

Section 8. Fly Vision Project

8.1 Fly Wet Practice Session

8.2 Fly Movie Design

8.3 Fly Wet Data Collection

8.4 Fly Data Analysis

Overview

Let's do a real experiment! -- Putting all the pieces together

We have assembled the fly labs and associated Matlab tutorials to give you the chance to actually do an experiment that is totally your own. We hope that you will see that, by the time this is done, you will have gone through each of the steps that are done in a systems neuroscience lab when a new experimental project is undertaken.

The overall goal of the fly lab sessions is to teach you the basics of studying and understanding the responses of sensory neurons. That is, you should think of yourself as a sensory physiologist trying to understand how some particular fly neurons process visual information. Throughout the course, you have already gained expertise in using electrophysiology to record neuronal activity. Now you get to take it to the next level. You get the opportunity to design your own visual experiment (i.e. visual stimuli made using Matlab – using basic design techniques covered in Matlab Tutorial project 2), collect neuronal responses to those stimuli, extract the spikes from single neurons in your recordings (using your Matlab spike detection algorithm – Matlab Tutorial project 1), and try to interpret what those spikes might be telling the fly about the visual world (using basic analyses covered in Matlab Tutorial project 3 as a starting point). Of course, at the end of the day, all scientists need to communicate their results to others if they expect their work to have any impact on the world. Thus, you must also clearly describe what you find in the form of a short scientific paper (Your Lab Report 2) and you have by now also had practice in this important aspect of science. Because there are essentially an infinite number of visual stimuli that can be shown to the fly, it is highly unlikely that any experiment you design will have been done before and this is a big part of the fun of these labs and the excitement of science.

Some Background Material

The “magic” of sensory systems results from their remarkable ability to transform raw sensory representations of the visual world into profoundly more useful forms of visual information representation. For example, our ability to perceive the visual world begins with two-dimensional patterns of neuronal activity on our retina (i.e. like the images in a camera). Those patterns of neuronal activity are then transformed in the nervous system to new forms of representation that are more closely related to our behavioral and cognitive goals. For example, neurons in the highest levels of the primate visual system are highly selective for specific visual objects (e.g. faces), but are tolerant to variables such as object position, size, pose, illumination, etc. The population of neurons at these higher levels of the nervous system contains much of the same information that entered the system at the retina (after all, visual information that does not pass through the retina cannot be created, only destroyed), but the information is in a different format. That is, it is re-presented in a different manner than the retina. This kind of representation is vastly more useful for behavior and memory than the original neuronal representation in the retina. Creating such useful forms of representation is the reason that our sensory systems have so many neurons working on this problem.

Given this, one of the primary goals of systems neuroscientists working in the sensory systems is to understand how neurons at each level of the system have recoded (i.e. transformed) the image data that impinges on the sensory system of interest (e.g. the retina, the cochlea, or the skin surface). From the point of view of any particular neuron under study, this means trying to figure out how that neuron transforms its sensory inputs to its output pattern of neuronal activity. Which sensory stimuli does it “like” (big response) and which stimuli does it “not like” (poor response)? Can we understand the rules that govern this behavior? The first fundamental concept in this regard is called the “receptive field” – the region of sensory space to which the neuron responds. But this is just the beginning of understanding how a neuron transforms the sensory information, the real magic is in understanding and describing its response to (ideally) any sensory pattern of input. This is a daunting task, and there is much, much more work to be done in this regard in all the sensory systems.

In 9.02, we will introduce you to the basic techniques that are used to assess the sensory function of neurons in the visual system. As a model, we will use the fly visual system because some neurons in the fly visual system are relatively easy to record from and have very interesting response properties.

As you know, the fly is often ... flying! As you might imagine, navigating around when you are quickly flying and turning in the real world is a very challenging task and it is far from understood how the fly achieves such grace in the air. It is clear however that navigating requires an assessment of motion across the retina because this can be used to determine heading and turning velocities. It is thus perhaps not surprising to learn that the fly has many neurons that are very sensitive to the direction and speed of visual motion across their eyes. As you might also imagine, visual sensitivity to motion is not something restricted to flies but is a

common feature of visual systems. Indeed, some of the most elegant recent work on the visual processing in the primate visual system has been carried out in areas that are very sensitive to motion and motion direction (e.g. area MT and MST, you will see some of this in your recitation sections).

Besides its importance in vision, motion sensitivity is one of the properties where computational approaches have made great strides in proposing possible mechanisms that could be used to compute motion (you will see some of this in another recitation section). Many of those predictions have been and are being tested in visual systems including the very same flies that you will use in the 9.02 fly labs.

Because there are essentially an infinite number of visual stimuli that can be shown to the fly, it is highly likely that any experiment you design will never have been done before and this is a big part of the fun. Nevertheless, there are few things about the particular fly neurons that you will likely find with your electrode that you should know to help you design your experiment:

The fly neurons you will likely record from are very sensitive to motion and tend to prefer motion in certain directions. Which direction(s)? What speed of motion is best? Motion detectors are often sensitive to transient changes in the luminance (brightness) of the visual field. Is that true of your neurons?

These same neurons often have very large receptive fields, often larger than the size of the video monitors you will use to present visual stimuli. That means that they will respond to (some) stimuli placed at most positions on the monitor (of course this depends on how your monitor is positioned next to your fly, but you get the idea). Thus, you should not expect to “map” a nice, small, circumscribed receptive field in your neurons, but that does not mean it is not worth trying, and you will likely find that, even though the fields are large, they are not uniform. Are they continuous or do they contain holes? Is there one maximal peak in the receptive field where the cell give a maximal response or are there multiple “hot spots” in the receptive field?

These are the very basics of fly visual responses, but these ideas plus the tools we have provided you (see Matlab Tutorial 2) should give you a basic idea as to what to expect of your neurons but also let you think outside the box and design virtually any visual stimuli of your choosing. Before you start this project, it might help to read one or more of the articles posted on the class site for this class to help you learn more about the fly visual system. Because we will spend the first lab class on the fly just trying to record neurons, you will get a better sense of these neurons, and you may have the chance to make an additional fly movie (besides the one due as part of this assignment), before you return to lab two weeks later to test your stimuli.

Besides creating your very own visual stimuli in this project (which we strongly encourage you to do), we would like to make sure each of you also includes some basic visual stimuli that are very likely to give you useful data. Specifically, you should use stimuli that contain some sort of motion and are as large as possible (i.e. cover as much of the screen as possible), and you should

systematically vary the direction of motion in at least 8, even spaced directions around the clock (e.g. 45 deg increments or less).

