

Video Article

Video-oculography in Mice

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Abstract

Eye movements are very important in order to track an object or to stabilize an image on the retina during movement. Animals without a fovea, such as the mouse, have a limited capacity to lock their eyes onto a target. In contrast to these target directed eye movements, compensatory ocular eye movements are easily elicited in afoveate animals^{1,2,3,4}. Compensatory ocular movements are generated by processing vestibular and optokinetic information into a command signal that will drive the eye muscles. The processing of the vestibular and optokinetic information can be investigated separately and together, allowing the specification of a deficit in the oculomotor system. The oculomotor system can be tested by evoking an optokinetic reflex (OKR), vestibulo-ocular reflex (VOR) or a visually-enhanced vestibulo-ocular reflex (VVOR). The OKR is a reflex movement that compensates for "full-field" image movements on the retina, whereas the VOR is a reflex eye movement that compensates head movements. The VVOR is a reflex eye movement that uses both vestibular as well as optokinetic information to make the appropriate compensation. The cerebellum monitors and is able to adjust these compensatory eye movements. Therefore, oculography is a very powerful tool to investigate brain-behavior relationship under normal as well as under pathological conditions (f.e. of vestibular, ocular and/or cerebellar origin).

Testing the oculomotor system, as a behavioral paradigm, is interesting for several reasons. First, the oculomotor system is a well understood neural system⁵. Second, the oculomotor system is relative simple⁶; the amount of possible eye movement is limited by its ball-in-socket architecture ("single joint") and the three pairs of extra-ocular muscles⁷. Third, the behavioral output and sensory input can easily be measured, which makes this a highly accessible system for quantitative analysis⁸. Many behavioral tests lack this high level of quantitative power. And finally, both performance as well as plasticity of the oculomotor system can be tested, allowing research on learning and memory processes⁹.

Genetically modified mice are nowadays widely available and they form an important source for the exploration of brain functions at various levels¹⁰. In addition, they can be used as models to mimic human diseases. Applying oculography on normal, pharmacologically-treated or genetically modified mice is a powerful research tool to explore the underlying physiology of motor behaviors under normal and pathological conditions. Here, we describe how to measure video-oculography in mice⁸.

Video Link

The video component of this article can be found at <http://www.jove.com/video/3971/>

Protocol

1. Preparation

The following experiments were conducted in accordance with The Dutch Ethical Committee for Animal Experiments.

1. Preparing mice for video-oculography. In order to measure eye movements of a mouse, the head of the mouse needs to be immobilized. Therefore, a pedestal construction is made on the skull of the mouse (**Figure 1**).
 1. Anesthetize the mouse by a mixture of isoflurane (isofluran 1-1.5%; Rhodia Organique Fine Ltd, France) and oxygen in a gas chamber. The excessive gas is scavenged. Maintain anesthesia via nose cone. Confirm depth of anesthesia via a toe pinch.
 2. Maintain the body temperature at 37 °C with the use of an anal thermosensor and a heating pad (FHC, Bowdoinham, ME).
 3. Protect the eyes by covering them with an eye ointment (duratears, Alcon, Belgium). Shave the dorsal cranial fur, and clean the surgical area with a rotation of scrub and betadine or chlorhexidine solution.
 4. Make a middle line incision to expose the dorsal cranial surface of the skull. Make the surface clean and dry.
 5. Apply a drop of phosphoric acid (phosphoric acid gel etchant 37.5%; Kerr, CA) on the dorsal cranial surface of the skull from bregma to lambda. Remove the etchant after 15 seconds and make the cranial surface clean with saline and dry again.
 6. Apply on top of this etched cranial surface a drop of OptiBond prime (Kerr, CA) and air-dry it for 30 seconds.
 7. Place a drop of OptiBond adhesive (Kerr, CA) on top of the OptiBond prime and cure with light for 1 minute (Maxima 480 visible light curing unit; Henry Schein, USA).

