Patient demographics

Patient demographics included the age at operation, etiology of the deafness, and preoperative developmental quotient. The preoperative developmental quotient (DQ) was evaluated using the Kyoto Scale for Psychological Development. The Kyoto Scale of Psychological Development is a widely used developmental test with satisfactory reliability and validity in Japan. This scale consists of three subtests: a test for the Postural-Motor Area (PM) of development, a test for the Language-Social Area (LS) of development, and a test for the Cognitive-Adaptive Area (CA) of development. In most patients, the DQ for the PM was above the upper limit, so we focused analysis on the DQ for the LS and the CA.

Implant devices

All of the implanted devices were multichannel devices. Patients were implanted with the Nucleus 22 between 1987 and 1999, and the Nucleus 24 between 2000 and 2007. After the approval of the HiRes90K and Combi40+/Pulsar devices in April 2008, the patients' families were allowed to choose the implant device for themselves.

Intraoperative findings

The surgical approaches utilized were collected from the operation records. The incidence of obstruction of the cochlea and perilymph/cerebello-spinal fluid gusher was also reviewed.

Postoperative development

In 36 patients, the DQ was obtained more than 6 months after the cochlear implantation surgery. In these patients, the DQ for LS and CA before and after the operation was compared using a paired *t* test. The correlation of preoperative DQ and the postoperative DQ for LS was evaluated by calculating Pearson's correlation coefficient.

Results

Patient demographics

The age at implantation ranged from 1 year and 2 months to 41 years and 11 months. The median age at implantation was 3 years and 5 months (Figure 1). The median age at implantation in the most recent 4-year interval was lower than in previous years (Figure 2).

In all, 101 patients were born deaf without any known etiologies. The other etiologies of deafness included: inner ear and/or internal auditory canal anomaly in 17 patients; meningitis in 8 patients; cytomegalovirus infection in 3 patients; rubella infection in 2 patients; drug-induced in 1 patient; bilateral sudden hearing loss in 1 patient; and Goldenhar syndrome in 1 patient.

The preoperative DQ for LS ranged from 13 to 117, with an average of 61. Only 20 patients (15%) were within the normal range (≥ 80). The preoperative DQ for CA ranged from 39 to 121, with an average of 88. Seventy-nine patients (59%) were scored in the normal range (≥ 80).

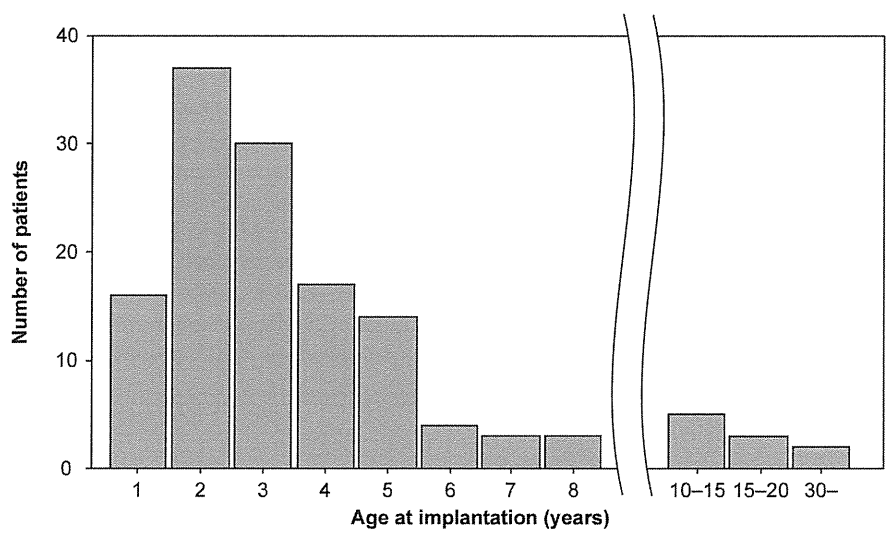

Figure 1. Age distribution at the time of cochlear implantation. The age at implantation ranged from 1 year and 2 months to 41 years and 11 months. The median age at implantation was 3 years and 5 months. Note that most patients underwent surgery at or before the age of 3 years.

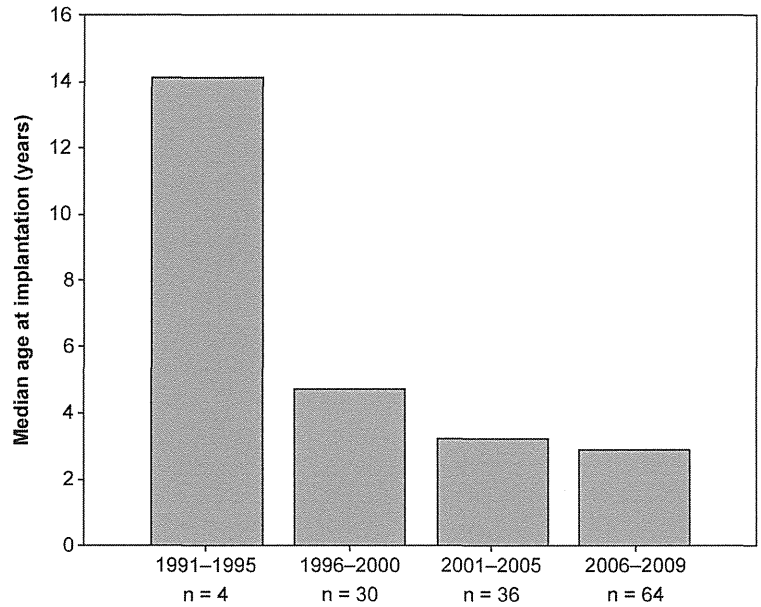

Figure 2. Change in the median age at the time of cochlear implantation over time. The age of cochlear implantation recipients is continually getting lower and the number of patients is getting larger. The median age at implantation in the most recent 4-year interval was lower than in previous years.

Implant devices

Twenty-five patients were implanted with the Nucleus 22 device, 105 patients with the Nucleus 24, and the other 4 patients with the HiRes90K. No patients were implanted with MEDEL devices.

Intraoperative findings

In 123 patients, the electrodes were inserted using the transmastoid facial recess approach. In four patients, partial removal of the posterior canal wall with subsequent reconstruction was needed because of poor mastoid development. In two patients, the posterior canal wall was totally removed and the external auditory canal was closed to control middle ear inflammation. In four patients, the electrodes were inserted through the external auditory canal. One patient had atresia, and the electrode was inserted after opening the tympanic cavity.

In four patients (3.0%), cochlear obstruction was identified during the operation. All had lost their hearing as a result of meningitis. In one of the four patients, the inferior portion of the basal turn of the cochlea was ossified, although the preoperative MRI showed a narrow but patent cochlea (Figures 3 and 4). Perilymph/cerebello-spinal fluid gusher was observed in six patients (4.5%). All six patients had inner ear anomalies. In four patients, the cribriform plate was absent (Figure 5). In the other two patients, the vestibular aqueduct was enlarged. In all six cases, the perilymph/cerebello-spinal fluid gusher was controlled with reverse Trendelenburg positioning, administration of mannitol, and subsequent electrode insertion and packing of the cochleostomy with the temporal muscle fascia.

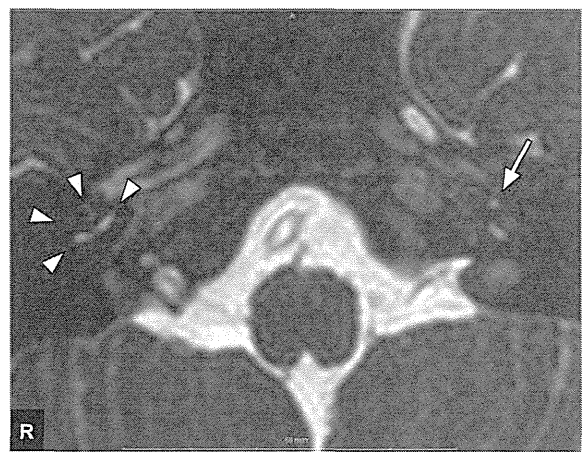

Figure 3. Preoperative high resolution T2-weighted MRI of a patient with an obstructed cochlea. The inferior portion of the basal turn of the cochlea was narrow but patent on the right side (arrowhead). Only the ascending portion of the basal turn of the cochlea was visible on the left side (arrow). In four patients, cochlear obstruction was identified during the operation. All had lost their hearing as a result of meningitis.

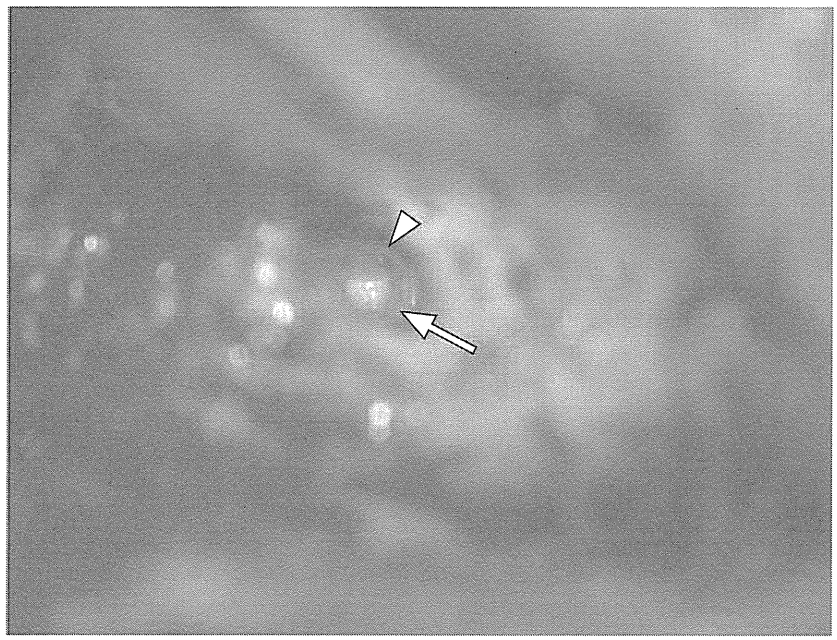

Figure 4. Intraoperative findings of a cochlear implantation surgery in the right ear. The inferior portion of the basal turn of the cochlea was totally ossified, although the preoperative MRI showed a narrow but patent cochlea. Note that the color of the scala tympani (arrow) and scala vestibuli (arrowhead) is different as a result of the different degrees of ossification.

