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Hearing Research 196 (2004) 33–38
HEARING RESEARCH
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Changes of 2-deoxyglucose uptake in the rat auditory pathway after bilateral ablation of the cochlea

S.H. Ahn [a], S.H. Oh [a], J.S. Lee [b], J.M. Jeong [b], D. Lim [a], D.S. Lee [b], C.S. Kim [a,*]

[a] *Department of Otolaryngology-Head and Neck Surgery, Seoul National University College of Medicine, 28 Yongon-dong, Chongno-gu, Seoul 110-744, South Korea*
[b] *Department of Nuclear Medicine, Seoul National University College of Medicine, Seoul, South Korea*

Received 3 June 2003; accepted 25 May 2004
Available online 19 July 2004

Abstract

It has been reported that the area of decreased glucose metabolism in the FDG-PET of prelingually deaf children correlates significantly with speech performance after cochlear implantation. In this study, we undertook to confirm changes of glucose metabolism in the cerebral cortex using an animal model with age-matching groups to completely exclude the influence of age differences between the deaf and normal-hearing groups.

The cochlea was ablated bilaterally at a postnatal 10–14 days in the deaf groups; 3–4 deaf and normal rats were included at each time point at 1, 2, 4 and 8 weeks and 7 months after ablation. After injecting 2-deoxyglucose intraperitoneally, digitalized autoradiographic images were obtained, and analyzed by using two different methods; 3-dimensional voxel-wise statistical analysis and conventional 2-dimensional densitometry. The hypometabolic area analyzed using 3-dimensional analysis and the differences of optical density between normal and deaf as determined by densitometry were widest and most prominent between 4 and 8 weeks after ablation. Differences were not significant before 2 weeks or after 7 months after ablation.

This result shows that the hypometabolic area becomes prominent after a critical period and it decreases as the duration of deafness increases. We believe that cross-modal plasticity may be the mechanism of changes in glucose metabolism and that this result reinforced the usefulness of evaluating hypometabolic area using FDG-PET in deaf children.

Keywords: Deaf; 2-deoxyglucose; Auditory cortex; Glucose metabolism; Cross-modal plasticity

1. Introduction

In the previous study using preoperative FDG-PET of prelingually deaf children, we found that the area of the decreased glucose uptake in temporal cortex is wider in younger patients and this area decreased as the age becomes older. And the hearing capability score was predicted independently and most strongly by the degree of metabolism (Lee et al., 2001). In this study, we hypothesized that a decrease in the hypometabolic area with age may be caused by the overtaking of the auditory cortex by other modalities. Moreover, this phenomenon may inhibit the re-colonization of the cortex to receive auditory input. However, the decreased metabolism was determined on the basis of the normal distribution of glucose uptake in normal adult because taking the FDG-PET images of normal children was impossible for ethical reasons. Therefore, we wanted to confirm whether the same phenomenon takes place in an animal model, using age-matched control to completely exclude the influence of aging between child and adult upon deaf and normal group comparisons.

Because the rat brain was too small for PET study, we used the 2-deoxyglucose (2DG) method which was used to map the functional activity of the central nervous system. In the auditory pathway, unilateral deaf

* Corresponding author. Tel.: +82-2-760-2440; fax: 82-2-745-2387.
E-mail address: chongkim@plaza.snu.ac.kr (C.S. Kim).

Abbreviations: 2-DG, 2-deoxyglucose; FDG-PET, fluorodeoxyglucose-positron emission tomography; IC, inferior colliculus; MGB, medial geniculate body

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doi:10.1016/j.heares.2004.05.012

models are frequently used to identify tonotopicity or effect of monoaural hearing, because straightforward comparisons can be made between the uptakes in the normal and deprived sides in the one animal. Although, they used a unilateral deaf model, Sasaki et al. (1980) reported the recovery of 2DG uptake in the auditory pathway from the cochlear nucleus to the MGB 1 month after the ablation of one cochlea. Heil and Scheich (1986) reported that after bilateral cochlear removal in chicks, central nuclei, including field L, showed strong but spatially restricted 2DG accumulation, in contrast to the absence of accumulation in the periphery, and mentioned the possibility of cross-modal plasticity. However, the images used were analyzed qualitatively by comparing densities by eye or by using a densitometer to compare selected areas. Therefore, results comparable with those of human FDG-PET studies are not available, and whether changes in the metabolic status of the auditory cortex occur according to the duration of deafness has not been investigated. Moreover, the main region of interest in these studies was the brainstem or midbrain and not the cerebral cortex.

