To address this, we performed a systematic study of the effects of NT-3 and BDNF on rat SGNs, from the late embryonic stage through maturation, in explant cultures.

Materials and methods

Animal dissection and explant culture

All animal procedures were approved by the local animal subjects committee (San Diego VA Medical Center) in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Explant cultures of SGNs were prepared from 95 Sprague-Dawley rats (Harlan, Indianapolis, IN, USA) at embryonic Day 18 (E18; the day the positive vaginal plug was considered as E0), postnatal Day 0 (P0, the day of the birth), P5, P10 and P20. The embryonic animals were obtained from timed pregnant rats anesthetized with an intraperitoneal injection of rodent cocktail (ketamine 12.5 mg/ml, xylazine 1.26 mg/ml, acepromazine 0.25 mg/ml; 0.4 ml/100g body weight). After decapitation of the pups and embryos, the mandible was removed and skulls were opened midsagitally. The brain was removed and the temporal bones were harvested and transferred to Petri dishes containing sterile phosphate buffered saline (PBS; pH 7.4). The membranous labyrinth was exposed by peeling off the bony or cartilaginous cochlear capsule. The spiral ligament, stria vascularis and organ of Corti were removed and then the spiral ganglion (SG) was separated from the modiolus using fine forceps. The SG of P10 and P20 were covered with a thin bony layer. Since the bone inhibits the SG from efficient attachment to the bottom of the well, special attention was paid to removing bony fragments as completely as possible in this procedure. The procedure used for dissection allowed for harvesting of the entire SG, from base to apex. The ganglion was then divided into eight explants of approximately equal size, which ensured that here was no sampling bias toward any part of the ganglion.

Each SG explant was placed on the center of one well of a 48-well tissue culture plate that had been precoated overnight at 4 °C with 10 μg/ml fibronectin from human plasma (Sigma, St. Louis, MO, USA) and with 20 μg/ml poly-L-lysine (Sigma, St. Louis, MO, USA) for 1 hour at 37 °C. To promote attachment of the tissue to the bottom of the wells, the explants were incubated overnight at 37 °C with 5% CO_2, in 80 μl of primary growth media consisting of Dulbecco's modified Eagle's medium (DMEM; Sigma, St. Louis, MO, USA), 10% fetal bovine serum (FBS) (Invitrogen Life Technologies, Grand Island, NY, USA), 10-mM HEPES buffer (Invitrogen Life Technologies, Grand Island, NY, USA) and 300 U/ml penicillin (Sigma, St. Louis, MO, USA). The explants were then cultured in 200 μl of maintenance medium consisting of DMEM, 10 μl/ml N2 sup-

plement (Invitrogen Life Technologies, Grand Island, NY, USA), 10-mM HEPES buffer, 300 U/ml penicillin and glucose (to achieve a final concentration of 6 g/l; Sigma, St. Louis, MO, USA) for an additional 72 hours. To compare the effects of neurotrophins, the cultures of each age were divided into 15 groups consisting of 8–12 explants each, and supplemented with either recombinant human NT-3 (EMD Biosciences, La Jolla, CA, USA) or recombinant human BDNF (Upstate Biotechnology, Lake Placid, NY, USA) at concentrations ranging from 0.1 to 100 ng/ml, with one group cultured without neurotrophin to serve as an untreated control, for a total of 75 groups across ages.

Immunohistochemistry

Immunostaining was performed using the avidin–biotin complex (ABC) method (Vectastain Elite kit; Vector Laboratories, Burlingame, CA, USA). SG explants were fixed by immersion in 4% paraformaldehyde in 0.1-M phosphate buffer for 30 minutes at room temperature (RT). After washes with PBS, endogeneous peroxidase activity was blocked by treatment with 0.3% hydrogen peroxide in methanol for 30 minutes at RT. The tissues were then incubated with PBS containing 1% glycine and blocking solution (PBS containing 4% FBS and 0.2% Triton X-100) for 30 minutes each at RT to reduce nonspecific antibody binding. The explants were then incubated with anti-neurofilament (NF) 200 mouse monoclonal antibody (1:500 in blocking solution; Sigma, St. Louis, MO, USA) or anti-active caspase-3 rabbit polyclonal antibody (1:1000 in blocking solution; Abcam, Cambridge, MA, USA) at 4 °C overnight. After three washes in PBS, the tissues were then incubated with biotinylated horse anti-mouse IgG or goat anti-rabbit IgG secondary antibody (Vector Laboratories, Burlingame, CA, USA) corresponding to the primary antibody for 1 hour at RT. After three washes in PBS, the tissues were then reacted with ABC solution (Vector Laboratories, Burlingame, CA, USA) for 30 minutes at RT, following the manufacturers' instruction. Immunoreactivity was visualized by diaminobenzidine (DAB; Vector Laboratories, Burlingame, CA, USA) reaction. After washing in distilled water, the tissues were coated with crystal mount (Fisher Scientific, Tustin, CA, USA) and air dried for several days. The primary antibody was omitted from the procedure as a negative control, which showed no labeling.

RNA isolation and polymerase chain reaction (PCR)

The SG tissue from E18, P0, P5, P10 and P20 animals were harvested in the same manner, as described above for explant culture, and immediately immersed and kept in RNA later (Qiagen, Valencia, CA, USA). Total RNA was isolated using a guanidine

thiocyanate/phenol/ chloroform-based extraction procedure (TRIzol Reagent; Invitrogen Life Technologies, Grand Island, NY, USA) according to the company's instructions. Reverse transcription was performed using Superscript First-Strand cDNA synthesis system for reverse transcription-polymerase chain reaction (RT-PCR) (Invitrogen Life Technologies, Grand Island, NY, USA) using oligo (dT) primers. Subsequent PCR amplification was performed using the Platinum Taq DNA Polymerase kit (Invitrogen Life Technologies, Grand Island, NY, USA) in a 50-μl solution, with 3 μl of the reverse-transcribed complementary DNA (cDNA), 1 × PCR buffer, 1.0-U Taq DNA polymerase, 200-nM dNTPs, 1.5-mM $MgCl_2$, 4% DMSO and 200-nM primers. Primer sequences for TrkB were sense: 5'-AGT CCA GAC ACT CAG GAT TTG TAC-3'; antisense: 5'-CTC CGT GTG ATT GGT AAC ATG-3'. Primer sequences for TrkC were sense: 5'-CAA CCA TGG CAT CAC TAC ACC-3'; antisense: 5'-TAA GAG GCT TGG AAT GTC CG-3'. Primer sequences for p75 were sense: 5'-GCA TAA GCC TGA AGC CAA CAC G-3'; antisense: 5'-CCC ACT CAT TCC AAC AGC AAG C-3'. Primer sequences for beta-actin were sense: 5'-GCT CGT CGT CGA CAA CGG CTC-3'; antisense: 5'-CAA ACA TGA TCT GGG TCA TCT TCT C-3'. The predicted fragment lengths of the PCR products were 522 base pairs (bp) for TrkB, 365 bp for TrkC, 583 bp for p75 and 353 bp for actin, respectively. The PCR reaction was performed on cDNA for 35 cycles at 94 °C for 15 seconds, 56 °C for 30 seconds and 72 °C for 1 minute with a final extension at 72 °C for 5 minutes. The omission of reverse transcriptase during reverse transcription served as a negative control to confirm the absence of genomic DNA contamination. Eight microliters of the reaction product were analyzed by agarose gel electrophoresis with ethidium bromide staining.

Image presentation and data analysis

Images of the immunostained explants were captured by a Spot digital camera (Diagnostic Instruments, Sterling Heights, MI, USA) attached to an inverted microscope (Olympus IX70) under brightfield illumination. Digital images were then assembled into panels using Adobe Photoshop 6.0 software (Adobe Systems Incorporated, San Jose, CA, USA). The images were not modified except for the minor adjustment of the brightness and contrast.

We used three parameters for evaluation of the effects of neurotrophins on SGNs: number of surviving neurons in each explant, number of neurites emanating from each explant and the length of neurite extension. We also evaluated the neurites visually for differences in other aspects of growth behavior.

To evaluate the SGN survival and the neurite number, each explant after immunostaining was examined

under an inverted microscope. The number of surviving neurons and the number of neurites were counted manually by adjusting the focus, if necessary, to assess the full depth of the explant. Our criteria of identifying surviving neurons were based on their immunostained morphology: (1) positivity for NF200 and (2) visible nucleus. The immunostained E18, P0 and P5 explants were sufficiently transparent that individual neurons could easily be identified and counted (Figure 1). For P10 and P20 explants, however, the tissue after immunostaining was not as transparent, and it was more difficult to count the surviving SGNs reliably. Therefore, we excluded these developmental ages from the analysis of SGN survival. Neurite number was determined by counting neurites emanating from each explant at its edge. Therefore, branching in more peripheral areas was not taken into account. Since we could not distinguish central axons from peripheral dendrites, we refer to them simply as neurites.

The magnitude of apoptosis in the culture was evaluated similarly by manually counting immunolabeled cells for active caspase-3 in each explant. This evaluation was performed only for E18, P0 and P5 cultures and could not be performed on P10 and P20 cultures because of the intransparency of older explants.

For the evaluation of neurite length extending from the explant, we used the five longest neurites from each explant for analysis. Using Spot advanced software (Diagnostic Instruments, Sterling Heights, MI, USA), each neurite was traced by drawing a line along the neurite, resulting in a series of short, linear segments. The lengths of these segments were then summed and calculated by the program. The selected neurites from all cultures in the same condition were pooled for analysis. When the explant had fewer than five neurites, all of the neurites were included in the analysis.

