Hearing Research 306 (2013) 145–155

Contents lists available at ScienceDirect

Hearing Research

journal homepage: www.elsevier.com/locate/heares

Research paper

Spiral ganglion neuron quantification in the guinea pig cochlea using Confocal Laser Scanning Microscopy compared to embedding methods

Antonina Wrzeszcz [a,*,1], Günter Reuter [a,1], Ingo Nolte [b], Thomas Lenarz [a], Verena Scheper [a]

[a] Department of Otolaryngology, Hannover Medical School, Carl-Neuberg-Str. 1, 30625 Hannover, Germany
[b] Small Animal Clinic, University of Veterinary Medicine Hannover, Bünteweg 9, 30559 Hannover, Germany

ARTICLE INFO

Article history:
Received 13 May 2013
Received in revised form
10 July 2013
Accepted 6 August 2013
Available online 20 August 2013

ABSTRACT

Neuron counting in the cochlea is a crucial but time-consuming operation for which various methods have been developed. To improve simplicity and efficiency, we tested an imaging method of the cochlea, and based on Confocal Laser Scanning Microscopy (CLSM), we visualised Rosenthal's Canal and quantified the spiral ganglion neurons (SGN) within.

Cochleae of 8 normal hearing guinea pigs and one implanted with a silicone filament were fixed in paraformaldehyde (PFA), decalcified, dehydrated and cleared in Spalteholz solution. Using the tissue's autofluorescence, CLSM was performed at 100fold magnification generating z-series stacks of about 20 slices of the modiolus. In 5 midmodiolar slices per cochlea the perimeters of the Rosenthal's Canal were surveyed, representative neuron diameters were measured and the neurons first counted manually and then software-assisted. For comparison, 8 normal hearing guinea pig cochleae were embedded in paraffin and examined similarly.

The CLSM method has the advantage that the cochleae remain intact as an organ and keep their geometrical structure. Z-stack creation is nearly fully-automatic and frequently repeatable with various objectives and step sizes and without visible bleaching. The tissue shows minimal or no shrinking artefacts and damage typical of embedding and sectioning. As a result, the cells in the cleared cochleae reach an average diameter of 21 μm and a density of about 18 cells/10,000 μm^2 with no significant difference between the manual and the automatical counts. Subsequently we compared the CLSM data with those generated using the established method of paraffin slides, where the SGN reached a mean density of 9.5 cells/10,000 μm^2 and a mean soma diameter of 13.6 μm.

We were able to prove that the semi-automatic CLSM method is a simple and effective technique for auditory neuron count. It provides a high grade of tissue preservation and the automatic stack-generation as well as the counter software reduces the effort considerably. In addition this visualisation technique offers the potential to detect the position and orientation of cochlear implants (CI) within the cochlea and tissue growing in the scala tympani around the CI and at the position of the cochleostomy due to the fact that the implant does not have to be removed to perform histology as in case of the paraffin method.

1. Introduction

The treatment of profoundly deaf patients has been revolutionised in the past decades by the introduction of cochlear implants (CI). Meanwhile cochlear implantation has become widely accepted as routine treatment for patients with complete and incomplete sensorineural hearing loss. However, there are still large individual differences in the level of success achieved with a CI.

Since the implant takes over the function of damaged hair cells by means of direct electrical stimulation of the spiral ganglion neurons (SGN), it was assumed that the success of CI's is also

Abbrevations: CLSM, Confocal Laser Scanning Microscopy; SGN, spiral ganglion neurons; CI, cochlear implant; PFA, paraformaldehyde; aABR, acoustically evoked auditory brainstem response; EDTA, ethylenediamine tetraacetic acid-disodium salt; MSBB, methyl salicylate benzyl benzoate; HE, hematoxylin and eosin

* Corresponding author. Tel.: +49 511 532 8284; fax: +49 511 532 3293.
E-mail address: wrzeszcz.antonina@mh-hannover.de (A. Wrzeszcz).

[1] The two authors contributed equally.

0378-5955/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.heares.2013.08.002

dependent on the number, survival and responsiveness of SGN available for electrical stimulation. After onset of deafness these neurons undergo degeneration, which progresses with ongoing deafness (Otte et al., 1978). Accordingly, in the literature the number of SGN was viewed as one of the crucial elements for the success of cochlear implantation (Lousteau, 1987; Incesulu and Nadol, 1998).

In current publications this hypothesis could not be confirmed distinctly in human patients. The high number of variables that can influence CI performance aggravate the comparisons between conditions of the cochlear tissues (nerve and hair cell survival, fibrosis and new bone) and implant function, particularly for complex functional measures such as speech recognition. Nevertheless, interpretation of correlations in animal models and within-subject across-site comparisons in humans suggest that conditions near each stimulation site have a strong influence on cochlear implant function (Pfingst et al., 2011). It is therefore still crucial to evaluate SGN number and survival.

