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Research Report

Nonlinear development of the populations of neurons expressing c-Fos under sustained electrical intracochlear stimulation in the rat auditory brainstem

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ABSTRACT

The immediate-early-gene *c-fos* is among the first genes to be expressed following sensory-invoked neuronal activity. Its gene product c-Fos forms the limiting monomer of the heterodimeric activator protein-1 transcription factor that triggers various genes involved in neuroplastic remodeling. This study investigated the pattern of c-Fos expression in anteroventral (AVCN) and dorsal cochlear nucleus (DCN) and central inferior colliculus (CIC) after 45 min, 73 min, 2 h, 3:15 h and 5 h of unilateral electrical intracochlear stimulation (EIS) at 50 Hz in anaesthetized rats. Following EIS, tonotopic c-Fos expression was observed for each stimulation time in ipsilateral AVCN, DCN bilaterally, and contralateral CIC. By counting c-Fos positive nuclei, we discovered temporal non-linearities in the size of the respective population of c-Fos expressing neurons. In all regions investigated, the populations significantly increased from 73 min to 2 h but decreased towards 3:15 h. In AVCN, the number rose again by 5 h of EIS. Remarkably, the same was noted for neurons with large nuclei in deep DCN. In both regions, the population of responsive neurons shifted spatially: In central AVCN, the density of c-Fos positive cells increased significantly from 2 to 5 h with medial and lateral regions remaining unchanged. In DCN, the density of large c-Fos positive nuclei fell in the upper and rose in the deep layers from 45 min to 5 h of EIS. In conclusion, spatiotemporally varying recruitments of neuronal subpopulations into cellular networks responding to specific patterns of sensory activity take place in the auditory brainstem.

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Abbreviations: ANOVA, analysis of variance; AVCN, anteroventral cochlear nucleus; AP-1, activator protein-1; cAMP, cyclic adenosine monophosphate; CRE, cAMP response element; CIC, central inferior colliculus; c, contralateral; c-Fos, product of the proto-oncogene *c-fos*; c-Jun, product of the immediate-early gene *c-jun*; Co, control; DAB, diaminobenzidine; DCN, dorsal cochlear nucleus; EABR, electrical auditory brainstem response; EIS, electrical intracochlear stimulation; GABA, gamma-aminobutyric acid; GAP-43, growth-associated protein-43; IC, inferior colliculus; IEG, immediate-early gene; i, ipsilateral; i.p., intraperitoneally; MIA, multiple-image-alignment; LTP, long term potentiation; P-CREB, phosphorylated cAMP response element binding protein; ROI, region of interest; SIE, sis inducible element; SRE, serum response element

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1. Introduction

Following sensory stimulation of the ear, effects may be observed not only on the electrophysiological level, but also on the molecular and morphological level in specific neuronal populations of the auditory brainstem of mammals. Modifications of enzymes or activation of genes playing potentially important roles in neuroplastic remodeling are among these molecular changes (Cole et al. 1989; Rampon et al. 2000).

One of the genes expressed following specific activation of primary sensory afferents is the proto-oncogene *c-fos* that encodes for a 62 kDa large protein with several regulatory elements in the 5'–3' promoter-region, among them a cyclic adenosine monophosphate (cAMP) response element (CRE), a sis 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). The phosphorylated cAMP response element binding protein (P-CREB) is one of the transcription factors acting on these elements (Sheng et al. 1988; Ginty et al. 1994; Illing and Michler 2001). *C-fos* is an immediate-early gene (IEG) that becomes transcribed as soon as 5 min after stimulus-onset, induced by growth factors, sensory stimulation, or internally evoked spiking activity (Greenberg and Ziff 1984; Peng et al. 1993; Chaudhuri 1997). The accumulated *c-fos* mRNA reaches its maximum 30–45 min after stimulus-offset, with an mRNA half-life of only 10–15 min (Müller et al. 1984; Sheng and Greenberg 1990). The c-Fos protein possesses a half-life of around 2 h (Curran et al. 1984; Müller et al. 1984).

