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Record visually-evoked eye movements in head-fixed adult mice using a hemispherical projection system

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We use this protocol and it's working

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Disclaimer

ETHICS DISCLAIMER

The **protocols.io** team notes that research involving animals and humans must be conducted according to internationally-accepted standards and should always have prior approval from an Institutional Ethics Committee or Board.

Abstract

This protocol describes how to record visually-evoked eye movements in head-fixed adult mice using a hemispherical projection system.

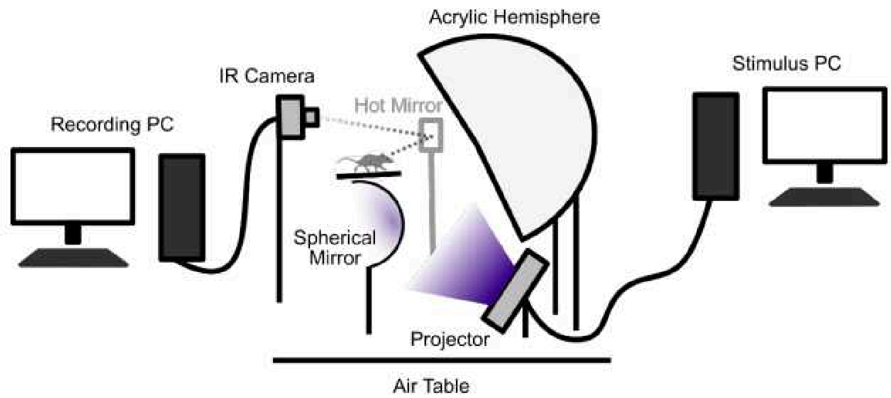

Figure 1. Schematic of rig setup.

Attachments

Hemispherical Projec...
178KB

Materials

Rig Parts List

• 24″ diameter acrylic hemisphere (California Quality Plastics)
• UV reflective paint with 50% internal reflection
• 6″ brass hemisphere (Wagner), silver-coated into a reflexive surface (custom performed by a silversmith)
• Optical breadboard/air table
• High speed infrared camera
• Hot mirror (Edmund Optics)
• 405 nm DLP Projector (LightCrafter, EKB)
• Infrared LEDs and diffuse infrared lighting
• Two computers (running either Linux or Windows). Use a powerful graphics card on the stimulus computer for best performance
• 2x serial to TTL cables (connected via jumper cables)
• Miscellaneous optical posts and parts, including to build a small platform for a mouse and a headplate holder (ThorLabs)

Software

• Meshmapper (https://paulbourke.net/dome/meshmapper/)
• Bassoon (https://sco]harris17.github.io/Bassoon/)
• Video acquisition software (StreamPix8 from NorPix with the DAQ Module

Troubleshooting

Rig Setup

1 Cover the concave surface of the acrylic hemisphere with an even coat of the UV paint.

2 Using optical posts, secure the hemisphere with several inches of clearance above the breadboard/air table - there must be sufficient space for the project to fit underneath the hemisphere.

3 Locate the point in space that marks the center of the hemisphere. Build a post with a platform and headplate holder such that when a mouse is head fixed on the platform, the eye that will be recorded from is as close as possible to this center point.

4 Secure the spherical mirror directly beneath the mouse's platform.

5 Position the projector under the acrylic hemisphere, and angle it to point towards the spherical mirror. This may require the use of optical posts. The goal is to reflect the projected image off of the spherical mirror and such that it casts a focused image across the concave surface of the hemisphere.

6 Connect the projector to the stimulus PC. Run Meshmapper to create a "warp file" that can then be used with Bassoon to linearize visual stimuli as they are projected onto the hemisphere.

7 Position the hot mirror on a post in front of the mouse's eye that will be recorded.

8 Place the camera on a second post behind the mouse's platform. Direct the camera toward the hot mirror, and adjust the angle of the hot mirror such that the reflection of the mouse's eye is focused on the camera. This setup allows direct imaging of the eye while not obscuring the mouse's field of view with the camera.

- For eye tracking calibration, a common approach is to place the camera on a swinging post that rotates around the virtual image of the mouse's eye. The infrared LEDs are aligned vertically and horizontally to the optical axis of the camera, and the eye is imaged from several angles before the beginning of each experiment to calibrate the system. This can be performed using Bassoon, and the mathematical methods are described extensively in [1-4].

9 Connect the camera to the recording computer and install the image acquisition software to acquire data during experiments.

10 Connect the stimulus and recording computers using the serial to TTL cables. This allows the recording computer to align timing information about the stimulus with the recording.

11 If needed, place diffuse LED lighting around the rig in order to improve the quality of the recorded image. Best results are achieved by performing experiments in a room devoid of ambient light.

Eye Tracking Experiments

12 Surgically secure a headplate to an adult mouse. Let the animal recover for at least 7 days before proceeding.

13 Once the animal has fully recovered from surgery, remove it from its cage and lightly anesthetize it in an isoflurane chamber.

14 Place the anesthetized animal on the rig and secure it tightly with the headplate holder. The image of the mouse's eye should be focused on the camera.

15 Give the animal several minutes to recover from the isoflurane before beginning experiments. The pupil will dilate as the animal wakes up.

16 Ensure that the pupil and corneal reflections are clearly visible from the camera before beginning experiments.

- Habituate each animal by head-fixing it on the rig for several minutes per day leading up to an experiment. No stimulus should be presented during habituation. Habituation improves the animal's comfort in the setup and the likelihood that animals will voluntarily keep their eyes open during experiments.

17 Initiate the recording software and present the stimulus. To elicit optokinetic eye movements in mice, drifting grating patterns can be used with a spatial frequency of approximately 0.15 cycles per degree and a speed of 10 degrees per second (though several combinations of spatial frequency and speed are sufficient). These parameters can be customized in Bassoon using the Moving Grating Direction stimulus.

- Eye tracking calibration procedures should be performed on the same day as experiments, and can be accomplished by using the Pupil Calibration stimulus in Bassoon.

18 Once the experiment is complete, save the recording videos and stimulus files from the recording and stimulus computers respectively.

19 Remove the mouse from the rig and place it back in its cage.

20 Data can be analyzed by following the steps described in https://github.com/UCSF-Nelson-Lab/QuickPhase-Analysis .

Protocol references

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