**NSCI 20100 Neuroscience Laboratory**

**Reaction Time**

**BSLC 322, January 26-28, 2022**

**Goals:** In this lab, you will use electro-oculogram (EOG) signals to measure saccadic reaction times. With these data, you will examine the effects of different stimulus conditions on those reaction times. In performing the measurements, you will gain experience with amplifying, filtering and processing microvolt analog signals.

**Safety:** There is alcohol in skin preparation pads for cleaning the skin before placing the EOG electrodes. Do not let any liquid from the pads get in your eyes. Once the EOG electrodes are installed, keep the leads protected and away from water and electrical sources until they are inserted into the isolation unit. No special personal protective equipment (PPE) is required or recommended.

**Data:** You will collect eye position traces for saccades under two stimulus conditions. You should collect data separately from both students in each lab team (complete data collection from the first before beginning the second). Clean the EOG electrodes between subjects as described in the procedures.

**Clean up:** When you have finished, you should clean the EOG electrodes as described in the procedures. Quit Matlab and collect any data files from the lab machine. You do not need to log out, reboot or shutdown the computer. Disconnect the electrical equipment so it is in the state in which you found it.

**Lab Report:** The primary question is whether saccadic reaction time for the gap and step conditions differ. Your report should describe the experiment and results. You should include an explanation based on the complete data set, and also describe and discuss differences you see between the complete set and the interim data sets. Your report should describe what the data suggest about interactions between fixation and saccadic eye movements and conclusions you might draw about parallel brain mechanisms controlling movements of the eyes.

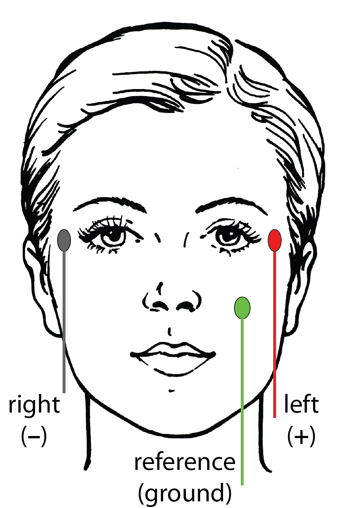
**Laboratory Procedures**

***Hardware:***

*Recording Equipment:* The hardware and cabling will need to be set up. The small switch on the C-ISO-256 pre-amplifier controlling low-pass filtering must be set to 2.5 kHz. The cable from the C-ISO-256 should be connected to left input (Channel 1) of the iWorx ETH-256 amplifier (multi-pin DIN input next to the Offset knob – not the BNC input). The initial settings for Channel 1 on the iWorx amplifier should be: “DC” HPF (high-pass filter); “50 Hz” LPF (low-pass filter); and “x10” gain. The settings for Channel 2 are irrelevant.

The iWorx C-ISO-256 pre-amplifier has a fixed internal gain of 400x. The settings on the amplifier are indicated by small red LEDs and are changed by pressing the appropriate black button. You may change these setting to improve the quality of your data, but they should be fixed during data collection from each subject (and noted in your lab report). Your calculation of EOG voltage must include the combined amplification of the amplifier (10x typically) and pre-amplifier (fixed at 400x). You should report all voltages as they appeared on the electrodes, not the values post-amplification.

Use a BNC cable to connect Output 1 of the ETH-256 to a LabJack U6 computer data acquisition unit. A BNC-to-wire adaptor will need to be installed to the LabJack’s analog input channel 0 (AIN0, red wire) and analog ground (GND, black wire) inputs and secured by gently tightening the screws to clamp the wires. The LabJack U6 should be connected to the iMac computer with a USB cable.

*EOG Electrodes*: You will record the EOG using three electrodes. Two active electrodes are positioned immediately lateral to the eyes, but not so close that they are jostled by blinking. The remaining reference electrode should be placed on the left check immediately below the eye.

You can put the electrodes on yourself using a mirror. It might be faster and easier for you and your partner to put them on each other.

Use an electrode prep pad to gently clean the skin at these three sites. Avoid getting alcohol from the pad in your eyes. Allow the skin to dry completely before attaching the electrodes.

