

Video Article

Analyzing Responses of Mouse Olfactory Sensory Neurons Using the Air-phase Electroolfactogram Recording

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Abstract

Animals depend on olfaction for many critical behaviors, such as finding food sources, avoiding predators, and identifying conspecifics for mating and other social interactions. The electroolfactogram (EOG) recording is an informative, easy to conduct, and reliable method to assay olfactory function at the level of the olfactory epithelium. Since the 1956 description of the EOG by Ottoson in frogs¹, the EOG recording has been applied in many vertebrates including salamanders, rabbits, rats, mice, and humans (reviewed by Scott and Scott-Johnson, 2002, ref. 2). The recent advances in genetic modification in mice have rekindled interest in recording the EOG for physiological characterization of olfactory function in knock-out and knock-in mice. EOG recordings have been successfully applied to demonstrate the central role of olfactory signal transduction components³⁻⁸, and more recently to characterize the contribution of certain regulatory mechanisms to OSN responses⁹⁻¹².

Odorant detection occurs at the surface of the olfactory epithelium on the cilia of OSNs, where a signal transduction cascade leads to opening of ion channels, generating a current that flows into the cilia and depolarizes the membrane¹³. The EOG is the negative potential recorded extracellularly at the surface of the olfactory epithelium upon odorant stimulation, resulting from a summation of the potential changes caused by individual responsive OSNs in the recording field². Comparison of the amplitude and kinetics of the EOG thus provide valuable information about how genetic modification and other experimental manipulations influence the molecular signaling underlying the OSN response to odor.

Here we describe an air-phase EOG recording on a preparation of mouse olfactory turbinates. Briefly, after sacrificing the mouse, the olfactory turbinates are exposed by bisecting the head along the midline and removing the septum. The turbinate preparation is then placed in the recording setup, and a recording electrode is placed at the surface of the olfactory epithelium on one of the medial turbinates. A reference electrode is electrically connected to the tissue through a buffer solution. A continuous stream of humidified air is blown over the surface of the epithelium to keep it moist. The vapor of odorant solutions is puffed into the stream of humidified air to stimulate the epithelium. Responses are recorded and digitized for further analysis.

Video Link

The video component of this article can be found at <http://www.jove.com/video/1850/>

Protocol

Part 1. The EOG recording setup

The recording apparatus consists of a recording electrode, reference electrode, air delivery tube, specimen stage, and dissecting microscope, all anchored on an air table within a Faraday cage. Micromanipulators are used for placement of the electrodes and the air delivery tube. A continuous air stream is bubbled through distilled water to add humidity before passing through the air delivery tube and over the specimen. A 60 mm culture dish filled with Sylgard to a depth of 6-8 mm is used as a mounting surface for the specimen. A well and a channel are hollowed out of the Sylgard in the mounting dish to provide a means to electrically connect the reference electrode to the specimen via modified Ringer's solution.

The recording electrode and the reference electrode are connected to an amplifier. Signals from the amplifier are sent to a digitizer and then to a computer. Software such as Axograph or pClamp can be used to control the stimulation protocol, to record the signal, and for subsequent analysis of the responses. An oscilloscope connected after the amplifier can be convenient for real time monitoring of the electrical potential while placing the recording electrode and during EOG recordings.

Delivery of odorant stimuli is controlled by a Picospritzer, which is connected to the same computer used for signal acquisition. The air pressure at the Picospritzer is set to 10 psi. A single air tank and regulator can be used to supply air to both the air table and the Picospritzer. A second air tank and regulator is used to provide air for the humidified air stream, as this requires a lower pressure and a large amount of airflow. Just

prior to delivering an odorant stimulus, the Picospritzer output is connected to an odorant bottle. The odorant bottle is then connected to the air delivery tube.

Part 2: Preparing electrodes

The recording electrode is a chlorided silver wire in a pulled glass capillary filled with modified Ringer's solution (135 mM NaCl, 5 mM KCl, 1 mM CaCl₂, 1.5 mM MgCl₂, 10 mM HEPES, pH 7.4, filter sterilized). The reference electrode is a chlorided silver wire.

