The Seoul Journal of Medicine
Vol. 33, No. 4: 307-315, December 1992

Estimation of Surviving Spiral Ganglion Cells by Electrically-Evoked Auditory Brainstem Response†

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= Abstract = **Electrically-evoked auditory brainstem responses (EABRs) were recorded from normal and artificially damaged cochleae in order to observe the general characteristics of EABRs and to know whether it is possible to estimate the survival of the spiral ganglion cells by EABR. Waves of EABR appeared 1.5 to 2.0 msec earlier than those of the acoustically-evoked brainstem response and wave I was not observed due to electrical stimulation, whereas wave III and IV were consistently obtained, giving input-output function curves. The analysis of EABRs from the normal and abnormal cochleae suggested that the input-output function curve is thought to reflect the survival rate of the spiral ganglion cells.**

Key Words: *Guinea pig, Electrical stimulus, EABR, Input-output function curve, Spiral ganglion cell*

INTRODUCTION

Cochlear implant has become a promising rehabilitation for bilateral profound deaf patients and the authors have experienced several clinical successes, using Nucleus 22-channel cochlear implants (Kim *et al.* 1989).

Since cochlear implant bypasses the organ of Corti by directly stimulating the primary auditory neurons through electrodes located in the scala tympani, effective stimulation is considered to be dependent on the survival of the auditory neurons. It is also agreed that many other factors influence the outcome of rehabilitation by cochlear implant, including the age of onset of deafness, the etiology of deafness, the motivation of the patients and their family, and so on. Among them, the etiology of deafness has been recognized as one of the most important factors because the survival state of the spiral ganglion of the cochlea is variable according to the cause of deafness (Otte *et al.* 1978; Lousteau 1987; Nadol *et al.* 1989).

We have a few criteria to select the candidates for cochlear implantation; medical, radiological and psychoelectrical criteria. Promontory stimulation test has been widely used, as a psycholelectrical evaluation method (House and Brackmann 1974; Hatsushika and Funasaka 1991). This test is to see if a patient can perceive any acoustical signal by giving electical stimuli around the cochlea. This simple test gives us very important informations about the patient, but has limitations when it is applied to patients who have been deaf for a long time or prelingually deaf, especially in pediatric age, since this test is based on subjective

Received September 1992, and in final form November 1992.

† This study was supported by a grant No. 890702 from the Korea Science and Engineering Foundation Reasearch Fund.

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responses. In this context, we need an objective method to evaluate the acoustic response to electrical stimulation.

Recently, many researchers have focused on electrically-evoked auditory responses (EABRs) which has been considered as one of the most promising methods to predict the outcome of the cochlear implantation.

The authors carried out an animal experiment to examine the general characteristics of eletrically-evoked auditory potentials and see if this can be used to estimate the survival state of the spiral ganglion cells.

MATERIALS AND METHODS

1) Experimental animals and surgical approach

All procedures of experiment were performed under general anesthesia by sodium pentothal (30mg/Kg). EABRs were observed from 22 ears of adult guinea pigs; Normal EABRs were obtained from 8 ears, and other 14 ears were destroyed by an ototoxic drug or freezing before measuring EABRs.

The bulla was opened by dorsal approach and the round window membrane was exposed. A bipolar concentric needle for electrical stimulation was placed in the scala tympani 4-5 mm from the membrane using a micromanipulator.

2) Electrical stimulation

We used charge-coupled biphasic single pulses for electrical stimulation. The amplitude of pulse(PA) ranged from 20 μA to 2 mA and the pulse width were from 60 μsec to 2 msec.

3) Observation of EABRs from normal ears

EABRs were obtained from the contralateral ear by RACIA APE 2020 brainstem audiometer, using stainless steel needle electrodes with the active one on the forehead. In order to reduce the influence of the artifact from the electrical stimulation, the stimualtion and the beginning of acquisition was linked by poststimulus synchronization. The sweeping window was 10 msec and about 300 to 500 responses were averaged. In all animals for normal response, their hearing were confirmed to be normal by auditory brainstem response.