Postoperative developmental quotient

In the 36 patients in whom postoperative DQ was measured, the average preoperative DQ was 60 for LS and 86 for CA, which was similar to the preoperative average of all 134 patients. The postoperative DQ for LS was 77, which was significantly higher than the preoperative value ($p < 0.01$, paired t test) (Figure 6), but still lower than the normal value (≥ 80). The correlation coefficient between pre- and postoperative

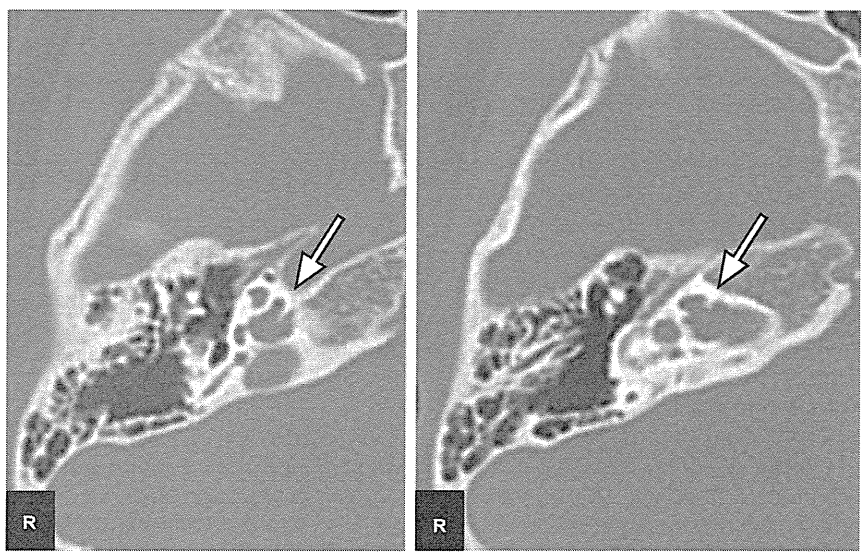

Figure 5. Preoperative high resolution CT scan of a patient. In this patient, two serial slices of CT images showed that the cribriform plate was absent (arrow). During the operation, perilymph/cerebello-spinal fluid gusher occurred after cochleostomy. All six patients showing perilymph/cerebello-spinal fluid gusher had inner ear anomalies.

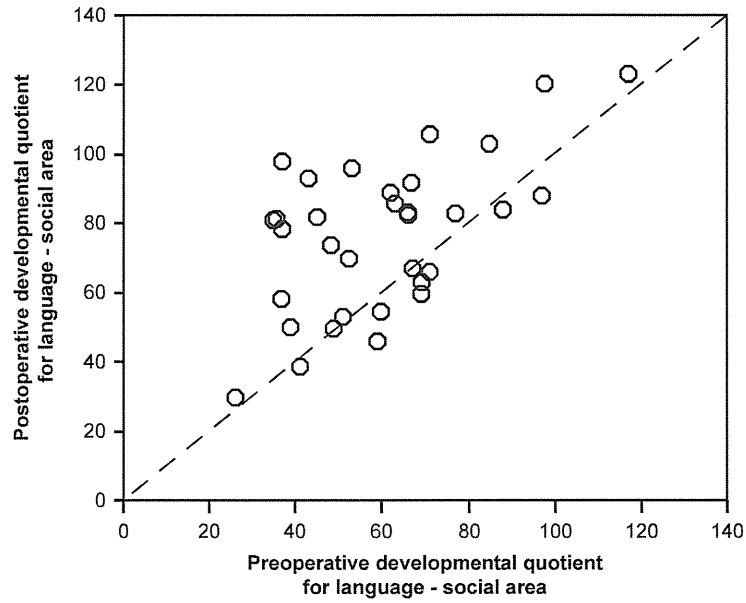

Figure 6. The pre- and postoperative developmental quotient (DQ) for the Language-Social Area (LS). The dashed line indicates where the pre- and postoperative DQ shows the same value. The average pre- and postoperative DQ for the LS were 60 and 77, respectively. The improvement in this area following cochlear implantation was significant ($p < 0.01$, paired t test).

DQ for LS was 0.57 (Pearson's correlation coefficient, $p < 0.01$). The postoperative DQ for CA was 90, and was not significantly changed from the preoperative value ($p = 0.11$, paired t test). However, the correlation coefficient between preoperative DQ for CA and postoperative DQ for LS was 0.71 (Pearson's correlation coefficient, $p < 0.01$) (Figure 7), meaning that the preoperative DQ for CA was strongly correlated with postoperative development in the linguistic area.

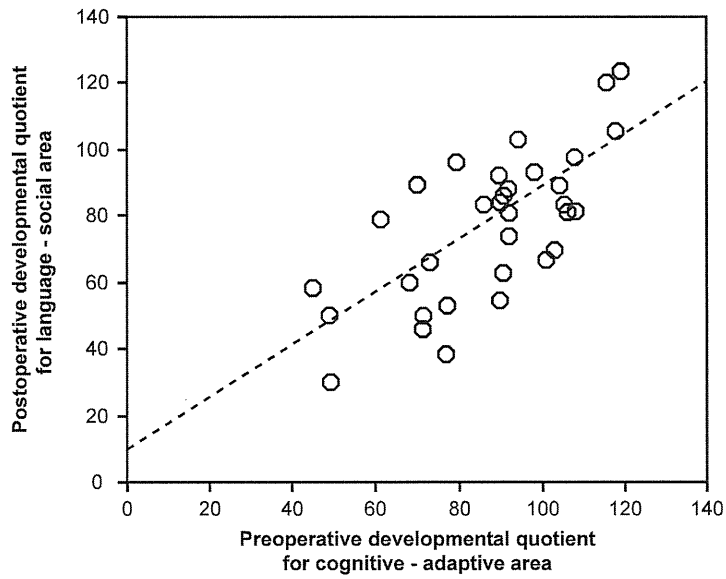

Figure 7. The preoperative developmental quotient (DQ) for the Cognitive-Adaptive Area (CA) and postoperative DQ for the Language-Social Area (LS). A strong correlation was observed ($r = 0.71$, $p < 0.01$, Pearson's correlation coefficient). The dotted line indicates the estimated regression line (regression coefficient = 0.79, intercept = 8.00). This means that the preoperative DQ for the CA was strongly correlated with postoperative development in the linguistic area.

Discussion

Cochlear implantation in patients with prelingual hearing loss is widely accepted [2,3]. Numerous studies have reported that the age at implantation is an important prognostic factor for linguistic development [4,5]. Excluding those patients who received operations during the first 5 years in which cochlear implantation was being performed at Kyoto University Hospital, most of the patients in our institute underwent operation at or before the age of 3 years. With the improvement in devices and coding strategies, old age is no longer a contraindication for cochlear implantation [6]. In such cases, however, the benefit is limited and the indications for cochlear implantation in prelingually deafened adults must be determined carefully.

In most patients, the standard transmastoid facial recess approach was utilized. In some cases, however, alternative procedures were needed. Previous studies reported the effectiveness of total or partial removal of the posterior canal wall and subsequent reconstruction with hard tissue [7] or soft tissue [8]. This procedure facilitates the approach to the round window and is useful in cases with poor mastoid development; four of our patients required this procedure. Radical mastoidectomy and closure of the external auditory canal is effective in controlling middle ear inflammation [9]. We adopted this technique in two cases with accompanying inflammation. The transmeatal approach was proposed as a minimally invasive surgery [10,11]. In addition to the minimal invasiveness, this approach is very useful in cases without mastoid development. We experienced four cases with little or no mastoid development; in these cases, the electrodes were inserted into the cochlea through the transmeatal approach.

Cochlear obstructions were found in four patients, and were apparent in all of the preoperative MRIs. In three cases, the degree of the obstruction was correctly predicted and the electrode was fully inserted after the removal of the lesions inside the cochlea. In the other case, shown in Figures 3 and 4, the preoperative MRI findings indicated that the basal turn was narrow but patent. The intraoperative findings, however, revealed that the inferior portion of the basal turn was totally ossified. The ossification was drilled out and the electrodes were fully inserted. Although the preoperative MRI predicts cochlear obstruction accurately in about 90% of patients [12], it is not perfect. Cochlear implant recipients with obstructed cochleae usually have successful outcomes. However, in patients with severe obstruction the number of active electrodes tends to be small, resulting in poor performance [13]. In patients deafened by meningitis, it is important to obtain the MRI just before cochlear implantation surgery.

In our series, perilymph/cerebello-spinal fluid gusher was observed in six patients. Although perilymph/cerebello-spinal fluid gusher is not always predictable [14], some CT findings strongly predict the incidence of the gusher; namely, an absent cribriform plate and/or an enlarged vestibular aqueduct. In all of our cases, the preoperative CT showed these findings. Perilymph/cerebello-spinal fluid gusher was alleviated by reverse Trendelenburg positioning, administration of mannitol, or just waiting. Once the perilymph/cerebello-spinal fluid gusher was alleviated, we inserted the electrode and sealed the cochleostomy with temporal muscle fascia. We did not encounter cases requiring spinal drain placement or middle ear packing.

The DQ for LS improved after cochlear implantation, as reported previously [2,3]. Developmental delay, especially in the area of cognition [15], adversely affects postoperative speech development [16]. In our study, the preoperative DQ for CA was strongly correlated with the postoperative DQ for LS. However, it is possible that the test to evaluate higher level language and social skills was difficult for them, even though they were achieving good speech development. Otherwise, patients with cognitive delay may eventually catch up with patients without such delay, since we examined the development only in the early stage. The effect of preoperative cognitive development on postoperative language improvement has yet to be explored.

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ORIGINAL ARTICLE

Multivariate analysis of hearing outcomes in patients with idiopathic sudden sensorineural hearing loss

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Abstract

Conclusions: Contralateral hearing loss is significantly correlated with poor hearing outcomes in patients with idiopathic sudden sensorineural hearing loss (ISSNHL). *Background:* The hearing outcome in patients with ISSNHL was analyzed using multiple variables. *Methods:* A retrospective chart review was conducted using 89 patients with ISSNHL. Patients within 40 dB HL of average hearing levels and/or patients whose hearing loss was restricted to low frequencies were excluded. The influence of pre-existing conditions on hearing outcome was analyzed using a polytomous universal model. Pre-existing conditions analyzed included hyperglycemia, hypercholesterolemia, hypertension, and contralateral hearing loss. In addition, the severity of hearing loss, age group, and the existence of vertigo were analyzed concomitantly. *Results:* Hearing recovery was significantly reduced in patients with a past history of contralateral hearing loss.

