Available online at www.sciencedirect.com
SCIENCE DIRECT®
Hearing Research 202 (2005) 63–73
HEARING RESEARCH
www.elsevier.com/locate/heares

A physiological place–frequency map of the cochlea in the CBA/J mouse

Marcus Müller, Karen von Hünerbein, Silvi Hoidis, Jean W.T. Smolders *

Physiologisches Institut II, J.W. Goethe-Universität Frankfurt, Theodor-Stern-Kai 7, 60590 Frankfurt am Main, Germany

Received 29 April 2004; accepted 17 August 2004
Available online 13 October 2004

Abstract

Genetically manipulated mice have gained a prominent role in in vivo research on development and function of the auditory system. A prerequisite for the interpretation of normal and abnormal structural and functional features of the inner ear is the exact knowledge of the cochlear place–frequency map. Using a stereotaxic approach to the projection site of the auditory nerve fibers in the cochlear nucleus, we succeeded in labelling physiologically characterized auditory nerve afferents and determined their peripheral innervation site in the cochlea.

From the neuronal characteristic frequency (CF) and the innervation site in the organ of Corti a place–frequency map was established for characteristic frequencies between 7.2 and 61.8 kHz, corresponding to locations between 90% and 10% basilar membrane length (base = 0%, apex = 100%, mean length measured under the inner hair cells 5.13 mm). The relation between normalized distance from the base (d) and frequency (kHz) can be described by a simple logarithmic function: $d(\%) = 156.5 - 82.5 \times \log(f)$, with a slope of 1.25 mm/octave of frequency. The present map, recorded under physiological conditions, differs from earlier maps determined with different methods. The simple logarithmic place–frequency relation found in the mouse indicates that mice are acoustic generalists rather than specialists.

Keywords: Mouse; Cochlea; Place–frequency map; Cochlear nucleus; Tonotopy

1. Introduction

Mice have gained a prominent role in auditory research because of the enormous possibilities opened by new methods in molecular biology and genetic engineering. Transgenic and gene targeting technologies in mice allow in vivo analysis of the functional role of specific genes. Genetically modified mice therefore have become a major source in acquiring more insights into the development and function of the auditory system. However, since the inner ear in mice is very small and difficult to handle when compared to cat or guinea pig, only limited information is available concerning basic physiologic parameters of the peripheral auditory system. One very important parameter is the projection of frequency along the cochlear partition, the cochlear place–frequency map, obtained under normal physiological conditions. The exact knowledge of the cochlear place–frequency map under such conditions is a prerequisite for the interpretation of normal and abnormal structural and functional features of the inner ear. Normal physiological conditions require an untouched cochlea, and when acoustic stimuli are applied for its

Abbreviations: ABR, Auditory brainstem response; BM, Basilar membrane; CF, Characteristic frequency; ECG, Electrocardiogram; FTC, Frequency tuning curve; HRP, Horse radish peroxydase; PB, Phosphate buffer; SD, Standard deviation

* Corresponding author. Tel.: +49 69 6301 6980; fax: +49 69 6301 6987.
E-mail address: Jean.Smolders@em.uni-frankfurt.de (J.W.T. Smolders)

0378-5955/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.heares.2004.08.011

determination they must not reach sound pressure levels that could damage the cochlea. A physiological place–frequency map can therefore only be established by labelling physiologically characterized auditory nerve fibers in vivo and tracing their peripheral course to the innervation site in the cochlea histologically. Such maps have been established in a number of species (cat: Liberman, 1982; mustache bat: Kössl and Vater, 1985; fat tailed gerbil: Müller, 1990; Müller et al., 1991a; rat: Müller, 1991a,b; mole rat: Müller et al., 1992; opossum: Müller et al., 1993; Mongolian gerbil: Müller, 1996), but not in the mouse. The small size of the mouse auditory periphery makes physiological recordings from the auditory nerve very difficult. Using a stereotaxic approach to the projection site of the auditory nerve fibers in the cochlear nucleus, we succeeded in labelling physiologically characterized auditory nerve afferents and determined a physiological place–frequency map in the normal mouse cochlea.

