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Immediate early gene expression invoked by electrical intracochlear stimulation in some but not all types of neurons in the rat auditory brainstem

Adrian Reisch [a], Robert-Benjamin Illing [a,*], Roland Laszig [b]

[a] Neurobiological Research Laboratory, Killianstr. 5, D-79106 Freiburg, Germany
[b] Department of Otorhinolaryngology, University of Freiburg, Killianstr. 5, D-79106 Freiburg, Germany

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Abstract

Specific patterns of sensory activity may induce plastic remodeling of neurons and the communication network they form in the adult mammalian brain. Among the indicators for the initiation of neuronal remodeling is the expression of immediate early genes (IEGs). The IEGs c-fos and egr-1 encode transcription factors. Following spectrally and temporally precisely defined unilateral electrical intracochlear stimulation (EIS) that corresponded in strength to physiological acoustic stimuli and lasted for 2 h under anesthesia, we characterized those neuronal cell types in ventral (VCN) and dorsal cochlear nucleus (DCN), lateral superior olive (LSO) and central nucleus of the inferior colliculus (CIC) of the rat brain that expressed IEGs. We found that EIS affected only specific types of neurons. Whereas sub-populations of glutamatergic and glycinergic cells responded in all four regions, GABAergic neurons failed to do so except in DCN. Combining immunocytochemistry with axonal tracing, neurons participating in major ascending pathways, commissural cells of VCN and certain types of neurons of the descending auditory system were seen to respond to EIS with IEG expression. By contrast, principal LSO cells projecting to the contralateral CIC as well as collicular efferents of the DCN did not. In total, less than 50% of the identified neurons turned up expression of the IEGs studied. The pattern of IEG expression caused by unilateral EIS led us to suggest that dominant sensory activity may quickly initiate a facilitation of central pathways serving the active ear at the expense of those serving the unstimulated ear.

Keywords: Auditory system; c-Fos; Cochlear implant; Cochlear nucleus; Egr-1; Immediate early genes; Inferior colliculus; Neuronal morphology; Plasticity; Superior olive

Introduction

A complex network of ascending, descending and commissural pathways interconnects several regions in the auditory brainstem. This network is designed to serve auditory preprocessing with respect to both spectral analysis and binaural integration. The neuronal cell types that constitute its communication lines and nodes of signal integration have been characterized by studies based on axonal tracing (Wenthold, 1987; Saint Marie et al., 1989) or on morphological characterization (Osen, 1969; Brawer et al., 1974; Oliver and Morest, 1984; Rietzel and Friauf, 1998). The major neurotransmitters of neurons in the adult auditory brainstem are glutamate, glycine and GABA, with glutamate largely mediating excitation and both glycine and GABA mediating inhibition (for review cp. Malmierca, 2003). The neuronal populations of the various auditory brainstem regions exhibit unique patterns of expression of the calcium-binding proteins (CaBPs), parvalbumin (PV), calretinin (CR) and calbindin-D28k (CB) in the adult rat (Friauf, 1993, 1994; Lohmann and Friauf, 1996). This class of proteins protects neurons against harmful consequences of overexcitation (Rogers, 1989) and contributes to synaptic plasticity (Molinari et al., 1996; Caillard et al., 2000).

Abbreviations: ABR, auditory brainstem response; CB, calbindin; CIC, central nucleus of the inferior colliculus; CR, calretinin; DCN, dorsal cochlear nucleus; EABR, electrically evoked auditory brainstem response; EIS, electrical intracochlear stimulation; GABA, gamma amino butyric acid; IEG, immediate early gene; LOC neurons, lateral olivocochlear neurons; LSO, lateral superior olive; PV, parvalbumin; VCN, ventral cochlear nucleus.

* Corresponding author. Neurobiological Research Laboratory, Universitäts-HNO-Klinik, Killianstr. 5, D-79106 Freiburg, Germany. Fax: +49 761 270 4075.
E-mail address: robert.illing@uniklinik-freiburg.de (R.-B. Illing).