Statistical analysis

The data for each experimental condition were compiled from at least four independent experiments, each of which contained two to three explant cultures for each given age and concentration of neurotrophin. Results were presented as the mean ± standard error of all samples. For neurite length, the average value of all the neurite length selected for analysis in each condition was designated as the maximum neurite length (MNL). Statistical analyses for survival, neurite number and MNL were performed using one-way analysis of variance (ANOVA), followed by Dunnet post hoc test with correction for multiple tests, using Statview 5.0 software to compare each neurotrophin subgroup with the corresponding untreated control group. Differences associated with p values < 0.05 were considered as statistically significant.

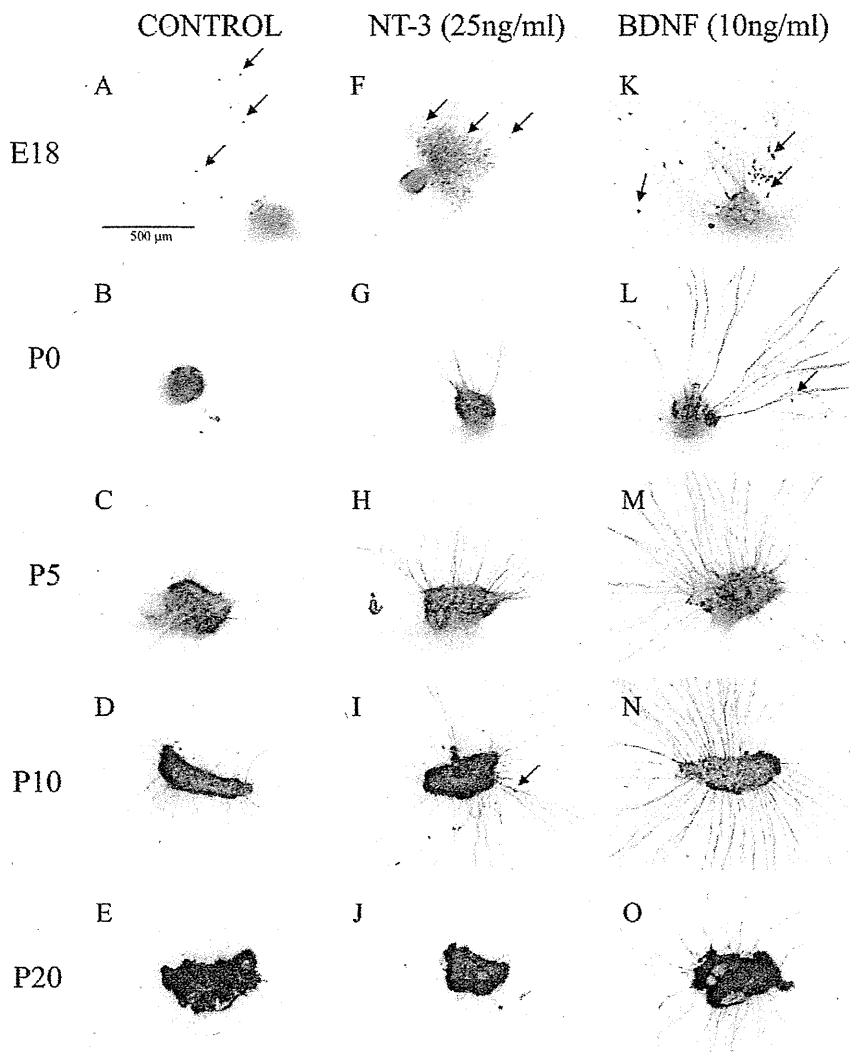CONTROL   NT-3 (25ng/ml)   BDNF (10ng/ml)

Figure 1.   Representative photomicrographs of SG explants from E18 to P20 cochlea. Each explant was cultured for 3 days in media without neurotrophin (A–E) or supplemented with either 25 ng/ml of NT-3 (F–J) or 10 ng/ml of BDNF (K–O), the lowest maximally effective dose of each factor. Explants were then fixed and immunostained with anti-NF200 antibody. In E18 cultures, large number of neurons migrated outside of the explants (arrows in A, F and K), whereas only a few such migrating neurons were observed at other ages (arrows in I and L). Neurites were thinnest at E18, thickest from P0–P10. Thickness was variable at P20. Neurite number and length declined significantly at P20. Responses to NT-3 and BDNF varied depending upon the ages of the culture, with NT-3 responses greatest at E18 (F) and BDNF dominant at later ages (L–O). Scale bar = 0.5 mm.

Results

General morphology of the rat SGN explants *in vitro*

Figure 1 illustrates the effects of NT-3 or BDNF on the appearance of SG explants at various ages. Generally, neurites extended from SG explants in a radial direction, as previously described [19,20]. In E18 explants, some neurons migrated from the explants (Figure 1A, F, K). Older SGNs exhibited little migratory capacity and remained inside the original explant (Figure 1B–E, G–J, L–O). We also observed an age-dependent difference in neurite thickness. E18 neurites appeared consistently thinner upon visualization (Figure 1A, F, K) when compared with P0, P5 and P10 neurites (Figure 1B–D, G–I, L–N). In contrast, P20 explants showed considerable variability in apparent neurite
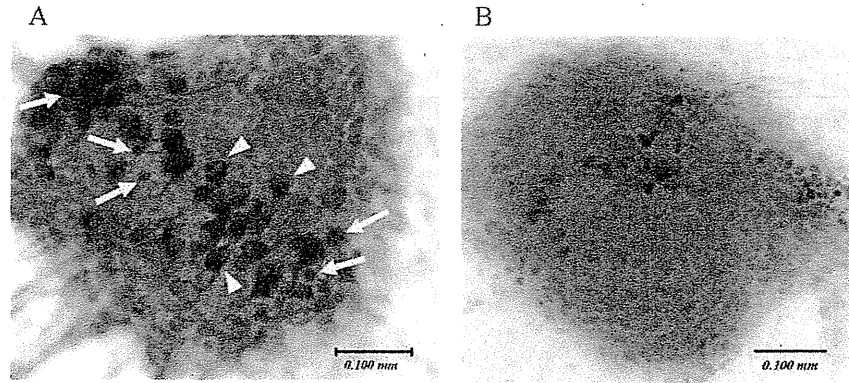
Figure 2. Degenerating neuronal cells in a P0 explant culture (A). The explant was maintained for 3 days in maintenance medium supplemented with BDNF (10 ng/ml), then fixed and immunostained with anti-NF200 antibody. Degenerating cells (white arrows) are small, exhibit condensed immunolabeling for NF200, lack neurites and do not show a clear contour of nucleus. Surviving SGNs (white arrowheads) show a clear, unstained nucleus and typically form neurites. Apoptosis in a P0 explant culture (B). The explant was immunostained with anti-active caspase-3 antibody. Scale bar = 0.1 mm.

thickness (Figure 1F, J, O). These age-related morphological differences in thickness did not appear to be affected by either neurotrophin.

An unusually large number of neurons showed morphological signs of apoptosis in P0 explants (Figure 2A). That is they showed anti-NF200 immunoreactivity, but the cell bodies were smaller and exhibited pyknotic nuclei. The average number of such cells per explant in P0 control explants (22.9 ± 7.7) was significantly greater than in E18 (11.7 ± 4.4, $p < 0.02$) or P5 (2.3 ± 0.6, $p < 0.01$) explants.

Effect of neurotrophins on neuronal survival

Survival of the SGN somata could readily be evaluated in E18, P0 and P5 explants. However, at older ages,

explant density precluded reliable visualization of cell bodies. A dose-response analysis of SGN survival, assessed by normalized SGN cell body counts (Figure 3), revealed that NT-3 exhibited a strong effect on neuronal survival at only E18. In contrast, BDNF supported survival at all the ages.

Caspase-3 activation in the explant cultures

Since morphology suggested some apoptosis, we immunostained E18, P0 and P5 control explants with anti-active caspase-3 antibody and counted the number of labeled cells in each explant (Figure 2B). The number of active caspase-3-positive cells/explant observed across all turns was 15.7 ± 1.6 at E18, 58.2 ± 13.0 at P0 and 38.7 ± 10.2 at P5. The number of labeled cells at P0
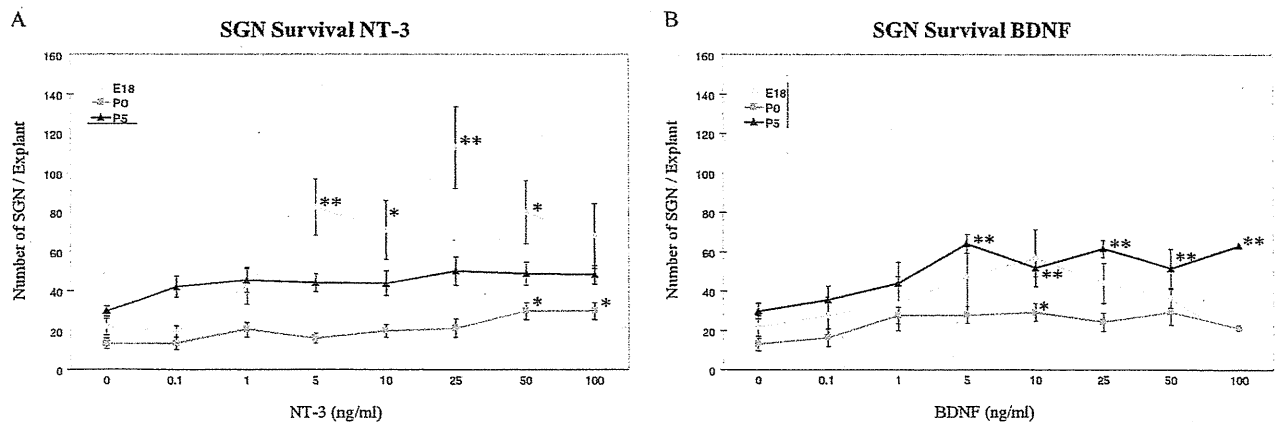

Figure 3. Dose-response effects of neurotrophins on SGN survival in E18, P0 and P5 explant cultures. Graphs show the average number of surviving SGNs observed after 3 days in maintenance medium with various concentrations of NT-3 (A) or BDNF (B). Error bars represent the standard error of the mean. Asterisks indicate a significant difference (*$p < 0.05$, **$p < 0.01$) compared with control culture (Dunnett post hoc test).

