

# The Brain and the Brain-less: A Lesson in Sensation and Perception

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## **Abstract**

Previous research on the photosynthetic algae *Euglena gracilis* has shown that Euglena can sense and respond to blue light, and does not seem to sense red or yellow light, due to the properties of its red eyespot. Euglena has only been known to show positive phototaxis when the intensity of blue light is within a critical intensity. To show the effect of blue light on Euglena, both positively and negatively, we used multiple different methods of exposing them to light. We found a strongly negative phototactic response under high-intensity beams of light, such as those from laser pointers or circuit LEDs above 20% brightness. There seemed to be a positive phototactic response when the brightness of the blue LED was lowered to 2%.

## **Introduction**

Neuroscience is a field of science thriving in the modern age. As knowledge within this field grows it is becoming imperative that the general public be educated in the fundamentals of neuroscience. BioBus Inc. is a non-profit organization whose mission is to communicate science to underprivileged communities and inspire them to pursue science. The organization has curricula for a vast array of scientific topics ranging from patterns in nature to the structure of the visual system. One aspect of neuroscience the organization is working on a curriculum for is sensation and perception. This topic deals with questions such as what is the purpose of the brain and whether brainless organisms can perceive. For this curriculum two activities were developed, one involving an organism without a brain and one involving the human brain.

A model organism that can be used to demonstrate sensation and perception in an organism lacking a brain is *Euglena gracilis*. It is a unicellular flagellate algae that can sense light utilizing an eyespot. This eyespot contains retinal, a form of vitamin A also found in the human eye (Gualtieri, 1992). Connected to the eyespot is the flagella; such a connection allows for the organism to exhibit phototaxis. Phototaxis is the movement of an organism in response to light. These responses can either be positive or negative. Positive phototaxis is movement of the organism towards the light source while negative phototaxis is movement of the organism away from the light source. If stimulated with blue light the algae will exhibit positive phototaxis. Red light yields no phototactic effect. *Euglena gracilis* will exhibit negative phototaxis to light that is too intense or light that is not intense enough (Giometto, 2015). The aim of this research was to reproduce these phototactic responses on the BioBus as an activity illustrating sensation and perception in an organism lacking a brain.

To illustrate sensation and perception in the human brain an EEG cap can be used. This device allows for the detection of alpha waves from the occipital cortex of the brain when the eyes are closed. These waves are the result of multiple neurons in the brain firing at the same time whilst the eyes are closed. When the eyes are closed there is no visual information being processed. This allows the neurons in the visual cortex to fire action potentials in synchrony. In contrast, when the eyes are open different neurons are responding to different stimuli within the field of view of the observer (EEG-Record from the Human Brain).