8. Cover the adhesive layer with a thin layer of Charisma composite (Heraeus Kulzer, Germany). Embed two connected nuts (diameter: 3 mm) in the composite. Cure the composite afterwards with light. When necessary, apply additional layers of composite and cure them with light.
 9. Administer buprenorphine (0.015 mg/kg, s.c) for post-operative analgesia. The animal should be back on its feet within approximately 5 min. Allow the mouse to recover in the home cage at room temperature for at least 3 days after the surgery.
2. Video-oculography setup for mice (**Figure 2**).
1. Place the mouse in the restrainer and fix his head to the restrainer by two screws (**Figure 1**). The mouse does not need to be anesthetized for this procedure. Restraining time should not exceed 1 hr/day.
 2. Mount the mouse head-and-body restrainer on an X-Y platform, which in turn is mounted upon the turntable (diameter: 60 cm). Using the X-Y platform the mouse head can be placed above the center of the turntable. The mouse can be moved over the pitch, yaw and roll axes. The head of the mouse is placed in the correct pitch, yaw and roll angle by aligning the eye using the visual image of the eye generated by the ISCAN system. Alternatively, the pedestal construction can be placed on the head of mouse in a stereotactic frame¹¹.
 3. The turntable is attached to an AC servo-controlled motor (Harmonic drive AG, the Netherlands) and the position of the turntable is monitored by a potentiometer (Bourns inc., CA) attached to the turntable axis.
 4. A cylindrical surrounding screen (diameter: 63 cm; height: 35 cm) with a random dotted pattern (each element 2°) covers the turntable; this drum is also equipped with an AC servo-controlled motor (Harmonic drive AG, the Netherlands). The position of the cylindrical screen is monitored by a potentiometer (Bourns inc., CA) attached to its axis and the screen can be lit by a halogen light (20 Watt). Both the surrounding screen and the turntable are driven independently.
 5. The movement of the turntable and surrounding screen is controlled by a computer that is connected to an I/O interface (CED limited, Cambridge, United Kingdom). Table and surrounding screen position signals are filtered (cut-off frequency: 20 Hz), digitized by the I/O interface and stored on this computer.
 6. The eye of the mouse is illuminated by three infrared emitters (600 mW, dispersion angle: 7°, peak wavelength: 880 nm, RS components, the Netherlands). Two infrared emitters are fixed to the turntable and the third emitter is attached to the camera. This third emitter produces a reference corneal reflection (CR), which is used during the calibration procedure and during the eye movement recordings.
 7. An infrared CCD camera equipped with a zoom lens (Zoom 6000, Navitar inc., NY) is attached to the turntable and is focused on the mouse head in the center of the turntable. The camera can be unlocked and can be yawed about the turntable axis over exactly 20° during the calibration procedure.
 8. The video signal is processed by an eye tracking system (ETL-200, ISCAN, Burlington, MA). The ISCAN system uses an algorithm to track the centers of the pupil and the reference CR. The system can track the pupil and reference CR in horizontal and vertical direction at a sample rate of 120 Hz.
 9. Reference CR position, pupil position and pupil size signals are digitized by the I/O interface and are stored in the same file as the table and surrounding screen position signals. The video pupil-tracking system induces a delay of the eye movement signals of approximately 27 ms.

2. Calibrating and Measuring Eye Movements Using Video Pupil-tracking

The eye tracking system captures the movement of the pupil as a translational motion. The translational motion of the tracked pupil contains a translational component due to axial difference between the rotational center of the eye and the anatomical center of the eye (i.e. center of corneal curvature), and a rotational component due to the angular rotation of the eyeball. By subtracting the reference CR from the pupil movement/position, the undesired translational component is eliminated from the signal, resulting in a translational motion that is only due to the rotation of the eyeball. Although they are often very small, this subtraction also eliminates the translations between the head and the camera. The residual isolated translational motion is converted into the angular rotation of the eyeball by the following calibration method^{8,12}. This calibration was performed prior to any eye movement experiment.

