Lab 1: Introduction to the Heart

Background
Pump, Pump, Pump. Your heart works continuously, day in and day out, to supply blood and oxygen through your body. Did you know that, like the brain and muscles, your heart uses spikes (electricity) to function as well? These electrical signals are regulated by your brain’s cardiovascular center (“heart center”) located in the medulla oblongata. The medulla is the last stop in your brain before you are in the spinal cord and it controls basic body functions such as breathing, heart rate, and blood pressure. Interestingly, your heart can beat without instructions from the brain! However, sometimes there are situations when your brain needs to change your heart rate! Your brain might decide your heart rate needs to change (like when you are exercising) or when hormones like epinephrine or adrenaline need to be released (like when you feel the emotions fear, excitement, and nervousness) your brain sends signals to your heart, changing both the rate of your heart’s beating and the strength of contraction.

In this lab, you will use the Heart and Brain Spikerbox to attach electrodes on your hands to measure your heart beat! The signal we will be recording is called an electrocardiogram, also known as an ECG (or EKG if you prefer the German spelling). Let’s find out if exercise changes our heart rate!

Materials
- Heart and Brain Spikerbox
  - 9V Battery (this powers the SpikerBox)
  - Electrode cable (orange)
  - Device-specific cable:
    - USB cable for computer link
    - Aux cable for non-USB devices
- 3 electrode stickers
- Spike Recorder App: backyardbrains.com/products/spikerecorder
- How-to Video: bit.ly/BYBIntroHeart (ignore the the word “arduino”, it is an older device, but the electrode setup still works)

Investigation 1: Find your Heart Rate
1. Place the patch electrodes on the insides of your wrists, with a ground on the back of your hand.
2. Clip the red alligator clips to the patches on the insides of your wrists and the black alligator clip to the ground on the back of the hand.
   a. Alternative electrode placement: Place the recording patch electrodes above and below your heart on your upper left chest, and another patch for your ground on your upper right chest. Connect the red alligator clips to the left electrode patches and the black alligator clip to the right electrode patch. (Don’t worry - you’re measuring your own electricity, not sending electricity into your body!)
   *Draw a picture of your setup below* (Drawing #1)
3. Connect your electrode cable to your SpikerBox and make sure the battery is correctly installed.
4. Connect your SpikerBox to your recording device, either using a USB cable to connect to your computer or a smartphone cable to your mobile device.

5. Open the SpikeRecorder software.

6. Turn on your SpikerBox.
   * Make sure you click the "USB" button if you’re on a computer. One way to test this is by clapping once. If the SpikeRecord shows a big spike, it’s not connected to your Spikerbox.

7. Getting a good quality signal is really important for this procedure. Test to see if you get a good ECG, watch your Spike Recorder and see if you see a regular heart beat.
   * Note: Small signal? Adjust the vertical zoom on SpikeRecorder by “pinching” and zooming in.

Other suggestions on getting quality signals:
   a. If you are using a laptop, avoid plugging it into the wall for power.
   b. Make sure to hold as still as possible while you are connected: muscle movements can be picked up, which causes interference with your ECG reading. Resting with your hands on your knees provides the most stable signal.
   c. Move your hands and arms and be able to recognize these movement artifacts (spikes on the screen) so you can ignore these noisy artifacts when analyzing your data.
   d. Feel free to experiment with where you place the ground electrode. You can also go into the SpikeRecorder settings and set the signal threshold from 70 to 1500, and check the box by "60hz" to filter out more noise.

NOTE: If your ECG signal appears upside down during recordings, simply swap the red alligator clips to the opposite patch electrodes.

   * Draw the ECG waveform (spike pattern) from the spike recorder* (Drawing #2)

8. If you want to record the data, press the red "record" button in the upper right side of the Spike Recorder Screen. The data will be saved as a .wav file.

Investigation 2: Resting Heart Rate

1. While watching your Spike Recorder app and using a timer, count the number of heart beats per minute. Repeat this three times.
   
   Tip: To save time, you can count for 30 seconds and multiple your number by two.

   * Record Results Below*

Investigation 3: Valsalva Maneuver

The Valsalva maneuver is used to clear the eustachian tubes in the ears. It can also be used by fighter pilots to keep from passing out during extremely high G-force maneuvers. It is usually done by closing the mouth while pushing air out, like if you were blowing up a balloon. Sometimes people will do this to “clear their eyes” after pressure changes from swimming or flying in planes. A side effect is that it affects the autonomic nervous system, meaning it can affect your heart rate. The Valsalva maneuver constitutes a simple test of the baroreceptor reflex function, which is your body’s way of controlling your blood pressure.

* Want to see a how-to video? Check out this video here: bit.ly/BYBValsalvaManeuver

Safety note: Each individual is different, so pay attention to how you feel, how fast your heart is beating, and how much you feel the changes in your body while attempting this procedure. If you have a heart condition or other autonomic nervous system disease, talk to your doctor before you conduct this exercise. If at any point in this procedure you feel dizzy, uncomfortable, or pain: STOP.
1. With the Heart and Brain box connected as in the previous exercise, check your signal is recording correctly.
   *Optional: If you have a lab partner, have them use the keyboard to add event markers when you start and when you exhale (breathe out) and begin to breathe normally.
2. Click record on your Spike Recorder app
3. Using a timer, take a deep breath, close your mouth and begin blowing air out without releasing the air in your lungs and hold for 15-20 seconds. *Click number “1” on keyboard*
   *TIP: If it feels weird to hold your breath and exhale at the same time, put the back of your hand to your mouth and blow out.
4. *Click number “2” on keyboard* Breathe out.
5. *Click number “3” on keyboard* Keep recording for at least 30 seconds while resuming normal breathing.
6. Stop the recording.
7. Open the recording and have a look at your ECG signal.
   *You are looking for 4 phases of this effect as described below.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Stage of Maneuver</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase 1</td>
<td>Onset of Strain</td>
<td>Exhale (mouth closed)</td>
</tr>
<tr>
<td>Phase 2</td>
<td>Continued strain</td>
<td>Intense Exhale (mouth closed)</td>
</tr>
<tr>
<td>Phase 3</td>
<td>Release</td>
<td>Exhale (mouth open)</td>
</tr>
<tr>
<td>Phase 4</td>
<td>Recovery</td>
<td>Normal breathing</td>
</tr>
</tbody>
</table>

8. Count the number of beats during each phase and divide the amount of time in each phase to standardize to beats per minute during each.
   For example if I counted 17 beats in the 15 seconds of Valsalva phase 1
   (17 beats /15 seconds  = 1.133 beats per second X 60 seconds = 68 BPM in phase 1)
   *Record results in Table 2 below*

Investigation 4: Exercise and Heart Rate

*Safety note: Each individual is different, so pay attention to how you feel, how hard you are breathing, how fast your heart is beating, and how much you feel the exertion in your muscles. If you have a heart condition or other chronic disease, talk to your doctor before you conduct intense exercise. If at any point in this procedure you feel dizzy, uncomfortable, or pain: STOP. Sit down, drink water, and contact your doctor.

1. Remove your alligator clips from your electrodes. Walk around casually for 5 minutes. After you stop, immediately re-attach the alligator clips and count the number of beats for 1 minute.
   *Record Results Below in Table 3*
2. Without exercising again, continue to take count your heart beats for the next three minutes. This will determine how quickly your heart returns to its resting rate.
   *Record Results Below in Table 3*
3. Remove your alligator clips from your electrodes. Perform 30 jumping jacks. Do the jumping jacks in a row without stopping. After you stop, immediately re-attach the alligator clips and count the number of beats for 1 minute.
   *Record Results Below in Table 3*
4. Without exercising again, continue to take count your heart beats for the next three minutes. This will determine how quickly your heart returns to its resting rate.

*Record Results Below in Table 3*

5. Now design your idea! What kind of exercise affects you? For example: aggressive hand exercises (squeezing a tennis ball, etc) running, recently consumed caffeine, holding your breath, deep slow breaths vs rapid ones, sitting vs standing, age, general athleticism, compare to your family members, etc.

