Increases resting-state PET activity after cochlear implantation in adult deafened cats

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design, data collection and analysis, decision to publish, or preparation of the manuscript.

Running title: Cortical awareness level was elevated after CI
Abstract

Background and Objectives:

For the hearing rehabilitation of patients with profound sensorineural hearing loss, cochlear implants (CIs) are widely used. However, the result of CI is variable, and central neural plasticity is considered to be a reason for this variability. We hypothesize that resting-state cortical networks play a role in the condition of profound hearing loss and are affected by CI. To investigate the resting-state neuronal networks after cochlear implantation, we acquired 18F-fluorodeoxyglucose (FDG)-positron emission tomography (PET) images in experimental animals.

Materials and Methods:

Eight domestic adult cats were enrolled in this study. The hearing threshold of the enrolled animals was within the normal range, as measured by auditory evoked potential. They were divided into two groups: control (n=4) and hearing loss (n=4) groups. Hearing loss was induced by co-administration of ethacrynate and kanamycin. FDG-PET was performed at normal hearing state and at 4 and 11 months after the deafening procedure. Cochlear implantation was performed in the right ear and electrical cochlear stimulation was performed for 7 months (from 4 to 11 months after the deafening procedure). Acquired PET images were analyzed and compared between the two groups at the three time points.

Results:

At 4 months after hearing loss, the auditory cortical area’s activity decreased, and the associated visual area activity increased. After 7 months of cochlear stimulation, the superior marginal gyrus and cingulate gyrus showed hypermetabolism, and these areas were components of the default mode network. The inferior colliculi showed hypometabolism.
Conclusions:

Resting-state cortical activity was elevated after cochlear stimulation in the default mode network component. This suggests that the awareness level was elevated after hearing restoration by the CI.

Key words: cochlear implants, postlingual deafness, Positron-Emission Tomography, auditory cortex, default mode network.
Peripheral sensory loss causes many changes in the central nervous system (CNS). This phenomenon is known as central neural plasticity. The changes due to sensory loss begin immediately after the loss of peripheral neural input and continue indefinitely (until death). The changes have been measured using various methods, including evoked potentials, electrophysiological studies, behavioral tests, molecular works, and acquiring images such as those from positron emission tomography (PET), functional magnetic resonance imaging (fMRI), functional near-infrared spectroscopy, and magnetoencephalography. Changes of the brain due to congenital or prelingual deafness are considered to be developmental deficits due to the loss of peripheral auditory signals. However, in this situation, the brain is not exposed to auditory signals during a development period. In the case of acquired or postlingual deafness, the central auditory system development was completed previously; thus, the signal pathway was intact before hearing loss (HL).

Postlingual HL also affects the central auditory system. However, in terms of development, individuals with prelingual deafness have more obstacles to overcome than those with postlingual deafness. Neuroimaging studies have revealed several changes in the CNS in postlingually deafened patients. Decreased glucose metabolism has been detected in both the anterior cingulate gyri and superior temporal cortices and in the right parahippocampal gyrus (8). White matter also changes with decreased fractional anisotropy and increased radial diffusivity after late-onset deafness (9). Animal studies show the same pattern of decreased metabolism in the auditory cortical area (10).

These changes in the central auditory system create very important considerations for hearing rehabilitation. Once changed, the neural system will not return to the previous state even after
hearing rehabilitation, and its return is affected by the duration of deafness. This duration period is known as the critical period (11, 12). Cochlear implants are used for hearing restoration in patients with postlingual profound deafness. Many researchers have attempted to determine the relationship between the duration of HL and the critical period of rehabilitation for cochlear implant users. As a result, the duration of deafness is known to be the most important prognostic factor for hearing rehabilitation with cochlear implants (13-15). The duration of deafness is negatively correlated with the postoperative auditory performance of cochlear implant users. Early hearing rehabilitation results in better auditory performance. Many studies have attempted to predict postoperative hearing results using preoperative diagnostic measurements. However, the results of cochlear implantation (CI) are variable, even if they do not occur too late after HL (16).

Several studies have reported that resting-state PET images can predict the results of CI (2, 15, 17). Resting-state brain glucose utilization is related to basal cellular function and considered an index of integrated local synaptic activity (18). Recently, some studies reported that long-term HL disrupted connectivity of brain networks and altered resting-state networks and activities. (19, 20).