For this purpose, the analysis was performed using SPM99, which enables 3-dimensional voxel-to-voxel statistical mapping, to determine the changes in the area of significant 2-deoxyglucose uptake reduction in the cortex of the deaf versus an age-matched normal group. And for confirmation of the result of 3-dimensional statistical analysis, we performed a traditional analysis using optical density of marked area.

2. Materials and methods

2.1. Surgery for the bilateral deaf model

Thirty-two Sprague–Dawley rats housed in cages in a specific pathogen free area were used in this study. Institutional guidelines regarding animal experimentation were followed throughout. For the purpose of our study, the most appropriate model was a prelingually deaf rat, and in terms of the calculation of deafness duration, the onset of deafness should be consistent between animals. Therefore, we ablated the bilateral cochlea surgically at 10–14 days after birth. In rats, major developmental changes in the auditory pathway are known to occur over about two weeks, beginning at around the postnatal 10th day (Clerici and Coleman, 1986). The age of surgery was determined to coincide with the critical period to mimic the prelingually deaf. Under anesthesia with isoflurane applied using a nasal cone, a posterior auricular incision was made and the bulla was identified. The cochlea could be found after sucking out the mesodermal tissue in the middle ear. It was uncapped using a pick and the fluid within the cochlea was sucked out with needle suction tip. The skin was sutured after irrigating the inner ear cavity with gentamicin. The deaf model was confirmed using auditory brainstem response and grossly destructed cochlea.

In the normal control group, the skin incision and the identification of the bulla surface was performed and the skin closed. To reduce the risk of conductive hearing loss, the bulla was not opened.

2.2. DG procedure

At 1, 2, 4 and 8 weeks and 7 months postoperatively, we examined 3–4 normal control and deaf rats, respectively. The rats received an injection of 16 μCi/100 g of 2-[1-14C]-deoxy-D-glucose (Perkin–Elmer^{TM}, in aqueous solution, 56 mCi/mM) intraperitoneally. The rats were exposed to ambient noise to analyze basal brain metabolism under normal condition as is done with patients receiving FDG-PET. After 45 min to 1 h, (Sokoloff et al., 1977) the animals were decapitated and their brains removed, frozen on dry ice and stored at −20 °C. Serial coronal sections of brain of 20 °C thickness were cut using a Leica cryostat at intervals between sections of 100 °C, from the frontal lobe to the IC level. About 100 sections were obtained from one animal. The slides were dried for one day and apposed to a BAS image plate (Bioimaging Analysis System, Fujifilm^{TM}) for 5 or 6 days and a digitized image was acquired using a FLA-2000 scanner (Fujifilm^{TM}), at a voxel size of 50 by 50 °C in 256 color, and the sections were stained with cresyl violet.

2.3. Data analysis

For 3-dimensional voxel-based statistical analysis, sequential slices were semi-automatically extracted from autoradiographic images containing coronal sections of rat brain. Using automatic image registration algorithms, adjacent slices were registered 2-dimensionally. The sections were realigned to the first slice by using the principal axes matching and mutual information maximization technique and reconstructed 3-dimensionally for further statistical analysis (Lee et al., 2002; Alpert et al., 1990; Maes et al., 1997). The images were anatomically standardized; that is, all brain images were spatially normalized onto one of a 7-month rat using SPM99 software (Statistical Parametric Mapping 99, Institute of Neurology, University College of London, UK) (Friston et al., 1995). After normalizing the global count, the voxel-wised *t*-test was performed on control and deaf rats at each time point. Voxels with a p value of <0.05 were considered statistically significant.

Additionally, in the cresyl violet stained slides, we defined the auditory cortex, the MGB, and the IC from three different section levels using Paxinos rat brain atlas (Paxinos and Watson, 1998). The optical density of the

delineated areas were then obtained in the corresponding autoradiographic images, and corrected using the optical density of the whole brain in each individual section. The average of three values from one site was taken as the value of that anatomic area, and this was compared by *t*-test between groups for both sides separately to confirm the 3-dimensional results. Results were plotted using mean difference and standard error of difference, which were obtained using SPSS 10.0.

3. Results

3.1. Auditory cortex

Fig. 1 is the result of 3-dimensional voxel-wise statistical analysis. The voxels of which globally normalized optical density of the deaf group found to be significantly lower than the corresponding voxels of the normal control are colored blue ($p < 0.05$). After a postoperative one week, no difference was observed in the cortices of the two groups. The hypometabolic area began to emerge two weeks after deafness in the right side. The blue area was widest at 4 weeks postoperatively and decreased thereafter to recover the normal level at 7 months postoperatively.

