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Research paper

Induction of single-sided deafness in the newborn rat and its consequence for cochlear nucleus volume development

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ABSTRACT

Aim of this study was to induce a single-sided deafness (SSD) in rats before hearing onset. Rats were operated at postnatal day 10 by approaching the tympanic cavity along a retroauricular path without manipulating ossicles or tympanic membrane. The ototoxic aminoglycoside neomycin was injected intracochlearly through the round window membrane on one side. When the animals have reached young adult stages, their hearing threshold was determined by their auditory brainstem response (ABR). Monaural deafening was considered successful when the hearing threshold was at least 95 dB above the threshold of the normal hearing ear. Growing up with one non-functional ear, rats developed a striking anatomical asymmetry of their cochlear nuclei (CN). The CN from age-matched normal hearing brains and from both sides of single-sided deaf brains were cut into series of frontal sections and their volumes calculated. No difference was detected between the volume of the normal hearing CN and the contralateral CN in SSD rats. By contrast, growth retardation was found for the ventral CN on the deaf side to result in a volume of only 57% compared to the normal hearing side. Marginal growth retardation was also observed for the dorsal CN on the deaf side. Thus, loss of sensory activation leads mainly, but not exclusively, to a reduction of tissue volume in the ventral CN of the deaf side, leaving the contralateral side apparently unaffected.

1. Introduction

Deafness experimentally induced by diverse procedures has been used to investigate neuro- and glioplasticity of the central auditory system in the mammalian brain. Different methods to achieve monaural deafening were published such as surgical cochleotomy (Argence et al., 2008; Illing and Horváth, 1995), intracochlear injection of aminoglycoside antibiotics (Argence et al., 2008), aspiration of cochlear contents (Hatano et al., 2009), or noise trauma (Michler and Illing, 2002). There are several animal-studies inducing binaural deafness by systemic injection of aminoglycoside antibiotics (Jakob et al., 2015; Matsuda et al., 1999; Osako et al., 1979; Rosskothen-Kuhl and Illing, 2012). For monaural deafening, different species of animals were used such as cats (Hardie and Shepherd, 1999), gerbils (Hessel et al., 1998), or rats (Argence et al., 2008; Hatano et al., 2009). In these studies on rats the integrity of the middle ear structures was not maintained. The major aim of the present study was to establish a method of unilateral deafening under preservation of the integrity of tympanic membrane and ossicular chain, maintaining the transfer function of the middle ear so that ABR measurement could be performed at later developmental stages to verify the success of the procedure. The second aim was the assessment of the effect of neonatal unilateral hearing loss for the subsequent growth of the rat CN up to maturation. As apparent from the literature, the reduced volume increase of the CN markedly varies between species, the age at induction of deafness, and the method of deafening.

Since the supply of patients suffering from single-sided deafness (SSD) with a cochlear implant becomes an increasingly widespread procedure for hearing rehabilitation including spatial hearing, basic

Abbreviations: ABR, auditory brainstem response; AVCN, anteroventral cochlear nucleus; BW, bony wall; CI, cochlea implant; CL, current level; CN, cochlear nucleus; Co, cochlea; contra, contralateral; dB, decibel; DCN, dorsal cochlear nucleus; EABR, electrical auditory brainstem response; EIS, electrical intracochlear stimulation; EP, evoked potential; ipsi, ipsilateral; MH, malleus handle; NH, normal hearing; n7, facial nerve; PVCN, posteroventral cochlear nucleus; RW, round window; S, stapes; SL, sensation level; SM, stapedius muscle; SSD, single sided deafness; TC, tympanic cavity; TH, threshold; TM, tympanic membrane; VCN, ventral cochlear nucleus

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data as collected here are mandatory to understand the state of the brain upon which the electrical stimulation is imposed.

2. Material and methods

2.1. Animals

This study is based on 79 Wistar rats of either sex. The study was carried out in strict accordance with EU Directive 2010/63/EU for animal experiments. Care and use of the animals as reported here were approved by the appropriate agency (Regierungspräsidium Freiburg, permission number 35-9185.81/G-10/116). Two groups of rats were formed: normal hearing rats (NH, n = 3) and single sided deafened rats (SSD, n = 64). For the SSD group, 6 litters with 4–14 pups were operated on the tenth postnatal day (P10). Mean body weight on P10 varied between 14.9 and 22.9 g.

For surgery, P10 rats were anesthetized by isoflurane (Forene 100% [V/V], Abbott GmbH & Co. KG, Wiesbaden, Germany). For measurements of the auditory brainstem response (ABR), rats were anaesthetized with an intraperitoneally injected mixture of ketamine (50 mg/kg, Bela-Pharm GmbH & Co. KG, Vechta, Germany) and xylazine (5 mg/kg, Rompun, Bayer-Leverkusen, Germany). Preceding transcardial perfusion, rats were given a lethal dose of sodium-thiopental (50–100 mg/kg i.p., Trapanal, Nycomed, Konstanz, Germany).

