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Recording potentials from scala media, saccule and utricle in mice

Huizhan Liu [a,b], Lidong Zhao [b,*]

[a] *Hair Cell Biophysics Laboratory, Department of Biomedical Sciences, Creighton University School of Medicine, Omaha, NE 68178, USA*
[b] *Department of Otolaryngology, Head and Neck Surgery, Institute of Otolaryngology, Chinese PLA General Hospital, Beijing, 100853, China*

Abstract

Objective: To describe a protocol for recording electrical potentials from the scala media, saccule, and utricle in mice.
Method: CBA/J mice were used and potentials were recorded with glass electrodes inserted through the basilar membrane using a patch clamp system.
Results: Resting potentials were successfully recorded from the scala media, saccule and utricle using described protocols.
Conclusions: With the method described, one can measure resting potentials from the scala media, saccule and utricle, as well as cochlear microphonics (CM) and even auditory nerve compound action potentials (CAP), in a single mouse.

Keywords: Electric potentials; Inner ear; Mice

1. Introduction

Cochlear bioelectrical activities, including the endocochlear potential (EP), cochlear microphonics (CM), summating potential (SP) and auditory nerve compound action potentials (CAP), are derived from different structures and cells in the cochlea. The endocochlear potential (EP) is a positive voltage of 80−100 mV seen in the endolymphatic space of the cochlea. The EP is only found in the cochlear portion of the inner ear. The endolymphatic space of the saccule, utricle and semicircular canals show much smaller resting potentials of only a few mV. Except for the EP, which is a resting potential mainly reflecting the function of stria vascularis, other cochlear potentials are auditory evoked responses from cochlear sensory hair cells or auditory nerve fibers of spiral ganglion neurons, respectively. Therefore, cochlear bioelectric activities recording is an ideal technique to study cochlear physiological functions (Rawdon-Smith and Hawkins, 1939). A number of animal models of inner ear diseases have already been established (MITF mice Tachibana et al., 2003), atoh1 mice (Fritzsch et al., 2005), GJB2 mice (Takada et al., 2014, etc) and the number is only growing larger. It is essential to use accurate detection methods for screening and determination in these disease models in order to reliably study cochlear electrophysiology under these conditions. This paper describes a protocol for recording the endocochlear potential (EP) and vestibular potentials from the murine inner ear.

2. Materials and methods

2.1. Animals

CBA/J mice were used for the study. Both strains were bred in-house and housed according to institutional protocols, with original breeding pairs purchased from Jackson Laboratory. Care and use of the animals in this study were approved by the Institutional Animal Care. All of the experimental mice had potentials of their scala media, saccule and utricle tested.

2.2. Instruments and reagents

The Following instruments were used during the recording process: Axopatch 200 (Axon), Axon digidata (Axon

* Corresponding author.
E-mail address: plagh@126.com (L. Zhao).
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Instruments 1322A), Stereo microscope (LEICA item number: 10446339), Glass electrode 1.5 mm 31 n (World Precision Instruments IB150F-3), Vertical pipette puller Model 720 (KOPF Instruments), 150 mmol potassium chloride (Sigma P-5405).

2.3. Glass electrode preparation

The glass electrodes are very important for the experiment. A high-melting point and thick initial wall are desired for the electrode. The electrode must be long enough for the contact with hair cells while as small as possible in diameter, and yet in an appropriate size to be held in still positions and controllable under a 10X microscope. Excessively large (micrometers) diameter may disturb the cell being tested and cause a leak of substances across the membrane. The electrode must also have its resistance value tested using a patch clamp. Resistance of 1–3 Ω is most ideal for the glass electrode. Resistance above or below this range will interfere with test results. A properly prepared glass electrode is essential for acquiring accurate EP results (Figs. 1 and 2).

2.4. Surgical procedure

Care must be taken due to the small size of murine inner ear. This method involves accessing the inner ear from the ventral side for recording scala media and vestibular potentials (Fig. 3).

The mouse was anesthetized with a combined regimen of ketamine (16.6 mg/ml) and xylazine (2.3 mg/ml) and placed on a polystyrene foam board. The base of the tail was secured with a piece of tape and a thin wire was hooked on the teeth to pull the body straight. The wire was then taped to the board and the front legs and rear legs were secured as well (Figs. 4–7).

