Ear tag system for recording head-twitch responses – Parts list and setup manual

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The system is built upon the equipment described in de la Fuente Revenga et al., *J. Neurosci. Methods*, 2020, with several new features and modifications:

- Scaled up to run four mice simultaneously
- A different magnet for the ear tags, after testing several types
- Minor modifications to MATLAB code for outputting results
- New additions to MATLAB code for controlling the DAQ
- Instructions for a high-speed video camera setup
- New additions to MATLAB code for controlling the camera

Several example recordings are included.

If you build and use this system, please consider citing:

- Shao LX et al., *Neuron*, 2021
  (Our lab's work on head-twitch response, using high-speed video camera)

- de la Fuente Revenga M et al., *J. Neurosci. Methods*, 2020
  (First description of the ear tag method)

- Halberstadt AL and Geyer MA, *Psychopharmacology*, 2013
 (First description of a magnet-based method)

Table of Contents

Overview

This document provides instructions for creating a setup to automatically quantify the head twitch response in mice via a magnetic ear tag. When administered psilocybin, LSD, DOI, or most other serotonergic hallucinogens, mice exhibit a characteristic head twitch caused primarily by the binding of the drug to the serotonin 5-HT$_{2A}$ receptor.

The conventional way to quantify these head twitches was to record a video of the mouse, which could then be visually analyzed by a trained observer. This method is tedious and time-consuming. A more streamlined method was described in 2013 based on a magnetometer (Halberstadt AL and Geyer MA, *Psychopharmacology*, 2013). This approach relies on the principle of electromagnetic induction. From Faraday's Law, a changing magnetic flux (magnetic field passing through an area) in a closed connecting loop of coil induces a momentary current within the coil. One way to change the magnetic flux to generate the induced current is by moving a magnet within the loop of coil. The induced electromotive force—i.e., the voltage source—responsible for the induced current to flow can then be quantified using a voltmeter. When a magnetic ear tag is attached to a mouse's head and the mouse twitches its head upon administration of the drug, the rapid shaking of the magnet leads to a characteristic voltage spike that can be identified by a computer software. To minimize the labor associated with surgically implanting a magnet, an improved method based on a magnetic ear tag was described in 2020 (de la Fuente Revenga et al., *J. Neurosci. Methods*, 2020).