4) Observation of EABRs from destroyed ears

In order to get EABRs from the ears with

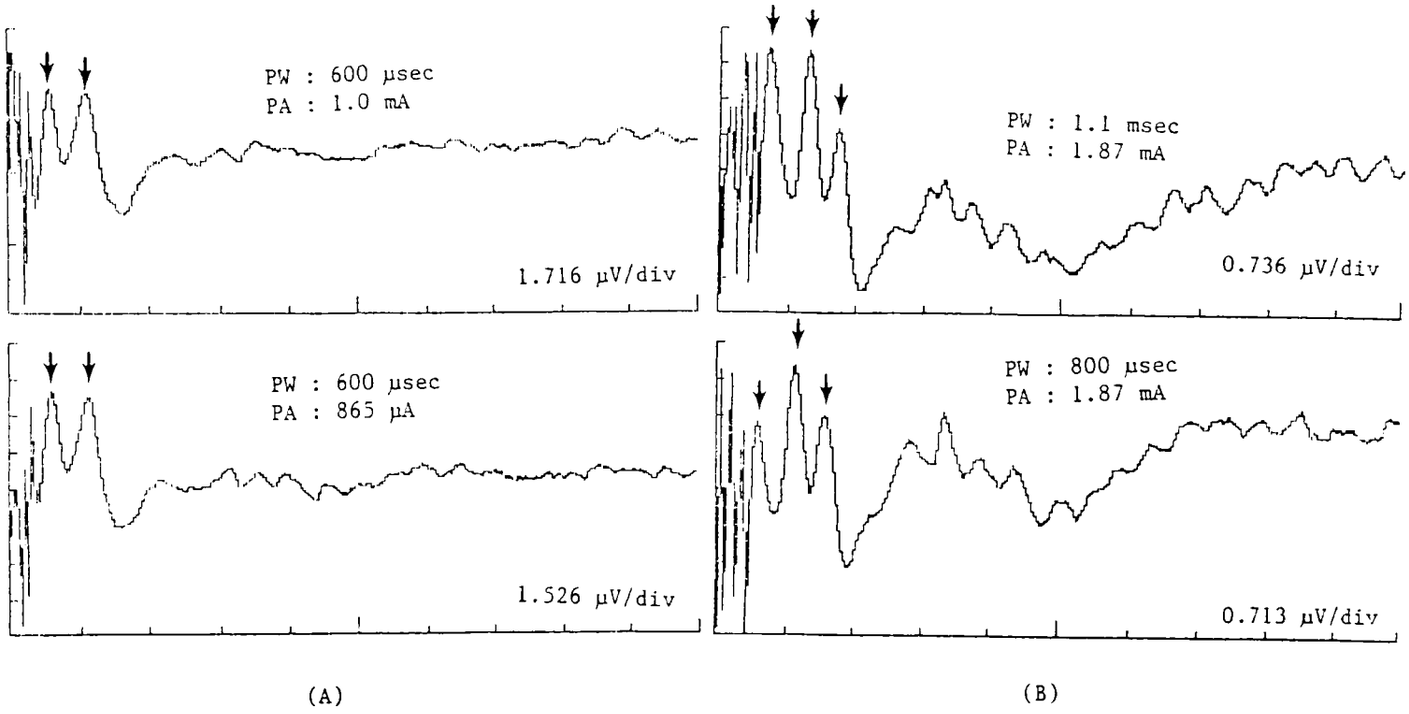

Fig. 1. Typical shapes of the EABRs in normal guinea pigs. The EABRs with only two peaks were usually observed (A), whereas the EABRs with three peaks appeared at certain conditions (B). (Abscissa: 1 msec/division)

various amounts of spiral ganglion cells, the cochleae were destroyed by the injection of kanamycin 50mg/kg or directly freezing the cochleae and adjacent structures using liquid nitrogen instilled in the bulla for 5 to 10 minutes (West *et al.* 1973; Smith and Simmons 1983; Lousteau 1987; Hatsushika and Funasaka 1989). EABRs were observed 4 weeks after the destruction from the kanamycin-injected group, or 2 to 5 days later in the frozen group.

5) Examination of the amount of the spiral ganglion cells.

Following the observation of EABRs, the animal was sacrificed and the temporal bones were taken after intracardiac perfusion of Haidenhain-Susa solution. The specimens were embedded in paraffin and 5 μm-thick sections were made parallel to the axis of the modiolus. The survival state of the spiral ganglion cells were examined under light microscope using hematoxylin-eosin staining. According to the survival state of the spiral ganglion cells, three groups were made; more than 60%, 60-30%, and less than 30%. The EABRS of each group were analyzed in terms of threshold, latency, input-output curve and the survival state of the ganglion cells.

RESULTS

1) Characteristics of the EABRs in normal animals

Eight normal ears were used in this observation. It is generally accepted that five waves are discriminated in auditory brainstem response. In EABR, wave I was not seen due to the electrical stimulation artifacts in the initial phase and it was usual to find only two (wave III, IV) or three (wave II, III, IV) waves (Fig. 1, Fig. 5). In a few cases, wave II, III, IV and V could be distinguished (Fig. 2(A)).

