub-dup sounds to determine if placement is correct.
- 5. Once lub-dup is distinctly heard, the partner will press the bell of the stethoscope against the subject (the subject should not touch the metal stethoscope in order to have clean ECG data).
- 6. The partner should press the event marker when lub is heard, release it when dup is heard. The subject should watch LS2 to determine if his or her partner has matched sets (heart sounds matching the correct electrical activity in the ECG, refer to Figure 8).
- 7. Keep recording until you have at least four matched sets (the heart sounds match the electrical activity in the ECG).



**Figure 8.** Sample recording of ECG recording (top channel) and Heart Sounds (bottom channel). This screenshot shows three matched sets with cursors in position to determine period between two successive R waves.

#### Results

- 1. Copy a screenshot of both channels from four cardiac cycles into your MS Word file.
- 2. Label P, Q, R, S, and T of one cardiac cycle and label the corresponding lub-dup sounds on the event marker channel.
- 3. Measure T2-T1 between two successive R waves to determine the ECG period.
  - In the analysis window, click Add Function, General, and T2-T1. Then, calculate HR (60/T2-T1) and record in Table 11. Repeat for two more periods to calculate an average HR and record in Table 11.
- 4. Repeat step 3 for the heart sounds HR from the event marker (from lub to lub) to determine the average and record in Table 11.

Table 11.	. ECG and	Heart Sound	ls Heart Rate	Data
-----------	-----------	-------------	---------------	------

Subject:				
Trial	ECG Data		Heart Sounds Data	
	Period ( )	Heart Rate ( )	Period ( )	Heart Rate ( )
1				
2				
3				
Average				

#### Discussion Questions

1. How does the ECG heart rate compare to the heart sounds HR? Excluding potential equipment malfunction and human error, if a patient had an abnormal ECG cycle, what could happen to the heart sounds? If the heart sounds were abnormal, what could happen to the ECG?

#### A.2 Notes for the Instructor

#### Activity Overview/Learning Goals

In this activity, students investigate electrical activity waves in the heart muscle using an ECG recording, and relate that activity to stages of contraction and relaxation of different parts of the heart (with the heart sounds generated by the closing of different valves). By the end of the activity, students should be able to recognize different parts of the ECG recording and identify at each stage what is happening in the cardiac cycle. They should also understand that the movement of electrical impulses through the heart from the pacemaker is what causes the cardiac muscle to contract in sequence.

#### Pre-laboratory Setup

For this module, the same electrode cable can be used that recorded EMG in the activities in Activity 1.3. The ECG recording is generated using all five leads that come with the cable while the EMGs in Activity 1.3 are made using only three of the cables. All of the cables are included with the purchase of the original cable. Each student station (consisting of a computer and an iWorx unit) needs one electrode cable with the six leads, 6 disposable electrodes and 2 alcohol swabs. This will allow you to record just the ECG signal. To record the heart sounds, you also need an event marker attached to the iWorx unit and a stethoscope to listen to the heart at each student station. See Tables 5 and 7 in Materials.

- 1. Plug the AAMI connector on the subject cable to Channels 1 & 2 and plug the event marker Channel 3 of the iWorx unit.
- 2. Open the LS2 program and load the custom settings for the Circulation activity.

#### In-lab Instruction

At the start of this module, we have a discussion in the pre-laboratory lecture about electrical activity in the heart, how it is generated and propagated through the heart muscle, and how this activity corresponds to contraction. Properly recording the heart sounds can be tricky as they are very faint sounds that can be difficult to hear, especially if the stethoscope is not placed in the correct position and in a loud classroom. We make sure to go over the steps of recording the heart sounds at the start of class with the students. To collect data have students load the custom settings for the ECG lab and connect the electrodes to the subject and follow the steps found in the Student Outline section. Students that have a history of cardiac problems should not be the subjects in this lab.

#### Troubleshooting

The LS2 troubleshooting tips to generate clean data are the same as found in the troubleshooting sections of Activity 1.3. It is important to eliminate noise and to make sure that the recording is not inverted.

# Appendix B Skeletal Muscle Physiology

#### **B.1 Student Outline**

Methods

#### Set-up Student Station

The student station should be set-up by the laboratory instructor. The EMG cable should be connected to Channel 1 & 2 of the iWorx unit and the connector of the hand dynamometer CH3 of iWorx. The Skeletal Muscle Physiology laboratory custom settings ("EMG-GripStrengthLS2") should already be open in LS2. Finally, the hand dynamometer has been calibrated for you.

