

Protocol on Startle Response Experiments

Table of contents

1. [Introduction](#)
2. [Experimental Design](#)
 1. [Background](#)
 2. [Setup](#)
 3. [Procedure](#)
3. [Software](#)
 1. [Recording](#)
 2. [Analysis](#)
4. [Troubleshooting](#)

Introduction

This is a guide for the startle response experiments. It will cover the setup and procedure of the experiment as well as the software used for recording and analysis. This repository also contains the example outputs from three separate recording sessions for reference.

Experimental Design

Background

Acoustic startle response (ASR) experiments are a useful tool in examining the auditory processing of mammals under various conditions and variations. Given a sudden, unexpected auditory stimulus, such as a loud noise within previous white background noise, muscles involuntarily contract, causing a measurable movement. There are a multitude of conditions that can impact the strength of this reflex, but for purposeful modulation, pre-pulse inhibition (PPI) is used to test for changes in this response. If a lesser sound which is not strong enough to in itself facilitate a startle response is played before the actual startle, one can see a decrease in the response. This "warning sound" can also be replaced with a gap in the constant background noise, and length and offset from the startle can be modulated. This allows for a very flexible experiment that does not require prior training of the animals. This is for example crucial when testing for early hearing loss in mouse models with ataxia 13, where any experiment that would require training would be too time intensive to be viable.

Setup

The setup consists of five parts:

- A double-layered sound-proof box with a pressure sensor
- A cage which holds the animal
- A box connecting the recording computer and the pressure sensor
- A computer with the recording software

- A computer for running the analysis software (and a USB-stick for data transfer)

The sound-proof box consists of two layers, an inner one containing the pressure sensor and cage, secured by a rotating lock, and an outer one which can be fastened using two latches on the left side (be careful not to hit the emergency power switch next to the latches, you don't want to be the first to do it).

The pressure sensor inside the chamber has pins inside it that hold the cage. Be careful not to press down onto the sensor, especially when putting down the cage, otherwise it could impact its calibration.

A box controlling the hardware is connected to the computer. It is below the chamber and has to be turned on before every experiment, with the switch being on the right side on the back of the device.

The connected computer has the "Startle Response" program installed and is used to record and store the data.

Due to the age of the recording computer, it is recommended to use a different computer for the analysis. This can be any other computer or laptop, though the more capable and up to date, the faster the analysis will be. A USB-stick will be needed to transfer the data between the two devices.

Procedure

To place the mouse into the cage for recording, take the cage out of the chamber, grabbing it by the tray, put it down onto a desk and open the lid. To get the mouse into the cage, carefully but without too much hesitation, grab it around the upper third of its tail, lift it into the cage and close the lid. Be sure to neither grab the tail too far to the base, as this would allow the mouse to use enough muscles to turn around, or too far towards the tip, as that could hurt the animal and also make placing it into the cage more difficult. Placing the cage inside the enclosure before moving the mice is also advisable to minimize risk of escaping. Now, put the cage with the animal back onto the sensor without pushing down on it. Close both chamber doors and secure the outer one using the two latches.

Start a timer for ten minutes. This is the habituation period for the mouse and is crucial to ensure that it is not affected by anything that has happened before.

Make sure that the box is turned on, indicated by a green light on its front. Do the same for the recording computer and open the Startle Response software, logging in without a username or password. Assuming everything is set up correctly, you can now begin the recording. Further details on using the software will be covered later.

Once the recording is done, open the chamber doors and remove the cage (this time being extra careful to get a good grip on the tray). Carefully move the mouse back into its container by placing the box inside and opening it, waiting for it to come out on its own. After you are done for the day, be sure to clean the tray of any excrements left behind.

You can now copy the recorded data onto a USB-stick and to the device used for analysis, which you can now begin.

Software

The software used consists of the TSE program for recording, a notebook for a preliminary cutting and analysis of the data and another for further evaluation such as significance tests and trend analysis.

Recording

The recoding software, although having a login screen, does not require a password. Once inside, there are several tabs to configure and conduct experiments.

These are the Help, Experiment, Analysis, Archive, System Test, Parameters and Exit tabs.