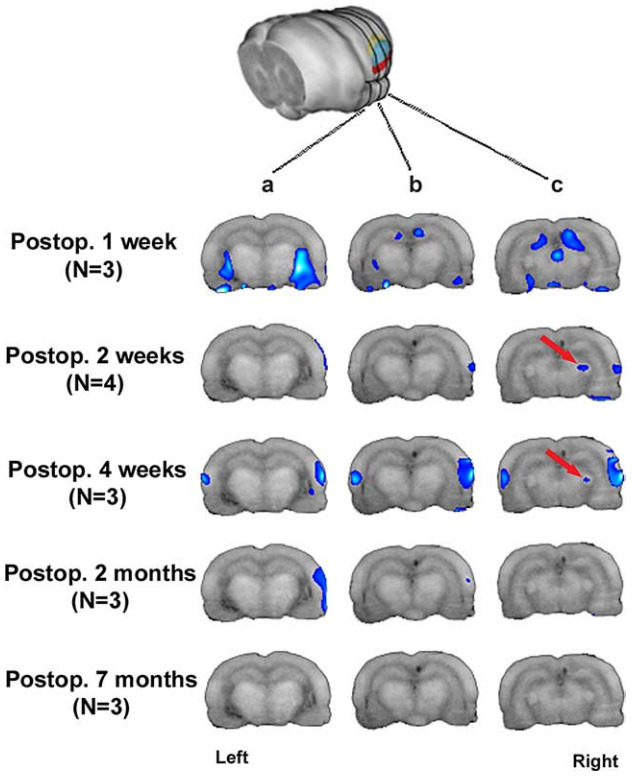

Fig. 1. The results of 3-dimensional voxel-wise statistical analysis. In the uppermost figure, the blue area represents the primary auditory cortex, the yellow and red colored area is the secondary auditory cortex. A small area of decreased glucose uptake starts to develop after 2 weeks. Four weeks after ablation, in both temporal cortices, a wide hypometabolic area was observed. This was reduced 2 months after ablation, and recovered to the normal level after 7 months. The medial geniculate body showed decreased glucose uptake in the right side at 2 weeks, but after 4 weeks, the hypometabolic area of the medial geniculate body had subsided (red arrow).

Using 2-dimensional optical density analysis, we confirmed that the decreased metabolism in deaf rats were most prominent at 4 and 8 weeks after deafness, and that in the right side, it showed a significant difference at 2 weeks postoperatively, although the difference was not large. This decreased metabolism was not significant before or after this period (Fig. 2(a)).

3.2. Medial geniculate body

The largest hypometabolic area was observed at 2 weeks postoperatively. This decreased at 4 weeks, and disappeared after 8 weeks (Fig. 1, arrow). The similar result was obtained by optical density measurements. A significant difference in glucose uptake was observed in the MGB at 2 weeks postoperatively (Fig. 2(c) and (d)), but after 4 weeks no significant differences were observed.

3.3. Inferior colliculus

At a postoperative 2 weeks, the glucose uptake of the IC in deaf rats decreased with a large mean difference (Rt:0.55, Lt:0.50). This decrease was large compared with that of the auditory cortex in which the mean difference at 4 weeks was 0.26 in the right side and 0.31 in the left. The difference in glucose uptake rapidly reduced thereafter and after 8 weeks no significant difference was observed (Fig. 2(e) and (f)).

Because the IC was at the caudal end of this series of frozen section, the 3-dimensional analysis of the IC was not possible due to image distortion.

4. Discussion

Changes in the glucose uptake in the auditory cortex after 4 weeks of deafness showed the same pattern as the changes in the hypometabolic area observed by FDG-PET in prelingually deaf children (Lee et al., 2001). The hypometabolic area of auditory cortex was widest at 4 weeks, and reduced as the duration of deafness increased. On the other hand, the difference in glucose uptake in the MGB and the IC was most prominent after 2 weeks and decreased thereafter. It was an interesting finding that there is latent period before the emergence of hypometabolism. In postoperative 1 week, there was no significant difference in auditory pathways despite of the diffuse hypometabolic area along the ventricle. The reason of hypometabolism in this area at postoperative 1 week is difficult to explain now. First of