#### Electrode Placement

- 1. Each student should try the following: with one hand resting on the other forearm, squeeze the dynamometer with just your fingertips (not the full palm). Feel the flexor muscles of the forearm contract and relax. These are the muscle fibers you will record EMG activity from.
- 2. The subject should remove all metal or electronics to avoid noise in the recording.
- 3. Use an alcohol swab to scrub regions inside both forearms from the elbow to the wrist where the electrodes will be placed.
- 4. After the areas dry, attach the disposable electrodes in the following way to both arms: one is near the wrist, the second is in the middle, and the third is about 2" from the elbow.
- 5. Snap the lead wires onto the electrodes on your dominant arm so that when your palm is facing up:
- "+1" lead attached to the electrode near the elbow, red
- "-1" lead attached to the electrode in the middle of the forearm, black
- "C" lead (the ground) is attached to the electrode on the wrist, green

#### Data Collection

- 1. Click Record and have the subject squeeze his or her fingertips around the hand dynamometer to observe the EMG signal and the Muscle Strength such that the EMG signal-to-noise ratio is observable. If not, troubleshoot by ensuring all metal has been removed from your station. If your EMG channel is erratic or flat, jiggle the thin cords until the signal returns.
- 2. When ready, set a Mark labeled "dominant", and hit enter.
- 3. The subject should squeeze the hand dynamometer three separate times, with about 2 seconds of relaxation between each (see Figure 9). Create a rhythm such that each successive squeeze becomes stronger but not longer. Watch the recording, and have your partner click the double display icon and AutoScale to help make sure each successive contraction is approximately two and three times stronger than the first contraction. Remain as still as possible during recording. Repeat as necessary.
- 4. Repeat "Data Collection" steps 1-3 for the non-dominant arm, using Marks labeled as "non-dominant" to indicate the new section of recording.
- 5. Stop the recording when you have collected the non-dominant hand data.



**Figure 9.** Sample recording of EMG activity (top channel) and Muscle Strength (bottom channel). The cursors are in place to determine the absolute area of the EMG signal for the first contraction set.

#### Results

- 1. Go to the Mark labeled "dominant" and click the AutoScale buttons for both channels.
- 2. Use the Double Cursors icon and Zoom Between Cursors icon to show only the above three muscle contractions for the dominant arm. AutoScale for an optimized view.
- 3. Copy a screenshot of both channels to your MS Word file.
- 4. Click the Double Cursors icon so that two blue vertical lines appear over the Muscle Strength channel on the Main window. Click the Analysis icon, do the following measurements in the analysis window and record in Table 12.

Abs Area on EMG (relative amount of electrical activity): Drag the left cursor to the beginning of the EMG activity in the top channel and the right cursor to the end. Abs Area will be displayed on the toolbar; record this value (no units). Muscle Strength: Drag the left cursor to the baseline in the bottom channel and the right cursor at the initial peak height, V2-V1, of Muscle Strength. V2-V1 will be displayed on the toolbar.

- 5. Repeat step 4 for the other two muscle contractions for the dominant arm.
- 6. Repeat steps 4 and 5 for the non-dominant arm

#### **Discussion** Questions

1. What is the relationship between the absolute area under EMG signals and the muscle contraction strength? Is the relationship similar for both arms? Is this what you expected? Why or why not?

Subject:			
Dominant Arm			
	1 <sup>st</sup> Contraction	2 <sup>nd</sup> Contraction	3 <sup>rd</sup> Contraction
Abs. Area EMG			
Muscle Strength ()			
Non-Dominant Arm			
Abs. Area EMG			
Muscle Strength ()			

#### **B.2** Notes for the Instructors

#### Activity Overview/Learning Goals

In this activity, students investigate electrical activity and muscle strength in the dominant and non-dominant arms using an EMG recording. By the end of the activity, students should understand that in order to increase skeletal muscle strength one of two things should occur: the number of actin and myosin heads that bind should increase or the frequency that actin and myosin bind and unbind per single action potential in that muscle fiber should increase.

#### Pre-laboratory Set-up

For this activity, the same electrode cable can be used that recorded EMG in the activities in Part 1, using only three of the cables. Each student station (consisting of a computer and an iWorx unit) needs one electrode cable with the 3 leads, 3 disposable electrodes, the hand dynamometer, the calibration weight and 2 alcohol swabs. This will allow you to record the EMG signal. In this lab, in addition to setting up the student station you also need to calibrate the hand dynamometer so that it can accurately convert the pressure changes applied to the dynamometer by the student to a measurement of strength. See Tables 5 and 8 in Materials Section.

