Experiment HP-11: Multisensory Reaction Times

Lab written and contributed by: Tracy M. Hodgson, Program in Biological Sciences, Northwestern University, Evanston, IL *and* Martha Cammarata, Department of Biology, North Park University, Chicago, IL

Equipment Required

PC or Mac Computer IXTA, USB cable, IXTA power supply HV stimulator lead wires EM-220 Event Marker

Experimental Considerations

Before you start collecting data, it is important to decide what type of information will be collected. Some factors to consider:

- 1. What information about your subject are you recording? These should be factors that will be pertinent to reaction time (age, height, weight, gender, etc...).
- 2. Once you decide what information to collect, use the Journal or Exel to design an a spreadsheet to enter your data. At a minimum, include a subject identifier, treatment condition (visual/auditory/somatosensory), and the reaction time.

| Subject ID | Treatment Condition | Reaction Time | Other subject info |
|------------|---------------------|---------------|--------------------|
| | | 5 | |

- 3. The settings file is set up to run 10 presentations of stimuli per reaction time experiment. When designing your own experiments you may decide to run more or fewer stimulus presentations per experiment.
- 4. As a group, discuss and decide:
 - Should the subject be allowed to have one or more practice trials?
 - How do you ensure the best possible stimulus for the subject (i.e. minimizing extraneous cues)
 - Should the stimulus cues be presented in a predictable fashion or with randomly spaced intervals? The settings file you are working with today will present stimuli at randomly spaced intervals, but you may decide to test predictable cues with the subjects for your experiments.

IXTA Isolated Stimulator

The IXTA has a high voltage stimulus isolator designed to deliver constant current to the nerve or muscle being studied. In situations where the resistance (R) along the path of the current increases, the voltage (V) increases to maintain the current (I in V = IR, Ohm's Law). The ability of the IXTA to adjust the voltage to deliver the required current is known as voltage compliance. The upper limit of this compliance by the IXTA is set at 100 Volts.

Constant current devices differ from constant voltage devices when presented with an increase in resistance, like the dehydration of the conductive gel under the electrodes. As pointed out earlier, a constant current stimulator is voltage compliant. In constant voltage stimulators, the current delivered to the tissue decreases as the resistance increases because the power supply of the constant voltage device is not designed to deliver additional current.

Although the IXTA can generate up to 100 Volts, the current delivered by the unit is limited to a maximum of 20 milliamperes, for a maximum duration of 10 milliseconds per pulse, and a maximum frequency of 50 pulses per second (Hz). At these levels, the maximum amount of power delivered by the IXTA will not cause injury or tissue damage.

The current is selected using the Stimulator Control Panel. The HV Stimulator can deliver a maximum output of twenty milliamperes

The duration, frequency, and number of stimulus pulses generated by the stimulator are also controlled by making changes to the values in the Stimulator Control Panel. The initial values of the pulses generated by the IXTA are programmed by the same settings file that configured the recording software. For example, if a pulse from the IXTA is programmed for a duration of 1 millisecond and a frequency of 1 Hz, the stimulator will generate a stimulus pulse with the same duration and frequency.

Equipment Setup

- 1. Plug the EM-220 into the EM port on the back of the TA.
- 2. Instruct the subject to remove all jewelry before beginning the experiment.
- 3. Connect the stimulator lead wires to the stimulator. Make sure you push the safety connector of each lead wire into the appropriate socket as far as possible.

Warning: Before connecting the IXTA stimulating electrodes to the subject, check the Stimulator Control Panel to make sure the amplitude value is set to zero (0).



Figure HP11-S1: The IXTA stimulating electrodes.



Figure HP-11-S2: The front panel of the IXTA with the stimulating electrodes connected correctly. The event marker will plug into the back into channel EM1.