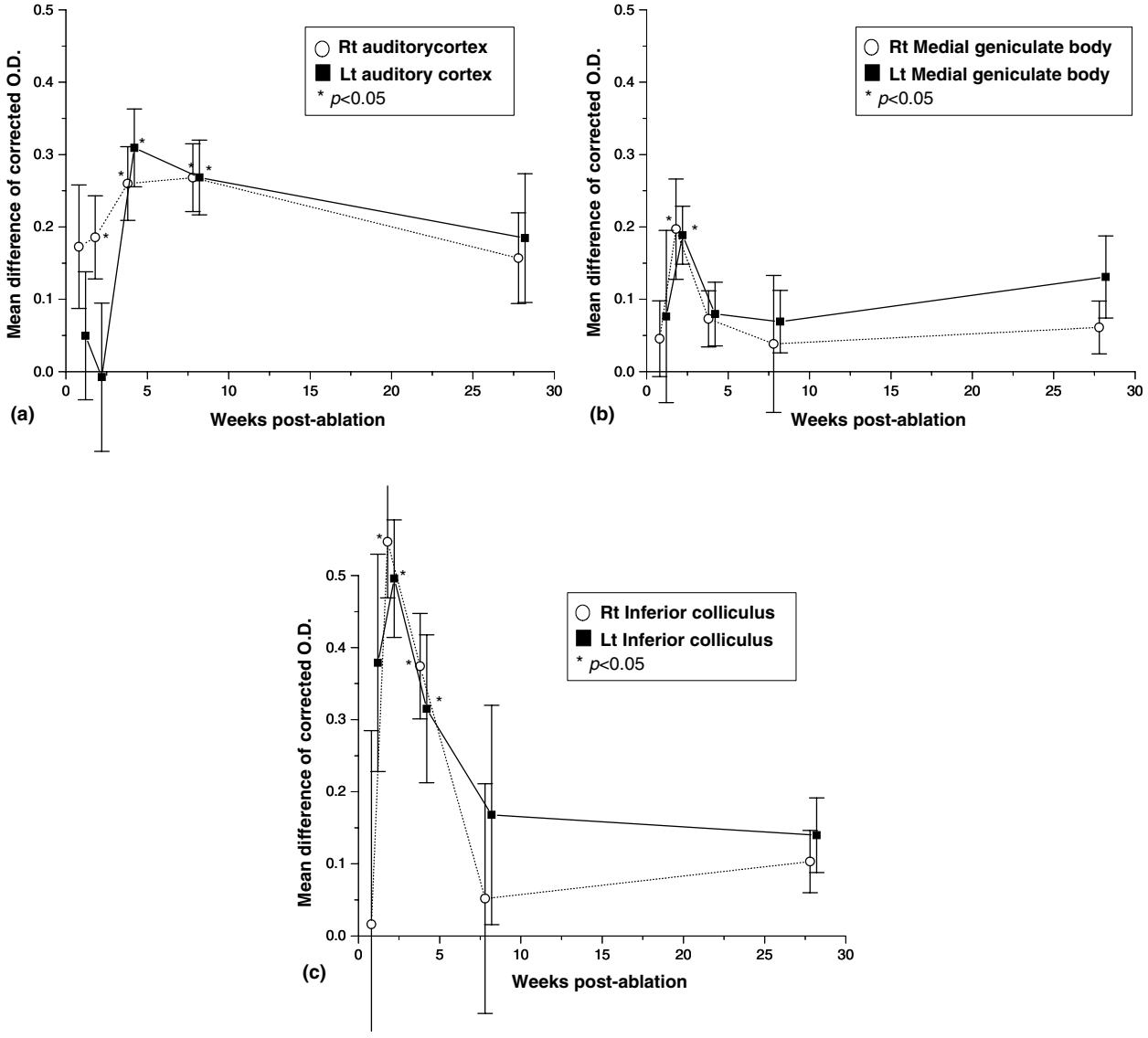

Fig. 2. The changes in the mean difference of the corrected optical density (OD); mean difference = mean of normal group – mean of deaf group (mean difference ± standard error of difference) a: auditory cortex, b: medial geniculate body, c: inferior colliculus.

all, the SPM is a program developed for analysis of functional images of human brain. So the adaptation of SPM in rat brain is a new trial and we performed the analysis using optical density in 2-dimensional images at the same time. At the auditory cortex and MGB, the result from two methods showed good correlation and we believe that the results of SPM in auditory pathways are reliable.