2.2. Surgical approach

Under general isoflurane anesthesia (1.5–2 Vol.%), the surgical procedure aimed to preserve the tympanic membrane and the ossicular chain for later measurement of ABR. Our approach formerly used for cochlear implantation and cochleotomy in adult rats (Illing and Michler, 2001; Jakob et al., 2015) is incompatible with this goal as tympanic membrane and ossicular chain are destroyed. Therefore, a retroauricular approach was used including identification of the facial nerve and its exit point from the skull. The facial nerve remained uninjured. Ventrorostal of its exit point the bony bulla of the middle ear was identified. As the bony wall in newborns is soft, a hole was made with a forceps ventral to the facial nerve exit point (Fig. 1A). The mucosa of the middle ear was opened by a needle and the round window niche exposed. The round window membrane was then perforated, releasing liquid (perilymph) from the inner ear. With a syringe, 10 μl of the ototoxic aminoglycoside antibiotic neomycin (10 mg/ml; Sigma–Aldrich Chemie GmbH, Steinheim, Germany) was gently injected intracochlearly until the liquid of the perilymph and the injected neomycin escaped from the cochlea. The injection was repeated twice, pausing for 1 min each. After 3 injections in total, the skin was surgically closed.

To identify critical parameters of the application method, pups (n = 12) were sham-operated, 7 by injecting buffered saline instead of neomycin into the inner ear, and 5 by injecting neomycin into the middle ear without perforating the round window.

2.3. Verification of hearing loss

After 6–8 weeks of development into sexual maturity, hearing thresholds of operated and untreated animals were tested by recording the ABR. For ABR recording, steel needle electrodes were placed subcutaneously at vertex and mastoids and a 20 Hz train of click stimuli was presented to each side separately through brass pipes equipped with a conical plastic tip positioned into the outer ear canal under general anesthesia. The ABR mean amplitudes were determined after 300 sweeps recorded in a frequency band of 0.1–3 kHz (Rosskothen-Kuhl and Illing, 2012). The minimal

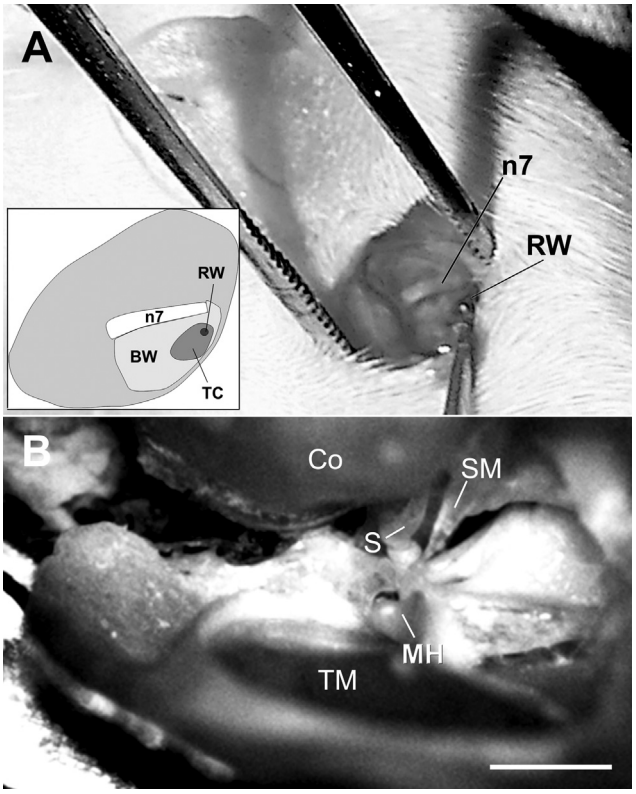

Fig. 1. A: Intraoperative view after a retroauricular incision. The operation area is held open by a forceps. The facial nerve (n7) is demonstrated, a needle points into the depth to the round window (RW). Inset: schematic sketch of the operation area seen in A. BW: bony wall of the tympanic cavity (TC). B: Temporal bone preparation in the adult rat after SSD surgery at P10. The intact tympanic membrane (TM) with malleus handle (MH) and stapes (S) are visible. On the head of the stapes, the tendon of the stapedial muscle (SM) is seen. Co: cochlear. Scale bar 1 mm.

loudness of the click stimuli producing an unequivocal response defined the ABR threshold. Sound pressure was stepwise increased, attempting to elicit an ABR visualized by an averager (Multiliner E; Evolution 1.70c; Toennies & Jäger GmbH, Höchberg, Germany). An indication for correct placement of the ear bar on the deaf side was the induction of cross hearing at 70–80 dB above threshold. By using masking noise on the hearing ear with 50–60 dB over threshold, cross hearing disappeared. During measurement, ambient noise was at a minimum level in a quiet environment, and the outer ear canal was tightly closed by the ear bar tips.

Thresholds were compared between the deaf ear, the hearing ear, and the ears of the normal hearing group. When acoustic stimulation failed to produce a positive ABR graph up to 95 dB above threshold on the operated side, stimulation was discontinued and the threshold was noted to be 100 dB or higher.

2.4. Electrical intracochlear stimulation (EIS)

This is described in detail in former research reports (Illing et al., 2002; Rosskothen-Kuhl and Illing, 2010). Electrical intracochlear stimulation was performed to verify the transfer function of the auditory nerve and the auditory brainstem following deafening. An electrode carrier was inserted into the scala tympani of the deaf or hearing side under microscopical view, with full insertion of two tip electrode rings that were connected to a Nucleus Implant Communicator kindly provided by Cochlear GmbH. The electrical auditory brainstem response (EABR) was recorded.