Hearing Research 333 (2016) 25–36

Contents lists available at ScienceDirect

Hearing Research

journal homepage: www.elsevier.com/locate/heares

Research paper

Cortical and thalamic connectivity of the auditory anterior ectosylvian cortex of early-deaf cats: Implications for neural mechanisms of crossmodal plasticity

M. Alex Meredith [a, *], H. Ruth Clemo [a], Sarah B. Corley [a, c, 1], Nicole Chabot [b], Stephen G. Lomber [b]

[a] Virginia Commonwealth University School of Medicine, Department of Anatomy and Neurobiology, Richmond, VA 23298, USA
[b] Cerebral Systems Laboratory, The Brain and Mind Institute, Natural Sciences Centre, University of Western Ontario, London, Ontario N6A 5B7, Canada
[c] University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

ARTICLE INFO

Article history:
Received 7 October 2015
Received in revised form
23 November 2015
Accepted 1 December 2015
Available online 24 December 2015

Keywords:
Hearing loss
Visual cortex
Somatosensory cortex
Thalamus
Sensory substitution

ABSTRACT

Early hearing loss leads to crossmodal plasticity in regions of the cerebrum that are dominated by acoustical processing in hearing subjects. Until recently, little has been known of the connectional basis of this phenomenon. One region whose crossmodal properties are well-established is the auditory field of the anterior ectosylvian sulcus (FAES) in the cat, where neurons are normally responsive to acoustic stimulation and its deactivation leads to the behavioral loss of accurate orienting toward auditory stimuli. However, in early-deaf cats, visual responsiveness predominates in the FAES and its deactivation blocks accurate orienting behavior toward visual stimuli. For such crossmodal reorganization to occur, it has been presumed that novel inputs or increased projections from non-auditory cortical areas must be generated, or that existing non-auditory connections were 'unmasked.' These possibilities were tested using tracer injections into the FAES of adult cats deafened early in life (and hearing controls), followed by light microscopy to localize retrogradely labeled neurons. Surprisingly, the distribution of cortical and thalamic afferents to the FAES was very similar among early-deaf and hearing animals. No new visual projection sources were identified and visual cortical connections to the FAES were comparable in projection proportions. These results support an alternate theory for the connectional basis for crossmodal plasticity that involves enhanced local branching of existing projection terminals that originate in non-auditory as well as auditory cortices.

1. Introduction

Individuals who experience profound sensory loss early in life often exhibit dramatic functional neurological changes that lead to perceptual and behavioral improvements in the remaining senses. Regarded as 'adaptive' or 'compensatory plasticity,' these behavioral effects have been reported for early-blind or early-deaf humans for a variety of sensory tasks (for review, see Merabet and Pascual-Leone, 2010; Frasnelli et al., 2011). In a broader context, the phenomenon where the representation of a damaged or lost sensory modality is replaced by the remaining, intact modalities is termed 'crossmodal plasticity' and this functional effect has been confirmed in experimental animals. In a seminal series of experiments on compensatory plasticity, visually-deprived cats demonstrated auditory localization behaviors which exceeded that present in normally-sighted controls. Furthermore, a region of normally visual cortex not only showed auditory crossmodal plasticity in visually deprived animals, but also contained auditory neurons with supranormal localization sensitivities (e.g., Rauschecker and Korte, 1993; Korte and Rauschecker, 1993).

Compared to the volume of studies of vision loss, few experimental investigations of the crossmodal effects of early deafness have been conducted, until recently. Congenitally deaf mice have been shown to exhibit both visual and somatosensory responses in the primary auditory (A1) area, as well as an expanded

* Corresponding author. Department of Anatomy and Neurobiology, Virginia Commonwealth University School of Medicine, 1101 E. Marshall St., Sanger Hall Rm. 12-067, Richmond, VA 23298-0709, USA.
E-mail address: mameredi@vcu.edu (M.A. Meredith).
[1] Present address: Department of Dermatology, University of North Carolina, Chapel Hill, NC 27516, USA.

http://dx.doi.org/10.1016/j.heares.2015.12.007
0378-5955/

representation of the primary visual area (Hunt et al., 2006). In early-deaf ferrets, auditory cortical fields including A1 and the anterior auditory field (AAF) exhibited somatosensory-evoked activity (Meredith and Allman, 2012). In congenitally deaf cats, visual crossmodal plasticity has been identified in the dorsal auditory zone (DZ) and the posterior auditory field (PAF; Lomber et al., 2010, 2011), but not in A1 (Kral et al., 2003), while both visual and somatosensory crossmodal reorganization has been demonstrated in the AAF and the auditory field of the anterior ectosylvian sulcus (FAES) of early-deaf cats (Meredith and Lomber, 2011; Meredith et al., 2011).