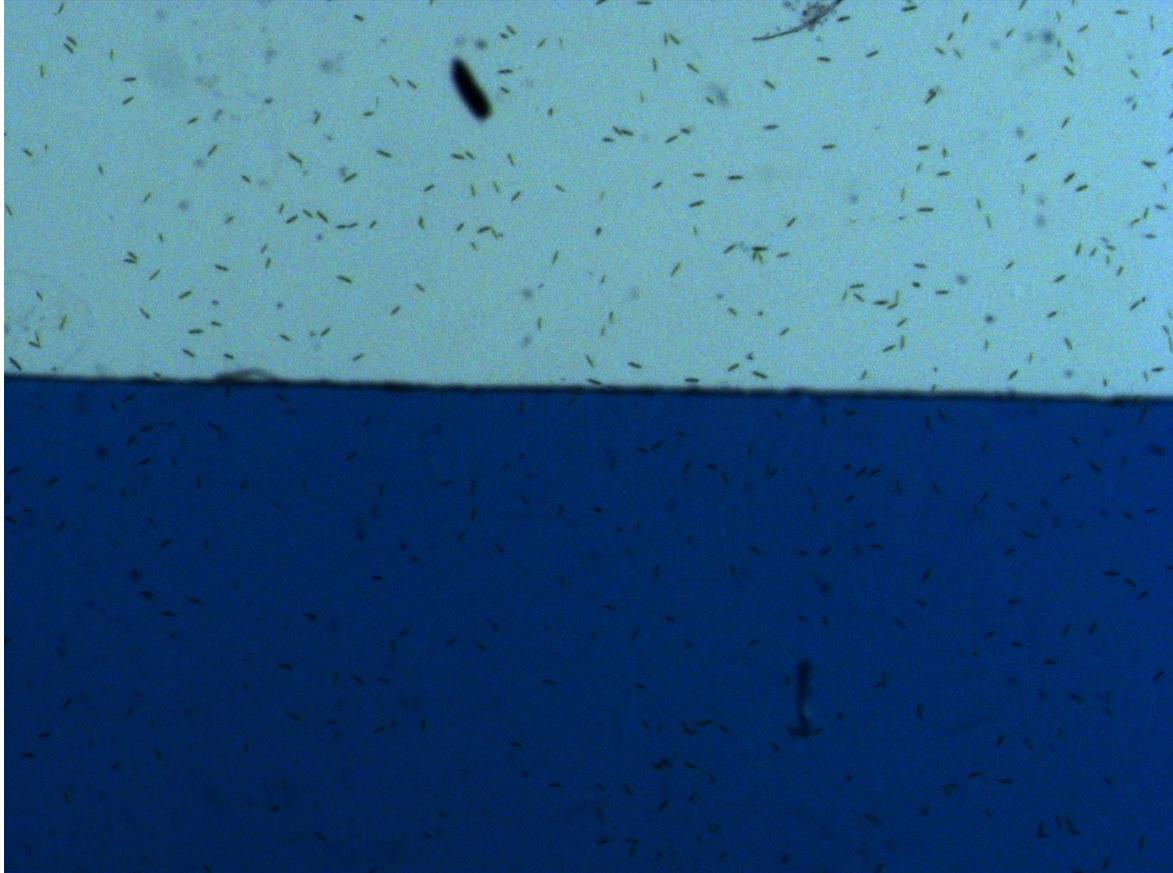
## **Methods**

### ***Optimization of Microscope***

For these experiments to be able to have quantifiable data, the camera on the stereomicroscope had to be changed to one that was capable of recording video. The camera that had been used prior to the change automatically adjusted the exposure and gain of the camera when the light intensity was changed, but this was a task that had to be undertaken manually. We spent a few days finding the correct light conditions for the images so that the Euglena could be seen, but also that the image would not be too noisy.

### ***Filter Paper***

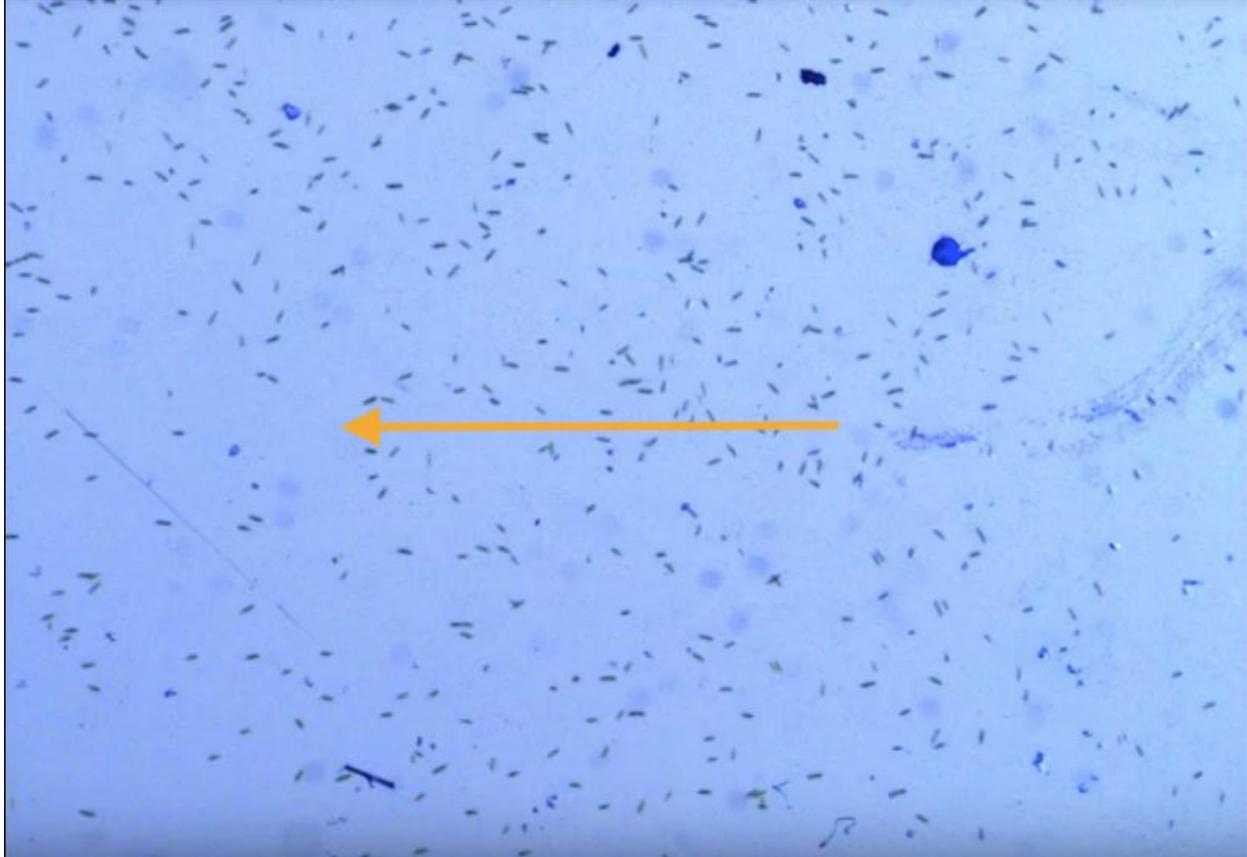
At different light intensities, we put red, green, or blue filter paper underneath half of the wet mount slide containing *Euglena gracilis*, with the expectation that the Euglena as a group would prefer one side to the other. Although the Euglena seemed to move one way or the other at first, we soon realized the change was negligent. The light that the Euglena was responding to was coming from the microscope, or the natural sunlight in the lab. The Euglena seemed to move parallel to the filter paper more often than they moved from one side or the other. They might have been moving away from the sunlight coming in through the windows, which was very strong because the Euglena no longer had the sea as a filter. The filter paper also may not have been letting enough light pass through it to attract the Euglena properly. In future experiments, the concept of a gradient could be explored. Maybe the Euglena would prefer some parts of the gradient to another, because the filter paper could have been too dark to let light through, or it might not have been the right shade of blue that the Euglena would be attracted to. With a gradient, we would be able to compare the concentration of Euglena in the different parts.