*Record Results Below in Table 3*

### Results Key

<table>
<thead>
<tr>
<th>Key</th>
<th>(Drawing #1) Drawing of Your Electrode Placement</th>
</tr>
</thead>
<tbody>
<tr>
<td>● Wrist or chest</td>
<td></td>
</tr>
<tr>
<td>● RED Electrodes (detail exactly where they are placed)</td>
<td></td>
</tr>
<tr>
<td>● BLACK (ground) Electrode (detail exactly where they are placed)</td>
<td></td>
</tr>
</tbody>
</table>

### (Drawing #2) Drawing of spike recording (from device screen)

<table>
<thead>
<tr>
<th>Table 1: Resting Heart Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condition</td>
</tr>
<tr>
<td>Resting</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2: Valsalva maneuver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condition</td>
</tr>
<tr>
<td>Beats per Minute</td>
</tr>
<tr>
<td>Decrease or Increase of heart rate?</td>
</tr>
</tbody>
</table>
### Table 3: Exercise and Heart Rate

<table>
<thead>
<tr>
<th>Condition</th>
<th>Immediately after exercise (bpm)</th>
<th>1 min after (bpm)</th>
<th>2 min after (bpm)</th>
<th>3 min after (bpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walking</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jumping Jacks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Design your own!</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Design your own!</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Reflection Questions

1. Most research suggests the average resting heart rate (RHR) is 66 beats per minute (bpm). Was yours around this? There’s lots of reasons why your heart beats faster or slower, what do you think might be causing yours? (Can you test to see if this is always the same number?)

2. Why does your heart rate increase when you exercise?

3. The Valsalva Maneuver is used by doctors to make the patient’s heart rate responds correctly. If a patient’s heart rate doesn’t change during this maneuver, what might be the problem?

4. Graph your results! Create a graph that shows your heart rate over time as you perform different exercises! Remember to label each line so it’s clear what exercise each refers to!

   *Hint: your y-axis (dependent variable) is what you measure and your x-axis (independent variable) is what stays the same (think “time”…) *There’s graph paper on the last page.

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**Keep going!** ⇒ Try out these other ideas to learn even more!

1. There are lots of different places you can put the electrodes for this experiment! What effect(s) do the different placements have on the signal you see? Is the signal different if you move the electrodes closer to one another? Closer to the heart?
2. Look at the effects of certain states on the amplitude and rate of heart action potentials. Recently run, recently drank caffeine, holding your breath, deep slow breaths vs rapid ones, sitting vs standing, age, general athleticism, etc.
3. If it’s cold or hot outside, try comparing heart rates when you’re inside vs when you’re exposed to the weather. Stay safe though!
4. What about before and after you’re eating? Why might that be? What, if anything, does the movement of your blood have to do with eating?
5. What other things do you think would affect heart rate? Experiment some, but make sure to stay safe!
Lab 2: Reaction Time

Background

René Descartes, a 17th century French philosopher and scientist, used to visit the gardens of a French Royal Palace where the French king (a prankster) had set up lifelike mannequins that would jump out and surprise garden visitors, along with other mechanical exhibits such as statues that would retreat as art lovers attempted to get close enough to see the details in the statues. René was curious how the system worked, and upon investigation, he found a hydraulic system of water pipes whereby stepping on a pressure plate caused almost "life-like" action in the mannequins.

Descartes used this experience and thought that, like the automated, hydraulic exhibits in the garden, the nervous system was controlled by "animal spirits" which would flow through the nerves of animals and people giving rise to automatic responses, or reflexes. Indeed, our body sometimes does react automatically, as if guided by a will that is not our own, but it still takes some time to process those commands. Reaction time tests help us understand how long it takes information to travel through our nervous system.

In this lab, we are going to look at the electromyographic (EMG) signal in the thousands of muscle fibers within your forearm to measure reaction time! We can record these signals using your Muscle SpikerBox Pro!

Materials

- Muscle SpikerBox Pro
  - 9V Battery (this powers the SpikerBox)
  - Electrode cable (orange)
  - Device-specific cable:
    - USB cable for computer link
    - Aux cable for non-USB devices
- 6 electrode stickers
- A partner! (it's hard to measure your own reaction time)
- Spike Recorder App: backyardbrains.com/products/spikerecorder

Note: This lab uses "event markers" which can only be used on laptops, PCs (Windows, MacOS, Linux). This lab is not compatible with mobile OS phones or tablets.

Setup: Measuring Reaction Time

1. Hook an arm up to the Muscle SpikerBox! The two red clips attach to the stickers on the user's forearm, and the one black clip attaches to the sticker on the back of the user's hand.
2. Connect your electrode cable to your SpikerBox and make sure the battery is correctly installed.
3. Connect your SpikerBox to your recording device using a USB cable (or aux audio cable if you have)
4. Open the SpikeRecorder software
5. Turn on your SpikerBox.
   * Make sure you click the "USB" button if you're on a computer. One way to test this is by clapping once. If the SpikeRecord shows a big spike, it's not connected to your Spikerbox.
6. Test your muscles by flexing the arm with the electrode stickers.
7. Next, test the event markers by typing any of the numbers "0" through "9" on your keyboard. Each time you push a key, you should see a colored line appear on the screen of the SpikeRecorder app.
Investigation 1: Hand Dominance and Decision Making

NOTE: Doing this procedure with a friend will be an easier and truer measure of your reaction time, since you can’t really “test” your own reactions.

Writing Hand

1. Sit at a table with your writing hand over the edge.
2. First we will test writing hand response. Your partner will cover their hand because they are going to type the number “1”. Their hand is covered so you don’t see their hand move before you see the “event marker” on the screen.
3. Click the “Record” button ➤
4. As soon as you see the “1” on screen, flex your arm (with the electrodes) as fast as possible. You have to react to the visual stimulus of seeing the event marker on the screen.
   *Record Results below*
5. Repeat the experiment four times total. Then switch places with your partner and redo it.
6. Open your recording using the three horizontal bars button ➤
   *Tip: You may need to find the “BYB” folder, find your date and time recording.
7. For each marker even, hold the RIGHT-CLICK and DRAG the mouse between the event marker and the beginning of the “arm flex” event. The time will be at the bottom in seconds.
   *Record Results Below*

Non-Dominant Hand

1. For this test, repeat the same procedure above just like you did for your writing hand, but this time, use the other hand (the one you didn’t test before).
2. Record the measurements and like before, repeat for 4 total trials, then switch places with your partner again.
   *Record Results Below*

Decision

1. For the last test, you will need to choose between two event markers. Your partner will (while covering their hand) either type the number “1” or the number “2”.
2. Click the “Record” button ➤
3. As soon as you see the “2” on screen, flex your arm (with the electrodes) as fast as possible. Do NOT react to the number “1”.
   *Record Results below*
4. Repeat the experiment four times total. Then switch places with your partner and redo it.
5. Open your recording using the three horizontal bars button ➤
   *Tip: You may need to find the “BYB” folder, find your date and time recording.
6. For each marker even, hold the RIGHT-CLICK and DRAG the mouse between the event marker and the beginning of the “arm flex” event. The time will be at the bottom in seconds.
   *Record Results Below*
Investigation 2: Designing Your Own Investigation!

The muscle spikerbox pro can record just about any muscle on your body. These are just some options you possess. Decide what you want to test today.

*Want some research ideas? Check out the “Keep Going!” section at the end of this handout.

1. What is your research question?

2. What data will you need to collect?

3. Design your own investigation **below** using the Muscle Spikerbox and reaction timer to generate evidence to answer your question:
### Results

#### Investigation 1: Investigate Your Own Question

<table>
<thead>
<tr>
<th>Partner</th>
<th>Writing Hand (seconds)</th>
<th>Non-Dominant Hand (seconds)</th>
<th>Decision (seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partner #1</td>
<td>Trial 1</td>
<td>Trial 1</td>
<td>Trial 1</td>
</tr>
<tr>
<td></td>
<td>Trial 2</td>
<td>Trial 2</td>
<td>Trial 2</td>
</tr>
<tr>
<td></td>
<td>Trial 3</td>
<td>Trial 3</td>
<td>Trial 3</td>
</tr>
<tr>
<td></td>
<td>Trial 4</td>
<td>Trial 4</td>
<td>Trial 4</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partner #2</td>
<td>Trial 1</td>
<td>Trial 1</td>
<td>Trial 1</td>
</tr>
<tr>
<td></td>
<td>Trial 2</td>
<td>Trial 2</td>
<td>Trial 2</td>
</tr>
<tr>
<td></td>
<td>Trial 3</td>
<td>Trial 3</td>
<td>Trial 3</td>
</tr>
<tr>
<td></td>
<td>Trial 4</td>
<td>Trial 4</td>
<td>Trial 4</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Investigation 2: Designing Your Own Experiment

1. Record your evidence on a Claim-Evidence-Reasoning ("CER") poster during the investigation.
   *NOTE: A template of the poster is on the last page of this handout*
2. (after experiment) Create a claim that answers your question
3. In your notebook record summaries of your team’s observations, questions, and results. Use this data to examine your model of reaction time.
Reflection Questions

Investigation 1
1. Which test did you have a faster reaction time on average? How much faster is it? Why do you think that is?

