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The pattern of Fos expression in the rat auditory brainstem changes with the temporal structure of binaural electrical intracochlear stimulation

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ABSTRACT

The immediate-early-gene c-*fos* with its protein product Fos has been used as a powerful tool to investigate neuronal activity and plasticity following sensory stimulation. Fos combines with Jun, another IEG product, to form the dimeric transcription factor activator protein 1 (AP-1) which has been implied in a variety of cellular functions like neuronal plasticity, apoptosis, and regeneration. The intracellular emergence of Fos indicates a functional state of nerve cells directed towards molecular and morphological changes. The central auditory system is construed to detect stimulus intensity, spectral composition, and binaural balance through neurons organized in a complex network of ascending, descending and commissural pathways. Here we compare monaural and binaural electrical intracochlear stimulation (EIS) in normal hearing and early postnatally deafened rats. Binaural stimulation was done either synchronously or asynchronously. The auditory brainstem of hearing and deaf rats responds differently, with a dramatically increasing Fos expression in the deaf group so as if the network had no pre-orientation for how to organize sensory activity. Binaural EIS does not result in a trivial sum of 2 independent monaural EIS, as asynchronous stimulation invokes stronger Fos activation compared to synchronous stimulation almost everywhere in the auditory brainstem. The differential response to synchronicity of the stimulation puts emphasis on the importance of the temporal structure of EIS with respect to its potential for changing brain structure and brain function in stimulus-specific ways.

Introduction

A cochlear implant is a powerful instrument replacing a natural auditory input with locally patterned electrical currents within the inner ear of deaf persons. Use of such a neuroprosthetic device is advisable in cases of hair cell loss in the inner ear as these cells are incapable of regeneration. Without sensory input to the auditory system, nerve fibers are at risk to degenerate. Electrical stimulation by cochlear implants, either monaurally or binaurally, may prevent degeneration of spiral ganglion cells (Leake et al., 1999) and preserves hearing. Binaural cochlear implantation in patients who are deaf on both ears became a common procedure in the last years. Spatial hearing requires binaural hearing. The different neuromolecular effects of binaural as compared to monaural stimulation of the auditory brainstem are not yet known.

The central auditory system consists of different major and minor brain nuclei which are connected by a complex network of ascending, descending, and commissural pathways. This system is designed to detect stimulus intensity, spectral composition, and binaural balance with an extraordinary sensitivity to temporal patterning. Sensory stimulation affects development and state of neurons forming the auditory system not only on a fast electrophysiological level but also by molecular and morphological changes which, under specific circumstances, could be long-lasting. Expression of the immediate-early-gene *fos* is used as a powerful tool to study neuronal plasticity.

There are at least two cellular signaling pathways activating *fos*: one by an increasing calcium level activating the Ca^{2+}/Calmodulin-dependent kinase (CaMK) IV, the other by increasing cAMP level and activation of the protein kinase A. The Fos protein may form the transcription factor activator protein 1 (AP-1) together with c-Jun. AP-1 has been implied in a variety of cellular functions like growth, apoptosis, and regeneration. Within minutes, *fos* mRNA is synthesized after specific sensory or electrical stimulation of neurons, with a maximum expression after 20 min (Greenberg and Ziff, 1984) and a half-life of 12–18 min (Shyu et al., 1989). Acoustic or electrical intracochlear

Abbreviations: ABR, auditory brainstem response; AC, auditory cortex; async., asynchronous; AVCN, anteroventral cochlear nucleus; bEIS, binaural EIS; c, contralateral; CIC, central core of the colliculus inferior; CN, cochlear nucleus; d, dorsal; DCN:, dorsal cochlear nucleus; DLL, dorsal nucleus of the lateral lemniscus; EABR, electrical auditory brainstem response; EIS, electrical intracochlear stimulation; i, ipsilateral; IC, inferior colliculus; ITD, intraural time difference; l, lateral; LSO, lateral superior olive; mEIS, monaural EIS; MNTB, medial nucleus of the trapezoid body; MSO, medial superior olive; NLL, nuclei of the lateral lemniscus; SL, sensation level; SOC, superior olive complex; SPL, sound pressure level; SPON, superior paraolivary nucleus; sync., synchronous; VCN, ventral cochlear nucleus; VLL, ventral nucleus of the lateral lemniscus.

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stimulation causes an increase of Fos expression in many neurons of the central auditory system (Ehret and Fischer, 1991; Friauf, 1995; Saito et al., 1999; Illing and Michler, 2001).

In contrast to the visual system where spatial analysis is performed by the eyes, the function of the cochlea is directed towards the analysis of the frequency composition of sound rather than sound localization. The spatial analysis of sound is performed by the central auditory system, particularly in the auditory brainstem where the superior olivary complex extracts interaural time difference (ITD) and interaural level difference (ILD) (Moore, 1991).

In the present study we examined differences between acute monaural and binaural electrical intracochlear stimulation in normal hearing and early postnatal deafened rats. When stimulating binaurally, stimulus trains were applied either synchronously or asynchronously. We aimed to determine whether binaural EIS induces the equivalent of the sum of two monaural EIS (one presented on the left, the other on the right cochlear) or if there is a binaural interference (potentiation or attenuation) resulting in non-linear effects with respect to neuroplastic changes induced on the central auditory system. We put special emphasis on a factor involved in binaural interaction: the synchrony and asynchrony of the electric binaural stimulation.