**FIGURE 1.** The blue filter paper at 50% light intensity. The top half of the image has no filter and is only the light provided by the microscope. The bottom half of the image is the blue filter paper underneath the slide. The movement of the Euglena is not in one net direction and is not observable mass behavior. The movement is completely random.

### ***Laser Pointers***

For the trials with the laser pointers, we constructed a black cardboard tube around the scope and stage of the microscope to decrease the influence that natural light would have on Euglena. When hit directly with the light beam from the laser, the Euglena exhibit a shock reaction, spinning to orient themselves before swimming quickly away from the light. The blue laser pointer showed very clear results of negative phototaxis at every microscope light intensity--the Euglena consistently fled from the light (Figure 1). This was only true for the blue light, however: with the red laser pointer, the Euglena seemed to move faster but their movement was much more random.



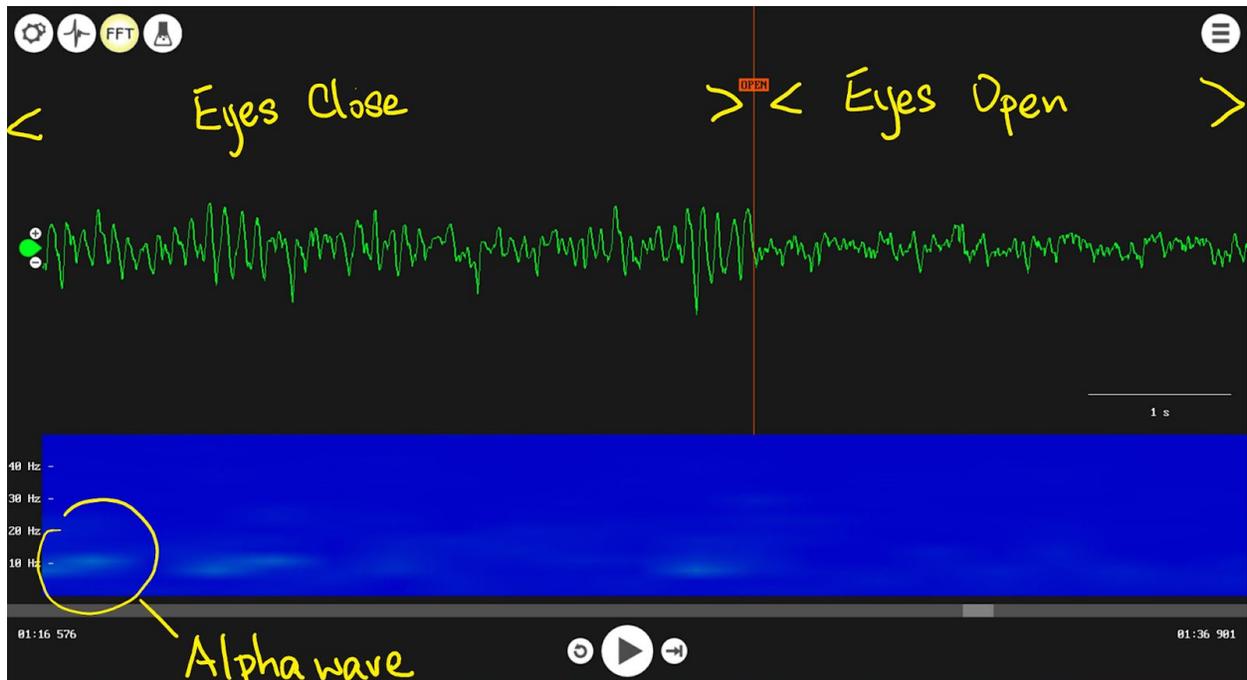
**FIGURE 2. The Euglena, in general, are oriented horizontally, and are all moving toward the left. The laser beam is on the right side of the image.**