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Expression of immediate early genes (IEGs) is an integral step in the molecular signal cascades triggered by specific and dominant patterns of neuronal activity to initiate long-term potentiation (LTP) known to underlie synaptic plasticity. The emergence of the IEG transcription factors Egr-1 (Sukhatme et al., 1988), also known as Zif-268 (Christy et al., 1988), NGFI-A (Milbrandt, 1987) or Krox 24 (Lemaire et al., 1988), and c-Fos (Morgan and Curran, 1989) is indicative for neurons in the adult mammalian brain to be transformed into a state of growth or remodeling (Cole et al., 1989; Rampon et al., 2000). A massive expression of c-Fos in the cochlear nucleus complex may be induced by stimulation with sound (Hillman et al., 1997) or after an electrical stimulation of the cochlea (Saito et al., 2000; Illing et al., 2002). Beyond the cochlear nucleus, neurons throughout the ascending auditory system including the superior olivary complex (Adams, 1995), the dorsal nucleus of the lateral lemniscus (Saint Marie et al., 1999a), the central nucleus of the inferior colliculus (CIC; Ehret and Fischer, 1991; Friauf, 1995), the medial geniculate body (Rouiller et al., 1992) and the auditory cortex (Zuschratter et al., 1995; Geissler and Ehret, 2004) may respond to acoustic or electric stimulation of the ear with a tonotopically specific up-regulation of c-Fos. After stimulating anesthetized cats with sound, IEGs were reported to be expressed in neurons of heterogeneous size in the cochlear nucleus and inferior colliculus as well as in small neurons of the lateral superior olive (LSO), suggested to contribute to the lateral olivocochlear (LOC) system (Adams, 1995). Studies on the awake rat claim that most cells of the cochlear nucleus with the exception of octopus cells up-regulate IEGs after sound stimulation (Saint Marie et al., 1999b; Yang et al., 2005). However, it is as yet unclear how large the share of the neurons is that responds to cochlear nerve activity, and whether the affected neurons constitute specific sub-populations related to their morphology, molecular type, or axonal projection.

In order to determine if afferent activity has general or specific consequences for the neural network of the auditory brainstem, we determined which neurons express IEGs upon strictly controlled electrical intracochlear stimulation (EIS) by their molecular or projectional type. We choose to employ EIS rather than acoustic stimulation to take advantage of a full control of the spectral and temporal pattern of electrophysiological activity in the system. With the present study, we aim to obtain knowledge about a basic topographic and cellular pattern of IEG activation. Once this has been defined, our experimental system will allow in follow-up studies to vary the temporal structure of the stimulus, an approach that has been utilized in slice culture experiments necessarily dealing with severely truncated and traumatized neuronal networks. By distinct contrast, we work upon an intact neuronal network. Combining EIS with the detection of IEG expression in characterized neurons we discovered that, within the tonotopic region related to the stimulation site, only specific types of neurons, and altogether less than half of the population of neurons present, responded to the activity in the sensory nerve with changes to their gene expression.

Parts of this study were published in abstract form (Reisch and Illing, 2005).

Materials and methods

Animals

Brains of 30 adult female Wistar rats aged 7–9 weeks were used. Care and use of the animals as reported here were approved by the appropriate agency (Regierungspräsidium Freiburg, permission number 37/9185.81/1/267.2). Rats were anesthetized with an intraperitoneal injected mixture of s-ketamine (50 mg/kg, Ketanest, Parke-Davis, Ann Arbor, MI) and xylazine (5 mg/kg, Rompun, Bayer-Leverkusen, Germany) for ear bone removal and tracer injection. For EIS, anesthesia was achieved with urethane (1.5 g/kg i.p.; Fluka AG, Buchs, Switzerland). Preceding transcardial perfusion, rats were given a lethal dose of pentobarbital (0.6 ml/kg of Narcodorm-n, Alvetra GmbH, Neumünster, Germany, i.p.).

Electrical intracochlear stimulation

To reduce the level of hearing-dependent spiking activity in the auditory system, the tympanic membrane was disrupted and the malleus was removed bilaterally 2 days before EIS. Judging from the auditory brainstem response (ABR), loss of middle ear transmission led to an immediate and sustained rise of hearing threshold by about 50 dB (Fig. 1A, upper and middle lane). We previously demonstrated that removal of the middle ear bones, without further stimulation, fails to up-regulate c-Fos expression (Illing et al., 2002). The cochlea was exposed using a retroauricular surgical approach. A hole was made into the bony wall of the basal cochlea to insert the electrode carrier (Illing and Reisch, 2006). We applied bipolar stimulation, using a cochlear implant (Model CI24M) run by the Nucleus Implant Communicator, both kindly provided by Cochlear AG (Basel, Switzerland). The electrically evoked brainstem response (EABR) was recorded by placing steel needle electrodes subcutaneously at vertex and mastoids to corroborate for the correct placement of the stimulation electrodes and to determine an appropriate current level. The EABR was visualized using an averager (Multiliner E; Evolution 1.70c; Toennies, Würzburg, Germany), calculating mean amplitudes over 500 sweeps in a frequency band between 0.1 and 3 kHz. We aimed to obtain an initial EABR amplitude in the range of 5 to 10 μV, corresponding to acoustically evoked amplitudes of 30 to 60 dB (Fig. 1A, lower lane). We applied EIS unilaterally (left side) in acute experiments for 2 h. Frequency of the biphasic stimulation remained constant at 50 Hz and pulse width was set to 50 μs, while the current level was varied between 260 and 950 μA between experiments to compensate for variations in electrode position. Throughout the period of stimulation, a duty cycle of 50% with 30 s stimulation alternating with 30 s pause was held. The ABR was recorded every 15 min to verify unchanged electrode positioning and sustained effective stimulation of the central auditory system (Fig. 1B). Despite serious efforts to keep stimulation conditions constant from experiment to experiment, the EABR was not always as complete as in Fig. 1A