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The relationship of the round window membrane to the cochlear aqueduct shown in three-dimensional imaging

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Abstract

The round window membrane and cochlear aqueduct complex in the guinea pig are reconstructed with 3D-imaging, using orthogonal plane fluorescence optical sectioning (OPFOS).

The 3D-images show that the periotic duct and the aqueduct are connected to a pouch-like extension of the round window.

The function of this may be regulation of aqueduct flow resistance under the influence of a pressure difference between inner ear fluid and middle ear.

Keywords: Round window membrane; Cochlear aqueduct; OPFOS; Perilymph; Flow resistance

1. Introduction

The cochlear aqueduct is a narrow channel between the subarachnoid space and the inner ear. In the inner ear its opening is in scala tympani, close to the round window membrane (Ghiz et al., 2001). The cochlear aqueduct was first noticed by Du Verney (1683), and about one century later Cotugno (1761) described it in detail.

In humans, it is about 1 cm long and has a smallest diameter of 0.14 mm. The aperture of the aqueduct at the dural end has the shape of a flat funnel (Gopen et al., 1997). In guinea pigs, the aqueduct is much shorter (about 2 mm) but not narrower than in humans (Ghiz et al., 2001).

The cochlear aqueduct is defined as the bony duct passing through the temporal bone from the inner ear on the lateral side to the dura on the medial side. The periotic duct tissue within the lumen of the cochlear aqueduct consists of fibroblasts and loosely arranged connective tissue (Nishimura et al., 1981). Pressure and fluid exchange is possible through the periotic duct (Palva and Dammert, 1969).

The round window membrane is fixated to the temporal bone. It has a pouch-like extension, just adjacent to the opening of the cochlear aqueduct. The membrane is a single cell-layer structure, while the extension is multi cell-layered, about 400 μm in length and 100 μm in diameter (Laurens-Thalen, 2004).

The cochlear aqueduct is thought to play a role in maintaining fluid and pressure balance between the inner ear and the cerebrospinal fluid (CSF). For sudden variations of CSF-pressure it behaves as a low-pass filter with a time constant of about 2 s in the guinea pig (Thalen et al., 2002).

Abbreviations: 3D, three-dimensional; OPFOS, orthogonal plane fluorescence optical sectioning; CSF, cerebrospinal fluid; EDTA, ethylenediaminetetraacetic acid; RITC, rhodamine-B isothiocyanate; He–Ne LASER, Helium–Neon Light Amplification by stimulated emission of Radiation; CCD, charge coupled device; IMOD, Image processing, MOdeling and Display program; 2D, two-dimensional; BCN, Behavioural and Cognitive Neuroscience

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Based on the finding that the resistance for fluid flow of the aqueduct is related to the position of the round window membrane, it is assumed that the permeability of the connective tissue (Duckert, 1974; Toriya et al., 1991a) inside the aqueduct changes if the position of the round window membrane changes (Wit et al., 2003). This assumption is supported by the fact that this connective tissue is attached to the pouch-like extension of the round window membrane (Toriya et al., 1991b).

In the present work, three-dimensional (3D) reconstructions are presented of the membranous aqueduct and the round window membrane together, with the aim to give insight in the morphology of the round window membrane–cochlear aqueduct region of the inner ear.

Orthogonal-plane fluorescence optical sectioning (OPFOS) microscopy was used to obtain these 3D-reconstructions (Voie et al., 1993; Voie and Spelman, 1995; Voie, 2002).

2. Material and methods

One healthy female albino guinea pig (Harlan Laboratories, UK) weighing 450 g was used. Animal care and use were approved by the Experimental Animal Committee of Groningen University, protocol No. 2883, in accordance with the principles of the Declaration of Helsinki.