2. What do you think is happening in our nervous systems when we are trying to react? What is different when we’re trying to DECIDE which event marker to react to? (during the “decision” test)

3. What are at least 3 other variables that may affect reaction time. (Examples: Warm arm and arm in ice water, heights of partners, ages of partners, etc)
   a. 
   b. 
   c. 

Investigation 2
1. What data supports your model?

2. Which data causes you to revise your model?

3. What new questions do you have that would help you further refine your model of the nervous system?

Keep going! ⇒ Try out these other ideas to learn even more!
1. Does your partner react quicker to the light, the sound, or the combination of both? When does your partner react the fastest and the slowest? Why do you think you got the results you did? Find more test subjects to create a larger sample size!
   a. Other ideas: Eyes closed reacting to sound (partner can slap the desk when entering event marker). Light could be a flashlight held by a partner. For sound you can also have your partner listening to earbuds (distracted, doing mental math, listening to classical vs heavy metal, etc)

2. Turn your Reaction Time lab into a quantitative, muscle physiology lab! You are in total control of the experimental variables. Compare sight, sound, decision making, and even different muscles...does a muscle further away from the brain take longer to react?

3. Do some web-searching and hunt for professional research that focuses on reaction times! We like this paper’s analysis of the difference between auditory and visual response times (spoiler alert in case you haven't performed the experiment yourself yet!)
### Backyard Brains

**Reaction Time**

#### Claim-Evidence-Reasoning Tool

<table>
<thead>
<tr>
<th>Question</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Evidence from data and observations</th>
<th>Science ideas or concepts or principles</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Include data tables or graphs if necessary</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Claim (Your claim should answer the question.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Explanation (Make sure to link your claim, evidence, and science ideas.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>
Lab 3: Reflexes and Reactions

RfBackground
Reaction time tests help us understand how long it takes information to travel through our nervous system. However, there are different “reactions” that people can make. Are these “automatic” reflexes faster, slower, or the same as “intentional” reactions, like grabbing something?

One example of a reflex is the patellar stretch reflex. Our spinal cord partners with sensors in our muscles, called muscle spindles, to keep track of where our bodies are in space and how stretched or contracted our muscles are. The way that these sensors interact with our spinal cord is through a reflex pathway. Stretching the muscle activates the muscle spindle at the end of the sensory neuron (embedded in your muscle) and starts the reflex.

For this experiment, we are going to look at the electromyographic (EMG) signal in the rectus femoris muscle, one of the muscles in your quadriceps. We elicit a reflex using a tap to the patellar tendon.

Materials
- Muscle SpikerBox Pro
  - 9V Battery (this powers the SpikerBox)
  - Electrode cable (orange)
  - Device-specific cable:
    - USB cable for computer link
    - Aux cable for non-USB devices
- 6 electrode stickers
- Partner (it’s hard to test your own reflexes)
- Reflex Hammer -or- straight tool (like a yardstick)
- A partner! (it’s hard to measure your own reflexes)
- Spike Recorder App: backyardbrains.com/products/spikerecorder
- How-to Video: bit.ly/BYBReflexes

Note: This next part uses “event markers” which can only be used on laptops, PCs (Windows, MacOS, Linux), and Android devices (not iOS phones or iPads). You will need a partner for help!

Investigation 1: Finding a Reflex
1. Have the subject sit on a sturdy surface high enough to allow their lower legs and feet to dangle freely.
2. Have the subject contract their quadriceps (knee extensor) muscle so you can place two on either side of the knee (see Figure 1). One electrode on the Vastus Medialis, one on the Vastus Lateralis.
3. Place a ground adhesive electrode on the back of the hand. (see Figure 1)
4. Clip the red leads of your orange muscle cable of the electrodes on the muscle of your thigh, and the black lead on the ground on your outer palm.
5. Plug your orange interface cable into the orange channel 1 on your SpikerBox.
6. Connect your SpikerBox to your recording device, either using a USB cable to connect to your computer or a smartphone cable to your mobile device.
7. Open the SpikeRecorder software
8. Turn on your SpikerBox.
Backyard Brains

*Make sure you click the "USB" button if you're on a computer. One way to test this is by clapping once. If the SpikeRecord shows a big spike, it’s not connected to your Spikerbox.

9. Getting a good quality muscle signal is really important for this experiment. Test to see if you get a good EMG signal with a fairly small contraction by asking the subject to straighten their knee against resistance.
   a. *Note: Poor signal? Adjust the vertical zoom on SpikeRecorder. If that still doesn’t help, adjust the electrode placement.*
   *Also, if you are still not getting a very clear signal, feel free to experiment with where you place the ground electrode. You can also go into the SpikeRecorder settings and set the signal threshold from 70 to 1500, and check the box by “60hz” to filter out more noise.*

10. Locate the part of the patellar tendon just below the kneecap and above where it inserts into the shinbone (see image). Tap (with the hammer, yardstick, or other tool) just **below** the kneecap.
   a. *Note: Avoid hitting the knee cap because...ouch! Use a quick, firm tap to the tendon to elicit an involuntary “leg-kick”. Experiment with small changes in location and tap force. Find the right spot that gets a “leg-kick”.

11. Once you have good electrode placement and apply a quick tap to the patellar tendon, you should see a spike!

*Record Electrode Placement below in Drawing 1*

Investigation 2: Measure Reflexes

1. Have a partner sit up high so their legs are dangling.
2. Have your partner close their eyes or look away so that they can't anticipate the reflex tap.
3. Give your partner some forceful taps below their kneecap. Once you find the sweet spot, you should be able to evoke the leg-kick reflex!
4. Click the “Record” button ⇒
5. At the **SAME TIME** as tapping your partner’s knee, type the number “1” on your keyboard (this creates an event marker in the recording)
   *NOTE: It will be difficult to time your event marker at the same time as you tapping the person’s knee. Do your best!
6. Repeat step 5 for a total of 5 trials.
   *Your data will be more accurate the MORE you trials to do!
7. Swap your electrodes to your other leg. Repeat steps 1-6. (Use event marker “2” for this leg)
8. Open your recording using the three horizontal bars button ⇒
   *Tip: You may need to find the “BYB” folder, find your date and time recording.*
9. Zoom in with touch controls or your mouse wheel. You can measure the reflex time by measuring the difference between the event marker “1” and when the muscles flex in response! RIGHT-CLICK and DRAG between the start of the knee reflex (muscle signal) and the event marker line.
   *Record Results Below in Table 1*
Investigation 3: Compare Against Reaction time

1. Have the subject contract their quadriceps (knee extensor) muscle so you can place two on either side of your OTHER knee (see Figure 1). One electrode on the Vastus Medialis, one on the Vastus Lateralis.
2. Place a ground adhesive electrode on the back of the hand (see Figure 1).
3. Clip the red leads of your orange muscle cable of the electrodes on the muscle of your thigh, and the black lead on the ground on your outer palm.
4. Plug your orange interface cable into the orange channel 2 on your SpikerBox. *NOTE: You will be recording one leg’s reflex from Channel 1 and the other legs reaction from Channel 2.
5. Click the “Record” button ⇒
6. Tell the research subject to close their eyes again. Tell them to kick their RIGHT leg when they feel the hammer tap the LEFT leg.
7. Open your recording using the three horizontal bars button ⇒
   *Tip: You may need to find the “BYB” folder, find your date and time recording.
8. RIGHT-CLICK and DRAG between the start of the LEFT knee reflex (muscle signal) and the RIGHT leg reaction (muscle signal). *Record Results Below in Table 2*

Results

<table>
<thead>
<tr>
<th>Key</th>
<th>(Drawing 1) Drawing of Subject’s Leg</th>
</tr>
</thead>
<tbody>
<tr>
<td>● Subject’s leg</td>
<td></td>
</tr>
<tr>
<td>● Vastus Medialis</td>
<td></td>
</tr>
<tr>
<td>● Vastus Lateralis</td>
<td></td>
</tr>
<tr>
<td>● Patellar Tendon ○ Where tapping caused a reflex</td>
<td></td>
</tr>
<tr>
<td>● Electrode adhesives (detail exactly where they are placed)</td>
<td></td>
</tr>
</tbody>
</table>
Table 1: Using the Reflex Hammer

For reference: 1 second = 1,000 milliseconds

<table>
<thead>
<tr>
<th>Tapping</th>
<th>Left Leg</th>
<th>Right Leg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tapping</td>
<td>Tapping</td>
</tr>
<tr>
<td>Tap #1</td>
<td></td>
<td>Tap #1</td>
</tr>
<tr>
<td>Tap #2</td>
<td></td>
<td>Tap #2</td>
</tr>
<tr>
<td>Tap #3</td>
<td></td>
<td>Tap #3</td>
</tr>
<tr>
<td>Tap #4</td>
<td></td>
<td>Tap #4</td>
</tr>
<tr>
<td>Tap #5</td>
<td></td>
<td>Tap #5</td>
</tr>
<tr>
<td>Average time</td>
<td></td>
<td>Average time</td>
</tr>
</tbody>
</table>