- 1. Plug connector of the EMG cable to Channels 1 & 2 of the iWorx unit and the connector of the hand dynamometer CH3 of the iWorx unit.
- 2. Open the LS2 program and load the custom settings for the Skeletal Muscle activity.

#### In Lab Instruction

In the pre-laboratory assignment, students are asked questions to ensure they understand we are studying absolute area (abs area) and not V2-V1 in this activity because we are not looking at individual action potentials but rather bursts of action potentials. We also stress the fact that the electrical activity (EMG), which is bursts of action potentials, causes the muscle contractions that they will be analyzing. At the start of class, we have a discussion in the background lecture about skeletal muscle microanatomy. We discuss minimal and maximum stimulus voltages and how this relates to the motor neuron(s). We make sure to go over the steps of software set-up and equipment calibration at the start of class with the students and stress the importance of using the figures provided in the student outline as guidance.

#### Troubleshooting

If the recording is "noisy" we review the check-list provided in the student outline of removal of all jewelry or other metal from their arms, wrists and hands, all non-lab electronics (i.e. cell phones), and the calibration weight as far away as possible when recording/collecting data. If your EMG channel is erratic or flat, jiggle the thin cords until the signal returns. Check the placement of the electrodes and ensure all the wires are correctly connected.

# Appendix C Resting Metabolic Rate (RMR)

## C.1 Student Outline

Methods

#### Set-up Student Station

This experiment is generally done in groups of 4-6, with one person being the subject and the RMR data shared amoung the group. The GTA or UA will run the gas analyzer (see Table 9 for materials) and then give the data on a USB drive to the group to work with.

Data Collection

- 1. To determine a true RMR, there are conditions that that subject should fufill prior to the class including not injesting food 12 hours prior to class, being physically and mentally rested, and carrying out the test in a 65-80°F room. The test can still be done if the conditions are not met, but the numbers will not represent a true RMR measurment.
- 2. Individuals who are ill or have pulmonary or cardiovascular disease should avoid serving as subjects.
- 3. The GTA will calibrate and run the gas analyzer.
- 4. The subject should affix the mask to their head. Ensure that the mask is tight enough to prevent air from escaping during breathing but loose enough that circulation is not cut off.
- 5. After calibration, the subject should sit quietly and breathe normally through their mouth and/or nose.
- 6. Click record and wait a minimum of 10sec for the Lung Volume STPD channel to zero. After 10sec, affix the ribbed tube attached to the mixing chamber to the clear outflow nozzle of the mask.



Figure 10. Screenshot of RMR recording. The channels from top to bottom are: Expired  $O_2$  Concentration, Expired  $CO_2$  Concentration, Air Flow, Lung Volume at Standard Temperature and Pressure Dry Air, and Absolute  $VO_2$ .

- 7. Continue sitting calmly for a minimum of 8 minutes. During this time, the air in the mixing chamber will be replaced by expired air. The data you get will look like the example in Figure 10.
- 8. While the subject is breathing into the gas analyzer, the other group members should fill in Table 13 with the information about the subject necessary to calculate the RMR. This includes: height, weight, gender, age and recent eating, sleeping and exercise information.
- 9. After 8 minutes stop the recording and save the data to a USB device as an .iwxdata file and return to your group's computer. This is the file that you will use to determine your RMR.

#### Results

- 1. Open LS2 and click on the File pull-down menu and open the data file.
- 2. Click the Advanced pull-down menu, select Metabolic, and then Mixing Chamber: Offline Calculations from the submenu.
- Under the Channels submenu, the CO<sub>2</sub>, O<sub>2</sub>, and Volume Channels should be set to "Expired CO<sub>2</sub> Concentration %", "Expired O<sub>2</sub> Concentration %", and "Lung Volume STPD" respectively. Heart rate and Energy should remain as "None."
- Click the Settings tab. Enter the subject's information (leave Protocol blank). Average (sec) should be 30. Insp. O<sub>2</sub> (%) should be 20.9 and Insp. CO<sub>2</sub> (%) should equal 0.04.
- 5. Click "Calculate".
- 6. A graph should appear on the right side of the window. You will notice that the graph has a sharp change at the beginning. Uncheck selected segments until the graph is relatively level.
- 7. Below the graph is a table. Click "copy" and then paste into MS Excel.
- 8. Find the values of Max Abs.VO<sub>2</sub> at the bottom of Column C (Note: This is not max VO<sub>2</sub>) and record that value in Table 13.
- 9. This is all the data you will need to collect using LS2 for RMR.

