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ALTERED VESICULAR GLUTAMATE TRANSPORTER DISTRIBUTIONS IN THE MOUSE COCHLEAR NUCLEUS FOLLOWING COCHLEAR INSULT

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Abstract—Vesicular glutamate transporters 1 and 2 (VGLUT1 and VGLUT2) have distinct distributions in the cochlear nucleus that correspond to sources of the labeled terminals. VGLUT1 is mainly associated with terminals of auditory nerve fibers, whereas VGLUT2 is mainly associated with glutamatergic terminals deriving from other sources that project to the cochlear nucleus (CN), including somatosensory and vestibular terminals. Previous studies in guinea pig have shown that cochlear damage results in a decrease of VGLUT1-labeled puncta and an increase in VGLUT2-labeled puncta. This indicates cross-modal compensation that is of potential importance in somatic tinnitus. To examine whether this effect is consistent across species and to provide a background for future studies, using transgenesis, the current study examines VGLUT expression profiles upon cochlear insult by intracochlear kanamycin injections in the mouse. Intracochlear kanamycin injections abolished ipsilateral ABR responses in all animals and reduced ipsilateral spiral ganglion neuron densities in animals that were sacrificed after four weeks, but not in animals that were sacrificed after three weeks. In all unilaterally deafened animals, VGLUT1 density was decreased in CN regions that receive auditory nerve fiber terminals, i.e., in the deep layer of the dorsal cochlear nucleus (DCN), in the interstitial region where the auditory nerve enters the CN, and in the magnocellular region of the antero- and posteroventral CN. In contrast, density of VGLUT2 expression was upregulated in the fusiform cell layer of the DCN and in the granule cell lamina, which are known to receive somatosensory and vestibular terminals. These results show that a cochlear insult induces cross-modal compensation in the cochlear nucleus of the mouse, confirming previous findings in guinea pig, and that these changes are not dependent on the occurrence of spiral ganglion neuron degeneration.

Key words: cochlear damage, cochlear nucleus, multisensory, plasticity, tinnitus, vesicular glutamate transporters.

INTRODUCTION

Vesicular glutamate transporters (VGLUTs) mediate the uptake of L-glutamate into synaptic vesicles. Three homologous subtypes (VGLUT1–3) have been identified in the mammalian CNS (Takamori et al., 2000, 2001, Fremeau et al., 2002), in which VGLUT1 and 2 have been studied most extensively. Occasionally, VGLUT1 and VGLUT2 co-localize, such as in the mossy fibers of the cerebellum (Hioki et al., 2003), however, generally, the spatial expression profiles of the two subtypes are distinct from each other throughout the brain (Fremeau et al., 2001; Kaneko et al., 2002; Hioki et al., 2003; Graziano et al., 2008; Balaram et al., 2015).

The cochlear nucleus (CN) is the first target of the VIIIth nerve and contains a number of different regions, which are defined by their relative locations and composition of cell types (Fig. 1). The dorsal part of the cochlear nucleus (DCN) consists of a molecular layer, a fusiform cell layer, and a deep layer (abbreviated as DCN1, DCN2, and DCN3, respectively). The ventral part of the cochlear nucleus (VCN) is subdivided in a posterior and an anterior region (PVCN and AVCN, respectively). In the mouse CN, the area where the auditory nerve enters the nucleus can be defined as a separate region, called the interstitial region (INT) or auditory nerve root (Lorente de Nó, 1933). The granule cell lamina (GCL) encapsulates the VCN on the dorsal and lateral sides and primarily contains granule and small cells. The CN is the first auditory brain station where auditory input is integrated with input from other sensory modalities, such as the somatosensory and the vestibular systems (Cant and Morest, 1978; Shore and Moore, 1998; Shore, 2005; Barker et al., 2012). Both VGLUT1 and VGLUT2 are highly expressed in the CN, with distinct distributions. These distinct expression profiles correspond to the source of the glutamatergic terminals (Zhou et al., 2007; Barker et al., 2012). VGLUT1 co-labels with synaptic terminals of type I auditory nerve

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Abbreviations: ABR, auditory brainstem response; ANF, auditory nerve fiber; ANOVA, analysis of variance; AVCN, anteroventral cochlear nucleus; CN, cochlear nucleus; DCN, dorsal cochlear nucleus; DCN1, molecular layer of the dorsal cochlear nucleus; DCN2, fusiform cell layer of the dorsal cochlear nucleus; DCN3, deep layer of the dorsal cochlear nucleus; EDTA, ethylenediaminetetraacetic acid; GCL, granule cell lamina; INT, interstitial region; PBS, phosphate-buffered saline; PVCN, posteroventral cochlear nucleus; SGN, spiral ganglion neuron; VCN, ventral cochlear nucleus; VGLUT1, vesicular glutamate transporter 1; VGLUT2, vesicular glutamate transporter 2.