To attach each electrode, lift the blue tab on one sticky pad to pull it and its paper face from backing. Attach the sticky side of the pad to the electrode face (larger side of the electrode, on which you can see the silver electrode). Remove the outer protective paper from the sticky pad and press the pad and electrode on the skin with the wire hanging downward. Once gentle pressure has been applied, the electrode should remain attached. Drape the wire over your shoulder or put it in a shirt pocket to keep it from getting pulled. Attach the remaining two electrodes. Insert a small amount of electrolyte gel inside the electrode using the syringe and blunt needle. Pass the blunt needle through the hole in the electrode and gentle push the plunger until gel starts to come out of the top of the hole. You should feel the (cold) gel contact your skin.

Plug the electrodes into the C-ISO-256 isolation unit. The jacks are color coded according to the diagram above (red-left, black-right, green-reference). Your electrodes might have different colors, but it does not matter. However, you should connect them according to this color coding (left-red, right-black, reference-green).

***Software:***

You will use Matlab applications to collect your data. The necessary software is installed and configured on each of the lab’s computers. Use the following procedures to run the software.

1) Log into the “labuser” account. There is no password for this account; it should not ask for one. If it does request a password, leave that field blank.

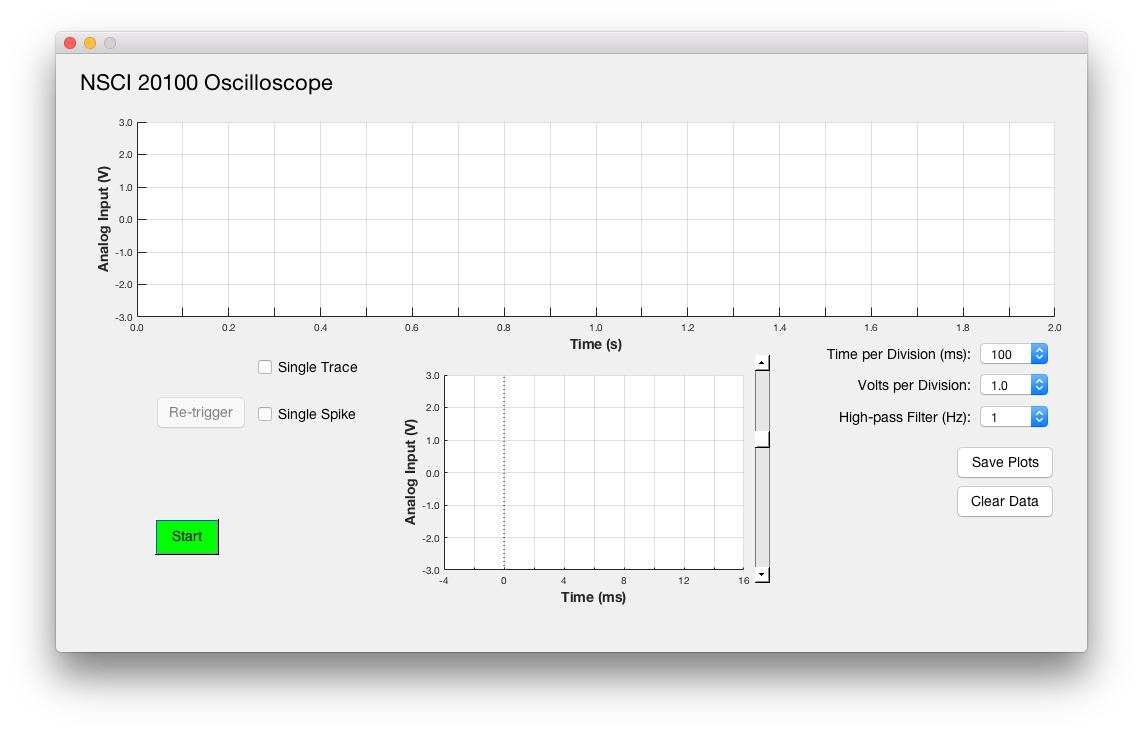
2) Launch Matlab by clicking on the Matlab icon in the dock at the bottom of the display. (Matlab might have a date appended to its name, such as “Matlab\_2021a”.)