1. Install silver wire into the electrode holder. For the recording electrode, one to two centimeters of wire should protrude from the end of the electrode holder. More wire can be left for the reference electrode.
2. To add the AgCl coat to the silver wire, position the wire in 0.1 M NaCl and connect the electrode holder to the positive terminal of a 1.5-9 V DC power source. The negative terminal of the power source should be electrically connected to the 0.1 M NaCl solution. Allow the chloriding reaction to proceed for 10 minutes. To equalize any static charge between the recording and the reference electrode, briefly touch the electrodes together before installing them on the recording apparatus.
3. Pull a glass capillary using a micropipette puller. The opening at the tip of the capillary should be around 5-10 microns in diameter.
4. Use a diamond pencil to score and break off the blunt end of the capillary so it is ~2 cm longer than the silver wire. Fire-polish the cut end with the butane torch.
5. Melt 0.5% agarose in modified Ringer's solution. Pull a small amount of molten agarose solution into the tip of the electrode using a transfer pipette.
6. Fill the pulled capillary about 1/2 of the way with modified Ringer's solution (a syringe that has been heated and pulled to have a long thin end is useful for this purpose). Gently flick the capillary to dislodge any air bubbles. Store the filled electrode in the storage jar with a small amount of modified Ringer's solution in the bottom until they are ready to be used. Once a tissue sample is prepared and ready for recording, install a filled capillary over the recording electrode wire.

Part 3: Preparing odorant solutions

The odorants amyl acetate and heptaldehyde evoke large responses and are thus good choices as EOG stimulants.

1. In microcentrifuge tubes, prepare a series of dilutions of odorant in dimethyl sulfoxide (DMSO). As a starting point for a dose-response curve, prepare 10-fold dilutions from 5 M to 5×10^{-6} M. Make fresh dilutions each day.
2. Further dilute the odorants 50-fold in water by mixing 100 μ L diluted stock in DMSO with 4.9 mLs water in 2 ounce bottles with silicone stoppers. Let the solutions equilibrate in the bottles for at least 30 minutes. Note that the vapor concentration of odorants in each bottle is unknown, but will vary as a function of the concentration of odorants in the liquid phase.
3. Insert two 18-gauge needles through the silicon stopper to provide input and output ports. The ports should be plugged when the bottle is not in use.

Part 4: Recording the EOG and analyzing data

1. Sacrifice a mouse by CO₂ euthanasia or anesthetic overdose followed by decapitation. Remove the skin overlying the skull and sagittally bisect the head along the midline.
2. Mount one half of the head, medial side up, on the mounting dish. Carefully remove the septum to expose the turbinates.
3. Place the dish with mounted tissue onto the recording stage. Align the stage so that the recording location on the turbinates is centered under the microscope.
4. Turn on the air tank to deliver humidified air to the turbinate surface. Position the air delivery tube so that it is approximately 10 mm away from the recording location. The flow rate is ~600 mL/min.
5. Set the amplifier to DC mode (AC amplification will induce artifacts in the EOG signal) with low-pass filter at 1 kHz, and gain at 100X.
6. Mount recording and reference electrodes on the micromanipulators.
7. Lower the reference electrode into the well on the mounting dish and cover with modified Ringer's solution such that it is electrically connected to the tissue.
8. Carefully lower the recording electrode onto the surface of turbinate IIb or III. The electrode should barely touch the surface of the olfactory epithelium! When the electrode comes in contact with the epithelium (i.e. completes an electrical circuit) a straight baseline will appear in the oscilloscope.
9. Attach an odorant bottle to the side port on the air delivery tube.
10. On the computer, initiate a stimulation protocol. The sampling rate for data acquisition should be 2 kHz or higher. The software will trigger an odor pulse and begin recording.
11. A typical stimulation protocol may be a 100-msec duration single pulse, paired 100-msec pulses separated by a 1-sec interval, or a 10-sec sustained pulse.
12. Allow some time between protocols so the tissue is minimally adapted. One minute is sufficient for liquid concentrations of amyl acetate and heptaldehyde up to 10^{-3} M; at higher concentrations allow 5 minutes. After delivering high odor concentrations (such as at the end of a dose-response curve) residual odor may remain in the tube. Wash the air tube with 95% ethanol and dry before continuing with additional tissue samples.
13. Axograph software provides tools for measuring key parameters of the EOG signal. Such parameters include the response amplitude, latency, time to peak, and time constants of termination. It may be desirable to digitally filter traces at 25 Hz before further analysis.