1. Adjust the mouse head position to the camera in such a way that the video image of the pupil is situated at the middle of the monitor and that the representation of the reference CR is located on the vertical midline of the eye preferably direct above the pupil. Minimize the movements of the reference CR due to angular camera rotations, which can be accomplished by placing the center of the corneal curvature over the camera/table axis.
2. Rotate the camera several times by +/- 10° (i.e. 20 degrees peak to peak) around the vertical axis of the turntable. Use the positions of the tracked pupil (P) and the reference CR recorded in the extreme positions of the camera rotation to calculate the radius of rotation of the pupil (R_p ; $R_p = \Delta/\sin(20^\circ)$; where $\Delta=(CR-P)$, see **Figure 3A**).
3. Due to the fact that the R_p value depends on the pupil size, a pupil size correction needs to be implemented¹² (**Figure 3B**). Repeat step 2.2 many times under various illumination conditions (i.e. manipulating the pupil size; **Figure 3C**) in order to determine the pupil size - R_p relationship and compose an R_p correction curve (**Figure 3D**). The R_p value also depends on the vertical eye position. When the experiment will cause vertical eye movements then a correction of the calibration for vertical eye positions is highly recommendable¹³.
4. Determine the angular position of the eye (E) by measuring the reference CR position, P position and the pupil size. The reference CR position is subtracted from the pupil position generating a translational free pupil position. By measuring the pupil size the R_p value can be extracted from the R_p correction curve and E can be calculated by using the following formula $E = \arcsin\{(\Delta 1)/R_p\}$ (**Figure 4A**; where $\Delta 1=(P_2-P_1)$ and P_1 and P_2 are corrected by subtraction of the reference CR).
5. A large repertoire of turntable and/or surrounding screen rotations can now be used to stimulate the oculomotor system. In order to perform video oculography in the dark, the mouse eye needs to be pretreated with a miotic drug to limit the pupil dilatation and allow pupil tracking under these circumstances. In our experiments, we use pilocarpine (4%, Laboratories Chauvin, France) to limit pupil dilatation in the dark.

3. Data Analysis

1. Eye positions, table positions and surrounding screen positions are all converted into angular positions (see **Figure 4B** and formula in 2.4). Eye signals are corrected for their delay of 27 ms induced by the imaging processing of the pupil-tracking system.
2. Angular positions of eye, table and surrounding screen are differentiated and filtered with a Butterworth low-pass filter using a cut off frequency of 20 Hz.
3. Saccades are removed from the eye velocity signal using a detection threshold of 40°/s. Data is removed starting from 20 ms before and up to 80 ms after crossing the detection threshold.
4. Table, surrounding screen and eye velocity signals are averaged using each individual cycle in the trail (**Figure 4C**).
5. Averaged signals are fitted with an appropriate function. In general, a sinusoidal velocity stimulation is used and the averaged cycles are fitted with sinus or cosinus function (**Figure 4C**). Then, the gain can be computed as the ratio of eye velocity to stimulus velocity, whereas the phase can be computed as the difference (in degrees) between the eye velocity and stimulus velocity.

4. Representative Results

Video-oculography can be used to investigate various forms of oculomotor performances (i.e. optokinetic reflex: OKR; vestibulo-ocular reflex: VOR; visually enhanced vestibulo-ocular reflex: VVOR) as well as motor learning (VOR adaptation; OKR adaptation). The OKR compensates for low-frequency disturbances using visual feedback. The OKR can be induced by rotating the well-illuminated surrounding screen (**Movie 1**). Rotating the surrounding screen over a frequency range of 0.2 -1.0 Hz with an amplitude of 1.6° shows how the optokinetic system is a more efficient compensatory mechanism in the low-frequency range than in the high-frequency range (**Figure 5A**). The VOR compensates for high-frequency head movements using signals from the vestibular organs. The VOR can be induced by rotating the animal (i.e. turntable) in the dark (**Movie 2**). Rotating the turntable over a frequency range of 0.2 -1.0 Hz with an amplitude of 1.6° demonstrates how the vestibulo-ocular system is more efficient in generating compensating eye movements in the high-frequency range than in the low-frequency range (**Figure 5A**). When the optokinetic and vestibulo-ocular system act in concert, images can be stabilized on the retina over a broad range of head movements. Rotating the turntable over a frequency range of 0.2 -1.0 Hz with an amplitude of 1.6°, while the surrounding screen is well-illuminated (**Movie 3**) shows how the eye generates "high gain" compensating movements over the entire frequency range (**Figure 5A**). All these gain and phase values are typical for mice, although gender¹⁴ and strain^{15,16,17} differences were reported.