Positioning of the Stimulating Electrodes

- 7. Use an alcohol swab to clean and scrub two areas on the back of the subject's hand. One area is in the center of the hand, and the second area is along the lateral edge of the hand between the base of the little finger and the wrist.
- 8. Obtain two disposable electrodes. Remove the plastic shield from one of the disposable electrodes. Attach the electrode to the center of the back of the hand. The electrode should be half way between the first knuckle of the middle finger and the wrist. This electrode is the positive stimulating electrode.
- 9. Remove the plastic shield from the second disposable electrode. Attach this electrode about one centimeter above the lateral edge of the hand, half way between the first knuckle of the little finger and the wrist. This electrode is the negative stimulating electrode.
- 10. Snap the ends of the H.V. Output lead wires onto the stimulating electrodes, so that:
 - the red (+) lead is snapped on the electrode in the center of the back of the hand,
 - the black (-) lead is snapped on the electrode at the lateral edge of the hand.
- 5. Make any changes to the parameters that are necessary and click on the **Apply** button to complete the changes.

Warning: Make sure the Amplitude on the Stimulator Control Panel is set to zero.

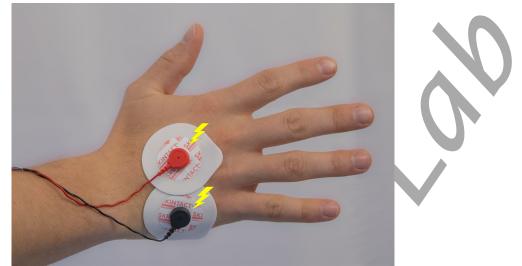


Figure HP-11-S3: A. Placement of the stimulating electrodes on the back of the right hand.

6. Click on the Record button. There should be no response from the subject since the current output is zero. Continue to record.

Note: As the current output is increased in the next couple of steps, ask the subject to indicate when he or she first feels any tingling under the negative stimulating electrode. Minor movement of the subject's finger can usually be seen when the stimulus current is raised another milliamp or two.

- 7. Enter the amplitude value into the Stimulator Control Panel and click APPLY. For each "1" amplitude increase it is equal to a current output of 2 milliamperes (mA). Remind the subject to indicate the first occurrence of tingling.
- 8. Make sure to check for tingling. If necessary, increase the current output in increments of 0.25mA until the subject feels a strong tingling sensation without too much finger movement.
- 9. If tingling does not occur at 2 mA, change the Amplitude by an additional 0.25 to increase the stimulus current by 0.5mA. Click the Apply button. Check for tingling. If necessary, increase the current output in increments of 0.25mA until the subject feels a strong tingling sensation without too much finger movement.
- 10. Find the lowest stimulus current that creates the most comfortable tingling sensation from the subject's finger.
- 11. Click Stop.
- 12. Set the current at where the subject felt a good tingling from the stimulation.

Experiment HP-11: Multisensory Reaction Times

Lab written and contributed by: Tracy M. Hodgson, Program in Biological Sciences, Northwestern University, Evanston, IL *and* Martha Cammarata, Department of Biology, North Park University, Chicago, IL

NOTE – This is a multi-day experiment. Students should be prepared to work on this for three lab periods.

Responding quickly and appropriately to external stimuli is an important component of life for any organism. The goal of this lab is to measure volitional reaction times in humans, which reflect the amount of time the nervous system requires to process sensory information (i.e. visual cues), select an appropriate motor command, and send that command to the skeletal muscles. During the first part of this lab, you will perform basic experiments to measure visual, somatosensory and auditory reaction times. Then, you will design your own multisensory reaction time experiment.

Day 1 - Exercise 1: Ruler Drop (you will not be using LabScribe for this portion of the experiment)

Aim: To perform a reaction time experiment using a ruler drop protocol, create bar graphs of class data, and analyze class data using T Tests in Excel or another statistical program.

Approximate Time: 20 minutes per subject

Procedure

- 1. Assign roles: experimenter, subject, and recorder for groups of 3, or experimenter and subject for groups of 2.
- 2. The experimenter holds the ruler near the 30cm mark and lets it hang vertically. Meanwhile, the subject places his/her thumb and index finger on either side of the 0 cm mark ready to catch it when it falls. His/her fingers should not touch the ruler.
- 3. Without warning, the experimenter lets go and the subject tries to catch the ruler as quickly as possible.
- 4. The recorder should note and record the level (in cm) just above the subject's finger where the ruler was caught.
- 5. Use the formula shown below to convert distance into time: $t = \sqrt{\frac{2d}{a}}$

Reaction time can be calculated as follows: t is reaction time (in seconds), d is the distance the ruler fell (in cm), and g is gravity (980 cm/s²).