Estimates of the cochlear place–frequency maps of mice explored with different methods are available, however, none of them satisfy the above-mentioned physiological conditions. Based on direct observations of the travelling wave in cochlear explants, Georg von Békésy (1944) determined the first place–frequency map of the mouse post-mortem. However, it has been shown in a number of species, for example in rat (Müller, 1991b), that the place–frequency map post-mortem deviates by 1–2 octaves from that determined under physiological conditions. Based on behavioral experiments, Ehret (1975) presented a map of the mouse cochlea. There are however theoretical assumptions in the construction of this map, which still have to be experimentally proven. Recently Ou et al. (2000) determined an anatomically based place–frequency map. After noise exposure the latter authors correlated histological lesions of cochlear hair cells with permanent threshold shifts obtained from auditory brainstem response (ABR) measurements. Inherent to this method, however, is the use of sound exposure levels that destroy the hair cells in the cochlea leading to abnormal cochlear function.

The most accurate way to obtain cochlear place–frequency maps is the injection of a tracer into single physiologically characterized auditory nerve fibers in vivo. However, in small mammals like the mouse, the cochlear nerve is very hard to access, especially when the experiment requires considerable survival time of the animal, as is the case in tracing experiments. We therefore used the method introduced in the horseshoe bat (Vater et al., 1985), injecting a neuronal tracer (HRP) into the cochlear nucleus at physiologically characterized sites in vivo. Subsequently the retrograde transport pattern of HRP into the cochlea was analysed. The physiological place–frequency map of the mouse inner ear determined this way, shows a simple linear relation of location along the cochlear partition as a function of log(frequency).

2. Materials and methods

2.1. Animals

Experiments were performed on CBA/J mice (*Mus musculus*) of either sex aged 6–13 weeks (average 7.7 weeks). The total number of animals used in this study amounted to 79. Animals were obtained from Charles River and kept in our animal housing. First and second generation offspring of these animals were also used. The care and use of the animals reported in this study was approved by the state authorities responsible (Regierungspräsidium Darmstadt).

2.2. Stimulation and recording

Electrophysiological recordings were performed in a custom-made sound proof chamber. For stimulus generation and recording of responses, a multi-function IO-Card (National Instruments) was used, housed in an IBM compatible computer. Sound pressure level was controlled with an attenuator (Tucker Davies Technologies (TDT) PA4) and a custom-made amplifier. Stimuli were delivered to the ear in a calibrated open system by a loudspeaker (TDT ESD1) placed 3–5 cm lateral to the animal's pinna. Sound pressure was calibrated on-line prior to each measurement with a microphone probe system (Bruel & Kjaer 4139 and 2636) placed near the animal's pinna. Brainstem responses were amplified by a custom-made low noise differential amplifier. Single unit activity was recorded with a BA1S (npi) amplifier. Signals were bandpass filtered and further amplified (TDT PC1; 0.3–5 kHz, 40 dB gain). Single-units responses were routed through a window discriminator and fed into the computer and an audiomonitor. ABRs were fed to the multi-function IO-board and sampled at a rate of 10 kHz. Custom-made software developed with National Instruments Measurement Studio (LabWindows CVI) was used in all experiments. For statistical analyses and curve fitting Systat 9 was used.

2.3. Audiograms

ABRs to clicks and tone bursts were recorded in anaesthetized animals prior to surgery and, in a few animals, also during and after the HRP application.

Tone pips of 3-ms duration (1-ms rise and fall time, cosine shaped) or clicks (100-μs width) were presented at a rate of 60/s. To record bioelectrical potentials, subdermal silver-wire electrodes were inserted at the vertex (active) and ventro-lateral to the measured ear (reference). After amplification and bandpass filtering (100 dB, 0.3–5 kHz), electrical signals were averaged over 32 repetitions of stimulus pairs with alternating phase. ABRs were recorded for frequencies between 2.0 and 45.2 kHz at a resolution of 2 steps per octave. For each frequency, ABRs