This document will describe how to build a magnetic ear tag system for recording the head-twitch response. The system is based on the de la Fuente Revenga et al. paper, with several modifications and additions. A completed setup looks like this:
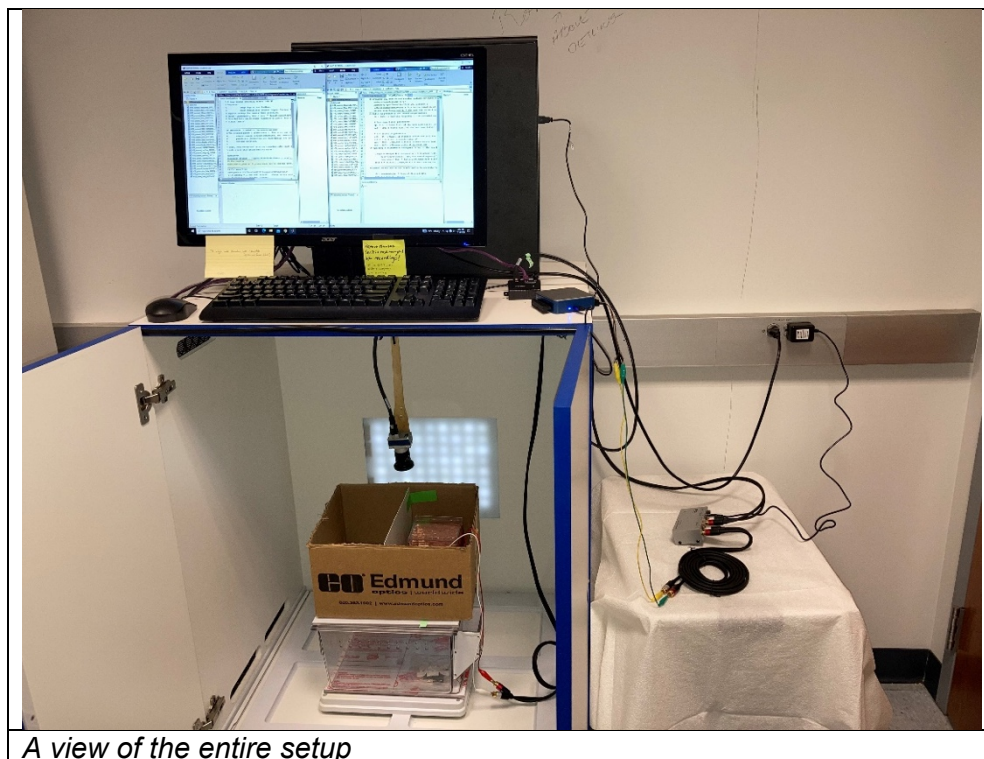
A view of the entire setup
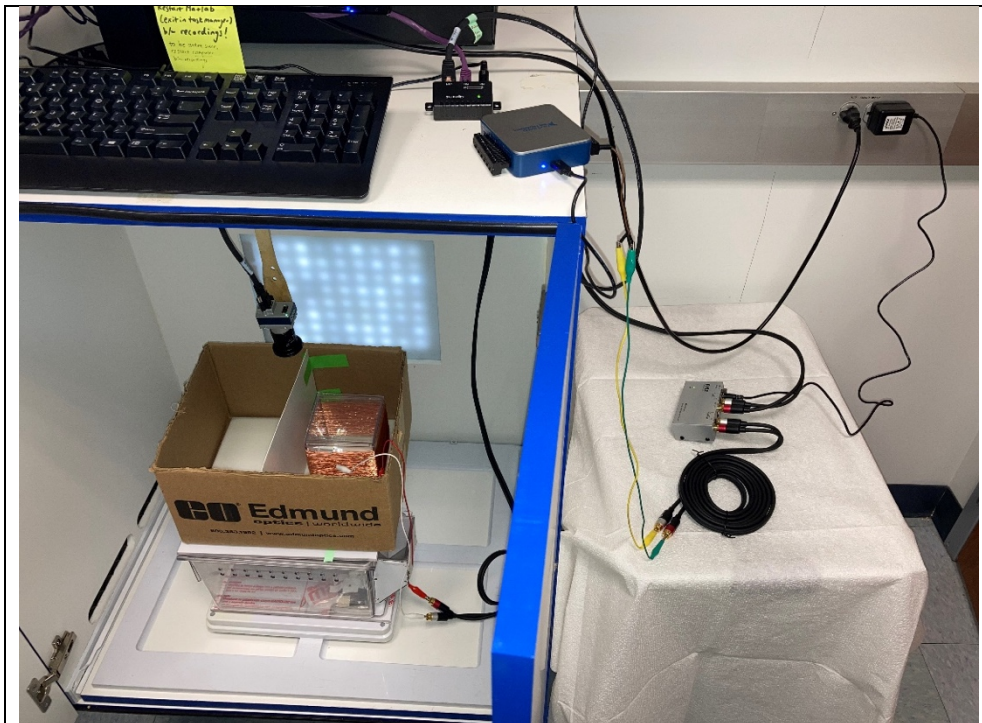