Keywords: *Contralateral hearing loss, pre-existing conditions, polytomous universal model*

Introduction

Idiopathic sudden sensorineural hearing loss (ISSNHL) is defined as inner ear hearing loss that develops abruptly without definitive causes. Although ISSNHL is one of the few sensorineural hearing disorders that can be cured, the hearing outcome differs greatly among cases. This diverse outcome is usually attributed to the heterogeneity of ISSNHL. No single pathophysiology can fully explain this diversity and various pathogeneses have been hypothesized to explain this disorder, including viral infections [1,2], genetic factors [3,4], and microvascular disturbance [1]. Given that different pathogeneses often result in different outcomes, prior studies have tried to correlate pre-existing pathological conditions with the hearing outcome of ISSNHL. For example, metabolic (e.g. hyperglycemia and hypercholesterolemia) and circulatory disorders (e.g. hypertension) may suggest underlying microvascular disorders as a cause of ISSNHL [5,6], while prior hearing disturbance in the contralateral ear may be suggestive of genetic factors [3]. However, the prognostic value of these factors differed from study to study. One reason for the inconsistencies may be the analytic procedures used. In previous studies aimed at evaluating prognostic values, the effects of single factors were analyzed. However, the results obtained from analyzing a single factor can be distorted by the existence of multiple background factors. Hearing level before treatment, age of the patient, and the existence of vertigo have all been reported to affect the prognosis of ISSNHL [7]; therefore, these factors should be analyzed concomitantly to more accurately assess the effect of pre-existing conditions on the hearing outcomes of ISSNHL. Since some of these factors are ordinal and the others are nominal, multifactorial analyses that can handle ordinal or nominal factors are needed. An analysis with a polytomous universal model is used to test for the effects of multiple independent factors on an ordinal dependent variable, which, in this case, is hearing outcome. These independent factors can be ordinal or nominal. Using this model it is possible to determine the significance of

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these independent variables. Thus, in this study we analyzed the influence of pre-existing pathological conditions on the post-treatment hearing results of ISSNHL using a polytomous universal model.

Material and methods

Patients

We conducted a retrospective chart review of patients who received initial treatment between January 2002 and December 2009 from the Department of Otolaryngology-Head & Neck Surgery, Kyoto University Graduate School of Medicine. One hundred and five ISSNHL patients who were more than 18 years of age visited the hospital within 2 weeks after the onset of hearing loss. Patients within 40 dB HL of average hearing levels at five frequencies between 250 and 4000 Hz and/or patients whose hearing loss was restricted to low frequencies were excluded from this study, because such patients were reported to have better hearing prognoses [8]. Some patients declined to receive the standard treatment provided by our hospital and they were also excluded from the analysis. In total, 89 patients with ISSNHL were included in the analysis. There were 50 men and 39 women ranging from 19 to 83 years of age (mean 53.0 years). All patients were hospitalized and administered corticosteroid intravenously (starting with 200 mg of prednisolone, tapered over 9 days), vitamin B12 perorally (1500 μg per day, during the follow-up) and vasodilators perorally (kallidinogenase 150 IU/mg per day, during the follow-up). Some patients were administered hyperbaric oxygen. Patient follow-ups were conducted until they showed complete recovery or their hearing level was stabilized for more than 1 month (fixed stage).

Analytic procedure

Auditory function was determined by pure-tone audiometry and was expressed by the pure-tone average (PTA in decibels) hearing level at five frequencies (250, 500, 1000, 2000, and 4000 Hz). The PTA before treatment was obtained at the first visit and the post-treatment PTA was obtained at the fixed stage.

Hearing outcome was analyzed based on the criteria prepared by the Acute Severe Hearing Loss Study Group [9]. Using these criteria, the outcome was graded into four classes: (1) complete recovery, recovery to a hearing level within 20 dB HL at all five frequencies between 250 and 4000 Hz, and/or recovery to the same hearing level as the 'good' side; (2) marked recovery, more than 30 dB recovery in the mean hearing level at the five frequencies tested; (3) slight recovery, recovery of 10–29 dB in the mean hearing level at the five frequencies tested; and (4) no response, recovery < 10 dB in the mean hearing level at the five frequencies tested. The hearing outcomes based on these criteria were analyzed using a polytomous universal model.

We recorded the following conditions for this analysis: hyperglycemia, hypercholesterolemia, hypertension, and a past history of contralateral hearing loss. In addition, severity of hearing loss, age group, and the existence of vertigo were also included in the analysis [7]. Hyperglycemic patients were defined as those who had been treated for hyperglycemia and/or whose fasting glucose at the first visit exceeded 100 mg/dl. The hypercholesterolemic patients included those previously treated for this condition and those with total serum cholesterol exceeding 240 mg/dl at the initial visit. Hypertensive patients were defined as those who had been treated for hypertension and/or whose blood pressure continued to measure 140/90 mmHg or higher before the administration of steroids. Patients with a past history of contralateral hearing loss were defined as those who had displayed distinct symptoms in the non-affected ear including hearing loss, tinnitus, and ear fullness before the onset of ISSNHL and whose PTA was above 40 dBHL. Sensorineural hearing loss compatible with normal age-related changes and/or hearing loss without subjective symptoms were not included.

In accordance with the criteria prepared by the Acute Severe Hearing Loss Study Group [9] the severity of hearing loss was described using the PTA at five frequencies (250, 500, 1000, 2000, and 4000 Hz): (1) moderate, PTA of 40–59 dB HL; (2) severe, PTA of 60–89 dB HL; (3) profound, PTA of > 90 dB HL. The patients were classified into three age groups: the young group consisted of those who were younger than 30, the middle-aged group was between 30 and 60, and those in the old group were over 60 years of age. The presence of vertigo was defined as rotatory sensation with nystagmus. Statistical analysis was conducted using SPSS software.

Results

The grand-averaged pretreatment hearing level was 79.1 dB HL, while the post-treatment hearing level was 44.6 dB HL. The averaged absolute hearing gain was 34.5 dB. Based on the criteria set by the Acute Severe Hearing Loss Study Group, 23 patients were evaluated as showing complete recovery, 35 patients

as marked recovery, 16 patients as slight recovery, and 15 patients as showing no response. The treatment results are shown in Figure 1.

The severity of the hearing loss before treatment was moderate in 18 patients, severe in 40 patients, and profound in 31 patients. Sixteen patients were classified as young, 37 patients were classified as middle-aged, and the remaining 36 patients were classified as old. The hearing outcomes according to pretreatment severity and age group are shown in Tables I and II, respectively. Among the 89 patients, 10 patients had hyperglycemia, 27 patients had hypercholesterolemia, 20 patients had hypertension, and 12 patients had contralateral hearing loss. Twenty-eight patients had accompanying vertigo.

The analysis of these treatment outcomes using the criteria prepared by the Acute Severe Hearing Loss Study Group showed that a past history of

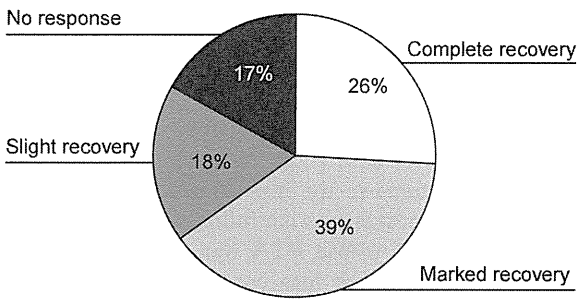

Figure 1. Overall treatment results for patients with idiopathic sudden sensorineural hearing loss. The hearing outcome was graded into four classes: complete recovery, recovery to a hearing level within 20 dB HL at all five frequencies between 250 and 4000 Hz, and/or recovery to the same hearing level as the 'good' side; marked recovery, more than 30 dB recovery in the mean hearing level at the five frequencies tested; slight recovery, recovery of 10–29 dB in the mean hearing level at the five frequencies tested; and no response, recovery < 10 dB in the mean hearing level at the five frequencies tested.

Table I. Summary of hearing outcome according to the pretreatment severity of hearing loss.

	Outcome				
Severity	Complete recovery	Marked recovery	Slight recovery	No response	Total
Moderate	7	2	5	4	18
Severe	13	13	7	7	40
Profound	3	20	4	4	31
Total	23	35	16	15	89

The severity was graded into three classes: moderate, pure-tone average (PTA) of 40–59 dB HL; severe, PTA of 60–89 dB HL; profound, PTA of greater than 90 dB HL.

Table II. Summary of hearing outcome by age group.

	Outcome				
Age group	Complete recovery	Marked recovery	Slight recovery	No response	Total
Young	5	9	2	0	16
Middle aged	7	12	11	7	37
Old	11	14	3	8	36
Total	23	35	16	15	89

The patients were classified into three age groups: the young group consisted of those who were younger than 30, the middle-aged group was between 30 and 60, and those in the old group were over 60 years of age.

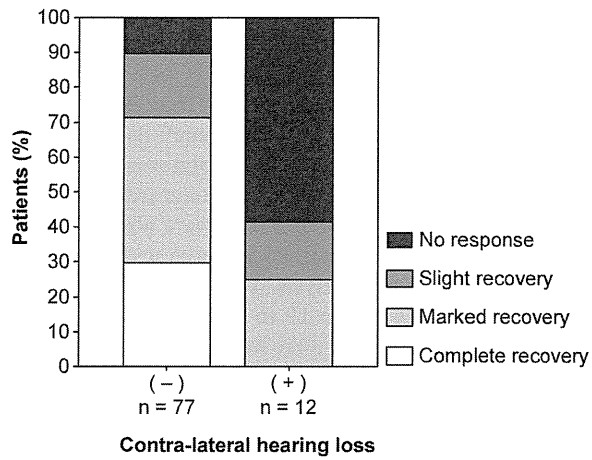

Figure 2. Hearing treatment results for patients with and without a past history of contralateral hearing loss. The treatment results from the groups with a past history of contralateral hearing loss were significantly worse than those without a past history of contralateral hearing loss.

contralateral hearing loss is significantly correlated ($p < 0.01$) with reduced recovery from ISSNHL. Figure 2 shows a statistically significant difference ($p < 0.01$, Mann-Whitney test) between the treatment results from the groups with and without a past history of contralateral hearing loss. The overall results of the analysis are shown in Table III.

Discussion

The effect of a past history of contralateral hearing loss on hearing outcomes following ISSNHL remains controversial. Stahl and Cohen have reported that the hearing outcome of ISSNHL is the same in patients with or without good hearing in the opposite ear [10]. In contrast, when Cvorović et al. analyzed 541 cases

Table III. Multivariate analysis of the results of treatment on hearing outcome (polytomous universal model).