### ***LED Circuits***

The Euglena showed consistent negative phototactic response to blue LED lights, and had no response to yellow, despite its intensity. Blue is one of the colors Euglena's eyespot is able to pick up, so it was probably deterred by the intensity of the light. The circuits were coded so that the lights could become dimmer. The coding language used for *Chibitronics* products dictates that the brightness of the lights used in the code be quantified on a scale from 0-100, with 0 being completely off and 100 being the brightest the light can possibly be. The only brightness thus far that has not garnered a completely negative response has been 2% brightness. Dimmer light seems to attract Euglena, probably because of the natural filter that the ocean offers them under normal circumstances. The Euglena's lack of response to yellow light indicated that the intensity of blue light is what they respond to, not the intensity of light in general. The 2% brightness has shown a positive phototactic response, but the experiment will need to be repeated, this time with the black tube to make sure that the outside influence of natural sunlight did not play a role, and that it was in fact positive phototaxis in response to blue light as opposed to negative phototaxis in response to sunlight. The results that we have are for a condition in which the LED is directly under the slide.

## EEG

The student will be hooked up to an EEG kit from Backyard Brain and be asked to repeatedly closed his eyes for 10 seconds and then open. The wave has a relatively synchronize rhythm and a high amplitude when student closes his eyes. On the other hand, the wave has a random frequency and a low amplitude when student opens his eyes(Figure 3).



**FIGURE 3.** This image was taken while the student being asked to open his eyes. The region on the left is the wave when eyes closed and the region on the right is when eyes opened. The green dots on the blue region at the bottom is the alpha wave being detected.

### **\*\*Chibitronics: How to Code**

Materials:

*Chibitronics: Love to Code*

Chibitronics copper tape\*

Chibitronics LED stickers

Chibitronics 6-pin circuit clip + corresponding USB wires

Laptop

\*this must be Chibitronics brand, because the width of the tape is important to the integrity of the circuits.

### *a. Building the Circuit*

Materials: USB power source (book or laptop), Chibitronics USB cord, copper tape, LED stickers, glass slides(2), transparent filter paper

Tape the two glass slides together, one on top of the other, and then tape the filter paper on top. Make sure that the edges are lined up. This will give the clip a solid surface to hold so that the circuit can close. Run two strips of copper tape parallel to each other, leaving a space between them about the width of a strip of the copper tape, from the base of the slide to . Building the circuit as if the clip is going to be on your left-hand side, the pointed part of the LED sticker should be facing downward. The pointed part should always be connected to the copper strip that will be connected to the ground, or “GND” on the circuit clip. The flat part will connect to the positive strip, either reading “+3V” on the right hand side of the clip, or the “0” pin on the left hand side.

When using only 1 LED sticker, only two strips of copper tape are necessary. One strip must be added for every additional LED sticker. The same ground strip must connect to all the stickers, but each sticker must have its own positive strip that will line up with a numbered pin. Be sure that the positive and ground strips are not connected, or the circuit will be nonfunctional. Also, if a circuit has multiple strips, fold the copper tape as opposed to separating it into smaller strips. The circuit will not work like this.

### *b. Uploading Code*

To power the circuit clips, plug the USB end of the red wire into either the battery pack in *Love to Code* and turn the pack on, or plug it into a laptop. Then, plug the micro-USB into the corresponding outlet on the clip.

To upload code, a laptop is required. Plug the micro-USB into the clip, then plug the USB and the audio jack into the laptop. Make sure the volume is turned all the way up. Log onto [ltc.chibitronics.com](http://ltc.chibitronics.com) to build the code. On the website are general guidelines, but you can also pull up examples of simple coding mechanisms. Once the code is done and ready to upload, hold down the button on the left-hand side, right next to the micro-USB outlet. Once the light above it, labeled “PROG” or program turns red, click the “upload” button. An orange bar with white sound waves will appear at the bottom of the screen, then the “PROG” light will turn green again. Once this is done, the code has been uploaded successfully and the clip can be unplugged.

### *c. Writing Code*

#### *1. 2-pin Alternating Blink*

```
int LED = LED;
int LED2 = 1;

void setup() {
  outputMode(LED);
  outputMode(LED2);
}

void loop() {
  setLevel(LED, 10);
  setLevel(LED2, 10);

  on(LED);
  off(LED2);
  pause(500);
  on(LED2);
  off(LED);
  pause(500);
}
```

## 2. 2-pin, Different Brightnesses

```
int LED = 0;
int LED2 = 1;

void setup() {
  outputMode(LED);
  outputMode(LED2);
}

void loop() {

  on(LED);
  setLevel(LED, 100);
  on(LED2);
  setLevel(LED2, 50);
```

## 3. 1-pin, Set Brightness to low Level

```
int LED = 0;
```

```

void setup() {
  outputMode(LED);
}

void loop() {

  int brightness = 2;

  while(brightness > 0){
    setLevel(LED, brightness);
    pause(200);
    brightness = brightness - 1;
  }

  if(brightness = 2){
    pause(200000);
  }
}

```

#### *d. Some Notes on the Coding Language*

It is hard to program the LED to a certain dimness once it gets below about 25%. This is why we must use a while loop to “fade” the light (even though it remains fixed).

