Section 9. Human Anatomy and Physiology

9.1 MR Neuroimaging

9.2 Electroencephalography

Overview

As stated throughout, electrophysiology is the key tool in current systems neuroscience. However, single- and multi-unit physiology, of the type applied thus far in the course, are not applicable in humans, leaving a crucial interpretive gap between our studies of animal model systems and the processing reality of the preparation of ultimate interest (us).

One time honored technique for trying to bridge this gap is electroencephalography (EEG). This technique allows measurement of electrical potentials on the surface of the scalp, reflecting underlying changes in neural activity (primarily but not exclusively, as we will discuss in class). This approach has proven essential historically for understanding how rhythmic activity in the brain, often referred to as 'brain state,' impacts perception, action and cognition.

In this laboratory, we will learn to use EEG, apply it to the recording of spontaneous and induced brain rhythms, and engage in the analyses typically applied of this kind of data. There will be a unique preparation for this lab: Yourselves!

In addition, we will show you how structural magnetic resonance imaging (structural MRI) can reveal the structure of brain tissue in a non-invasive manner. This opens up a range of possibilities including gross anatomy in humans, and coupling with functional studies (e.g. neurophysiology and functional MRI).

9.1 MR Neuroimaging

The broad goal of this laboratory is to give you hands-on experience with the primary means of data acquisition in magnetic resonance (MR) imaging (scanning human or animal subjects) and with the primary data analysis approach (using software to visualize and quantify data sets).

The specific goal of this laboratory is to demonstrate functional MRI (fMRI), the primary tool for identifying brain areas which are particularly engaged during stimulation, motor behavior or a cognitive task. In the laboratory, you will then have the opportunity to view structural MRI datasets from three species (rat, squirrel monkey and human). The software package we will use for these studies is NeuroLens, which also allows a Region of Interest (ROI), such as a particular brain structure, to be highlighted and its volume calculated.