The independent control over the turntable and the surrounding screen enables us to confront the mice with a mismatch between visual and vestibular information. After a long-term and uniform exposure of mismatched visual and vestibular information, the VOR of the mouse will change to compensate for the altered visual input (VOR adaptation; **Movie 4**). Rotating the turntable out of phase (i.e. 180°) with the surrounding screen (1 Hz, 1.6°) increases the VOR gain (**Figure 5B**). The maximal change in VOR gain, when using a one trial learning paradigm, is often reached after 30 minutes.

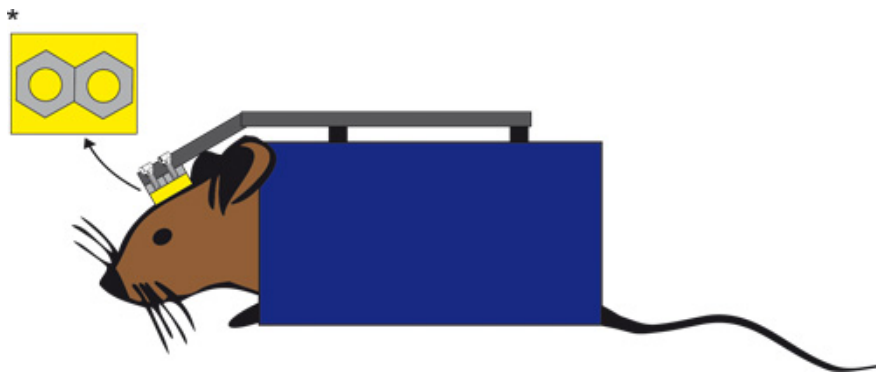


Figure 1. Schematic drawing of the mouse head-and-body restrainer. The body of the mouse is restrained using a plastic cylindrical tube with a diameter of 35 mm. The head of the mouse is immobilized by connecting the pedestal of the mouse to the iron bar with two screws. The iron bar makes an angle of 30 degree in order to position the head of the mouse in the normal pitch during ambulation. *, top view of the pedestal containing two nuts.