You will be using two applications in the lab: Oscilloscope and SaccadeRT. You must not run them simultaneously. Matlab will let you do this, but it will generate hardware contention. You must quit one application before launching the other. You do not need to quit Matlab to go between the applications.

***Signal Assessment:*** Before attempting to collect data, you will use the Oscilloscope application to assess the quality of your signal. You want to see little 60 Hz noise (16.7 ms period) and clear upward and downward deflections of the voltage trace when making large leftward and rightward saccades. You should strive for 60 Hz noise of <50 µV (pre-amplification – after 400x preamplification and 10x amplification you should have 60 Hz noise < 200 mV on the Oscilloscope display). You should also familiarize yourself with the consequences of shaking the electrode wires, clenching your teeth or blinking while recording EOGs.

3) When it launches, Matlab will display a large, multi-paneled window. You will first measure your 60 Hz noise using an application called “Oscilloscope”, which you can launch by typing its name in the Matlab “Command Window” at the bottom of the Matlab window.

4) The Oscilloscope application will take several seconds to launch. Once it has finished launching, you will see a new window as shown below. When you press the **Start** button, it should begin displaying the analog signal from the EOG electrodes. You should use this display to adjust the EOG cables and equipment layout to minimize the 60 Hz noise (16.7 ms period) on the signal. Large saccades to the left or right should produce vertical deflections that are much larger than any noise. If you are wearing the electrodes, you can see the signal by holding your gaze fixed on the display window while rapidly rotating your head to the left and right (thereby changes the position of your eyes in their orbits).



5) When you are satisfied with your signal, you can quit the Oscilloscope application by closing the EOG control panel window using its close button (red button in the upper left corner). Leave Matlab running: you will need it for collecting data.

***Running the SaccadeRT***

Launch the SaccadeRT application by typing its name in the Matlab command window. It will take several seconds to launch. Once the SaccadeRT application has finished launching, you will see two new windows.

../../Screen%20Shot%202017-12-11%20at%2018.30.33.png

The stimulus display will appear as a long, thin horizontal dark gray window at the bottom of the monitor. The control/display panel (GUI) will appear above. You should familiarize yourself with the controls and displays and run test trials before you start collecting data you plan to use. You can clear any test data before collecting data you plan to analyze and present.

The task involves following the small white dot with your eyes. The white dot will jump left or right at random by 5° at semi-periodic intervals. You should simply do your best to keep your gaze on the dot. Shift your gaze to follow the dot as quickly as you can after it moves, but remain relaxed. It is important that you do not look away from the dot while you are collecting data. Pause the data collection to take frequent breaks, but always pause the task before you stop following the dot.

**Settings:** Before collecting data, you must adjust several task settings. You must enter the **Viewing Distance**. Find a comfortable viewing position and measure the approximate distance from your eyes to the screen. This should generally be 50 to 75 cm. You must enter this value before data collection to ensure that the stimulus steps are calibrated in units of degrees of eye rotation.

The application has a digital notch filter that will remove frequencies close to 60 Hz from the eye position velocity traces. You will get better data with this filter turned on (using the **Filter 60 Hz** checkbox).

You should adjust the **Saccade Threshold** so that you reliably detect most of the saccades without allowing baseline noise to trigger spurious saccade detection. It is better to miss some saccades than to include noise in the baseline as saccades.

**Saccade Detection:** A saccade is detected when the position trace exceeds the **Saccade Threshold** after the stimulus step for five consecutive eye position samples (which are collected at 1 kHz). (If too few trials have been run for the application to establish a calibration, it will assume that the maximum voltage in the trace corresponds to the stimulus step size on that trial.) The application then searches back in time from the first sample above threshold to the point where the velocity last reversed sign, and counts that as the start of the saccade. It then searches forward in time to the first point where the velocity reverses sign again, and count that as the end of the saccade. The start and end of the first saccade detected after target onset are marked with vertical dotted lines in  most position and velocity traces (if a saccade is detected). No lines will appear when no saccade is detected. The times of the stimulus changes are marked with vertical the dash-dotted lines these plots.