- 6. Repeat this procedure to get at least 10 data points (replicates) with the same subject.
- 7. Repeat steps 2-7, but give a cue ("get ready") 1-2 seconds before dropping the ruler.
- 8. Switch roles and repeat, acquiring 10 cued and 10 uncued reaction times for each subject in your lab group.

- 9. Enter an ID (could be initials or a number), biographical information and the mean cued and uncued reaction times for each subject in the Excel worksheet on your computer desktop. We will graph and statistically analyze this data later in the lab period.
- 10. Follow the directions in the **Appendix** for calculating a T-Test with Excel.

Day 2 – Multisensory Reactions

- 1. Perform experiments measuring reaction time to visual, auditory and somatosensory stimuli using the LabScribe data acquisition system.
- 2. Formulate hypotheses and create an experimental protocol with your lab group, and discuss your protocol with your professor or TA before leaving lab.
 - **Experimental considerations:** Before you start collecting data, it is important to decide what type of information you will be collecting. Some factors to consider:
 - What information about your subject do you want to record? These should be factors that you think will be pertinent to reaction time (i.e. age, height, weight, etc).
 - Once you decide what information you want to collect, you can design an excel spreadsheet where you will record your data. At a minimum, you will need to include a subject identifier, treatment condition (i.e. visual/auditory), and the reaction time.
 - The settings file you are using today is set up to run 10 presentations of stimuli per reaction time experiment. When you design your own experiments you may decide to run more or fewer stimulus presentations per experiment.
- 3. As a group, you can discuss and decide:
 - Should the subject be allowed to have one or more practice trials?
 - How do you ensure the best possible stimulus for the subject (i.e. minimizing extraneous cues)
 - Should the stimulus cues be presented in a predictable fashion or with randomly spaced intervals? The settings file you are working with today will present stimuli at randomly spaced intervals, but you may decide to test predictable cues with the subjects for your experiments.

Hardware Setup -

Using the IXTA

• The cables should be plugged into the front of the IXTA into the two ports on the HVS.

We will be using the stimulators to provide either a visual stimulus (a square wave on the LabScribe acquisition screen), a somatosensory stimulus (a slight pulse on the skin when the electrodes are attached to the forearm) or both, so they should be adjusted to produce the minimum amplitude necessary for the subject to detect the stimulus. If the stimulus amplitude is too high, the somatosensory stimulus will be a distracting shock rather than a simple touch, which will affect reaction time.

Exercise 1: Reaction Time and Visual Cues

Aim: To measure the reaction time of a subject to a visual cue.

Approximate Time: 15 minutes

Procedure

- 1. Read all instructions carefully before beginning to record.
- 2. Assign roles within your lab group:
 - Research subject Person whose reaction time will be measured
 - Experimenter/Computer Tech Will control the stimulator, run the computer and assist the subject
- 3. Information for the subject:
 - Instruct the subject to sit in a chair and face the computer screen.
 - He or she should position a hand on the keyboard in a manner that enables the subject to push the F1 key as quickly as possible.
 - Watch the right side of the computer screen for a sqaure wave to appear and quickly press the F1 key on the keyboard when the signal generated by the stimulator first appears.
- 4. Directions for the other student(s):
 - Annotate the data trace with the subject's name and the treatment condition (i.e.
 <Subject's Name> Visual Cues). Marks can be added to a data file in one of three ways:
 - Type in a comment in the space next to the Mark button, then, while the trace is running, click **Mark** when you come to a point in the trace where you want the comment to be.
 - Click the **Mark** button during data acquisition, wherever you want a comment to appear later. After you finish acquiring data, go into the menu and click on "**Marks**". Highlight the mark you want, type your comment in the blank space next to the EDIT button, then click the **EDIT** button, then **OK**.
 - You can also click the **Mark** button after data acquisition is completed to place a comment go to a single cursor, place it where you wish the comment to go, then click the Mark button.

