Mantis Shrimp Punch Instructor's Guide

Overview

In this lab you will empower students to ask and answer questions about neuroscience by having them build their own equipment to examine the biology that underlies behavior. This lab will:

1. Teach students how to handle and anesthetize mantis shrimp
2. Develop and Perform a chronic implant surgery for EMGs
3. Record EMGs of the mantis shrimp strike
4. Analyze your data in Python

Objectives

Before doing this lab you should understand:

- What EMG is and how it relates to movement
- Understand the Mantis Shrimp hunting behaviour
- Basics of Mantis Shrimp anatomy

After doing this lab you should be able to:

- Understand how to record an EMG
- Synchronize relevant behaviours to electrophysiological data
- Describe the mantis shrimp use power amplification
- Explain how to analyze electrophysiology data

Equipment

Pt1

- Spikerbox Pro
- USB to microUSB cable
- Dip sockets
- An old toy speaker
- Backyard Brains muscle cable (x2)
- Soldering iron
- Liquid electrical tape
- Small scissors
- Insulated silver wire (0.005” bare, 0.0070” coated, A-M Systems)
- Soldering flux
- Lighter
- Coarse sandpaper
- Helping hands

Pt2

- Ice
- Small scissors
- Two pairs of forceps
- Superglue
- Marine epoxy or dental cement (powder component, perm-reline/liquid component, mixing container, disposable wooden mixer)
- Hypodermic needles, between 22 ga and 31 ga
- Silly putty
- Paper towels/kimwipes

Pt3

- Computer with the Backyard Brains App and Spyder IDE installed
- Helping hands

- Scissors and fabric

Pt 4

- Anaconda or miniconda installation of Spyder IDE and dependencies (Python 3.6). In anaconda terminal (or regular terminal on unix-based operating systems):
```
conda install spyder
conda install matplotlib pandas seaborn scipy beautifulsoup4 tkinter
```

Time: 4 days (1 hour per part), or 1 day given a long lab time.

Difficulty: Hard

Introduction

Mantis shrimp evolved a unique way of interacting with the world around it compared to other carnivores in the animal kingdom. While most animals use talons, teeth, claws, or stingers to capture prey and aggress other individuals, mantis shrimp evolved the ability to punch. And you know what? They got good at it. Though brief, the impact has the same peak force as a tiger's bite, and bigger ones can break aquarium glass.

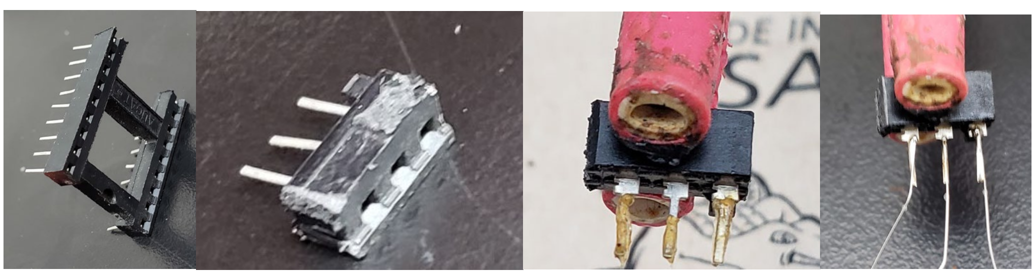
How do they muster such energy? The appendage they use to punch is arranged like a crossbow. A small muscle called the flexor flexes, cocking the propus against the merus, and it won't move until the flexor is relaxed. While the flexor is activated, the extensor starts twitching, each twitch bending the meral saddle more and more. Once sufficiently bent, the flexor releases and the propus and dactyl heel fly out towards the target.

For this experiment, the extensor's EMGs will be recorded during this infamous strike. A chronically implanted EMG "backpack" will be made and implanted to accomplish this. This backpack can be plugged in and out, allowing for mantis shrimp to be kept alive over the course of multiple days and even after the study is over.

Part 1: Making the chronic implant

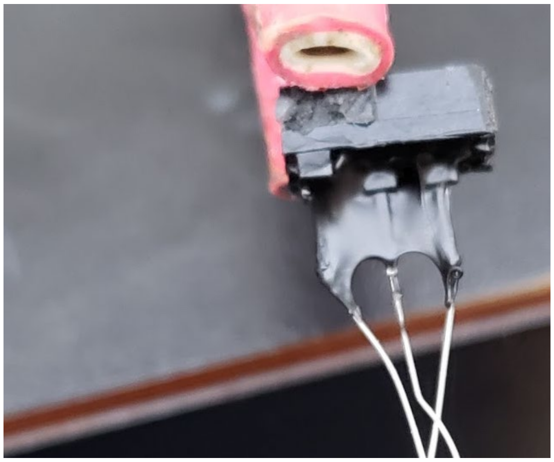
1. Cut three approximately 1.5-inch lengths of insulated silver wire. Strip a few millimeters of both ends of the wire by holding the wire above the flame of a lighter until the insulation retracts down the wire. The remaining silver might form a tiny ball at the end due to surface tension.

2. Take a line of dip sockets and carefully cut off three pins. Apply flux to each lead. In one hand, hold the stripped end of a piece of wire against the flux, and with the other hand solder it in place using a very small amount of solder. A helping hands tool may be useful.

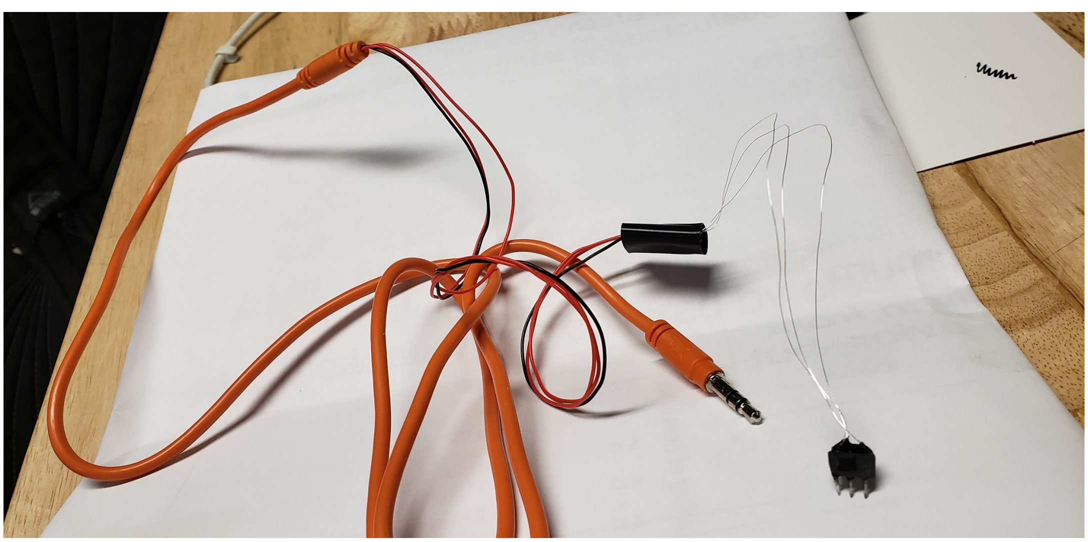


3. Use liquid electrical tape or your preferred method of insulation, cover the exposed leads, it will take at least 10 minutes to dry. Make sure the entire insulated length is covered. If metal is

exposed on the plastic from where the pins were cut, insulate as well.