Parameter	B	SEM	Wald	*p* value
Severity				
Moderate	−0.28	0.60	0.22	0.64
Severe	−0.11	0.50	0.05	0.82
Profound	0.00			
Hypercholesterolemia				
−	0.09	0.45	0.04	0.85
+	0.00			
Age group				
Young	−0.18	0.64	0.08	0.78
Middle-aged	0.69	0.49	1.97	0.16
Old	0.00			
Hyperglycemia				
−	−0.20	0.67	0.09	0.77
+	0.00			
Hypertension				
−	−0.26	0.52	0.24	0.62
+	0.00			
Vertigo				
−	−0.91	0.48	3.71	0.05
+	0.00			
Contralateral hearing loss				
−	−2.51	0.66	14.33	<0.001*
+	0.00			

The analysis of these treatment outcomes showed that a past history of contralateral hearing loss was significantly correlated with reduced recovery. B, regression coefficient; SEM, standard error of the mean; Wald, Wald statistics.
*$p < 0.01$.

they concluded that patients with previously diagnosed hearing loss in the opposite ear had poor hearing results [7] and the correlation between hearing recovery in the affected ear and hearing in the opposite ear was significant. In our study, similar to the study of Cvorović et al., we demonstrated poor hearing outcomes in ISSNHL patients with prior contralateral hearing loss. This discrepancy between studies may be explained by the patient selection criteria. In the present study we excluded those with low-frequency hearing loss in order to exclude patients with delayed endolymph hydrops. Patients with previous hearing loss often develop endolymph hydrops in the opposite ear (delayed endolymph hydrops), and short-term hearing recovery is good in such patients. Cvorović et al. included only a small number of patients with low-frequency hearing loss (4.7%) [7], whereas in the study by Stahl and Cohen, five of nine patients showed up-slope hearing loss and two patients experienced the recurrence of hearing loss [10], suggesting that these patients were affected by endolymph hydrops. These data could explain the more favorable hearing results reported in that study for patients with contralateral hearing loss.

Microvascular disease is one candidate for the pathophysiology of ISSNHL. Metabolic and circulatory diseases including hyperglycemia, hypercholesterolemia, and hypertension are well known risk factors for microvascular diseases. The levels of triglycerides, total cholesterol, and lipoprotein A are often significantly higher in patients with sudden deafness than in control subjects [11]. However, the influence of such metabolic diseases on the hearing outcomes of ISSNHL is still controversial. Orita et al. reported that ISSNHL patients with hyperglycemia and hypercholesterolemia had improved hearing results [5]. In the present study, however, we did not find that such diseases were correlated to the outcomes of ISSNHL. This discrepancy may be explained by the different treatment regimens employed. Orita et al. used prostaglandin and hyperbaric oxygen administration, which may be beneficial in patients with microvascular diseases [5]. Another study using a similar treatment regimen to ours reported that ISSNHL patients with hyperglycemia, hypercholesterolemia, and hypertension showed poor hearing results [12]. These results suggest that modification of the treatment regimen may be preferable in ISSNHL patients with risk factors for microvascular diseases.

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Mechanical stress-induced reactive gliosis in the auditory nerve and cochlear nucleus

Laboratory investigation

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Object. Hearing levels following microsurgical treatment gradually deteriorate in a number of patients treated for vestibular schwannoma (VS), especially in the subacute postoperative stage. The cause of this late-onset deterioration of hearing is not completely understood. The aim of this study was to investigate the possibility that reactive gliosis is a contributory factor.

Methods. Mechanical damage to nerve tissue is a feature of complex surgical procedures. To explore this aspect of VS treatment, the authors compressed rat auditory nerves with 2 different degrees of injury while monitoring the compound action potentials of the auditory nerve and the auditory brainstem responses. In this experimental model, the axons of the auditory nerve were quantitatively and highly selectively damaged in the cerebellopontine angle without permanent compromise of the blood supply to the cochlea. The temporal bones were processed for immunohistochemical analysis at 1 week and at 8 weeks after compression.

Results. Reactive gliosis was induced not only in the auditory nerve but also in the cochlear nucleus following mechanical trauma in which the general shape of the auditory brainstem response was maintained. There was a substantial outgrowth of astrocytic processes from the transitional zone into the peripheral portion of the auditory nerve, leading to an invasion of dense gliotic tissue in the auditory nerve. The elongated astrocytic processes ran in parallel with the residual auditory neurons and entered much further into the cochlea. Confocal images disclosed fragments of neurons scattered in the gliotic tissue. In the cochlear nucleus, hypertrophic astrocytic processes were abundant around the soma of the neurons. The transverse diameter of the auditory nerve at and proximal to the compression site was considerably reduced, indicating atrophy, especially in rats in which the auditory nerve was profoundly compressed.

Conclusions. The authors found for the first time that mechanical stress to the auditory nerve causes substantial reactive gliosis in both the peripheral and central auditory pathways within 1–8 weeks. Progressive reactive gliosis following surgical stress may cause dysfunction in the auditory pathways and may be a primary cause of progressive hearing loss following microsurgical treatment for VS. *(DOI: 10.3171/2010.2.JNS091817)*

KEY WORDS • auditory nerve • auditory brainstem response • cochlear nucleus • hearing loss • reactive gliosis • vestibular schwannoma

A GROWING body of evidence indicates that a significant number of patients with VS experience progressive hearing loss following microsurgical intervention. A controlled prospective study has not been performed, and the existing reports are characterized by substantial interpatient variation with respect to hearing level, definition of hearing preservation, and length of the follow-up period. However, hearing loss ranges from 15–40%, within mean follow-up periods of 5–9 years.[5,9,18,32,58,59] In one report as many as 56% of patients (14 of 25) had experienced a decline in hearing quality in the surgically treated ear 20 years after surgery, with only 1 patient experiencing a similar loss in the contralateral ear.[47] Although several explanations have been proposed,

Abbreviations used in this paper: ABR = auditory brainstem response; CAP = compound action potential; CPA = cerebellopontine angle; CR = compression-recording; EDTA = ethylenediaminetetraacetic acid; GFAP = glial fibrillary acidic protein; IAA = internal auditory artery; IAC = internal auditory canal; IAM = internal auditory meatus; IPL = interpeak latency; PBS = phosphate-buffered saline; PNS = peripheral nervous system; TSF = tractus spiralis foraminosus; TZ = transitional zone; VS = vestibular schwannoma.

This article contains some figures that are displayed in color online but in black and white in the print edition.

none has been demonstrated experimentally, and the primary cause remains unknown.

There are several potential causes of hearing loss following surgery. During surgery the contents of the IAC and the surrounding structures in the CPA are mechanically manipulated, which may cause axon degeneration and/or constriction of the arterial blood supply to the cochlea along with fibrosis and scarring in the CPA.[20,47] These reported pathological processes are likely to act together with a combinatorial effect.

Reactive gliosis has not been proposed as a cause, but it is often associated with trauma to the CNS, including that caused by ischemia, radiation, genetic disorders, or chemical insult.[6,13,21,38] A considerable number of quiescent astrocytes can resume proliferation, become hypertrophic, and upregulate GFAP.[19,28,39,52,56]

The mechanical effects of surgery are complex but can be broken down in terms of various forces, such as stretching, laceration, and compression.[7] Evaluating the effects of compression is thus clinically relevant. In this report, we demonstrate for the first time that mechanical compression applied to the auditory nerve induces reactive gliosis not only in the auditory nerve but also in the cochlear nucleus. Various aspects of hearing loss that occur after surgical treatment for VS can best be explained in terms of reactive gliosis combined with additional pathophysiological mechanisms described previously. We emphasize the need to investigate pathological changes not only in the axons but also in the astrocytes to thoroughly understand the mechanisms responsible.

Methods

Inducing Auditory Nerve Degeneration by Compression

Animal experiments were conducted in accordance with the Guidelines for Animal Experiments at Kyoto University. In our experimental model, the axons of the auditory nerve were quantitatively compressed in the CPA without permanent compromise of the blood supply to the cochlea. This was achieved by intraoperative monitoring of CAPs from the auditory nerve as reported elsewhere.[30,43] Briefly, male Sprague-Dawley rats weighing 400–450 grams each were anesthetized by an intraperitoneal injection of ketamine (100 mg/ml; Sankyo Co.) and xylazine (9 mg/ml; Bayer). After exposing the trunks of the seventh and eighth cranial nerves through right retromastoid craniectomy, simulating the same procedure in humans, an L-shaped stainless-steel wire was placed on the auditory nerve as the CR electrode to compress the auditory nerve and simultaneously record the CAPs (Fig. 1). The only device that directly touched the auditory nerve throughout the experiment was this CR electrode. In several rats, the auditory nerve was identified in the CPA and the wound was closed without compressing the nerve. In these rats, the ABRs were completely preserved, and the histological analyses revealed normal cochleae, auditory nerves, and brainstems (data not shown).

In the experimental animals, as the CR electrode was advanced, the auditory nerve was gradually compressed between the tip of the electrode and the rostroventral edge of the IAM (Fig. 1). The cochlear portion of the eighth cranial nerve is located posterodorsally at the IAM level, so the cochlear nerve was directly and highly selectively injured by the CR electrode with the vestibular and facial nerves preserved anatomically.[43] First, the CR electrode was advanced at the speed of 1 μm/second (Positions 1, 2, and 3) until the CAP flattened (Position 4), termed the "flat point," and was maintained at the flat point for 1 minute before the electrode was finally advanced to compress the auditory nerve. Rats in which the CAP recovered within 60 seconds after the flat point was reached, while the CR electrode was maintained at the flat point, were included in this study to ensure that the IAA was still functionally intact after the flat point had been reached. The flattening of the CAP was caused by impairment of the blood supply to the cochlea through the IAA. Recovery of the IAA caused the CAP to reappear spontaneously, while the CR electrode was maintained at the flat point. The critical time of cochlear ischemia that allows for complete recovery of the CAP has been reported to be 5 minutes or more.[2,40] In this experimental model, the cessation of blood supply to the cochlea never exceeded 1 minute in either of the advancements of the CR electrode.

In Group A, the CR electrode was advanced 200 μm from the flat point to compress the auditory nerve. Six rats in this group were studied 1 week after compression and another 6 rats were studied 8 weeks after compression.

In Group B, the electrode was advanced 400 μm, thus increasing the compression damage to the nerve. The same numbers of animals were studied at the same time intervals.

In both groups, the speed of CR electrode advancement was 10 μm/second during the second advancement (the compression procedure); 60 seconds after the start of the second advancement, the electrode was withdrawn at 110 μm/second. The temporal bone on the right side was treated and the left side was used as a control.