Mouse video-oculography setup

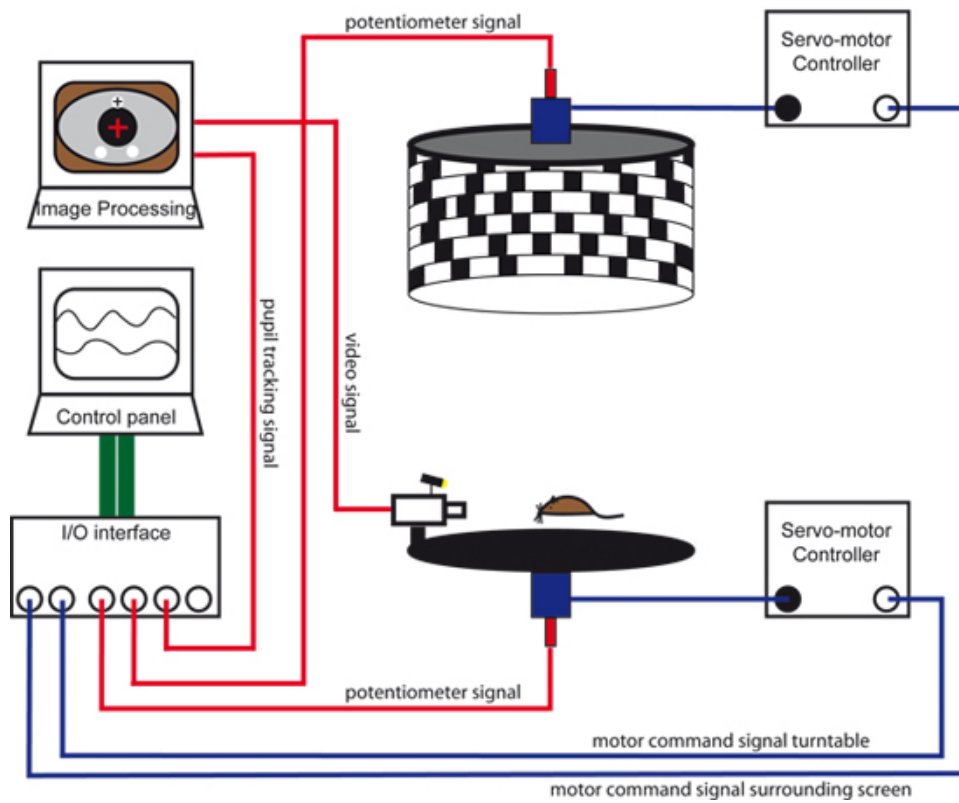


Figure 2. Schematic drawing of the mouse video-oculography setup.

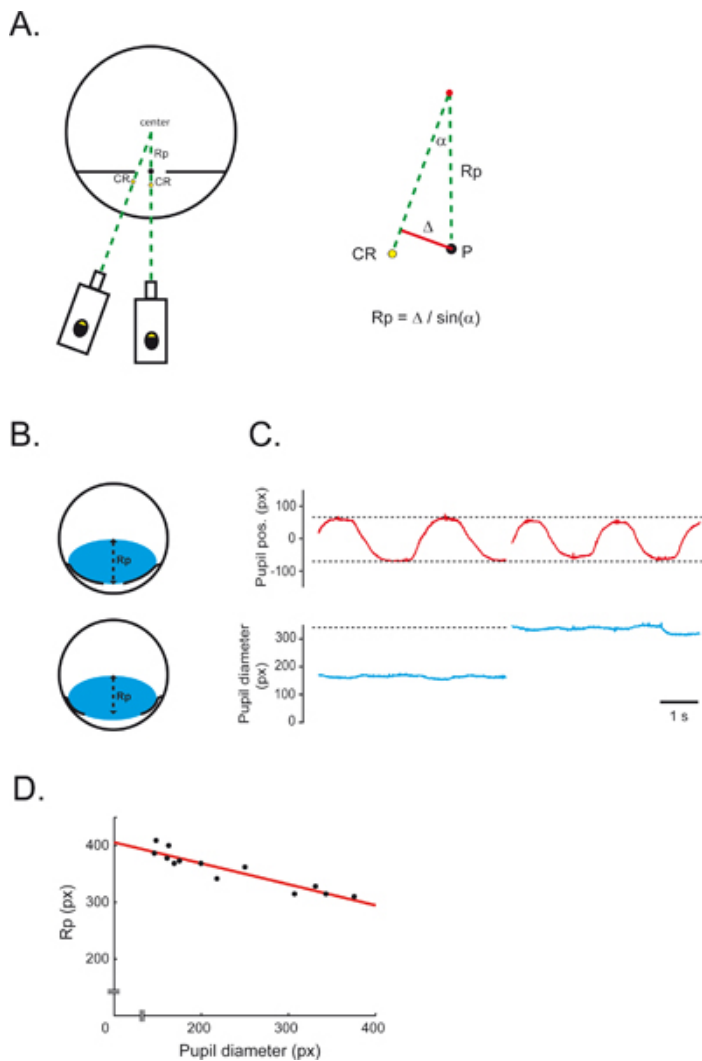
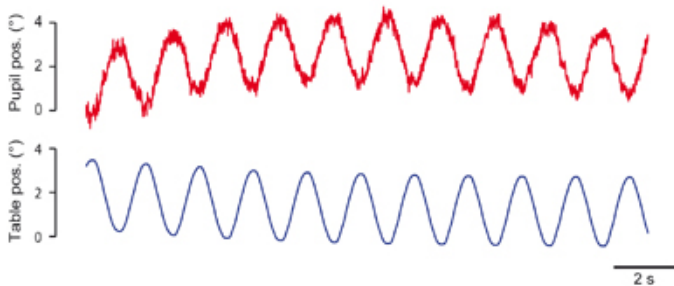