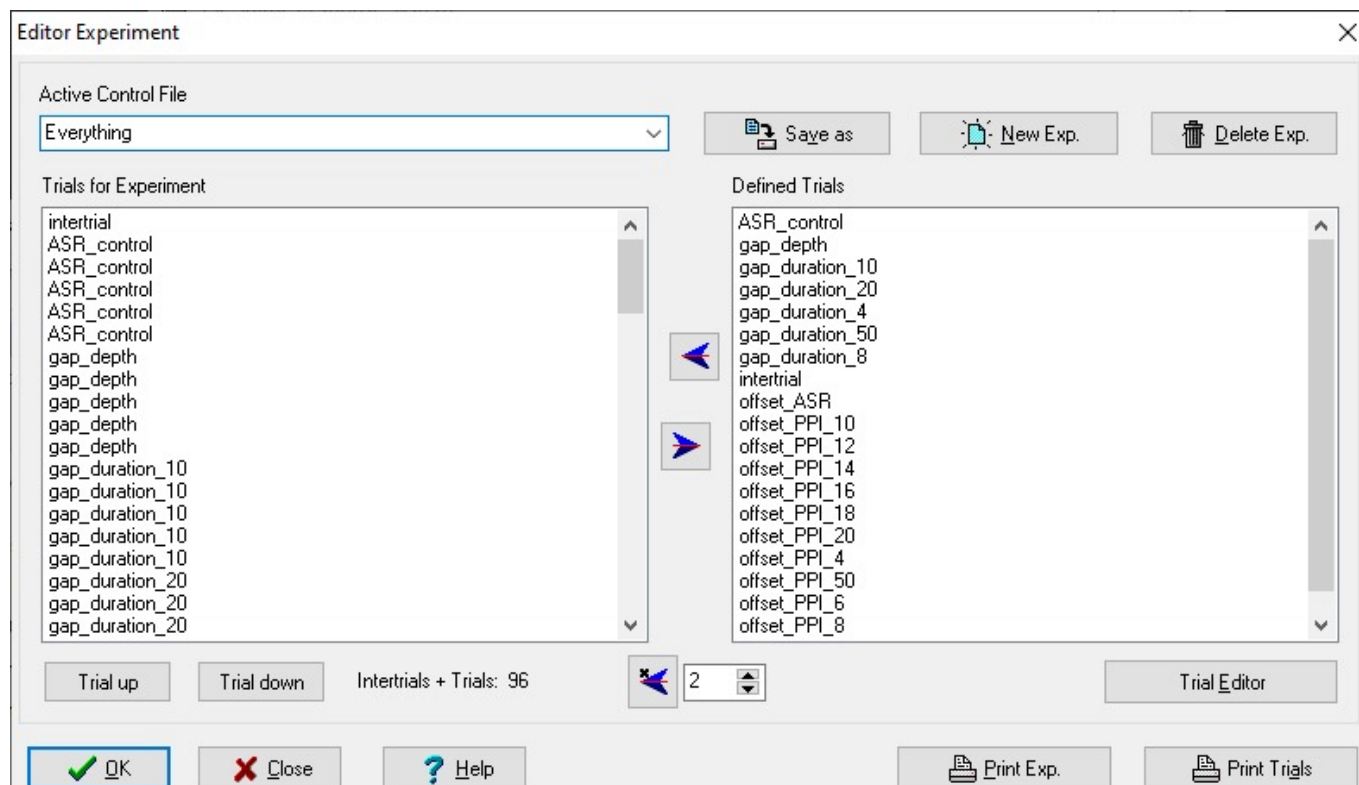
- Help: link to guides
- Experiment: configure trials and experiments and start measurements
- Analysis: select and export data
- Archive: configure location of saved data
- System Test: check system functions
- Parameters: configure export parameters
- Exit: exit the software

The most commonly used tabs are Experiment, Analysis and Archive.

Experiment Tab

Here you can configure and run experiments. Under **Experiment**, you can find the setup for loading and executing an experiment file containing the trials, both of which can be configured in the **Editor**. To begin an experiment, select the right experiment file, whether or not and in what way you want the trials to be randomized, applying the randomization if applicable. Then, tick **Box 1** and input any relevant information. Note that the field **Animal No.** cannot start with a 0 like some animal numbers do, so it is recommended to use the **Group** or other fields if you wish to save the animal's full number. The **Comment** is useful for saving the date of the recording.

The **Editor** allows you to configure the trials and the experiments they make up.