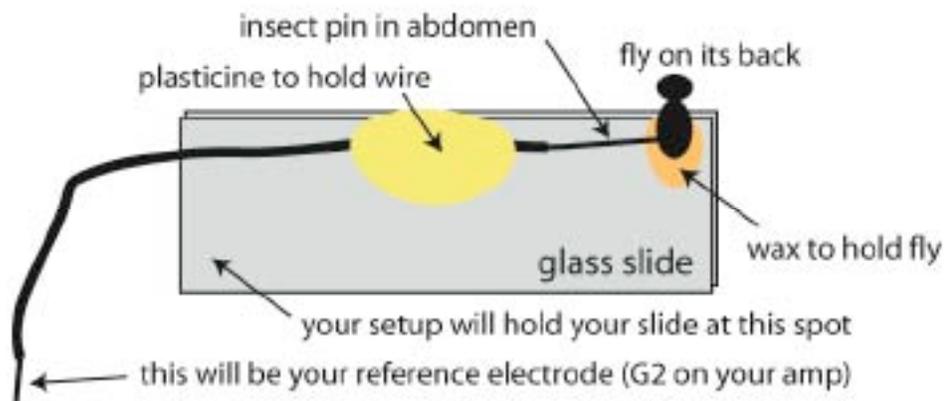
Good luck and have fun!

8.1 Fly Wet Practice Session

Prepare A Fly for Recording

1. Take one of the vials provide at your bench, remove the cover and catch a fly from the container.
 - Keep your thumb over the vial at all times prior to anesthesia so that the fly does not escape.
 - Be sure to close sleeve when you are finished so that no flies escape.
2. Anesthetize the fly by inserting the regulator handle into the vial, while keeping your thumb in place, and slowly releasing carbon dioxide into it until the fly stops moving.
3. Bring the fly back to your bench and QUICKLY, clip its wings and legs.
 - The anesthesia is temporary; if it does wear off before you have finished with this procedure, repeat step #2 and continue.
 - Be sure not to leave any stumps of legs or wings when you cut, i.e. cut as close to the body as possible. Any remaining appendage of piece of one can cause noise issue with your prep.
4. Take a slide and drip a small amount of wax on the upper right corner.
5. When you are ready to mount the fly onto the slide, grab the fly body (ventral side up) with forceps, reheat the wax on the slide, and use this to glue the fly's body to the slide.
 - We want to glue down the body only, NOT the head.
 - Be sure to place the head well above the top edge of the slide.
6. Rotate the fly head as far forward such that the fly "chin" is touching its "chest."
 - You want the head to actually rotate to expose the back of the head. You are not simply pushing forward, so this may take some practice. If you need help, consult the teaching staff.
7. Once the fly is chin to chest, add some melted wax to the front of the head to secure it in this position.
 - Be careful that you do not use too much wax or it will run up over the eyes.
8. Place the lower edge of the slide in a block of plasticine and put the fly under a dissection scope. Adjust the scope and the position of the fly under it until you can clearly see the back of the head.
9. Using the scalpel, remove the exoskeleton behind the RIGHT eye.

10. Using the forceps, clear fat and air trachea beneath the exoskeleton until you reach the visual neural structures.
 - Go slowly and ask the teaching staff for assistance if you are no sure where you are. It is easy to go to far and to remove the neural structures, ask for help if you need it.
11. Periodically add saline to your dissection area to facilitate structure visibility.
12. Upon completing the dissection, add saline to your dissection area to prevent drying.
13. Take the fly back to your bench, insert a reference electrode in the abdomen, and secure it with tape or plasticine, as shown below
 - Be careful not to dislodge the fly from the wax during this process.



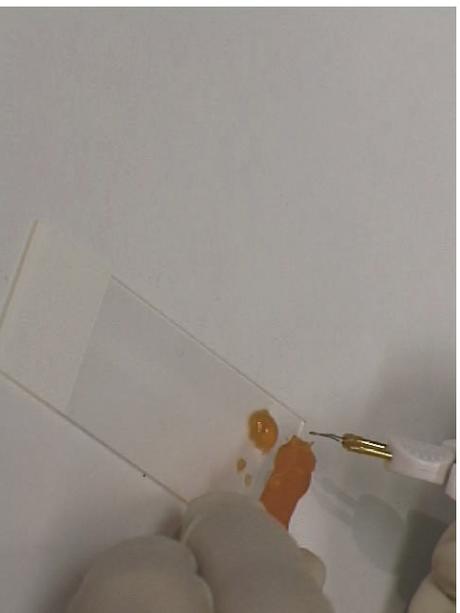
Recording from Neurons in the Fly Visual System

1. Mount the glass slide in your recording setup, and firmly tighten the slide in place.
2. Obtain an electrode from the teaching staff, and mount it on your micromanipulator, making sure that the connection end of the electrode makes good contact with the attached wire.
 - Be careful with the recording tip of the electrode.
3. Connect your recording wires to the recording equipment: amp G1 to electrode wire, amp G2 to reference wire via alligator clip.
4. Adjust the microscope and lighting to clearly visualize the target area on the fly.
5. CAREFULLY use the micromanipulator course knob to adjust the position of the electrode relative to your target such that your electrode aims directly at the lobula plate.
 - Very generally, we are aiming for the medial portion of your dissection area.
 - Use the microscope for this process. You will not be able to see the electrode tip clearly until you are very close to the fly.
6. Add saline to your dissection prep if it appears dried out.
7. CAREFULLY advance the electrode into the saline behind the eye using the micromanipulator fine adjustment knob.
 - Remember you are aiming approximately at the lobula plate.

8. Turn on your audio, check noise levels and adjust grounds if needed to reduce noise.
 - As in previous labs, you should be able to determine the “normal” saline noise from other noise and make adjustments accordingly.
9. If you need to, make a final adjust the electrode position to aim the electrode at or medial to the lobula plate.
10. Advance the electrode slowly into the neural tissue, listening to the audio monitor for neural activity.
11. Once the electrode has settled, wave your finger in front of the fly eye to try to activate neurons while you are listening.
12. Try to adjust the electrode to get a large action potential on your oscilloscope that you can modulate with visual stimuli.
 - If you are unable to get any response, then try backing out the electrode a bit. As in previous lab sessions, you may need to finagle to get a signal.
13. Qualitatively ‘map’ the response of this visual neuron.
 - Can finger motion change the cells firing rate at all?
 - Which part of the visual field is best?
 - Which direction of finger motion is most preferred by the cell (if any)?