A closeup view of the box & coil attached to the phono preamp and DAQ. The wires connecting the box to the phono preamp and the wire connecting the camera to the power injector go through a hole in the top right of the crate.
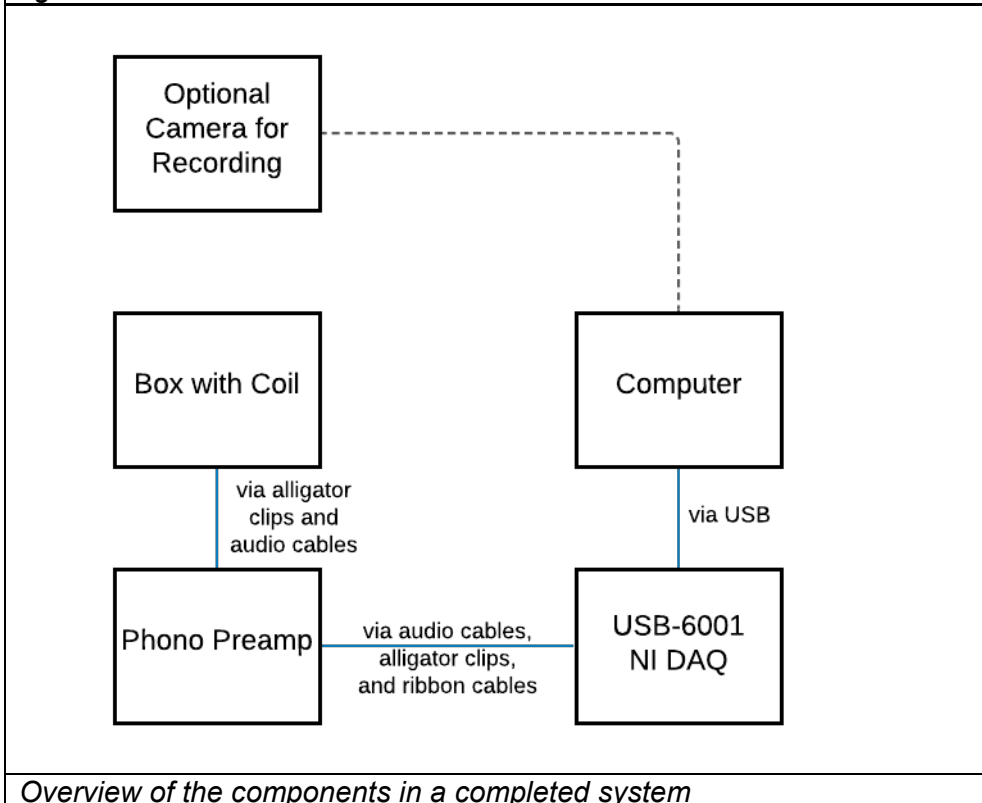
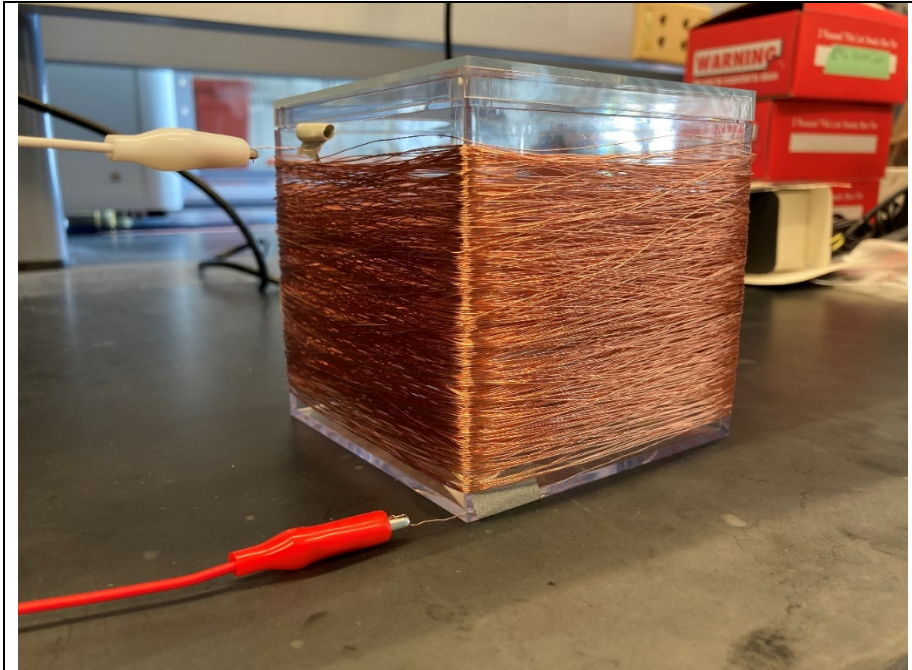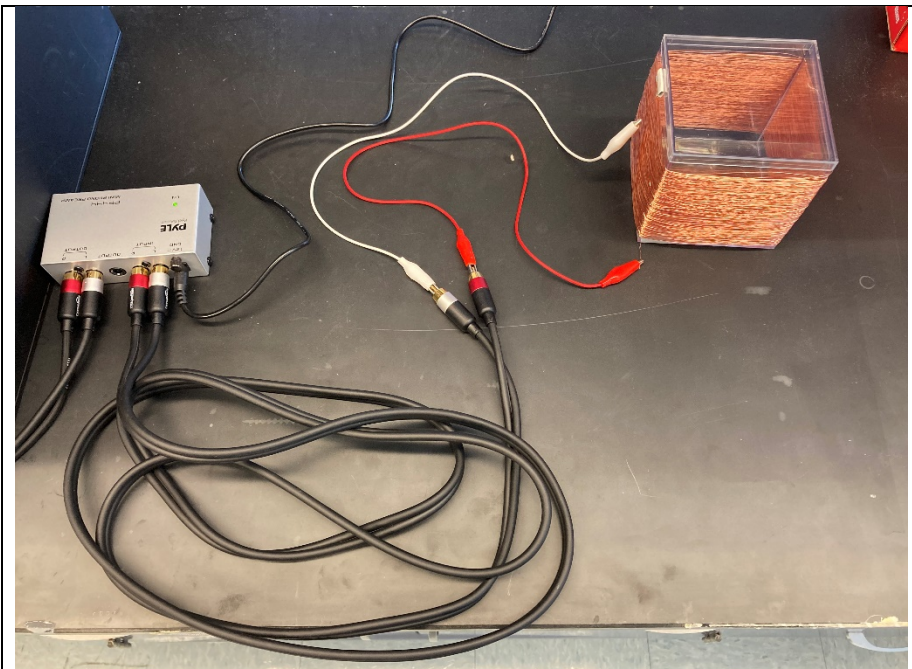*Overview of the components in a completed system*