Table 2: Compare Against Reaction time

For reference: 1 second = 1,000 milliseconds

<table>
<thead>
<tr>
<th>Tapping</th>
<th>Reflex Leg</th>
<th>Reaction Leg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tapping</td>
<td>Tapping</td>
</tr>
<tr>
<td>Tap #1</td>
<td></td>
<td>Tap #1</td>
</tr>
<tr>
<td>Tap #2</td>
<td></td>
<td>Tap #2</td>
</tr>
<tr>
<td>Tap #3</td>
<td></td>
<td>Tap #3</td>
</tr>
<tr>
<td>Tap #4</td>
<td></td>
<td>Tap #4</td>
</tr>
<tr>
<td>Tap #5</td>
<td></td>
<td>Tap #5</td>
</tr>
<tr>
<td>Average time</td>
<td></td>
<td>Average time</td>
</tr>
</tbody>
</table>

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Supplementary Material for Developing and Implementing Low Cost Remote Laboratories for Undergraduate Biology and Neuroscience Courses
Reflection Questions

1. Which leg had a faster reflex, why do you think that is?

2. This is the same test that is done when you go to the doctor’s office. Doctors use it as a quick test to determine spinal pathway health. What does this test for neuromuscular diseases?

3. What are some other reflexes you think you could test?

4. Does the speed/amplitude of the response change depending on the state someone is in? For example, if they are tired, have a lot of energy, had coffee recently, etc. Why do you think these do or don’t affect the reflex?

5. When comparing the reaction time of flexing your knee to the reflex, which leg was faster? Why do you think that is?

Keep going! ⇒ Try out these other ideas to learn even more!

1. What are some other reflexes you think you could test?

2. Don’t hurt yourself or anyone else investigating this, but does the speed or amplitude depend at all with the force you strike the knee with? Why might this be or not be?

3. Does the speed/amplitude of the response change depending on the state someone is in? For example, if they are tired, have a lot of energy, had coffee recently, etc. Why do you think these do or don’t affect the reflex?

4. Don’t hurt yourself or anyone else investigating this, but does the speed or amplitude depend at all with the force you strike the knee with? Why might this be or not be?

5. For this experiment, the person having their reflex activated normally keeps the leg relaxed, but what if they try to resist the reflex? Does that affect the speed or amplitude at all, or does the reflex even happen? Why might this be?

6. We have noticed two things worth further examination. The reflex time tends not to be very variant in a single person during a session (always ±2 ms), but the reflex time between individuals can range from 13 ms to 35 ms. Since this is an unconscious reflex, what may be the reasons for such variability between individuals? Age, fitness, athletes (runners vs weightlifters, etc) may all be factors. Also, the reaction component tends to be more varied within a person (from 130 - 200 ms), as this requires conscious control and “attention” which increases time variability between trials. Start collecting data from all the people you know!

7. Is there a statistical difference in your results? You can use a student’s t-test to find out! (read more here: backyardbrains.com/experiments/p-value)
Lab 4: Muscle Movement

Name _____________________________

Background
When your brain decides to move a muscle, signals from your neurons travel from your motor cortex (called "upper motor neurons") to your spinal cord, where they connect with "lower motor neurons" (also called "alpha motor neurons"). These motor neurons then connect with muscle to make a "motor unit." A motor unit consists of a single motor neuron and the multiple muscle fibers it controls. A muscle fiber is a very special type of cell that can change its shape due to myosin/actin chains sliding across each other. A single motor neuron can connect with multiple muscle fibers. In general, a large, powerful muscle like your bicep has motor neurons that innervate thousands of muscle fibers, whereas small muscles that require a lot of precision, such as your eyeball muscles, have motor neurons that only control ~10 muscle fibers. When you contract a muscle, this is the result of many muscle fibers firing action potentials and changing shape.

In this lab, we are going to look at the electromyographic (EMG) signal in the thousands of muscle fibers within the bicep and tricep muscles in your arm. We can record these signals using your Muscle SpikerBox Pro!

Materials
● Muscle SpikerBox Pro
   ○ 9V Battery (this powers the SpikerBox)
   ○ Electrode cable (orange)
   ○ Device-specific cable:
     ■ USB cable for computer link
     ■ Aux cable for non-USB devices
● 6 electrode stickers
● Spike Recorder App: backyardbrains.com/products/spikerecorder
● How-to Video: bit.ly/BYBMuscleMovement

Investigation 1: Bicep Muscle
1. Remove the sticky backing from your Large Muscle electrodes, and place these surface electrodes over the largest part of your bicep. Exact placement is not critical. (see Figure 1)
2. Connect the red recording alligator clips from your electrode cable to these patch electrodes
3. Connect the black ground alligator clip to an electrode patch on the back of your hand.
4. Plug your orange interface cable in the orange channel 1 (left channel) on your SpikerBox.
5. Turn on the SpikerBox and listen to the sound that occurs. Listen for changes in the sound, for instance when you flex your bicep. *Don’t hear any sound? Make sure your device is on and turned up by turning the volume knob.
6. Connect your SpikerBox to your recording device using a USB or smartphone cable.
7. Open up the SpikeRecorder app so you can see the spikes on your screen. *Make sure you click the "USB" button if you’re on a computer. One way to test this is by clapping your hands once. If the SpikeRecord shows a big spike, it’s not connected to your SpikerBox.
8. Flex your muscle three times, each time drawing what the signal looks like on your screen. **Draw a picture below***(Drawing #1)
9. Pick up something heavy and put it down three times, each time drawing what the signal looks like on your screen. **Draw a picture below** (Drawing #2)

10. Pick up something heavy and HOLD it for 30 seconds. Draw what the signal looks like on your screen. **Draw a picture below** (Drawing #3)

---

**Investigation 2: Agonist/Antagonist Muscle Pairs**

1. Place two electrode stickers over the largest portion of the triceps and connect the **channel 2** red cables to them.
   (see lower electrodes on **Figure 2**)
   *Reminder: Your bicep cable should be connected to **channel 1**

2. Clip the second black lead alligator clip to the first one or place one additional sticker electrode on the back of your hand nearby wherever you placed your other ground for the bicep.

3. Look at your Spike Recorder app, you should see a new button color channel on your screen.
   *Help: If you don’t, click the USB button two times. If that doesn’t create two channels (green and red), you can open the settings (“gear”) and manually change the second channel to a non-black color.

4. While sitting at a table and gently lifting up with the palm of your hand, Flex your **bicep** (don’t flip your table!). **The color signal that moves the most is measuring your biceps**. Note which color that is, this should still be channel 1.

5. Flex your **tricep** by sitting at a table and pushing down with the back of your hands. **The color signal that moves most is measuring your triceps**. Note which color it is.

The two muscles (biceps and triceps) are an **antagonistic pair**. That is, if one extends a limb during its contraction, the other will return the limb to its original position when flexed. In each pair, depending on the movement, one muscle plays the role of the “agonist” and the other muscle plays the role of “antagonist”. The agonist is a muscle that contracts to cause the movement. The antagonist is an opposing muscle that relaxes relatively to stretch. These two roles, agonist and antagonist, can be exchanged back and forth. For example, if the bicep is an “antagonist”, it relaxes (low signal) while the tricep contracts (bigger/louder signal) as the “agonist”. In this activity you will record the **Root Mean Square** value (RMS), which represents the square root of the average power of the EMG signal for a given period of time, basically means it is a way to quantify the measure of muscle activity!

6. Click the "Record" button ⇒

7. Using the event markers (use the numbers keys on the keyboard), perform these different actions.
   a. WAVE at someone
   b. Perform a FIST PUMP
   c. Perform some push ups
   d. **Design your idea!** What other actions can you perform that will test the agonist/antagonist pair of muscles (check with your instructor)?

8. Stop recording

9. Open your recording using the three horizontal bars button ⇒
   *Tip: You may need to find the “BYB” folder, find your date and time recording*
10. Right-click and hold to highlight the tallest (high amplitude) signal for part a “waving”.

*Record Results Below in Table 1*

Investigation 3: Coactivation

For this investigation, we will measure the phenomena known as “coactivation” between the biceps and triceps. It is sometimes also known as muscle co-contraction, since two muscle groups are contracting at the same time.

