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THE MORPHOLOGICAL CHANGES IN THE VESTIBULAR SENSORY EPITHELIA FOLLOWING ELECTRICAL STIMULATION

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Abstract

The morphological changes of the vestibular sensory epithelia of the guinea pig following electrical stimulation were investigated using scanning electron microscope.

Positive and negative square wave pulse stimulation was given through a silver ball electrode placed on the round window membrane for one hour. The current intensities used were 100, 200 and 300 μA.

While the direct current stimulation at intensities of 100 or 200 μA did not cause any significant changes, severe damage of the utricular macula and the ampullar crista of the lateral semicircular canal was observed at 300 μA. The degenerative changes such as fusion of sensory hairs, protrusion of the cuticular plate and loss of sensory cells were found on both the utricle and the semicircular canal. In the most severely damaged area, the sensory epithelial surface was badly torn apart.

In the clinical application of direct current to the inner ear for relieving tinnitus, special attention should be paid to the vestibular organ.

KEY WORDS: Morphological Changes, Scanning Electron Microscopy, Vestibular Organ, Utricle, Lateral Semicircular Canal, Direct Current, Electrical Stimulation

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Introduction

It has been established that electrical stimulation of the inner ear elicits hearing sensations in the profoundly deaf human [3]. Since the 1960's many investigators have developed prosthetic devices (cochlear implants) for deaf patients [7,11,16]. An attempt has also been made to electrically stimulate the inner ear for suppression of tinnitus [12,13]. Yet, very little is known about the effect of electricity on the vestibular organ. In this paper the morphological changes of the vestibular organs (utricle and lateral semicircular canal) induced by direct current electrical stimulation are investigated using scanning electron microscope.

Materials and Methods

Healthy young guinea pigs (250-450 g body weight) with normal Preyer's reflex were used for this experiment. After intraperitoneal injection of pentobarbital sodium (25-30 mg/kg), a silver ball electrode was placed on the round window membrane of each cochlea through a small hole in the tympanic bulla. The electrode impedance was measured and maintained from 10 to 20 kΩ in order to keep uniform contact of the electrode across the subjects. The electrode wire was fixed to the bulla with dental acrylic during stimulation. All procedures were performed using aseptic techniques. The electrode wire was 0.127 mm in diameter with one flamed to produce a small ball about 0.3 mm in diameter. The Teflon insulation extended to about 2 mm from the ball. A needle electrode, inserted into the posterior neck muscle, was used as the reference electrode. Electrodes were implanted in both ears of each animal. One side was electrically stimulated while the other side served as a control.

Two types of stimulus currents were used, positive or negative square wave pulses (Figure 1). All stimulation waveforms were generated by a constant current stimulator.

The electrical stimulation commenced immediately after electrode placement. The current levels were either 100, 200 or 300 μA. During stimulation, the current level was monitored by

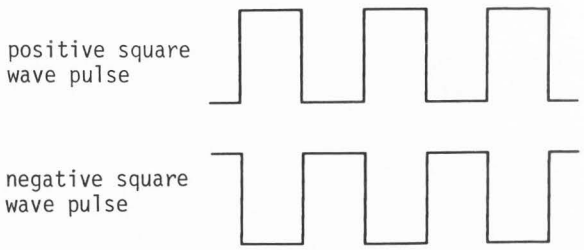

Figure 1 Diagram of stimulus wave forms. duration: 1 msec, frequency: 500 Hz

oscilloscope. After 1 h of stimulation, electrodes were removed and the incised skin was sutured.

The animals were sacrificed one week after stimulation. The middle ear was opened in order to check the presence of infection. If any evidence of infection was observed, the subject was eliminated from the investigation. The inner ear was fixed with 2.5 % phosphate buffered glutaraldehyde by cardiac perfusion under deep general anesthesia. After removal of the temporal bone, the round window and the oval window were perforated and perilymphatic space was gently perfused with the same fixative. The temporal bones were dissected in the fixative (2.5 % glutaraldehyde), to remove the utricle, the lateral semicircular canal (l.s.c.c.) and the cochlea. The reason that only the utricle and the l.s.c.c. were removed for observation is to avoid mechanical damage to the lower turn of the cochlea which is also our interest for observation. Changes in the cochlea will be reported in a separate publication.

The specimens were conductive stained by 2 % tannic acid for 2 h and postfixed by 1 % OsO_4 solution for 1 h. Following dehydration with graded ethanol, the specimens were treated with isoamyl acetate, critical point dried (CO_2) and coated with platinum.

Results

Table 1 presents a summary of the stimulation level and the presence of the morphological changes observed in each animal. While at intensities of 100 and 200 μA no remarkable change was found in the utricle or l.s.c.c., damages of various degree were observed in the animals stimulated at 300 μA.

The utricle of an animal (No.19) stimulated with the negative square wave (300 μA) is shown in Figure 2. In nearly half of the utricular macula, the sensory hairs were lost and many globular substances were found. In close proximity to the most severely damaged portion, degenerative changes, such as fusion and loss of the sensory hairs, were observed (Figure 3). At the top portion of the crista of the l.s.c.c., remarkable loss and fusion of the sensory hairs were found (Figure 4). In another ear stimulated with the same wave form (No.20, negative, 300 μA), the epithelial surface was badly torn apart (Figure 5). Most sensory hairs were missing throughout the macula and a few remaining hairs showed severe morphological changes (Figure 6). In the l.s.c.c. of the same animal, loss of the hairs and globular substances were observed (Figure 7). Figure 8 is the utricle from an ear stimulated with 300 μA positive wave (No.14). Loss of the sensory hairs, giant hair formation and protruding of the cuticular plate were observed. In the animals No.15, 16 and 17, fusion of sensory hairs and loss of the sensory epithelia were found, but the damaged areas were much smaller than those observed in the animals No.14, 19 and 20. There was no distinct relationship between the area of the damage and the electrode position.