Section 1: Box, Coil, and Phono Preamp

The mouse with the magnetic ear tag will roam around in the plastic box wrapped around with coil. The preamp amplifies the signal.

Closeup of the plastic box wrapped with coil, the ends of which are attached to alligator clips

Connecting the wires around the plastic box to the phono amp input using audio cables

Parts List (to make 1 box)
- Clear plastic box cube (4'' x 4'' x 4'')
    - https://www.amazon.com/Clear-Plastic-Box-Cube-Square/dp/B0883FNSFJ/
- 30 AWG enameled copper wire
    - https://www.amazon.com/BNTECHGO-AWG-Magnet-Wire-Transformers/dp/B00UWCXRK6
- Phono preamp (PP444, Pyle)
    - https://www.amazon.com/Pyle-Phono-Turntable-Preamp-Preamplifier/dp/B004HJ1TTQ
- Two test lead wires with alligator clip on each end
    - https://www.amazon.com/WGGE-WG-026-Pieces-Colors-Alligator/dp/B06XX25HFX
- Set of 2-male to 2-male RCA audio stereo cables, 4 feet long
    - https://www.amazon.com/AmazonBasics-2-Male-RCA-Audio-Cable/dp/B01D5H8P0G

Assembly
1. Wrap all ~820 feet of the copper wire around the clear plastic box, ensuring that both ends of the wire are easily accessible.
2. Connect the alligator clips to the two ends of the wire. Attach the alligator clips on the other side of the wires to the ends of the audio cables.
3. Plug the audio cables into the phono preamp, which should be plugged into the wall socket.

Section 2: Data Acquisition Device (DAQ)

A data acquisition device (DAQ) is used to acquire the voltage signal with a computer.
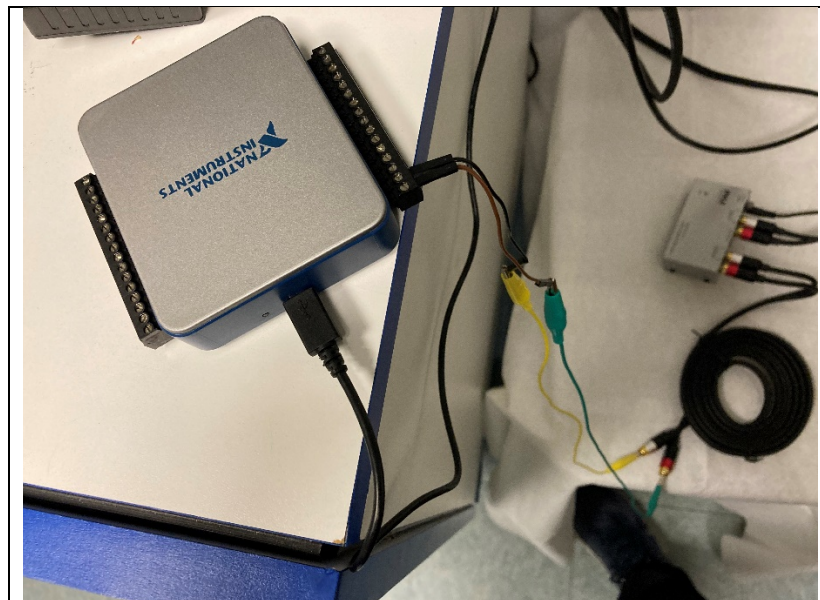
DAQ attached via ribbon cables and alligator cables to the phono preamp. A USB cable connects to the computer.

Closeup of the DAQ with ribbon cables

Parts List
- National Instrument USB-6001 DAQ (20 kS/s sampling rate, 4 differential analog-input channels)
  - https://www.ni.com/en-us/shop/hardware/products/multifunction-io-device.html?modelId=124894
- Two test lead wires with alligator clip on each end
  - https://www.amazon.com/WGGE-WG-026-Pieces-Colors-Alligator/dp/B06XX25HFX
- Set of 2-male to 2-male RCA audio stereo cables, 4 feet long
  - https://www.amazon.com/AmazonBasics-2-Male-RCA-Audio-Cable/dp/B01D5H8P0G
- Male-to-male ribbon cables
  - https://www.amazon.com/Elegoo-EL-CP-004-Multicolored-Breadboard-arduino/dp/B01EV70C78/

Assembly
1. Plug the USB cable from the DAQ into the computer.
2. Connect the audio cables and alligator cables to the ribbon cables.
3. Use a flathead screwdriver to insert the ends of the ribbon cable into the analog input (AI) channels. In the image above, the ribbon cables are attached to the analog input channel 0 (in the + and – ports).
4. Use the MATLAB script described in Section 5 to allow the DAQ to record voltage signals.

Notes
- If the voltage signal from the DAQ appears to stay the same and does not have any peaks, make sure that the phono amp is plugged in and attached to the DAQ. The voltage signal from a head twitch is not detectable without the phono preamp.
- While other DAQs may work, the NI DAQ is especially helpful because it can be directly controlled via MATLAB.

