BRAIN RESEARCH 1435 (2012) 40–55

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BRAIN RESEARCH

Research Report

The impact of hearing experience on signal integration in the auditory brainstem: A c-Fos study of the rat

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ARTICLE INFO

Article history:
Accepted 20 November 2011
Available online 1 December 2011

Keywords:
c-Fos
Cochlear nucleus
Inferior colliculus
Plasticity
Electrical intracochlear stimulation
Kanamycin

ABSTRACT

In this study we investigated the pattern of c-Fos expression in anteroventral (AVCN) and dorsal cochlear nucleus (DCN) and central inferior colliculus (CIC) following electrical intracochlear stimulation (EIS) of anesthetized adult rats that were neonatally deafened. The animals never experienced acoustic sensations as their hair cells were destroyed by daily kanamycin injections between postnatal days 10 to 20, resulting in a rise of hearing threshold by about 90 dB. Unilateral EIS was applied through a cochlear implant inserted into the medial turn of the left cochlea and lasted for 45 or 73 min, 2, 3:15, or 5 h. Following EIS at 50 Hz, a high number of c-Fos positive nuclei were observed showing only marginal tonotopic order in ipsilateral AVCN, in DCN bilaterally, and in contralateral CIC. Quantifying the number of c-Fos positive nuclei in ipsilateral AVCN, we found a steady increase with stimulation time. By contrast, the population of neurons expressing c-Fos in DCN and CIC revealed a transient maximum at 73 min. A direct comparison with our previous study (Rosskothen-Kuhl, N., Illing, R.-B., 2010. *Nonlinear development of the populations of neurons expressing c-Fos under sustained electrical intracochlear stimulation in the rat auditory brainstem*. Brain Res. 1347, 33–41) reveals that absence of hearing experience has far-reaching consequences for the interneuronal communication within networks of the auditory brainstem. When hearing fails, EIS entails expression of c-Fos in populations of neurons that are much larger than normally, essentially disregard tonotopic order, and lack much of spatio-temporal variations seen in hearing-experienced rats.

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Abbreviations: ABR, auditory brainstem response; ANOVA, analysis of variance; AVCN, anteroventral cochlear nucleus; AVCNc, AVCN contralateral to stimulation; AVCNi, AVCN ipsilateral to stimulation; AP-1, activator protein-1; ATF-2, activating transcription factor-2; BDNF, brain derived neurotrophic factor; bFGF, basic fibroblast growth factor; cAMP, cyclic adenosine monophosphate; CB, cerebellum; CRE, cAMP response element; CIC, central inferior colliculus; CICc, CIC contralateral to stimulation; c, contralateral; c-Fos, product of the proto-oncogene *c-fos*; c-Jun, product of the immediate-early gene *c-jun*; Co, control; CoOP, control group of sham-operated rats with or without electrode insertion; c-sis, simian sarcoma virus homolog; Cx, neocortex; d, dorsal; DAB, 3,3′-diaminobenzidine tetrahydrochloride; dB, decibel; DCN, dorsal cochlear nucleus; DCNc, DCN contralateral to stimulation; DCNi, DCN ipsilateral to stimulation; EABR, electrical auditory brainstem response; Egr-1, early growth response protein-1; EIS, electrical intracochlear stimulation; EP, evoked potential; GABA, gamma amino butyric acid; GAP-43, growth-associated protein-43; GFAP, glial fibrillary acidic protein; IEG, immediate-early gene; i, ipsilateral; i.p., intraperitoneal; l, lateral; LTP, long term potentiation; mRNA, messenger ribonucleic acid; p-CREB, phosphorylated cAMP response element binding protein; P, postnatal day; ROI, region of interest; SIE, sis inducible element; SPL, sound pressure level; SRE, serum response element; VCN, ventral cochlear nucleus

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doi:10.1016/j.brainres.2011.11.046

1. Introduction

Sensory stimulation of the mammalian inner ear affects neurons forming the central auditory system, not only on the electrophysiological level but also on their molecular and morphological organization. Modifications of enzymes and transcription of genes result in an altered molecular profile of nerve and glial cells, both playing important roles in neuroplastic remodeling (Cole et al., 1989; Freeman, 2010; Rampon et al., 2000).

One of the first genes expressed in neurons following specific activation of primary sensory afferents is the proto-oncogene *c-fos* that encodes for a 62 kDa large protein. c-*fos* has several regulatory elements in its promoter-region, among them a cyclic adenosine monophosphate (cAMP) response element (CRE), a c-sis (Simian sarcoma virus homolog) inducible element (SIE), a serum response element (SRE), and an activator protein-1 (AP-1) like sequence (Curran et al., 1984; Ginty et al., 1994; Herdegen and Leah, 1998). As a transcription factor found to participate in various systems of neuroplasticity, the phosphorylated cAMP response element binding protein (p-CREB) is able to induce *c-fos* expression through the CRE site (Ginty et al., 1994; Montminy et al., 1986; Sheng et al., 1988).