We hypothesize that CI will change the resting-state cortical networks compared with the deaf state. Most of the animal studies were performed using a congenital deaf cat model. So far, no studies have been conducted on the acquired hearing loss model. Thus, we attempted to measure the time interval between electrical cochlear stimulation and changes in resting-state network activity using an acquired deafness model. To investigate resting-state neuronal networks after CI, we acquired 18F-fluorodeoxyglucose (FDG)-PET imaging in experimental animals.
Materials and Methods

1. Animals and hearing measurement

Eight domestic cats were included in the study. Seven were male, and one was female. All cats were adults (body weight 2.7 kg ~ 5.5 kg, age 12–24 months) and had normal hearing thresholds (under 20 dB SPL at click stimulation). All cats showed normal external auditory canals and tympanic membranes on otoscopic examination. The animals were divided into two groups: HL control (n=4) and CI group (n=4). Hearing thresholds were measured using auditory brainstem response (ABR) to click sounds. Subdermal needle electrodes were located below the ear and vertex. The Intelligent Hearing System (HIS Inc., Miami, Florida, USA) was used to measure the ABR threshold. The stimulus rate was 19.1/s using the click sound in rarefaction mode. The response was amplified, band-pass filtered (100-1500 Hz), and averaged to create overstimulation (512 sweeps per stimulation). Hearing thresholds were measured at normal hearing status and during the deafening procedure. Deafness was confirmed 2 weeks after the deafening procedure. All experiments were approved by the Institutional Animal Care and Use Committee (IACUC Number 35-2015-00-84).

2. Deafening procedure

All animals were deafened by co-administration of kanamycin and ethacrynic acid, following a previously reported method (21, 22). All procedures were performed under anesthesia using Zoletil (Virbac Laboratories, Carros, France). The cats were anesthetized with an intramuscular injection of 0.2 cc Zoletil. Before the deafening procedure, hearing thresholds were measured to confirm the normal threshold range. For deafening, kanamycin (kanamycin
sulfate, Yuhan, Seoul, Korea) was dissolved in normal saline and injected subcutaneously into the posterior neck area (500 mg/kg). After 30 min, ethacrynic acid (ethacrynate sodium, MSD, West Point, PA, USA) dissolved in normal saline was continuously infused intravenously at a rate of 1 mg/min using an infusion pump. The ABR threshold was measured continuously during the infusion of ethacrynic acid using 60-, 70-, 80-, and 90-dB SPL click sounds repeatedly. When the ABR wave V disappeared at the 90 dB SPL click sound, the infusion was stopped. Two weeks later, ABR was measured using a 90 dB SPL click sound to confirm the deafened status.

3. Cochlear implantation and stimulation

CI was performed in the right ear 3 months after the deafening procedure in the CI group (n=4) (Fig 1A). A Nucleus 422 device (Cochlear Ltd., Australia) was used for implantation. A retroauricular incision was performed under deep anesthesia using isoflurane. The bulla was exposed and opened using a drill. The round window (RW) was identified under microscopic view, and an incision was made to the RW membrane. The electrode was introduced slowly via the RW until resistance to the insertion occurred. The RW was sealed with soft tissue, and the electrode was fixed using fibrin glue. The ground electrode was located between the adjacent neck muscles. The internal device is located at the vertex area under the periosteum without bone drilling (23). The wound was closed using subcutaneous and skin sutures. Impedance was checked and neural response telemetry (NRT) was performed. Postoperative radiography was performed to verify the electrode position.

Four months after the deafening procedure, electrical stimulation was started for 8 h per day, 5 days per week. Environmental sounds, including voice, laboratory noise, and radio, were used for the stimulation. Mapping was performed using Custom sound 4.1 (Cochlear Ltd,
Australia) to determine the stimulation level every month during the stimulation period. Impedance was measured to check the electrode, and NRT was obtained monthly to determine the threshold level using the standard clinical settings in the program. Stimulation was based on measured T-NRT levels.

4. PET scan and image analysis

FDG-PET images were acquired using an Inveon scanner (Siemens Inc., Knoxville, TN, USA). Images were acquired three times at the normal hearing state (before deafening procedure of baseline), four months after the deafening procedure immediately before electrical stimulation, and eleven months after the deafening procedure (7 months after stimulation for CI group). Before imaging acquisition, food was restricted for 8 h. Anesthesia was induced by intramuscular injection of Zoletil (1 mg/kg), and 2% isoflurane in 100% oxygen (Forane solution; Choong Wae Pharma, Seoul, South Korea) was used to maintain anesthesia during the image acquisition. FDG (1 mCi/kg) was administered intravenously. After 30 min of uptake, images were acquired for 40 min. Transmission PET data were acquired for 15 min for attenuation correction (24). Emission list-mode data were sorted into 3D sinograms and reconstructed using 3D reprojection (3DRP) algorithms. The image matrix was $128 \times 128 \times 159$, the pixel size $0.77 \text{ mm} \times 0.77 \text{ mm}$, and the slice thickness $0.79 \text{ mm}$. The image acquisition room had an environmental noise of 60 dB SPL. Facility lighting was purposely dimmed to prevent visual stimulation. Anatomical brain areas were determined using the MRI-based cat brain atlas (25, 26).