The pauses between blinks are measured in milliseconds. For the experiments, which are generally done over the span of a few minutes, that is why the numbers are so big.

The code legend is as follows:

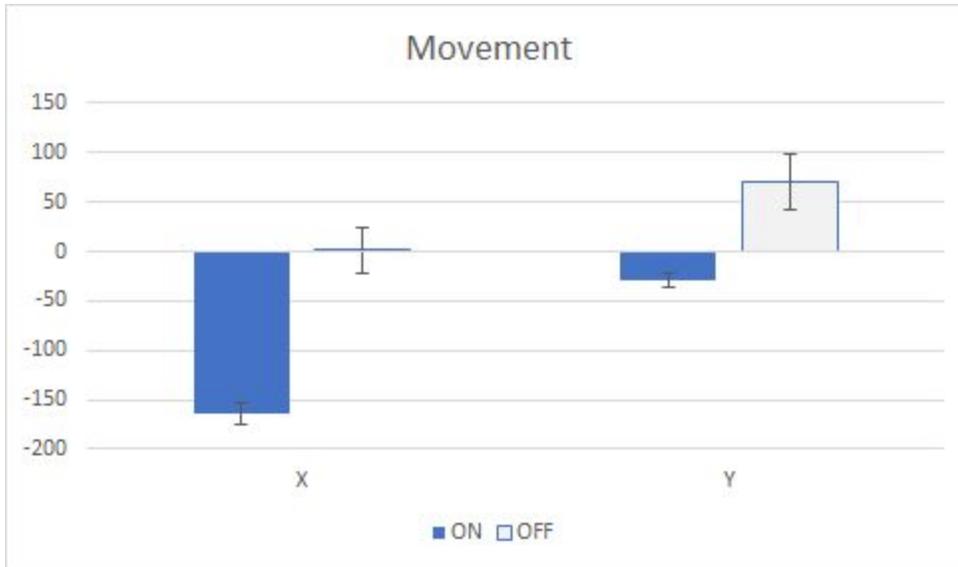
- a. “Int LED = 0;”: the “int” means “initial.” You are setting all the pins you would like to use (0, 1, 2, etc.) and assigning them variables, such as “LED” or “LED2.” It is labeled “initial” because declaring variables is necessary even before setting the basic parameters of the code.
- b. Setup: “outputMode(LED);”: Setup denotes what must be declared before you can program it. Basically, you need to know that the LED will be an output variable before you can program it to output anything. “outputMode” is somewhat self-explanatory; the LED will be an output. Whatever the result of the code, the LED will be doing it.
- c. Loop: The loop function dictates that whatever the program states must be repeated. This is why blink codes blink continuously, and other codes just repeat when they finish out.
- d. “setLevel(LED, 10);”: this sets the brightness of the light. The format is “setLevel(variable, brightness)” because the pin that the variable represents is setting the light to a certain brightness. The brightness must be between 0-100, 0 being off, and 100 being the brightest possible.
- e. on/off: turns the LED on or off, respectively.

- f. `“pause(X);”`: indicates that whatever was coded before it stays like that for however long is designated between the parentheses. The pause function is quantified in milliseconds, so for a pause of one second, you would enter `“pause(1000);”`.
- g. `“int brightness = X;”`: while still within the loop function, the `“initial”` marks that this must be declared before any other part of the program is written. This is the brightness that the LED will start out at. This is only applicable when there is one pin, otherwise it will have to be differentiated.
- h. `“While(brightness > 0);”`: dictates that as long as one condition is fulfilled, something will happen, and if another condition is fulfilled, something else will happen. So, as long as the brightness is greater than zero, everything in the brackets following this statement will happen.
- i. `“{ setLevel(LED, brightness); pause(200); brightness = brightness - 1; };”`: sets the LED variable to a brightness of `“brightness”`, and dictates that the brightness is constantly fading down.
- j. `“if(brightness = 2) { pause(200000); }”`: this function effectively prevents the fade that was coded for with the `“while”` loop. Since the LEDs did not seem to respond to being programmed so low with a `“setLevel”` function, the while loop was necessary. The if statement commands that while the brightness is on 2%, there will be a 200-second pause. The pause is necessary for the loop to continue.