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A   SGN Neurite Number NT-3

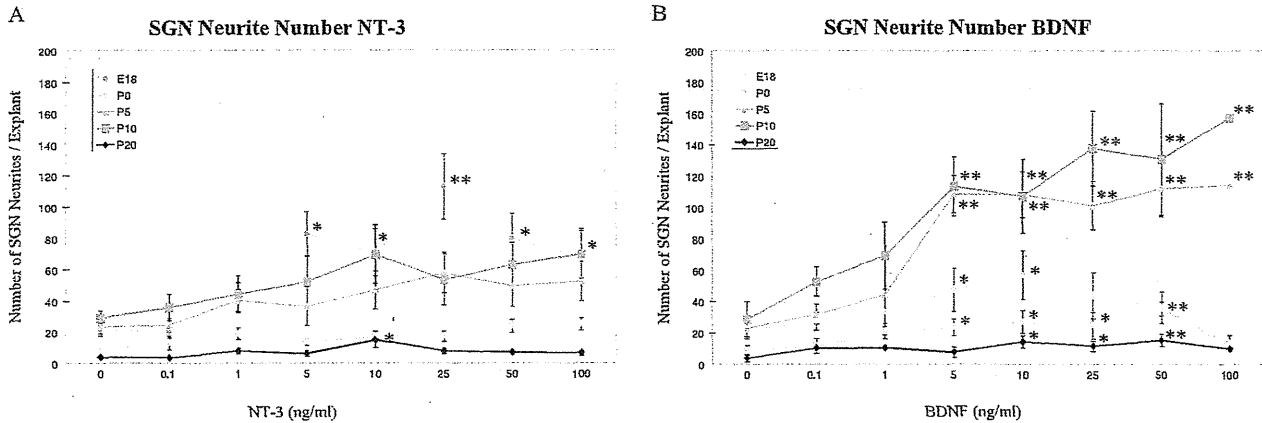**B**   SGN Neurite Number BDNF

Figure 4.   Dose-response effects of neurotrophins on the neurite number observed in SG explant cultures at E18, P0, P5, P10 and P20. Graphs show the average number of neurites emanating from each explant after 3 days in maintenance medium with various concentrations of NT-3 (A) or BDNF (B). Error bars represent the standard error of the mean. Asterisks indicate a significant difference (*p < 0.05, **P < 0.01) compared with the control culture (Dunnett *post hoc* test).

was significantly larger than the other ages (p < 0.01), representing ~10% of all neurons present.

When P0 explants were treated with NT-3, significantly fewer cells (33.7 ± 5.6) were caspase-positive (p < 0.03). However, although fewer cells were caspase-positive after BDNF treatment (38.8 ± 4.9), this difference was not statistically significant (p = 0.073). Neurotrophin treatment had no effect on caspase positivity for either E18 or P5 explants.

Effect of neurotrophins on neurite number

Quantitative analysis for the number of neurites extending from each explant also demonstrated clear

age-dependent differences (Figure 4). In untreated explants (neurotrophin concentration 0), the fewest neurites were observed at P0 and P20, and the most at P5 and P10. NT-3 had a large and significant effect on neurite number only at E18, although a more modest increase was seen at one NT-3 dose at P20. In contrast, BDNF increased neurite number at all ages, although it exhibited an especially strong effect at P5 and P10.

Effect of neurotrophins on neurite length

As shown in Figure 5, there was also a strong effect of age on neurite length. The shortest neurites were observed on untreated explants from P20 rats, and the
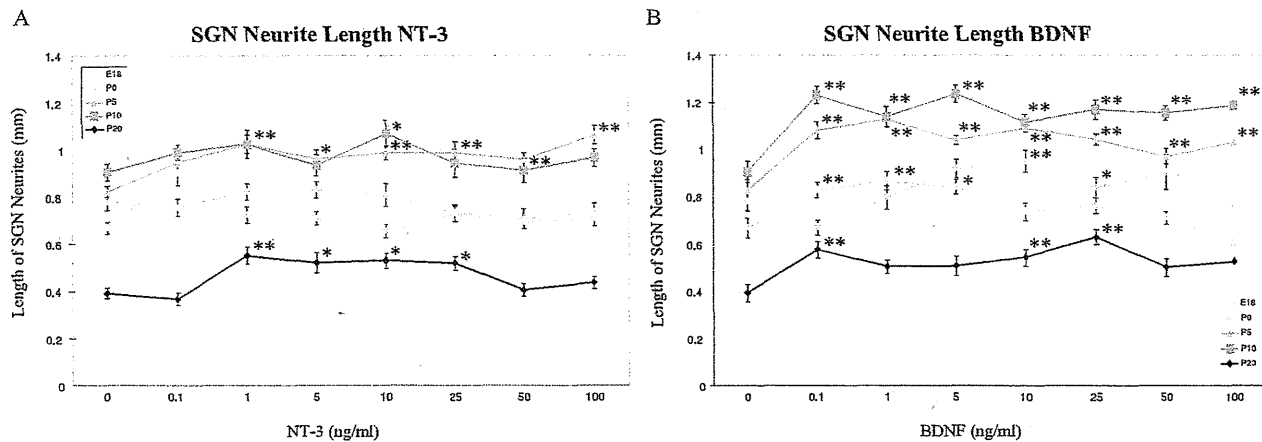
A   SGN Neurite Length NT-3

B   SGN Neurite Length BDNF

Figure 5.   Dose-response effects of neurotrophins on the neurite extention observed in SG explant cultures at P0, P5, P10 and P20. Graphs show the average length of longest neurites selected from each explant (5 neurites/explant) and pooled in the same culture condition. Each explant was cultured for 3 days in maintenance medium with various concentrations of NT-3 (A) or BDNF (B). Error bars represent the standard error of the mean. Asterisks indicate a significant difference (*p < 0.05, **p < 0.01) compared with the control culture (Dunnett *post hoc* test).
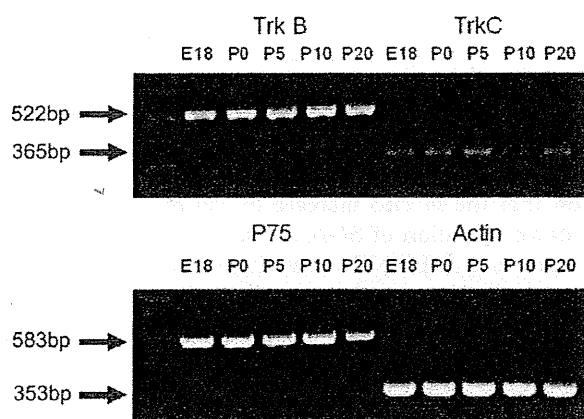
Figure 6. Expression of mRNA for TrkB, TrkC and p75 receptors in SG during rat development. Ethidium bromide-stained agarose gel demonstrating RT-PCR products for TrkB (522 bp), TrkC (365 bp) and p75 (583 bp) throughout the developmental period examined (E18–P20). DNA ladder and the expression of mRNA for actin (internal control) are also shown.

longest at P5 and P10. These age differences were generally maintained during neurotrophin treatment. However, NT-3 treatment produced a modest increase in neurite length at P5, P10 and P20, and no effect at E18 or P0. BDNF treatment produced moderate increases in neurite length at all ages tested, with the greatest effect at P5, P10 and P20.

Expression of neurotrophin receptors in developing SGNs determined by RT-PCR

We assessed the expression of messenger RNA (mRNA) for TrkC, TrkB and p75, the receptor proteins for NT-3 and BDNF, in SG across age using RT-PCR. As shown in Figure 6, we observed robust PCR products for the mRNA of each receptor throughout the developmental period analyzed. Assuming that the mRNA is translated, these data suggest that changes in Trk or p75 receptors may be unlikely to mediate changes in the responsiveness of SG explants. However, it must be noted that the PCR products potentially represent expression by both neuronal and nonneuronal elements within the ganglion. Therefore, variation in expression between neurons and other cell types is certainly possible.

Discussion

The aim of the present study was to investigate the age-dependence of SGN survival, neurite outgrowth and neurite extension regulation by NT-3 and BDNF. Our results demonstrate that both NT-3 and BDNF support these aspects of SGN development in a dose- and stage-

dependent manner and that the response pattern to neurotrophins varies between each of these parameters. The findings suggest that BDNF and NT-3 mediate separate ontogenetic events at different developmental stages.