To date, one of the most comprehensively studied auditory regions to demonstrate crossmodal plasticity is the FAES. In hearing cats, the FAES contains a mixture of auditory (~77%) and non-auditory (~33%; mostly in the form of auditory-visual, and auditory-somatosensory multisensory neurons; Meredith et al., 2011) and many FAES neurons are characterized by sensitivity to acoustic location (Clarey and Irvine, 1990a; Korte and Rauschecker, 1993; Xu et al., 1998; Las et al., 2008) and sound movement (Jiang et al., 2000). Connections from auditory cortical sources dominate inputs to the FAES, especially from areas AAF and DZ (Lee and Winer, 2008) while non-auditory afferents arrive largely from somatosensory area SIV (Meredith et al., 2006) and the visual lateral suprasylvian areas (Clarey and Irvine, 1990b). The FAES is the major source of auditory corticotectal projections (Meredith and Clemo, 1989; Chabot et al., 2013) and, therefore, plays an important role in mediating superior colliculus (SC) function and behaviors (Meredith and Clemo, 1989; Wallace et al., 1993; Malhotra et al., 2004; Meredith et al., 2011). Accordingly, reversible deactivation of the FAES in hearing cats blocks accurate orienting and localization behaviors to auditory stimuli (Malhotra et al., 2004; Meredith et al., 2011). In early-deaf cats, auditory-evoked activity in the FAES is replaced by visual (~70% of neurons) and somatosensory (~30%) responses (Meredith et al., 2011). Although a visuotopic organization was not observed, visual receptive fields displayed complex response properties such as direction and velocity preferences and, collectively, represented the central and contralateral visual field. Ultimately, the crossmodal visual representation in the early-deaf FAES is critical for visuomotor function, since reversible deactivation resulted in the loss of accurate orienting and localization behaviors to contralateral visual cues in early-deaf, but not hearing controls (Meredith et al., 2011). However, little is known about the connectional basis subserving deafness-induced crossmodal plasticity in the FAES.

The mechanisms underlying the phenomenon of crossmodal plasticity have long been the subject of discussion and speculation. In a review, Rauschecker (1995) summarized the logical possibilities that could provide a connectional substrate for the phenomenon: crossmodal plasticity could result from the recruitment of new projections from novel areas, by increased projections from existing sources, or by the 'unmasking' of existing crossmodal inputs. The present experiment sought to test these possibilities by making tracer injections into the crossmodally-reorganized FAES of early-deaf cats to identify the distribution and proportional strength of input sources to the region, and comparing these results to data obtained by similar tracer injections made into FAES of hearing animals.

2. Materials and methods

All procedures were performed in compliance with the *Guide for Care and Use of Laboratory Animals* (National Institutes of Health, publication 86-23), the National Research Council's *Guidelines for Care and Use of Mammals in Neuroscience and Behavioral Research* (2003) with prior approval by the Institutional Animal Care and Use Committee at Virginia Commonwealth University. Also, all procedures were conducted in accord with the Canadian Council on Animal Care's *Guide to the Care and Use of Experimental Animals* (Olfert et al., 1993) with prior approval from the University of Western Ontario Animal Use Subcommittee of the University Council on Animal Care.

2.1. Ototoxic procedures

All animals were obtained from pregnant mongrel cats to avoid potential genetic influences on neural connectivity that may be coupled with congenitally deaf lineages. At 6–8 days postnatal (near hearing onset for cats), each animal was deafened using the ototoxic protocol of Xu et al. (1993). Inhalation anesthesia (isofluorane) was used to permit catheterization of the saphenous or jugular vein. A single, subcutaneous dose of kanamycin (300 mg/kg) was then administered followed by the intravenous injection of ethacrinic acid (100 mg/kg). Following recovery, the animals were returned to their mother as quickly as possible where they were housed until they were weaned (~6 weeks postnatal).

2.2. Hearing evaluation

At 4–6 weeks postnatal, treated animals had their hearing tested using standard Auditory Brainstem Responses (ABR, Fig. 1A). Under ketamine (30 mg/kg) and acepromazine (5 mg/kg) anesthesia, a calibrated auditory click (at least 2000 trials each, 0.1 ms square-wave click, rarefaction) delivered through a minispeaker positioned in front of the ear was used as the auditory stimulus. The full range of stimulation intensities was run for one ear before presenting the tests to the other ear. Subdermal recording leads were inserted at sites superior to the mastoid processes of the right and left ears, at a mid-cranial scalp location, and at a mid-back position. Electrical activity recorded by the leads was routed through an amplifier to a computer for signal averaging and storage. Animals with an ABR threshold of >80 dB SPL, like that illustrated in Fig. 1B, were considered profoundly deaf, as defined by the World Health Organization (1991). However, two of the cases showed a partial hearing decrement and the ototoxic procedure was repeated followed by a second ABR test. In these cases, hearing threshold met the criterion of >80 dB SPL hearing threshold before the age of 50 days postnatal, which is before the critical period of auditory maturation in cats (Kral et al., 2005; Kral, 2013). Treated animals were raised until maturity (>6 months of age) when data collection occurred. All ototoxically treated animals failed to startle or react to loud sounds, nor could they be aroused from sleep without tactile stimulation. In addition, mature animals with normal ABRs (hearing threshold ~15 dB SPL; see Fig. 1A), were used as hearing controls.

2.3. Neuroanatomical procedures

Adult cats were anesthetized (sodium pentobarbital, 30 mg/kg i.v.) and their heads were secured in a stereotaxic frame. Under aseptic conditions, a unilateral craniotomy and durotomy was made to expose the AES cortex, which is known to exhibit variable positions and configurations on the lateral surface of the cortical hemisphere (Clemo and Stein, 1983, 1985). An electrode carrier was used to support the syringe (Hamilton 5 μl; 31 gauge needle) containing the tracer biotinylated dextran amine (BDA; 10 kMW, lysine fixable 10% in PBS, or a 50/50 mix of 10 kMW and 3k MW BDA, 10% in PBS). The carrier was angled 53–60° (from vertical) with 35–40° cant (anterior-to-posterior from the coronal plane) and the needle tip was inserted at a point 0.8–1.5 mm anterior to the vertical limb of the AES to a depth of 5.25–5.7 mm. The tracer