Figure 3. Calibration of the video pupil-tracking system. A) The camera is rotated several times by $\pm 10^\circ$ (i.e. 20 degrees peak to peak) around the vertical axis of the turntable. The tracked pupil (P) and the reference corneal reflection (CR) recorded in the extreme positions of the camera rotation are used to calculate the radius of rotation of the pupil (R_p). B) The radius of the pupil diameter is dependent on the size of the pupil. C) Example showing the effect of pupil size on pupil position during the calibration procedure (both measured in pixels (px)). D) Relationship between R_p and pupil diameter measured in a single mouse. The thirteen different pupil diameters were accomplished by altering the intensity of the surrounding light.

A.



B.



C.

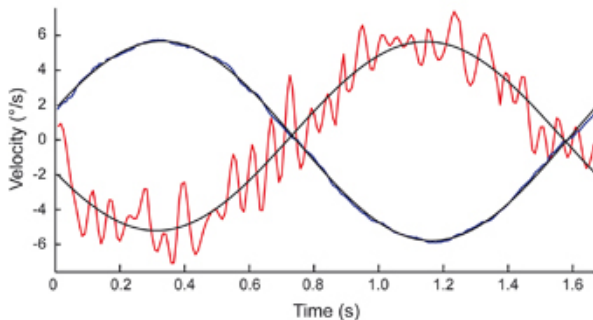


Figure 4. Measuring and analyzing eye movements using video pupil-tracking. A) The angular pupil position is calculated from radius of the pupil (R_p) and the position of the Pupil (P ; corrected for CR position). B) Example of compensatory eye movement induced by stimulating the vestibular and visual system (visual enhanced VOR). The turntable was rotated sinusoidally at 0.6 Hz with an amplitude of 1.6° , while the surrounding screen was well-illuminated. C) Analyses of the recording shown in B). Graph shows the averaged velocity trace of the turntable (blue) and pupil (red). These averaged traces were fitted with a sinusoidal function (black).

