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Research Paper

Effects of surgical lesions on choline acetyltransferase activity in the cat cochlea☆

Mark J. Frilling [a], Gregory J. Wiet [b], Donald A. Godfrey [a, *], Judy A. Parli [c], Jon D. Dunn [d], C. David Ross [c]

[a] Division of Otolaryngology and Dentistry, Department of Surgery, and Department of Neurology, University of Toledo College of Medicine and Life Sciences, Toledo, OH, USA
[b] Department of Otolaryngology, Head and Neck Surgery, The Ohio State University and Nationwide Children's Hospital, Columbus, OH, USA
[c] Department of Physiology, Oral Roberts University School of Medicine, Tulsa, OK, USA
[d] Department of Anatomy, Oral Roberts University School of Medicine, Tulsa, OK, USA

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ABSTRACT

Although it is well established that the choline acetyltransferase (ChAT, the enzyme for acetylcholine synthesis) in the mammalian cochlea is associated with its olivocochlear innervation, the distribution of this innervation in the cochlea varies somewhat among mammalian species. The quantitative distribution of ChAT activity in the cochlea has been reported for guinea pigs and rats. The present study reports the distribution of ChAT activity within the organ of Corti among the three turns of the cat cochlea and the effects of removing olivocochlear innervation either by a lateral cut aimed to totally transect the left olivocochlear bundle or a more medial cut additionally damaging the superior olivary complex on the same side. Similarly to results for guinea pig and rat, the distribution of ChAT activity in the cat outer hair cell region showed a decrease from base to apex, but, unlike in the guinea pig and rat, the cat inner hair cell region did not. As in the rat, little ChAT activity was measured in the outer supporting cell region. As previously reported for whole cat cochlea and for rat cochlear regions, transection of the olivocochlear bundle resulted in almost total loss of ChAT activity in the hair cell regions of the cat cochlea. Lesions of the superior olivary complex resulted in loss of ChAT activity in the inner hair cell region of all cochlear turns only on the lesion side but bilateral losses in the outer hair cell region of all turns. The results are consistent with previous evidence that virtually all cholinergic synapses in the mammalian cochlea are associated with its olivocochlear innervation, that the olivocochlear innervation to the inner hair cell region is predominantly ipsilateral, and that the olivocochlear innervation to the outer hair cells is bilateral.

1. Introduction

The olivocochlear system, or olivocochlear bundle (OCB), has long been recognized to provide the primary efferent innervation of the cochlea to affect the sensitivity of its coding of auditory signals (Warr, 1992; Guinan, 2006).

The OCB originates bilaterally from the superior olivary complex (SOC) in the brain stem and consists of lateral and medial efferent systems. The lateral olivocochlear (LOC) system consists of neurons with somata laterally located in the SOC and thin, unmyelinated axons, while medial olivocochlear (MOC) neurons have somata more medially and rostrally located in the SOC and thicker, myelinated axons. Both MOC and LOC axons travel dorsomedially in close proximity to the fourth ventricle upon leaving the SOC. At this point, about 70% of MOC fibers in the cat decussate to the opposite side of the brain stem and about 30% remain ipsilateral. A few MOC neurons have bilateral projections. About 80% of LOC fibers remain ipsilateral, and about 20% cross to the opposite side in the cat. All of these fibers traverse the facial nerve root before forming the compact OCB in the vestibular nerve root. The LOC fibers comprise about 60% of the OCB in the cat (Warr, 1992).

☆ The experimental procedures for this study were completed while most of the authors were members of the Department of Physiology (GJW, DAG, JAP, CDR) or Anatomy (JDD) at Oral Roberts University School of Medicine, Tulsa, Oklahoma.

* Corresponding author. Department of Neurology, Mail stop 1195, 3000 Arlington Avenue, University of Toledo College of Medicine, Toledo, OH 43614, USA.
E-mail address: Donald.godfrey@utoledo.edu (D.A. Godfrey).

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Abbreviations in text

ChAT	choline acetyltransferase
IHC	inner hair cells
IntraCN	intra-cochlear nucleus
LOC	lateral olivocochlear
MOC	medial olivocochlear
OCB	olivocochlear bundle
OHC	outer hair cells
OSC	outer supporting cells
r	correlation coefficient
SOC	superior olivary complex

In the cochlea, the LOC fibers have been shown to predominantly synapse with the type I afferent nerve fibers underneath the inner hair cells (IHC), whereas the MOC fibers primarily synapse on the bases of the outer hair cells (OHC). The LOC fibers form the inner spiral bundle and the tunnel spiral bundle under the inner hair cells of the organ of Corti of the cat (Ginzberg and Morest, 1984; Liberman et al., 1990). In the cat, the fiber population of the inner spiral bundle peaks in mid-cochlear regions; synapses per auditory nerve fiber peak in more apical parts, but the number of auditory nerve fibers decreases apically (Liberman et al., 1990). The MOC neurons travel as upper tunnel radial fibers in the organ of Corti before reaching the OHC. In the cat, few if any MOC fibers travel in the inner spiral bundle (Liberman et al., 1990). Efferent innervation of the OHC is greatest in the mid-to-high frequency region of the cochlea, with a decline in innervation both apically and basally (Liberman et al., 1990; Warr, 1992).