Section 3: Ear-tagging the Mouse

A magnet is superglued onto an ear tag, which is then attached to one of the ears of each mouse.
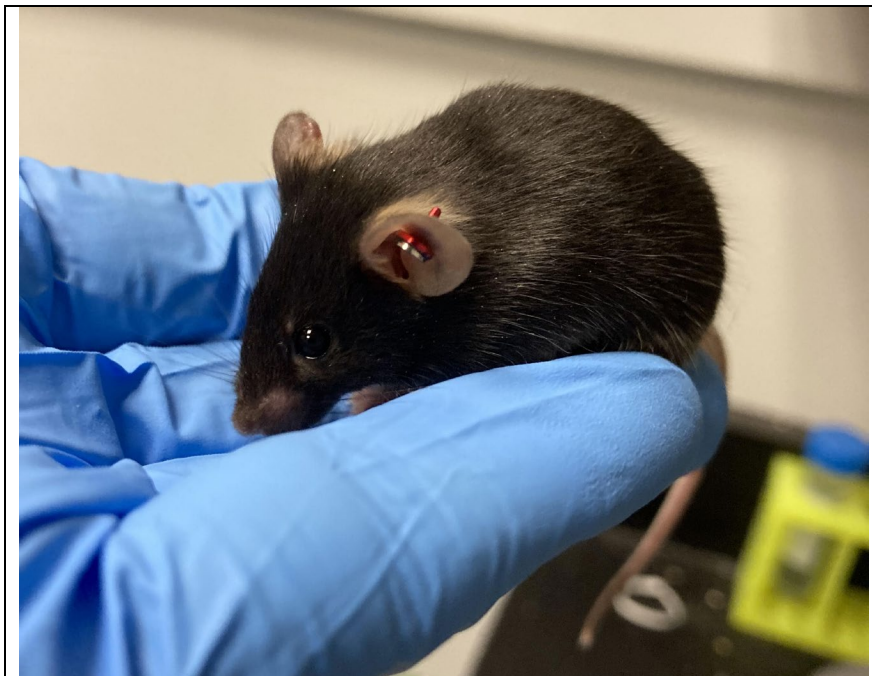
Ear tag with magnet attached to a mouse's ear

Parts List
- La Pias Aluminum Ear Tags (Item 56780-100 from Stoelting)
  - https://stoeltingco.com/Neuroscience/Ear-Tags--Mouse~9785
- Ear Tag Applicator (Item 56791 from Stoelting)
  - https://stoeltingco.com/Neuroscience/Ear-Tags--Mouse~9785
- Neodymium Magnets (Product D1005-10 from SuperMagnetMan; N45 magnet with diameter 3mm and thickness 0.5mm)
  - https://supermagnetman.com/products/d1005-10?_pos=1&_sid=55a9637bc&_ss=r
- Superglue (Ultra Gel Control, #1739050, Loctite)
  - https://www.amazon.com/Loctite-Control-4-Gram-Bottle-1739050/dp/B00ELV2D0Y
- Nitrocellulose Marker
  - https://www.amazon.com/Colortone-Touch-up-Marker-Opaque-Lacquer/dp/B0731FS9YS
- Plastic Forceps
  - https://www.finescience.com/en-US/Products/Forceps-Hemostats/Standard-Forceps/Plastic-Forceps/11700-00

Assembly
1. Use the superglue to attach the male end of the ear tag to the magnet. Coat the magnet with the nitrocellulose marker (in order to prevent irritation to the mouse's ear caused by the magnet). Wait a few hours to dry.

2. When it is time to ear tag the mouse, place the female end of the ear tag onto the red slot of the applicator and the male end of the ear tag with the magnet on the other slot. Briefly place the mouse under anesthesia (via isoflurane) and apply force to the handles of the applicator to position the ear tag in the middle part of the mouse's ear (avoiding the cartilage) such that the magnet faces inward.
3. Allow around 2 days for the animal to rest before experimenting (to minimize excessive ear grooming).

Notes
- The neodymium N45 magnet (product D1005-10 from Super Magnet Man with a diameter of 3mm and a thickness of 0.5mm) was selected based on trying out several types of magnets. Initial trials used the neodymium N50 magnet (product D1007 from Super Magnet Man with a diameter of 3 mm and a thickness of 1 mm), but when magnets were attached to both ears of one mouse, the two ears stuck together above its head. Furthermore, when the magnet was attached to one ear of each mouse within group housing, ears of two mice would get attached, preventing the animals from moving independently and irritating their ears. In these cases, sometimes the mice would pull each other's ear tags off completely. For the N45 magnet, the voltage signal was comparable to the thicker magnets and the mice rarely got magnetized to one another. When they did, the animals could separate themselves.
- When mice are group-housed, ensure that the same pole on each magnet faces the outward direction to prevent the mice from getting magnetized to each other.
- After supergluing the magnet to the ear tag, wait a few hours before applying the ear tag on the mice. If the glue is not dry enough, the magnet might get stuck to the magnetic applicator instead of the ear tag. Furthermore, after applying the ear tag to the mouse, use plastic forceps (instead of magnetic ones) to separate the magnet on the ear tag from the applicator.
- If the mouse is not given enough time to rest before experimenting, it might excessively groom its ear, leading to potential false positives.