Representative Results

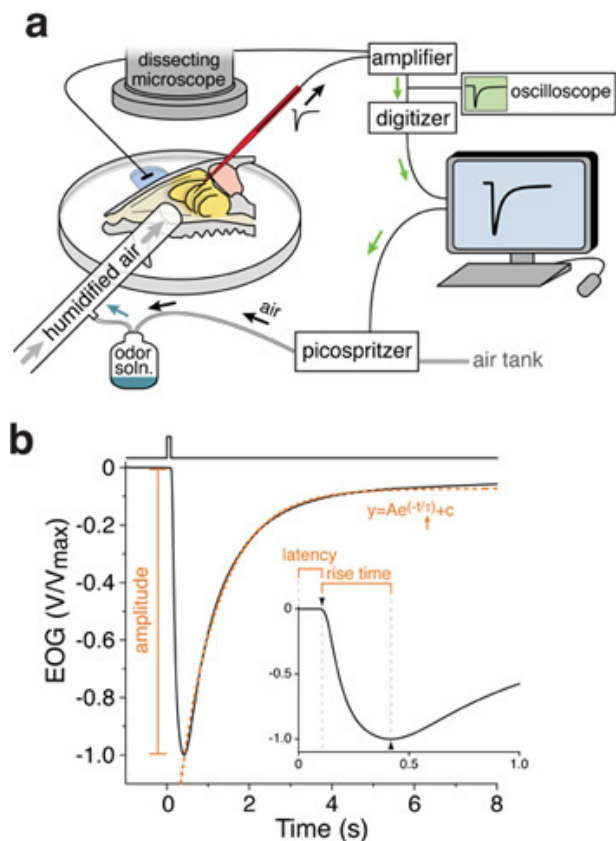


Figure 1. Parameters for EOG analysis. Several parameters of the EOG are particularly useful for comparison of responses between mice, including the response amplitude, the latency (the time between when the stimulus is administered and the response begins), rise time (the time between the start of the response and the peak), time to peak (the time from the start of the stimulation to the peak of the response), and time constant of termination (τ , determined by fitting the decay phase of the response to a single exponential equation). For comparison of kinetic parameters such as latency, rise time, and time constant of termination, it is advisable to normalize the peak amplitude of the responses prior to analysis.