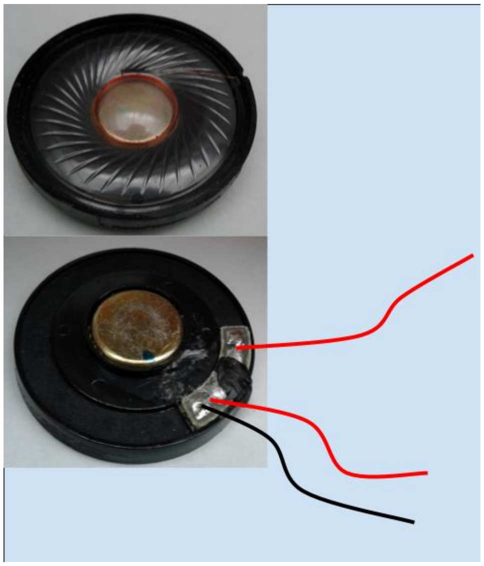


4. To make the plug and microphone, cut off the alligator clips and strip the ends of the wires on each of the Backyard Brains muscle electric cables.
5. For the plug, make another set of three stripped silver wires. These should be at least 8 inches long. Cut another three pins from the dip socket line.
6. Sand the female side of the three dip socket pins until metal is exposed. Apply flux to the metal and **solder one end of each of the stripped silver wires** to it. A helping hands may be useful.
7. Finally, **solder each of the other ends of the stripped silver wire** to each of the stripped wires on the muscle electric cable. Make sure that the black wire is soldered to one of the side pins, not the middle. The black wire is ground, and future steps are made easier if the black wire is soldered to one of the side connectors instead of the middle one.
8. Insulate all exposed metal and solder with liquid electrical tape.



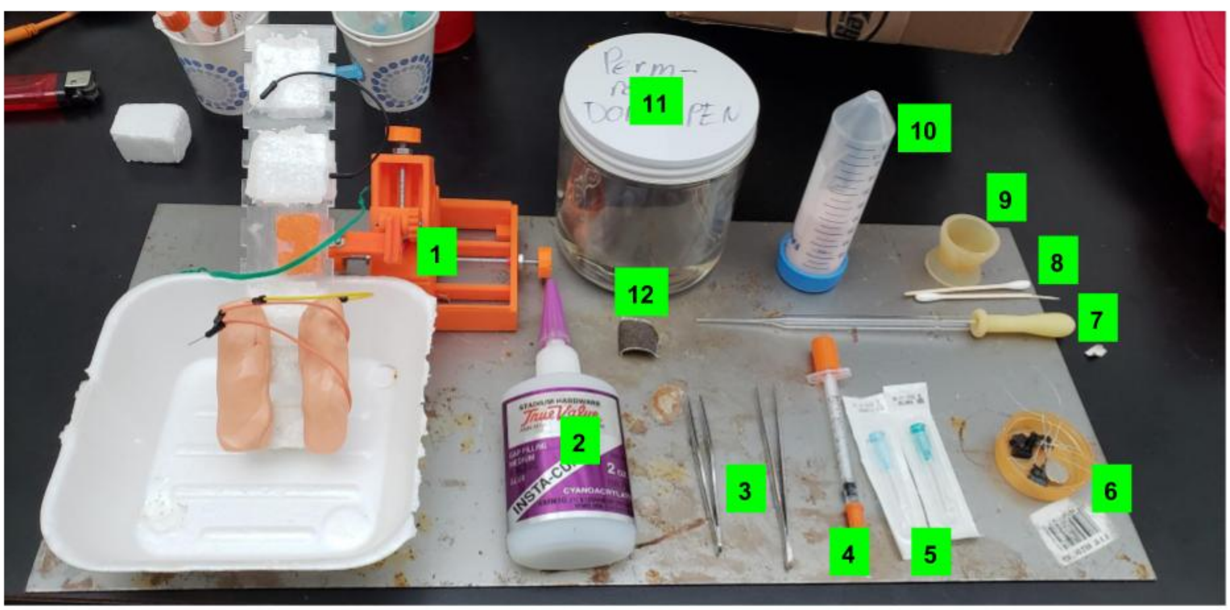
9. To make the microphone/hydrophone, strip the microphone so that it looks like it does below. Solder one red wire to each, and solder the black wire to one of them. This allows the hardware on the Spikerbox Pro to function as a simple microphone.

   Cover all metal and soldered parts with liquid electrical tape. Finally, waterproof by covering with a plastic glove.



Part 2: Surgery

1. For convenience, items pictured here can be kept in cups with magnets at the bottom, so they stay upright on the stainless steel tray.



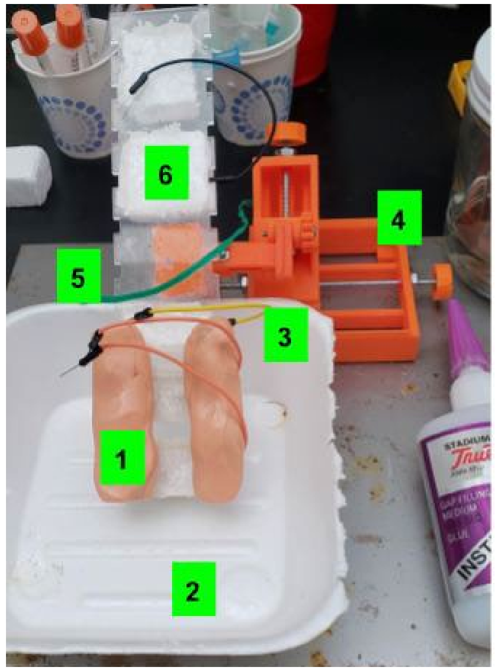
Parts shown:

01. Leverable stereotax (see below)
02. Superglue
03. 2 pairs forceps
04. High gauge (30+ ga) needle, new
05. 2 Medium gauge (21-25 ga) needles, new. One is for application of superglue, the other is for making small holes in cuticle.
06. Backpack, already fabricated
07. Pasteur pipette
08. Cotton swab, cracked down the middle for fine application of dental cement.
09. Silicone mixer
10. Dental cement powder
11. Perm-reline, A.K.A. dental cement liquid component
12. Coarse sandpaper
13. Keep paper towels handy.
14. Small scissors (not pictured)

Dental cement can be mixed in a glass, not plastic, container, and cleaned after each mixing with ethanol. Excess dental cement can be tossed in the trash.