A pair of small scissors was used to make a midline incision in the neck and the thyroid was exposed. The trachea was transected at the bottom of cartilage tracheal ring (Figs. 8 and 9). While the thyroid was retracted to the side with sutures, muscles below the thyroid were carefully separated and muscles over the middle ear removed. Care was taken to prevent any excessive bleeding, which can be fatal for the mouse, and the middle ear was exposed (Fig. 10).

Under a stereomicroscope, the transparent band on top of the middle ear was located, through which a hole was opened with micro forceps. Care was taken on probing depth not to damage the inner ear and stapedial artery. The hole was widened to expose the round window while care was taken not to damage the stapedial artery and nerves. A dark band near the basilar membrane in the hook region was identified, where hair cells are located (Fig. 11).

Fig. 1. The length of the glass electrode.

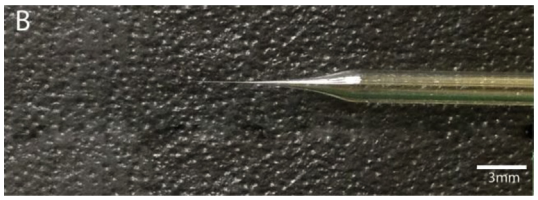

Fig. 2. The tip of the glass electrode.

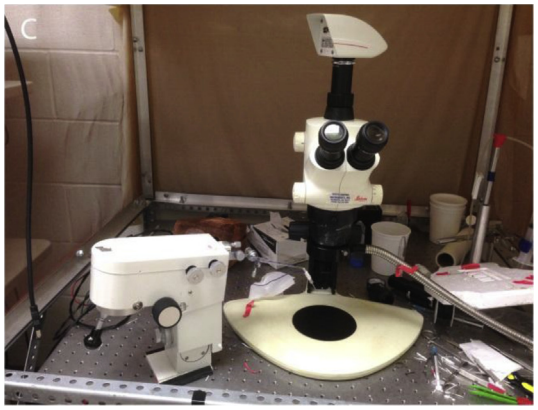

Fig. 3. Equipment for the experiment: stereomicroscope, Leica micromanipulator, Ferrari cage and isolation table.

Fig. 4. The base of the tail taped down.

2.5. Recording potentials from the scala media

A silver ball electrode was placed near the basilar membrane to record the CM and CAP with the TDT system

Fig. 5. A thin wire hooked on the teeth and pulled to straighten the body. The wire is then taped to the board.

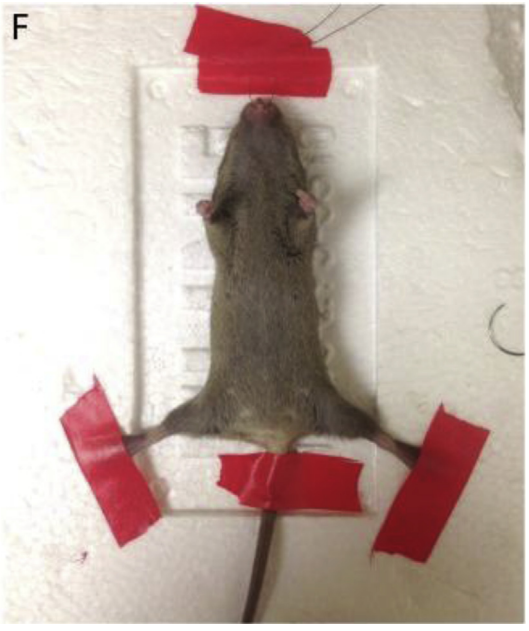

Fig. 6. Rear legs secured.

(Fig. 12). To record the EP from the scala media, the reference silver ball electrode was placed firmly against the inner ear bone near the round window. The Leica micromanipulator was gently rotated and, with the electrode in contact with the basilar membrane, the zero line was adjusted and baseline recorded. The glass electrode was inserted through the basilar membrane to access the scala media. During this insertion, the potential reading was initially −80 to −100 mV, followed by +80 to +100 mV readings as the electrode entered the scala

Fig. 7. Front legs secured.