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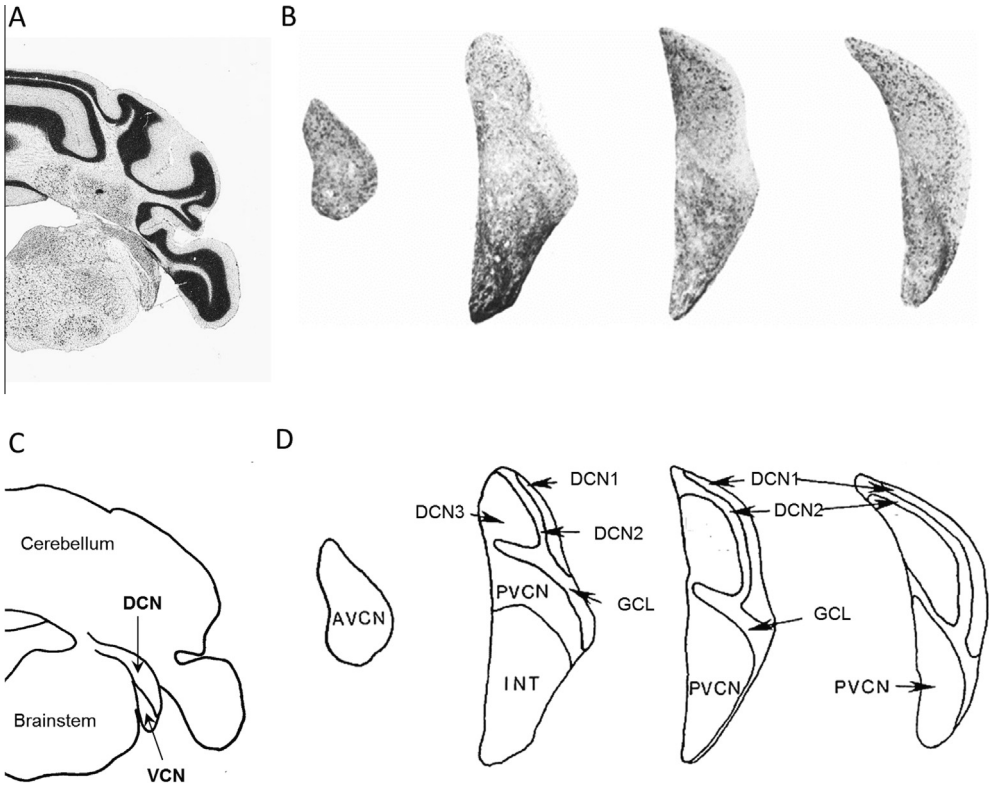

Fig. 1. The mouse cochlear nucleus. (A) Nissl staining of a coronal section of the mouse brain (Mikula et al., 2007), showing the location of the cochlear nucleus in the brainstem. This section was located at approximately 5.88 mm posterior to bregma and 2.08 mm posterior to the interaural axis. (B) Coronal sections along the rostrocaudal axis of the mouse CN, stained with Cresyl Violet and Luxol Fast Blue, modified from (Martin, 1981). (C) Schematic drawing of the section shown in panel A, presenting the location of the dorsal and ventral cochlear nucleus in the mouse brainstem. (D) Schematic drawings of the sections shown in panel C, modified from (Martin, 1981). Regions where photomicrographs were taken for VGLUT analysis are illustrated with arrows and include the molecular layer, fusiform cell layer, deep layer of the DCN (DCN1, DCN2, and DCN3, respectively), GCL, INT, AVCN, and PVCN.

fibers (ANFs) and is mostly expressed in the magnocellular region of the ventral CN (VCN) and the deep layer of the dorsal CN (DCN3) (Zhou et al., 2007; Gomez-Nieto and Rubio, 2009). On the other hand, VGLUT2 co-labels with non-auditory terminals, including those from the somatosensory and the vestibular system, and is mostly expressed in GCL, where these terminals primarily innervate granule cells (Haenggeli et al., 2005; Zhou et al., 2007; Zeng et al., 2011; Barker et al., 2012). Thus, studying VGLUT1 and VGLUT2 expression provides insights into the magnitude and spatial distributions of both auditory and non-auditory innervation of the CN.

Previous studies in guinea pig have shown that the expression profiles of VGLUT1 and VGLUT2 in the CN are affected by cochlear damage. After kanamycin injections (Zeng et al., 2009, 2012), VGLUT1 is down-regulated in the CN, as would be expected from the loss of afferent input from the ANFs. Interestingly, VGLUT2 is up-regulated after cochlear damage, which reflects a reactive re-innervation of the CN by inputs from the somatosensory system (Zeng et al., 2009, 2012).

This cross-modal plasticity in the CN after cochlear damage is of potential importance for 'somatic' tinnitus, in which patients can modify their tinnitus through head and neck maneuvers, such as in jaw clenching (Levine et al., 2003), or when their tinnitus can be attributed to an insult in the head and neck region (Lockwood et al., 1998; Pinchoff et al., 1998, Levine, 1999). Indeed, animals with behaviorally confirmed tinnitus have altered functional auditory–somatosensory integration in the CN (Dehmel et al., 2012; Koehler and Shore, 2013). To further study the role of cochlear damage-induced upregulation of VGLUT2 terminals in the CN in somatic tinnitus, conditional VGLUT2 knock-out studies in mice are of high importance (Brown et al., 2008). Therefore, the current study investigates whether the changes in VGLUT1 and VGLUT2 expression in the CN previously shown in guinea pigs (Zeng et al., 2009, 2012) are also evident in mice following cochlear insult induced by intracochlear kanamycin injection.

EXPERIMENTAL PROCEDURES

Six adult C57Bl6 mice (Charles River Inc.) of 5 weeks of age were used in this study. Mice were socially housed in a 12:12-h light/dark cycle and had *ad libitum* access to food and water. Four animals were unilaterally deafened by intracochlear kanamycin injection and two age-matched animals were used as controls. VGLUT1 and VGLUT2 densities were assessed in the ipsilateral and contralateral CN of deafened animals and in both CNS of control animals. All procedures were approved