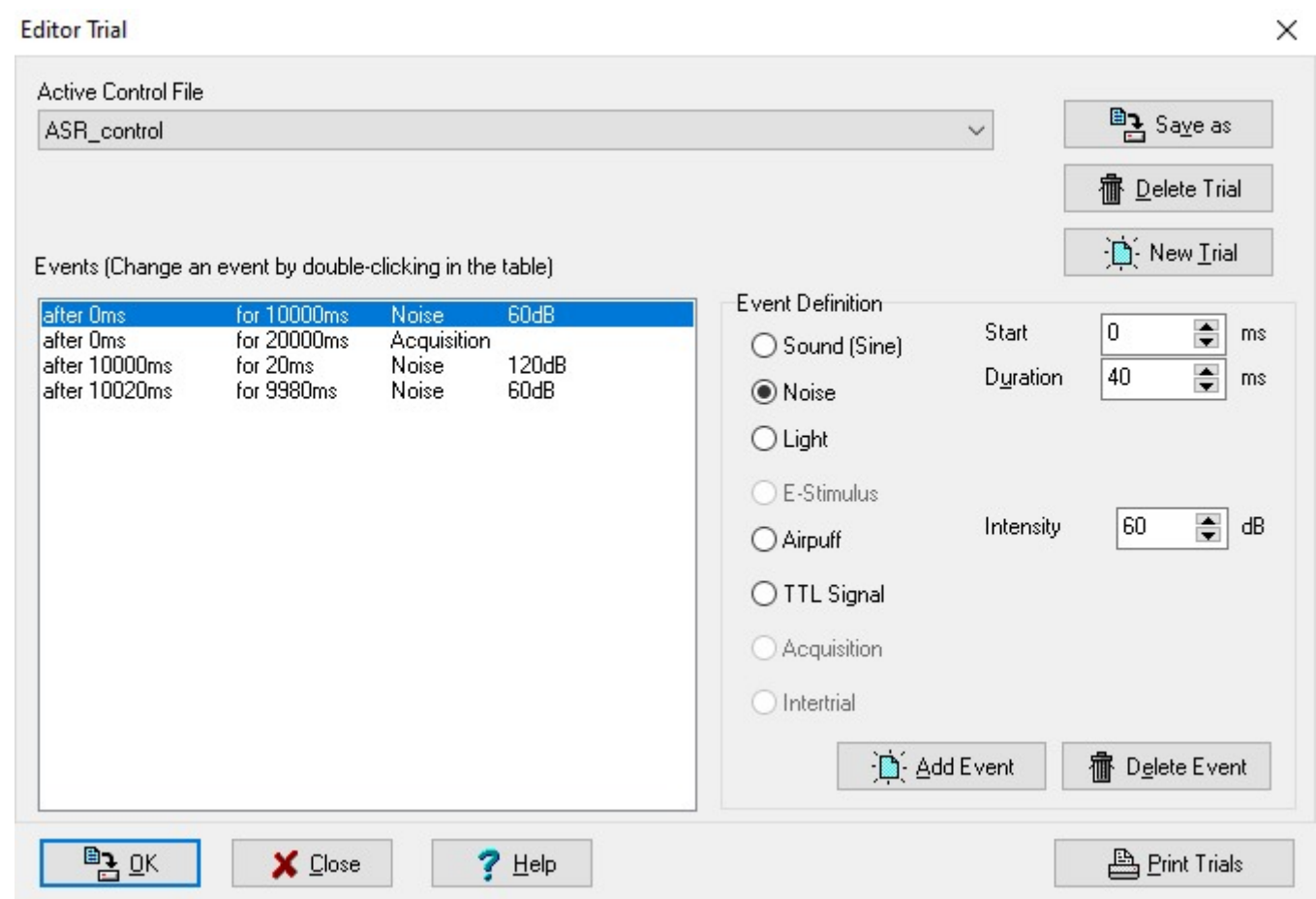


Fig. 1: Experiment (top) and Trial Editor (bottom). Trials can be created and edited in the trial editor to then be moved into experiments. One experiment can hold multiple instances of the same trial. The trial shown goes on for 20 seconds, playing a 60dB background noise for the first 10 seconds, then playing the 120dB startle noise for 20ms before switching back to the background noise for the remainder of the 20 seconds.

An experiment always contains one "intertrial" at the beginning, a short, empty trial needed for the software, and five instances of each trial. A trial, accessible via the Trial Editor, is usually 20 seconds long and begins with the command to begin recording immediately for the duration of the trial, so 20000ms of Acquisition starting at 0ms. The background noise is also started from the beginning and usually goes on for around 10 seconds before trial specific events happen, though this time too can vary. Note that for software reasons, the noise has to be before the acquisition, even though both start at the same time.

Under Experiment, you can now start the recording. Select the experiment you want to run and whether or not to shuffle the trials, then tick Box 1, configure the animal and trial parameters accordingly and run the experiment.