1. Sit in a relaxed position, with your elbow bent to 90° with the palm facing upwards. Use the other hand to grasp the wrist of the arm you have the electrodes attached to. (see Figure 3)

2. Activate the biceps and triceps alternately as you did for investigation 1.
   a. Activating Bicep = Try to do a “bicep curl” with the arm with the electrodes (your other arm will “resist” this by pushing down on your wrist).
   b. Activating Tricep = Flex your tricep (your other arm will “resist” this by pulling up on your wrist).

3. Practice this alternating pattern until it feels to you that both muscles are being equally activated in turn.

4. Click the “Record” button ⇒

5. Perform the alternating pattern of activation for 20 to 30 seconds of at least 3 full contractions of each muscle in turn. Use the event markers to keep track of each muscle that you are activating.

6. Stop recording

7. Open your recording using the three horizontal bars button ⇒

8. Scroll through the recorded data and observe the EMG traces for both the biceps and triceps.

Note that when the biceps muscle is activated forcefully, there is a minor increase of activity in the triceps. You should also notice there is a minor increase of activity in the biceps trace when the triceps are activated. This phenomenon is called “coactivation”. Its meaning is not well understood, although it perhaps serves to stabilize the elbow joint.

8. Measure and insert into the table the RMS values for biceps and triceps during your alternating contraction of biceps and triceps.
## Results

<table>
<thead>
<tr>
<th>(Drawing #1) Drawing of flexing Bicep spike recording (from device screen)</th>
<th>(Drawing #2) Drawing of picking up heavy object spike recording (from device screen)</th>
<th>(Drawing #3) Drawing of HOLDING heavy object spike recording (from device screen)</th>
</tr>
</thead>
</table>

*Label when you flex your bicep!

### Table 1: Agonist/Antagonist Muscle Pairs

<table>
<thead>
<tr>
<th>Action</th>
<th>Spike Recording Drawing (label both the RED and GREEN channel drawings)</th>
<th>RMS Amplitude (mV/second)</th>
<th>Which muscle is the AGONIST?</th>
<th>Which muscle is the ANTAGONIST?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waving</td>
<td>Bicep</td>
<td>Tricep</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fist Pump</td>
<td>Bicep</td>
<td>Tricep</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Push Up (pushing up)</td>
<td>Bicep</td>
<td>Tricep</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Push Up (lowering)</td>
<td>Bicep</td>
<td>Tricep</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Design your own!</td>
<td>Bicep</td>
<td>Tricep</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Coactivation:

<table>
<thead>
<tr>
<th>Contracting Muscle</th>
<th>Contraction 1</th>
<th>Contraction 2</th>
<th>Contraction 3</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biceps RMS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triceps RMS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biceps RMS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triceps RMS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Reflection Questions

1. Describe how your bicep and tricep (antagonistic muscle pairs) are working at the elbow during the up and down movements of a press up.

2. If a friend told you they wanted to get bigger biceps, what exercises would you recommend? Which ones wouldn't be as helpful?

3. Is the arm the only place we see a muscle pair act like this? (Feel free to Google!)

4. Why do you think the co-activation of the opposite muscle group is necessary for normal joint operation? (Feel free to Google!)

Keep going! ⇒ Try out these other ideas to learn even more!

1. What happens if you attach the 2nd channel to someone else's bicep? Can you find ways to compete and change each other’s bicep contraction signals? (Hint: What happens during arm wrestling?)
2. Find someone to perform a **hands-free** “arm wrestling” match. Winner goes to whoever can keep their spikes longer! Add more science to your arm wrestling: Hit record during the “wrestling match” and then analyze your results. Look at RMS values and length of muscle contractions to get real data!
3. Do you think you could record the activity of other muscles with this setup? What about your leg muscles? Back? Chest? Forearm? Try them all out!
4. You are recording through the skin, and from several muscle fibers simultaneously. How would the recordings differ if you were recording right next to one of those cells? Inside the cell? Would you see the same number and type of spikes? How would the amplitude change?
Lab 5: Muscle Fatigue

Name _____________________________

Background
Why does lifting a 30 lb weight over and over get harder and harder to lift? Our muscle system is the largest system in our body (40%-50% of our weight). This system includes your heart, which is a pump made of specialized cardiac muscle, and the smooth muscles in your guts, allowing food to move. But to make voluntary actions such as lifting a soldering iron or kicking a soccer ball, you use your skeletal muscles! Your skeletal muscles allow you to do all the wonderful movements with which you pass your days. Each of your muscles is subdivided into functional groups of muscle fibers called motor units. A motor unit is a motor neuron and all of the muscle fibers it controls. To achieve great things, like lifting a heavy weight, motor units join together in a coordinated way to supply the force required to achieve greater strength. Muscle Fatigue occurs when the muscle experiences a reduction in its ability to produce force and accomplish the movement.

In this lab, you will use the Muscle SpikerBox Pro to record your bicep or forearm muscles while doing muscle contractions until fatigue (“failure”) occurs. You will measure the EMG amplitude during the contractions to learn about changes in muscle cells during fatigue.

Materials
- Muscle SpikerBox Pro
  - 9V Battery (this powers the SpikerBox)
  - Electrode cable (orange)
  - Device-specific cable:
    - USB cable for computer link
    - Aux cable for non-USB devices
- 6 electrode stickers
- Timer
- Weights of some kind (books, canned food, etc) in the 5-20 pound range
- A reusable shopping bag or backpack.
- Spike Recorder App: backyardbrains.com/products/spikerecorder
- How-to Video:
  - Video 1 - bit.ly/BYBMuscleFatigue1
  - Video 2 - bit.ly/BYBMuscleFatigue2

Investigation 1: Isometric Biceps Hold
1. Place two electrode stickers on two sides of your bicep on your dominant arm (see picture above). Add a ground electrode to the back of the hand. Repeat this on your other arm.
2. Connect the red recording alligator clips from your electrode cable to these patch electrodes on the dominant arm.
3. Connect the black ground alligator clip to the electrode patch on the back of your hand.
4. Plug your orange interface cable in the orange channel on your SpikerBox.
5. Turn on the SpikerBox and listen to the sound that occurs. Listen for changes in the sound, for instance when you flex your bicep.
   *Don’t hear any sound? Make sure your device is on and turned up by turning the volume knob.
6. Connect your SpikerBox to your recording device using a USB or smartphone cable.
7. Open up the SpikeRecorder app so you can see the spikes on your screen.
   *Make sure you click the “USB” button if you’re on a computer. One way to test this is by clapping once. If the SpikeRecord shows a big spike, it’s not connected to your Spikerbox.
8. Select a dumbbell or other weight that is at about 60% of your maximum lifting weight. Depending on your strength, this will be ~10-25 lbs (~5-12 kg).

9. Click the “Record” button ⇒

10. Click the number “1” on your keyboard (this creates an event marker in the recording)

11. Start a timer for 60 seconds

12. With your back to a wall to control your posture and arm position, hold the weight in your dominant hand for as long as you can, with your elbow at a 90 degree angle. This is called an "isometric" contraction since your muscles are working, but your joints are not moving.

   *Note: you will probably find that your wrist gets tired faster than your bicep. You can avoid this problem by hanging the bag or backpack off your wrist rather than holding a dumbbell in your hand (see video 1 for help)

13. Every 10 seconds, click the number “2” on your keyboard.

14. When the timer goes off, click the number ”3” on your keyboard. (this creates an event marker in the recording)

15. Repeat steps 9-13 for your non-dominant arm (you will have to switch the electrodes to your other bicep

   a. Use the event marker keys “4” to start, “5” every 10 seconds, and “6” at the end for your non-dominant arm.

   *Tip: If you get sweaty or the electrode stickers stop sticking, use new ones before continuing.

16. Stop recording

Background knowledge: In this investigation, you will record the Root Mean Square value (RMS), which represents the square root of the average power of the EMG signal for a given period of time, so basically it is a way to quantify the measure of muscle activity!

17. Open your recording using the three horizontal bars button ⇒

18. Starting at marker "1", hold the RIGHT-CLICK and DRAG the mouse between the event marker "1" and event marker "2"

   *If you can’t see both event markers on the screen, pinch to zoom OUT to show more time on the screen.