Material and methods

Animals

This study is based on the analysis of 38 brains from adult Wistar rats of either sex, aged 7 to 14 weeks. Experiments were carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. 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). There were two groups of rats: normal hearing ('hearing') rats and early postnatal deafened ('deaf') rats. Inspection of the ear canal and the tympanic membrane, Preyer's reflex (response to hand clap), and auditory brainstem response (ABR) verified for the hearing group that they were normal hearing on both sides. Rats were anesthetized 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) for ABR measurements and ear bone removal. For EIS anesthesia was achieved with urethane (Ethyl carbamate, 1.5 g/kg i.p., Fluka AG, Buchs, Switzerland). Preceding transcardial perfusion, rats were given a lethal dose of sodium-thiopental (50–100 mg/kg i.p., Trapanal, Nycomed, Konstanz, Germany).

Early postnatal induction of bilateral deafness

Between postnatal days 10 and 20 (P10 to P20 inclusive, Tab.1), rats received a daily injection of kanamycin (i.p., 400 mg/kg body weight; Sigma, Taufkirchen, Germany; Rosskothen-Kuhl and Illing, 2012). As a consequence, hearing threshold rose by 80 dB SPL (sound pressure level) or more due to hair cell destruction.

Verification of hearing loss

Hearing thresholds of untreated animals and kanamycin treated animals were tested for Preyer's reflex and ABR threshold. For the ABR recording, steel needle electrodes were placed subcutaneously at vertex and mastoids and a 20 Hz train of click stimuli was presented to one side through a brass pipe equipped with a conical plastic tip into the outer ear canal. The SPL was stepwise increased, attempting to elicit an ABR graph visualized by an averager (Multiliner E; Evolution 1.70c; Toennies & Jäger GmbH, Höchberg, Germany). The threshold of the ABRs was set as hearing threshold when the first unequivocal response merged as distinguishable curve (sensation level SL). During measurement, ambient noise was at a minimum level in a quite environment and the ear canal was tightly closed by the ear bars. In the untreated, normal hearing group, the first unequivocal ABR potentials were found 5 dB above threshold corresponding to 10 dB SPL. When acoustic stimulation failed to produce a positive ABR graph up to 95 dB above threshold, stimulation was discontinued and the threshold was noted to be 100 dB or higher. The ABR mean amplitudes were determined after 300 sweeps recorded in a frequency band of 0.1 to 3 kHz (Rosskothen-Kuhl and Illing, 2012).

Ear bone removal

The tympanic membrane was disrupted and the malleus was removed bilaterally in the hearing rats three days before EIS or implantation without stimulation (control) to reduce the level of hearing-dependent spiking activity in the auditory system. Judging from the auditory brainstem response (ABR), loss of middle ear transmission led to an immediate and sustained rise of hearing threshold by 50 dB (Illing and Michler, 2001). With this intervention, hearing-induced Fos expression was almost entirely absent (Illing et al., 2002).

Electrical intracochlear stimulation (EIS)

The cochlea was exposed using a retroauricular surgical approach and the round window membrane was gently perforated. The electrode carrier was inserted through the round window into the scala tympani under a microscopical view on one or both sides, with full insertion of two tip electrode rings that were connected to a Nucleus Implant Communicator kindly provided by Cochlear GmbH.

For controls (implantation without stimulation), brains of 6 hearing and 6 deaf animals were analyzed. An electrode carrier was inserted monaurally or binaurally (3 animals each) into the cochlea for 2 h through the round window, without being used for stimulation. In case of binaural insertion the electrodes were held in position by filling the bulla with a 4% agar solution.

For EIS, brains of 3 hearing and of 4 deaf rats were stimulated monaurally (mEIS). Eight brains of hearing and 11 brains of deaf rats were stimulated binaurally (bEIS). Animals were sacrificed and perfused after 2 h of stimulation. In the mEIS group, bipolar stimulation consisted of a sustained 50 Hz train of biphasic stimuli with a phase width of 50 μs and 20 μs phase gap (Fig. 1). Animals experiencing bEIS were divided in two groups. In one group ($n = 4$ for hearing rats and $n = 7$ for deaf rats), rats were stimulated synchronously (sync.), i.e. stimuli on both cochleae were given in phase. The other group ($n = 4$ for hearing and $n = 4$ for deaf rats) animals were stimulated asynchronously (async.), i.e. the stimulus occurred exactly alternating, or interdigitating, between ears (Fig. 1).

The electrical auditory brainstem response (EABR) was recorded with steel needle electrodes placed subcutaneously at vertex and mastoids to corroborate for the correct placement of stimulation electrodes and to determine an appropriate current level. The EABR was visualized using an averager (Multiliner E, Evolution 1.70c, Toennies, Germany), calculating mean amplitudes over 500 sweeps in a frequency band of 0.1 to 3 kHz. We aimed to obtain maximal EABR amplitudes of 8 μV ± 10% by adjusting the current level to match acoustic stimuli of about 85 dB SPL (Fig. 2). For bEIS this was done for each ear separately. In conjunction with the basal position of the stimulation electrode, moderate current levels served to induce a local intracochlear stimulation of spiral ganglion cells in the basal cochlea rather than a global activation of the auditory nerve. The current level required to achieve this varied between 220 and 240, corresponding to currents ranging from 861 to 1291 μA, compensating for unavoidable variations in the precise distance of the electrodes from the modiolus. For bEIS, adjustments were done for the right and left ear to obtain matching amplitudes