One focus of various research groups all around the world is the protection or even regeneration of SGN to improve the cochlear implants' outcome. Nowadays it is widely accepted that neurotrophic factors such as glial cell line-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF) as well as electrical stimulation, if applied as single and combined stimuli directly and delayed after the onset of deafness, protect SGN from degeneration (Staecker et al., 1996; Ylikoski et al., 1998; Gillespie and Shepherd, 2005; Scheper et al., 2009). Research is extended to evaluate the optimal drug concentrations and delivery methods in vivo (Yagi et al., 2000; Garnham et al., 2005; Borenstein, 2011; Wise et al., 2011).

To verify if a suggested therapy has an effect on the SGN survival, next to electrophysiological measurements, histology has to be performed to evaluate the number of surviving SGN. Therefore the counting of primary auditory neurons in the cochlea is crucial to establish novel therapies for inner ear treatment. Different histological methods such as embedding the cochlea into paraffin (de Franceschi et al., 2011), celloidin (Hinojosa and Nelson, 2011) or Araldite resin (Zilberstein et al., 2012) to subsequently generate histological sections (Fig. 1A) are used. Embedding the cochlea into matrixes like OCT (Sly et al., 2012) or epoxy resin and sectioning it on a cryostat or grinding it (Fig. 1B) with documentation of every slice, respectively, are common methods for SGN evaluation. Electron microscopy provides high resolution images of the detailed cell morphology (Kellerhals, 1967). All those methods are time-consuming and elaborative, moreover, they are disruptive to the three-dimensional structures of the organ and result in severe shrinkage and damage artefacts of the delicate tissues. The cochlea has a complex spiral-shaped structure, consisting of large fluid-filled spaces and a wide variety of tissue types ranging from dense bone to fragile membranous structures and the delicate organ of Corti containing many different cell types.

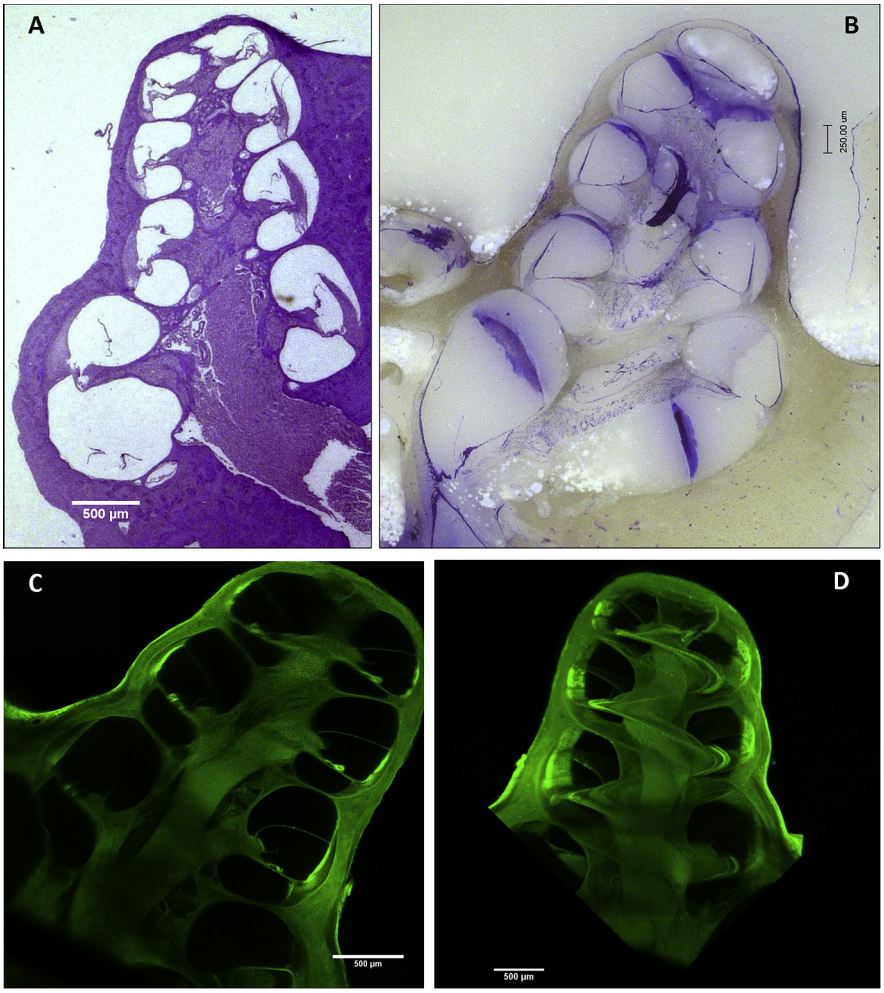

Fig. 1. (A)Paraffin-embedded cochlear section; (B) epoxy resin-embedded and ground section, (C) one optical section and (D) whole 3D optical volume from CLSM scan, 5x objective, 10 µm step size (green = autofluorescence). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)