*Neuroscience* 313 (2016) 184–198

## RESEARCH PAPER

# COUNTER-REGULATION OF THE AP-1 MONOMERS pATF2 AND FOS: MOLECULAR READJUSTMENT OF BRAINSTEM NEURONS IN HEARING AND DEAF ADULT RATS AFTER ELECTRICAL INTRACOCHLEAR STIMULATION

**A.-K. RAUCH,[†] N. ROSSKOTHEN-KUHL [*†] AND R.-B. ILLING**

*Neurobiological Research Laboratory, Department of Otorhinolaryngology, University of Freiburg, Germany*

**Abstract—Expression of the immediate-early gene *fos* (also known as *c-fos*) and phosphorylation of the product of the early response gene *atf2* (pATF2) in the adult auditory brainstem can be modulated by electrical intracochlear stimulation. The Fos and pATF2 proteins are competitive monomers of the heterodimeric activator protein-1 (AP-1) transcription factor that triggers the expression of genes related to neural plasticity. Our previous findings showed that the stimulation-induced spatio-temporal pattern of Fos expression in the adult auditory system depends on hearing experience. In this study, we aimed to identify a possible correlation of pATF2 and Fos expression. Adult normal hearing and neonatally deafened rats were unilaterally stimulated with a cochlear implant (CI) for 45 min, 73 min, or 2 h. The numbers of Fos- and pATF2-positive neurons in the anteroventral cochlear nucleus (AVCN), the lateral superior olive (LSO), and the central inferior colliculus (CIC) were evaluated. Following stimulation, an increased Fos expression was demonstrated in all these regions in hearing and deaf rats. However, in neonatally deafened rats, significantly more Fos-positive neurons emerged that did not obey a tonotopic order. Independent of hearing experience, Fos expression correlated with a locally matching decrease of pATF2 expression in AVCN and LSO, but not in CIC. We suggest that these changes in gene expression result in a shift of AP-1 dimer composition from ATF2:Jun to Fos:Jun. This change in AP-1 constellation is expected to invoke different transcriptional cascades leading to distinct modes of tissue reorganization and plasticity responses in the mature central auditory system under stimulation.** 

**Key words: neuroplasticity, hearing experience, cochlear implant, immediate-early gene, activator protein-1.**

## INTRODUCTION

Neuroplastic remodeling is a process by which the central nervous system responds to dynamic changes of neural activity with lasting structural and functional adaptations. Following changes in sensory input, immediate-early gene (IEG) activation is an initial step toward neuroplastic remodeling in the auditory system of the mature mammalian brain (Christensen et al., 2013; Lanahan and Worley, 1998; Sheng et al., 1993).

Acoustical or electrical stimulation of the auditory system induces expression of the IEG *fos* (also known as *c-fos*) and its protein product in distinct neurons of the auditory brainstem (Ehret and Fischer, 1991; Reisch et al., 2007; Rosskothen-Kuhl and Illing, 2010). Stimulating normal hearing rats electrically by way of a cochlear implant (CI) results in a tonotopic but spatio-temporally dynamic pattern of the product of the proto-oncogene *c-fos* (Fos) expression (Illing et al., 2002; Rosskothen-Kuhl and Illing, 2010). When maturation of a functional Organ of Corti is halted before hearing onset, stimulation-induced Fos expression is significantly stronger and the population of responding cells disregards tonotopic order (Rosskothen-Kuhl and Illing, 2012). For the auditory brainstem we have shown that the Fos protein is the limiting monomer for the heterodimeric Fos:Jun activator protein-1 (AP-1) transcription factor complex (Rosskothen et al., 2008). Apart from being an

*Corresponding author. Address: Neurobiological Research Laboratory, Department of Otorhinolaryngology, University of Freiburg, Killianst. 5, 79106 Freiburg i. Br., Germany. Tel: +49-761-270-42650.
E-mail addresses: ann-kathrin.rauch@uniklinik-freiburg.de (A.-K. Rauch), nicole.rosskothen@uniklinik-freiburg.de (N. Rosskothen-Kuhl), robert.illing@uniklinik-freiburg.de (R.-B. Illing).
[†] Contributed equally.

*Abbreviations:* ABR, auditory brainstem response; ANOVA, analysis of variance; AP-1, activator protein-1; ATF2, activating transcription factor-2; AVCN, anteroventral cochlear nucleus; AVCNi, AVCN ipsilateral to stimulation; c, contralateral; CI, cochlear implant; CIC, central inferior colliculus; CICc, CIC contralateral to stimulation; c-Jun, product of the immediate-early gene *c-jun*; Co, control; CREB, cAMP response element-binding protein; DAB, 3,3′-diaminobenzidine tetrahydrochloride; dB, decibel; EABR, electrically evoked auditory brainstem response; ECM, extracellular matrix; EIS, electrical intracochlear stimulation; Fos, product of the proto-oncogene *c-fos*; Gap43, growth-associated protein-43; i, ipsilateral; IEG, immediate-early gene; i.p., intraperitoneal; LSO, lateral superior olive; LSOi, LSO ipsilateral to stimulation; LTP, long-term potentiation; MAPK, mitogen-activated protein kinase; NGF, nerve growth factor; pATF2, (phosphorylated) activating transcription factor-2; ROI, region of interest; SPL, sound pressure level; VCN, ventral cochlear nucleus.