Fig. 8. Midline incision in the neck with the thyroid exposed.

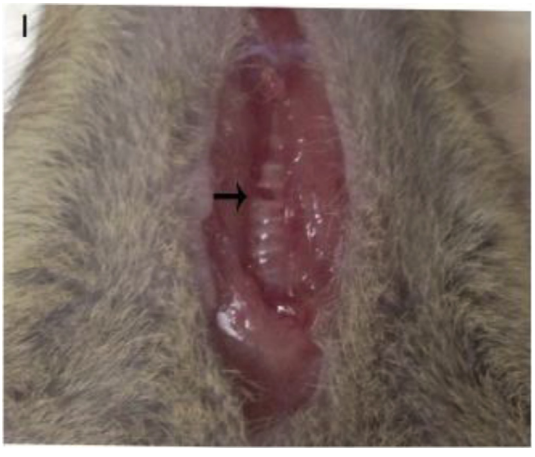

Fig. 9. Transsection at the bottom of cartilage tracheal ring (arrow head).

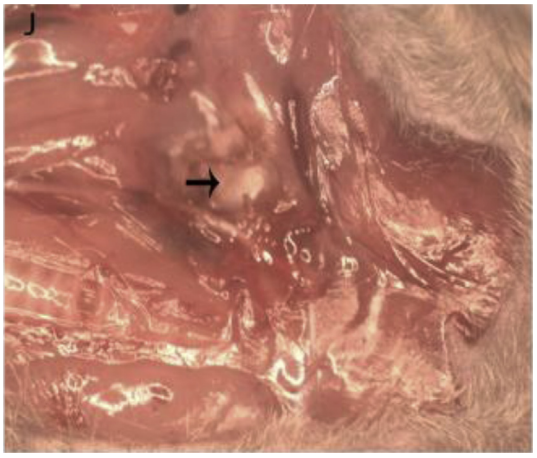

Fig. 10. Middle ear exposed (arrow head).

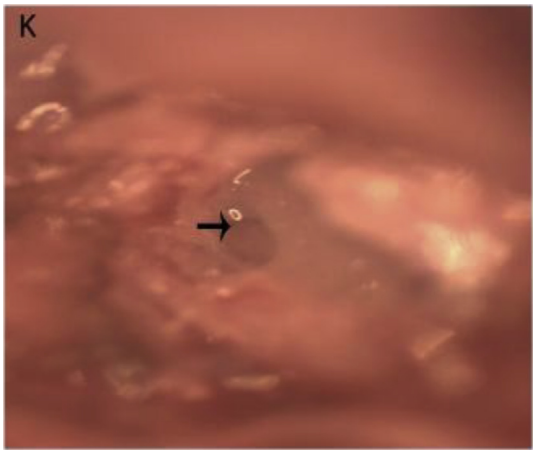

Fig. 11. Location of the basilar membrane near the hook region.

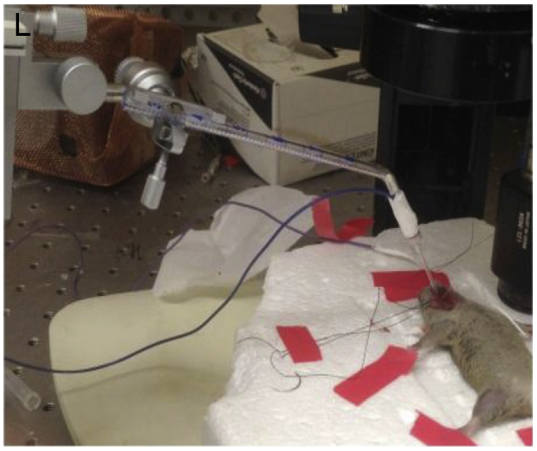

Fig. 12. Position of the mice in relation to the glass electrode.

media. The −80 mV reading appeared when the electrode was at the location of hair cells.

Recording potentials from the saccule: After recording scala media potentials, the glass electrode was directed downward and advanced through the vestibular membrane, with the reading going back to the zero line. The glass electrode was then advanced through the membrane of saccule and into the cavity of saccule. During this process, there were no negative potentials but a +5 to +10 mV reading when the electrode was inside the saccule. This is the potential of the saccule (Figs. 13–16).