Section 4: Optional Video Recording Setup

A camera can be helpful to verify that the magnetic ear tag setup works as designed. A camera able to record a high frame rate (around 180 fps) is necessary to visualize the high-frequency head twitches. We used a Dalsa Genie Nano GigE PoE camera connected to a power injector, attached via ethernet port to a Lenovo ThinkStation P620 which has the Aquantia AQC107 NBase-T/IEEE 802.3bz Ethernet Controller. We control this camera via a MATLAB script.

Two angles of the box and camera in crate with backlight turned on
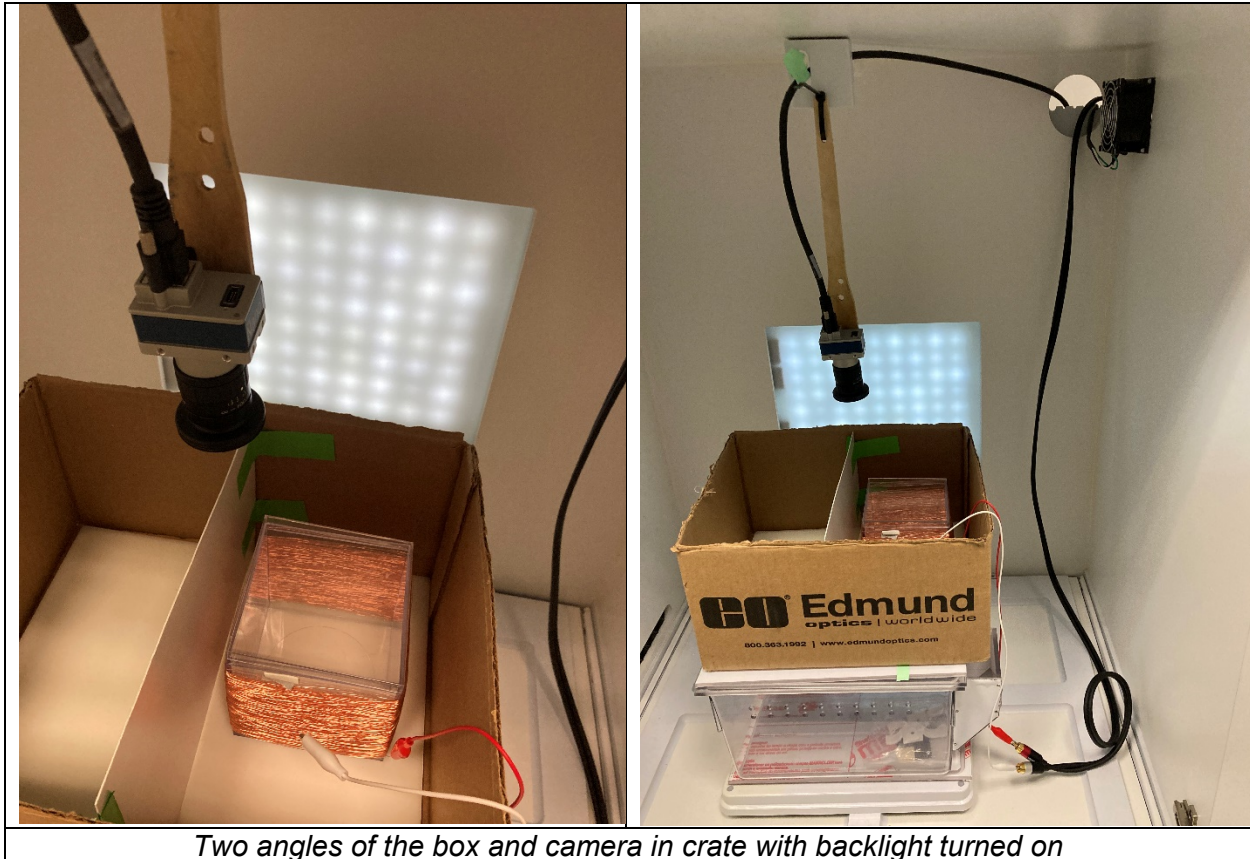
Parts List
- Dalsa Genie Nano-M1280 GigE PoE Camera with camera cable (1/2" sensor, 1280x1024 pixels, 213 fps in burst mode, monochrome)
    - https://www.edmundoptics.com/p/m1280-12-monochrome-dalsa-genie-nano-poe-camera/4048/
- Lenovo ThinkStation P620
- PoE injector, with ethernet cable (single port, 15W at 48V, 0.3 A, power always-on)
    - https://www.amazon.com/Single-Port-Injector-Power-Ethernet/dp/B07KFKYF79
- Chamber for video recording (We used the Open Field Test Package w/NIR Lighting for Rat)
    - https://www.med-associates.com/product/rat-open-field-test-package-with-nir-lighting/
- Sapera CamExpert image acquisition software

Assembly
1. Connect the camera to the PoE injector and the PoE to the computer. A popup should appear on the monitor indicating that the camera has connected.
2. Use the MATLAB script described in Section 5 to preview the camera and make recordings.