http://dx.doi.org/10.1016/j.neuroscience.2015.11.025
0306-4522/

initial marker for neural sensory activation (Pinna et al., 2006; Tachikawa et al., 2013), Fos participates in regulating the expression of genes associated with neuroplasticity (Cohen and Greenberg, 2008; Illing, 2001; Vonhoff et al., 2013). Williams et al. (1991) demonstrated that Fos expression comprises an early response to sciatic nerve injury, followed by the expression of growth-associated protein 43 (Gap43) in the same cell region, a protein associated with neuronal growth and synaptic plasticity (Benowitz and Routtenberg, 1997). Weber and Skene (1998) have shown that among the genes controlled by AP-1 is Gap43, and its expression is at least partly dependent on the activation of its AP-1 binding site (Diolaiti et al., 2007). Nerve growth factor (NGF)-regulated AP-1 activity and subsequent Gap43 transcription have been linked to neuronal differentiation and neurite outgrowth. This supports the view of Fos as a mediator of the AP-1 gene regulatory network responsible for neural plasticity (Miller et al., 2010). Furthermore, Fos has been identified as relevant candidate for long-term potentiation (LTP) (Hartwig et al., 2008; Sanyal et al., 2002; Seoane et al., 2009), and its expression has been linked to synaptogenesis (Kleim et al., 1996) mediated by the AP-1–CREB (cAMP response element binding protein) pathway.

The early response protein activating transcription factor-2 (ATF2) is another optional monomer for the dimeric AP-1 complex. ATF2 is ubiquitously expressed with predominance in the brain (Takeda et al., 1991) and plays an important role in oncogenesis (Breitwieser et al., 2007; Lau and Ronai, 2012; Li et al., 2010; Kim et al., 1996), cellular differentiation, neural development (Ackermann et al., 2011; Breitwieser et al., 2007; Maekawa et al., 1999; Reimold et al., 1996), and neuroprotection (Han et al., 2009). The function of ATF2 depends on its subcellular localization, phosphorylation state, and dimerization (Berger et al., 2003; Gupta et al., 1995; Lau and Ronai, 2012), and includes participation in neural plasticity (Pearson et al., 2005).

Fos and ATF2 monomers may dimerize with the IEG c-Jun to form a heterodimeric AP-1 complex. Common characteristics for AP-1 proteins are the activation through a mitogen-activated protein kinase (MAPK) in order to initiate gene transcription. While the Fos:Jun dimer plays an important role in neural plasticity by way of extracellular matrix (ECM) degradation, for instance by activating matrix metalloproteinase nine (Kuzniewska et al., 2013; Meighan et al., 2006; Van Dam and Castellazzi, 2001), the target genes of the ATF2:Jun dimer may promote neural stability by way of genes coding for specific ECM components (Van Dam and Castellazzi, 2001). Moreover, the ATF2:Jun complex triggers the expression of *c-jun* (Fu et al., 2011; Yamasaki et al., 2009) and genes for MAPKs of neurons and glial cells (Breitwieser et al., 2007). The ATF2:Jun heterodimer was also shown to bind to the product of the immediate-early gene *c-jun* (c-Jun) promoter in the presence of NGF, a factor essential for neuronal survival (Kristiansen et al., 2010).

In general, heterodimerization of Fos with c-Jun leads to an AP-1 complex of enhanced stability and DNA binding affinity (Shaulian and Karin, 2001). By contrast, the transcriptional activity of ATF2 homodimers is poor, but ATF2 heterodimers (e.g. ATF2:CREB or ATF2:Jun) display a higher transcriptional activity (Lau and Ronai, 2012). Whereas the transcriptional activity of ATF2:Jun heterodimers is specifically regulated by phosphorylation of ATF2 (pATF2) on Thr69 and Thr71 (Lau and Ronai, 2012; Liss et al., 2010; Gupta et al., 1995), regulation of Fos:Jun heterodimer transcriptional activity appears to be regulated by Fos availability (Liu et al., 2006).

Little is known about the conditions under which Fos and ATF2 compete for forming heterodimeric AP-1 complexes with Jun. We expect that examining differential expression patterns and regional specificity of AP-1 monomers will provide insights into the functional state of the central auditory neurons that were or were not exposed to sensory-evoked firing activity. This we investigated here in hearing and deaf rats to learn if molecular plasticity is different in brains for which specific sensory activity was or was not available through development. The underlying hypothesis was that functional conditions can be defined under which AP-1 is differentially composed in order to promote, in one variant, neural plasticity, for instance by way of ECM degradation, whereas in another variant, AP-1 would promote neural stability by triggering genes coding for specific ECM components.

## EXPERIMENTAL PROCEDURES

### Animals and anesthesia

Thirty-two mature female Wistar rats aged 6–12 weeks were used. Animal care and experiments as stated here were approved by the appropriate local agency (Regierungspräsidium Freiburg, permission number 37/9185.81/G-10/83). Anesthesia for auditory brainstem response (ABR) measurements and ear bone removal was induced by the intraperitoneal (i.p.) injection of a ketamine (50 mg/kg body weight; Ketamin, Bela-Pharm GmbH & Co. KG, Vechta, Germany) and xylazine (5 mg/kg body weight; Rompun, Bayer-Leverkusen, Germany) mixture. For electrical intracochlear stimulation (EIS), rats were anesthetized by urethane (i.p., 1.5 g/kg body weight; Fluka AG, Buchs, Switzerland). Groups of normal hearing and neonatally deafened rats were compared. Within each group, control experiments were conducted by implanting a CI for 2 h without EIS.

### Neonatal deafening

Rats ($n$ = 16) were neonatally deafened by daily i.p. injections of kanamycin (400 mg/kg body weight; Sigma, Taufkirchen, Germany) from postnatal day P10 to P20, inclusively, causing hair cell destruction in response to the exposure of the ototoxic antibiotic (Matsuda et al., 1999; Osako et al., 1979). Deafness was determined by failure of eliciting an auditory brainstem response to 90 decibel (dB) above the normal hearing threshold.