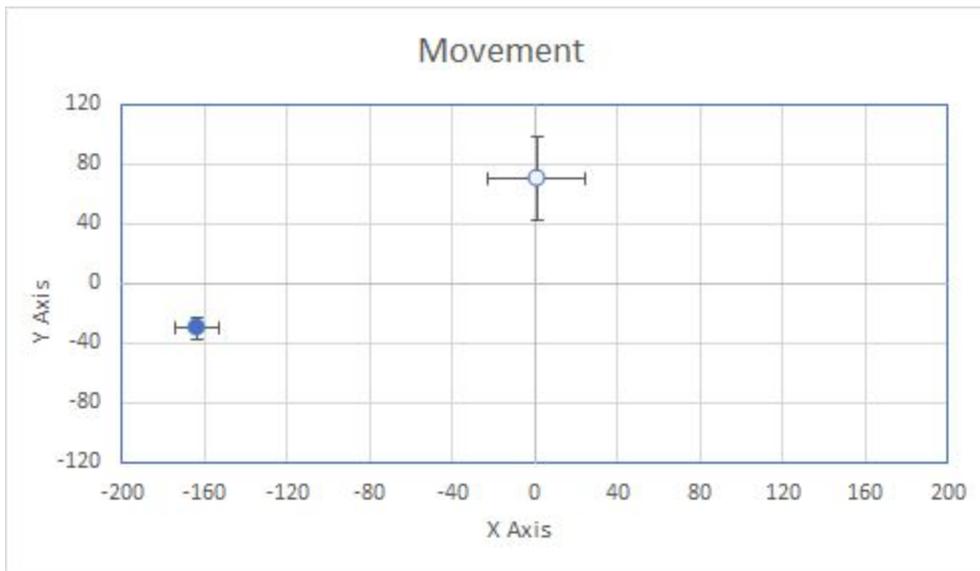
## **Results**

### ***Euglena***

The use of the blue laser pointer was the first thing that showed a clear mass migration of *Euglena*, and it was migration away from the light. Those that were in close contact with the beam had a shock response at first and began spinning around to reorient themselves, but fled as soon as they were able to. This was consistent across all trials, even changing which side of the same slide the beam was pointing toward. The *Euglena* moved away very consistently. With the red laser pointer, the *Euglena*'s movement stayed random. There was no mass movement in either direction



**FIGURE 4: Average displacement (+/- SEM) of Euglena in both the X and Y axis, expressed in pixels. One pixel is approximately 6.5 microns. Blue bars represent displacement when the blue LED was on and white bars represent the displacement when the blue LED was off.**

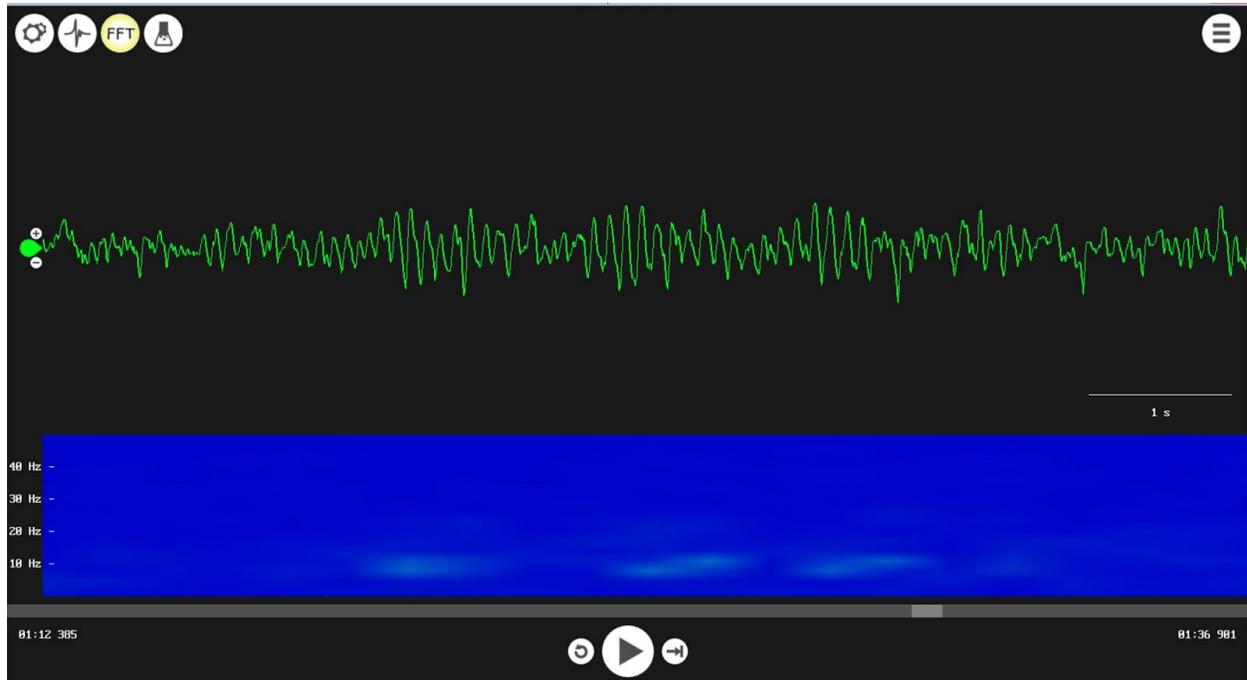


**FIGURE 5: A two dimensional graph of the average displacement (+/- SEM) of *Euglena gracilis***

Illumination of the Euglena by a blue LED directly below the slide yielded a significant net movement away from the LED. Displacement in the x direction averaged at 163.8 pixels away from the light source and 30.1 pixels towards the Northern portion of the slide, each with a standard error of 10.47 and 7.1 respectively. Following termination of illumination the net movement ceased and random movement of Euglena commenced. Displacement in the x