If your lab cannot obtain dental cement, marine epoxy dries over the course of a few hours instead of 5 minutes for dental cement, but it does begin to harden over the course of 5-10 minutes, enough to keep the implants in the animal. Marine epoxy is a two-part adhesive that needs to mixed on a paper surface. It is quite feasible to perform surgeries with marine epoxy successfully, but things tend to stick less well. Animals may have to be re-anesthetized to fix wires that come out of the cuticle more often than with dental cement. For marine epoxy, putting the mantis into an empty tank with only saltwater and an airstone for an hour before returning it to its home tank allows the dental cement to dry in an environment with as few obstacles as possible.

Finally, super glue and marine epoxy must not be directly applied to the mantis shrimp. Instead, extrude each substance onto a piece of paper. In the case of marine epoxy, mix it with a syringe tip. Using the same tip, apply small globs of adhesive as required.



Leverable stereotax:

01. Silly Putty
02. Water bath
03. Jump cables
04. Stereotaxis apparatus with four degrees of freedom (see http://www.grifiti.com/nootle/grifiti-nootle-magnetic-mini-ball-head-camera-stand.html for a good alternative)
05. Twist tie
06. Plank (1.5” x 7 in”)

Superglue small blocks of styrofoam to the bottom of the plank. These will act as a sort of corkboard for plugging jumper wires into, a very convenient method for quickly restraining an unruly mantis shrimp.

2. Depending on the size of the mantis shrimp, put ice in a large cup or a bucket (if less than 5 inches, put in cup, otherwise put in bucket), and allow the mantis shrimp to cool down for two minutes. If it is still responsive after 2 minutes, wait another minute before picking it up, and repeat as necessary. Keep the ice nearby throughout the surgery.

3. Working quickly, place the mantis shrimp on your stereotax. The mantis shrimp’s pleopods should be mostly immersed in aerated water. The restraint you use for the animal depends on whether you are using a large or small mantis shrimp. For large mantis shrimp, you will want to rely on twist ties to hold it against the plank, as well as jumper wires plugged into the styrofoam. For smaller mantis shrimp, arrange one egg’s worth of silly putty

Big mantis shrimp:

Smaller mantis shrimp:

The anesthesia only works for a minute or two, which should be enough time to get a good restraint. Once the anesthesia wears off, mantis shrimp often strain mightily to escape. If the mantis shrimp escapes, simply pick it up, re-anesthetize it, and restrain it again, more tightly than before.

4. Use a small square of coarse sandpaper to *lightly* score the carapace, pictured below.
5. Dabbing some superglue on the mixing paper, use your mixing needle to apply a layer on top of the scored carapace. Place the backpack on top of the layer with thumb and forefinger in the orientation below. Mix dental cement or marine epoxy and apply it on the sides, top, and back of the backpack. Leave the front, where the dip socket's holes are, free of adhesive.

6. Make sure the animal is breathing: are its pleopods undulating in the water?

7. Implant ground

    a. Take the higher gauge (smaller tip) needle and place it against a part of the carapace caudal to the backpack. Roll the needle between the dominant hand's thumb and forefinger, gently keeping it against that same spot. Your fingers will travel down the syringe due to the pressure are exerted on the needle. Use the non-dominant hand's index finger to stabilize the syringe by touching the upward-facing base. If the needle isn't digging in to the cuticle properly, try the lower gauge needle.

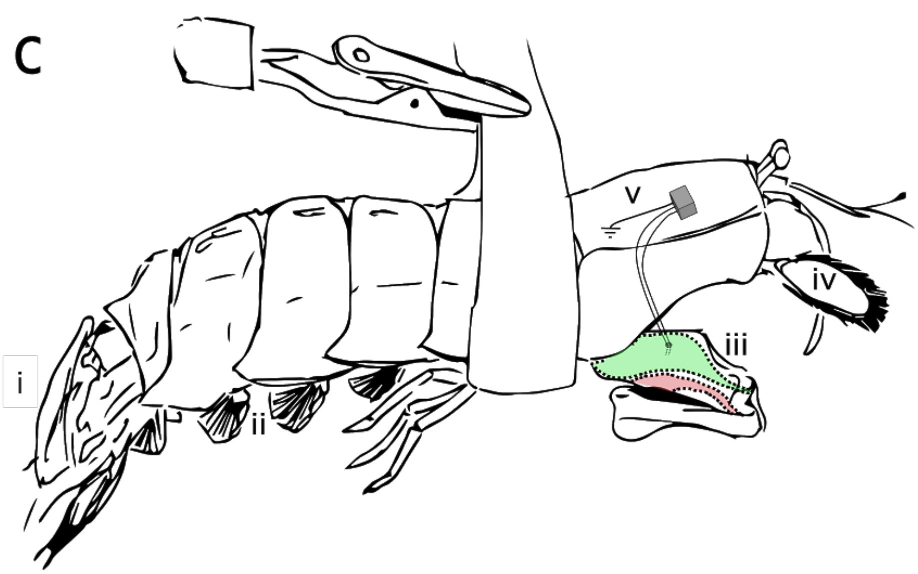
    b. Repeat until a hole has opened on the cuticle. The higher gauge needle might enter the flesh, in which case it can be gently taken out with no harm done. The lower gauge needle will make a hole from which a blob of fluid will seep.

    c. If a surface tension ball of silver is on the end of any wires on the backpack, cut it off (but leave the stripped silver). Take a forceps in each hand, and make a millimeter-long bend in the silver wire ground. This will act as an anchor, making it harder for the wire to slip out.

    d. Angle the silver wire's anchor into the hole, making sure the entire length of bare silver is inside the cuticle. Use one or both forceps as you see fit to make the entry as smooth as possible.

    e. With the mixing needle, apply a layer of superglue around where the ground wire enters, and then a layer of dental cement or marine epoxy. **Be careful not to let any adhesive drip onto joints. If any does, quickly wipe it away.**

8. Implant signal electrodes in merus, targeting the extensor.



    a. Score merus with sandpaper.

    b. Using the same method as above, open a hole in the cuticle in the region of the extensor.

    c. For each remaining probe wire, make an anchor and insert into the hole, making sure the insert the probes in different directions to minimize probability of them touching.