As an immediate-early gene (IEG), *c-fos* is transcribed in various types of neurons in the mammalian central nervous system as soon as 5 min following novel sensory stimulation, evoked spiking activity, or the action of growth factors (Chaudhuri, 1997; Greenberg and Ziff, 1984; Peng et al., 1993). The number of accumulated *c-fos* messenger ribonucleic acid (mRNA) molecules reaches a maximum 30–45 min after stimulus-offset, with an mRNA half-life of 10–15 min (Müller et al., 1984; Sheng and Greenberg, 1990). By comparison, the c-Fos protein has a half-life of around 2 h (Curran et al., 1984; Müller et al., 1984).

The c-Fos protein is a monomer of the heterodimeric Fos-Jun AP-1 transcription factor which has an 8-fold higher DNA binding affinity than the homodimeric Jun–Jun complex (Abate et al., 1990). As c-Jun p38 but not c-Fos is abundant in the AVCN of normal hearing rats that were not exposed to distinct changes in auditory nerve activity, the induction of c-Fos expression appears to determine availability of the high-activity variant of AP-1 (Rosskothen et al., 2008).

In the central auditory system, c-Fos expression may be induced by acoustical or electrical cochlear stimulation (Ehret and Fischer, 1991; Illing and Michler, 2001; Illing et al., 2010; Jakob and Illing, 2008; Sato et al., 1993). In previous studies of hearing-experienced rats with electrical intracochlear stimulation (EIS) at varying intracochlear sites we saw a tonotopic c-Fos expression in all major areas of the auditory brainstem, including the anteroventral cochlear nucleus (AVCN), the dorsal cochlear nucleus (DCN) and the central inferior colliculus (CIC) (Illing et al., 2002; Rosskothen et al., 2008; Rosskothen-Kuhl and Illing, 2010). Reisch et al. (2007) showed that, within the tonotopically appropriate regions, only specific molecular and projectional types of neurons develop c-Fos immunoreactivity after EIS.

In our study preceding and complementing the present work, we analyzed and discussed the effect of EIS on the pattern of c-Fos expression in normally hearing-experienced rats. Stepwise increasing stimulation time, we found spatio-temporally varying recruitments of neuronal subpopulations into cellular networks responding to activity in the sensory channel with c-Fos expression (Rosskothen-Kuhl and Illing, 2010). The question addressed in the present study was, how the interneuronal networking as visualized by the pattern of c-Fos expression might change when stimulation is applied upon a hearing-inexperienced central auditory system.

2. Results

2.1. *Effect of neonatal kanamycin treatment on the auditory brainstem response of mature rats*

Compared to normal hearing wild-type Wistar rats with large, clearly differentiated acoustically evoked auditory brainstem responses (ABR), the kanamycin treated group showed no or only minimal ABRs at high sound pressure levels (SPL) (Figs. 1A, B). Correspondingly, hearing rats showed a strong hand clap startle (Preyer's reflex) while deaf rats entirely failed to do so.

Following neonatal kanamycin treatment, 9 rats showed a hearing threshold above 95 decibel (dB) bilaterally, 10 animals showed a hearing threshold between 75 dB and 95 dB for one ear but more than 95 dB on the opposite side, and another 11 rats showed a hearing threshold between 75 dB and 95 dB bilaterally. In case of failing to find a hearing threshold less than or equal to 95 dB, we discontinued stimulation and set the threshold to 100 dB for calculations. On average, then, due to kanamycin treatment, the hearing threshold increased by at least 92.8 dB for the left ear and 92.0 dB for the right ear.

Applying EIS to hearing-experienced and hearing-inexperienced rats, both groups showed a well differentiated electrical auditory brainstem response (EABR) under identical stimulation conditions. The amplitudes of peaks III–V were slightly reduced for the neonatally deafened rats as compared to the hearing animals (Figs. 1C, D).

2.2. *Controls*

Nuclei of neurons translating the c-Fos protein turn black after 3,3′-diaminobenzidine tetrahydrochloride (DAB)-peroxidase-nickel staining (Figs. 2A, B, C with upper panel of inset, 3A–D and left panel of inset, 5A with upper panel of inset, B). In deaf control animals receiving no surgical treatment, c-Fos expression remained below detection level in AVCN on either side of the brainstem, showing a near-total absence of staining (matching to Fig. 2B, inset). The same was true on either side for unilaterally sham-operated rats with or without insertion of the electrode carrier so that we combined both sides of the AVCN from all of these different control experiments together to control group Co (Fig. 2D).

In the DCN of neonatally deafened rats, two control groups need to be distinguished. First, control animals without any surgical treatment (Co) showed low levels of c-Fos expression bilaterally (Figs. 3E, 4). Second, sham-operated rats in which the cochlea was opened showed a significant bilateral increase of c-Fos expression against Co, mostly in the deep