A switch in neurotrophin dependence for SGN survival

We found that SGNs change their dependence for survival from NT-3 at E18 to BDNF at older ages. Although this report appears to be the first longitudinal study to show such a shift in mammals, it is consistent with previous reports from individual developmental stages. Pirvola et al. [21] observed greater survival-promoting effect of NT-3 over BDNF on rat embryonic (E13) cochleovestibular ganglion neurons, whereas others [22–24] reported greater BDNF dependence for postnatal SGNs. Developmental changes in neurotrophin dependence occur in other parts of the peripheral nervous system as well: trigeminal ganglion neurons switch from NT-3 and BDNF to NGF [15], whereas a subpopulation of DRG neurons switch from NGF to GDNF [16].

Our data are also similar to the developmental changes in the chick cochlea described by Avila et al. [17]. In their report, the chick cochlear neurons in culture predominantly depend on NT-3 for their survival in the early embryonic period. The response is maximum at E7 and decreased thereafter, being negligible from E13 to hatching. In contrast, the effect of BDNF for survival is more delayed and peaked at E9–E11 and although diminishing from then onward, remains in a significant range until hatching. This is roughly comparable with the timing of neurotrophin dependence in the rat, suggesting that age-dependent support of primary auditory neuron survival by NT-3 followed by BDNF may be a common molecular mechanism shared by birds and mammals.

Changes in survival response of SGNs to neurotrophins during development could be due to the selective death of a subset of neurons that respond preferentially to NT-3. For example, based on knockout mouse data, it has been suggested that developing type I SGNs depend preferentially upon NT-3 for their survival, whereas type II neurons depend upon BDNF [25]. Alternatively, our observation could be due to changes in the neurotrophin responsiveness of individual neurons. We do not have evidence to reach a definite conclusion regarding this point. Because rat SGNs upregulate peripherin in culture [26], we could not distinguish between type I and type II neurons. However, Mou et al. [27] found that the survival of dissociated postnatal (P1–P10) type I and type II mouse SGNs was preferentially enhanced by BDNF when compared with NT-3, in agreement with our results at

P5 and P10. With respect to other potential subtypes of SGNs, previous immunohistochemical examinations showed that all SGNs in both embryonic and postnatal mammals uniformly express both TrkB and TrkC receptors and that there appear to be no distinct subsets of neurons based on Trk expression [22,28]. This suggests that the overall population of SGNs may switch their dependence for survival from NT-3 to BDNF based on changes in the intracellular responses to TrkB and TrkC stimulation, rather than on receptor expression.

Effects of neurotrophin concentration

For each of the measures employed, a neurotrophin concentration effect was noted. This typically consisted of an increase in neuronal survival, neurite number or neurite length with increasing dose. In most cases, the response appeared to saturate at between 5 and 10 ng/ml. An exception was NT-3, where 25 ng/ml produced significantly greater SGN survival and neurite number than either lower or higher concentrations. The response saturation may reflect maximal utilization of all Trk receptors at relatively low concentrations. Another possibility is that neurotrophin receptors were downregulated at higher ligand concentrations, as has been observed in other systems (e.g. [29]). In this case, greater amounts of ligand may have been required to produce the same effect. In general, we did not observe systematic changes in threshold dose across age. When an effect was robust, it typically showed a similar minimal effective dose at all ages (e.g., Figure 4, BDNF; Figure 5, NT-3 and BDNF). Only when responses were minimal did we see variation in threshold dose. These data suggest that neurotrophin receptors are not expressed on SGNs gradually with age. Rather, age-dependent changes observed in some SGN responses may be related to changes in intracellular signaling in response to receptor activation.

A critical period of SGN death

Comparing the total number of rat SGNs/cochlea *in situ* (18 000–25 000; [4]) and the small numbers of surviving SGNs after 4 days *in vitro* in our culture system (20–120 neurons/explant in about 1/8 of the whole SG), considerable cell death occurred when SGNs were placed into explant cultures at any age. However, P0 cultures exhibited a significantly lower number of surviving SGNs, both in the presence or absence of neurotrophins than did either in E18 or P5 explants. P0 explants also exhibited significantly higher levels of caspase activity, indicating apoptosis. Interestingly, the dose response of SGNs to NT-3 for survival appeared to shift to higher concentrations, and the enhancement of survival at P0 was saturated at a lower magnitude (2.3-fold compared with control explants) than in E18 culture (more than

fivefold compared with control explants; Figure 3A). At the same time, BDNF influence on survival remains low (Figure 3B). It is significant that the period of culture for P0 ends at the time equivalent to P4 *in vivo*. This corresponds to the peak of naturally occurring cell death in the rat SG *in vivo* [4]. Our findings in culture suggest that the *in vivo* increase in cell death may reflect a downregulation of SGN sensitivity to NT-3, without an increase in BDNF sensitivity, resulting in neuronal apoptosis.

The expression of p75 by SG explants suggests another potential pathway for the regulation of cell death and survival. Stimulation of the p75 receptor by the proforms of neurotrophins is well known to mediate apoptosis, including in the SG [30,31] and has also been implicated in regulation of neurite length [32]. Since we applied mature neurotrophins to our cultures, this could not have resulted directly from our experimental manipulations. Although neurotrophins are not expressed in the SG of neonatal or adult rats *in vivo* [33], the potential for autocrine neurotrophic effects in SGNs [34] should be considered. The low level of survival of SGNs that we observed in untreated SGNs suggests that there is not extensive production of neurotrophins in our cultures. However, Zha et al. [35] have reported that neonatal SGNs can express neurotrophins, at least in culture. Thus neurotrophin genes expressed in their proforms could potentially mediate apoptosis *in vitro* via an autocrine process. Stimulation of p75 by mature neurotrophins is also well known to modulate the response of Trk receptors to mature neurotrophins [30]. Thus, changes in the interaction of p75 and Trk receptors across age could also contribute to altered responses to neurotrophins. On the basis of our PCR results, the expression of p75 mRNA did not appear to vary across age. Assuming that this mRNA was translated, any age-related changes would not be based on differential expression but could reflect downstream signaling changes.

The effects of neurotrophins on neurite number

Quantitative assessment of explants indicated a larger number of neurites in the presence of NT-3 than BDNF at E18 (Figure 1F and K, Figure 4). In contrast, at P0 neither neurotrophin had a strong effect. However, the number of neurites emanating from P5 and P10 SG explants was much more strongly enhanced by BDNF than NT-3. Thus, the effects of neurotrophins on neurite number resemble those observed for survival in E18, P0 and P5 cultures.

Our neurite outgrowth index (the ratio of neurite number/number of SGNs in each explant) demonstrated that none of the neurotrophin subgroups, either

for NT-3 or for BDNF, had significantly different ratios compared with the untreated control in P0 culture. In contrast, the BDNF subgroups at higher concentrations (10 ng/ml and 50 ng/ml) had significantly greater index values compared with the control group in P5 culture. This finding suggests that the modest increase in neurite number induced at P0 by neurotrophins reflects primarily an increase in surviving SGNs, whereas the effects on neurite number at P5 and P10 are mediated primarily by neuritogenesis.

Comparison of neurotrophic effects *in vitro* with the expression of neurotrophins and developmental events *in vivo*

The period of E18 culture (equivalent to E18–E21 *in vivo*), the earliest developmental stage we examined, corresponds to the period when the afferent fibers of SGNs reach the cochlear sensory epithelium [1,36]. By this stage, expression of both NT-3 and BDNF extends throughout the organ of Corti longitudinally [37], implying that both neurotrophins are available for all SGNs. NT-3 is more strongly expressed than BDNF [37–39] and distributed more widely in the sensory epithelium, since both hair cells and supporting cells express this neurotrophin, whereas BDNF is more restricted to hair cells [37,39]. This is consistent with our observation of greater enhancement of SGN survival by NT-3 than BDNF in E18 explants.

It should be noted, however, that there is a discrepancy between our result and the results of gene deletion studies [37]. Although mice null for the NT-3 gene show a considerably reduced number of SGNs in the basal cochlea (less than 20% of wild type) at birth [25,40], replacement of the NT-3-coding sequence with that for BDNF almost completely rescues the loss of basal turn SGNs (85%) by NT-3 absence [37,41]. Similar rescue in the number of SGNs has been demonstrated in mice for which the coding part of the BDNF gene was replaced with that of NT-3 [42]. These results suggest that NT-3 and BDNF can be functionally equivalent for the survival of SGNs prenatally [37,42]. The discrepancy between this finding and ours may be related to the mode of exposure of SGNs to neurotrophins: in the *in vivo* condition, neurotrophins are supplied to the SGNs basically through the targets of their neurites, and concentrations at these targets may be very high. In the *in vitro* condition, the entire SGN is exposed to the neurotrophins and concentration is uniform. The synergistic effects of neurotrophins with other survival factors *in vivo* must also be considered.

The period of P0 explant culture (equivalent to P0–P4 *in vivo*) corresponds not only to that of naturally occurring cell death [4,5] as mentioned above, but also to a relatively low level of responsiveness to NT-3

and BDNF with respect to survival, neurite number and neurite length. Interestingly, the expression of BDNF in the cochlear sensory epithelium, which disappears in the early postnatal period [10,39], reappears at P6–P7 in hair cells and supporting cells [10], which is temporally coincident with strong effects of BDNF, which we observed on survival, neurite number and neurite extension. SGNs may, therefore, tailor their responsiveness to coincide with developmental trends in neurotrophin availability.