9. With mixing needle, cover the hole and scored region around it with a thin layer of superglue. Once dry, mix dental cement and cover that same area. If the antennal scale is getting in the way, hold it up and out of the way with a needle skewered into the silly putty.

Be careful to not coat the wires in dental cement or marine epoxy. The wire a few millimeters above at the entrance to the cuticle can be covered in these substances, but any more than that puts the wire at risk of being snapped, as dental cement and marine epoxy are brittle when coating fine wires.

10. Carefully remove the animal from its restraints. Once it realizes that it is free, it may thrash around, but it probably will not leave the water. Pour the water bath containing the animal back into the tank, or grab the animal swiftly and firmly and place it back in the tank.

Part 3: The experiment

1. Connect the backpack plug to channel 1 on the Spikerbox Pro, and connect the micro/hydro-phone to channel 2. Connect the Spikerbox Pro to the computer by USB and open the Backyard Brains app. Make sure the red channel is for audio and the green channel is for EMG. The red channel should show a waveform when you snap your fingers near the microphone, and the green channel should respond when a moist finger touches the dip socket leads.

2. Cut a strip of fabric, two inches wide, half an inch high, with a notch in the middle to wrap around the mantis shrimp. For a bigger animal (at least a half foot), make the restraint proportionally bigger.

3. Fill an appropriately-sized water bath (see above for example baths for large and small animals, note that the smaller animal simply uses the same bath as is used for surgery) with aerated water. You want the animal's tail to have enough space to maneuver and stretch out.

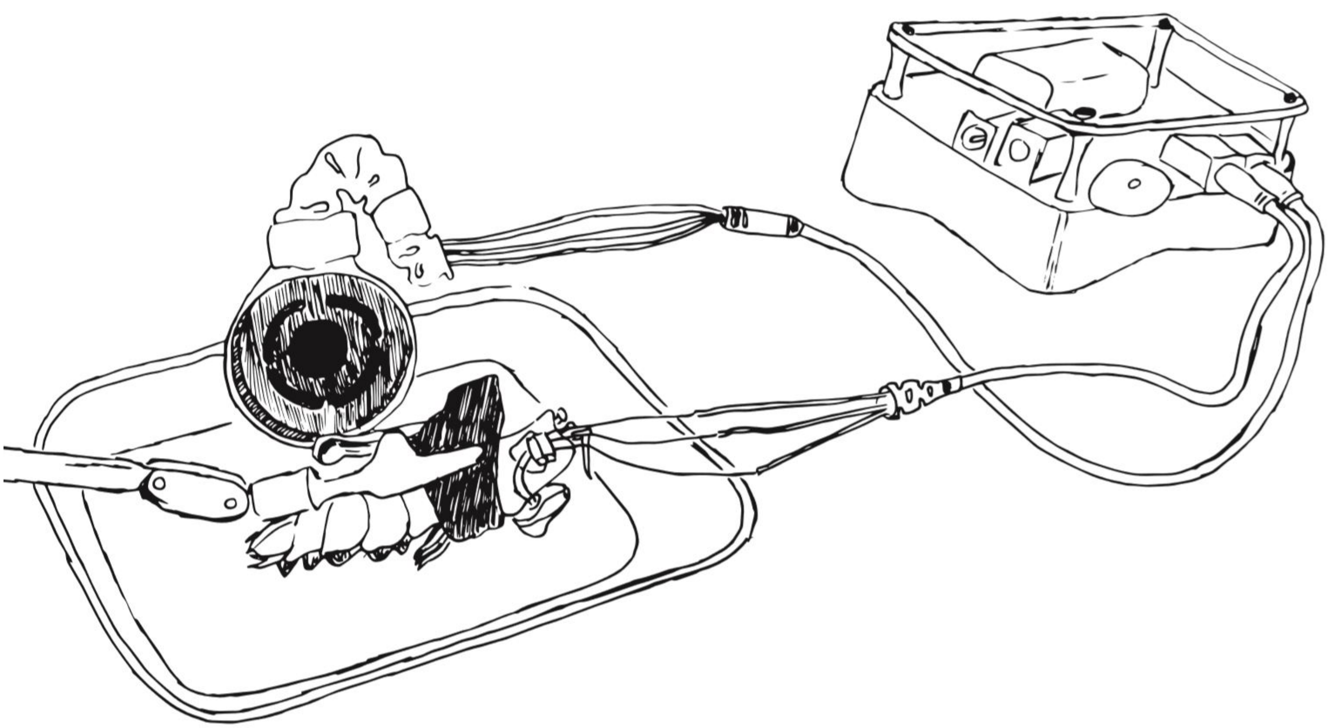
4. The blue implement in the background of the middle picture above is the hydrophone, the microphone soldered to the audio cable and wrapped in a glove. Position it half in, half out of the water.

5. Wrap the anesthetized animal as shown above and clamp, using a **stable** helping hands tool.

6. The stimulus can be a pencil, q-tip, pen, frozen shrimp, live shrimp, or a rolled-up piece of paper towel. A rolled up piece of paper towel was found to be a particularly effective stimulus, since it unravels in the water as shown below.

   Make sure your hands do not introduce electrical noise into the water. Humans are giant antennas for 60Hz/50Hz noise, so hold conductive stimuli (ie, wet or metal stimuli) with wooden or plastic implements. Do not touch the water while recording.

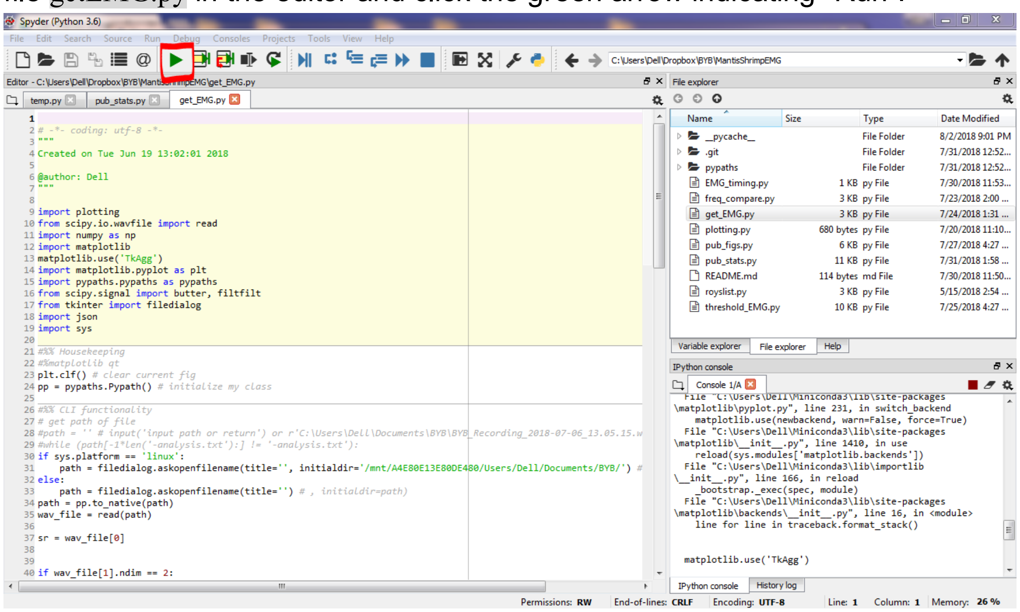
7. Make sure both channels are working, the Backyard Brains app running, and that you have a way of presenting stimuli. Grasp the backpack on the animal's carapace and plug in the cable, making sure the ground lead of the cable goes into the ground socket, which should be on the side. It may take a few tries to grasp the backpack firmly and plug the probe in.



