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The hemicochlea preparation of the guinea pig and other mammalian cochleae

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Abstract

The hemicochlea and its slice preparation is a novel method that allows access to various cochlear structures without the physical distortion that typically occurs from tissue dehydration during the embedding process. Therefore, the hemicochlea preparation provides an excellent model to study (1) cochlear morphology during cochlear development, (2) malformation caused by genetic defects, (3) changes related to diseases, (4) sensory physiology, (5) cochlear micromechanics, and (6) the expression of proteins by immunohistochemistry. This paper describes in detail the method of slicing hemicochleae for different mammalian species, including mice, rats, gerbils, guinea pigs, pigs, and human temporal bones. Furthermore, guinea pig cochleae are used as an example to provide cochlear dimensions of important anatomical structures. The values obtained in eight guinea pig hemicochleae are compared to published values, and upon review, discrepancies do exist. For example, gelatinous structures, such as the tectorial membrane, appear larger in the hemicochlea when compared to traditional embedding. Dimensions obtained for selected cochlear structures at different locations along the guinea pig cochleae provide an improved basis for cochlear models.

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1. Introduction

It is important to examine cochlear morphology from many perspectives, including comparative anatomy, developmental changes, genetic malformation, disease related changes, sensory physiology and cochlear micromechanics, including cochlear modeling. A traditional method to investigate cochlear morphology is to fix, decalcify, dehydrate and embed the tissue. After embedding the cochlea in either plastic, paraffin or sucrose the specimen is serial-sectioned and placed on glass slides for staining. These procedures are laborious and may take several weeks or months. Moreover, tissue dehydration is accompanied by tissue shrinkage and distortion. According to the literature, tissue shrinkage is expected to be 5–25% (Edge et al., 1998; Lim, 1980). Distortion of the tectorial membrane and the basilar membrane caused by dehydration are generally obvious.

In addition to the “classical” histological techniques, other methods have been developed to examine fresh tissue. For example, Lim (1980) studied tectorial membranes from chinchilla cochleae after acutely isolating them. Also freshly isolated sections of the mammalian cochlea or temporal bone preparations have been used to study cochlear mechanics (Chan and Hudspeth, 2005; Gummer et al., 1996; Karavitaki et al., 1996; Markin and Hudspeth, 1995; Strelioff and Flock, 1984; Ulfendahl et al., 1989). A different method, the hemicochlea, was introduced to study fresh, unfixed and hydrated cochlear tissue (Edge et al., 1998; Hu et al., 1999; Richter et al., 2000, 1998). The term hemicochlea is derived from the fact that the experimental preparation is the result of sectioning an excised cochlea into two halves along its mid-modiolar plane. Unlike slice preparations, where a wedge of tissue is maintained for

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acute examination, the hemicochlea represents an intact half cochlea, where the tonotopic features of apical, middle and basal regions can be preserved and studied.

Slicing of pristine hemicochleae is non-trivial and the method varies from species to species. This paper describes the methods of slicing hemicochleae in mice, rats, gerbils, guinea pigs, pigs, and in human temporal bones. In addition, it will be demonstrated that this hemicochlea method provides anatomical measurements that differ from published data, as exemplified by the guinea pig tectorial membrane.

2. Methods

The method for sectioning hemicochleae in different mammalian species is detailed in the following sections. The method will then be used to systematically examine cochlear dimensions of the guinea pig. Video images from eight guinea pig hemicochleae were taken and cochlear dimensions of the non-dehydrated specimen were measured.