NOTE: It is crucial to annotate your chart frequently! You will need to come back to specific places in the chart when you analyze the data later on in this exercise.

5. The stimulator is set to fire when the sequence is set to "Run".

- 6. Click the Macro tab.
 - Choose the sequence called "Visual + Somatosensory" and then
 - Click OK. At the top of the main acquisition screen, to the right of the row of icons should be a button labeled with the sequence you are currently using, in this case "Visual and/or Somatosensory".
 - When you click "Record", the words "Visual and/or Somatosensory" should turn bright green. If they don't, click on "Macro" again click run and start the recording again.

| 10 | Run Sequence | sensory Auditory Visual Somatosensory |
|----|---------------|---|
| | Stop | ALL T2-T1(60 msec - 30 msec)= |
| | Edit Sequence | V2-V |
| - | Open File | |
| | Save File | V2-V1= |

Figure HP-11-L1: Sequences menu selection.

- 7. Click on the Record button.
- 8. Type **<Subject's Name> Visual Cues** in the Mark box. Click the mark button.
- 9. Instruct the subject to press the F1 key to mark the recording as soon as he or she sees the visual cue (square wave) on the right side of the computer screen.
- 10. Instruct the subject that the exercise has begun and that a visual cue could appear on the screen at any time. Sometimes immediately after pressing record, a cue appears, this one can be ignored.
- 11. The stimulator is set to deliver ten visual cues to the subject. The cues are variable, but generally not be less than one second nor more than ten seconds apart.
- 12. After the tenth cue, click Stop to halt recording.
- 13. Select Save As in the File menu, type a name for the file. Click on the Save button to save the data file.

Human Psychophysiology – Multisensory Reaction Times – Labs

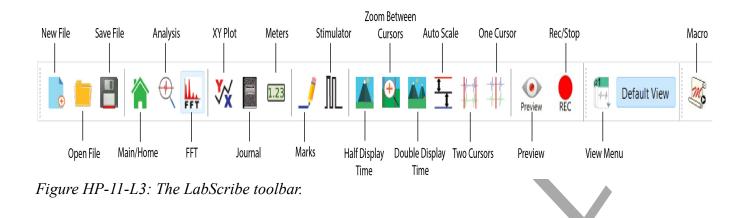
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| Reaction Time | BNC \oplus \oplus \bigcirc f_x | | | | | | | | | | V2-V1= -0.00504 |
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| 5.1- | | | | | | | | | | | |
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Figure HP-1-L2: Three visual cues, each followed by the subject's response, are displayed on the Main window. Each visual cue (square wave) is made by the stimulator; each response mark is made by the subject pushing the F1 key on the keyboard.

Data Analysis

- 1. Scroll to the beginning of the data recorded for Exercise 1 to display the entire set of data recorded.
- 2. Use the Display Time icons to adjust the Display Time of the Main window to show both the visual cues made with the stimulator and the marks made by the subject's response on the Main window.





- 3. Click on the Analysis window icon. **NOTE**: The AutoFind function will not work properly from the Main acquisition screen you MUST be in the Analysis screen).
- 4. Toward the upper left of the screen, make sure "T2-T1" is showing. If not, choose it from the Add Functions General menu.
- 5. Make sure there are two cursors on screen. Place both cursors in front of the first stimulus square wave to be analyzed.
- 6. Click the "Marks" icon to open the Marks Dialog.
 - In the "Marks" Dialog, change the names of **all** events to be analyzed to something other than "Mark" (doesn't matter what it is, could be one letter or the initials of your subject, for example; it does need to be the same name for every event in a trial/block of data).
 - Click OK. Verify that the names of the marks in the Analysis window have changed then **SAVE** YOUR DATA FILE AGAIN.