EABRs were seen about 1.5-2.0 msec earlier than the brainstem responses by the acoustic stimulation. This is partly due to bypassing the mechanical transduction in the cochlea in EABR (Since the poststimulus

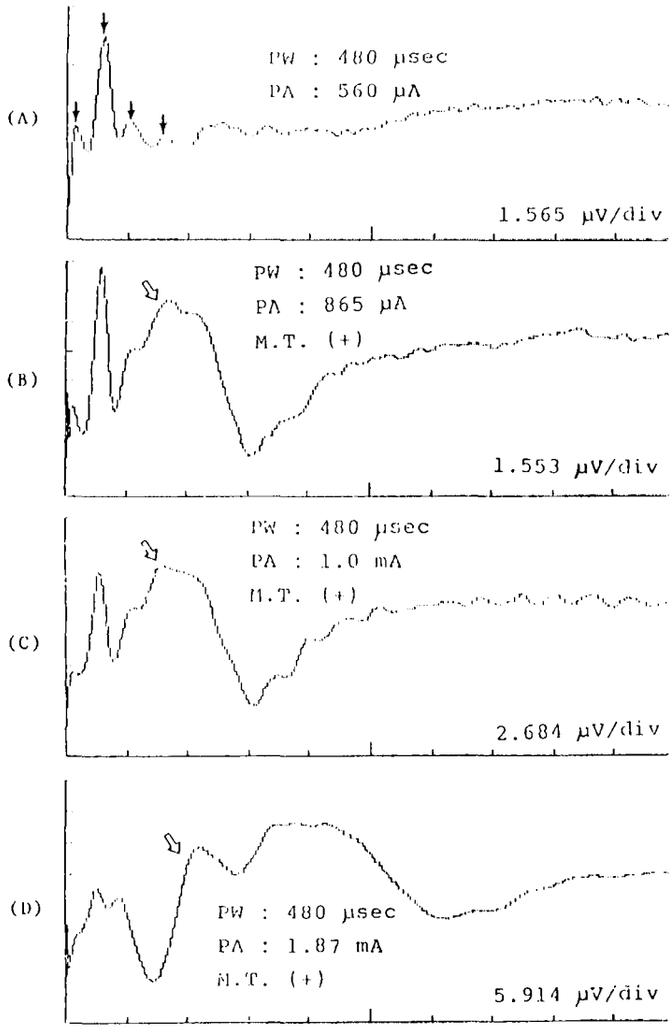

Fig. 2. Myogenic responses.
(A): EABR wave II, III, IV and V (small arrows) without contamination of myogenic responses.
(B), (C): Myogenic responses distorted the EABR. Visible muscle twitchings began to appear at (B).
(D): Myogenic responses overwhelmed the entire waveform. Blank arrows: myogenic responses. M.T.: muscle twitching

synchronization was employed, the apparent latencies were shorter by 0.5 msec). The order of the amplitude of the EABR was several μV and the waves were definitely distinguished from the other nonspecific parts.

Sometimes myogenic responses from the facial and neck muscles disturbed the recog-

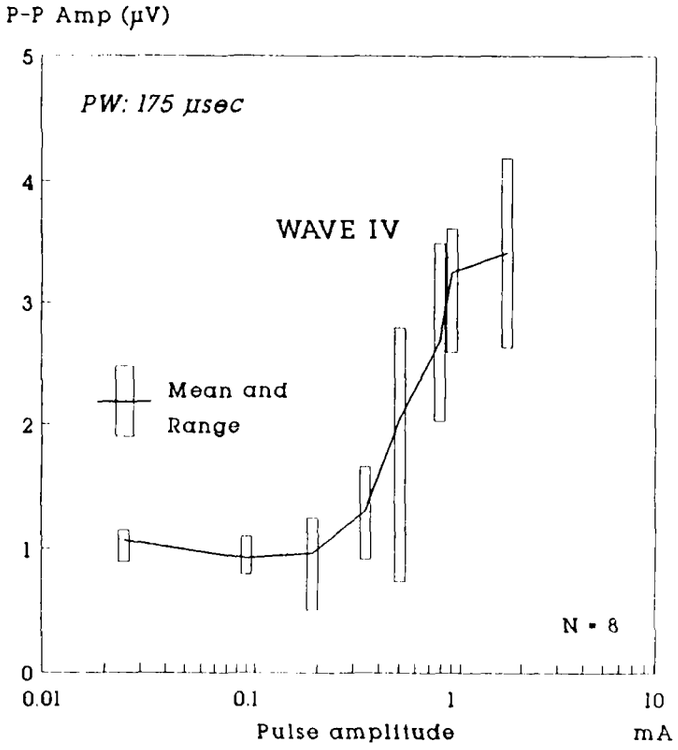

Fig. 3. Input-output function curve of wave IV according to the change of pulse amplitude with the pulse width was fixed at 175 μsec in normal guinea pigs. (P-P Amp: Peak-to-peak amplitude)