In the rats, the immature afferent connections invades IC (Gabriele et al., 2000), and MGB (Asanuma et al., 1988) by the day of birth. The IC gets adult-like afferent dense patches at postnatal 12 days before auditory experience (Gabriele et al., 2000). The neurons of the rat MGB show rapid growth from postnatal 5–7 days involving synaptogenic activity and from postnatal 11–16 days after the onset of hearing (Clerici and Coleman, 1998). The thalamocortical axons arrive at their target cortical layer 4 at postnatal 3 days in rats and areas of cortex can be detected (Pallas, 2001). But the rapid functional maturation of auditory cortex occurs during the second and third postnatal weeks (Metherate and Aramakis, 1999). So the maturation of brain auditory pathway by intrinsic factors may be completed before cochlear ablation in this study. This study shows the changes in metabolism when the extrinsic factors have been deprived. It is well known that, the auditory experience during the early postnatal development is important for the functional maturation of auditory pathway. Because the time of operation is just the time that rats begin to experience the sound, it takes some time for the functional maturation of IC, MGB and auditory cortex. And the latent period of no difference from the normal subjects suggest that the differences in glucose metabolism in auditory pathways become de-

tectable after completion of functional maturation. If we hypothesize that pathways complete development from periphery to central, the structures that finish the maturation earlier, i.e., IC and MGB, may show the hypometabolism in earlier period after cochlear ablation than auditory cortex that may need more time for maturation.

According to the articles about the predictive factors of cochlear implantee, the age at implantation and duration of deafness are considered the most significant in terms of the outcome of cochlear implantation in prelingually deaf children (O'Donoghue et al., 2000; Kirk et al., 2002). On the other hand, the postlingually deaf patients showed faster improvement with good performance after cochlear implantation in general (Oh et al., 2003). This suggests that patients who have no experience of hearing have a mechanism of speech learning that differs from those who once were able to hear. As mentioned earlier, the extent of the hypometabolic area was found to be a strong and independent prognostic factor in cochlear implantation when multiple regression analysis was performed; this included the age at implantation as one of the factors (Lee et al., 2001). We believe hypometabolism in the cortex of the prelingually deaf indicates that areas pre-assigned for speech perception are saved and not invaded by another modality. Moreover, to hinder the recruitment of the auditory cortex after cochlear implantation, the mechanism of cross-modal plasticity, which creates the changes in the extent of hypometabolic area, may involve a reorganization of long-range subcortical connectivity. This form of plasticity seems to be limited to developing organisms and this change reliant on the stabilization of normally transient redundant pathways (Bavelier and Neville, 2002). The possibility of recruiting primary cortices associated with deprived modalities has been largely investigated using blind or deaf models. Rebillard et al. (1977) reported that cats that were cochleoectomized before the age of 3 weeks showed visually evoked potentials in A1. However, in congenitally deaf cats, visually evoked potentials in A1 were recordable in only one out of three cats (Rebillard et al., 1977) or were not be detactable (Kral et al., 2002). This inconsistent recruitment of A1 in genetically deaf individuals may be due to large inter-individual differences in the timing of cochlear degeneration (Rebillard et al., 1976). However, in the congenitally blind mole rat, a projection from the IC to the non-degenerated visual thalamus and the recruitment of the occipital cortex by auditory stimuli were identified (Kubo et al., 1997). It has long been known that the MGB receives somatosensory and visual-motor inputs and that lateral and external cortex of the IC receives the somatosensory input (Malmierca et al., 2002). The peripheral lesions may unmask hidden inputs (Rauschecker, 1995) and it may be possible for these natural minor pathways to substitute the IC, MGB and finally the auditory cortex making a change in subcortical connectivity. And this phenomenon can explain the earlier recovery in IC and MGB than that in auditory cortex.

So, as we know, the early rehabilitation is critical for the restoration of deprived sensory modality, and in the patients at the age during or immediately after the critical period. And we believe that the evaluation of the area of hypometabolism can suggest whether the auditory cortex is saved or not from colonization by other modalities in prelingually deaf patients.

5. Conclusion

In this study, we found that changes in the hypometabolic area created by deafness are associated with a critical period and that the changes of hypometabolic area having been observed in prelingually deaf patient was reproducible in deaf animal models using age-matched controls. Our findings reinforce the possibility of the usefulness of FDG-PET as an important preoperative evaluation tool in the cochlear implant candidate.

Acknowledgements

This work was supported by Grant No. 1999-2-213-003-3 from the Basic Research Program of the Korea Science and Engineering Foundation.

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