Notes
- Make sure to follow the steps in the GigE Vision Quick Start Configuration Guide (https://www.mathworks.com/matlabcentral/answers/uploaded_files/41167/GigEVisionQuickStart.pdf). In specific, ensure that the correct driver from the manufacturer is

installed. The driver we used is the Marvell AQtion Windows 64-bit driver (https://www.marvell.com/support/downloads.html). In addition, ensure that the firewall is disabled as the firewall may prevent the camera from connecting to the computer. To do so, type in the command line (with administrator controls) `netsh advfirewall set allprofiles state off`.
- Ensure that the chamber light is on when recording.

Section 5: Computer Control

Both the DAQ and camera are controlled by MATLAB scripts.

Parts List
- The following MATLAB add-ons are required for control of the DAQ:
  - Signal Processing Toolbox
  - Data Acquisition Toolbox
  - Data Acquisition Toolbox Support Package for National Instruments NI-DAQmx Devices
- The following MATLAB add-ons are required for control of the GigE camera:
  - Image Acquisition Toolbox
  - Image Processing Toolbox
  - Image Acquisition Toolbox Support Package for GigE Vision Hardware
  - Image Acquisition Toolbox Support Package for Teledyne DALSA Sapera Hardware

Assembly
1. Download and install all required add-ons.
2. To control the DAQ:
   a. Open /code/DAQ_code/DAQ_HTR_Code
   b. The first section allows the user to enter the number of seconds the session will run for. All succeeding sections (adapted from de la Fuente Revenga et al., Sci. Rep., 2019) apply filters and thresholds to the raw data, correct the raw voltage signal to baseline, and transform the signal to absolute value. As is currently written, the top threshold value is given as 0.225 V, the minimum distance between events is 3000 ms, and the maximum width of an event is 1000 ms. These values worked well for us with few false positives and negatives but could be altered if needed.
   c. Pasted on the graph will be the number of peaks (head twitches) recorded.
   d. Pasted in the command window will be the time points of the head twitches.
   e. Save and export the data.mat file. Do not run the script again unless the data.mat file has been saved and exported; otherwise, the data file will be overwritten.

3. To control the camera:
   a. Within the /code/HTR-recording-master folder, there is a file titled "master_head_twitch" that controls both the preview functionality and the recording functionality of the camera. When running this file, make sure that all files within HTR-recording-master folder and the subfolders within are also added to the current MATLAB path. For instance, the "bin" folder has two important functions ("newid" and "stoploop") that are necessary for the program to run.

4. To control the DAQ and camera simultaneously:

a. While it is possible to institute a trigger so that the DAQ and camera can run at the exact same time, we have found that is sufficient to open two windows of MATLAB and run the DAQ script soon after running the camera recording script.

b. First, open the "master_head_twitch" code and place the mouse pointer in the section titled `%% For recording:`. Run the section (not the entire script). Immediately afterward, in a separate instance of MATLAB, run the entire DAQ script.

5. To analyze the data in a separate computer:

a. Load the "data.mat" file by dragging the file into the command window. Then, highlight all sections in the DAQ code beginning with `%% Defining processing and detection parameters` but do not highlight the section beginning with `%% Creating DAQ session tracking running voltage for specified period of time` as this section controls the DAQ itself. Right-click on the highlighted section and press "Evaluate Selection."

Notes
- Ensure that all necessary folders and subfolders (such as the "bin" file and "Boubat-UpdatableText-90a229b" file) are added to the correct path.