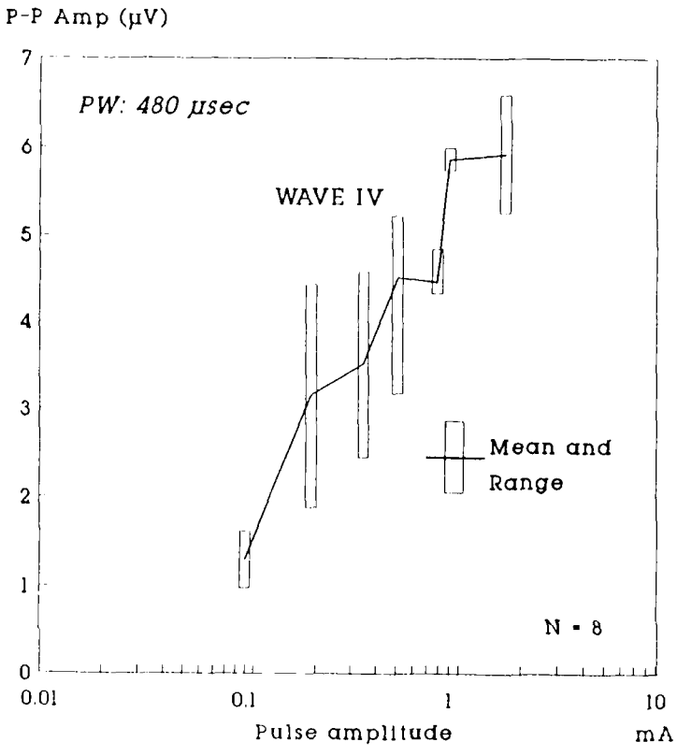

Fig. 4. Input-output function curve of wave IV according to the change of pulse amplitude with the pulse width fixed at 480 μsec in normal guinea pigs. (P-P Amp: Peak-to-peak amplitude)

nition of the EABRs. In Fig. 2, Wave II, III, IV and V were clearly presented at low amplitudes of the stimulus (Fig. 2(A)), but a big myogenic response appeared when the amplitude of the stimulation was increased.

By plotting the input-output function cur-

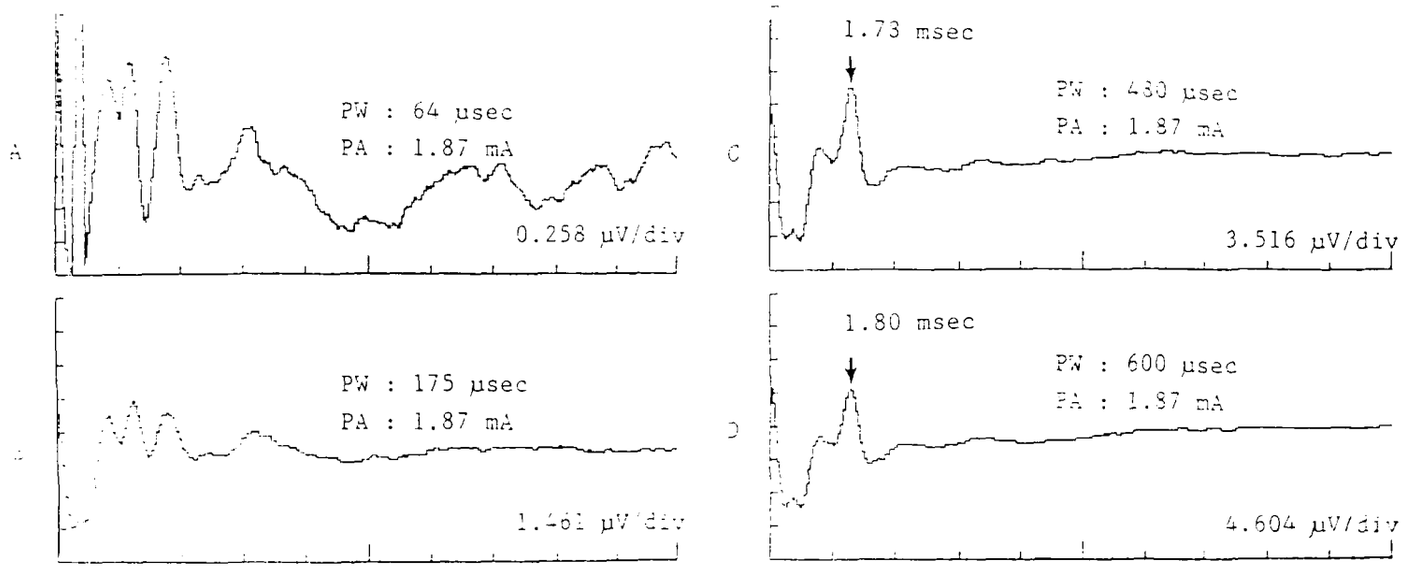

Fig. 5. Variation of the EABRs according to the change of the parameters of the stimulus in the same animal: At the same pulse amplitude, three peaks were observed by shorter pulses, whereas only two peaks were recognized by longer pulses. (Abscissa: 1 msec/division)

