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Brain Research 1031 (2005) 39–55

BRAIN RESEARCH

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Research report

Electrical stimulation of the cochlear nerve in rats: analysis of c-Fos expression in auditory brainstem nuclei

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Accepted 19 October 2004
Available online 28 November 2004

Abstract

We investigated functional activation of central auditory brainstem nuclei in response to direct electrical stimulation of the cochlear nerve using c-Fos immunoreactivity as a marker for functional mapping. The cochlear nerve was stimulated in the cerebellopontine angle of Lewis rats applying biphasic electrical pulses (120–250 μA, 5 Hz) for 30 min. In a control group, bilateral cochlectomy was performed in order to

Abbreviations: BI_DCN_L, left dorsal cochlear nucleus (control group); BI_DCN_R, right dorsal cochlear nucleus (control group); BI_DLL_L, left dorsal nucleus of the lateral lemniscus (control group); BI_DLL_R, right dorsal nucleus of the lateral lemniscus (control group); BI_IC_L, left inferior colliculus (control group); BI_IC_R, right inferior colliculus (control group); BI_ILL_L, left intermediate nucleus of the lateral lemniscus (control group); BI_ILL_R, right intermediate nucleus of the lateral lemniscus (control group); BI_LSO_L, left lateral superior olive (control group); BI_LSO_R, right lateral superior olive (control group); BI_LTB_L, left lateral nucleus of the trapezoid body (control group); BI_LTB_R, right lateral nucleus of the trapezoid body (control group); BI_MSO_L, left medial superior olive (control group); BI_MSO_R, right medial superior olive (control group); BI_MTB_L, left medial nucleus of the trapezoid body (control group); BI_MTB_R, right medial nucleus of the trapezoid body (control group); BI_VCN_L, left ventral cochlear nucleus (control group); BI_VCN_R, right ventral cochlear nucleus (control group); BI_VLL_L, left ventral nucleus of the lateral lemniscus (control group); BI_VLL_R, right ventral nucleus of the lateral lemniscus (control group); BI_VTB_L, left ventral nucleus of the trapezoid body (control group); BI_VTB_R, right ventral nucleus of the trapezoid body (control group); CIC, central nucleus of the inferior colliculus; DAB, 3-3′diaminobenzidine; DCIC, dorsal cortex of the inferior colliculus; DCN, dorsal cochlear nucleus; DLL, dorsal nucleus of the lateral lemniscus; ECIC, external cortex of the inferior colliculus; EL_DCN_L, left dorsal cochlear nucleus (group with electrical stimulation); EL_DCN_R, right dorsal cochlear nucleus (group with electrical stimulation); EL_DLL_L, left dorsal nucleus of the lateral lemniscus (group with electrical stimulation); EL_DLL_R, right dorsal nucleus of the lateral lemniscus (group with electrical stimulation); EL_IC_L, left inferior colliculus (group with electrical stimulation); EL_IC_R, right inferior colliculus (group with electrical stimulation); EL_ILL_L, left intermediate nucleus of the lateral lemniscus (group with electrical stimulation); EL_ILL_R, right intermediate nucleus of the lateral lemniscus (group with electrical stimulation); EL_LSO_L, left lateral superior olive (group with electrical stimulation); EL_LSO_R, right lateral superior olive (group with electrical stimulation); EL_LTB_L, left lateral nucleus of the trapezoid body (group with electrical stimulation); EL_LTB_R, right lateral nucleus of the trapezoid body (group with electrical stimulation); EL_MSO_L, left medial superior olive (group with electrical stimulation); EL_MSO_R, right medial superior olive (group with electrical stimulation); EL_MTB_L, left medial nucleus of the trapezoid body (group with electrical stimulation); EL_MTB_R, right medial nucleus of the trapezoid body (group with electrical stimulation); EL_VCN_L, left ventral cochlear nucleus (group with electrical stimulation); EL_VCN_R, right ventral cochlear nucleus (group with electrical stimulation); EL_VLL_L, left ventral nucleus of the lateral lemniscus (group with electrical stimulation); EL_VLL_R, right ventral nucleus of the lateral lemniscus (group with electrical stimulation); EL_VTB_L, left ventral nucleus of the trapezoid body (group with electrical stimulation); EL_VTB_R, right ventral nucleus of the trapezoid body (group with electrical stimulation); IC, inferior colliculus; ILL, intermediate nucleus of the lateral lemniscus; LL, lateral lemniscus; LSO, lateral superior olive; LTB, lateral nucleus of the trapezoid body; MSO, medial superior olive; MTB, medial nucleus of the trapezoid body; PB, phosphate buffer; PBST, phosphate buffer containing bovine serum albumine and Triton-X; SOC, superior olivary complex; TB, trapezoid body; VCN, ventral cochlear nucleus; VLL, ventral nucleus of the lateral lemniscus; VTB, ventral nucleus of the trapezoid body

