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Brain Research 1062 (2005) 92 – 100
BRAIN RESEARCH
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Research Report

Expression of Trk receptors in otolith-related neurons in the vestibular nucleus of rats

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Accepted 25 September 2005
Available online 26 October 2005

Abstract

The expression of the three Trk receptors (TrkA, TrkB, and TrkC) in otolith-related neurons within the vestibular nuclei of adult Sprague–Dawley rats was examined immunohistochemically. Conscious animals were subjected to sinusoidal linear acceleration along either the anterior-posterior (AP) or interaural (IA) axis on the horizontal plane. Neuronal activation was defined by Fos expression in cell nuclei. Control animals, viz labyrinthectomized rats subjected to stimulation and normal rats that remained stationary, showed only a few sporadically scattered Fos-labeled neurons. Among experimental rats, the number of Fos-labeled neurons and their distribution pattern in each vestibular subnucleus in animals stimulated along the antero-posterior axis were similar to those along the interaural axis. No apparent topography was observed among neurons activated along these two directions. Only about one-third of the Trk-immunoreactive neurons in the vestibular nucleus expressed Fos. Double-labeled Fos/TrkA, Fos/TrkB and Fos/TrkC neurons constituted 85–98% of the total number of Fos-labeled neurons in vestibular nuclear complex and its subgroups x and y. Our findings suggest that Trk receptors and their cognate neurotrophins in central otolith neurons may contribute to the modulation of gravity-related spatial information during horizontal head movements.

Theme: Motor systems and sensorimotor integration
Topic: Vestibular system

Keywords: Fos; Trk receptor; Vestibular nuclei; Utricle; Immunocytochemistry

1. Introduction

The neurotrophin family, which includes nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin (NT)-3, and NT-4/5 [2,25], are known to be crucial for cell regulation and differentiation in developing animals [19], as well as synaptic plasticity and neuronal survival in adult animals [2,25,35,37,59]. The action of neurotrophin is mediated by its binding to high affinity tyrosine kinase receptors (Trk). NGF signals through TrkA receptors, while BDNF and NT-4/5 signal through TrkB receptors. NT-3, however, primarily signals through TrkC receptor [2,25,59]. The biological effects of neurotrophins in the development of the inner ear have been extensively investigated [15,18,19], BDNF and NT3 are expressed in the inner ear. In rat embryos, these neurotrophins are important for the establishment of contacts between neuron and target cell in the inner ear [40,46,56]. At the postnatal stage, however, the expression of BDNF and NT3 is restricted to inner hair cells [40]. In the adult rat, neurotrophin receptors p75, TrkB, and TrkC are expressed in the

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doi:10.1016/j.brainres.2005.09.025

vestibular ganglion [40]. Moreover, exogenous BDNF and NT3 protect adult vestibular ganglion neurons and hair cells from ototoxins [25,36,61], suggesting their role in modulating neuronal survival. Neurotrophin receptors are also widely distributed within the central nervous system [23,25,27]. Specific neuronal populations require certain neurotrophin(s) and Trk receptor(s) to maintain their normal functions [23,25]. For example, animals lacking one of the neurotrophins or the cognate Trk receptors displayed loss of subpopulations of sensory neurons within the dorsal root ganglia [25]. Within the vestibular system of adult animals, neurotrophins and their cognate receptors play an important role in the plasticity and the maintenance of central vestibular neurons [21,27,50,59,60]. In guinea pigs, elevated levels of BDNF protein were observed in the lateral vestibular nucleus on both sides of the brainstem after hemilabyrinthectomy [50]. In addition, NT3-knockout mice exhibited a delay in vestibular compensation [21]. These findings suggest the participation of BDNF and NT-3 in plasticity during vestibular compensation. In adult rats, neurons in various subnuclei of the vestibular nuclear complex were found to express Trk receptors A–C [60], but the functional entities of these neurons have not been identified.