Step 1. Prepare a fly for dissection.

The goal is to catch and anesthetize the fly, clip its wings and legs, and securely attach it to a glass slide.



1.1 Check that your physiology setup is in order and that you have all of the dissection equipment that you need. Melt some wax on upper right edge of a slide.



1.2 The flies are in a 'fly house' in teh lab. With the help of a TA, use a glass vial to capture a single fly.



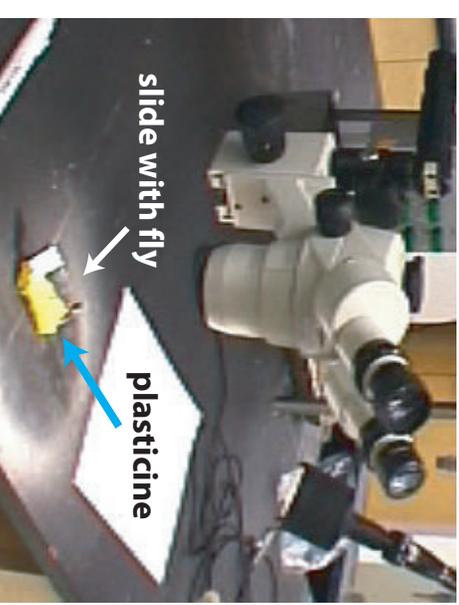
1.3 Anesthetize the fly with carbon dioxide. Use gentle pressure and keep your thumb over the vial opening.



1.4 Use forceps and scissors to quickly clip the fly's wings and legs. If this takes too long, the fly may begin to wake up and you will need to place it in the vial and re-anesthetize.



1.5 Re-heat the wax on the slide and place the fly on its back in the wax. It is VERY important that the head extends well beyond the edge of the slide. It is helpful to have someone heat the wax while another places the fly.



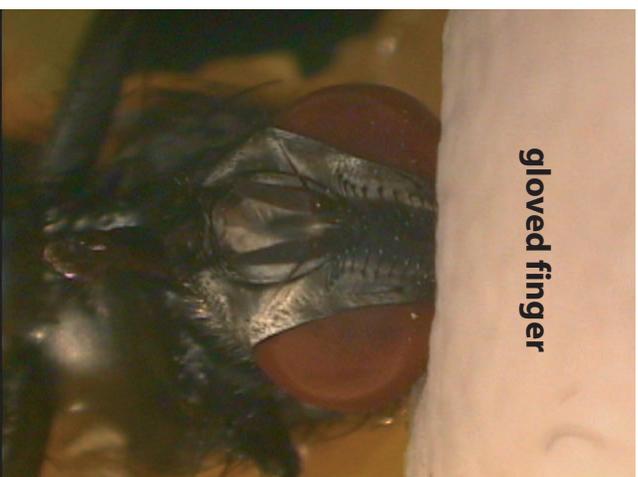
1.6 You can now place the lower edge of the slide firmly in a block of plasticine (to hold it) and place the prep under the dissection microscope to begin your dissection.

Step 2: Secure the fly head in a flexed position so that you have clear access to the the back of the head.

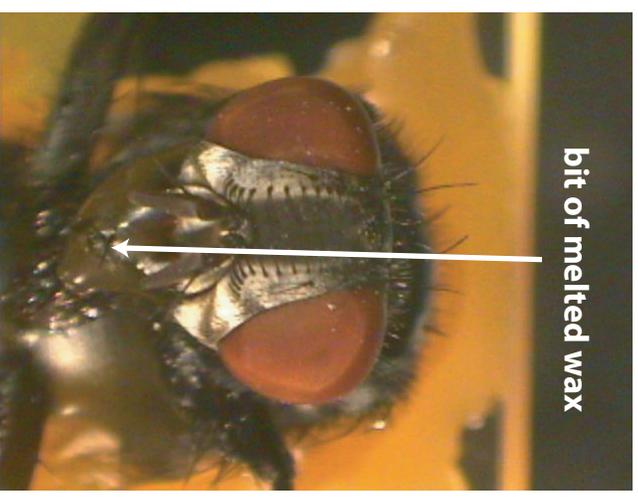
This is a very important step. If you do not complete this step successfully, you should start again with a new fly (rather than continuing with a poorly positioned head).



2.1 Place the glass slide to which the fly is attached in a block of plasticine to hold it upright. Your fly should look like this (above) under the dissection microscope.

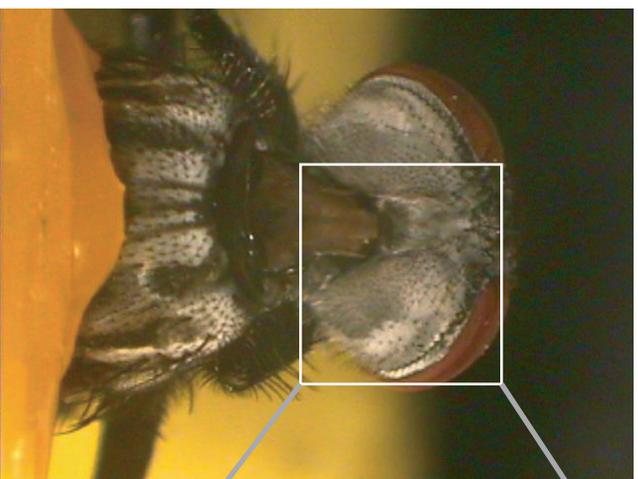


2.2 Use your finger to gently flex the fly's head forward as you watch under the microscope. Practice this. The goal is to FLEX the head, not push it down toward the thorax (think of putting your chin on your chest).



2.3 Pick up a small bit of wax in the tip of your (cold) wax pen. As you hold the fly head flexed (step 2.2), put the tip of the pen at the indicated point and melt the wax to hold the head in as shown.

2.4 Turn the slide around on the block of plasticine and look at the back of the head. It should look something like this. Note that the head is well above the edge of the slide and that it is flexed far forward so that you have clear access to the exoskeleton behind the esys



2.5 Zoom in using higher magnification on the right side of the head. Your prep should look like this. You are now ready for step 3.

Step 3: Remove the exoskeleton behind one of the eyes.