8. Press record on the app and present stimuli. Do not make a lot of noise because the hydrophone is recording audio both inside and outside of the water. The punch makes a popping sound that will be used for data analysis later on.
    a. Space stimulus presentations out by 5-10 minutes because the animals will quickly habituate to a stimulus quickly otherwise. Make a single recording per stimulus presentation.
    b. Some mantis shrimp strike stimuli readily and some do not. Discuss why this might be during the 5-10 minutes in-between trials. Discuss within your group what might be done to elicit behavior in non-striking animals, and why your approach did or didn't work.

Part IV: Data analysis in python

1. Open Spyder IDE.
2. First, you need to find the part of your data that contains the data you want. For this, we will open the file getEMG.py in the editor and click the green arrow indicating "Run".



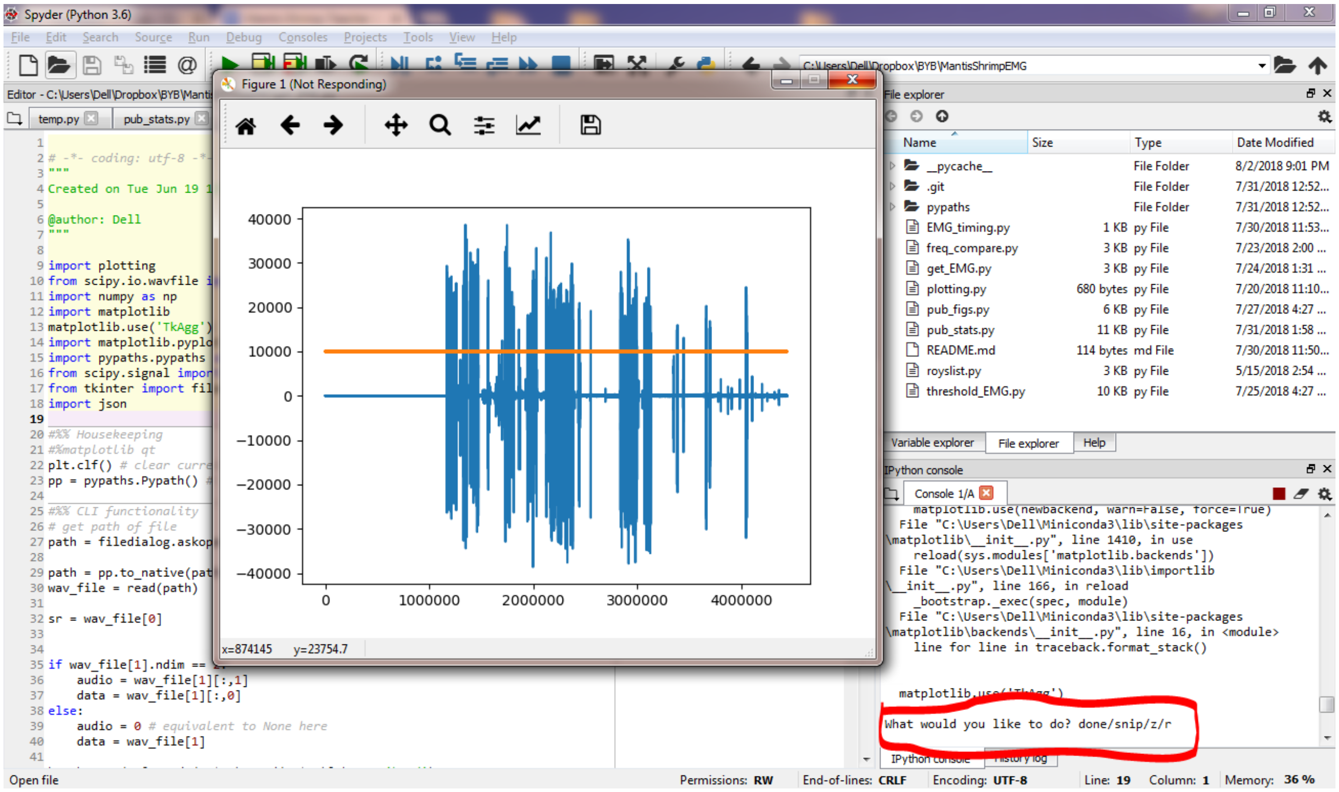
3. A new window open, prompting you to select the WAV file in your Documents/BYB/ folder.
4. The file selection window will close and be replaced by a new window with a visualization of your entire recording. In the Spyder console, there are a few command-line interface-style options:

    Typing done and pressing enter will end the analysis.

    Typing snip and pressing enter will select the portion of the recording that is currently displayed, which you usually don't want to do immediately.

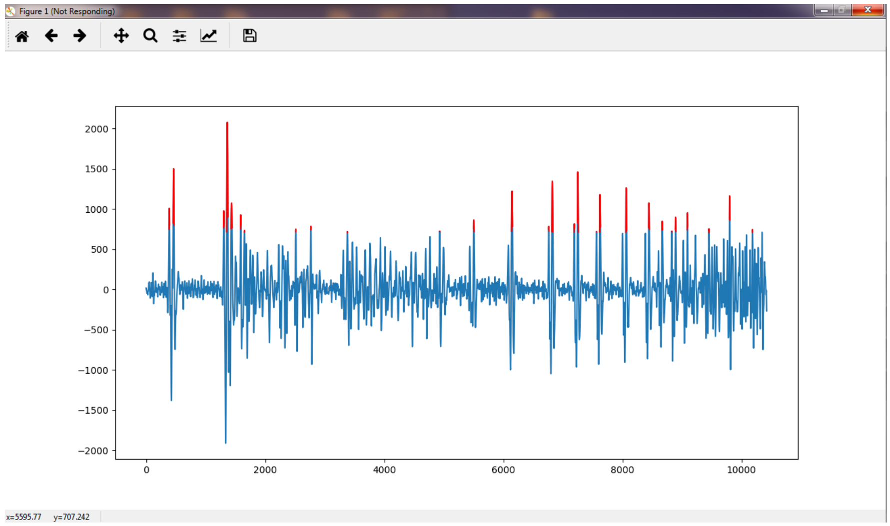
    Typing z (for zoom) and pressing enter will allow you to zoom into the recording and select an area of interest. Left click on the left and right of the area of interest within the windows. This will resize the window around the selected region. Type z and left click again to regine the connection . See the github README for more information on how to zoom in a matplotlib window.

