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Recording potentials from scala media, saccule and utricle in mice

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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

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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.

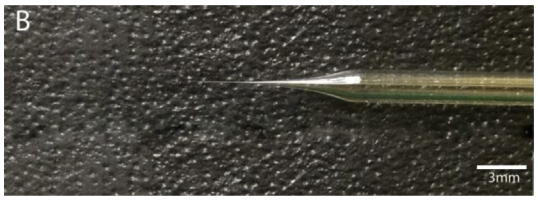

Fig. 2. The tip of the glass electrode.

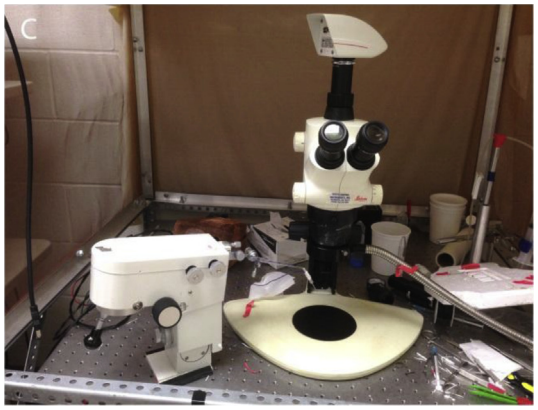

Fig. 3. Equipment for the experiment: stereomicroscope, Leica micromanipulator, Ferrari cage and isolation table.

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