P20 cultures, the oldest stage we examined, correspond to P20–P24 *in vivo*. Almost all of the major developmental events for SGNs are complete and hearing function has matured by this age [1]. Although the intrinsic capacity for neurite growth has considerably declined in this period, SGNs still retained the capacity to respond to both NT-3 and BDNF. This finding is consistent with the enhancement of survival and neurite regrowth of adult SGNs by neurotrophins *in vivo* [43–46]. NT-3 is highly expressed in inner hair cells and their supporting cells [36,39,47,48], so responsiveness to this neurotrophin is not surprising. However, BDNF expression is almost absent in the target field of adult SGNs [10,33,39]. Moreover, BDNF expression is observed in SGNs themselves [49] and may be acting in an autocrine manner [49].

Mechanisms for differential regulation by neurotrophins

Our RT-PCR results demonstrate that three kinds of neurotrophin receptors, TrkC, TrkB and p75, all of the neurotrophin receptors involved in the signaling of NT-3 and BDNF, are expressed in SG throughout the developmental period we examined. These data are in good agreement with previous immunohistochemical and *in situ* hybridization studies [9,22,28,33,37,47].

Our results appear in line with the results of transgenic mouse studies in which the coding part of the NT-3 gene is replaced by BDNF [37,50] and *vice versa* [42]. These studies suggest that although NT-3 and BDNF can be functionally equivalent for the survival of SGNs prenatally [37,42], they have distinct roles for the axon guidance and innervations in the cochlea [50]. These findings cannot be explained simply by the expression pattern of each neurotrophin receptor. As noted above, the biological responses to neurotrophins are presumably regulated by molecular cascades downstream of Trk/p75 signaling. It has been shown that multiple signal transduction pathways are involved in neurotrophin-mediated biological effects on neurons, which may contribute to age-dependent differential regulation of SGN biological responses to neurotrophins.

Conclusion

The present study demonstrates that neurotrophins regulate developing rat SGNs in an age-dependent manner. The temporal patterns of responsiveness of SGNs to NT-3 and BDNF presented here correspond well to the expression pattern of the two neurotrophins in cochlear sensory epithelium *in vivo* and also correlate with the time course of developmental events in the SG, such as neuronal cell death and the remodeling of afferent innervation. Our data, therefore, suggest multiple, age-specific roles for NT-3 and BDNF in the ontogeny of cochlear innervation.

Declaration of interest

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総　説

聴覚に関わる社会医学的諸問題
「加齢に伴う聴覚障害」

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要旨：加齢に伴う聴覚障害では，末梢聴覚，中枢聴覚，認知の三つの機能が複合的に障害されている。老人性難聴では聴力は高音域から閾値上昇し，難聴の進行は年と共に加速し，個人差が大きいことが知られる。語音明瞭度は聴覚レベルに応じて悪化するが，高齢になるほど聴力レベルよりも悪化する傾向にある。耳音響放射や聴性脳幹反応は主に聴力レベルに応じて障害されるが，年齢自体の影響も見られる。Gap detection などで評価できる時間分解能も加齢により悪化する。難聴のために日常生活上の会話に不自由を感じる場合には補聴器装用が治療の第一選択となる。補聴効果が無くなった場合は人工内耳が高齢者においても有用であるが，装用開始年齢が高齢であるほど術後の聴取成績が悪い傾向にある。加齢に伴う聴覚障害に対しては不要な強大音曝露の回避や動脈硬化の予防や治療などが有用と考えられる。また聴覚に基づく認知訓練が時間分解能の改善に役立つ可能性も示唆されている。

ーキーワードー

老人性難聴，聴覚検査，人工内耳，時間分解能

はじめに

　老人性難聴は加齢に伴い生じる聴覚障害の総称であり，純音聴力閾値の低下，（特に騒音下での）語音聴取能の低下，聴覚情報の中枢処理遅延，音源認知の障害などが知られる。この結果，日常会話，音楽聴取，社会生活活動などにおいて困難を感じることになる。

　加齢に伴う聴覚障害には，大きく分けて三つの要素，すなわち①末梢聴覚の機能低下，②中枢聴覚の機能低下，③認知機能全般の低下，が関与している。高齢者が聞き取りの障害を訴える場合にはこれら末梢・中枢・認知の三機能が複合的に障害されていると考える必要がある[1]。日常診療でよく耳にする「雑音の中でうまく会話が聞き取れない」という訴えは，若年の内耳性難聴症例でも同様に雑音下聴取が障害されることから，末梢聴覚の機能低下が主に影響していると考えられる。一方，空間聴や音源分離の機能が脳幹にあることから中枢聴覚の障害も（雑音下）聴取を低下させる原因となる。また認知機能の低下も音源への注意に影響し，雑音下聴取がより困難になると推測されている。実際，難聴者や雑音下聴取の悪い被験者は認知機能が低いとの報告も存在している[2]。また逆に難聴の程度と認知症の相関[3,4]も認められ，うつ[5]，社会的孤立，低い自己評価[6]との相関も報告されている。

　加齢による聴覚障害の評価においては，蝸牛障害を少なからず伴うため，聴覚中枢だけを評価することは困難とされている。本稿ではこの限界を考慮した上で，加齢に伴う聴覚障害につき，老人性難聴を中心にレビューする。

疫学およびリスク因子

　2003年JAMAに掲載されたYuehら[7]の総説では，65歳以上の25～40％，75歳以上の40～66％，85歳以上の80％以上において，加齢に伴う難聴があると推定されている。韓国の2000年の報告[8]では，500，1,000，2,000，4,000Hzの6分法平均気導聴力レベルが27dBHL以上の割合は65歳以上で37.8％，41dBHL以上は8.3％である。内田ら[9]は「国立長寿医療センター　老化に関する長期縦断疫学研究（NILS-LSA）」データを検討し，WHOの聴力障害基準に従い，500，1,000，2,000，4,000Hzの会話音域4周波数平均気導聴力レベルを基準とした良聴耳聴力レベルが25dBHLを超えた場合を「難聴あり」として，第6次調査（2008-2010年）参加者の難聴有病率を集計した。その結果を図1に示すが，難聴有病率は60～64歳までは徐々に増加し，65歳以上で急速に増加する傾向が見て取れる。なおいずれの年代においても男性の有病率が女性より高いが，有意差は65～69歳でのみ見られている。この結果をもとに65歳以上の全国難聴有病者数を推計すると1655万程度であり，また「耳疾患の既往なし」「職場騒音の就労歴なし」と答えた者のみの集計結果から計算すると1569万人超と推計されるという。この数は膨大であり，加齢に伴う難聴にどのように対処していくかが国民的課題である事が認識される。

　一方，この高い難聴の頻度は先進国にのみ見られる可能性も高い。Rosenら[10]はスーダンに住むMabaan族の聴力検査を行い，高齢に至るまで聴力が明らかに良く保たれていることを報告している。この地域は特に静かな場所であり，人種の差（肌色の差）や遺伝的素因の影響もあると思われるが，先進国における老人性難聴では純粋な老化に加え，環境要因が大きく影響していることが示唆される。

　一般に老人性難聴の発症や程度に影響する因子としては，遺伝要因のほか，人種差，騒音曝露歴，喫煙，飲酒，糖尿病・循環器疾患等の合併，性ホルモンなどが挙げられている[11]。遺伝的要因の関与は0.35-0.55と推定されており，また黒人の方が白人より難聴の程度が軽い事も知られている[12]。NILS-LSAにおいては，動脈硬化や肥満に関与する遺伝子多型の関与が示唆されている[13]。遺伝子多型ではエンドセリン1，脂肪酸結合蛋白2，ミトコンドリア脱共役蛋白2などが報告されている[12,14]が，その多くが動脈硬化や酸化ストレスと関与するものであることは興味深い。遺伝外要因については，NILS-LSAでは騒音暴露歴，内頸・腎動脈の動脈硬化，糖尿病などの関与が報告されている[13]。文献的には，高血圧，心血管疾患，脳血管疾患，喫煙，糖尿病，騒音曝露等との相関が報告されている[12]。

加齢に伴う聴覚変化の特徴

1）純音聴力検査

　多数例を用いて加齢に伴う純音聴力閾値の変化を調べた報告はいくつかある[15-18]が，ほぼ同様の傾向である。図2に立木ら[19]の検討における，30歳代から5歳ごとの年齢別聴力（平均）を示す。この図からわかる特徴は二つある。一つは聴力が初期には高