Your ultimate aim is to record from neurons that are driven by light arriving at the eye contralateral to your dissection (that is, the eye on the opposite side of your dissection). The goal of this step is to remove as much of the exoskeleton as you can without damaging the underlying structures.



scalpel blade

3.1 Use the scalpel to just pierce the exoskeleton at the point indicated. It is very thin. Do not cut deeper than you need



3.2 Cut near the margin of the eye, but do not cut the eye. Keep the tip just under the exoskeleton (no deeper).



3.3 Continue your cut as far lateral as you can. These cuts do not have to be made with one sweeping stroke -- you can "punch" along the tline to make the cut if that is easier.



3.4 Now go back to your starting point and cut parallel to the midline. Do not cut too deep!



3.5 Continue the cut along the ventral side. You do not need to fully connect to the end of the other cut, just get close.



3.6 Gently grab the edge of the exoskeleton with a fine pair of forceps.



3.7 Pull off the piece of exoskeleton. It is OK if some of the underlying fatty-looking material comes off with the exoskeleton.



3.8 Your prep will hopefully look like this. The white, stringy material is not neural -- it is fat and air tubes.

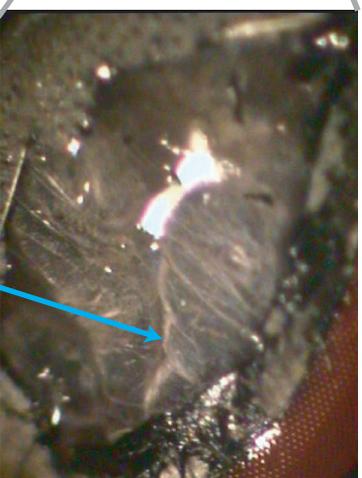
Step 4: Remove air trachea and fatty tissue beneath the exoskeleton.

The goal is simply to remove the extra white "stuff" that you will find beneath the exoskeleton. Some of this will likely come out with your bit of removed exoskeleton. The main thing that can go wrong here is that you probe too deeply and injure or remove the underlying neural tissue.

Using fine forceps, try to push the material off to the sides or grasp it and pull bits out. Concentrate your efforts near the medial edge, but err on the side of too little dissection rather than too much.



4.1



4.2 Not the white stringy structures. These are not neural process, but are air trachea.



4.3



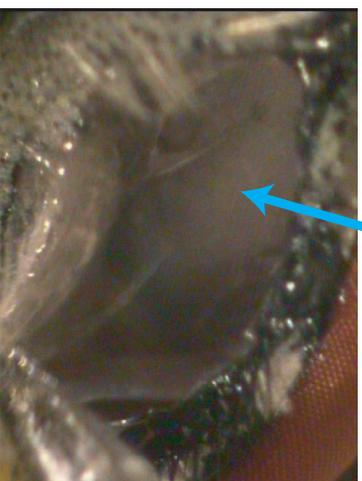
4.4 Adding a drop of saline can often help at this point.



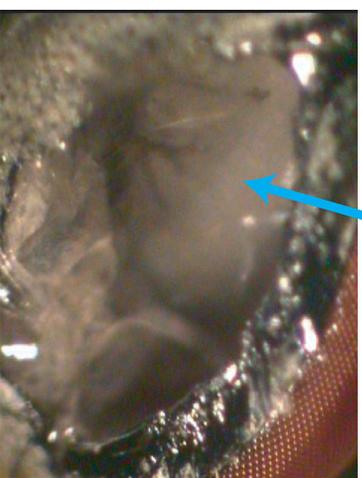
4.5



4.6



4.7



4.8

neural tissue

neural tissue

neural tissue

Step 5: Setup the fly in your recording rig, visualize neural structures, and place a recording electrode.

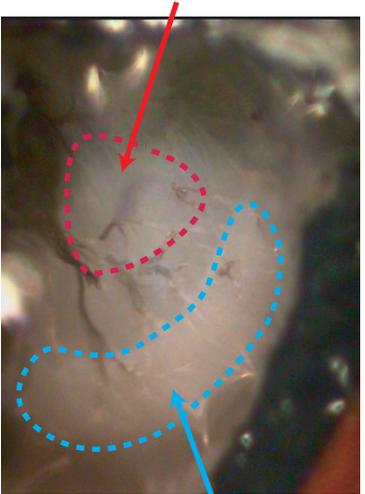
* before proceeding to your rig, you should place a reference electrode in the abdomen and secure it to the glass slide (see lab handout)



5.1 Note that saline has been applied. You should add saline from time to time to prevent the tissue from drying out.



5.2 Closer view.



lobular plate

medulla



5.3 Still closer. Structures are outlined above. If you have trouble visualizing neural structures, try adjusting your light. If you do not see everything perfectly, you can still try to record.



electrode tip

5.4 One electrode placement: The arrow is aligned along the electrode entering from the right. The electrode tip is in the saline and just about to contact the tissue. It is at this point that you should turn on your amplifier, etc.



5.5 Another electrode placement. There is no magic spot, but you should aim your electrode near or medial to the lobular plate (even more medial than shown here), listen closely for neural activity, and not advance the electrode much beyond first contact with the tissue. (please see your lab handout for more details)

8.2 Fly Movie Design

****** There is no formal protocol for this lab session, because each group will not make the same movie, therefore you will not all follow the same procedure. By the end of this session your lab notebook should include the interesting experimental question that you can ask of a fly motion-sensitive neuron, design a strategy to make a movie to carry out that experiment and to analyze the data that you will get in the next lab. **ALL OF THIS SHOULD BE APPROVED BY THE TEACHING STAFF BEFORE YOU LEAVE THE LAB TODAY.**

****** Prior to next week's lab, we will take your group's movie and generate a more compressed movie will be ready for you to play in the next lab when you record voltage signals from your fly neurons. Thus, your movie must be turned in by this coming Monday at lecture.

****** You must also save and share (with your team) a single copy of the Matlab code your team used to make the movie (see FAQ below.) This code does not have to be turned in, but we are more than happy to review it with you.

Ideas for Experimental Design

****** Hopefully you already have your own ideas of things you might like to try in the fly.

1. Spatial organization of the neuron's receptive field.
 - Do all points in the field prefer the same direction of motion?
2. Temporal organization of the neuron's receptive field.
 - e.g. How long does the fly neuron respond to brief pulses of motion?
 - Can the response to longer motion durations be predicted by the response to brief durations?
3. Speed tuning.
 - We showed you that fly neurons generally have a preference for motion direction. Do they also have preferences for motion speed?
4. Adaptation is a simple form of memory. Do these neurons adapt?
 - That is, if a fly neuron "sees" one direction of motion for a long time, does it change the way it responds to that direction or other directions of motion?
5. Early in your design process, in addition to talking amongst yourselves, please speak with one or more of the teaching staff to discuss your experimental questions, your plans for testing them, and your thought on how you will analyze your data.