Typing r (for reset) and pressing enter will display the entire recording again. If you have zoomed in and have decided that the region you are looking at is not so interesting after all, this command will let you search for EMG spikes somewhere else in your recording. Often, the EMG region that looks like a nice spike burst ends up being noise, so this command is used quite often.



Once a good enough region has been isolated, containing only EMG spike bursts, type snip and press enter. This will save that region for future analysis, and give you the option of zooming in on another region within the recording, or ending this stage of analysis for that data file. If you want to find another region, type z and press enter, otherwise type done and press enter.

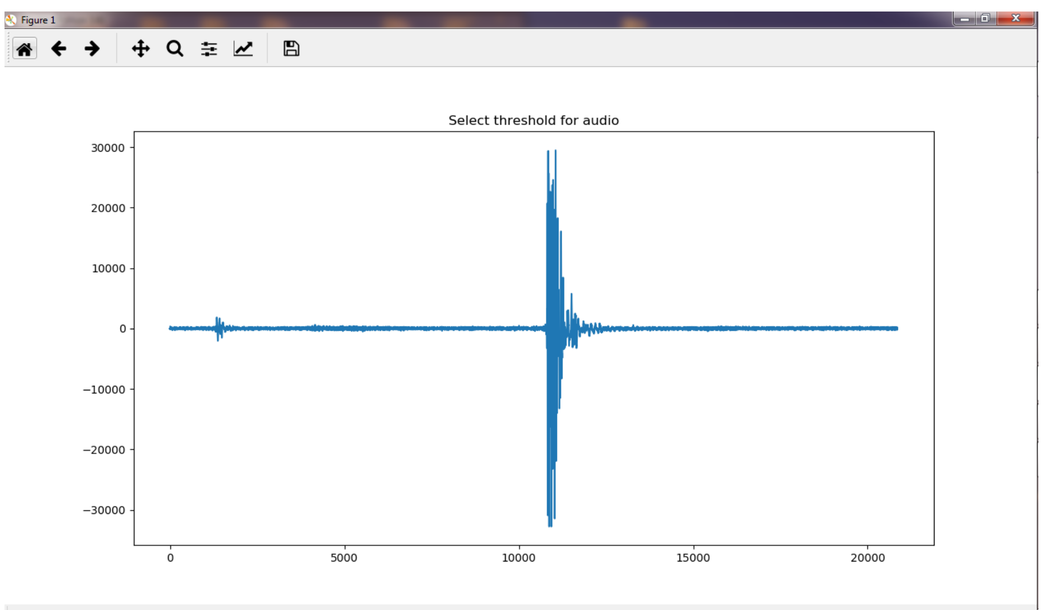
5. Once this script has been run, a new JSON analysis file with the filename BYB_recording_<timestamp>_analysis.json has appeared in the Documents/BYB folder. JSON files are a convenient way to serialize, or convert data into text that can be stored data on disk, which you can open back up in the future and use as Python variables.
6. Next, open the threshold_EMG.py file in the editor and click run. This time, select the **JSON analysis file in the window that pops up, not the WAV file.**



Another window will pop up with a visualization of your EMG region, as above. Now, you have to isolate each spike to collect its waveform, without including noise. To do that, this script uses a technique called thresholding to find “threshold crossings” that only include the tips of spikes. From there, the program isolates the points around the spike and stores it for later analysis.

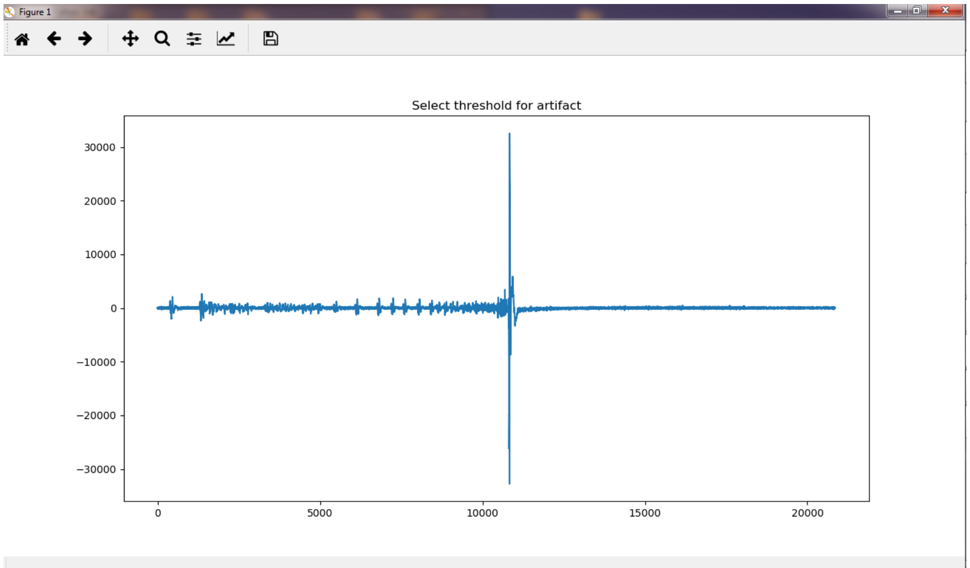
To do this thresholding, left click in this window at a point with a y-value you think is appropriate as a threshold. All points above this threshold will be colored red. If you think this would work well as a threshold, enter y in the Sypder console. Otherwise enter n to try again.

7. Next, a window audio will pop up with a section of the audio waveform that was collected at the same time as your EMG region. Click a point with a y-value that clearly intersects the sharp spike of the pop sound of the strike.