2.1. Fixation procedure

The animal was terminated by lethal administration of sodiumpentobarbital. After decapitation the bulla was dissected and fixated in a 10% formalin solution, neutral buffered. Then the bulla was rinsed in aqua-dest. Decalcification in ethylenediaminetetraacetic acid 10% solution (EDTA; Sigma, ED5SS, pH 7.4) took place at a temperature of 50 °C in a microwave oven (T/T MEGA microwave histoprocessor, Milestone) in eight sessions of 6 h. After decalcification the bulla was again rinsed with aqua-dest and dehydrated in a graded seven-step ethanol series (30%, 50%, 70%, 90%, 96%, 100%, 100%).

Spalteholz fluid, a 5:3 solution of methyl salicyclate (Sigma, M-6752) and benzyl benzoate (Sigma, B-6630) (Spalteholz, 1914), was thereafter used to achieve transparency of the specimen. The clearing process consisted of application of a succession of Spalteholz–ethanol solutions, 24 h each. The Spalteholz fluid fraction in the clearing session was 25%, 50%, 75%, 100%, 100%, respectively. Hereafter the specimen was dyed in a fluorescent dye bath of Rhodamine-B Isothiocyanate (RITC; Sigma, R-1755). RITC absorbs maximally at 570 nm and emits at 595 nm. The dye bath was prepared by dissolving 1.0 mg/ml RITC into ethanol, followed by dilution in Spalteholz fluid to a final dye concentration of 5×10^{-4} mg/ml (Voie et al., 1993; Voie, 2003). The specimen was dyed for 4 days.

2.2. OPFOS imaging system

An overall schematic of the OPFOS imaging system is given in Fig. 1. The beam of a green 2.0 mW He–Ne LASER (Research Electro Optics R-30972; wavelength 543 nm) is expanded into a 3 cm diameter parallel bundle. The 30× beam expander consists of two independently positionable lenses with focal lengths of

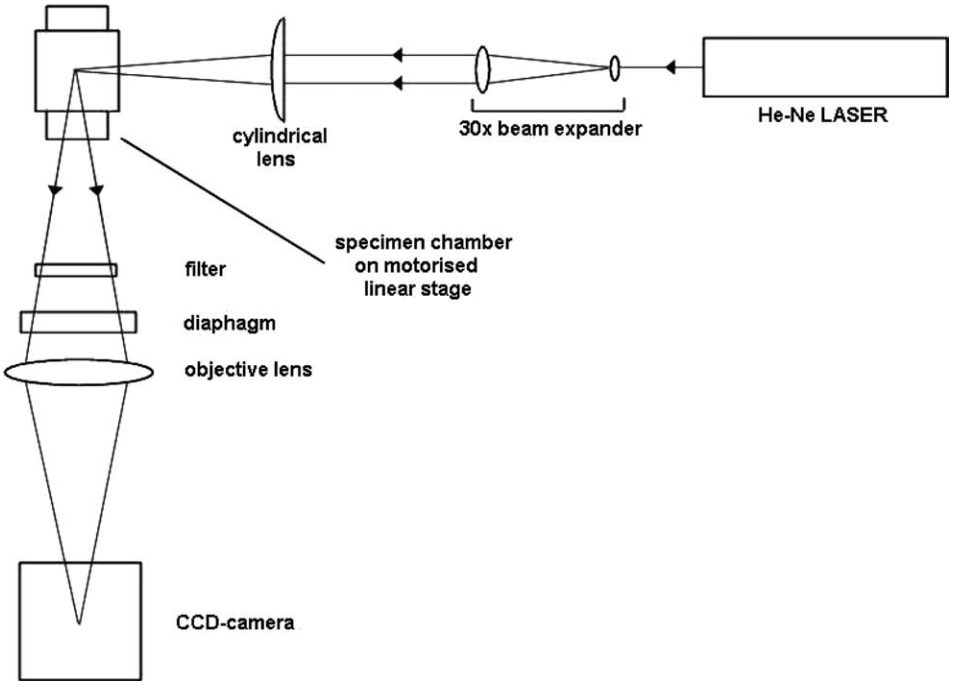

Fig. 1. Schematic of the OPFOS set-up.