Building your Group Movie

1. Create a movie that will test your experimental question.
 - The movie should:
 - Length: no more than ~ 60 sec (for one full run) – we will repeat your entire movie

10 times when we collect data.

Size: 320x240 pixels

Plan to run at 30 frames per second.

You should show some planning and creativity in experiment design.

2. You should create your movie using a Matlab function and name that function `generateFlyMovie.m`. That function should take no arguments and should return your movie cube. This file will be submitted to and used by the teaching staff to generate your movie. You **MUST** be sure that it works before submitting.

-You learned how to do this in Matlab tutorial 2.

3. **ALL MOVIES MUST contain FULL-FIELD drifting grating stimuli presented at at least 8 different directions (equally-spaced around the clock). We suggest you use the first ~8 seconds of your movie for these stimuli (e.g. 500 ms on, 500 ms off). The purpose of these stimuli is to measure the direction tuning of the neuron you record in the fly. The results of this tuning must be included in your lab report and will demonstrate that you have successfully sorted spikes and counted them in the proper time windows.**

4. Make sure you comment this file to explain what visual stimuli are being shown at which portion of the movie.

5. You can write other functions to help make any stimuli you want, including beyond those provided as part of Tutorial 2. However, those functions should also be turned in with your main movie function and with your movie.

Creating the Movie Cube

1. In your `generateFlyMovie()` function, you'll want to start off with something like:

```
totalStimulusLength = 20    % stimulus length in seconds
FPS = 30                  % number of frames per second
MAXX = 320
MAXY = 240
myMovieCube = zeros(MAXY, MAXX, FPS*totalStimulusLength, 'uint8')
```

2. Do not make the length any longer than you need to – this will just chew up time and memory.

Submitting Your Experimental Movie

****Turning in only your code is unacceptable. If you cannot generate a working movie, it is your job to seek help and figure out how to do so.**

1. Save your movie cube with the saveMovieCube function:

```
>> saveMovieCube(myMovieCube, 'myfilename.yuv')
```

2. We recommend you save your movie cube with the 'yuv' extension so it is easier for us to work with. The filename should be something very specific to your group.
3. The resulting file might be large, up to 200 MB. Please copy this along with the code you wrote to generate the movie to the USB Flash drive provided to you by the teaching staff and submit it.
4. The 'yuv' movie file and your code is due in lecture the Monday before the next fly wet lab (less than a week from today)!!! This is all you need to turn in.
 - We do not need your code (but you and your team do).

Frequently Asked Questions

1. Can our movie be shorter than 60 seconds?
 - Yes. The best experiments are not always the longest ones. The goal is to design a movie which tests a hypothesis.
2. Can our movie be longer than 60 seconds?
 - Sure, but don't make it too long. You will be showing your movie to the fly 10 times (do you know why?), so a 60-second stimulus will actually take a full 10 minutes to run on lab day.
 - Also, you may find that you run into memory limitations on Matlab when you are trying to generate movies that are much longer than 60 seconds – this depends on the RAM of the machine you are using.
3. I want to show an image/movie to the fly but there is no function for it! What can I do?
 - You're free (and encouraged) to improvise, innovate, and create any sort of visual stimulus you want for the fly. Just make sure you also include the 8 full-field drifting grating stimuli (to measure direction tuning) and that you can explain why you want to use a particular stimulus. Also, you will need to submit ALL the Matlab code necessary to generate your movie, including any extra functions you write.
4. Why should I save my code?
 - When we show the fly the movie, and record the resulting neural data, you're going to want to go back and figure out "stimulus A was shown first for 30 frames, stimulus B for the next 60", and so on. Your source code serves as a record to let you later reconstruct what was going on during the experiment.
 - You will be working in teams of two or three to create your project movie and to get the neuronal in the lab. Because all team members should work on the analysis of this data relatively independently, it is essential that your code be well- documented, so everyone on the team can understand the different parts of your movie.

8.3 Fly Wet Data Collection

**Here, your goal is to get real neuronal data to answer your experimental question.

Familiarize Yourself with the Data Collection Equipment

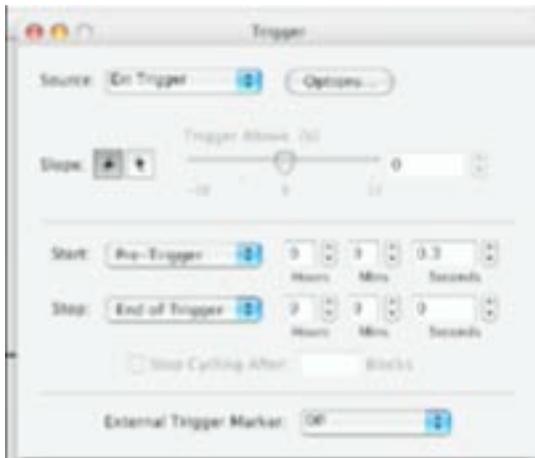
**Before working with an actual fly, work through the record/save procedure once to familiarize yourself with the equipment. In particular, go ahead and take one practice collection of data (even though it will just be noise) so that you have tested that everything works.

1. Make sure cables are connected properly.
 - The output of the converter box labeled “START” should be connected to ‘channel 2’ input of the ADC system.
 - The output of the converter box marked “TRIGGER” should connect the ‘trigger’ input of the ADC system.
 - The output of your amplifier should be connected to the ‘channel 1’ input of the ADC system (as well as to the oscilloscope and audio monitor).
2. Launch chart by double-clicking on the fly-lab icon on the OS X desktop of the data acquisition computer.
 - This will run chart using the pre-configured chart settings.



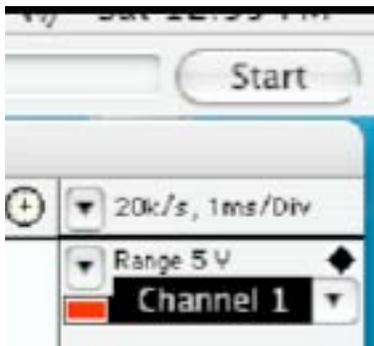
© ADInstruments. All rights reserved. This content is excluded from our Creative Commons license. For more information, see <http://ocw.mit.edu/help/faq-fair-use/>.