*Head-twitch responses logged by the MATLAB program over an example session*
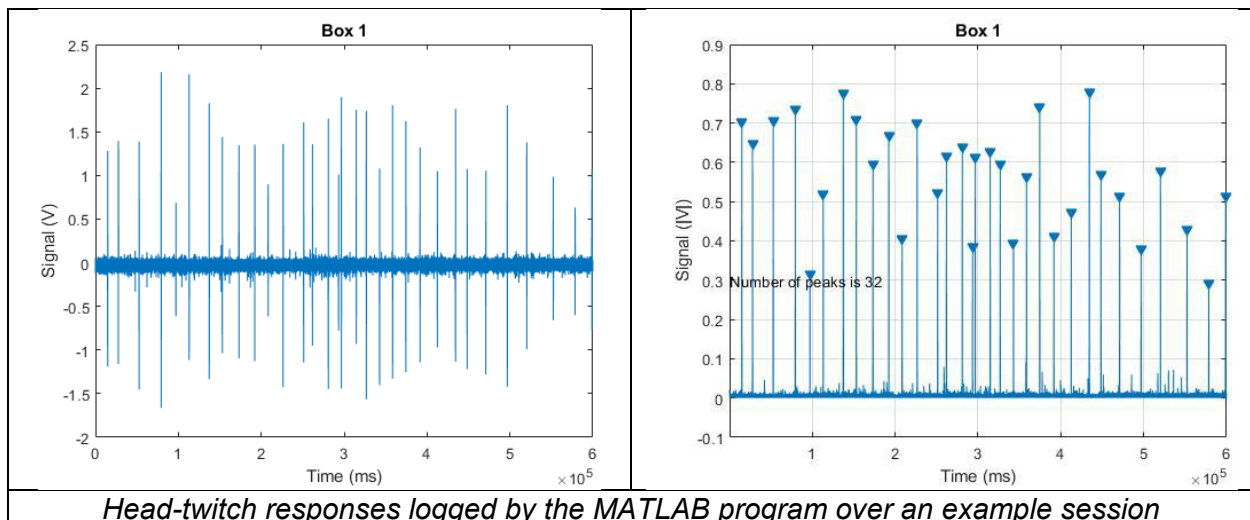
Addendum: Scaling Up

It is useful to run multiple mice simultaneously, which can be accomplished by scaling up the setup. Similar steps as described in this manual can be applied to add more boxes.

- To run the code for two boxes, run /code/DAQ_code/DAQ_2_Boxes. Be sure to specify which analog inputs are being used.
- Similarly, run /code/DAQ_code/DAQ_3_Boxes or /code/DAQ_code/DAQ_4_Boxes to run the code for three or four boxes, respectively.

The 4-box version of the setup is shown in the picture below:

Setup in which the head twitches of four mice can be automatically quantified simultaneously
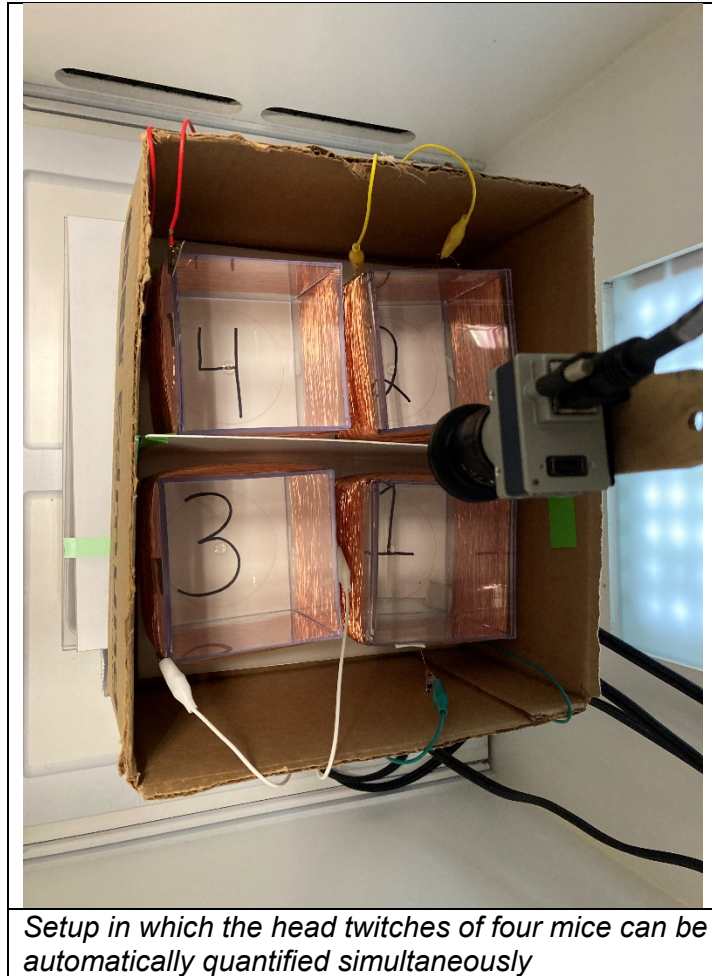
Addendum: Performance

Using a set of data including 243 visually identified head-twitch responses from videos, acquired from 4 conditions (saline, 1 mg/kg psilocybin, 5 mg/kg MeO-DMT, and 10 mg/kg 5-MeO-DMT), we compared the manually identified HTR versus events detected by the magnetic ear tag system.

Summary of the performance