Trial Monitor

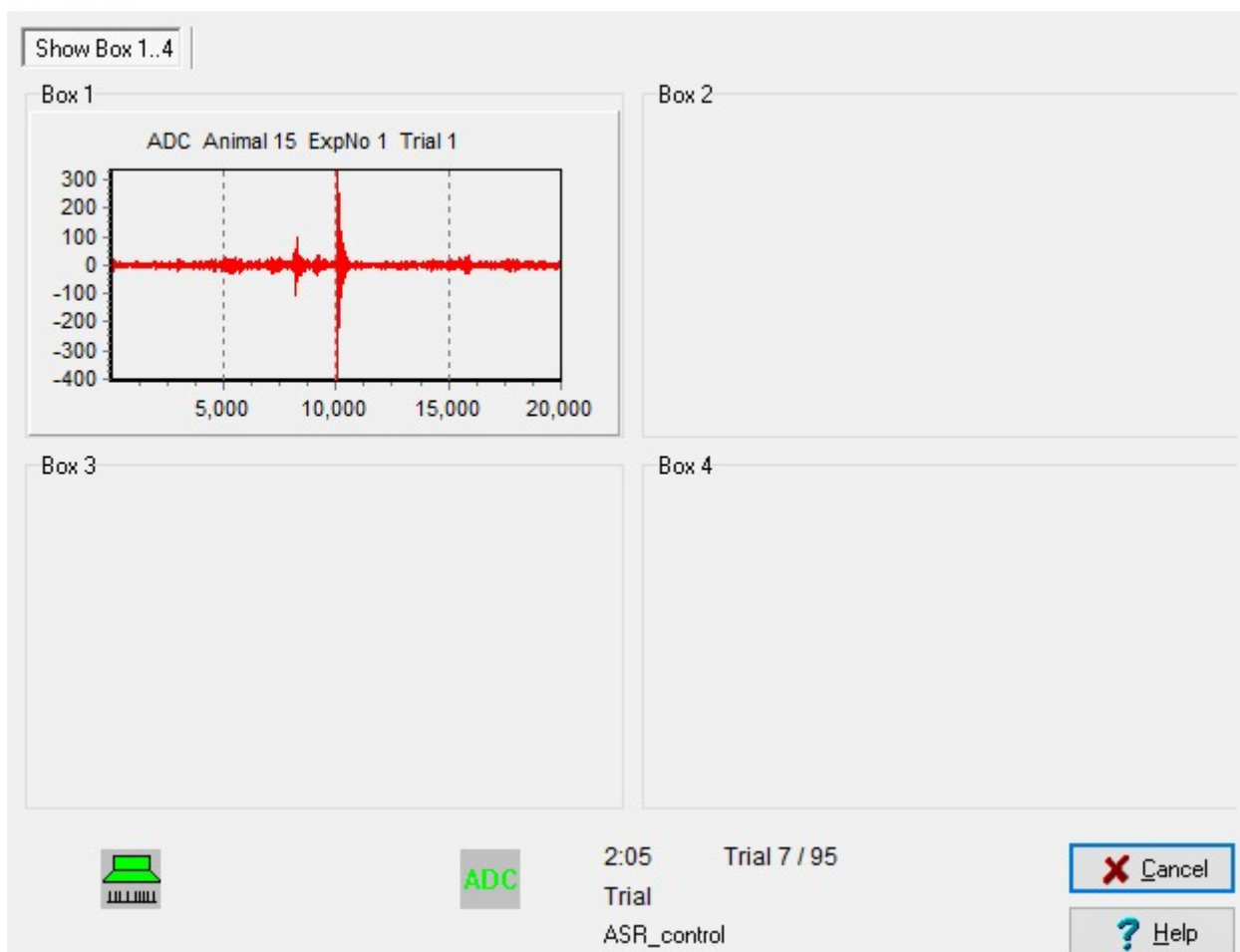


Fig. 2:

Pop-up during recording. The image in Box 1 (top) shows the current measurement, the green icons (bottom left) indicate that both recording and stimulation are working. Information on the progress of trials is also provided (bottom right).

If everything is working, you should now see a live visualization of the recording as well as indicators for the noise and recording. If you are running a lot of trials at once, it may be advisable to roughly calculate how much time everything will take (usually 20 seconds per trial) and set a timer accordingly. Besides ensuring that both the sound and recording is running, you do not necessarily need to be present for the duration of the experiment. For example, with 19 different trials with 5 repetitions at 20 seconds each, this would take a little over half an hour per animal, or 45 minutes including the 10 minute habituation period and the time needed to transfer the animal. Knowing this can be useful in order to plan recordings ahead of time.

Analysis Tab

The analysis tab is where you select and export your data. **Data Selection** shows all recorded data in the top field, by default sorted in the order it was recorded in. Either double-clicking on one of the lines or using the **ALL** or **Filter** options copies a recording into the bottom field. When exporting data, only these recordings will be exported.