3. Check that the trigger settings for the data acquisition program (called ‘Chart’) are configured properly. On the menu bar, go to Setup > Trigger and make sure the settings look as below.
 - The point of a ‘trigger’ is to let one instrument (or computer) tell another instrument to ‘start now.’ That is, the ‘trigger’ provides a way to synchronize the display system with the ADC system (voltage data).



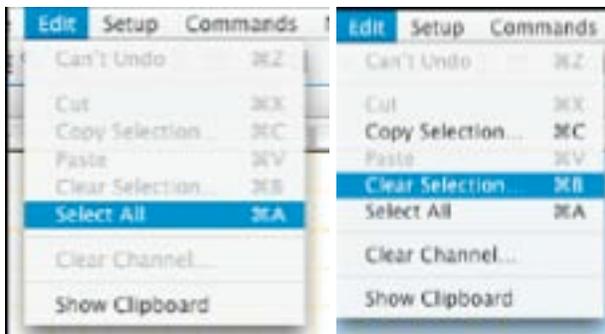
© ADInstruments. All rights reserved. This content is excluded from our Creative Commons license. For more information, see <http://ocw.mit.edu/help/faq-fair-use/>.

4. Check to make sure the sampling rate and voltage ranges are correct.
 - You want to sample at 20ksp/s (kilosamples per second).
 - For channel 2 (the start channel), you want a 5 V range, and for channel 1 (the neural data channel) you want a 500 mV Range.



© ADInstruments. All rights reserved. This content is excluded from our Creative Commons license. For more information, see <http://ocw.mit.edu/help/faq-fair-use/>.

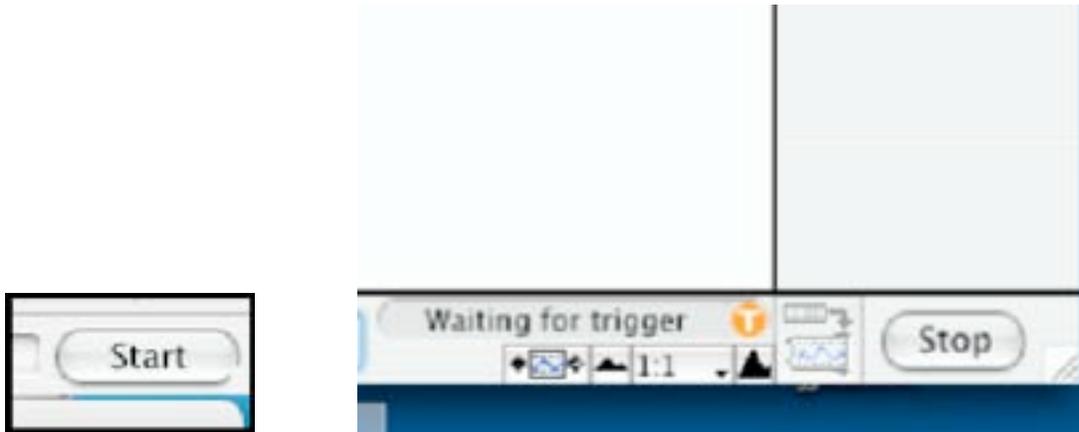
5. Clear out any previous data in the chart by selecting all, and then hitting clear.



© ADInstruments. All rights reserved. This content is excluded from our Creative Commons license. For more information, see <http://ocw.mit.edu/help/faq-fair-use/>.

Testing Voltage Data Recording

1. Make sure the chart buffer is clean (on the data acquisition computer).
2. Click on the 'start' button; the lower-left hand corner should say "waiting for trigger" as shown below:



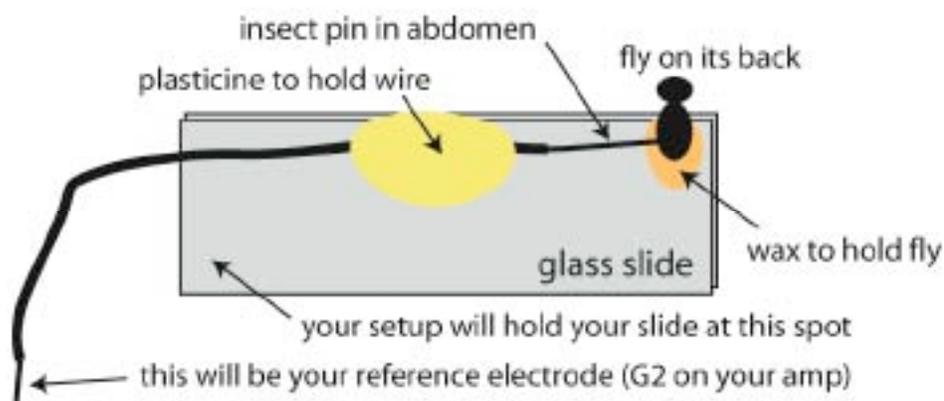
© ADInstruments. All rights reserved. This content is excluded from our Creative Commons license. For more information, see <http://ocw.mit.edu/help/faq-fair-use/>.

3. Find your movie on the list of movies on the display computer.
4. Open the VLC movie player, and drag and drop your movie in the player.
5. Be sure that the volume on the computer is at max.
 - The movie you have created contains two audio channels, one which is used to indicate the start of a movie run and one which is used to trigger the data recorder.
6. Set the movie player to full screen.
7. Double-click on your movie to play it.
 - When the movie begins to play, you should be able to see the data acquisition computer collecting "data."
8. Once you have verified how the data acquisition will work, you are ready to get a fly.

Prepare A Fly for Recording

1. Take one of the vials provide at your bench, remove the cover and catch a fly from the container.
 - Keep your thumb over the vial at all times prior to anesthesia so that the fly does not escape.
 - Be sure to close sleeve when you are finished so that no flies escape.
2. Anesthetize the fly by inserting the regulator handle into the vial, while keeping your thumb in place, and slowly releasing carbon dioxide into it until the fly stops moving.
3. Bring the fly back to your bench and QUICKLY, clip its wings and legs.
 - The anesthesia is temporary; if it does wear off before you have finished with this procedure, repeat step #2 and continue.