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doi:10.1016/j.brainres.2004.10.024

assess the basal expression of c-Fos in the auditory brainstem nuclei. The completeness of cochlear ablations and the response of auditory brainstem nuclei to electrical stimulation were electrophysiologically verified. C-Fos immunohistochemistry was performed using the free floating method. In anaesthetized animals with unilateral electrical stimulation of the cochlear nerve, increased expression of c-Fos was detected in the ipsilateral ventral cochlear nucleus (VCN), in the dorsal cochlear nucleus bilaterally (DCN), in the ipsilateral lateral superior olive (LSO) and in the contralateral inferior colliculus (IC). A bilateral slight increase of c-Fos expression in all subdivisions of the lateral lemniscus (LL) did not reach statistical significance. Contralateral inhibition of the nuclei of the trapezoid body (TB) was observed. Our data show that unilateral electrical stimulation of the cochlear nerve leads to increased expression of c-Fos in most auditory brainstem nuclei, similar to monaural auditory stimulation. They also confirm previous studies suggesting inhibitory connections between the cochlear nuclei. C-Fos immunoreactivity mapping is an efficient tool to detect functional changes following direct electrical stimulation of the cochlear nerve on the cellular level. This could be particularly helpful in studies of differential activation of the central auditory system by experimental cochlear and brainstem implants.

Theme: Sensory Systems
Topic: Auditory systems: central anatomy

Keywords: c-Fos; Immediate early genes; Brainstem auditory system; Cochlear nerve; Electrical stimulation

1. Introduction

Immunohistochemical staining of the immediate early gene product c-Fos has been widely used as an indicator of neuronal activation induced by a variety of external stimuli [18,19]. It may be considered as a functional marker with cellular resolution [33], providing distinct advantages over electrophysiological mapping which lacks spatial resolution, and over time-consuming and costly autoradiographic procedures.

Several studies in the past investigated c-Fos expression within the auditory system following acoustic stimulation [1,2,5,6,8,9,14,24,26,28,31]. Increased c-Fos immunoreactivity was consistently found in neurons of the dorsal cochlear nucleus (DCN) and inferior colliculus (IC). Effects of cochlear ablation on c-Fos immunoreactivity in the central auditory nuclei have been investigated in other studies [16,25]. C-Fos expression in the auditory pathway elicited by electrical stimulation of the cochlea was assessed in an animal model of cochlear implantation. These studies found c-Fos immunoreactivity mainly in the DCN and the inferior colliculus with a pattern that resembled the results after acoustic stimulation [12,20,29,35,37].

It has been shown that electrical stimulation of the central auditory system by auditory brainstem implants evokes useful hearing sensations in profoundly deaf patients even if the connection between cochlea and cochlear nucleus, the cochlear nerve, was interrupted bilaterally [7,11,17].

Recently, we have reported on the cellular basis of activating central auditory structures by electrical stimulation—an issue that had not been analyzed before. Our study showed increased c-Fos immunoreactivity in the ipsilateral VCN and bilateral DCN after unilateral electrical stimulation of the cochlear nerve with a broad distribution of c-Fos positive neurons in all tonotopic areas of the ventral and dorsal cochlear nucleus [21].

The present study follows up on this topic and provides an analysis of c-Fos expression in supra-cochlear auditory brainstem nuclei following unilateral electrical stimulation of the cochlear nerve in rats.

2. Materials and methods

2.1. Animals

Following contralateral cochlear ablation, the right cochlear nerve was electrically stimulated in 10 adult female Lewis rats. A control group (n=10) underwent bilateral cochlear ablation in order to investigate the basal expression of c-Fos in the cochlear nuclei. Cochlear ablations were performed to eliminate any uncontrolled auditory input that could lead to c-Fos expression in auditory brainstem nuclei. Animals were kept in a sound shielded room in a holding cage where they could freely move before the beginning of the experiments. All surgical procedures were performed under deep anesthesia with ketamine and medetomidine. The depth of anesthesia was monitored by cutaneous nociceptive and corneal reflexes, respectively.

2.2. Cochlear nerve stimulation

Stimulation of the cochlear nerve was performed 14 days after contralateral cochlear ablation to ensure that no contralateral auditory input could influence the activation of c-Fos in auditory brainstem nuclei. Following a retroauricular incision, a lateral suboccipital craniectomy was performed on the right side. After exposure of the cerebellopontine angle, the cochlear nerve was identified. A concentric Tungsten electrode was attached to the nerve and stimulated with biphasic electrical pulses of 120–250 μA at a rate of 5 Hz for 30 min. The stimulus intensity was