Data Selection ✕

Available Data Records D:\2025\15

Animal	No.	Group	Trial	Exp.	Date	Comment
15	1		1	1	4/14/2025	14.04.2025
15	2	15	1	1	4/14/2025	14.04.2025
15	3	15	1	1	4/14/2025	14.04.2025
1	4	15	1	1	4/15/2025	15.04.2025
1	5	15	1	1	4/16/2025	15.04.2025
15	6	15	1	1	4/16/2025	16.04.2025

Selected Data Records (1)

Animal	No.	Group	Trial	Exp.	Date	Comment
15	6	15	1	1	4/16/2025	16.04.2025

Sort

☐ Anim.
☒ No.
☐ Group
☐ Trial
☐ Exp.
☐ Date
☐ Comm.

ALL

None

Filter

No Filter.

Fig. 3:

Data Selection screen. Recording number 6 has been selected and is ready to be exported.

Export Analog Data exports the files in the way specified in **Parameters** -> **Export Parameters**. By default, this should be the CSV files we need for the analysis. Should you also wish to save Excel files, you can change the export parameters accordingly. Although more pleasant to look at, these are substantially slower to process and are thus not suitable for analysis.

Archive Tab

Data Directory defines the directory all recording data is saved to, while the **Reindexing** option allows you to update the **Data Selection** whenever you change directory. The **Data Selection** window also has this option.

Recorded data is saved onto the **Data (D:)** drive. Make sure to make a folder for your recordings and set it as the new data directory before starting your line of experiments.

Should you ever wish to back up your experiments and trials, they can be found in the TSE folder on drive C.

Analysis

In order to familiarize yourself with the analysis software, it would be best to go through both notebooks once to know what outputs to expect.

To now use your own data, you first have to set up the input folder. Create a new folder in the same one as the notebooks, for example named after the year of the recording, the operator, etc. Inside this folder, create new folders, one for each animal, and name them accordingly. Within these folders, place all CSV files that contain recordings from the respective animal. Depending on how you did the recordings, this can be just one file. The animal folder and CSV file names will be used internally to label slices, but are otherwise not important for anything other than debugging. Lastly, in the "Peakfinder" notebook, change the variable

`input_dir` of the second codeblock to the name you gave to the folder containing the animal folders.

There are two different notebooks used for analysis, the first one, "Peakfinder", taking the raw data and turning it into excel files containing the reaction time, the time until the peak, the difference between the former two and the strength of the peak, all averaged across the different experiments. The second notebook, "Analyzer", takes these files and runs several tests on them, examining changes across animals and experiments. Especially the latter can and should be edited by you to fit your specific needs.

Additionally, there are two dictionaries you need to adjust. The `startle_time` dictionary links the trials to the times in which the startle is played within them. You will need to insert your own, should they not already be included. If there be multiple startles in one trial, insert them as a list.

The second dictionary is `sex`, wherein you need to link the animals by number to their sex. Note that the animal numbers are shortened to their final digits, so there may be overlap with older entries when you enter your own.

Other settings you may want to adjust are `output_dir`, which defines where you want the output files to go, `verbose`, which when set to `False`, mutes all output except for error messages, and `excel_output`, which when `False` makes the program output CSV files instead of excel files. CSV files are faster to read by other programs, however they are less readable to humans due to their lack of neat looking columns and rows. With the output files containing only a small low amount of data, this should not make a noticeable difference. Further details on the code are included in the notebooks markdown sections and comments.

The notebook for trend analysis is currently incomplete and will be updated at a later time.

Troubleshooting

Oh my god it escaped!

Don't panic. The mouse may be faster and more agile than you, but (hopefully) it is not smarter. Stay calm, and attempt to scoop it up again as done before. It will likely move towards something it perceives as cover - a pile of cables, a dark corner, a bag or jacket, etc. Carefully check all of these spots until you find it, and avoid loud noises or rash movements to not scare it further.

Recording Software Error

Call customer support.

Analysis Software Error

Usually, any error the notebooks put out is well documented. Check if the files look ok (expected file size, normal column organization, no corruption etc.), whether or not the startle times are set correctly, and if in applicable, look up the error code. If the code block handling the creation of the library which will be turned into the output files says that it encountered a lot of bad peaks, examine the respective recording, either directly in the raw file or via the `Debugging` code block at the very end of the first notebook. If the peak really is too early, late or non-existent, proceed as is fit for your use case, adjusting whether or not to discard them in the configuration block at the beginning of the notebook. If you suspect that something is wrong with the code or need help understanding a part of it, feel free to reach out to me directly or via another group member. If you happen to have a fix for it and know your way around GitHub, you could even submit a pull request.