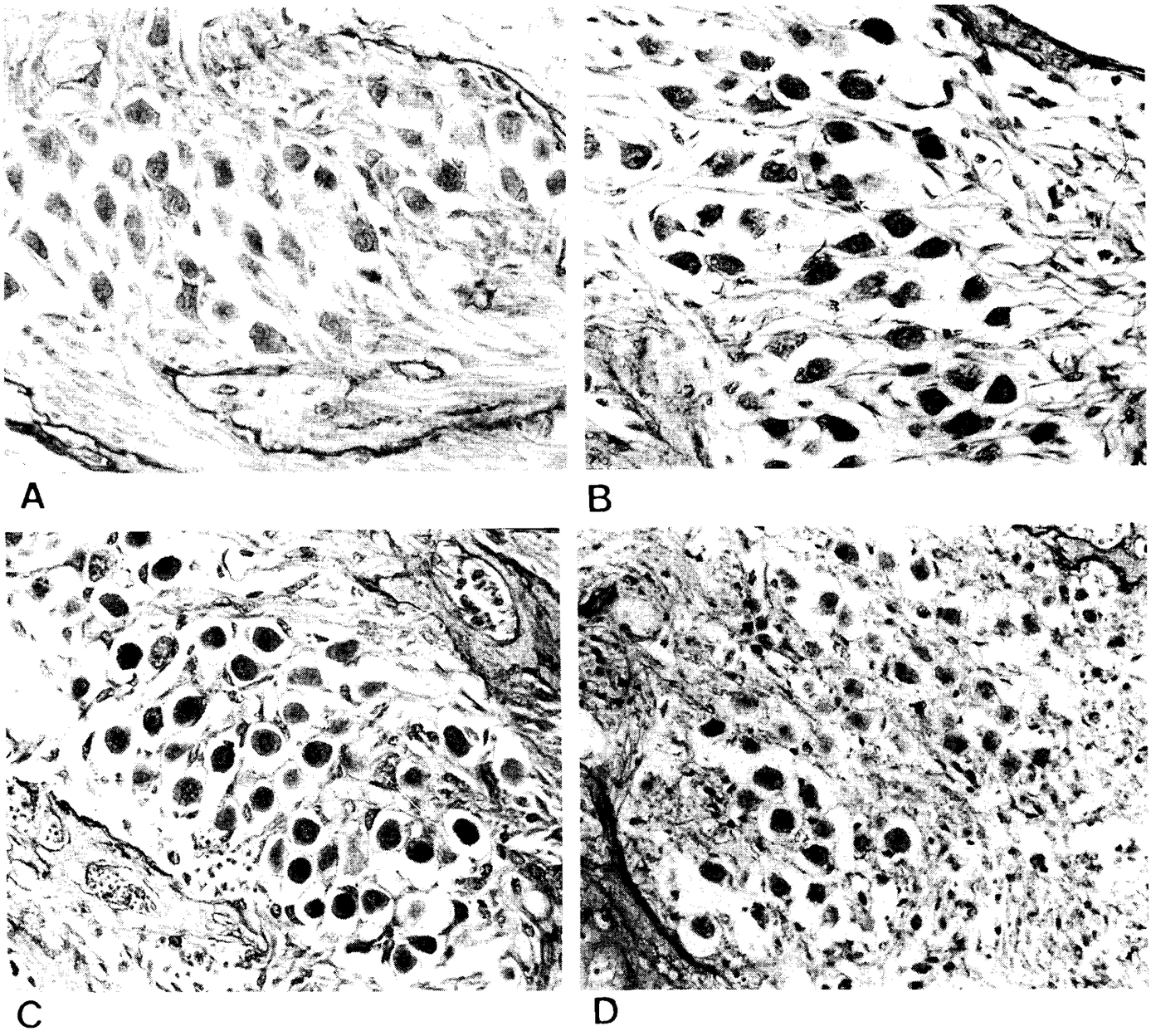

Fig. 6. Sections of the spiral ganglions from guinea pigs, showing various amounts of surviving spiral ganglion cells. (Hematoxylin- eosin staining, ×400)

A. Normal: Nearly all ganglion cells showed well-demarcated nuclear membranes and nucleoli with plump cytoplasms.

B. Example of 60-90% survival group: Some nucleoli demonstrated dense pyknotic change.

C. Example of 30-60% survival group: More cells displayed pyknotic changes in their nuclei and about half of the ganglion cells were lost.