Recordings of ABRs and CAPs of Auditory Nerve

Auditory brainstem responses were recorded between the base of the earlobe of the operative side (right) and the vertex, with the ground electrode at the base of the forelimb. Click stimuli (100 dB sound pressure level) were presented to the right ear at a rate of 9.5 pulses/second through a tube earphone or a hollow ear bar driven by a 100-μsec rectangular pulse wave fed by a stimulator. Evoked potentials were amplified with a bandpass of 50 Hz to 3 kHz and averaged using a processor (Synax 1100, NEC Medical Systems) with a sampling interval of 20 μsec and 500 data points in each recording. The responses to 100 successive clicks were averaged for ABR recordings and stored in a computer. Alternating clicks were used to stimulate the ABR. During the first and second compression procedures, the CAPs were recorded between the tip of the CR electrode and the vertex, with the ground electrode placed at the base of the forelimb. For CAP recordings, the acoustic nerve potentials evoked by 5 successive clicks were averaged and stored in a computer. This rate led to a continuous CAP recording rate

of 1 potential every 2.4 seconds before the flat point. The click was picked up at the exit from the earphone with a microphone (ACO 4016, ACO Pacific) connected to the earphone and amplified with a microphone amplifier (MA3, Tucker-Davis Technologies). It was subsequently transferred to an oscilloscope (Iwatsu DS-8812, Iwatsu Electric) for fast Fourier transform analysis, revealing that the frequency of the tones included in the click ranged up to approximately 5 kHz. In contrast to absolute values of amplitudes of ABR, IPLs are relatively independent of the intensity of the stimulus.[11] We measured IPL between Waves I and II (I–II IPL) and IPL between Waves II and IV (II–IV IPL) to investigate the state of nerve impulse conduction from the cochlea to the brainstem. For statistical analyses, unpaired t-tests were performed using Microsoft Office Excel 2007.

Immunohistochemical Analysis

To prepare the temporal bones, each rat was placed in a state of deep anesthesia and was perfused transaortically with 4% paraformaldehyde in 0.01 M PBS at pH 7.4. The temporal bones were decalcified with 10% EDTA and HCl solution (pH 7.4) for 2–3 weeks.

After decalcification, the temporal bones were embedded in OCT compound (Sakura Finetechnical) and frozen. Serial 8-μm sections were then cut for immunohistochemical analysis. Midmodiolar sections included 4 good cross-sections of the Rosenthal canal (basal, lower middle, upper middle, and apical) and the widest part of the auditory nerve. These sections were mounted on glass slides, washed in PBS, and dried at room temperature for 30 minutes. They were permeabilized and then blocked with 10% goat serum in PBS-T (PBS containing 0.2% Triton X-100) at room temperature for 30 minutes.

To visualize astrocytes in the auditory nerve and cochlear nucleus, anti-GFAP rabbit polyclonal antibody (× 500; DAKO) was applied to the sections and incubated at 4°C for 12 hours followed by washing in PBS-T 3 times for 5 minutes each. A secondary antibody (Alexa Fluor 488 goat anti–rabbit IgG antibody × 500, Molecular Probes, diluted with 10% goat serum in PBS-T) was applied to the sections at room temperature for 1 hour followed by washing in PBS-T twice for 5 minutes each time.

To visualize neurons, anti–beta III-tubulin mouse monoclonal antibody (× 500; Covance Research Products, Berkeley) was used as the primary antibody and Alexa Fluor 594 goat anti–mouse IgG antibody (× 500, Molecular Probes) as the secondary antibody.

For nuclear staining, the sections were incubated in 4′,6-diamidino-2-phenylindole (DAPI, 2 μg/ml, Molecular Probes) solution at room temperature for 15 minutes. After being rinsed in PBS, the samples were mounted onto glass slides and coverslipped with VECTASHIELD mounting medium (Vector Laboratories). A fluorescence microscope system equipped with appropriate filters (Olympus BX50 and BX-FLA) was used for observation, and samples were photographed with a digital camera (Olympus DP10). Confocal images were obtained with a confocal laser-scanning microscope (TCS-SP2 Leica Microsystems). Images used for the figures were processed with Photoshop (version 6.0, Adobe Systems).

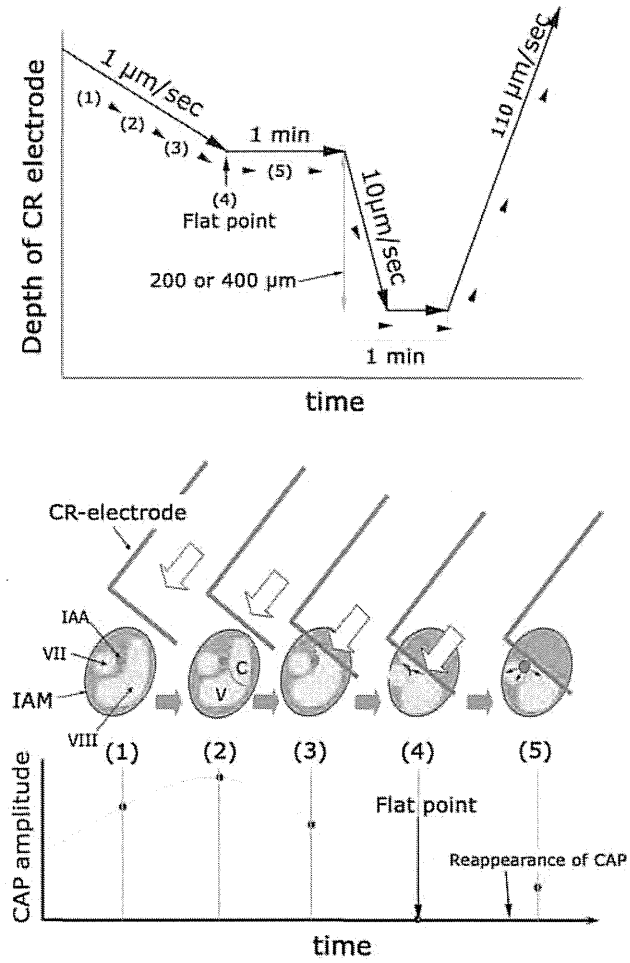

FIG. 1. Mode of advancement of the CR electrode **(upper)** and relationship between electrode depth and amplitude of the auditory nerve CAP during the first advancement of the electrode **(lower)**. **Upper:** First, the CR electrode was advanced at 1 μm/second (CR electrode Positions 1, 2, and 3) until the CAP flattened (Position 4). The CR electrode was maintained at the flat point for 1 minute and then was advanced 200 μm (in Group 1 rats) or 400 μm (in Group 2 rats) at 10 μm/second to compress the auditory nerve; 60 seconds after the start of this advancement, the electrode was withdrawn at 110 μm/second. **Lower:** The IAC contents at the IAM level of the right ear, viewed from the brainstem side toward the cochlea. The CAP amplitude increased as the CR electrode came close to the auditory nerve (Positions 1 and 2) and decreased as the CR electrode continued to be advanced and began to directly compress the auditory nerve due to conduction block of nerve impulses (Position 3). The CAP flattening was caused by impairment of the blood supply to the cochlea through the IAA (Position 4, *small black arrow* indicates collapsed IAA). However, pulsation of the IAA would push the surrounding nerve tissue aside *(small black arrows)*, causing the caliber of the IAA to normalize gradually and the CAP to reappear spontaneously, while the CR electrode was maintained at the flat point (Position 5).

Results

Controls

In a previous paper we demonstrated that a TZ, the interface between the PNS and the CNS portions of the auditory nerve, can be observed even in routine H & E

staining.[44] In our present study the TZ was more clearly observed with anti-GFAP immunostaining because astrocytes are found only in the CNS portion of the auditory nerve (Fig. 2).

At the fundus of the IAC, multiple tiny osseous canals, called the TSF, allow the axons to pass from the Rosenthal canal along the auditory nerve (modiolus) toward the CNS (Figs. 2 and 3).[50]

Throughout the control specimens, the length of the astrocytic processes toward the basal cochlear turn tended to be longer than those toward the middle and apical cochlear turns (Fig. 2 right). It was noted, however, that these astrocytic processes never entered into the TSF in any cochlear turn, even in the basal cochlear turn in controls. With the exception of those in the basal turn, the length of the astrocytic processes never exceeded approximately 75 μm.

Electrophysiological and Morphological Changes After Compression

In rats the CNS portion of the auditory nerve is relatively long, and hence, the TZ is situated within the IAC as in humans (Figs. 2 and 3).[15,53] Because of this anatomical relationship, the compression in the CPA cistern always injured the CNS portion where astrocytes are abundant.

Group A. One week after compression, the general shape of the ABR was preserved but the peak amplitudes were attenuated and the latencies of Waves II, III, and IV were prolonged (Fig. 4). The I–II IPL increased from 0.34 ± 0.03 msec before compression to 0.40 ± 0.03 msec (mean ± SD). The II–IV IPL increased from 0.64 ± 0.03 msec to 0.66 ± 0.04 msec (Fig. 5). The I–II IPL was significantly prolonged after compression ($p < 0.05$) but the II–IV IPL was not. After compression, an unlabeled region was observed just beneath the compression site (Fig. 6). Within this region, GFAP immunoreactivity was lost, indicating the mechanical disruption of the astrocytes. The shape of the TZ was essentially unchanged and the astrocytic outgrowth at the TZ was limited. In the cochlear nucleus we did not observe any change in GFAP staining (data not shown).

Eight weeks after compression the general configuration of the ABR was preserved but the peak amplitude was attenuated and the latencies of Waves II, III, and IV were prolonged (Fig. 7). The latency of the I–II IPL increased from 0.34 ± 0.02 msec to 0.41 ± 0.03 msec ($p < 0.05$) and the II–IV IPL decreased from 0.64 ± 0.01 msec to 0.63 ± 0.03 msec (not significant) (Fig. 5). There was no significant difference in the I–II IPLs between 1 week and 8 weeks after compression. Labeling for GFAP showed that the astrocytic processes elongated enormously from the TZ toward the PNS portion of the auditory nerve (Fig. 8A and B). The length of a substantial number of astrocytic processes was more than 200 μm. The elongated processes ran in parallel with the residual auditory neurons. They entered much further into the TSF in the basal portion of the cochlea compared with the middle cochlear turns (Fig. 8B and C). At the compression site, small, unlabeled areas were observed. Confocal images disclosed a dense meshwork of gliotic tissue at and in the vicinity of the lesion epicenter and fragments of neurons were scattered in this gliotic tissue (Fig. 8D). In the cochlear nucleus, hypertrophic astrocytic processes were abundant around the soma of the neurons (Fig. 8E *single asterisks*) in comparison with the control (Fig. 8F), and in a limited area they formed a meshlike structure of gliotic tissue (Fig. 8E *double asterisks*).