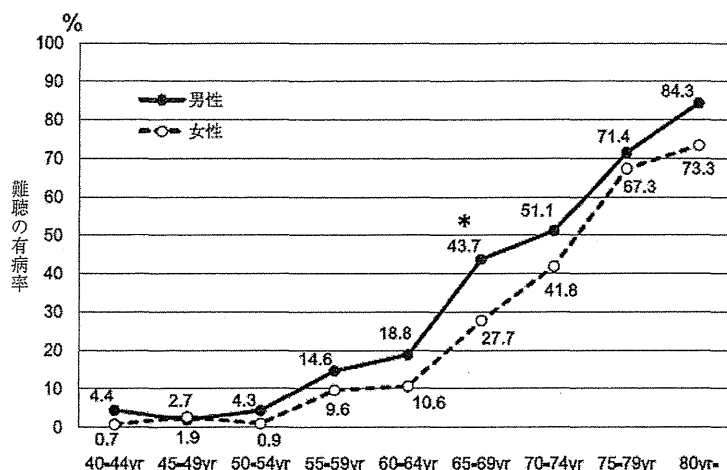

図1　NILS-LSAにおける難聴の有病率　＊：p＜0.05（文献9より改変）

周波数域から障害され，難聴が進行するにつれ低音から中音域まで障害されることである。二つめは聴力の進行が年をとるほど加速する傾向にあることであり，その傾向は高周波数ほど明らかである。またこの他に三つめの特徴として難聴に個人差が大きいことも挙げられる。さらに男女差が存在する事も良く知られている。図3に男性と女性の年齢別平均聴力を示す[17]。同年齢では男性が女性よりも聴力が悪い傾向にある[9,17]が，最近では以前の報告より男女差は縮まりつつあるとされ[15]，男女の生活様式の近似化などの影響も示唆される。

2）語音明瞭度

本邦において語音明瞭度と年齢の関係を調べた報告，特に感音難聴者を対象とした報告は多くない。下田[18]は65歳以上の360耳（65-69歳160耳，70-74歳92耳，75-79歳64耳，80歳以上44耳）の語音弁別能について，57S語表を用いて1000Hz純音のMCLレベルで聴取した時の語音明瞭度として調べた。これら4年代群では平均聴力は年齢とともに低下し，語音弁別能もそれぞれ81.0％，71.3％，67.2％，55.9％であった。すべての群で平均聴力レベルと語音弁別能に有意な相関がみられたが，若年齢群の高音漸傾型感音難聴者120例と比較すると聴力閾値上昇に伴う語音弁別能の悪化がより顕著であった。すなわち，語音明瞭度は聴力閾値上昇に伴って悪化するが，さらに年齢の要素も加わるという結果であった。下田[18]は，方向感も加齢に伴い悪化するが，方向感の成績と語音弁別能は従属事象であると報告している。前田ら[20]も感音難聴者329例の平均聴力レベルと語音明瞭度の関係を年齢別に検討し，平均聴力レベルが30-60dBHL台では年齢が上昇すると明瞭度が低下する傾向にあり，30，40，50dB台では70歳代と80歳代で有意差があり，60dBHL台では60歳代と70歳代で有意差があったと報告している（図
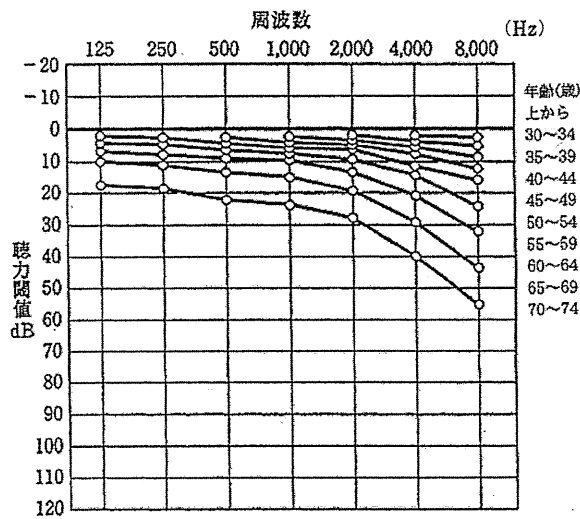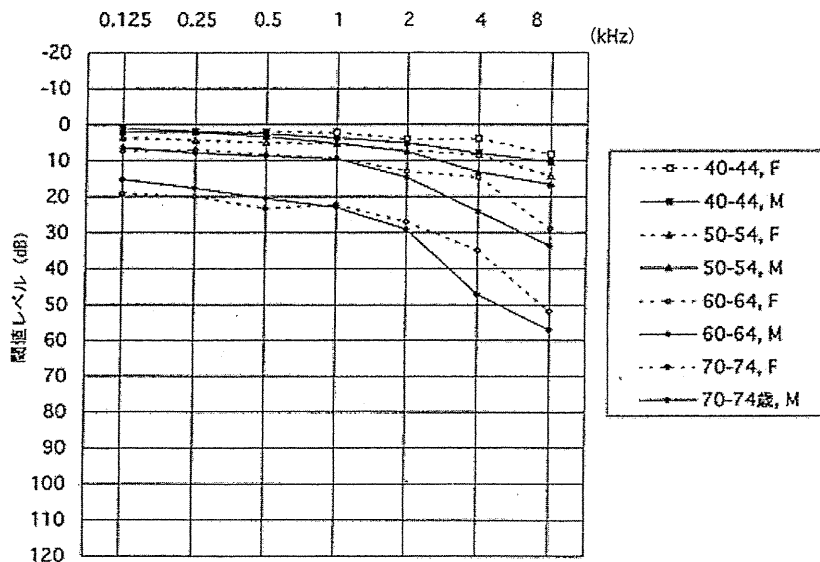

図2　年齢ごとの平均聴力（文献19より改変）

図3　加齢による聴力の変化の男女の違い（文献17より改変）
どの年齢群でも男性の方が女性より聴力が悪い傾向にあるが，その差は大きくない。

騒音下において語音聴取能が悪化することはよく知られた現象であり，海外では多くの研究があるが，本邦での報告は少ない。廣田ら[21]は平均聴力レベル40dBHL以下の高齢者48例（60，70，80歳台がそれぞれ16例）と聴力正常成人（20-22歳）で騒音下の聴取能を調べた。この対象では，67S語表の語音弁別能に若年者と高齢者の差は少なかったが，最高語音明瞭度条件で騒音を負荷すると，高齢者は若年者に比べ，より少ない音圧でのスピーチノイズ負荷で明瞭度が低下し，最高語音明瞭度より20-25dB低い音圧条件での語音明瞭度では騒音負荷による明瞭度低下はより顕著であった。Frisnaら[22]は若年（18-39歳）正常聴力10例，高齢（60-81歳）40例（正常聴力10例，高音域難聴30例（聴力閾値の上昇程度により10例ごと3群にさらに分類））を対象に，spondee wordと2種類のtarget word（それが推測されやすい文章と推測されにくい文章の中の2種類）を聞かせ，speech reception threshold（SRT）を求めた。なおこの対象者では単語聴取は全例96-100％の正答であった。その結果，SRTは聴力正常者では若年と高齢者で差はなかったが，高齢者では高音域の閾値が上昇するとSRTも上昇した。さらにSRTの30dB上の音圧での聴取成績が50％になるmulti-talker noiseの音圧を求めたところ，正常聴力者では若年者が高齢者よりもS/N比が低く，年齢が騒音下の聴取に影響することが示された。高齢者の群間比較では高音域の聴力閾値が上昇するほどS/N比が増加し，特に推測しにくい文章中のtarget wordでその傾向が明らかであった。この結果は年齢の他に聴力閾値上昇も騒音下の聴取に影響することを示唆している。

3) 耳音響放射

加齢により耳音響放射に影響が出る事はよく知られ，自発耳音響放射（spontaneous otoacoustic emissions（SOAE））の出現率も加齢とともに減少する。例えばMazelováら[23]は高音漸傾型感音難聴を示す高齢者30例（67-93歳）と若年30例（19-27歳）を比較したところ，若年では53％の耳にSOAEがみられたが，高齢者では3耳のみ（5％）であったとしている。
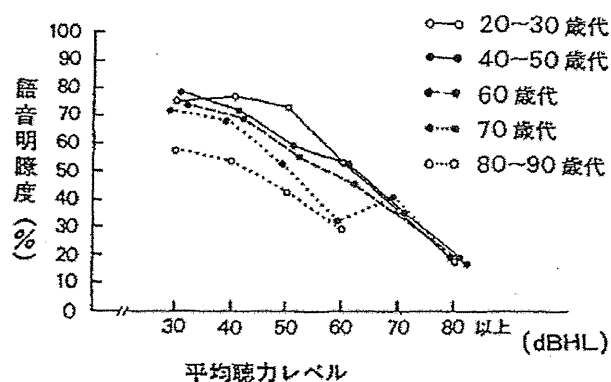

図4　年代別における平均聴力レベルと語音明瞭度の関係（文献20から改変）
70歳以上の群は若年齢群と比較すると平均聴力に比べて語音明瞭度が悪い傾向にある。

誘発耳音響放射（transitory evoked otoacoustic emissions（TEOAE））についても加齢とともに反応レベルが低下し，検出率が下がることが知られている。上述のMazelováら[23]の検討ではreproducibilityが60％以上を反応ありとすると，若年者の97％で反応が得られたのに対し，高齢者では55％であり，TEOAEレベルも高齢者で有意に減少していた。同様の報告は他にも多く，例えばBonfilsら[24]は60歳までは100％でTEOAEは記録できたがそれ以降は35％であったと報告している。本邦でも同様の傾向が報告されており，例えば大内ら[25]は125Hz〜8kHzの7周波数の聴力レベルがすべて30dBHL以内の41名52耳（6〜73歳：平均40.0歳）を対象として，500Hz〜4kHzのトーンバースト刺激によるTEOAEに対する加齢の影響を検討したところ，TEOAEの最大振幅に対する加齢の明らかな影響は認められないが，加齢に伴い見かけ上の閾値は明らかに上昇すると報告している。