Starting NeuroLens

1. Open the NeuroLens program by double-clicking on the NeuroLens icon on the desktop.

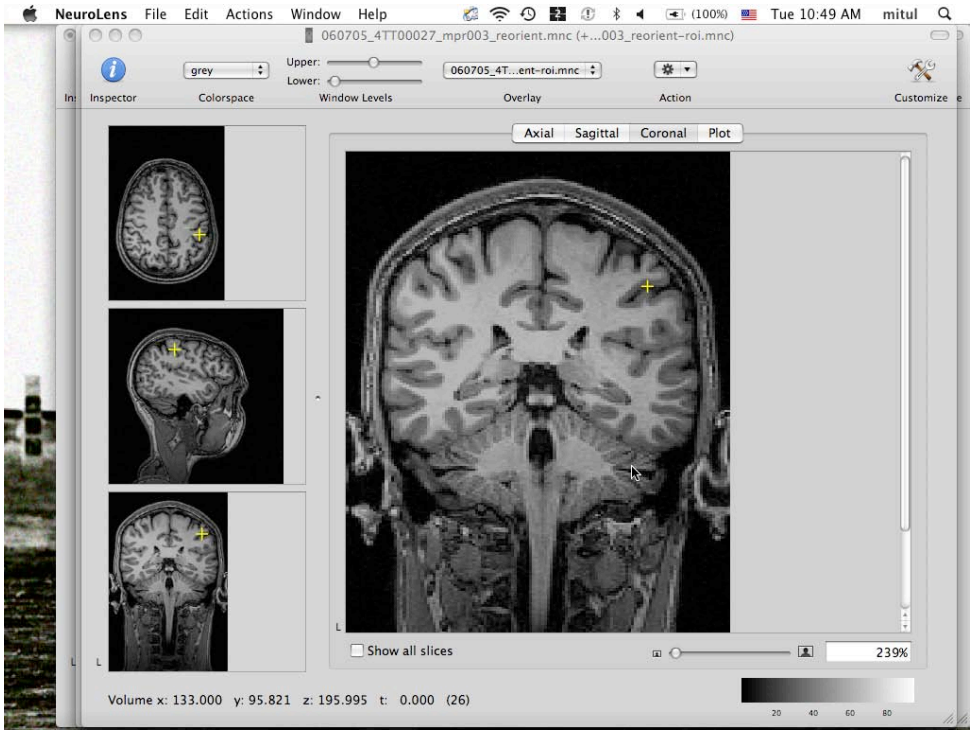
Loading Data Sets

1. To open data sets in NeuroLens, go to the 'file' tab on the top of the screen and scroll down to 'Open…'

2. Select the desired data set (rat, monkey or human). The screen should look as below for a human data set.

3. The data will then appear on the screen.

4.



Viewing the Data

1. You can change the displayed plane of section by clicking on the Axial (Horizontal), Sagittal or Coronal tabs above the large brain slice.

2. To change the place where that plane of section is taken, click on the images to the left.

   -For example, if a Coronal slice is displayed in the large-view screen, you can click on the Axial image on the left side (the top image), and the point where you click in that image will resection the brain and provide a new Coronal image corresponding to the point where you clicked.

Painting regions of interest (ROI)

**'Region of interest (ROI)' is a phrase widely used to refer to a part of a brain that is, well, of interest. For example, one might identify an ROI as being a region of the hippocampus activated in a previous study (e.g., 'try to remember a map of the London streets'), and one might look at this ROI to examine whether there is any indication of functional activation there using a new task (e.g., 'recall every passenger that was in your cab today').

1. Call a slice to the center (large) screen that shows the brain area of interest. For example, a coronal section that shows the part of the hippocampus you would like to measure.

2. Go to the 'file' tab at the top of the screen, and open 'Create Overlay.'

3. Hold down the shift key, and with the mouse draw a freehand curve around the ROI.

4. Click on the blue 'I' button in the top left corner (the 'inspector')

5. In the screen that appears, click on the 'ROI' tab at the top of the screen.

6. In the screen that appears, click on the 'compute ROI statistics.'

Calculating Anatomical Data

1. The screen that now appears should provide you with (almost) all of the information you will need to compute the volume of the ROI defined. To do this, you will also need to know the voxel size in the image and the slice thickness, data that the instructor or TA will provide.

2. A voxel is the resolution that an MR image was sampled at, the unit of measurement for a given scan. If an image was taken with slices oriented in the coronal plane, and the image in this plane is 10 cm x 10 cm, and the data were sampled at a resolution of 100 x 100 voxels, that means that each voxel is 1 mm (.1 cm) across in each of these dimension.

3. The thickness of the slices sampled (how long a voxel is in the third dimension) can vary from these voxel dimensions. For example, if the slice thickness is 5 mm in the previous example, then the area of a voxel would be 1mm x 1mm x 5mm.

4. With this information, then, one can calculate the area in an ROI defined in a given plane. If there are 50 voxels in the ROI in this example, then the area would be: 50 x 1mm x 1mm x 5mm, or 250mm3.

5. To measure the volume of a structure that spans several planes, you will need to paint an ROI on each. This is best done along a single axis (paint an ROI on each e.g. sagittal section that contains the structure; don't paint on some sagittal sections and some coronal).

9.2 Electroencephalography

** This protocol has been derived from the A/D Instrument Student Protocol for EEG (2005).

**Each student should assume the role of the subject and of the researcher at least once during this session.

Familiarize Yourself with the Equipment

1. At your station you should have a data acquisition computer connected to an A/D converter. The converter should be connected to a bioamplifier. Check that these connections are made.
   -The bioamplifier will retrieve electrical signals from the subject and send them through the A/D converter for digital processing.
2. Locate the bioamplifier cable and make sure that it has three flat electrode leads attached to it, one in the earth (ground), one in CH1 negative and one in CH2 positive. Be sure that this cable is plugged into the bioamplifier.