Group B. One week after compression, the ABR was hardly discernible (Fig. 9). Immunohistochemically, a large area unlabeled for GFAP was observed at the compression site (Fig. 10), and it was much larger than that observed in Group A (Fig. 6). The IAC was filled with swollen auditory nerve tissue, a finding not observed in any of the rats in Group A at either time point or in the Group B rats 8 weeks after compression (see below). Astrocytic outgrowth from the TZ was, however, limited (Fig. 10 *large arrowheads*) and in the cochlear nucleus there was no obvious change in GFAP staining (data not shown).

Eight weeks after compression, the peaks of the ABR could not be identified (Fig. 11). The growth of astrocytic processes was much more extensive than in Group A at 8 weeks postcompression (Fig. 12). The length of many processes was more than 300 μm. The astrocytic outgrowth was most evident at the basal portion of the cochlear turn, where the processes elongated and occupied all the orifices of the TSF. Confocal images showed that they ran parallel with the residual auditory neurons within the TSF. In the lesion epicenter, dense gliotic tissue surrounded neural tissue fragments. Similar dense gliotic tissue occupied the cochlear nucleus, where the neurons were tightly surrounded by ramified gliotic tissue. This pathology was only rarely observed in the Group A animals (Fig. 8E). The transverse diameter of the auditory nerve at and proximal to the compression site was reduced considerably, and this finding was more pronounced in this subgroup than in the Group A rats killed at 8 weeks (Figs. 12A and 8A, respectively).

Discussion

In this study we demonstrate for the first time that compression of the auditory nerve induces reactive gliosis not only in the auditory nerve but also in the cochlear nucleus. This can occur even with minimal degradation of the ABR. Thus, reactive gliosis should potentially be regarded as a "third causative factor," in addition to neural and vascular factors, for hearing loss following surgical treatment for VS.

Glial Scar Formation and Degree of Injury

Glial scars are formed in the adult CNS following various insults and constitute a physical and molecular barrier unfavorable to axon survival and regeneration.[6,41,49] In our present study, the gliotic tissue was observed at the lesion epicenter and in the vicinity of the compression site 8 weeks postcompression. Normal tissue architecture was lost, and fragments of auditory neurons were surrounded by reactive astrocytes (Figs. 8D and 12D). Reported ultrastructural findings of degenerating and degenerated

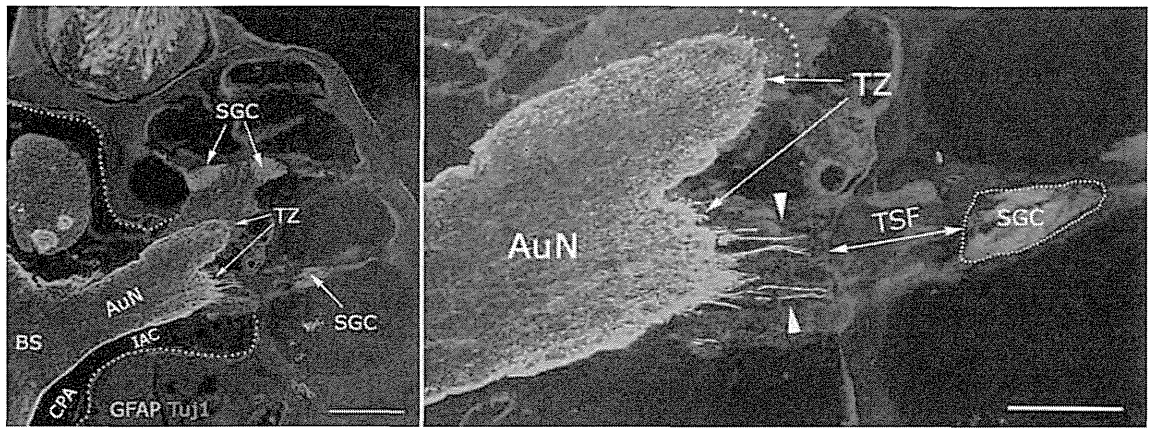

FIG. 2. Photomicrographs showing the TZ of the normal auditory nerve. The interface between the central and peripheral portions of the auditory nerve is clearly observed with anti-GFAP immunostaining *(green)* because of the presence of astrocytes only in the CNS portion of the auditory nerve. Note that the astrocytic processes toward the basal cochlear turn are longer than those toward the other cochlear turns (*arrowheads* in the **right panel**). The astrocytic processes never entered into the TSF in any cochlear turn even in the basal cochlear turn in controls. In this rat, the length of the astrocytic processes from the TZ was less than 75 μm, the longest distance of astrocytic extension in controls in all the cochlear turns except the basal turn (*dotted line* in the **right panel**). The *dotted line* in the **left panel** indicates the border between the intra- and extracranial compartments. Anti-GFAP and anti–beta III-tubulin (clone Tuj1) immunostaining. Bar = 500 μm (left) and 250 μm (right). AuN = auditory nerve; BS = brainstem; SGC = spiral ganglion cell.

axon terminals surrounded and phagocytosed by reactive astrocytes after deafferentation may correspond to our results.[4,10,17,23]

Our results also show that the higher level of compression applied to animals in Group B caused greater degradation of the ABR, increased astrocytic outgrowth from the TZ, higher levels of gliosis in the cochlear nucleus, and larger areas lacking GFAP labeling close to the compression site. In spinal cord injury, the hemorrhagic zone at the lesion epicenter cavitates as a result of necrosis several days after trauma.[22,55] Hemorrhagic foci have been observed previously within the auditory nerve trunk following mechanical trauma.[45] This study shows that they decrease between 1 and 8 weeks after surgery, suggesting invasion by reactive astrocytes. The observed swelling of the auditory nerve within the IAC was much less for low levels of compression and after the longer survival period. Hence, it is likely that swelling occurs only in acute stages of severely compressed auditory nerves, and that it is caused by edema as observed in the optic nerve.[25]

Astrocytic Proliferation and ABR Deterioration

In small experimental animals, Wave I of the ABR is generated from the extracranial (intratemporal bone) portion of the auditory nerve, Wave II reflects synaptic activity in the cochlear nucleus, and the subsequent waves reflect electrical activity in the pons/upper brainstem.[33,46] Because the compression site in our experiments was situated at the IAM, the IPL between Waves I and II was prolonged, but from Wave II through Wave IV the latencies were unaffected.

In our present study, the astrocytic processes elongated conspicuously from the TZ toward the PNS portion of the auditory nerve, ran parallel with the residual auditory neurons, and entered into the TSF, particularly

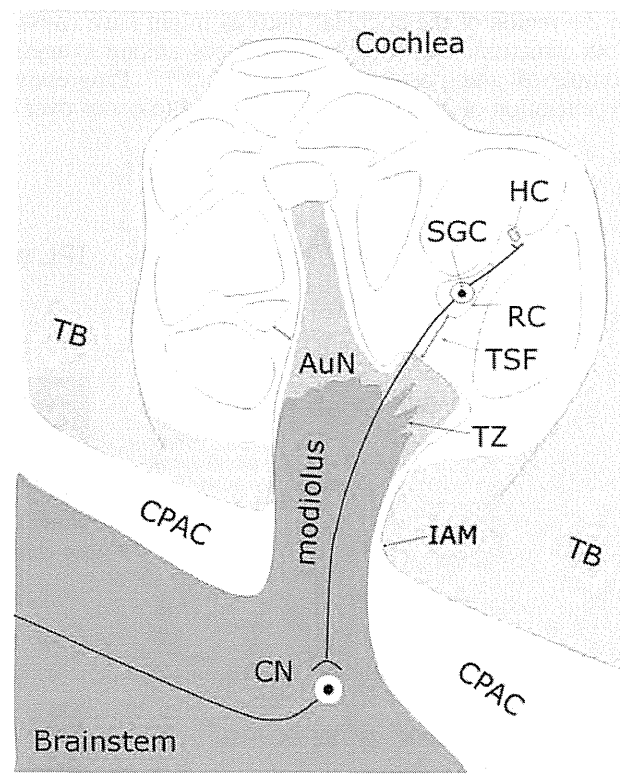

FIG. 3. Schematic illustration showing the anatomical relationships between the auditory nerve and the surrounding structures. The auditory nerve is a bundle of bipolar neurons that form synaptic contacts with the hair cells peripherally and cochlear nucleus cells centrally. The cell bodies of the auditory neurons (spiral ganglion cells) are housed in the Rosenthal canal. The TSF is an osseous canal through which the axons of the auditory nerve pass from the Rosenthal canal to the axis of the auditory nerve (modiolus). CN = cochlear nucleus; CPAC = CPA cistern; HC = hair cell; RC = Rosenthal canal; TB = temporal bone.

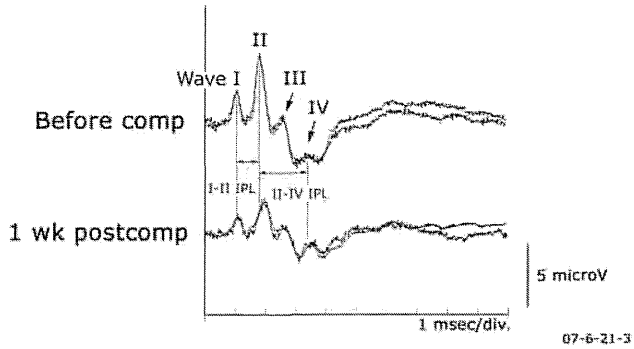

FIG. 4. Auditory brainstem responses in a Group A rat, before and 1 week after compression. The general configuration of the ABR was maintained after compression, but the amplitude of each peak was attenuated and the latencies of Waves II, III, and IV were prolonged. The IPL between Waves I and II (I–II IPL) and that between Waves II and IV (II–IV IPL) before compression are indicated by *doubleheaded horizontal arrows*. Comp = compression.

in the basal region of the cochlea. Massive proliferation of astrocytic processes within the modiolus may physically compress the adjacent nerve fibers, especially within the narrow canals of the TSF. If so, then this could be a cause of hearing loss. Moreover, enhanced glial activity in the region of the cochlear nucleus might have caused both structural and functional changes among synaptic complexes and postsynaptic neurons.[14,16,48] Progressive degeneration of axons has been reported to occur over 8 months and more than 1 year after peripheral insult to the auditory nerve/cochlear nucleus (noise-induced hair cell damage) and spinal cord injury, respectively.[35,57] Thus, the attenuation of Wave II of the ABR may have been caused both by reactive gliosis related to cochlear nucleopathy and by reduction of auditory nerve activity in the cochlear nucleus.