歪成分耳音響放射（distortion product otoacoustic emissions（DPOAE））も加齢により反応が低下することが知られている[23,26]。高橋ら[27]は10歳から69歳までの32名58耳でDPOAEを解析（f2/f1=1.2，P1=P2）し，60歳代では70dBSPLなどの高い入力音圧では良好に検出されるが，低い入力音圧ではノイズレベルまで低下すること，純音聴力レベルでは有意差が無い場合でも10歳代と50歳，60歳代との間でDPOAE検出閾値に有意な差があることを報告している。

耳音響放射の反応低下には年齢自体が影響するのか，加齢に伴う聴力閾値の上昇が影響するのか，議論のあるところである。これまでの多くの報告ではその聴力が若年者と差が無いとされている高齢者でもわずかながら難聴が存在していることや報告の症例数が少ないことが結果や解釈の違いに影響している。例えばTEOAEに関する71耳（正常聴力47耳，老人性難聴24耳）を検討した最近の報告では，TEOAEは年齢より相関が強いが，聴力レベルも一定の影響をするとされている[28]。しかし多数例の報告では年齢自体より，聴力閾値の影響が大きいとされている。Clientoら[26]はFramingham cohortに参加した486例（男性209例36-82歳，女性277例31-80歳）を調べ，DPOAEの振幅低下は年齢より聴力閾値に有意に相関すると報告した。Hothら[29]は通常臨床でのさらに多数例（0歳から90歳までの5142名）のデータを用い，聴力，TEOAE，DPOAEの得られた5424耳を解析した。1-4kHzの聴力閾値が10dBHL以下の群と年齢相当に閾値上昇した群に分けたところ，どちらの群でもOAEの振幅は年齢とともに悪化したが，聴力正常群では3，4kHzでのDPOAEでのみ反応低下が明らかであったのに対し，難聴群ではTEOAEの振幅低下は明らかで，DPOAEは全周波数で反応が低下し高周波数ほど顕著であったと報告している。これら多数例の検討結果から，少なくともDPOAEにおける高齢者の反応低下は年齢よりも聴力閾値上昇を主に反映することが示唆される。

4) 聴性脳幹反応

加齢の聴性脳幹反応（auditory brainstem response（ABR））に対する影響は30年ほど前を中心に多くの検討がなされたが，その結果は方法や対象によりやや異なっている[30]。ABRにおける閾値は聴力閾値の上昇により上昇するが，両者の差は若年者に比べ高齢者で大きいと報告されている。例えば1，2，4kHzのトーンピップを用いた場合の差は若年者では12，7.5，8dBであったのに対し高齢者では17.5，18，21dBであったという[30]。この理由としてラセン神経節細胞数の低下や神経興奮同期性の低下が考えられている。また各波の振幅についても高齢者で低下するという報告が多い[30]。

各波の絶対潜時については高齢者で延長する傾向にあるという報告が多い。一方波間潜時については報告間に差が大きい。若年者に比べて高齢者ではV波潜時もI-V波間潜時も延長するという報告[31,32]もあるが，波間潜時は延長しないという報告も多い[30,33]。横小路と加我[34]は難聴を訴える70歳以上の高齢者47人94耳を検討し，I-V波間潜時が延長していたのは5耳（5.3%）であったと報告している。また波間潜時の延長があるという報告においても，それが年齢によるものか聴力閾値の上昇によるものか，異なった意見が多い（詳細はBoetther[30]の総説を参照されたい）。

5) Temporal Gap Detection 検査等

高齢者では通常末梢聴覚障害を伴うため中枢聴覚機能のみを評価するのが難しい。上述した日常臨床で用いられている聴覚検査では高齢者の聴覚中枢のみを評価することはできない。主に蝸牛の基底板振動に基づくと考えられる聴覚の周波数分解能とは異なり，聴覚の時間分解能は蝸牛神経以降の中枢機能に基づいていると考えられており，聴覚心理学実験では末梢機能に関わらず聴覚中枢の時間情報処理能は加齢に伴い低下し，それが語音明瞭度に影響するとされている。一般的な検査ではないが，両耳間時間差（ITD），Binaural Masking Level Difference（BMLD），Temporal Gap Detection（GD），Voice Onset Time（VOT）などによる評価で時間分解能の障害傾向が報告されている[35]。前二者は両耳の時間情報比較，後二者は単耳の時間分解能であり，BMLDとVOTはspeechを，ITDとGDはnon-speechを見ているが，高齢者では感音難聴の有無によらず，これらすべてが障害されるとされる。なお単耳の時間分解能の低下は語音聴取低下の原因の一つになるが，non-speechとspeechの時間分解能低下には異なるメカニズムが存在すると考えられている。

GD検査は，広帯域雑音の中間に時間的な無音区間を挿入して，この無音区間（Temporal Gap）が検知できるかどうかを計測する，非語音を用いた聴覚心理学的なタスクであり，正常若年者では（条件にもよるが）通常3-5ms程度の短いTemporal Gapまで検知できる。Mazelováら[23]は高音漸傾型感音

難聴を示す高齢者では検出時間が長く，SRT と相関するとした。Moore ら[36]は，純音聴力検査上閾値上昇のない高齢者と軽度閾値上昇のある高齢者のGD 検査を施行し，閾値上昇のない高齢者においても若年者より検出閾値の増加がみられ，軽度難聴の高齢者と差がなかったと報告している。このように純音聴力検査閾値の上昇がほとんどない高齢者を用いることにより，末梢聴覚の影響を（ある程度）除いて中枢の影響を評価する手法は聴覚心理学的によく行われており，他の研究[35]でも GD 閾値の増大が報告されていることから，末梢聴覚の障害の有無に関わらず，中枢聴覚における時間分解能は加齢とともに低下していると考えられる。この GD 閾値の上昇がどのように高齢者の語音聴取能に関わっているかは明らかではないが，雑音下での聞き取りにおいて，雑音レベルが短い瞬間での語音聴取（hearing in temporal dip）の低下に関わっていることが示唆されている[36]。

GD 以外の聴覚心理学的タスクでも加齢変化が検討されている。例えば非語音タスクでは，刺激音の長さの弁別や両耳間時間差による方向感の認知が加齢性に低下することが確認されており，語音タスクでは VOT の実験で高齢者は感度が低いことが報告されている。子音 [p] と子音 [b] は唇の閉鎖が開放された瞬間から母音が発生されるまでの時間VOT が長いか短いかによって違いが聴き分けられているが，高齢者ではたとえオージオグラムが正常に近くてもこのような破裂音・破擦音を VOT で聞き分ける感度が低くなり，パ行 [p] とバ行 [b]の聴き違えがおこると説明されている[35]。

Gap 検知は蝸牛神経の発火頻度の増減で示されるミリセカンド単位の時間分解能を反映していると考えられている[37]が，聴覚中枢は蝸牛神経の発火のマイクロセカンド単位の位相差（時間差）をとらえて処理する能力があり，たとえば両耳間時間差検知（interaural time difference, ITD）は右聴覚路と左聴覚路でのわずかな位相のずれを聴覚中枢（上オリーブ複合体）が検知することで音の方向感を生成している[37]。これらのマイクロセカンド単位の位相（時間）の差は，temporal fine structure（TFS, 時間微細構造）とよばれ，ヒトや動物における TFS の聴覚処理能力について様々な検討が繰り返されてい

る。我々はこの TFS を手掛かりにピッチ感覚を引き起こすといわれる音刺激を用いて，前期高齢者群において ITD 検知閾および TFS 検知閾の低下を比較したところ，両耳の TFS を手掛かりとして方向覚をひきおこす ITD 検知閾は有意な低下を示さなかったのに対し，片耳の TFS を手掛かりにピッチ知覚をひきおこす TFS 検知閾値が有意に低下していた（Ochi et al, 投稿中）（図 5）。後期高齢者では ITD 検知閾も有意に低下すると言われており[38]，TFS の時間処理能の加齢に伴う低下はより複雑な形で起こることが示唆される。

6）人工内耳聴取成績

一般に高齢者においても人工内耳装用により良好な聴取成績が得られると報告されている。しかし多数例での検討では加齢による影響が見られている。Lenarz ら[39]は後天性難聴1,005例の成人を18～39歳（220例），40～59歳（420例），60～69歳（235例），70歳以上（130例）に分け，聴取成績を調べた。なおこれらの群間には術前聴力レベルと失聴期間に差は無かった。その結果，人工内耳装用後の2年間の聴取成績の向上は，70歳以上の高齢者もその他の年齢群と同様であり，静寂下での単音節や文章聴取にも
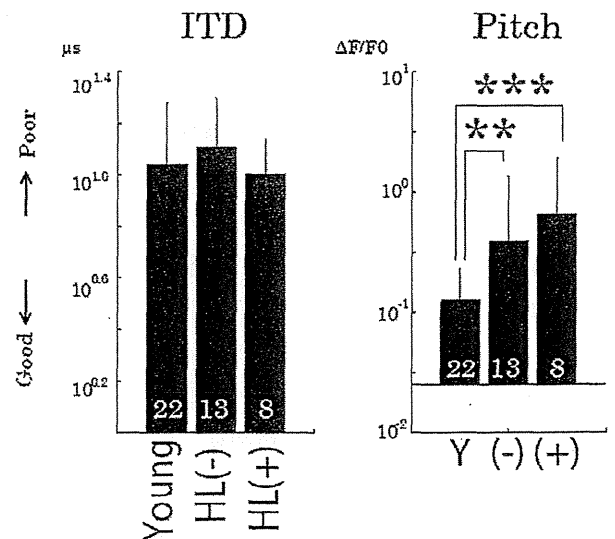