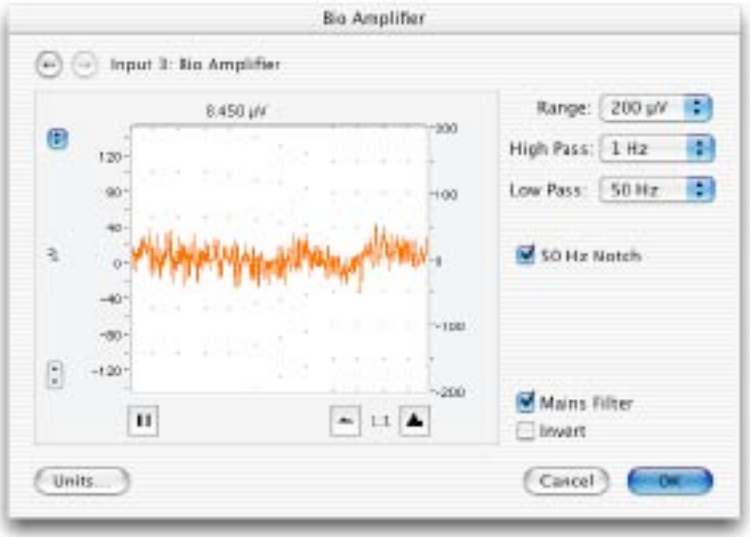
Preparing the Subject

1. Take the flat ends of the electrode leads and fill them with conductive paste.
2. Take at least one of the alcohol pads provided and wipe the subject's forehead and a spot on the back of one side of the head.
3. Carefully place the flat lead, paste side down, for CH1 positive on the wiped spot on the back of the head, for CH1 negative on the forehead of the same hemisphere, and for earth on the opposite side of the forehead.
   -The recording leads (positive and negative) will collect data from the entire hemisphere in this orientation.
4. While holding the leads in place, have a lab mate take the swim cap provided and place it on the subject's head over the leads.
   -The swim cap should provide sufficient contact to maintain the position of the leads.
5. Once the swim cap and leads are in place, you are ready to start data collection.
6. In order for this experiment to be most effective, the subject must remain relaxed throughout.

**Note: After our initial experiments, you are welcome to play with the lead positions and place them however you want. However, you must remember to wipe down the head with an alcohol pad and replace the conductive paste as necessary to receive a good signal.

Preparing for Data Collection

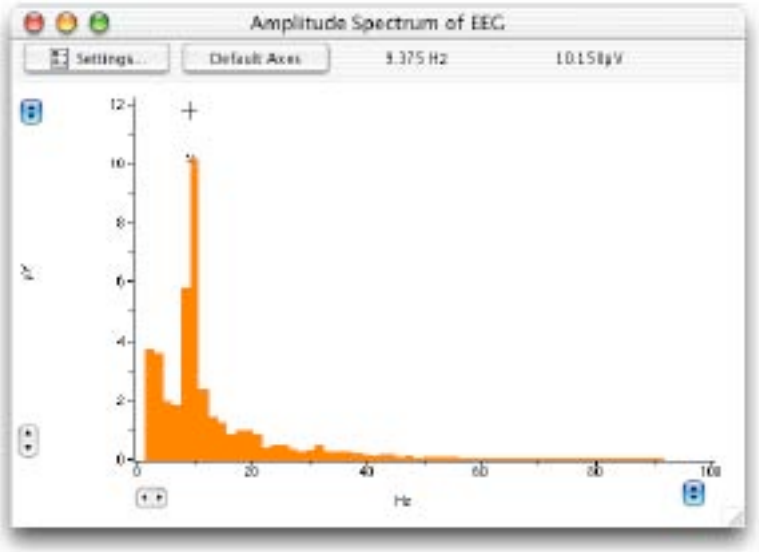
1. Open the Chart program icon if it has not already been done.
2. Go to the file menu and select the "experiments gallery."
3. In this dialog, select the "electroencephalography" experiment, then click open.
4. Two windows should appear. One that will actively record the data, and one that shows an amplitude spectrum (see examination of alpha waves for picture).
4. Click on "BioAmplifier" from the channel function menu. Review the settings, shown below, and then click okay.