Within Group A, statistically significant differences in the I–II IPLs were not observed between 1 and 8 weeks after compression. However, some auditory nerve degeneration must have developed without being detected in the ABR recordings. Our fast Fourier transform analysis revealed that the click that we used included frequencies up to approximately 5 kHz. The stimulator used was designed for use in humans, and its power spectrum normally stimulates the apical, middle, and upper basal turns of the cochlea; in rats, however, it stimulates only approximately one-quarter of the length of the cochlea.[11,37,60] Thus, the ABRs in our experiments did not cover the potential electrophysiological changes associated with the auditory nerve dysfunction due to the massive outgrowth of astrocytic processes in the lower apical, middle, and basal turns. In one study on rats in which the cochlea was surgically removed, GFAP immunoreactivity increased in the cochlear nuclei 2 days after the surgery, remained intense for 3–8 days, and then declined by Day 21.[12] In another study, the GFAP reaction occurred on Day 1, increased in intensity at Days 4–21, and then remained elevated until Day 45 in the cochlear nucleus (the longest observation time in the study).[8] Our results suggest that

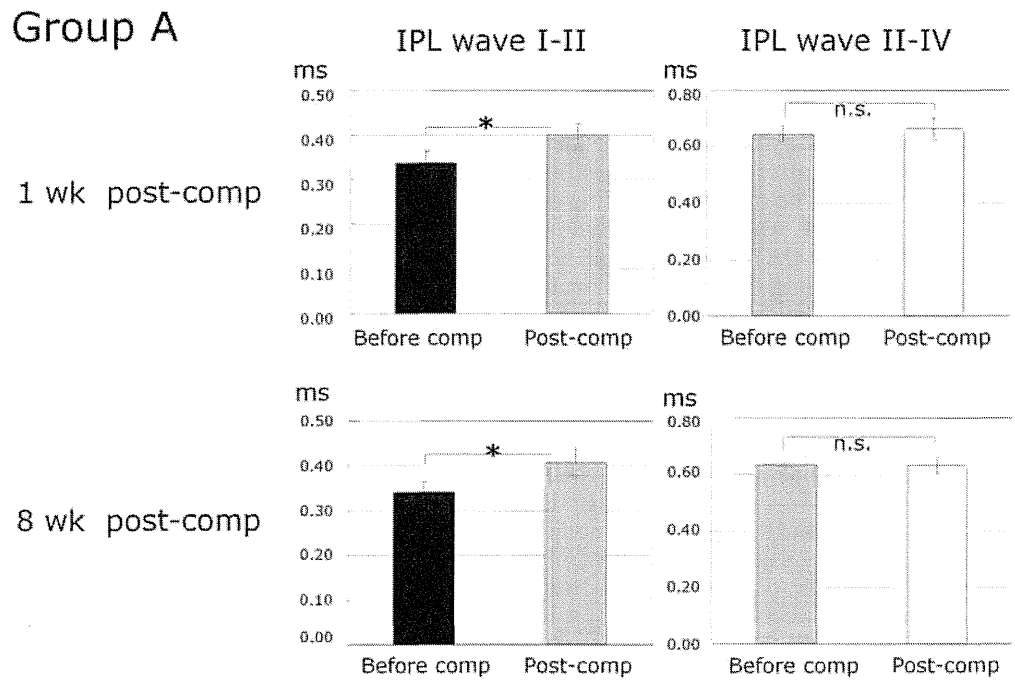

FIG. 5. Bar graphs showing the mean IPLs (± 1 SD) between Waves I and II (I–II IPL) and between Waves II and IV (II–IV IPL), 1 week and 8 weeks after compression in Group A. The I–II IPL was significantly prolonged after compression but the II–IV IPL was not. There was no significant difference between the I–II IPL at 1 week and that at 8 weeks postcompression. ms = msec; n.s. = not significant. * $p < 0.05$.

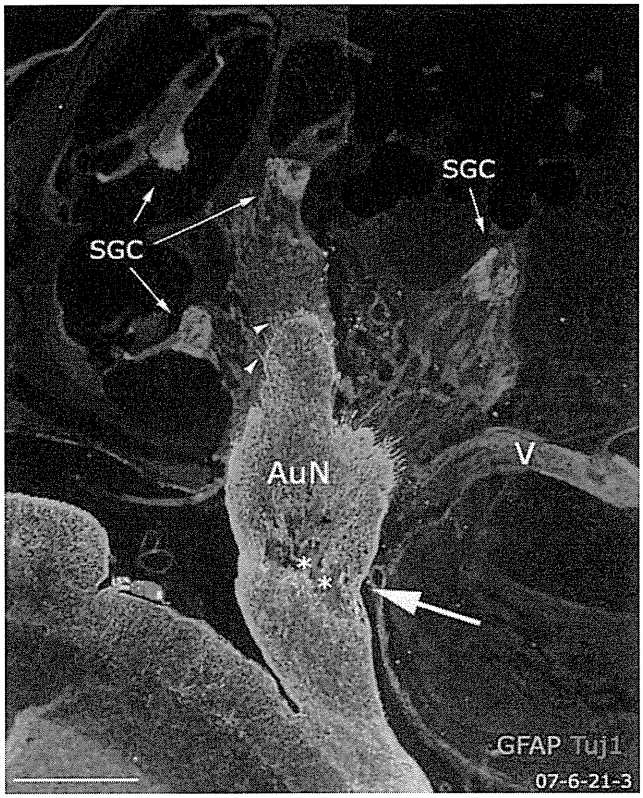

FIG. 6. Photomicrograph showing morphological changes in the auditory nervous system 1 week after compression in Group A (the same rat as in Fig. 4). A GFAP-negative region *(asterisks)* was observed at the compression site *(arrow)*. The shape of the TZ was essentially unchanged *(arrowheads)*. Anti-GFAP and anti–beta III-tubulin (clone Tuj1) immunostaining. Bar = 500 μm. V = vestibular nerve.

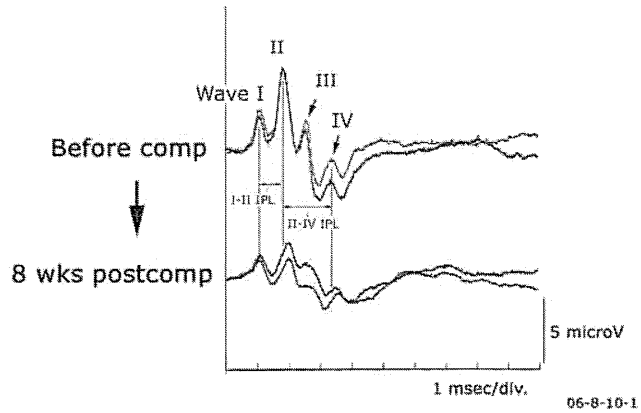

FIG. 7. Auditory brainstem response in a Group A rat, before and 8 weeks after compression. The amplitude was attenuated, but each peak of the ABR was preserved while the latencies of Waves II, III, and IV were prolonged. The I–II IPL and II–IV IPL before compression are indicated by *doubleheaded arrows*.

reactive gliosis continues at least to the 8th week post-compression, and longer-term studies are needed to describe the full consequences of the response.

Clinical Extrapolations

Various clinical observations can be explained by reactive gliosis combined with the previously reported pathophysiological mechanisms. Several reports have demonstrated that the presence of adhesion in the interface between the auditory nerve and the tumor is the most significant negative prognostic factor in hearing preservation surgery, regardless of tumor size.[24,36,51,61] The less adhesion, the less mechanical force needed to separate the auditory nerve from the tumor surface, leading not only to less trauma-induced auditory nerve degeneration but also to less reactive gliosis.

"Cochlear nucleopathy" may "naturally" occur as a VS increases in volume. As the cochlear nuclei are located at the entrance of the fourth ventricle[1] and the shape of the fourth ventricle is inevitably distorted in accordance with tumor growth, the cochlear nuclei cannot escape from the effects of mechanical stress and reactive gliosis. In patients with neurofibromatosis Type 2, the outcome of auditory brainstem implant placement was less favorable in those cases in which the VS compressed and distorted the brainstem than in those in which it did not.[31] In the former, reactive astrocytic proliferation in the cochlear nuclei may have modified synaptic organization leading to less effectiveness of the implant, although larger tumors can be expected to cause more advanced degeneration than smaller ones.

There are some caveats with respect to extrapolation from our results to the situation in human patients. First, the length of the auditory nerve differs markedly between rats and humans. In rats the cisternal portion is approximately 0.5 mm at most (Fig. 2), whereas in humans it is approximately 10–15 mm.[26,34,54] Reactive gliosis may be more severe where the compression site is so much closer to the brainstem.[29,42] Second, in our study the changes to the ABR were created on purpose by traumatizing the "normal" auditory nerve. Under clinical conditions, trauma to the normal auditory nerve may be very rare. In the clinical setting, the ABR configuration in patients with VS is often already distorted before surgical intervention, with the tumor mass causing auditory nerve dysfunction through mechanical compression. This is especially the case with respect to the intracanalicular portion of the auditory nerve. In a study in which the intracanalicular pressure was directly measured in the patients with VS, the pressure within the IAC was significantly elevated, and the authors concluded that pressure from tumor growth in the IAC might be responsible for hearing loss.[3] The morbid auditory nerve in the patients with VS could be significantly more sensitive to the same insult than the normal auditory nerve.[27] Third, we observed ABR decline and remarkable astrocytic proliferation 8 weeks after compression. In contrast, delayed hearing loss has been reported years after surgery in patients who have undergone VS surgery with preserved hearing.[5,9,18,32,58,59] Therefore, our results may be better applied to "subacute" hearing loss in VS surgery. Typically, these patients wake up with hearing after surgery but suffer hearing loss 1–2 months later. However, the time course in gliosis may be different in humans and rats, and it is important to carry