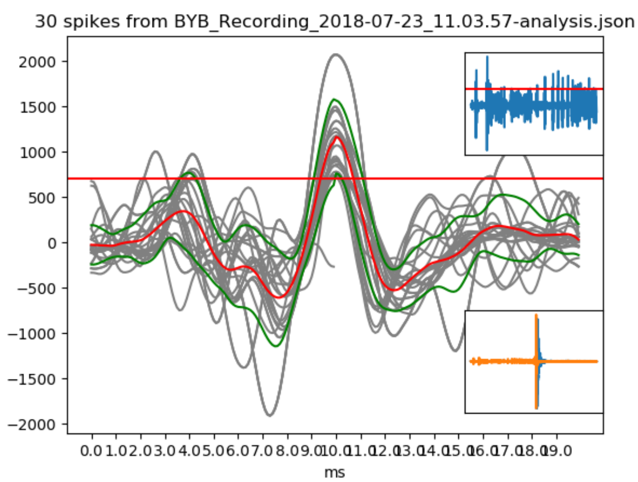
Sometimes, the audio does not contain a clear audio trace of the pop. That is alright, in that case just make sure you threshold at least some points.

8. Finally one more window will pop up. This is a trace of the EMG. It looks different because the artifact phase, which looks a bit like the spiky audio waveform of the 'pop', is a lot bigger than the EMG twitch spikes themselves.



9. For each EMG region that you selected for that recording (i.e., in that WAV file), you will repeat this process. If you have one region per recording, the program will update its JSON file and terminate.

Also, as a result of this thresholding, the program has spit out a PNG file in the Documents/BYB folder



The main figure is a summary of all the spikes, with each in grey, the average in red, and one standard deviation plotted in green. In the top right, we see all the spikes together in the trace, and on the bottom right, we see what thresholding the audio and artifact has created: two cross shapes, which tell us about how clear our "auxiliary" data are. These tell us that the region that we isolated are most likely taking place right before a strike, and the spikes in the main figure are actual extensor activity.

Now you can move on to the next step!

10. If you open your JSON file, you will see several fields, including x_regions, spikes, onset, offset, etc. Having run the getEMG.py and threshold_EMG.py scripts, we have accumulated a lot of information about the recording. Now it's time to visualize it! First, run pub_figs.py and select the relevant **JSON analysis file.** Take a look at the resulting PNGs in the Documents/BYB folder.
11. After analyzing a few more recordings, make an excel file named dataannotations.xlsx, and format it as below.

	A	B	C	D
1	**title**	**organism**	**notes**	**include**
97	C:\Users\Dell\Documents\BYB\BYB_Recording_2018-07-16_13.35.29.wav	Mantis Shrimp		
98	C:\Users\Dell\Documents\BYB\BYB_Recording_2018-07-16_13.49.48.wav	Mantis Shrimp		
99	C:\Users\Dell\Documents\BYB\BYB_Recording_2018-07-16_13.54.54.wav	Mantis Shrimp		
100	C:\Users\Dell\Documents\BYB\BYB_Recording_2018-07-16_14.42.40.wav	Mantis Shrimp		
101	C:\Users\Dell\Documents\BYB\BYB_Recording_2018-07-16_14.49.31.wav	Mantis Shrimp		
102	C:\Users\Dell\Documents\BYB\BYB_Recording_2018-07-16_14.57.53.wav	Mantis Shrimp		
103	C:\Users\Dell\Documents\BYB\BYB_Recording_2018-07-18_13.00.00.wav	Mantis Shrimp		1
104	C:\Users\Dell\Documents\BYB\BYB_Recording_2018-07-18_13.01.11.wav	Mantis Shrimp		
105	C:\Users\Dell\Documents\BYB\BYB_Recording_2018-07-18_13.06.28.wav	Mantis Shrimp		
106	C:\Users\Dell\Documents\BYB\BYB_Recording_2018-07-19_15.12.27.wav	Mantis Shrimp		
107	C:\Users\Dell\Documents\BYB\BYB_Recording_2018-07-19_15.14.13.wav	Mantis Shrimp		
108	C:\Users\Dell\Documents\BYB\BYB_Recording_2018-07-19_15.17.55.wav	Mantis Shrimp		
109	C:\Users\Dell\Documents\BYB\BYB_Recording_2018-07-19_15.21.57.wav	Mantis Shrimp		
110	C:\Users\Dell\Documents\BYB\BYB_Recording_2018-07-19_15.22.45.wav	Mantis Shrimp		
111	C:\Users\Dell\Documents\BYB\BYB_Recording_2018-07-19_15.31.26.wav	Mantis Shrimp		
112	C:\Users\Dell\Documents\BYB\BYB_Recording_2018-07-23_10.36.59.wav	Mantis Shrimp	Good data!	1
113	C:\Users\Dell\Documents\BYB\BYB_Recording_2018-07-23_10.42.36.wav	Mantis Shrimp	Good data!	1
114	C:\Users\Dell\Documents\BYB\BYB_Recording_2018-07-23_10.48.31.wav	Mantis Shrimp	Good data!	1
115	C:\Users\Dell\Documents\BYB\BYB_Recording_2018-07-23_10.56.22.wav	Mantis Shrimp	Good data!	1
116	C:\Users\Dell\Documents\BYB\BYB_Recording_2018-07-23_11.03.57.wav	Mantis Shrimp	Good data!	1
117	C:\Users\Dell\Documents\BYB\BYB_Recording_2018-07-23_11.15.35.wav	Mantis Shrimp	Good data!	1
118	C:\Users\Dell\Documents\BYB\BYB_Recording_2018-07-23_11.31.49.wav	Mantis Shrimp	Good data!	
119	C:\Users\Dell\Documents\BYB\BYB_Recording_2018-07-23_11.38.47.wav	Mantis Shrimp	Good data!	1
120	C:\Users\Dell\Documents\BYB\BYB_Recording_2018-07-23_11.53.07.wav	Mantis Shrimp	Good data!	1
121	C:\Users\Dell\Documents\BYB\BYB_Recording_2018-07-23_12.01.44.wav	Mantis Shrimp	Good data!	1
122	C:\Users\Dell\Documents\BYB\BYB_Recording_2018-07-23_12.10.20.wav	Mantis Shrimp	Good data!	1
123	C:\Users\Dell\Documents\BYB\BYB_Recording_2018-07-23_13.35.50.wav	Mantis Shrimp		
124	C:\Users\Dell\Documents\BYB\BYB_Recording_2018-07-23_13.49.36.wav	Mantis Shrimp		