D. Example of 0-30% survival group: Most of the ganglion cells revealed degeneration and a considerable part of the ganglion was already replaced by fibrosis.

ves, we observed two different types in response pattern (Fig. 3). With the pulse width fixed at a certatin condition (175 μsec in Fig. 3), there were no significant increases of the response amplitudes at lower stimuli, whereas a steep slope of the increment of the response was seen at higher stimuli more than 200 μA. But no flat portion in Fig. 3 was observed, when the pulse width was fixed at 480 μsec (Fig. 4).

At a fixed pulse width, the latencies of the

waves were shortened as the stimulations were increased. On the contrary, when the intensity of the electrical stimulation was increased by changing the pulse width at a fixed pulse amplitude, the latencies of the last wave seemed to be elongated in spite of the increased intensity, or the number of the peaks was reduced (Fig. 5).

The change of the waves according to the stimulation rate was minimal, except for the increase of the latency at higher stimulation rates.

2) The patterns of EABRs and the state of the spiral ganglion

In addition to the above-mentioned normal responses, EABRs were also obtained from 14 animals whose cochleae were destroyed by the injection of kanamycin or by direct freezing with liquid nitrogen.

On the histologic examination, the normal animals showed ganglion cells with plump cytoplasms and definite nuclear membranes and nucleoli (Fig. 6A). However, the cochleae destroyed prior to the observation of EABRs revealed not only the normal-looking ganglion cells but also degenerated cells with the pyknosis of nuclei and shrunken cytoplasms. Some specimens disclosed severe degeneration, showing extensive fibrosis without normal ganglion cells (Fig. 6D).

Two observers evaluated the survival state of the spiral ganglion cells of each cochlea, and four groups were made according to the survival state as follows: 3 cochleae in 0-30% group, 5 in 30-60% group, and 6 in 60-90% group plus 8 normal cochleae without prior manipulations. The input-output function curves were plotted for the average values of each group and the patterns were compared with the survival states (Fig. 6 & 7).

The threshold was a poor indicator of the survival state of the ganglion cells because in the group with 30-60% survival the responses could be obtained at lower stimulations than the group with higher survival or even normal group (Fig. 7). The latency itself could not be used as an indicator for estimating the survival state either, because of the unexpectable wave forms as explained above in Fig. 5.

Meanwhile, suprathreshold EABRs depicted as the input-output function curves were very good predictors of the survival state of the gangion cells. In the groups with a higher survial of the ganglion cells, bigger waves were presented and the amplitude also rapidly increased as the intensity of the stimulation was increased, whereas smaller waves were observed and the slopes of the function curves were less steep in the groups with lower survivals. These curves were not overlapped each other and well correlated with the survial states of the gaglion cells (Fig. 6 & 7).

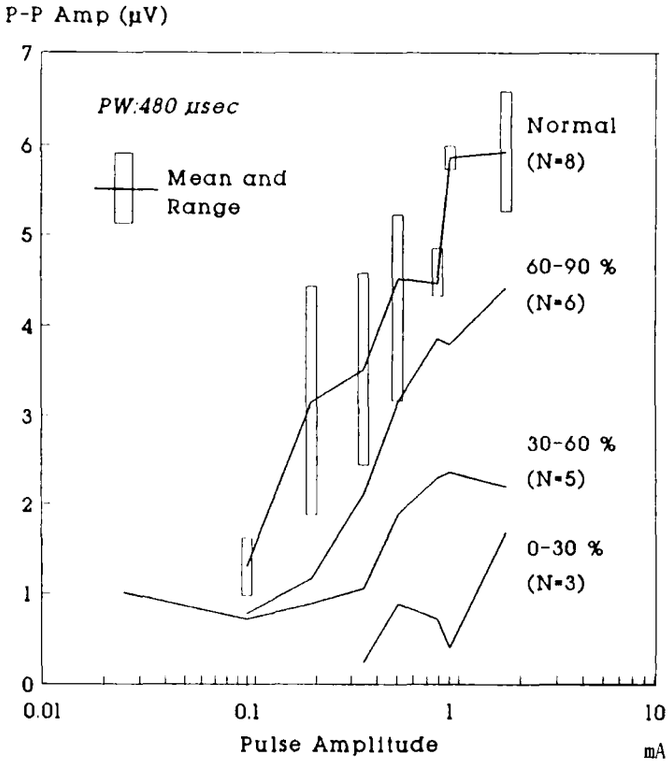

Fig. 7. Input-output function curves of wave IV with normal and varying degrees of spiral ganglion cell survivals.

DISCUSSION

It is not easy to get EABRs and we have to exercise much caution in analyzing the response because the waves were frequently influenced by artifacts from the eletrical stimulation itself or myogenic responses and vestibular responses, especially when monopolar stimulation was used (Hatsushika and Funasaka 1989).