In the ascending auditory system, c-Fos expression can be induced by acoustical or electrical intracochlear stimulation (Ehret and Fischer 1991; Sato et al. 1993; Illing and Michler 2001; Jakob and Illing 2008; Illing et al. 2010). An implicit assumption was that the number of c-Fos positive neurons grows with time of stimulation, with a saturation level that is reached sooner or later. Here we show that this assumption needs revision.

In previous studies, we verified tonotopic c-Fos expression following electrical intracochlear stimulation (EIS) in almost all major core 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). Reisch et al. (2007) have shown that, within the tonotopically appropriate regions, only specific types of neurons develop c-Fos immunoreactivity after EIS.

By closer analysis, we noticed that the neuronal population of the auditory brainstem developing c-Fos expression under sustained stimulation does not simply rise with time but revealed an unexpected, non-linear progression, in number as well as in pattern.

2. Results

2.1. Controls

As a consequence of the binding of antibodies directed against c-Fos, nuclei of neurons containing this protein turn black after diaminobenzidine (DAB)-peroxidase-nickel staining (Figs. 1A, C, upper panel of inset, 2A inset, C, 3B). In control animals bilaterally deafened by ear bone removal 3 days before brain fixation, c-Fos expression lay below detection level on either side of the AVCN, independent of the type of sham-operation or insertion of the electrode carrier. For DCN and CIC a low level of c-Fos positive nuclei was detected under control conditions, whereas no significant differences were observed between several types of controls (Figs. 2E–J, 3C). Consequently, all control animals within our study were grouped as controls (Co, Figs. 1D, 2E–J, 3C).

2.2. C-Fos expression in the AVCN after various duration of EIS

Following EIS with stimulation periods of 45 min, 73 min, 2 h, 3:15 h, and 5 h, tonotopic c-Fos expression was observed in the AVCN on the stimulated side (Fig. 1A–D). Excluding 45 min of EIS, the detected c-Fos level was always significant higher than under control conditions ($p<0.001$; Fig. 1D). Contralateral to stimulation, c-Fos positive nuclei were rare after any period of time (Fig. 1B, inset, D).

In the AVCN ipsilateral to EIS, however, despite identical set-up and stimulation parameters as well as comparable electrical auditory brainstem responses (EABRs) that allowed to distinguish 4 to 5 troughs (cp. Reisch et al. 2007; Jakob and Illing 2008), the number of emerging c-Fos positive neurons did not continuously increase with stimulation duration (Fig. 1D).

Initially, there was a significant increase of c-Fos expression from 45 min to 2 h of sustained stimulation ($p<0.001$; Fig. 1D). However, there was a significant decline in the number of stained neuronal nuclei between 2 h and 3:15 h of EIS ($p<0.001$; Fig. 1A, B, D). From 3:15 to 5 h of stimulation, the number rose again to reach levels similar to that found after 2 h of EIS ($p<0.001$; Fig. 1C, D).

In addition to counting c-Fos positive nuclei in the sections throughout AVCN (Fig. 1A, dashed line), we determined the density of c-Fos positive nuclei after 2 and 5 h of stimulation in the lateral, central, and medial parts of AVCN that corresponded tonotopically to the intracochlear site of stimulation (Fig. 1C, marked regions of interest, ROIs). Independent of stimulation time, the highest density of c-Fos positive nuclei was found in the lateral third as compared to the central and medial regions ($p<0.001$; Fig. 1E). After 2 h of EIS, a significant difference in the density of c-Fos positive nuclei existed between the central and medial part, with a lower density centrally ($p<0.05$). This difference had vanished by 5 h of EIS (Fig. 1E). Comparing nuclei counts at 2 and 5 h, a significant increase of the c-Fos density has occurred in the central part of the ipsilateral AVCN ($p<0.05$ by *t*-test), whereas medial and lateral regions remained unchanged (Fig. 1E).

2.3. C-Fos expression in the DCN after various duration of EIS

Compared to AVCN, non-linear c-Fos expression could be observed on both sides of the DCN following different stimulation times (Fig. 2E). For each point in time, the c-Fos level was higher ipsi- than contralaterally ($p<0.001$ for 2 h ipsilateral (i) vs. contralateral (c); $p<0.05$ for 73 min and 5 h; all by *t*-test) and bilateral significant higher compared to control