   (You will see the “scale” line on the bottom-right get smaller)

19. Repeat step 18, except this time start at the first “2” (this was at 10 seconds into the experiment) and go to the next “2” (which would have been 20 seconds into the experiment)

20. Record the RMS value from the EMG signal on your recording. (The RMS value will be on the right-side of the screen)

   *Record Results Below in Table 1*

21. Repeat steps 18-20, for your non-dominant hand. (if you followed the tip, you will be looking for the “3”, “4”, and “5” event markers)

   *Record Results Below in Table 1*
Investigation 2: Measuring Bicep Fatigue

1. Click the “Record” button ⇒
2. Click the number “1” on your keyboard (this creates an event marker in the recording)
3. With your back to a wall to control your posture and arm position, hold the weight in your hand for one minute, with your elbow at a 90 degree angle.
4. Every 10 seconds, click the number “2” on your keyboard.
5. When the timer goes off, click the number “3” on your keyboard.
6. Open your recording using the three horizontal bars button ⇒
7. Starting at the first spike, counting the firing rate during the first 10 seconds (between event marker “1” and “2”) *Record Results Below in Table 2*
8. Repeat step 10 for the next five 10-second intervals, for a total of one minute. *Record Results Below in Table 2*
9. Now construct a graph that shows the spiking rate during muscle fatigue. *Record Results Below in Table 2*
10. Label the x and y axes (include units)

Results

Table 1: Isometric Biceps Hold

<table>
<thead>
<tr>
<th>Time Range</th>
<th>Dominant arm (RMS Values)</th>
<th>Non-dominant arm (RMS Values)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 10 seconds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 - 20 seconds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 - 30 seconds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 - 40 seconds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40 - 50 seconds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 - 60 seconds</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2: Measuring Bicep Fatigue

<table>
<thead>
<tr>
<th>Time Range</th>
<th># of Spikes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 10 seconds</td>
<td></td>
</tr>
<tr>
<td>10 - 20 seconds</td>
<td></td>
</tr>
<tr>
<td>20 - 30 seconds</td>
<td></td>
</tr>
<tr>
<td>30 - 40 seconds</td>
<td></td>
</tr>
<tr>
<td>40 - 50 seconds</td>
<td></td>
</tr>
<tr>
<td>50 - 60 seconds</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
</tr>
</tbody>
</table>

Reflection Questions

1. Which hand did you have a higher amplitude (height) while holding the weight? Why do you think that is?

2. After measuring the muscle fatigue over time, what trends or patterns in your data did you observe? What do you think these tell you about muscle fatigue?

3. Muscles store energy until it is used up. Each muscle cell contains extra energy "storage" of glycogen, which your muscles break down so they can be used. What evidence do you have in your data to indicate that your muscles may have begun to use glycogen during this activity?

4. Do some additional Googling or reading. Where does glycogen come from? Is it protein, carbohydrate, fat or something else? What foods end up making glycogen? Is there a way to give your muscles more energy?
Keep going! ⇒ Try out these other ideas to learn more!
1. Sometimes, when hiking in your favorite park (like the Wonderland Trail or Torres del Paine), you find, even if you are not very fit, you can hike for 6-10 hours. However, if you tried to lift a 100 pound (45 kg) barbells repeatedly, you would rapidly get tired within 5-30 reps over a couple minutes depending on your athletic ability. Why is the time scale of fatigue so different in these two activities?
2. How can two muscles that are about the same size be so different in their fatigue properties? We didn’t cover it here, but you can begin reading about slow twitch and fast twitch muscle fibers to learn more.
3. Are there muscles that are very fatigue resistant? Can you find examples? Can you test them?
4. Work out your biceps for a month. Measure your fatigue time and EMG changes before the period of training and after the period training using the same test load/force.
Lab 6: ‘Fight or Flight’ Response

Background
Most of our labs have dealt with the *voluntary* nervous system or perception - that is, things we do willingly and consciously. But now let’s think about the “involuntary” part of the nervous system, the *autonomic nervous system*. The autonomic nervous system controls things we are both aware and unaware of but generally do not have much control over - digestion, homeostasis, sweating, blood pressure, heart rate, and many others. It is traditionally divided into two systems, the *sympathetic* nervous system (which activates the “fight or flight” response) and the *parasympathetic* nervous system (which activates the “rest and digest” response).

To activate your sympathetic nervous system, we will use an "ice water" stimulus. This is often used in pain studies as humans can tolerate it but does not result in psychological damage. As you maintain the hand in the ice water and your hand begins to hurt, your sympathetic nervous system "fight or flight" response will activate. We will also examine another effect on the heart rate, called the “diving reflex”. When a seal lion or other marine mammal dives, its heart rate decreases, but do other mammals, like humans, have this same reflex?

In this lab, we will use the *Heart and Brain Spikerbox* to record the signal called an electrocardiogram, also known as an ECG (or EKG if you prefer the German spelling). Let’s find out how our autonomic nervous system controls our heart rate!

Safety note: For students under the age of 18, perform this investigation with an adult or instructor present. Because each individual is different, pay attention to how you feel, how much “pain” you can take from the ice water’s coldness, and how fast your heart is beating. This investigation will work even with a slight amount of pain caused by the cold, so don’t push it beyond your comfort level! If you have a heart condition or other chronic disease, talk to your doctor before you conduct this investigation. If at any point in this investigation you feel dizzy, uncomfortable, or intense pain: STOP. Sit down, drink water, and contact your doctor.

Materials
- Heart and Brain Spikerbox
  - 1 9V Battery (this powers the SpikerBox)
  - Electrode cable (orange)
  - Device-specific cable:
    - USB cable for computer link
    - Aux cable for non-USB devices
- 3 electrode stickers
- Timer
- A large bowl of ice water
- A snorkel (if available) or wet & cold washcloth
- Spike Recorder App: [backyardbrains.com/products/spikerecorder](http://backyardbrains.com/products/spikerecorder)
Investigation 1: Ice Water and Heart Rate

1. Fill a large pot, bucket, or large bowl 3/4 full with ice.
2. Add cold water. The ice water will ensure that the mixture is always 32° F (0° C).
3. Place electrode patch stickers on your upper forearms, and one on the back of your hand.
4. Connect the red alligator clips with the electrodes on your upper forearms, and the ground alligator clip to the ground electrode on the back of your hand.
5. Plug your orange interface cable in the orange channel on your Heart and Brain SpikerBox.
6. Plug one end of the USB cable to the Heart and Brain SpikerBox and the other end into the computer, or use the aux cable to connect to the audio input of your device.
7. Open the SpikeRecorder software, and connect to the USB port in the settings menu if necessary.
   *Note: if the electrocardiogram appears upside down, swap the two red alligator clips to opposite forearms.
   (see Figure 1)

   Note: This next part uses “event markers” which can only be used on laptops, PCs (Windows, MacOS, Linux), and Android devices (not iOS phones or iPads). If conducting this on a phone, you will need to record the data in “real time” so make sure to have a friend, family member, or be able to monitor your heart rate while conducting this investigation.

8. Click the “Record” button ⇒
9. Type the number “1” on your keyboard (this creates an event marker in the recording)
10. Start a timer and just rest for 60 seconds while your heart rate finds a normal pace.
11. Place their hand in the ice water, but leaving the upper forearms exposed so that the electrodes are NOT submerged.
12. When you begin to feel “really” cold, click the number “2” on your keyboard.
13. Click the number “3” on your keyboard right as you remove your arm from the ice water.
14. Allow your hand to return to “normal” temperature and Repeat the procedure at least two more times to get an average.
15. Open your recording using the three horizontal bars button ⇒
   *Tip: You may need to find the “BYB” folder, find your date and time recording
16. For each marker event, count the number of peaks there are in the EKG oscillations for one minute to get your heart rate (beats per minute)
   *Record Results Below* (Table #1)

Investigation 2: Activating the “Dive Reflex”

1. Dump out the ice water in the pot above and replace it with more tolerable cold water (no ice).
2. Click the “Record” button ⇒
3. Type the number “1” on your keyboard (this creates an event marker in the recording)
   *NOTE: If you are not on a device with event markers, you may need to use a timer note the “recording” times at the top of your SpikeRecorder app so you know where your experiment starts, stops, or where key points are. ⇒
4. Start a timer and just rest for 60 seconds while your heart rate finds a normal pace.
5. Type the number “2” on your keyboard
6. Hold their breath and submerge their face under water for as long as you comfortably are able to.
   *Be careful! It is not a competition to hold your breath the longest or see how much “pain” you can take. Just do it as long as is comfortable.
7. Type the number “3” on your keyboard
8. Now use a snorkel -or- turn your head slightly so your head is still in the water while you keep breathing.
9. Repeat steps 2-7, but hold your face under the water while breathing through the snorkel.
   *Alternative: You can use cold wet washcloth across the face here instead of the snorkel.
10. Type the number “4” on your keyboard
11. Hold your breath instead but DO NOT put your head under water. Hold it as long as you comfortably are able to.
12. Open your recording using the three horizontal bars button ⇒
   *Tip: You may need to find the “BYB” folder, find your date and time recording
13. For each marker even, count the number of QRS peaks there are in the EKG waveforms for one minute to get your heart rate (beats per minute)
   *Record Results Below* (Table #2)
Results

Table 1: Ice Water and Heart Rate

<table>
<thead>
<tr>
<th>Condition</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Rate at Rest</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart Rate at moment hand began to feel cold</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart Rate at moment before hand was taken out</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Activating the “Dive Reflex”

<table>
<thead>
<tr>
<th>Condition</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Rate at Rest - marker “1”</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart Rate - marker “2”</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ Holding breath</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ Underwater</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart Rate - marker “3”</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ Breathing air</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ In water</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart Rate - marker “4”</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ Holding breath</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ NOT underwater</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Reflection Questions

1. Why do you think ice water triggers the “fight or flight” response? What does your heart rate increase?

2. Looking at the “autonomic nervous system” diagram on the right, what other reactions are happening in your body (besides increased heart rate) while your sympathetic nervous system is activated?