With an electrophysiological approach, the number of lateral and descending vestibular neurons responding to otolithic stimulation along the interaural direction was twice of those along the antero-posterior direction [7,26,29,30]. In the medial vestibular nucleus and y group subnucleus, however, a predominance of neurons responding to the antero-posterior direction was found [9,49]. These findings suggest regional distribution of central otolith neurons distinguishable by their response vectors. These electrophysiological data randomly sampled from a large number of animals are inadequate for an estimate of the quantitative distribution of otolith-related neurons that encode specific spatial orientations in individual animals. Recently, *c-fos* expression triggered by constant velocity off-vertical axis rotation has proven effective for documenting the location of central otolith neurons that encode all horizontal orientations [10,32]. We aim therefore to use *c-fos* expression as a marker of vestibular nuclear neurons that encode anterior–posterior or transverse orientations and thus to map their distinct anatomical distribution, if any. In addition, it remains to be determined whether or not these central otolith neurons show differential expression of Trk receptors for possible modulation of gravity-related spatial information during horizontal head movements. In the present study, the otolithic origin of Fos expression in test animals was confirmed by labyrinthectomized and/or stationary controls. With double immunostaining, we demonstrated that a vast majority of Fos-immunoreactive (-ir) vestibular nuclear neurons expressed Trk receptors, and that these neurons, functionally activated either by antero-posterior or interaural linear acceleration on the horizontal plane, showed overlapping distribution.

2. Materials and methods

2.1. Sinusoidal linear acceleration

Conscious adult Sprague–Dawley rats ($n = 40$), weighing 220–250 g, were used. All animal protocols and procedures described were performed in compliance with the *Principles of Laboratory Animal Care* (NIH publication no. 86-23, revised 1985) and were approved by the University of Hong Kong Committee on the Use of Live Animals in Research. In the present study, 12 rats were subjected to sinusoidal linear acceleration along the antero-posterior (AP group; $n = 6$) or interaural (IA group; $n = 6$) axis on the horizontal plane. The remaining rats were divided into one of the six control groups: (i) normal rats, restrained but not subjected to horizontal linear acceleration along the AP direction (Control 1; $n = 4$) or the IA direction (Control 2; $n = 4$); (ii) bilateral labyrinthectomized rats, restrained but not subjected to horizontal linear acceleration along the AP direction (Control 3; $n = 4$) or the IA direction (Control 4; $n = 4$); and (iii) bilateral labyrinthectomized rats, subjected to horizontal linear acceleration along the AP direction (Control 5; $n = 6$) or the IA direction (Control 6; $n = 6$).

As described in previous studies, each conscious experimental rat was enclosed in a perspex restrainer with the head of the animal cushioned against a silicone head-mask supported externally by an acrylic tube [10,32]. The head-mask was designed with aims to maintain the animal's head on the stereotaxic horizontal plane and to cushion the head so as to prevent possible movement during horizontal linear acceleration. The perspex restrainer housing the conscious rat was placed on a cart that was coupled to a set of parallel linear rails attached to a supporting frame. Then the animal was subjected to sinusoidal linear acceleration at a frequency of 1.5 Hz for 90 min. This stimulus paradigm provided the optimal condition for Fos expression. The animals expressed no signs of struggle or stress in the course of stimulation. Their behavior after stimulation was calm and showed no obvious sign of ataxia. After stimulation, the animals were sacrificed and perfused immediately.

2.2. Immunofluorescent double labeling for Fos protein and Trk receptors

Following stimulation, the animals were deeply anesthetized with pentobarbital sodium (0.1 ml/100 g, 50 mg/ml, i.p.; Abbott Laboratories, USA) and perfused with 150 ml of 0.05 M phosphate-buffered saline (PBS, pH 7.2–7.4) via the ascending aorta. Rats were then decapitated, and brains were removed and immersed in a fixative containing 0.5% paraformaldehyde and 75% saturated picric acid in 0.1 M phosphate buffer (pH 7.4) for 6 h, followed by postfixation in the same fixative but containing 4% paraformaldehyde for 2 h. Finally, the brains were placed in 0.05 M PBS containing 30% sucrose overnight at 4 °C. Frozen coronal