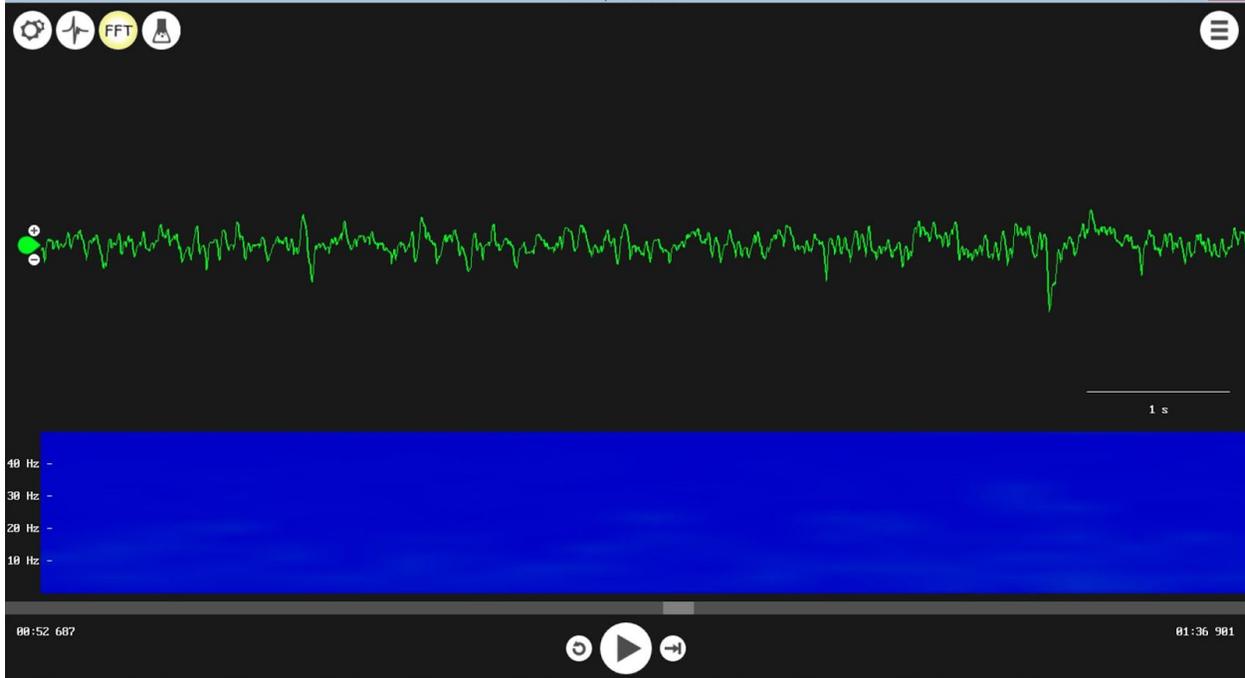
direction while the LED was off averaged at 1 pixel away from the light source and 70.5 pixels towards the southern portion of the slide. Each condition had a standard error of 23.65 and 28.26 respectively (Figure 7 and 8). Statistical analysis with a t-test yielded significantly different migration between both the experimental and control groups ( $p < 3 \times 10^{-5}$ ). Movement was tracked for a period of 15.54 seconds.