#### Calculations

Calculate the following values and record in Table 13: Surface Area (BSA) =  $\sqrt{((\text{Height in cm*Weight in kg)/3600)}}$ Observed Resting Metabolic Rate (ORMR) (Max Abs. VO<sub>2</sub> (LO<sub>2</sub>/min) \* 60 (min/hr) \* 4.859 (kcal/LO<sub>2</sub>))/BSA (m<sup>2</sup>) Predicted Basal Metabolic Rate (PBMR) Male: ((14.4\*Weight) + (3.13\*Height) +113)/24 Female: ((10.4\*Weight) + (6.15\*Height)-282)/24

Table 13. RMR Data.		
Subject:		
Age:	Sex:	
Height:	Weight:	
Surface Area (BSA):	Eat recently? (y/n)	
Restful sleep? (y/n)	Recent Exercise? (y/n)	
Measurements and Calculations		
Max Abs. $VO_2$ ( ):	PBMR ( ):	
ORMR ( ):	% RMR:	

-----

#### Discussion Questions

- 1. What was the %RMR of PRMR for your subject? Was it within the 10% margin of error? Explain why or why not for your particular subject.
- 2. List two reasons why the ORMR as determined in this exercise is not a true BMR.

#### C.2 Notes for the Instructor

# Activity Overview/Learning goals

In this activity students are able to see how the gas analyzer, an example of indirect calorimetry, can be used to determine their own RMR. They have to think about all the factors that contribute to the resting metabolic rate (such as ingested food, activity and temperature). Students will also be able to determine if their measured RMR is within the normal healthy range for a person of their age.

#### Pre-laboratory Set-up

The set-up for this lab requires the GTAs to load the custom settings for LS2 on one computer station attached to the gas analyzer. The custom LS2 settings for this activity are called "RMR-GA300-LS2." The equipment for the gas analyzer activity needs to be set up and calibrated before the students arrive. Setting up the gas analyzer equipment requires two

different calibration steps. Calibrating the spirometer only needs to happen once per laboratory section, while calibrating the gas analyzer itself needs to happen between each subject. This involves calibrating the spirometer flow head using a 3L calibration syringe and gas analyzer using a known mixture of  $12\% O_2$ ,  $5\% CO_2$ , and  $83\% N_2$ .

Calibration can take up to an hour depending on the familiarity of the staff with the equipment, while between students calibration takes 5-10 minutes. Set up and calibration for the gas analyzer is very techincal. We have created a tutorial video that we have for the teaching staff on calibrating the gas analyzer.

#### In-lab Instruction

The gas analyzer requires the instructor to run the equipment and monitor the student during the experiment. We run this activity in groups of 4-6, with one student as the subject. It takes 8-10 minutes to record data from each subject, so during this time we explain the different components of the equipment and generate discussion with the small group about how the data we are generating relates to RMR and indirect calorimetry. After collecting data, the student groups can work independently to calculate their RMR. As designed, this module requires at least two instructors to run, one to work the equipment and the other to help the students with calculations. Students enjoy being the subject for this module because they get to see first-hand their own RMR.

#### Troubleshooting

We have a tutorial video for staff on how to set up and troubleshoot the gas analyzer.

# Appendix D Respiratory Volumes and Capacities

**D.1** Student Outline *Methods* 

#### Set-up Student Station

The student station should be set-up by the laboratory instructor. The spirometer should be connected to CH4 of the iWorx unit. The Respiratory Volumes and Capacities laboratory custom settings ("Breathing-Gravity-LS2") should already be open in LS2. Finally, the spirometer has been calibrated for you.

- 1. Firmly push the two air flow tubes onto the two outlets on the FH-300 flow head.
- 2. Firmly push the other ends of the two air flow tubes onto the two outlets on the SP-304 spirometer unit.