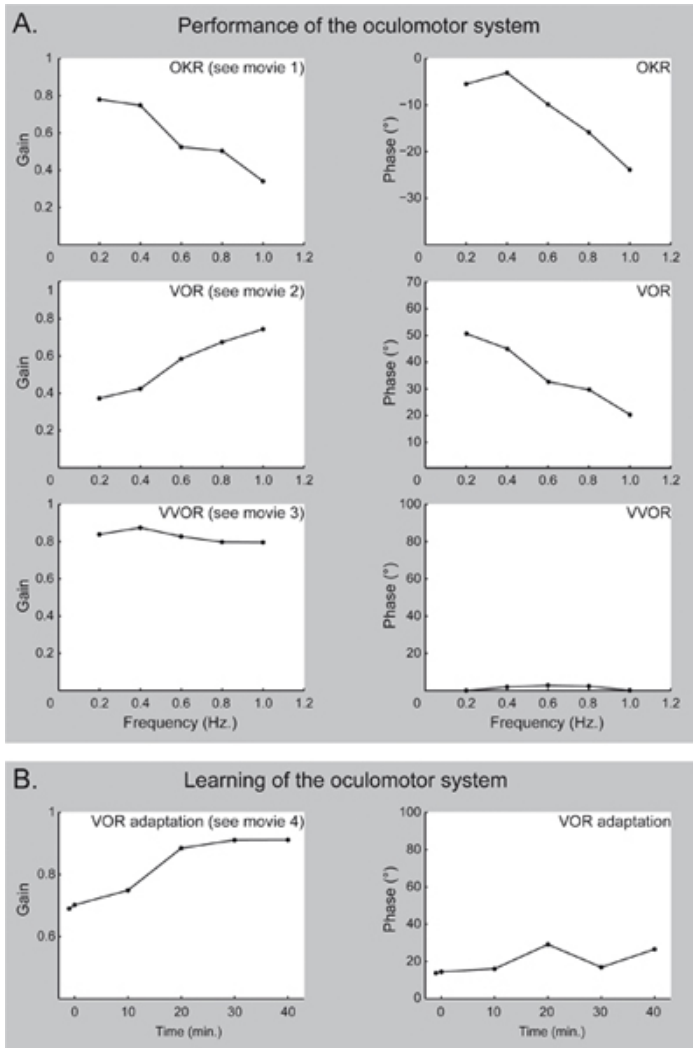


Figure 5. Performance and learning of the oculomotor system measured in one C57Bl6 mouse. A) Eye movements are generated by rotations of the surrounding screen (optokinetic reflex: OKR, top panels), by rotating the mouse in the dark (vestibulo-ocular reflex: VOR, middle panels) and by rotating the mouse in the light (visually-enhanced vestibulo-ocular reflex: VVOR, bottom panel) with frequencies ranging from 0.2 to 1.0 Hz at an amplitude of 1.6°. The gain of the reflex was computed as the ratio of eye velocity to stimulus velocity (left panels) and phase of the reflex was computed from the phase difference between the eye velocity and stimulus velocity (right panels). B) Motor learning was accomplished by adaptively increasing the VOR using an out of phase training paradigm. The mouse was subject to a visuovestibular training paradigm in which the rotation of the mouse was out of phase (180°) with the rotation of the surrounding screen (both rotating at 1.0 Hz, 1.6°) for forty minutes. Every 10 minutes the VOR was tested (1.0 Hz, 1.6°). In this mouse the out of phase training increased the VOR gain.

Movie 1. Animation showing the paradigm that induces OKR in mice [Click here to view movie.](#)

Movie 2. Animation showing the paradigm that induces VOR in mice. [Click here to view movie.](#)

Movie 3. Animation showing the paradigm that induces VVOR in mice. [Click here to view movie.](#)

Movie 4. Animation showing the visuovestibular out of phase training paradigm that induces VOR adaptation (increase) in mice. [Click here to view movie.](#)

Discussion

In order to obtain high-quality video eye movements recordings in mice several requirements are necessary. The calibration procedure needs to be performed in the above mentioned standardized manner. For example off-center calibration, when the pupil is not positioned on the vertical midline with the reference CR during the calibration procedure, will result in an underestimation of RP and consequently an overestimation of the eye movement. Furthermore, we recommend integrating the pupil size correction method in the calibration procedure¹², because trials that show a very stable pupil size are very rare. Even a small stressor during the trial can already alter the pupil diameter substantially.

When designing an eye movement experiment, the following factors need to be taken into account or controlled for because they are known to affect the eye movement response: age^{13,18}, gender¹⁴ and strain^{15,16,19}. Furthermore, the experimental animal should have pigmented irises

since pupil detection and tracking is impossible when the contrast between pupil and iris is too low, like in the BALB/c mouse. Extremely nervous or anxious animals need to be trained, prior to the experiment, to get used to the experimental set up and the restrained condition. This animal handling procedure results in less closure or semi-closure of the eyes and prevents the generation of eye fluids during the experiment, and consequently a better pupil tracking is accomplished.

Finally, acquiring and analyzing the data requires two to three hours per animal. Therefore, eye movement recordings will likely remain a specific procedure applied to selected mice and is not suitable as a high throughput screening test.

Disclosures

No conflicts of interest declared.

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