The artifacts from the electrical stimulation are seen in the very early phase of the response, usually before wave III. Several methods have been tried to eliminate this artifact. The examples are as follows; reducing the averaging window (Chourd *et al.* 1979; Gardi 1985; Miyamoto 1986), alternating the polarity of the electical stimulation (Lusted *et al.* 1984), using a very short (Gyo and Yanagihara 1980) or triphasic pulses (Marsh *et al.* 1981), contralateral acquisition of the response (Starr and Brackmann 1979; Miyamoto 1986; Stypulkowski *et al* 1986), and employing stimulus artifact suppression circuitry (Black *et al.*, 1983; Shepherd *et al.*, 1983) or acoustic tracer (Simmons and Glattke 1972).

The authors were effectively able to obtain EABRs, basically using poststimulus synchronization in which the acquistion was triggered 0.5 msec after the electrical stimulation.

Myogenic responses are usually seen around 3 to 4.5 msec or later (Starr and Brackmann 1979), although some reports discussed the influence of the facial myogenic response to the wave III or IV (van den Honert and Stypulkowski 1986). These waves can be discriminated by rapid increase of the wave size with stronger stimulations and the fixed latency in spite of changing the intensities of the simulations.

During our experiment, if myogenic responses were present, these waves were seen only at stronger levels of stimulation, usually higher than 700 μA. At the same time, twitchings of the facial or cervial muscles were noted. Sometimes these waves were seen in the early phase within 2-3 msec, enough to disturb the EABRs.

Another possible artifact is the vestibular response. This is thought to be an early phase response (within 2.0 msec) and cannot be eliminated without cutting the vestibular nerve (Starr and Brackmann 1979; van den Honert and Stypulkowski 1986; Hatsushika and Funasaka 1989). Although the shape of this response is quite similar to that of wave II or III of EABRs, Dobie and Kimm (1980) already demonstrated that the chief components of the EABRs were originated from the cochlear nerve by transecting the vestibular nerve in monkeys.

The authors chose the waves between 2.0 and 3.0 msec which were not relatively influenced by above-mentioned artifacts, to plot the input-output function curves. As shown in Fig. 3, two kinds of response patterns were noted; a flat portion below the 200 μA of the eletrical stimulation without much changing of the response amplitudes with the increment of the stimulation, and a steep slope at higher stimulation intensities with a rapid increase of the response amplitude.

These patterns are believed to come from the hair cells (electrophonic response, the former flat portion) and the nerve (electroneural response, the latter steep portion) (Simmons and Glattke 1972; Yamane *et al.* 1981; Black *et al.* 1983; Shepherd *et al* 1983; Lusted and Simmons 1984). When a cell is located in an electrical field, the change of the currency polarity (AC) can cause a cell to vibrate (Brownell 1984). The electrophonic response is from the mechanical vibration of the hair cells at a lower intensity of the stimulation, and the eletroneural response is from the excitation of the cochlear nerve fibers by the eletrical stimulation of higher intensities. The former response is not dependent on the intensity of the stimulation, but the latter showed a rapid increase of the response along the increment of the stimulation intensity. As demonstrated in this experiment, the threshold and the latency of EABRs are not good indicators of the survival state of the spiral ganglion cells (van den Honert and Stypulkowski 1986; Hatsushika and Funasaka 1989). Smith and Simmons (1983) reported that the elevation of the threshold was observed when the survivng cells were less than 5%, and it was impossible to estimate the state of the spiral ganglion if there were more than 10% surviving cells. But it would be reasonable to hold the cochlear implantation if there is no response at all or the threshold is too high (Hatsushika and Funasaka 1989).

The change of the latency of a specific

wave is not expectable as shown in Fig. 5, probably due to the late deflection of the polarity of the wide biphasic eletrical pulse. So the absolute latency cannot be compared among different stimulation conditions.

The input-output function curves from the suprathreshold EABRs can reflect the survival state of the spiral ganglion. Merzenich and White (1977) showed the size of the EABRs and the slope of the curve were dependent on the number of the stimulated nerve fibers, and Smith and Simmons (1983) reported similar results in cats as is demonstrated in this experiment.

However, it is too early to conclude the clinical significance of the EABR because there is a possibility that the shape of the function curve can be affected not only by the number of the ganglion cells but also by the state of the brainstem. Especially, the late-appearing wave IV might be more influenced by higher auditory organ (Stypulkowski *et al.* 1986). In addition, since it is almost impossible to get EABRs from intracochlear stimulation before the operation, we have to develop a more reliable technique to obtain EABRs from the extracochlear stimulation in order to apply these findings to the patients.