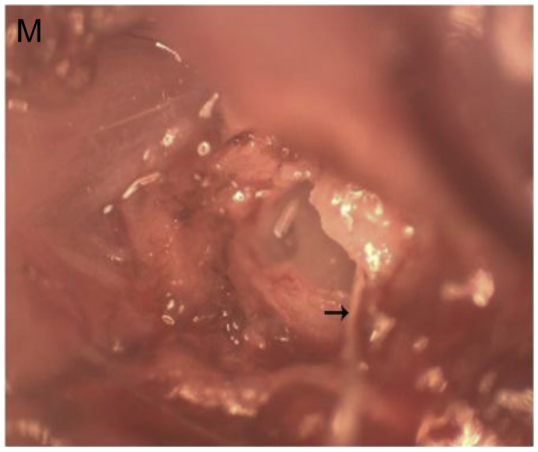

Fig. 13. The glass electrode in the cochlear (arrow head).

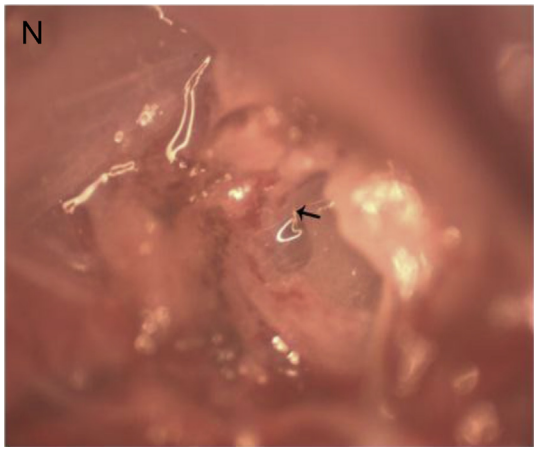

Fig. 14. Position of the glass electrode in the basilar membrane near the hook region (arrow head).

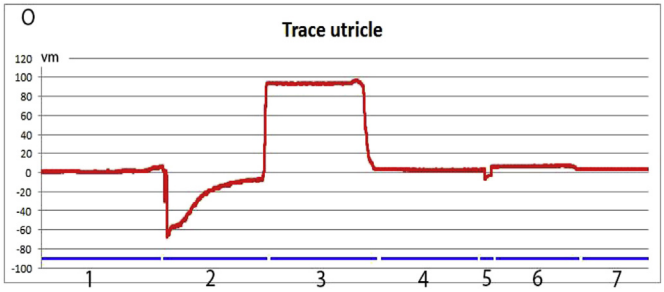

Fig. 15. Potentials of the scala media and utricle. From left to right: the glass electrode in contact with the basilar membrane; glass electrode in the basilar membrane; glass electrode into the scala media; glass electrode into the scala vestibule; glass electrode into the macula of utricle; glass electrode into the utricle; and glass electrode back to the scala vestibule.

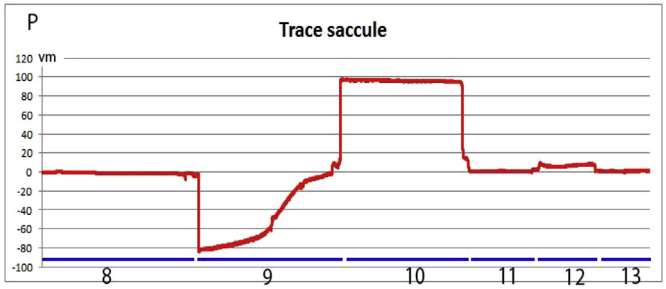

Fig. 16. Potential of the scala media and saccule. From left to right: the glass electrode in contact with the basilar membrane; glass electrode in the basilar membrane; glass electrode into the scala media; glass electrode into the scala vestibule; glass electrode into the saccule; and glass electrode back into the scala vestibule.

Recording potentials of the utricle: Following recording potentials of the scala media, the glass electrode was directed downward and advanced through the vestibular membrane into the utriclar macula to access the cavity of utricle. During this process, there was a −3 to −5 mV potential reading after returning to zero line, followed by +5 to +10 mV readings as the electrode entered into the utricle.