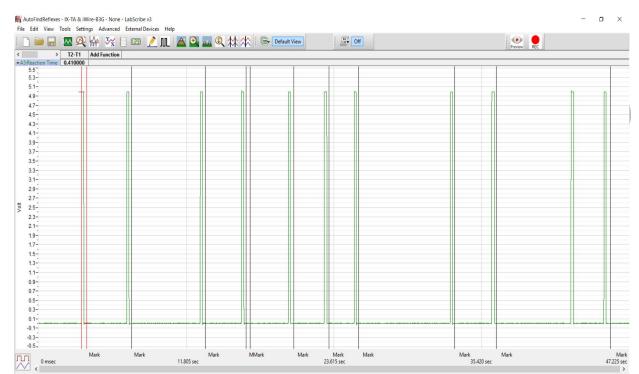


Figure HP-11-L4: A visual cue, followed by the subject's response, are displayed on the Analysis window. The two cursors are positioned at the beginning of the visual cue and on the mark for measurement of the subject's reaction time (T2-T1) in this trial.

- 7. Click on "Tools", then "AutoFind".
 - At the top of the AutoFind dialog, next to "New Cursor 1 Position", choose "Next Positive Threshold".
 - In the same line, to the right, next to the "+" sign, type in 0.05 seconds.
 - Next to "Channel", choose "Visual Cue" square waves should appear in the box below.
 - Set Thresholds: move the two horizontal blue lines anywhere within the stimulus square waves one near the top of the waves, the other near the bottom.
 - Set "New Cursor 2 Position" to "Mark". In the same line, to the right, next to the "+" sign, type in 0.05 seconds.
 - In the line below, to the right of the word "Mark", type in the **first letter** of the name of the events (marks) you wish to analyze. **NOTE**: If any events are unmarked or have a different name within the events you are measuring, the AutoFind function will stop at that point.
 - Set "Repeat Until" to "Document Limits" to analyze every event in a data file (across blocks), or choose "Block Limits" to analyze only those events in a single block (delineated by the thick black lines that indicate where data acquisition was started then stopped).
 - Click the Export button and choose "Export to File", then create a file on the desktop for the text file of reaction times for this experiment.

- Click "Find".
- Copy and paste the reaction times (T2-T1) from the text file on the Desktop into an Excel spreadsheet.
- 8. Once the reaction times in all ten trials have been measured and recorded, open the Journal or use Excel. Use the values to determine the mean reaction time of the subject. Discard the longest and shortest times from the data set, and determine the average of the eight remaining reaction times. Record the mean reaction time for this exercise in Table HP-11-L1.

Exercise 2: Reaction Time and Auditory Cues

Aim: To measure the reaction time of a subject to an auditory cue.

Approximate Time: 15 minutes

Procedure

- 1. Follow the procedure described in the Visual Reaction time experiment with the following modifications:
 - The computer screen should be out of view of the subject (either covered with paper or turned away from the subject)
 - The computer tech should ensure that the data trace is correctly annotated with **<Subject's name> Auditory cues**.
 - To deliver an auditory cue, under LabScribe Preferences, click on "Macros".
 - Choose the sequence called "Visual Auditory Somatosensory".
 - Make sure that the appropriate Macro is showing in the Main acquisition screen at the top. It may be necessary to click "**Run**" within the menu. The macro will present the square waves paired with a computer generated tone.
 - Choose a .wav file from your computer to generate the sound you want the subject to hear. (See notes below for adding .wav files).
- 2. Click on the Record button.
- 3. Instruct the subject that the exercise has begun and that an auditory cue could be heard at any time.
- 4. The stimulator is programmed to deliver ten auditory cues to the subject. The cues are variable but should not be less than one second nor more than ten seconds apart.
- 5. Instruct the subject to press the F1 key to mark the recording as soon as he or she hears the auditory cue.
- 6. After the tenth cue, click Stop to halt recording.
- 7. Select Save in the File menu.

Data Analysis

- 1. Use the same technique explained in Exercise 1 to measure and record the reaction times of the subject presented with auditory cues.
- 2. Enter the mean reaction time for this exercise in Table 1.

Questions

- 1. How does the subject's mean reaction time to visual cues compare to his or her mean reaction time to auditory cues?
- 2. What would cause a longer reaction time to one type of cue as compared to another?
- 3. How do your subject's mean reaction times compare to those of other subjects?
- 4. Do all subjects respond more quickly to the same cue?

Exercise 3: Somatosensory Reaction Time

Aim: To measure the reaction time of a subject to a more complex somatosensory stimulus.