2.1. Common instrumentation and procedures

Independent of the animal's age, hemicochleae can be sliced in the mouse, rat, gerbil, and guinea pig using the vibratome. The same holds for the cutting of cochlear slices. In contrast to the aforementioned species, pig and human cochleae require a diamond wire saw for cutting. In our laboratory, we use a modified vibratome (Vibratome 1000+, Pelco 102, TedPella, USA) or a diamond wire saw (SXJ-2, Precision wire saw, MTI Corporation). Initially, the vibratome's minimum factory setting for the blade's advance was too fast, and poor cuts resulted. The advance speed was changed by soldering a 10 kΩ resistor in series to the 25 kΩ potentiometer used to set the blade's advance speed. Cochleae are fixed to the vibratome tray with cyanoacrylate (Superglue-gel, Loctite, Henkel, OH, USA) or with paraffin wax. For additional stabilization of the specimen while slicing, forceps are placed behind the cochlea to generate a slight counter-pressure to the blade's push. Care must be taken not to loosen the cochlea during the cutting procedure. For illumination and visualization, all hemicochleae are placed in high vacuum grease (Dow Corning, Midland, MI) in perilymph-filled petri dishes on the stage of an upright microscope. The grease allows for the orienting of the hemicochlea for each turn. The microscope is placed on a vibration isolation table (Newport, model RS4000) and is equipped with 10*x*, 25*x* and 40*x* water-immersion objectives. Despite most of the organ of Corti tissues being light transparent, different translucent structures can still be visualized by oblique illumination with five red light emitting diodes (LEDs, P408-ND, Digi-Key Corp., Thief River Falls, MN) from below (Edge et al., 1998; Kachar, 1985). Blue and white LEDs (P465-ND and 365-1177-ND Digi-Key Corp., Thief River Falls, MN) will work as well. For example, blue LEDs can be used as radiation source for FITC (fluorescein isothiocyanate) imaging. Pictures are taken with a CCD camera (GP-MF602, Panasonic), which is attached to one port of the viewing head.

2.2. Harvesting the cochlea

2.2.1. Gerbils (Meriones unguiculatus)

Animals (ages 1 day–2 years) are injected intraperitoneally with a lethal dose of sodium pentobarbital (180 mg/kg body weight). Following decapitation, the head is divided in the medial plane and the bullae are removed. Next, one of the bullae is opened and the cochlea is exposed. With two forceps the cochlea is dissected from its surrounding bony structures. Cochleae from animals older than 28 days typically yield an increased risk of fracturing throughout the specimen due to higher calcification. Special care must be taken when the cochleae are separated from the semicircular canals as the cochlear wall is often damaged during this procedure. The semicircular canals are cut individually with a malleus knipper and no further separation of the remaining parts from the cochlea should be attempted.

2.2.2. Mice and rats

Harvesting the cochlea in mice and rats is similar to the methodology as described above for gerbils. Animal ages can be from day of birth to adult. After euthanizing the animal with a single sodium pentobarbital injection (200 mg/kg body weight), the animal is decapitated and the head is divided along the medial plane. One of the half-heads is held with a pair of forceps to allow a second pair of forceps to enter the outer ear canal. With this second pair of forceps, the annulus of the bony outer ear canal is grabbed and pulled caudally. The bulla separates along fissure lines and exposes the cochlea and semicircular canals. The dissection leaves the semicircular canals in place, allowing the semicircular canals to act as a natural shim.

2.2.3. Guinea pigs

Guinea pigs are euthanized with sodium pentobarbital 200 mg/kg body weight and are decapitated. Animal ages can be from day of birth to adult. Again, the head is cut in the medial plane and the bullae are removed. Once the bullae are separated from the head, they are opened carefully and the exposed cochlea can be dissected. Similar to the gerbil, each semicircular canal should be dissected individually. Compared to gerbils, the guinea pigs' bullae and cochleae tend to be more calcified, which increases the risk of inadvertent fractures throughout the cochleae. Therefore, only little parts of bone should be dissected with a malleus knipper at a time. It is helpful to remove as much bone as possible surmounting the apex and base of the cochlea in order to better orient the specimen on the vibratome. After immersion in artificial perilymph (described below) the cochlea can be cut with a vibratome. Cutting the cochlea with a diamond wire saw is also possible but has disadvantages over using the vibratome. First, the diamond wire saw removes tissue from the cochlea according to the wobble and the diameter of the diamond wire. The smallest wire diameter commercially available to us was 180 μm. Second, the cut surface tends to collect "bone dust" from the cutting procedure despite constant irrigation. Therefore, it is recommended that the vibratome be used to slice guinea pig cochleae.

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