図5　若年（Young）群，高齢で難聴のない群（HL－），高齢で軽度感音難聴群（HL＋）群における両耳間時差検知（ITD）とピッチ感覚
ITD タスクでは各群に差がないが，ピッチ感覚タスクでは有意差が見られる。
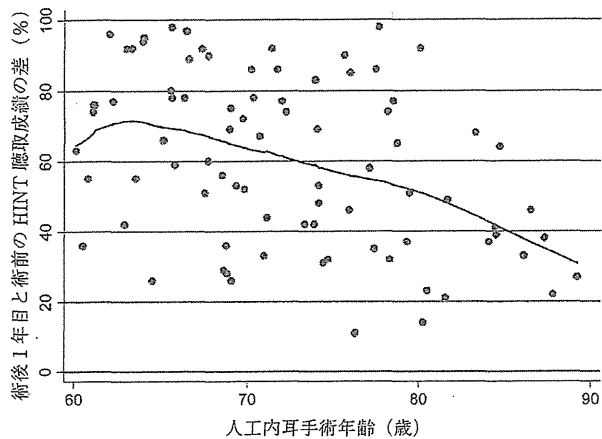** : p<0.01, *** : p<0.001

図6　人工内耳手術後1年目と術前における HINT 聴取成
　　績の差と手術年齢との関係（文献40より改変）
　　手術年齢が高くなるほど術後の HINT 聴取成績の改
　　善が少ない傾向にある。

差は無かったが，騒音負荷条件（S/N 比＋10dB）
での文章聴取は70歳以上の高齢者群で有意に悪化し
ていた。Lin ら[40] は60歳以上で人工内耳手術を受け
た445例中，術前と手術1年後に同じ条件で HINT
（hearing in noise test）を受けた83例を解析したと
ころ，高齢者になるにつれて術後の成績が悪いこと
（図6），手術前の聴取能が良いほど（40％以下の症
例に対して40-60％の症例において）術後聴取成績
が良いことを示した。これらの結果は，高齢人工内
耳装用者の騒音下での語音聴取には年齢の影響は無
視できないほど大きいことを示しており，高齢者の
騒音下の語音聴取において後迷路・中枢聴覚の変化
や注意・記憶と言った認知機能低下の影響が大きい
事を示唆している。

　なお高齢の人工内耳装用者において，装用年数が
増すにつれ，すなわちより高齢になるにつれて聴取
成績が低下するのかよくわかっていない。Dillon
ら[41] は65歳以上で人工内耳手術を受け10年以上装用
した14例につき，静寂下と騒音負荷での単語聴取成
績を調べたところ，装用開始1年から5年の間に聴
取成績はさらに向上し，5年から10年の間は安定し
ていたと報告している。この結果は高齢者において
も術後の装用経験により聴取成績は向上し，加齢に
よる学習効果に対する悪影響は無い事を示唆してい
る。ただし，さらに高齢になると聴取能が低下して
くるかどうかについては今後検討が必要である。

老人性難聴の予防と対策

　ヒトの老人性難聴の発症機序は動物とは必ずしも
同じでないと考えられるが，疫学データや動物実験
の結果からは，加齢に伴う蝸牛内の酸化ストレスに
よりミトコンドリア DNA 変異が蓄積し，またミト
コンドリア機能も悪化し，その結果有毛細胞，ラセ
ン神経節細胞，血管条など聴覚機能に重要な細胞が
障害を受けて難聴が進行性に生じると考えられてい
る[12]。この仮説に従うと，蝸牛内における過剰な
free radical 産生の予防が重要ということになり，
不必要な強大音への暴露を若いうちから極力避ける
ことが勧められる。また騒音環境では蝸牛の酸素消
費，すなわち血流の増加が必要となるが，動脈硬化
があると十分な血流供給が障害されて相対的虚血，
となり，さらに騒音曝露終了後の再灌流による free
radical の過剰産生を引き起こしやすいと考えられ
る。この意味から動脈硬化を予防することも重要と
考えられ，生活習慣（食事，塩分，運動，禁煙など）
に対する指導も必要である。高脂血症，糖尿病，高
血圧症などを持病に持つ患者またはその予備軍では
内科的治療などの早期介入も勧められる。free radi-
cal を除去するサプリメント，特にミトコンドリア
内で作用するものの摂取も予防効果が期待されてい
るが，ヒトにおける老人性難聴予防効果のエビデン
スはまだ無く，その検証は今後の課題である[12]。な
おこれらの予防的対応には難聴の進行を遅らせると
いう意味もあり，難聴が生じた後であっても一定の
効果が期待される。

　老人性難聴が生じて生活上の不具合を感じる場
合，すなわちコミュニケーション障害が生じる場
合，補聴器の活用が勧められる。アメリカの退役軍
人を対象として Hearing Handicap Inventory in the
Elderly（HHIE）や Geriatric Depression Scale など
を用いて補聴器装用後1年間の経過を見た検討で
は，補聴器装用は認知機能，社会性，感情，鬱傾向，
コミュニケーションに明らかに有益であったと報告
されている[42]。横断研究である Blue Mountains Eye
Study に参加した60歳以上1328名の検討でも，両側
軽度難聴（500～4000Hz の平均が 25-40dBHL）者
で鬱の傾向が強く，1日1時間以上補聴器を装用す
る者では有意に鬱傾向は抑制されていると報告され

ている[43]。このように補聴器の有用性については言うまでもないが，特に「騒音下での聞き取りが悪い」ということに配慮し，ノイズリダクション機能を活用することが重要である。なお補聴器を両側に装用するか一側にするかという点について，一般的には両耳聴効果や騒音下聴取の改善を考慮して両耳装用が有利と思われるが，一側装用の方が聞き取りやすいという患者にも時に遭遇する[44]。Henkin ら[45]は28例（平均72.8歳）の軽度から高度の両側感音難聴者に対し，右耳装用，左耳装用，両耳装用の三条件において，背後から与えた騒音（S/N 比＋10dB）下で正面から 70dBSPL で与えた単語の聴取成績を評価したところ，71％の患者で両耳装用よりも片耳装用において成績が良かったと報告している。このように日常生活場面を想定した研究は本邦でも積極的に行う必要があろう。

　会話においては時間分解能が障害されていることを念頭に置き，「ゆっくりと話してもらう」ように指導する。また顔が良く見える位置で会話すると視覚情報も活用できる。時間分解能の機能低下は補聴器では補えないが，聴覚を用いた認知訓練である程度改善する可能性がある。Anderson ら[46]は55-70歳の67例を対象に，トレーニング群では家庭で8週間の Brain Fittness cognitive training を用いて聴覚に基づいた認知訓練を行い，対照群は科学や歴史などの教育DVDを見て（注意してDVDを見るため）Multiple Choice Question に答える訓練を行った。評価は6つの formant を持つ170msの [da] を刺激音とした脳幹反応（静寂下と騒音下（S/N 比＋10dB）で記録），騒音下の単語聴取（S/N 比 0～25dB），短期記憶などで行った。その結果，トレーニング群では騒音下の formant transition に対応する時間が早くなり，短期記憶が向上し，騒音下聴取成績が改善し，脳幹反応のピークの騒音負荷による遅れも減少したが，対照群では効果は見られなかった。この結果は，聴覚に基づいた認知訓練が加齢に伴う時間分解能低下をある程度改善させうる可能性を示唆している。

ま　と　め

　本稿では，加齢に伴う聴覚障害について，老人性難聴を中心に，また主に臨床に用いられている検査を中心にレビューした。加齢に伴う聴覚障害は末梢聴覚障害に加えて中枢聴覚・認知の障害も加味され，極めて複雑な障害となっている。今後超少子高齢化を迎える日本において，高齢者の聴覚障害を適切に評価して対策を講じることがますます重要となってくる。騒音下での両耳聴や補聴器装用効果など日常生活を想定した評価・研究，老人性難聴予防に関する基礎研究・介入研究，日本人向けの聴覚認知訓練の開発など，我々の取り組むべき課題は多く残されている。

Age-related auditory disorder

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Age-related auditory disorder is a complex disorder characterized by a decline in peripheral and central auditory and cognitive functions. Hearing thresholds, which begin to be elevated from higher frequencies, vary significantly among the subjects and the speed of the threshold elevation increases with age. Speech perception is affected in subjects with presbycusis, due mainly to their hearing loss, but is more severely so in patients of advanced age. Otoacoustic emissions and auditory brainstem responses are also impaired, mainly reflecting the subjects' hearing threshold elevations, and less significantly, their age. Auditory temporal processing, which can be evaluated by psychoacoustic tests such as the gap detection test, is also deteriorated in elderly subjects. For elderly subjects with difficulty in speech communication in daily life, hearing aid (HA) is the treatment of choice. When HAs no longer provide benefit, cochlear implantation is the treatment of choice; excellent results of cochlear implantation have been demonstrated even in eld-

erly subjects, although those who are older at implantation tend to show lower speech understanding scores postoperatively. It is considered important to avoid unnecessary exposure to loud noises and to prevent/treat atherosclerosis in order to prevent age-related auditory disorder. Auditory-based cognitive training may be useful to restore age-related deficits in temporal processing.

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