- Be sure not to leave any stumps of legs or wings when you cut, i.e. cut as close to the body as possible. Any remaining appendage of piece of one can cause noise issue with your prep.
- 4. Take a slide and drip a small amount of wax on the upper right corner.
- 5. When you are ready to mount the fly onto the slide, grab the fly body (ventral side up) with forceps, reheat the wax on the slide, and use this to glue the fly's body to the slide.
 - We want to glue down the body only, NOT the head.
 - Be sure to place the head well above the top edge of the slide.
- 6. Rotate the fly head as far forward such that the fly "chin" is touching its "chest."
 - You want the head to actually rotate to expose the back of the head. You are not simply pushing forward, so this may take some practice. If you need help, consult the teaching staff.
- 7. Once the fly is chin to chest, add some melted wax to the front of the head to secure it in this position.
 - Be careful that you do not use too much wax or it will run up over the eyes.
- 8. Place the lower edge of the slide in a block of plasticine and put the fly under a dissection scope. Adjust the scope and the position of the fly under it until you can clearly see the back of the head.
- 9. Using the scalpel, remove the exoskeleton behind the RIGHT eye.
- 10. Using the forceps, clear fat and air trachea beneath the exoskeleton until you reach the visual neural structures.
 - Go slowly and ask the teaching staff for assistance if you are no sure where you are. It is easy to go to far and to remove the neural structures, ask for help if you need it.
- 11. Periodically add saline to your dissection area to facilitate structure visibility.
- 12. Upon completing the dissection, add saline to your dissection area to prevent drying.
- 13. Take the fly back to your bench, insert a reference electrode in the abdomen, and secure it with tape or plasticine, as shown below
 - Be careful not to dislodge the fly from the wax during this process.



Recording from Neurons in the Fly Visual System

1. Mount the glass slide in your recording setup, and firmly tighten the slide in place.
2. Obtain an electrode from the teaching staff, and mount it on your micromanipulator, making sure that the connection end of the electrode makes good contact with the attached wire.
 - Be careful with the recording tip of the electrode.
3. Connect your recording wires to the recording equipment: amp G1 to electrode wire, amp G2 to reference wire via alligator clip.
4. Adjust the microscope and lighting to clearly visualize the target area on the fly.
5. CAREFULLY use the micromanipulator course knob to adjust the position of the electrode relative to your target such that your electrode aims directly at the lobula plate.
 - Very generally, we are aiming for the medial portion of your dissection area.
 - Use the microscope for this process. You will not be able to see the electrode tip clearly until you are very close to the fly.
6. Add saline to your dissection prep if it appears dried out.
7. CAREFULLY advance the electrode into the saline behind the eye using the micromanipulator fine adjustment knob.
 - Remember you are aiming approximately at the lobula plate.
8. Turn on your audio, check noise levels and adjust grounds if needed to reduce noise.
 - As in previous labs, you should be able to determine the “normal” saline noise from other noise and make adjustments accordingly.
9. If you need to, make a final adjust the electrode position to aim the electrode at or medial to the lobula plate.
10. Advance the electrode slowly into the neural tissue, listening to the audio monitor for neural activity.
11. Once you have isolated a robust neural signal, you are ready to collect data.

Obtain a Set of Voltage Data

1. Optimize the position of the display monitor relative to the fly eye.
 - Be careful NOT to jostle the prep during this process.
2. Using the ruler provided, calculate the screen size and the distance of the fly to the screen.
 - You will use this information to calculate visual degrees.
3. If necessary, troubleshoot your prep for noise prior to data collection.
 - One strong noise source is the display monitor. If it becomes a problem, place a faraday cage cover in front of it and ground it.
4. Repeat the steps listed for **Testing Voltage Data Recording** with one difference- drag and drop your movie into the play ten times to get one set of visual data.
 - Connections between the audio output from the movie (display computer) to the ‘trigger’ of the ADC system (data acquisition computer), will automatically start data acquisition on the data acquisition computer at exactly the same time.
 - The trigger is actually sent at the beginning of each of the ten presentations of the movie,

but this is just a detail.

-At the end of the ten movie presentations you can save the data on the data acquisition computer knowing that it is synchronized with your movie.

5. Sit back and enjoy doing science while your movie is running.

**Be very careful not to touch anything that might disturb the preparation – the prep is very sensitive to even tiny vibrations -- you do not want random noise bursts in your data because you were tapping out a songs on your iPod instead of carefully monitoring what was going on with your data collection.

Saving Your Data

1. At the conclusion of your movie presentation, you should have ten runs recorded, visible at the top of Chart.



© ADInstruments. All rights reserved. This content is excluded from our Creative Commons license. For more information, see <http://ocw.mit.edu/help/faq-fair-use/>.

2. Now, save those runs by clicking File, Save-As.

3. You should save your data file with a name that is likely to be unique and that you can remember.

-We will post your final processed data with the same name.

-For example:

i. username: Your group name.

ii. setup: the name of the setup or group name.

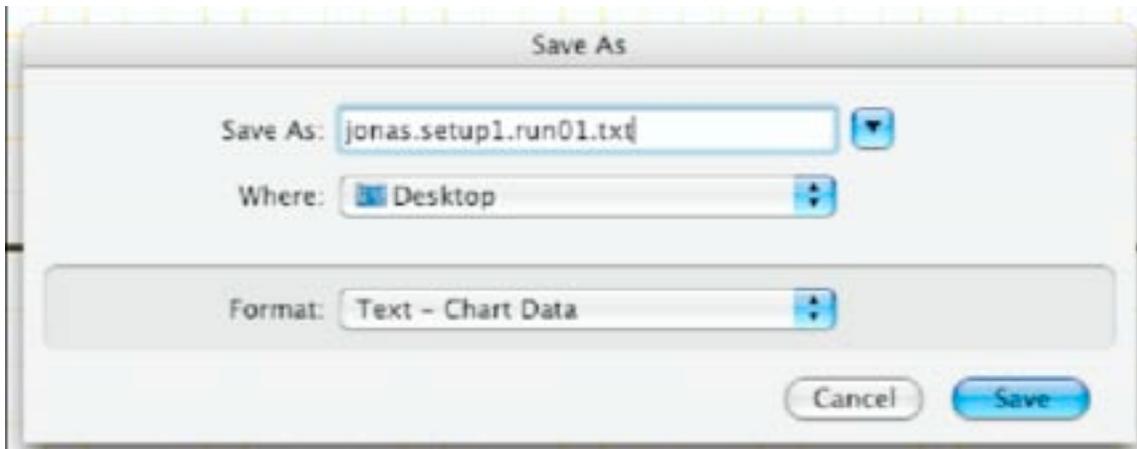
iii. The “try” number. Your movie will automatically be played 10 times. However, you want to try your movie more than once (see below), the first time would be 01, the second 02, the third 03, etc.