Two different stimulus conditions will be randomly interleaved on different stimulus steps: gap and step. In the step condition, the fixation point simply disappears from one position and reappears at another. In the gap condition, the fixation point disappears and reappears at a new position after a 200 ms gap. Reaction times will be plotted in the two right plots, separately for steps and gaps. Once enough trials have been collected, these plots will display the distribution mean, SD and SEM

The gap histogram plot will also include a t-test statistic. This is the result of comparing the gap reaction time distribution to the step reaction time distribution using a two-sample one-tailed t-test. The value shown is the p (probability) value resulting from the test. Statistical significance is typically taken to be p<0.05 or p<0.01.

**Breaks**: While you do the task, you should take frequent breaks, but you should not leave the task running when you pause. You should be tracking the spot with your eyes whenever the task is running. You can use the **Start/Stop** button to toggle the task run state. For convenience, you can also use the space bar to toggle the run state if the control panel is front-most. This allows you to pause the task without looking at the mouse or Start/Stop button on control panel. If you are distracted by the plots updating, you should move that window so that the plot are offscreen.

**Chart

Description automatically generatedControlling the EOG Task**

The following controls are found at the bottom left of the Control Panel.

**Start (Stop):** Toggles whether the task is running. You can also use the space bar when the control window is front-most.

**Viewing Distance (cm):** The distance from your eyes to the screen. This value must be set before data collection to ensure that your position and speed measures are properly calibrated.

**Saccade Threshold (deg):** The program detects the start and end of each saccade using a movement threshold (dashed horizontal blue line in the plot labeled “Most recent position trace”). You can adjust that threshold here. If your filtered trace is noisy, you will need to set the threshold higher to avoid spurious detections. If your filtered traces are less noisy, you can set the threshold lower to get more precise measurements of the saccade dynamics.

**Stop when n =:** Your lab report requires that you compare distribution statistics after collecting varying numbers of samples. The value you enter here will make the application stop running once it has collected a certain number of samples for both connections. This will save you monitoring progress and trying to pause execution at the correct moment.

**Clear Data:** This button can be used to discard the current data. You should save any data you care about before clearing.

**Save Plot:** This button will allow you to save a PDF file with the current contents of the display plots. You must use this control to get figures you can use in your lab report. Each plot is saved with a file name that includes the time of day.

**Save Data:** This button will allow you to save your data in a Matlab ‘mat’ file. Save data can be reloaded using the “Load Data” button.

**Load Data:** This button will allow you to load data you have previously saved using the Save Data button. Note that any loaded data will overwrite current data, causing it to be lost.

The following displays are plotted in the control/display panel:

**Most Recent Position Trace:** The most recent position trace is displayed. The difference between the two EOG electrodes is plotted as a function of time. Rightward and leftward eye movements correspond to up (positive) and down (negative) on this and other displays. A dotted-dashed line marks the stimulus movement. The target onset will be marked with a black vertical dash-dotted line. The fixation offset will be marked with a red vertical dash-dotted line, and will appear before the target onset by 200 ms in the gap condition. On step trials no fixation offset line will appear (it is the same as target onset). If a saccade is detected, its duration will be marked by vertical lines. Traces are offset vertically so that the average of the pre-movement portion of the trace lies at y = 0 V (0°).

**Most Recent Velocity Trace:** The most recent velocity trace is displayed. This curve is the derivative of the most recent position trace plotted in Most Recent Position Trace. Calibration is in V/s until enough traces have been collected to establish a voltage-to-position calibration, after which it is plotted in units of deg/s. Other formatting follows that for the most recent position trace.

**Clean up**

When you have finished collecting and saved your data, you can terminate the SaccadeRT application by either 1) closing the SaccadeRT control panel window using its close button (red button in the upper left corner), 2) closing the Matlab window using its close button, or 3) making Matlab quit using Quit in the File Menu (or the keyboard equivalent, command-Q). In any case, you will be asked whether you are sure you want to quit. All unsaved data will be lost when you quit.

Immediately after removing the EOG electrodes, wash out the gel or cream using distilled or deionized water. Do not use tap water. A cotton swab can be used to remove the main bulk of the gel. Do not scratch the metal surface of the electrode. You can remove any gel from your face with a wet paper towel.