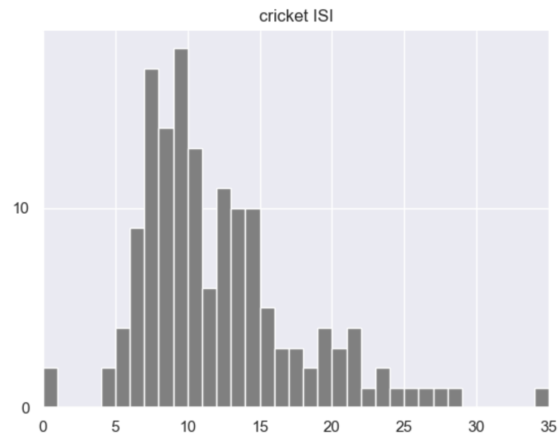
Sheet1 | Sheet2 | update new sheets

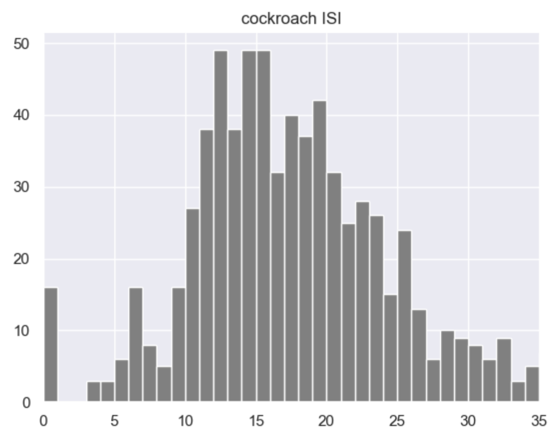
Ready

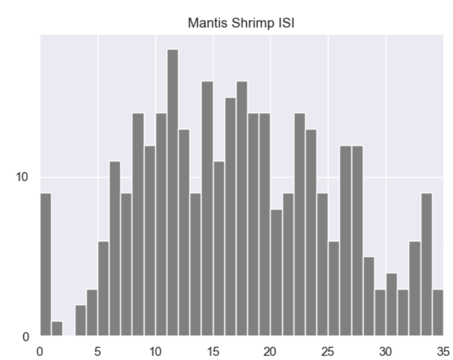
Each row is information about an entire recording. The first column has the name of the .wav file that was analyzed. The second column is the animal from which the recording was taken (ie, the species name for a cross-taxa experiment or the name of the individual organism for an experiment in only mantis shrimp). The notes column can have anything you deem important about the recording. Finally, the fourth column contains numbers delimited by spaces corresponding to the nth EMG regions you want to include for overall analysis within that file. (i.e., if you are interested in the first, third, and fourth region of recording x, type 1 3 4. Format this column as text.)

12. Run pub_stats.py.

Since this study used three animals, three figures are produced summarizing the interspike interval for all recordings in each species. A interspike interval histogram simply show the distribution of delays between spikes in each recording. If spikes are very spaced out in the recording, the histogram will have a peak on the right, and if spikes are very close together in the recording, the histogram will have a peak on the left.

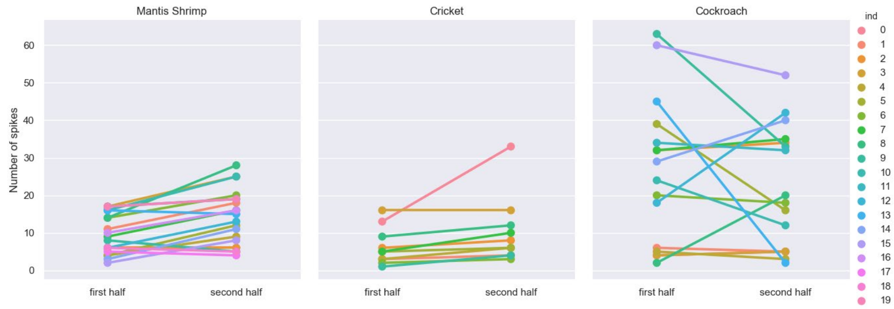




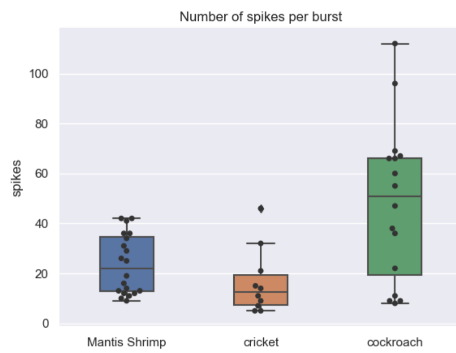


Do interspike intervals between species seem to be the same or different?

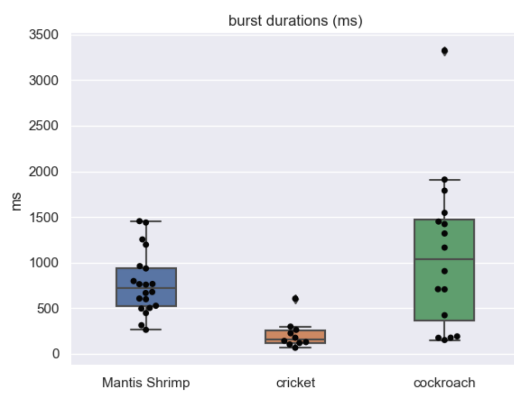
A few other graphs are generated, too. First, a graph showing the number of spikes in the first and second half of each recording for each organism roughly indicates how the rate of firing changes throughout the recording.



Next, another graph simply shows the total number of spikes in each recording. In effect, it summarizes the previous figure.



Finally, another figure shows the total length of time each EMG spike burst is from beginning to end for each organism.



Answering questions about data by programming analyses is a crucial skill for any scientist to learn. Students are encouraged to ask more questions about their data and tinker with this script to answer them. The JSON files this script analyzes contain a wealth of information from which an immense number of subtleties can be pulled out.

Chronic electrode & Hydrophone	Company	Catalog number	Unit Price	Qty
0.1” pitch dip socket strip	TE Connectivity	816-AG12D-ES-LF	$2.85	1
Silver wire, 0.005” bare, 0.0070” coated, 25’	A-M Systems	786000	$76.00	1
Liquid electrical tape, black	Star brite	071247841541	$6.98	4 oz
Muscle Cable	Backyard Brains	BYP0075	$15.00	2
4W Microphone	PUI Audio, Inc.	AS07708PS-2-WR-R	$4.67	1

Stereotax Materials	Qty
Silly Putty	28 g
Water bath	> 300 ml
Jumper cables or pipe cleaners	4
3D Printed Micromanipulator	1
Plank (1.5” x 7” x 0.1”)	1

Surgery Materials	Qty
Superglue	1
Marine epoxy or dental cement	1
Forceps	2 pairs
High gauge needles (30+ ga)	2
Low gauge needles (21- ga)	2
EMG backpack	1
Coarse sandpaper	1 sheet
Scissors	1 pair
Paper towels	1 roll

Table 1. Itemized list of raw materials needed to construct the equipment for this laboratory exercise.