#### Data Collection

- 1. The subject should remove all jewelry or other metal from their face, neck and hands, and all non-lab electronics, such as a cell phone, as far away as possible.
- 2. The subject should be seated quietly and insert a cardboard mouthpiece to the flow head (but do not allow the cardboard to touch the membrane within the flow head). Hold the flow head such that the outlets are in the upright position in front of his/her face, but do not start breathing into the flow head yet.
- 3. Click Record and wait at least 10sec until the Lung Volumes channel zeroes.
- 4. The subject to insert the mouthpiece into their mouth and plug his/her nose while breathing into the flow head and their partner should insert a mark called "seated tv".
- 5. Record 3 TV breaths.
- 6. Then the subject should inhale as deeply as possible (lift chest and tilt shoulders slightly back), pause, then exhale as quickly and completely as possible (crunch slightly forward), pause, then resume 3 TV breaths (see Figure 11).



Figure 11. Sample recording of air flow (top channel) and lung volume (bottom channel.) Cursors are in the correct position to measure FVC.

#### Results

- 1. Click the AutoScale buttons for both channels.
- 2. Use the Double Cursors icon and Zoom Between Cursors icon to show all three trials of your data. AutoScale for an optimized view.
- 3. Insert a new Mark labeled "seated 1" just before the visually best/easiest to analyze of the three trials. This is the one you should analyze.
- 4. Copy a screenshot of both channels into MS Word.
- 5. Click the Double Cursors icon so that two blue vertical lines appear over the Lung Volume channel on the Main window. Click the Analysis icon, do the following measurements for the bottom channel and record in table 14.

Some of the values may be negative due to cursor placement. Record all values as positive. Also, if the procedures are confusing, place cursors to measure V2-V1 or T2-T1 of the appropriate terms.

- Tidal Volume (TV): Drag the left cursor to the beginning of inhalation (upward slope) and the right cursor to the peak of the last TV before forced inhalation. V2-V1 is already displayed in the toolbar.
- Breath Period: Leave the right cursor in position from TV and drag the left cursor to the previous TV peak. T2-T1 is already displayed in the toolbar.
- Forced Expiratory Volume in 1 second (FEV1): Drag the left cursor to the forced inhalation peak and the right cursor to a T2-T1 of 1 second and record V2-V1.
- Functional Vital Capacity (FVC or measured VC): Leave the left cursor in position from the FEV1 and drag the right cursor to the forced exhalation trough and record V2-V1.

#### Calculations

Calculate the following values and record in Table 14: Breathing Rate (BR) = (1 breath/period in sec)\*60 sec/min FEV1/FVC and indicate whether value is  $\geq 0.8$  (normal) Predicted VC: For Males: VC = (0.052 x H) - (0.022 x A) - 3.6 For Females VC = (0.041 x H) - (0.018 x A) - 2.69 H is the subject's height (cm) and A is the subject's age (years.) % deviation from measured FVC: (FVC – Predicted VC)/Predicted VC

Table 14. Respire	atory volumes and Capacities Calculations.
Subject:	
Age:	
Height:	
	Measurements and Calculations
TV ()	
Breath Period ()	
FEV1 ()	
FVC ()	
FEV1/FVC	
Predicted VC	
%VC	

# Table 14. Respiratory Volumes and Capacities Calculations.

#### Discussion Questions

1. What is the general effect of age on predicted VC? Of gender? Of height?

### **D.2** Notes for the Instructors

#### Activity Overview/Learning Goals

In this activity students investigate respiratory volumes and capacities that provide information about the respiratory efficiency of the body and the elasticity of the pulmonary components. By the end of the activity, students should understand that there are differences in these volumes and capacities based on sex, age, size, fitness and build of the subject being tested.

#### Pre-laboratory Set-up

For this lab, each student station (consisting of a computer and an iWorx unit) needs a spirometer with flow head tubing, a flow head and a disposable cardboard mouthpiece. The spirometers also need to be calibrated. See Tables 5 and 10 in Materials Section.

1. Plug the connector of the spirometer unit into CH4 of the iWorx unit.

2. Open the LS2 program and load the settings for the Respiratory Volumes and Capacities activity ("Breathing-Gravity-LS2.")

#### In lab Instruction

At the start of class, we review and discuss all of the respiratory volume and capacity terms, both at rest and exercise and how they would be affected with different body positions. While the students can analyze lung capacity in different body positions, at rest and after exercise, we will only focus on the seated position here today to determine vital capacity and

forced expiratory volume after one second of the subjects. These measurements are important for clinical assessment of spirometry.

#### Troubleshooting

If the recording is "noisy" we review the check-list provided in the student outline of removal of all jewelry or other metal from their arms, wrists and hands, and all non-lab electronics (i.e. cell phones) as far away as possible when recording/collecting data.