Choline acetyltransferase (ChAT), the enzyme responsible for the synthesis of acetylcholine, has been shown to be a definitive marker for cholinergic neurons (Godfrey et al., 1985). The high ChAT activity in the OCB is consistent with all LOC and MOC fibers being cholinergic (Godfrey et al., 1984), and the OCB fibers exhibit immunoreactivity to ChAT (Altschuler and Fex, 1986; Eybalin, 1993). Some LOC neurons have also shown immunoreactivity to the neurotransmitters enkephalin and calcitonin gene-related peptide in conjunction with ChAT, whereas MOC neurons have shown immunoreactivity only to ChAT (Altschuler and Fex, 1986; Eybalin, 1993).

Quantitative assessment of the density of acetylcholine metabolic enzymes (acetylcholinesterase and ChAT) in the cochleas of the guinea pig (Godfrey et al., 1976) and rat (Godfrey and Ross, 1985; Godfrey et al., 1986) have shown high activities of these enzymes in the IHC and OHC regions. In the cat, ChAT in the cochlea as a whole has been associated with its OCB innervation (Jasser and Guth, 1973), but there has not previously been an analysis of the hair cell regions of the organ of Corti. To specifically evaluate the cholinergic OCB innervation of the hair cell regions of the cat cochlea, ChAT activity was measured in microdissected samples of the organ of Corti of 13 cats, some of which had surgical cuts in the brain stem that damaged the olivocochlear system. A preliminary presentation of the results has been made (Wiet et al., 1989).

2. Methods

2.1. Preparation of temporal bones

These experiments were carried out on temporal bones obtained during a previous study on the cochlear nucleus of cats (Godfrey et al., 1990a). The temporal bones were isolated and quickly frozen after the removal and freezing of brain tissues. The cats are identified by the same letters as in the previous study, except for two cats for which brain tissue was not included in that study (Table 1).

The results presented here are derived from 13 cats of both sexes weighing 2–4 kg. Placement of surgical cuts and animal treatment, which was in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals, have been described, and maps of the lesion locations have been published (Godfrey et al., 1990a) except for cats C and E, with sham lesions, and cat D2, which was not included in the previous study. The lesion locations for the 9 lesioned cats included in the previous study are shown in abbreviated form in Fig. 1, and a more comprehensive map of the lesion location for cat D2 is shown in Fig. 2. The cats can be separated into 4 groups: 3 sham-lesioned cats, with exposure of the left cochlear nucleus but no cut made (C, E, I), 5 cats with transection of the left OCB (A, D2, F, H, J), 3 cats with left superior olivary complex (SOC) damage as well as transection of most or all of the left OCB (B, G, K), and 2 cats with cuts within the left cochlear nucleus to separate its rostral from its caudal parts (intra-cochlear nucleus (intraCN) cuts) (D, L). Post −surgery survival times were 7–10 days. Brain parts and temporal bones were frozen in Freon (Curtin Matheson “Freeze It”) cooled to its freezing point (−130 °C) with liquid nitrogen. Temporal bones were trimmed close to their cochlea portions before freezing approximately an hour post-mortem. Cochleas were freeze-dried (Thalmann, 1976) for 9–14, usually 10 days at −40 °C and then stored under vacuum at −20 °C until dissected.

Table 1
Information about the lesions in the cats that provided cochlear tissues.

Cat	Lesion
A	Complete lateral cut through left brain stem, just medial to the cochlear nucleus, completely transecting the left OCB, shown in Fig. 1 of Godfrey et al. (1990a)
B	Large damage to the left side of the brain stem, completely transecting the left OCB and encroaching partially on the left SOC, shown in Fig. 7 of Godfrey et al. (1990a)
C	Sham lesion; left cochlear nucleus exposed but no cut made, not included in Godfrey et al. (1990a)
D	Cut within left cochlear nucleus, shown in Fig. 8 of Godfrey et al. (1990a)
D2	Complete lateral cut through left brain stem, just medial to the cochlear nucleus, completely transecting the left OCB, not included in Godfrey et al. (1990a) but shown in Fig. 2
E	Sham lesion; left cochlear nucleus exposed but no cut made, not shown in Godfrey et al. (1990a)
F	Cut through dorsolateral part of left brain stem, completely transecting the left OCB, shown in Fig. 5 of Godfrey et al. (1990a)
G	Cut through left brain stem, transecting the left OCB and encroaching partially on the left SOC, transecting its contralateral projections, shown in Fig. 7 of Godfrey et al. (1990a)
H	Cut through dorsolateral part of left brain stem, completely transecting the left OCB, shown in Fig. 5 of Godfrey et al. (1990a)
I	Sham lesion; left cochlear nucleus exposed but no cut made, shown in Fig. 1 of Godfrey et al. (1990a)
J	Cut through dorsolateral part of left brain stem, completely transecting the left OCB, shown in Fig. 5 of Godfrey et al. (1990a)
K	Cut through left brain stem, transecting the left OCB and encroaching partially on the left SOC, transecting its contralateral projections, shown in Fig. 7 of Godfrey et al. (1990a)
L	Cut within left cochlear nucleus, shown in Fig. 8 of Godfrey et al. (1990a)

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