3. Results

The procedures of setup and operation for recording potentials from the scala media, saccule, and utricle in the mice were photographed and thoroughly documented. Refer to the figures for a depiction of the setup, operation and potential recordings.

4. Discussion

This paper describes a protocol of setup and operation for recording scala media and vestibular potentials in mice. Most classical studies have measured scala media potentials in the guinea pig or rat (Morizono et al., 1980; Konishi, 1979), where a hole has to be drilled on the bony wall of the cochlea. This can be difficult and is apt to damaging the spiral ligament. Besides, potentials of the saccule and utricle cannot be recorded through the same opening and the EP recorded through the classical method is usually relatively low. The approach described in the present report represents an improvement in that it allows recording the EP through the round window membrane, avoiding an opening on the bony wall of the cochlear. The glass electrode can be inserted directly into the scala media to record EP while potentials in the saccule and utricle can be recorded with the same microeletrode on the same animal. Also, the methods reported here can be used to record CM and CAP in mice (Wang et al., 2004). Thus, the described technique makes it possible to examine functions of different parts of the cochlea, including the stria vascularis and hair cells, as well as their interactions, using a single mouse.

The EP and potentials from the saccul and utricle recorded using our methods are consistent with previous reports. These potentials are important for studying functions of the stria vascularis and dark cells of vestibular organs. Although the scala media, saccule and utricle contain the same perilymph, there are noticeable differences in their resting potentials (Lundquist, 1976; Sitko et al., 1976). Therefore, our results also support the notion that potentials of the sacla media, saccule and utricle have no direct relationship with endolymphatic potassium (Morizono et al., 1980).

With the development of genomics and genetic technology, numerous animal models with genetic hearing loss or equilibrium impairment have been developed to study the biological mechanism of genetic mutations (Friedman et al., 2007). While the phenotype of these animal models may be different, relevant potentials must be tested to evaluate functional changes caused by the gene modification. Classical methods to record potentials from different inner ear compartments separately are labor intensive and time consuming. A convenient and practical method to quickly determine functions of different portions of the cochlea in mice is needed. The methods described in this report serve to meet such needs and can therefore be extremely useful in future inner ear studies using mice.

Acknowledgements

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References

Friedman, L.M., Dror, A.A., Avraham, K.B., 2007. Mouse models to study inner ear development and hereditary hearing loss. Int. J. Dev. Biol. 51 (6–7), 609–631.
Fritzsch, B., et al., 2005. Atoh1 null mice show directed afferent fiber growth to undifferentiated ear sensory epithelia followed by incomplete fiber retention. Dev. Dyn. 233 (2), 570–583.
Konishi, T., 1979. Some observations on negative endocochlear potential during anoxia. Acta Otolaryngol. 87 (5–6), 506–516.
Lundquist, P.G., 1976. Aspects on endolymphatic sac morphology and function. Arch. Otorhinolaryngol. 212 (4), 231–240.
Morizono, T., Rybak, L.P., Asp, S., 1980. Endocochlear potential and potassium concentration in endolymph and perilymph of the chinchilla. Arch. Otorhinolaryngol. 229 (2), 149–153.
Rawdon-Smith, A.F., Hawkins, J.E., 1939. The electrical activity of a denervated ear : (section of otology). Proc. R. Soc. Med. 32 (5), 496–512.
Sitko, S.T., Strelioff, D., Honrubia, V., 1976. Source and maintenance of the endocochlear potential. Trans. Sect. Otolaryngol. Am. Acad. Ophthalmol. Otolaryngol. 82 (3 Pt 1), 328–335.
Tachibana, M., Kobayashi, Y., Matsushima, Y., 2003. Mouse models for four types of Waardenburg syndrome. Pigment. Cell Res. 16 (5), 448–454.
Takada, Y., et al., 2014. Connexin 26 null mice exhibit spiral ganglion degeneration that can be blocked by BDNF gene therapy. Hear Res. 309, 124–135.
Wang, T., et al., 2004. The effect of tripterygium wilfordii polyglycosidium on the compound action potential of skeletal muscle in experimental animals with myasthenia gravis. Sichuan Da Xue Xue Bao Yi Xue Ban 35 (3), 379–381.