REFERENCES

Battmer RD, Lehnhardt E, Laszig R. Promontoriumstet und Electrocochleografie im Hinblink auf die Indikation zum Cochlear Implant. HNO 1986; 34:139-42

Black RC, Clark GM, O'Leary SJ, Walters C. Intracochlear electrical stimulation of normal and deaf cats investigated using brainstem response audiometry. Acta Otolaryngol (Stockh) Suppl. 1983; 399:5-17

Brownell WE, Bader CR, Bertrand D, *et al.* Evoked mechanical responses of isolated cochlear outer hair cells. Science 1984; 227: 194-6

Burton MJ, Miller JM, Kileny PR. Middle latency responses. I. Electrical and acoustic excitation. Arch Otolaryngol Head Neck Surg 1989; 115:59-62

Chourd CH, Meyer B, Donadieu F. Auditory brainstem potentials in man evoked by electrical stimulation of the round window. Acta Otolaryngol (Stockh) 1979; 87:287-93

Dobie RA, Kimm J. Brainstem response to electrical stimulation of the cochlea. Arch Otolaryngol 1980; 106:573-7

Gardi JN. Human brain stem and middle latency responses to electrical stimulation: Preliminary observations. Cochlear Implants, Raven Press, New York, 1985; 351-63

Gyo K, Yanagihara N. Electrically and acoustically evoked brainstem responses in guinea pig. Acta Otolaryngol(stockh) 1980; 90:25-31

House WF, Brakmann OE. Electrical promontory testing in differential diagnosis of sensorineural hearing impairment. Laryngoscope 1974; 4:2163-71

Hatsushika S, Funasaka S. Estimation of surviving auditory nerve by electrically-evoked auditory brainstem response (EABR). (in Japanese) J Otolaryngol Jpn 1989; 92:1005-11

Kim CS, Kim JY, Sung MW, Hong SH, Han MH, Yoon MS, Song JW. Clinical trial of Nucleus 22 channel cochlear implant in two totally deaf patients. Korean J Otolaryngol 1989; 32:174-91

Lousteau RJ. Increased spiral ganglion cell survival in electri- cally stimulated, deafened guinea pig cochleae. Laryngoscope 1987; 97:836-42

Lusted HS, Simmons FB. Comparison of electrophonic and auditory-nerve electroneural responses. J Acoust Soc Am 1988; 83:657-61

Lusted HS, Simmons FB. Interaction of cortical evoked potentials to electric and acoustic stimuli. J Acoust Soc Am 1984; 76:449-55

Lusted HS, Shelton C, Simmons FB. Comparison of electrode sites in electrical stimulation of the cochlea. Laryngoscope 1984; 94:878-82

Marsh RR, Yamane H, Potsic WP. Effect of site of stimulation of the guinea pig's electrically evoked brain stem response. Otolaryngol Head Neck Surg 1981; 89:125-30

Merzenich MM, White MW. Cochear implant, the interface problem. In Hambrecht FT, Resnick

JB (Ed) Functional Electrical Stimulation Marcel Dekker, Inc. New York 1977.

Miyamoto RT. Electrically evoked potentials in cochlear implant objects. Laryngoscope 1986; 96:178-85

Nadol JB, Young YS, Glynn RJ. Survival of spiral ganglion cells in profound sensorineural hearing loss : Implications for cochlear implantation. Ann Otol Rhinol Laryngol 1989; 98. 411-6

Otte J, Schuknecht HF, Kerr AG: Ganglion cell populations in normal and pathological human cochleae. Implications for cochlear implantation. Laryngoscope 1978; 88:1231-46

Shepherd RK, Clark GM, Black RC. Chronic electrical stimulation of the auditory nerve in cats-physiological and histopathological results. Acta Otolaryngol (Stockh), Suppl 1983; 399:19-31

Simmons FB, Glattke TJ. Comparison of electrical and acoustical stimulation of the cat ear. Ann Otol Rhinol Laryngol 1972; 81:731-8

Smith L, Simmons FB. Estimating eighth nerve survival by electrical stimulation. Ann Otol Rhinol Laryngol 1983; 92:19-23

Starr A, Brackmann DE. Brainstem potentials evoked by electrical stimulation of the cochlea in human subjects. Ann Otol Rhinol Laryngol 1979; 88:550-6

Stypulkowski PH, van den Honert C, Kvistad SD. Electrophysiologic evaluation of the cochlear implant patient. Otolarygol Clin North Am 1986; 19:249-53

van den Honert, Stypulkowski PH. Characterization of the electrically evoked ABR in cats and humans. Hearing Res 1986; 21:109-216

Yamane H, Marsh RR, Potsic WP. Brainstem response evoked by electrical stimulation of the round window of the guinea pig. Otolaryngol Head Neck Surg 1981; 89:117-24

West BA, Brummett RE, Himes DL. Interaction of kanamycin and ethacrynic acid. Arch Otolaryngol 1973; 98:32-7