4. In your lab notebook, you should write down what is different for each of the tries.

-For example, maybe try01 was the first neuron you recorded, and try02 was the second.

jonas.	setup1.	try01.	txt
Your username	the number of the setup you're on	You may try to collect more than one data file .	the file extension

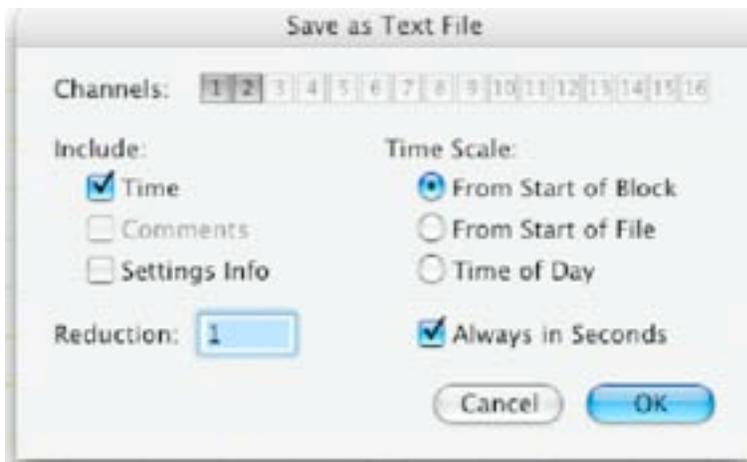
-This is to help you and us keep track of who recorded what and where.



© ADInstruments. All rights reserved. This content is excluded from our Creative Commons license. For more information, see <http://ocw.mit.edu/help/faq-fair-use/>.

****Note:** Be sure to save your data as a .m file! Saving files in this format saves teaching staff data processing time. The sooner the data becomes processed, the sooner you get it back!

5. You will then be prompted for additional settings. Make sure they look like this and hit OK.



© ADInstruments. All rights reserved. This content is excluded from our Creative Commons license. For more information, see <http://ocw.mit.edu/help/faq-fair-use/>.

-The saving may take a LONG time, so please be patient. Now would be a good time to check that you have good notes on the arrangement of the fly relative to the screen, etc.

Obtain Another Set of Voltage Data

1. Once your data has been saved, clear the buffer in the chart program (data acquisition computer) in preparation for the next try at data collection.
2. If you still like the neural signal, maybe you want to carefully change the arrangement of the screen relative to the fly to get a better response.
3. If your neural signal is losing its robustness, you may want to change your electrode position to get a stronger signal.

4. When you like your rearrangement of either the screen or the electrode or any other change you make, repeat the movie playing and acquisition steps to collect more data.

Final Steps before You Leave Today

1. Transfer your group's saved data file(s) collected on Powerlab while you were showing the movie to the fly from the data acquisition computer desktop to a provided USB memory stick
 - The file(s) should be clearly named as described.
 - Be sure to save all files as .m
2. Give this USB stick to the lab manager.
3. Be sure to have lots of clear notes in your lab notebook, particularly on,
 - How the visual stimulus was arranged relative to the fly.
 - Your qualitative judgment of the neuron you recorded (e.g. tested with your moving finger)
 - Any worries or things you should look out for when analyzing the data (e.g. noise bursts, the file ended early, etc.)
4. We will take your data file and convert it to a format that you can easily load into Matlab and manipulate in the same way that you manipulated data in Tutorial 3.
 - You should have this within a couple days of the end of lab.

8.4 Fly Data Analysis

******You will use this session to get advice and help on analyzing the data you collected in fly lab last week. Upon starting today, you should know what your group's experimental goal is, what your group's movie stimulus does and how you plan to analyze your data. You should have received a your converted data from the teaching sta

******By the end of this session you should have successful output of your spike sorter on your fly voltage data, sketches (hand drawings) of figures that you will try to make to show your results, a clear plan on how to analyze your spike data (above) to make those figures and thoughts on the possible things you might see in those figures and how you would interpret them differently in each case. **ALL OF THIS SHOULD BE WRITTEN IN YOUR NOTEBOOK AND SHOULD BE APPROVED BY A STAFF MEMBER BEFORE YOU LEAVE THE LAB TODAY.**

******Each student should work independently on group data. It is appropriate to share insights and ideas on how to proceed, but each student should process their group data file from start to finish, including results and figures, and the lab report as a whole.

Spike Sorting

1. You learned how to do spike detection in Matlab Tutorial 1. However, that was relatively clean data.
2. Your data file may be much messier and may contain many artifacts that you should try to deal with.
 - For example, if the fly moves, this creates a lot of noise in the voltage trace, and a simple spike detector might count this noise as spikes.
3. You should NOT blindly run a spike detector, but should examine your data closely and make sure the spike detector is doing something reasonable.

******Please ask the staff for help if you are not sure about how to proceed. It is impossible to fully recover from very noisy data, so the most important thing to think about is how your choices might bias your final results.

Data Analysis

1. We expect you to ask one or more intelligent questions about the response of your fly neurons and to use your data to try to answer that question(s).
2. The groundwork for this was laid down two weeks ago in the fly movie design lab, so now it is a matter of implementing that, dealing with any surprises in your data, and thinking

about how you will present your results (figures and text).

****Please discuss these issues with the staff. This day is meant to help you with lots of one-on-one feedback. If you are thinking clearly about where you are heading after the end of today, your lab report will almost write itself.**

Last Minute Notes on Lab Report 2

1. What you should strive for is a paper where one of your classmates could read it, understand why you did what you did, what you did, what you found, and what you think it means.
-That is what a scientific paper is -- nothing more, nothing less. It really is that simple.
2. There are no hard and fast rules on length, and we will not explicitly grade on length, but a ballpark estimate should be approximately 3-5 figures (including at least one methods figure) and approximately 5-10 pages double spaced (not counting figures, captions or references).
3. Remember, the goal is to motivate your experimental question (Introduction), explain what you did (Methods), show what you found (Results), and speculate on why it may or may not be important (Discussion).
4. Clarity and conciseness are paramount, and these come from having a clear idea of exactly what you are trying to say in each section (above) and in each paragraph of each section.

MIT OpenCourseWare
<http://ocw.mit.edu>

9.17 Systems Neuroscience Lab
Spring 2013

For information about citing these materials or our Terms of Use, visit: <http://ocw.mit.edu/terms>.