Examination of Alpha Waves

**Alpha waves occur between 8-13Hz.

1. The subject should have their eyes closed at the start of this experiment.
2. Click the start button in chart to start recording data.
3. Type "open" and when ready, ask the subject to open their eyes while you press enter.
   -This will allow the researcher to have a comment attached to the data so that they know when eye opening commenced.
4. Type "closed" and when ready, ask the subject to shut their eyes while you press enter.
5. Repeat steps #3-4 two more times, pausing at least ten seconds in between each eye change.
6. Click stop to cease recording data.
7. Scroll back to the portion of your recording data where the subject's eyes are closed.
8. Drag on this data to highlight it.
9. With the spectrum window, the highlighted data is displayed by frequency using a fourier transformation, which should look like this,



10. If you accidentally close this window, go to the Windows menu, and choose "spectrum."
11. If you cannot see the frequency range of alpha clearly, then you can expand the horizontal axis to better visualize it.
12. Repeat steps #7-9 for the other data ranges in which the eyes are open and for the other closed eye ranges.
    -Do you see the alpha frequency equally when the eyes are closed and open, or does it occur more with one state? Why is that?
13. While you are searching for the alpha waves, are they any other waves that you observe occurring often?
    -Beta, 13-30Hz
    -Theta, 4-8Hz
    -Delta, 0.5-4Hz

Presentation of an Auditory Stimulus

1. Connect the audio monitor to the first output on the A/D converter.
2. In chart under the setup menu, click on "stimulator." Alter the settings of the stimulator until you are able to generate a sine wave from the audio monitor that will play while you record data.
   -Start with sine wave, 50Hz, amplitude 3-5V. This will definitely give you a sound but you may need to optimize for the subject, which is why we recommend altering.
3. Have the subject sit with their eyes closed and with the ear opposite to the recording electrodes next to the audio monitor speaker.
   -Be sure that the volume on the audio monitor is not too high so that you do not disturb other groups and so that you do not injure the subject.
4. Let the subject relax for about a minute, then start recording on the chart program while the audio stimulus plays.

5. Stop recording and perform a fourier transformation on the recorded data.
6. Determine if you can locate alpha or any other waveforms in this data.
   -Gamma waves appear at 40-50Hz and typically occur with auditory stimulation.
7. Change the stimulator settings such that there is a delay between the onset of recording and the onset of the auditory pulse.
8. Repeat steps #3-4. When you stop recording the electrical activity of the subject, perform a fourier transformation first on the data that you collect before the onset of the stimulus and then on the data that you collect after onset.
9. Determine if you can locate alpha, gamma or other waveforms in either set of data.
   -If you see alpha before the stimulus presentation, and lose it after, what does this indicate about alpha generation?

Other Experiments

**These experiments are suggestions. You do not need to perform all of them and you are welcome to add your own experiments at any time. This time is your last in the lab, so have fun!

1. Alter the position of the electrodes so that you focus on one specific area, e.g. visual cortex or auditory cortex. Repeat alpha and auditory stimulation experiments from these new lead positions.
2. The alpha wave is coupled to attention, but not necessarily visual attention. While the subject has their eyes closed, ask them to think about a specific visual or auditory image and to alert you when they perform this task (so that you can note its start in the chart data). Compare the frequency distributions before and after these "thoughts."
3. Continue to alter your audio stimuli further to determine if there is any significance to the type of electrical responses obtained with each stimulus.
4. Have your subject attempt to sleep or to meditate while you record data from a hemisphere.
   -Theta and delta waves are most apparent during these states.
5. Have the subject close their eyes, and stimulate with touch the side of the body where you have recording leads attached, then repeat the experiment with touch to the side of the body opposite the leads. Determine if there are any changes to waveforms with this stimulation.

9.17 Systems Neuroscience Lab

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