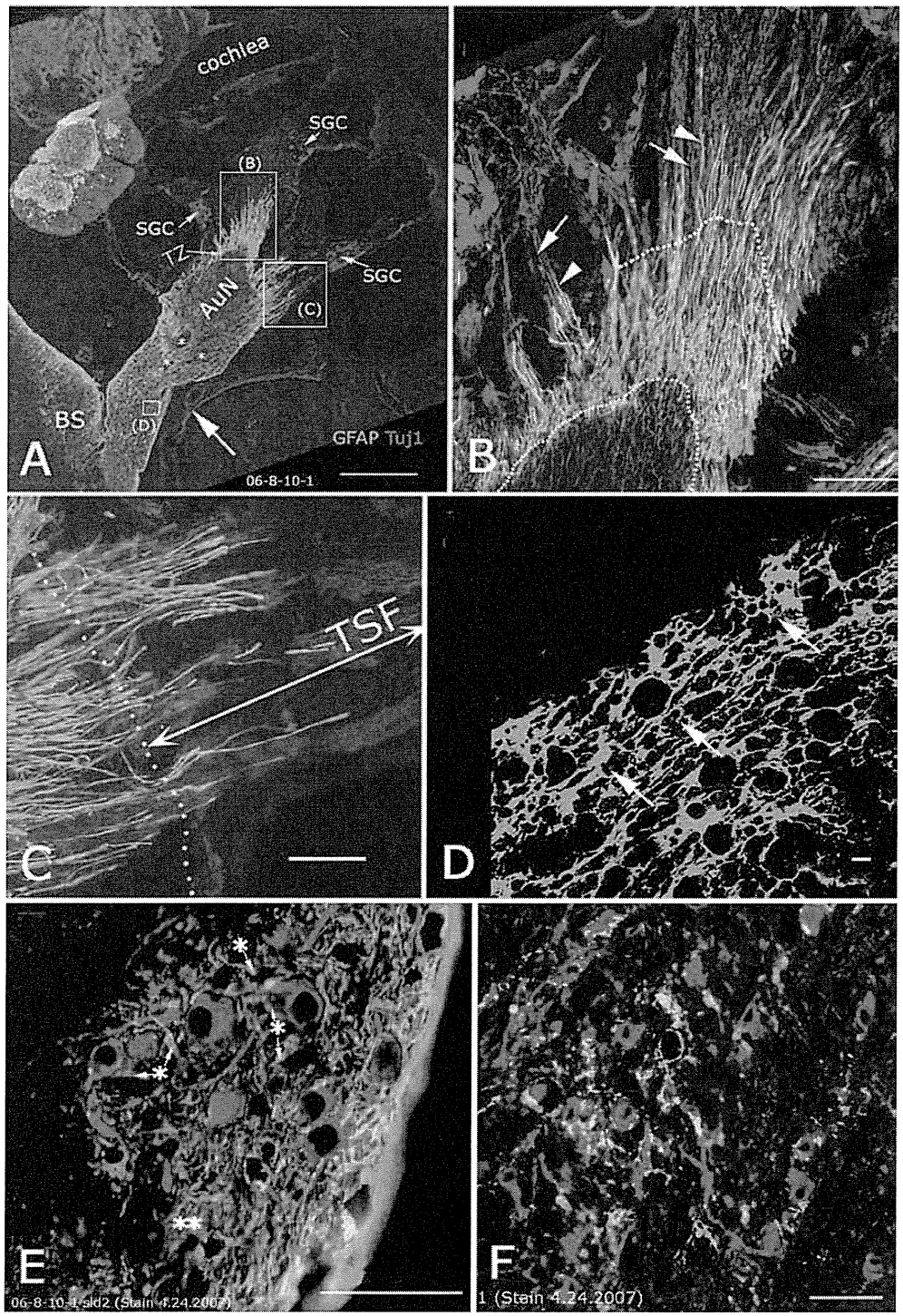

FIG. 8. Morphological changes in the auditory nervous system 8 weeks after compression in Group A (the same rat as in Fig. 7). The astrocytic processes elongated enormously from the TZ toward the periphery (**A and B**). The length of many astrocytic processes was more than 200 μm from the TZ (*dotted lines* in **B**) and they ran parallel with the residual auditory neurons (the *arrowhead* in **B** indicates an astrocytic process and the *arrows*, auditory neurons). The astrocytic processes penetrated the TSF more deeply in the basal portion of the cochlea **(C)** than in the middle portion **(B)**. (Panels **B and C** are enlargements of areas indicated by "(B)" and "(C)" in **panel A**.) At the compression site (*large arrow* in **A**), small, unlabeled areas were observed (*asterisks* in **A**). At and in the vicinity of the lesion epicenter, a dense meshwork of gliotic tissue containing the fragments of neurons (*arrows* in **D**) was observed (the area indicated by "(D)" in **panel A** is enlarged in **panel D**). Hypertrophic astrocytic processes were observed in the cochlear nucleus (*single asterisks* in **E**). Meshlike structure of gliotic tissue was occasionally seen (*double asterisks* in E). **F**: Cochlear nucleus region in control. Anti-GFAP and anti–beta III-tubulin (clone Tuj1) immunostaining. Bar = 500 μm (A), 100 μm (B), 100 μm (C), 10 μm (D), 50 μm (E), and 50 μm (F).

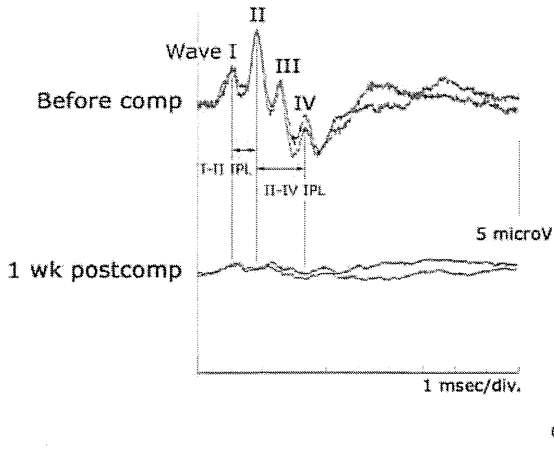

FIG. 9. Auditory brainstem responses in a Group B rat before and 1 week after compression. All the components of ABR were hardly discernible after compression. The I–II IPL and II–IV IPL before compression are indicated by *doubleheaded arrows.*

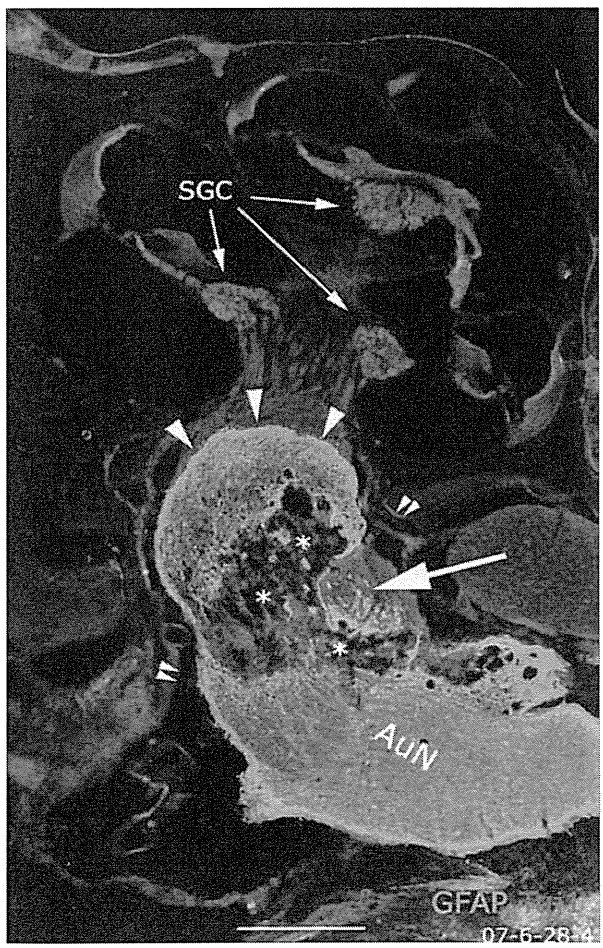

FIG. 10. Morphological changes in the auditory nerve 1 week after compression in a Group B rat (the same animal as in Fig. 9). A large area unlabeled for GFAP *(asterisks)* was observed at the compression site *(arrow)* (compare with Fig. 6). The IAC was filled with swollen auditory nerve tissue. Astrocytic outgrowth from the TZ was limited *(large arrowheads). Small double arrowheads* indicate the IAM. Anti-GFAP and anti–beta III-tubulin (clone Tuj1) immunostaining. Bar = 500 μm. Original magnification × 2.

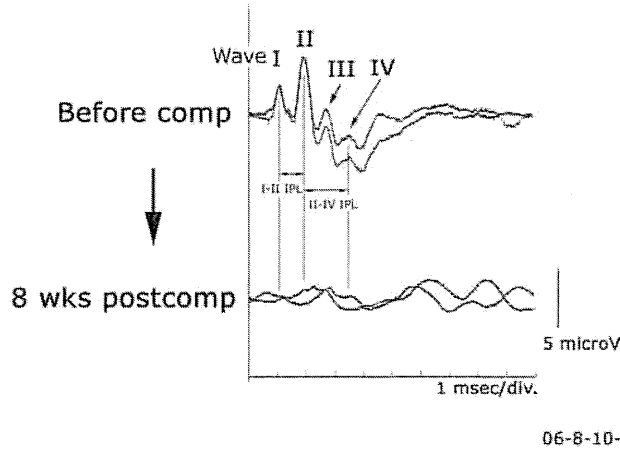

FIG. 11. Auditory brainstem responses in a Group B rat before and 8 weeks after compression. The waveform was not visible after compression. The I–II IPL and II–IV IPL before compression are indicated by *doubleheaded arrows.*

out short- and long-term studies of sequential ABR tracings following surgery in human patients.

Conclusions

We applied compression, a constituent mechanical factor in complex operative procedures, to the auditory nerve of rats while recording ABRs to measure the related hearing loss quantitatively. We found for the first time that a substantial reactive gliosis occurs in both the peripheral and central auditory pathways within 1–8 weeks and is associated with significant degradation of the ABR. This finding warrants further research to test the possibility that in the longer term the gliosis may correlate with and may even cause continued hearing loss. Reactive gliosis may be a primary cause of progressive hearing loss following microsurgical treatment for VS.

Disclosure

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper. This study was supported by the Ministry of Education, Culture, Sports, Science and Technology (Japan), the General Insurance Association of Japan, and the Univers Foundation (Japan).

Author contributions to the study and manuscript preparation include the following. Conception and design: T Sekiya. Acquisition of data: T Sekiya, M Matsumoto, K Ono, S Kada, H Ogita, RT Horie, A Viola. Analysis and interpretation of data: T Sekiya, K Ono, MC Holley. Drafting the article: T Sekiya. Critically revising the article: T Sekiya, MC Holley. Reviewed final version of the manuscript and approved it for submission: T Sekiya, M Matsumoto, K Kojima, K Ono, YS Kikkawa, S Kada, H Ogita, RT Horie, A Viola, MC Holley, J Ito. Statistical analysis: T Sekiya. Study supervision: J Ito.

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