## EEG



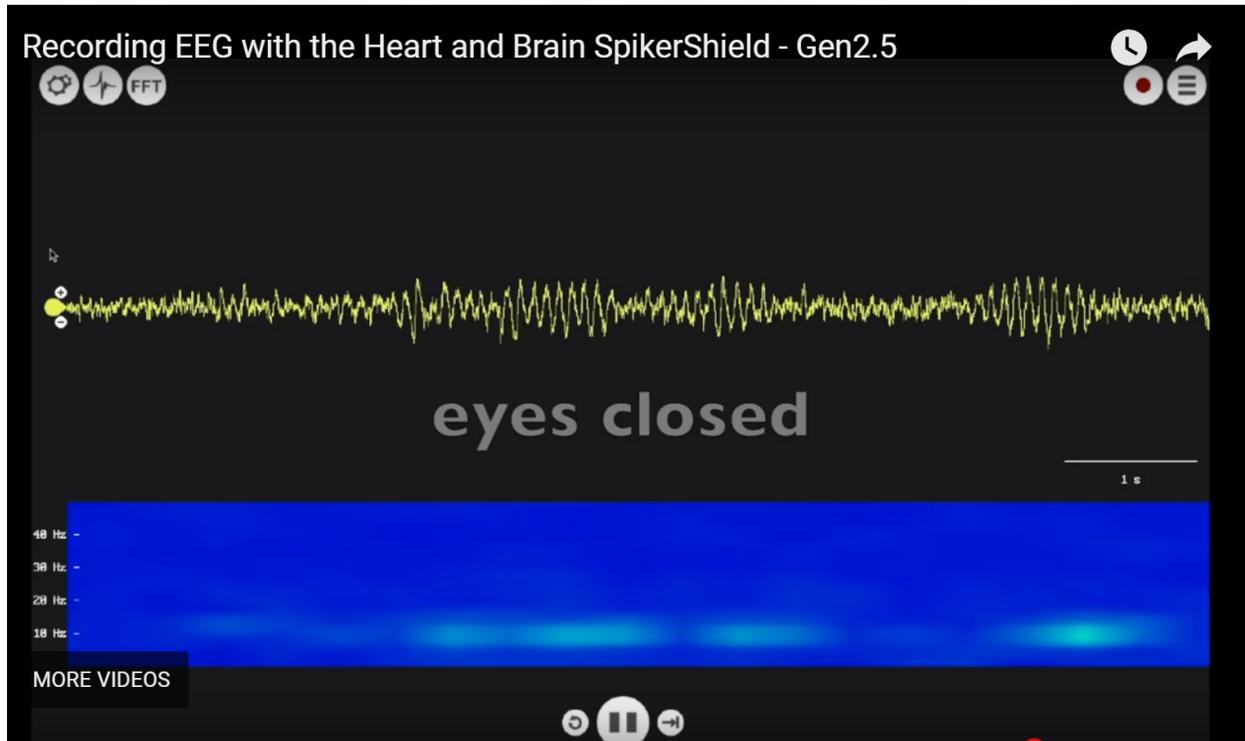
**FIGURE 6.** Image taken during student closed his eyes.

When student closes his eyes, the visual cortex is not receiving complex information. Instead, visual cortex only receives signal inputs from the thalamus at 10 Hz and all the neurons are trend to fire at the same times. At this point, the alpha wave is strong enough to be detected as it shows on the diagram. The more consistent cycles and frequency will have a stronger alpha wave.



**FIGURE 7. Image taken during student opened his eyes.**

When student opens his eyes, each individual neuron is processing different light frequency. Since the EEG kit can only detect the average population neural activity, the high and low firing rate in neurons are canceling out each other during the measurement. Therefore, the amplitude of the wave with eyes open is relatively low.



**FIGURE 8.** Image taken from the Backyard Brain video when eyes closed.

In conclusion, we can see the alpha wave from our experiment during student closed his eyes, but it is not as strong as the video example showed from the Backyard Brain. Further practise with the EEG kit still needed.

## Discussion

### *Euglena*

The *Euglena* exhibited an immense negative phototactic result when the LED was placed directly below the slide. This is illustrated by the significant shift in the x and y directions. This mass movement may have been caused by the intense light from the LED being directly below the slide. Following termination of illumination of the LED light, no further significant movement was detected in a particular direction and the movement of the *Euglena* became random. Ambient light from the environment and light from the stereomicroscope not being uniform may have been factors influencing the phototactic responses of the organisms. Future experiments could explore how concentrations of various ions in nutrient media *Euglena* are grown in could influence their phototactic responses. Furthermore, different colors of light such as green could also be tested.

## EEG

During our practices, we found out the EEG kit did not show a consistent wave on student with long hair in contrast to students with short hair. That might be due to long hair interfering the contact in between the electrodes on the EEG headband and the scalp, which causes a lot of noisy waves. Furthermore, it took a while to wait for the alpha rhythm to show up after eye closure with the short hair student. We noticed when the student stayed still at a comfortable position, stopped talking and covered up his eyes, the alpha waves were more likely to be detected when the student closed his eyes. Therefore, student's body posture and movement, and light intensity in the room should be considered during the EEG experiment on the Biobus. In addition, it is easier to stick the ground electrode behind student's ear after rubbing that skin area with alcohol. And make sure to put sufficient gel on the headband electrodes.

## References

**Experiment: EEG-Record from the Human Brain. (n.d.). Retrieved from <https://backyardbrains.com/experiments/eeg>**

**Giometto, A., Altermatt, F., Maritan, A., Stocker, R., & Rinaldo, A. (2015). Generalized receptor law governs phototaxis in the phytoplankton *Euglena gracilis*. *Proceedings of the National Academy of Sciences*, 112(22), 7045-7050. doi:10.1073/pnas.1422922112**

**Gualtieri, P., Pelosi, P., Passarelli, V., & Barsanti, L. (1992). Identification of a rhodopsin photoreceptor in *Euglena gracilis*. *Biochimica Et Biophysica Acta (BBA) - General Subjects*, 1117(1), 55-59. doi:10.1016/0304-4165(92)90162-n**