Approximate Time: 20 minutes

Note: Disconnect the subject from the IXTA prior to powering off the device.

Procedure

- 1. Follow the procedure described above with the following modifications:
 - The computer screen should be out of view of the subject (either covered with paper or turned away from the subject).
 - The computer tech should ensure that the data trace is correctly annotated with **<Subject's name> Somatosensory cues**.
 - To deliver a somatosensory cue, under LabScribe Preferences, click on "Sequences". Choose the sequence called "Visual and/or Somatosensory".
 - Attach electrodes to the subject's forearm.
 - Before acquiring data with LabScribe you will need to adjust the amplitude of the stimulus to the point where the subject can just feel the stimulus, but does not experience a "shock".
 - The stimulus is programmed in to the Stimulus toolbar.
 - Click the Record button on LabScribe to run the sequence. While the sequence is running, if needed adjust the current on the stimulator until the subject reports that they can feel the stimulus, but that it is not painful. You are now ready to record reaction times for the somatosensory stimulus.

2. Click the record button to run the sequence.

Data Analysis

- 1. Use the same technique explained in Exercises 1 and 2 to measure and record the reaction times of the subject presented with somatosensory stimuli.
- 2. Enter the mean reaction time for this exercise in Table 1.

| Cue | Mean Reaction Time of Your Subject (ms) | Mean Reaction Time of All Subjects (ms) | Shortest Mean Reaction Time in Class (ms) | Longest Mean Reaction Time in Class (ms) |
|---------------|---|---|---|--|
| Visual | | | | |
| Auditory | | | | |
| Somatosensory | | | | |
| Hypothesis | | | | |

Day 3 - Creating your Own Multisensory Reaction Experimental Procedure

Aim: To design your own multisensory reaction time experiment.

Approximate Time: 60 minutes, including experimental design

Procedure

- 1. Discuss with your group members and design your own multisensory reaction experiment.
- 2. Now that you've completed some basic experiments, brainstorm factors that could influence reaction times to unimodal as compared to multimodal stimuli. Then, devise hypotheses and experimental protocols to test at least one of these factors.Include an educated guess as to where in the nervous system this factor exerts its effect.
- 3. Create one (or more if necessary) hypotheses for your multisensory experiment how will each factor affect reaction time? Produce a sentence or two with an educated guess as to where in the nervous system you think these factors are exerting their effects and how (changing synaptic interactions in some way?).
- 4. Create an experimental protocol a set of instructions that is detailed enough so that anyone else who reads your protocol could perform your experiment. In your proposal, be sure to indicate:
 - How many experimental subjects will you use? Since the class is small, you will probably need to recruit all your fellow students plus the TAs as subjects.
 - How many replicates will you perform on each subject?
 - What are your control experiments?

- What equipment do you need?
- How will you graph and analyze the data?
- 5. Perform your experiment and present your findings as required by your professor.

Suggestions for multisensory experiments:

- Experiment with the effect of predictable cues (e.g., every 5 seconds) vs. cues at randomly spaced intervals. Do you observe any learning effects? In other words, do your subjects' reaction times shorten with repeated trials, especially if the cues are predictably spaced?
- Is there a difference between reaction time to visual as compared to auditory and somatosensory stimuli? In other words, does sensory modality affect reaction time?
- How does the reaction time to bimodal stimuli compare with unimodal or even trimodal stimuli? Is there a particular combination of sensory modalities that produces the shortest/longest reaction time (visual-auditory vs. visual-somatosensory for example)?

Creating your own Sequences and Settings Files in LabScribe

You can perform your experiments using the Settings file already created in LabScribe, but you may want to design your own set of sequences for the presentation of stimuli. If so, you will need to create at least one Settings file in LabScribe.

Creating Settings Files in LabScribe

Once you have chosen all the parameters for your settings file using the LabScribe-Preferences menus (see below), Click "Save As", choose your file location (on the Desktop or in a folder), then be sure to choose the settings file extension (.iwxset) from the drop down menu at the bottom of the File screen. When you want to use your settings file, you MUST launch LabScribe from your settings file.