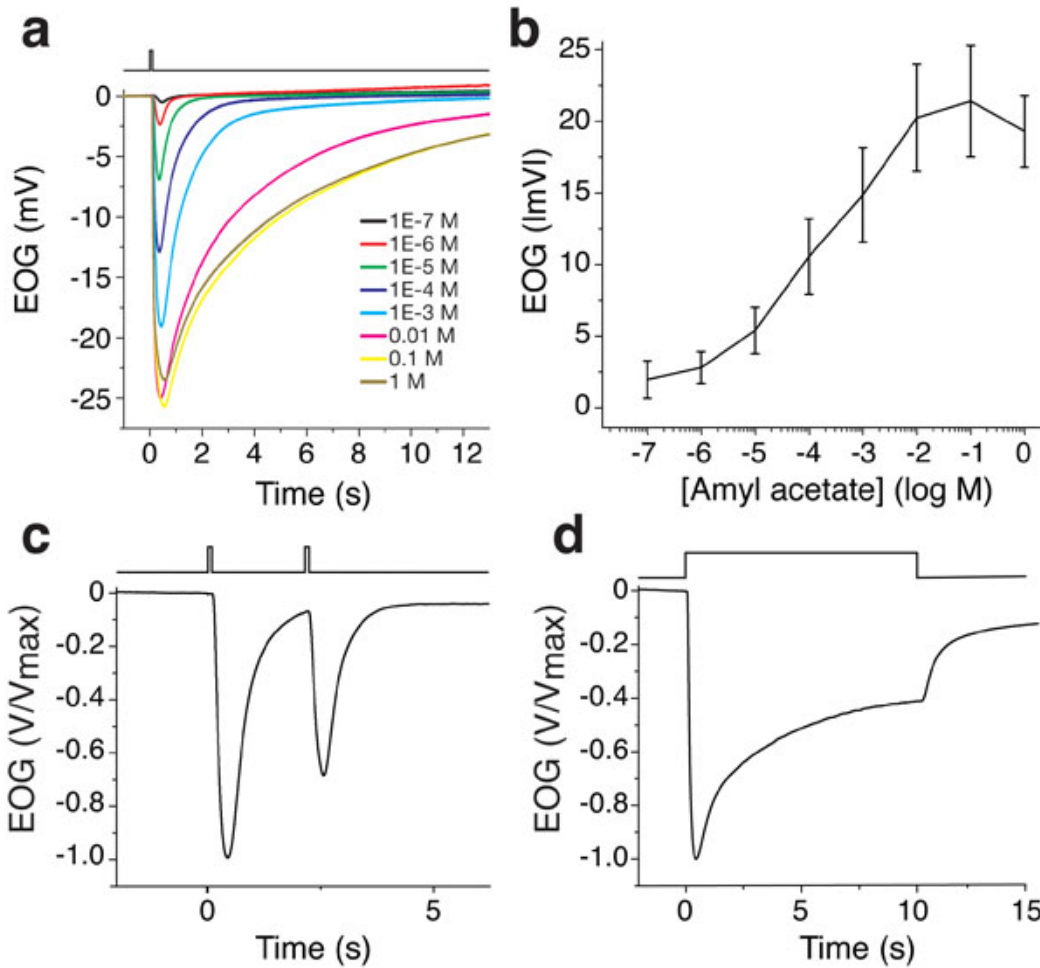


Figure 2. Representative EOG signals under different stimulation protocols. (a) Examples of EOGs from a mouse in response to stimulation with increasing concentrations of amyl acetate. The black line at the top of the panel indicates the timing and duration of odorant stimulation. The concentrations in the legend are the concentrations of the liquid solution. (b) A dose-response relation averaged from five mice. Error bars are 95% confidence intervals. A decline in the peak amplitude is often observed at very high odor concentrations. (c) An example of an EOG in response to a paired-pulse stimulation. A single short pulse of odorant elicits adaptation lasting for several seconds. (d) An example of an EOG in response to a 10-sec sustained odorant stimulation. The EOG shows desensitization during continuous odorant presentation.

Discussion

With the setup described in this protocol, the odorant stimuli at the surface of the olfactory epithelium will be consistent between tissue preparations, allowing for comparison between wild type and mutant mice, even though the exact odorant concentration and dynamics are unknown. Several factors, particularly the recording location and the flow rate of humidified air, cause variations in the EOG. Care should be taken to record from similar positions on the same turbinate to minimize variation. This can easily be achieved by consistently recording from the same side of the head and keeping the footprint of the microscope, odor delivery tube, and micromanipulators on the air table unchanged between tissue samples. In addition, tissue samples should be immediately placed into the humidified air stream after dissection to prevent excessive drying of the tissue.

EOG recordings on mice can also be carried out with a liquid perfusion apparatus on prepared mouse turbinates^{7, 14, 15}, or by leaving the head intact and inserting the electrode into a small hole drilled above the turbinates^{16, 17}. Each variation of EOG recording has its own strengths: air-phase recordings on tissue preparations as described in this protocol require a minimal amount of setup and are the easiest to conduct; recordings using a liquid perfusion apparatus facilitate the use of pharmacological reagents, although the hydrophobic nature of many odorants complicates odor delivery; lastly, recordings in which the head is left intact can be used in 'artificial sniff' experiments, although electrode placement is more difficult than when the turbinates are fully exposed.

Disclosures

Mice were handled and euthanized with methods approved by the Animal Care and Use Committees of The Johns Hopkins University.

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