3. Using one of the reactions you chose in #2, explain why you think your body reacts this way. (Do some research!)

4. Why do marine mammals evolve the “diving reflex”? Why does this change in heart rate help them?

5. Thinking about evolution, why might humans still have the “diving reflex”?

6. During the “Dive Response: Did the “head underwater” or “hold breath” investigation lead to a higher heart rate? Do some research and explain why you think that is.

Keep going! ⇒ Try out these other ideas to learn more!

Note: Remember to consult with your instructor before exploring other research questions that may affect your heart rate.

1. Are there differences between your left and right arm as it relates to cold tolerance and ultimately, heart rate?
2. Can the “flight or fight” response be triggered by other responses besides cold water?
3. Are there differences between athletes and people with normal or low levels of fitness?
4. Are there differences in age, differences between male and female students, etc.
5. Does the temperature of the ice matter? Try several different trials with varying temperatures to see if this affects heart rate.
6. We previously studied the effect of exercise of heart rate. Why would or would not this stress response caused by ice increase the heart rate through different mechanisms than exercise?

Note: If you repeat enough observations, you can do statistical hypothesis testing, using a student’s t-test (read more here: backyardbrains.com/experiments/p-value)
Lab 7: See Your Own Brain

Name _____________________________

Background
Your skull and skin in your head protect the valuable, wonderful brain of yours, but they are also excellent electrical insulators, making it difficult to record from individual neurons in the human brain. To record individual neurons, your electrodes have to be in direct contact with the neural tissue. In EEG research, the common positions we record from identify the region they are sitting over. Today we are recording from O₁ and O₂ where the ‘O’ stands for **Occipital Lobe**, the visual processing center!

When you close your eyes, your visual cortex is not receiving complex information from your eyes (only darkness), and the visual cortex enters an idling (like a car) and produces "alpha waves" which we can detect non-invasively (that’s correct - no brain surgery today).

In this lab, we will use the **Heart and Brain Spikerbox** to record the signal called an **electroencephalogram**, otherwise known as an **EEG**.

Materials
- Heart and Brain Spikerbox
  - 9V Battery (this powers the SpikerBox)
  - Electrode cable (orange)
  - Device-specific cable: USB cable for computer link
  - Aux cable for non-USB devices
- EEG headband
- 1 electrode sticker
- Bottle of electrode gel
- Spike Recorder App: backyardbrains.com/products/spikerecorder
- How-to Video: bit.ly/BYBSSeeBrain

Investigation 1: Record an EEG
1. Put the orange EEG headband on your head, with the two electrodes centered on the top-back of your head (this is where your visual cortex is). The smooth side of the electrodes should touch your scalp.
   *Tip: This investigation works even if you have hair, but moving your hair "out of the way" of the electrodes will be helpful.*
2. Add globs of electrode gel underneath the smooth metal patches in the headband.
3. Now, add an electrode sticker on the bony part right behind your ear (the mastoid process). (See “Figure 1”)
   *Tip: Adding some conductive gel to this electrode before applying to the ear bone will improve the stability of your signal.*
4. Place the red alligator clips* on the back of your head on the metal parts of the headband, and the black alligator clip on the ground behind your ear.
   *Which red clip is in which location doesn’t matter.*
5. Connect your electrode cable to your SpikerBox and make sure the battery is correctly installed.
6. Connect your SpikerBox to your recording device, either using a USB cable to connect to your computer or a smartphone cable to your mobile device.
7. Open the SpikeRecorder software.
8. Turn on your SpikerBox.
   *Make sure you click the "USB" button if you're on a computer. One way to test this is by clapping once. If the SpikeRecord shows a big spike, it's not connected to your Spikerbox.
9. Hold still and relax while you are attempting to record EEGs - muscle movements can also be picked up, which causes interference with your EEG reading.
   *Tip: Have your laptop and SpikerBox far from any electrical outlets, away from any fluorescent lights, etc. Also have your laptop running on battery power alone. If the signal seems excessively noisy and unstable, add more conductive gel between the headband electrodes and your scalp, and more gel to the electrode placed behind the end. Use the video above as a guide.
10. Click the “+” button on the left side of the signal or “pinch and zoom” to increase the height of the signal on your screen. (See “Figure 2”)
11. Open your eyes for 10 seconds, then blink a few times, then close your eyes for 10 seconds. Repeat a couple times. (Obviously, doing this investigation with a friend will be easier and more fun, since you can't see the signal appear when your eyes are closed.)
   a. If you are by yourself, you should record the data, you can press the red record button on the Spike Recorder software.
      *Tech Tip: Each event (i.e. eye opening) can be recorded with an “event marker”, created by pressing a number on your keyboard.
   b. See our troubleshooting guide for tips if you are having trouble locating the alpha rhythms

*Draw a picture below* (Drawing #1)

Investigation 2: Alpha Rhythms of the Visual Cortex

1. Click the “Record” button ⇒
2. Open and close your eyes, alternating every 10 seconds. Mark an event for each time you open and close your eyes (Event marker created by pressing a number on your keyboard)
3. Repeat step 1, two more times.
4. Stop recording.
5. Open your recording using the three horizontal bars button ⇒
   *Tip: You may need to find the "BYB" folder, find your date and time recording
6. Count the number of downward peaks there are in the EEG oscillations starting from the beginning of the time bar to the end.
   *Tip: There should be roughly 10 oscillations in that 1-second time window, then we write 10 per second as “10 Hz” (Hz = 1/s). These are Alpha Rhythms!
   *Record Results Below* (Table #1)
7. Continue experimenting with Alpha Rhythms by testing these different conditions:
   a. Without opening and closing your eyes, but turning the lights on and off in your room. (Use event markers when you turn lights on and off)
   b. With the lights off and eyes closed, try to "look" for something in the darkness.
   c. With the lights off and eyes closed, think “really hard” about being in a bright room.
   *Record Results Below* (Table #1)
Results

Drawing 1: Record an EEG

<table>
<thead>
<tr>
<th>Condition</th>
<th>Drawing of SpikeRecorder signal</th>
<th>Label: Alpha Waves, Amplitude, Event Markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eyes open (10 seconds)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(spike signal from device screen)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Label the amplitude (height)!</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eye Lids Closing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(spike signal from device screen)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Label the amplitude (height)!</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eyes closed (10 seconds)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(spike signal from device screen)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Label the amplitude (height)!</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Alpha Rhythms of the Visual Cortex

<table>
<thead>
<tr>
<th>Condition</th>
<th>Drawing of SpikeRecorder signal</th>
<th>Label: Alpha Waves, Amplitude, Event Markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eyes open for 10 seconds, Eyes closed for 10 seconds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eyes closed, turning lights on and off</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lights off, eyes closed; “looking” for something</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lights off, eyes closed; thinking “really hard”</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Reflection Questions

1. The reason behind Alpha Rhythms lies with how "synchronous" the brain activity is. The more synchronous (i.e., firing at the same time) the neurons in your brain are, the less data processing is occurring. This leads to the odd result that the stronger the electrical signal we can record on the surface of your scalp, the less interesting things your brain is doing. Knowing this, why are we only able to detect alpha rhythms when our eyes are closed?

2. Beta rhythms are rhythms with a higher frequency (increased spikes per second) than alpha rhythms (see image to right). They are associated with anxiety, focus, panic, and worry. Why might this be the case?