Creating Your Own Sequences in LabScribe

The easiest way to do this is to add a new Sequence to the settings file "SomatosensoryReflexes", and then rename and resave the settings file. The SomatosensoryReflexes settings file already has the Stimulator settings needed to control the stimulator for your experiments. :

- 1. Go to Edit Preferences Macros.
- 2. Select "New", then type a new name in the box.
- 3. The "Wait" button sets the delay before a stimulus is presented.
 - Select "sec", then the number of seconds you want for a delay, then click "Wait". The wait time will appear in the Sequence window.
- 4. Click on "Fire", then the "Add State to Sequence" button this will signal the stimulator to produce a pulse, which will show up on the recording screen as a square wave.
- 5. If you want to add a computer generated tone to your Sequence, scroll down the list of states on the left side of the Sequence menu to find **Sound.wav** files.

- Click on any of the Sound files shown (they will all play the same tone), then "Add State to Sequence".
- 6. Continue adding "Wait ______ sec" states, "Fire" commands and Sound.wav states to your sequence. When you are finished click "OK", then **be sure to save the** <u>Settings</u> file again.
- 7. If you want to change the sound the computer makes, several different sounds are available as .wav files on your computer or your can look online and save the .wav files to your LabScribe folder.

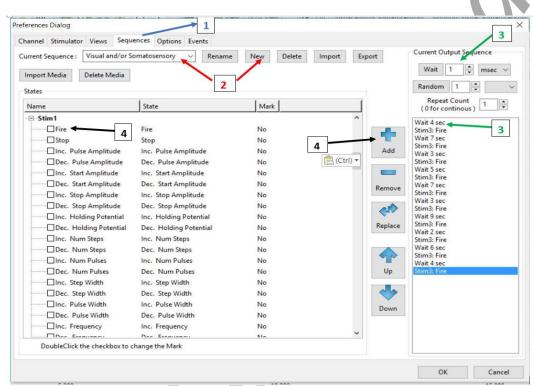


Figure HP-11-L5: Creating Sequences.

| hannel Stimulator Views Sequ | ences Options Events | | | |
|------------------------------------|------------------------|---------------------|---------|---------------------------|
| Current Sequence : Auditory Visual | Somatosensory ~ Rename | New Delete Import E | xport | Current Output Sequence |
| Import Media Delete Media | | | | Wait 1 🔹 msec ∨ |
| States | | | | Random 1 🔹 🗸 |
| | 1 | lu i | i l | Repeat Count |
| Name | State | Mark | | (0 for continous) |
| Start Recording | Start Recording | No | | Wait 4 sec |
| Stop Recording | Stop Recording | No | - | Sound1.wav 4 5 |
| Update | Update | No | | Stim3: Fire Wait 3 sec |
| Media | 5 | | Add | Sound2.wav |
| Sound1.wav | Random gif | Yes | | Wait 5 sec Stim3: Fire |
| Sound2.wav | Random png | Yes | | Wait 7 sec |
| Sound3.wav | Random jpg | Yes | Remove | Sound2.wav |
| - E Colour | | | | Stim3: Fire Wait 3 sec |
| - E Dig. Output | | | (| Stim3: Fire |
| E Stim1 | | | 4. | Sound3.wav |
| Fire | Fire | No | Replace | Wait 9 sec Stim3: Fire |
| Stop | Stop | No | | Sound3.wav |
| Inc. Pulse Amplitude | Inc. Pulse Amplitude | No | | Wait 2 sec |
| Dec. Pulse Amplitude | Dec. Pulse Amplitude | No | | Wait 6 sec Sound1.way |
| Inc. Start Amplitude | Inc. Start Amplitude | No | Up | Stim3: Fire |
| Dec. Start Amplitude | Dec. Start Amplitude | No | | Wait 4 sec Stim3: Fire |
| Inc. Stop Amplitude | Inc. Stop Amplitude | No | | Stim3: Fire |
| Dec. Stop Amplitude | Dec. Stop Amplitude | No | David | |
| Inc. Holding Potential | Inc. Holding Potential | No | Down | |
| Dec. Holding Potential | Dec. Holding Potential | No | | |
| | Inc. Num Stone | No | | |

Figure HP-11-L6: Adding sound.wav files to the Sequence.

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