To identify the regional differences in cerebral glucose metabolism, voxel-wise statistical analysis using SPM 8 (http://www.fil.ion.ucl.ac.uk/spm) was used. For SPM analysis, the brain region of interest was extracted, and a study-specific brain template was constructed.
Individual PET data were spatially normalized by placing affine and non-linear transformations onto a study-specific brain template, and then overlaying them onto a study-specific brain template (27). The voxel size of spatially normalized images was 0.3 mm × 0.3 mm × 0.3 mm. A 3-mm Gaussian smoothing kernel was applied to enhance the signal-to-noise ratio. Count normalization was performed.

PET image comparisons were performed between baseline and 4-months after deafening to evaluate the effects of hearing loss to the central auditory system in both groups. Another comparison was performed between images of 4-months after deafening and those of 7-months after electrical stimulation to evaluate the effect of electrical stimulation in the CI group. Paired t-tests were used to identify regional differences between different time points within groups with a threshold of $p < 0.005$ (uncorrected).

Results

1. Deafening procedure

Baseline ABR showed that the thresholds were below 20 dB SPL in all animals (Fig 1B). After the deafening procedure, all animals showed no response to 90 dB click sounds (Fig 1C). This hearing level was confirmed by follow-up ABR 2 weeks later, with no response to the 90 dB SPL click sound in all animals.

2. CI and stimulation

The electrode array was introduced using an RW approach. The mean number of the introduced electrodes was 11.5 (range 10-14). The implantation was performed without any special events. Simple radiography showed that the electrode array was well introduced in the
cochlea in all animals (Fig 1D). The impedance data showed that each electrode state was normal (Fig 1E). NRT was administered to all the animals.

3. PET scan

Four months after deafness, glucose metabolism was decreased in both primary auditory areas in both groups (Figure 2A).

Four months after deafness, the suprasplenial gyrus (visual area 17) showed increased glucose metabolism (Figure 2B, $p<0.005$). This area is known as the visual-associated cortex (26).

After 7 months of electrical stimulation, glucose metabolism in the bilateral primary auditory cortical area was improved and normalized compared with the baseline of normal hearing states. The inferior colliculus (IC) showed a decreased metabolism (Figure 3A). This hypometabolism was not detected 4 months after deafness. The superior marginal gyrus still showed increased metabolism, and the cingulate gyrus showed hypermetabolism (Figure 3B, $P<0.005$).
Discussion

This study aimed to show resting-state cortical activity after cochlear stimulation in an acquired deafness state. In this study, electrical cochlear stimulation restored glucose metabolism in the bilateral auditory cortex. A study using electroencephalography showed that cochlear stimulation rapidly improved bilateral auditory cortical activity in postlingually deafened patients (1).

After deafness, glucose metabolism decreased in both the auditory cortex and inferior colliculus. This pattern matches that of previous reports from humans and cats (2, 10). In this study, we found increased metabolism in the superior marginal gyrus 4 months after deafness. This area is known as visual area 17 and is considered to be a visually associated area (26). In a previous report, this visual-associated area could be activated for compensatory changes (10).

Changes in glucose metabolism after CI have been previously reported in postlingually deaf human participants. To evaluate the stimulating state, glucose metabolism was compared between the switch on and switch off state of the cochlear implant in postlingually deaf patients. In that study, the bilateral auditory area showed increased metabolism regardless of the implanted side, and it was positively correlated with speech perception outcomes, and negatively correlated with the duration of deafness (28-30). This trend was also observed in prelingually deaf patients. Sound stimulation with a cochlear implant device activates glucose metabolism in both auditory cortical areas (31).

An $[^{15}\text{O}]\text{H}_2\text{O}$ PET study was performed to evaluate brain activity at rest, and that study showed increased blood flow in the right inferior occipital gyrus, extending to the inferior temporal region and in the left posterior cingulate gyrus of experienced cochlear implant users (32). Inexperienced cochlear implant users elicited more resting-state activities in the
visual-associated cortex. This group also pointed out that a high CI outcome depends on intramodal compensation of the visual cortex and cross-modal reorganization of the superior temporal gyrus (2).