3. Using the image on the right, you can see the common EEG wave patterns, from highest frequency to lowest. Deep sleep, drowsiness, eyes closed, and focus can be assigned to each of these wave patterns. Match the following activity with the wave pattern:

   1. Deep Sleep a. Delta
   2. Drowsiness b. Alpha
   3. Eyes closed c. Beta
   4. Awake, focused d. Theta

Keep going! Try out these other ideas to learn even more! (check with your instructor first)

1. Is this alpha rhythm generated in the visual cortex? Try moving the headband around your head to see where the rhythm is the strongest.
2. What effect do certain conditions have on aspects of the alpha wave? Ex- age, male vs female, how much sleep they've had, whether they've had caffeine recently.
3. We say here that the alpha rhythms really don't reflect what someone is thinking, but is that really true? Try recording alpha rhythms from a person while they're thinking different thoughts. How about while doing math problems or listening to music? Does it have any effect? Why or why not?
4. If you're recording from someone and are able to consistently get alpha rhythms, try seeing how long after they close their eyes the rhythms appear, and how long after they open their eyes, the rhythms disappear. Does the amount of time affect if alpha rhythms are present? Why or why not?
5. What rhythms do you see during sleep? If you record your sleep using the SpikeRecorder app, you can see if your rhythms change!
6. What about while listening to music? Try a song that is familiar to the volunteer and one that they have never heard. Are there any differences?

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1 Low noise low power instrumentation amplifier for biomedical application, - Scientific Figure on ResearchGate. Available from: https://www.researchgate.net/figure/The-typical-normal-brain-rhythms-with-their-amplitude-levels_fig3_303487700
Lab 8: Measuring Eye Movements

Name _____________________________

Background

While it is well known that your heart and brain generate electrical potentials, you may not know that your EYE has a potential as well! It does not change quickly in the form of "impulses" like your heart and brain, but it does have a voltage difference we can measure. Specifically, the front of the eye, where the cornea is located, is more positive than the back of the eye (where the retina is). By placing the electrodes on our BYB headband on either side of the eye, we can detect left vs right movement. By having one electrode above the eye and another electrode below the eye, we can detect up and down movements along with blinks.

In this lab, we will use the Heart and Brain Spikerbox to measure the movement of our eyes by recording an electrooculographic signal, otherwise known as an EOG.

Materials

- Heart and Brain Spikerbox
  - 9V Battery (this powers the SpikerBox)
  - Electrode cable (orange)
  - Device-specific cable:
    - USB cable for computer link
    - Aux cable for non-USB devices
- 3 electrode stickers
- Tape measure, yard stick, or ruler (longer is better)
- Can, cup, baseball or similar-sized object
- Spike Recorder App: backyardbrains.com/products/spikerecorder
- How-to Video: bit.ly/BYBEOG

Investigation 1: Eye Movements (Left and Right)

1. Take your BYB headband, and place it such that the electrodes are positioned on either side and eye (see Figure 1)
   a. Alternate hookup: You can use two sticky electrodes in the same position at figure 1 above your eyebrow instead of using the headband.
2. Now, add an electrode patch on the bony protrusion behind your ear (this bone is your mastoid process; see Ground in image).
   *Note: An alternative electrode setup is to put both electrodes patch electrodes on either side of the temples, on the outer side of the eyes.
3. Place the red alligator clips on the electrodes around the eye, and the black alligator clip on the ground behind your ear. Which red is in which location around the eye does not matter for this experiment.
4. Connect your electrode cable to your SpikerBox and make sure the battery is correctly installed.
5. Connect your SpikerBox to your recording device, either using a USB cable to connect to your computer or a smartphone cable to your mobile device.
   *Tip: Like with the brain, you will have to monitor electrical "noise" in this experiment. Have your laptop and SpikerBox far from any electrical outlets, away from any fluorescent lights, etc. Also, have your laptop running on battery power if it has enough charge.
6. Open the SpikeRecorder software.
7. Turn on your SpikerBox.
   *Make sure you click the "USB" button if you’re on a computer. One way to test this is by clapping once. If the SpikeRecord shows a big spike, it’s not connected to your Spikerbox.

8. This signal is prone to EMG artifacts from facial muscles. Try clenching your teeth, blinking, or raising your eyebrows and be able to identify these as artifacts, not usable data.

9. Move your eyes to the **left**. You should see a signal deflection in one direction followed by a rapid deflection in another direction. (see example signals in Figure 2)
   *Record Results Below in Table 1*

10. Move your eyes to the **right**.
    *Record Results Below in Table 1*

11. Blink
    *Record Results Below in Table 1*

### Investigation 2: Saccade Calibration

Your eye is used to calibrating when objects are moving. For example, if you try reading this paragraph while moving your head slowly left and right, your eyes automatically adjust to help you keep reading! Our eyes constantly flicker back and forth (involuntary movements known as **saccades**) so we’re now going to test your **saccade** eye movements. We’ll find out how far apart your eyes need to be before an eye potential can be measured.

1. Using a yardstick, you will be moving a can, cup, or similarly sized object 20 centimeters per trial. You will ultimately create an experimental setup that looks like this:

   ![Diagram of calibration setup](image)

   "0 cm" will be your center. "+" indicates to the **right** and "+" indicates to the **left**.

   *Tip: If you don’t have a long yardstick, you may want to use tape to clearly mark these measurements or place objects at each point (Ex: placing a cup at each 20 cm increment)

2. Place your object "+20 cm" (20 centimeters to your right) and "-20 cm" (20 centimeters to your left).

3. Sit in a location so that your eyes are centered at the "0 cm" location.

4. Click the "Record" button ⇒

5. Type the number "0" on your keyboard (this creates an event marker in the recording)
   *NOTE: If you are not on a device with event markers, you may need to use a timer note the “recording” times at the top of your SpikeRecorder app so you know where your experiment starts, stops, or where key points are. ⇒
6. Look at the “0 cm” marker. Do **NOT** look left or right. Wait for about 3 seconds.

   *This will be your control*

7. Type the number “1” on your keyboard

8. Look **right** at the object at the “+20 cm” location. (do this 3 times)

9. Type the number “2” on your keyboard

10. Look **left** at the object at the “-20 cm” location. (do this 3 times)

11. Repeat steps 9-10 for the objects at the other locations. (use different event marker numbers)

   *Tip: Table 2 below has the labeled event markers so you don’t have to remember all of the numbers.*

   a. For more trials, repeat steps 10-13 more times to get more accurate data.

12. Open your recording using the three horizontal bars button ⇒

   *Tip: You may need to find the “BYB” folder, find your date and time recording*

13. Starting at marker “0”, hold the RIGHT-CLICK and DRAG the mouse between the event marker “1” and event marker “1”

   *If you can’t see both event markers on the screen, pinch to zoom OUT to show more time on the screen.
   (You will see the “scale” line on the bottom-right get smaller)*

14. Repeat step 13, except this time start at the first “1” (this was at 10 seconds into the experiment) and go to the “2” (which would have been 20 seconds into the experiment)

15. Record the RMS value from the EMG signal on your recording. (The RMS value will be on the right-side of the screen)

   *Record Results Below in Table 2*

---

**Results**

**Table 1: Eye Movements**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Drawing of SpikeRecorder signal</th>
<th>Label: Signal, Amplitude, Event Markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eye moves left</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eye moves right</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blinking</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2: Saccade Calibration → Record below: Did the movement trigger a potential above the threshold?

<table>
<thead>
<tr>
<th>Condition</th>
<th>-80 cm</th>
<th>-60 cm</th>
<th>-40 cm</th>
<th>-20 cm</th>
<th>0 cm</th>
<th>+20 cm</th>
<th>+40 cm</th>
<th>+60 cm</th>
<th>+80 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Event Marker</td>
<td>8</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>0 (control)</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>7</td>
</tr>
</tbody>
</table>

Trial 1 (RMS Values)

Trial 2 (RMS Values)

Trial 3 (RMS Values)

Reflection Questions

1. Do the potentials look different when your eyes are open or closed? Why or why not?

2. Your saccades allow for your eyes to scan scenes and help your brain produce a detailed image. Why do you think this constant movement of the eyes is helpful to producing an image? Why is it better than just our eyes staying still? (Don’t know? Do some research!)

Keep going! ⇒ Try out these other ideas to learn more!

1. Is the amplitude of the potential affected by how far you move your eyes? How quickly? What other variables do you think could have an effect, and why?

2. How does placement of the electrodes affect the potential generated? If the electrodes are over your left eye, do your right or left movements produce higher RMS values? What if you switch the electrodes to above your other eye? What if you close one eye while you repeat investigation 2?

3. Try movements that just require your eyelids-closing your eyes (slowly), squinting, opening them wide. Why or why don’t we see an EOG for these, or if you do, how does it compare to the earlier ones seen?

4. How about holding your gaze far to the left, right, up, or down? Looking far in one direction will trigger a potential, but does keeping your focus keep triggering new potentials? If so, how long does it keep firing?

5. Does whether or not someone needs glasses have an effect? Why or why not? If so, what is the effect?

6. How do you know that the differences in investigation 2 are statistically significant? If you repeat enough observations, you can do statistical hypothesis testing, using a student’s t-test (read more here: backyardbrains.com/experiments/p-value)