The inferior colliculus (IC) showed metabolic changes after cochlear stimulation in this study. At 4 months after deafness, IC areas are not changed significantly compared to baseline. However, the auditory cortical area showed less activation than baseline. This discrepancy between the auditory cortex and IC may result from the short duration of deafness. At 11 months after deafness, IC areas showed significantly decreased metabolism even with 7 months of electrical stimulation. The activity of the right IC was lower than that of the left side (Figure 3A), which may be due to the cochlear stimulation being performed on the right side, resulting in more stimulation to the left IC than the right IC. However, to confirm this, we need long-term stimulation and observation. In this study, IC changes followed those of the auditory cortical area.

After cochlear stimulation, we observed increased metabolism in the cingulate gyrus. The cingulate gyrus is considered a component of the default mode network (DMN) (33). The DMN is known as a hypermetabolic area when the brain is in a resting state and not in task scan mode. Three major divisions of DMN in humans are the ventral and dorsal medial prefrontal cortex, posterior cingulate cortex, and precuneus (34). DMNs have been identified in other species, including monkeys (35), rats (36), mice (37), and cats (38).

The association between the cingulate cortex and HL has been reported in several studies. Postlingually deaf patients showed significant decreased glucose metabolism in both anterior cingulate gyri, superior temporal cortices, and the right parahippocampal gyrus in the glucose PET study (8). In older people with mild to moderate sensorineural HL and abnormal distortion product otoacoustic emission (DPOAE), the anterior and posterior cingulate gyri
showed significantly decreased cortical thickness compared with those in people with normal hearing and DPOAE (39). In contrast, hypermetabolism of the cingulate gyrus was observed in a study with profound HL patients using PET, and the authors considered that this phenomenon was a compensatory mechanism for deafness to be aware of the stimulation (32). Another study showed dissociated functional coupling patterns in the anterior cingulate cortex and its subdivisions in patients with bilateral long-term sensorineural HL using resting-state fMRI. That study showed the connectivity between the cingulo-opercular network DMN and the auditory network (40). The cingulo-opercular network activity has been associated with increased cognitive effort, and it was increased in listening in noise condition. Speech recognition is improved at elevated cingulo-opercular activity (41).

In our results, increased metabolism in the cingulate gyrus was considered evidence of activation of the DMN after cochlear stimulation. This suggests that the awareness level in a resting state was elevated after hearing restoration by the CI.

Conclusion

The authors suggest that electrical cochlear stimulation can elevate the level of awareness at the resting state, and this could activate the DMN. Our study showed increased metabolism in the cingulate and supramarginal gyri after cochlear stimulation. These areas are considered to be DMN areas in humans. This indicates that cochlear stimulation activates and restores the DMN. For accurate evaluation of DMN activity, PET scans should be performed in both modes. This study did not acquire a PET image of the device according to mode, and this is a
limitation of this study. However, the CI group showed alterations in the DMN area after cochlear stimulation in the resting state.

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Table 1. Areas of significant changes after hearing loss and cochlear stimulation.

Figure 1A. Experimental scheme of deafening, cochlear implantation, cochlear stimulation, and PET scan. PET 1st scan was performed at normal hearing states for the baseline. 2nd scan was performed 4 months after hearing loss to evaluate the effect of deafness to the brain. 3rd scan was performed 7 months after cochlear stimulation to evaluate the effects of cochlear stimulation. Cochlear implantation was performed during the 2nd and 3rd months after deafening procedure.

Fig 1B. Auditory brainstem response (ABR) shows a threshold of 20 dB SPL at normal hearing states (from experimental animal number #1).

Figure 1C. After deafening procedure, ABR shows no response to the 90 dB click sound (from experimental animal number #1).

Figure 1D. Skull AP view after cochlear implantation. At the center of the figure, introduced electrodes were identified (from CI group number #2).

Figure 1E. Impedance measurement immediately after implantation. Eleven electrodes were introduced in the cochlea (from CI group number #2).

Figure 2. Changes after hearing loss (comparison between normal hearing (baseline) and hearing loss (4th month)).
2A. Both primary auditory cortical areas show decreased glucose metabolism in hearing loss state compared with normal hearing state.

2B. The suprasplenial gyrus (visual area 17) shows increased glucose metabolism after deafness.

Figure 3. Changes after cochlear stimulation (comparison between hearing loss (4th month) and after cochlear stimulation (11th month).

3A. Glucose metabolism was decreased at both inferior colliculi after 11 months of deafness.

3B. Cingulate gyrus (left panel) and superior marginal gyrus (right panel) show increased glucose metabolism after 7 months of stimulation.
References


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Figure 1B

40dB

30dB

25dB

20dB

15dB
Figure 1E

Impedance

- Common Ground Electrode
- Monopolar 1
- Monopolar 2
- Monopolar 1+2

Electrode No
Figure 2A
Figure 3A