In the control ear of each animal, normal anatomy was preserved both in the utricle and the l.s.c.c. (Figure 9).

Table 1 A summary of the stimulation level and the presence of the morphological changes in each animal. P:positive, N:negative

No.	stimulation level(μA)	utricle	l.s.c.c.
1	100(P)	-	-
2	100(P)	-	-
3	100(P)	-	-
4	100(P)	-	-
5	100(P)	-	-
6	100(P)	-	-
7	100(P)	-	-
8	100(N)	-	-
9	200(P)	-	-
10	200(P)	-	-
11	200(P)	-	-
12	200(P)	-	-
13	200(N)	-	-
14	300(P)	+	+
15	300(P)	+	+
16	300(P)	+	+
17	300(P)	-	+
18	300(P)	-	-
19	300(N)	+	+
20	300(N)	+	+
21	300(N)	-	-

Discussion

There have been many morphological studies of the cochlea following electrical stimulation [1,4,6,9,14,15,17]. And it has been reported that application of the direct current to the inner ear induces morphological damage of the organ of Corti [1,6]. On the other hand, very few studies of the vestibular organs have been

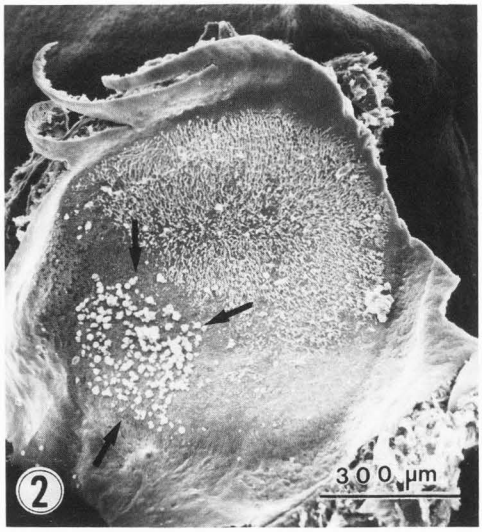

Figure 2 The utricle of animal No.19. Negative current (300 μA) was applied. In nearly half of the utricular macula, the sensory hairs are lost and many globular substances are found (arrows).

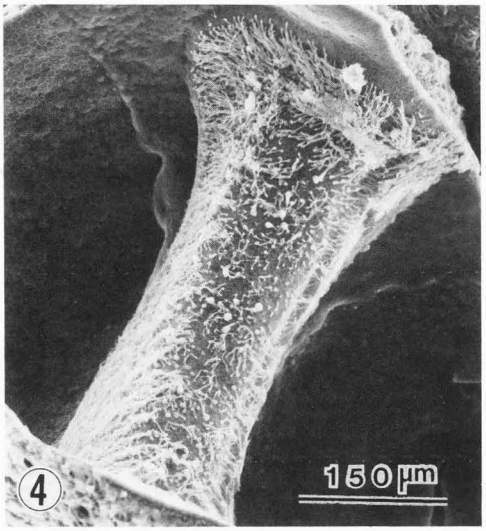

Figure 4 The l.s.c.c. of animal No.19 stimulated with negative direct current (300 μA). At the top portion of the crista, extensive loss of the sensory hairs is observed.

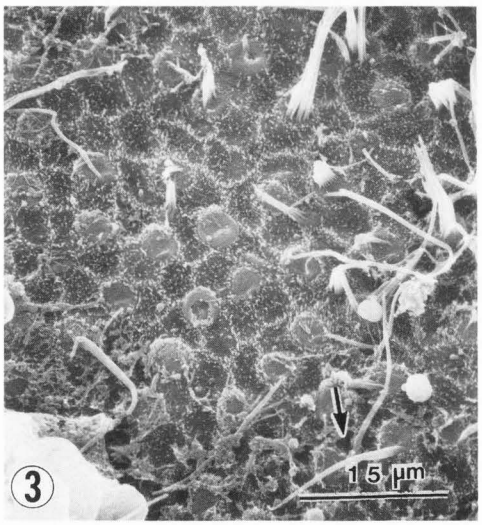

Figure 3 The utricular macula (No.19). In close proximity to the most severely damaged portion, fusion (arrow) and loss of the sensory hairs are observed.

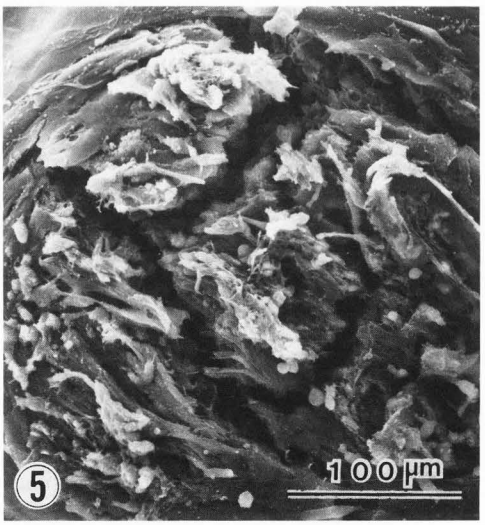

Figure 5 The utricular macula from animal No.20 stimulated with negative current (300 μA). The epithelial surface on the macula is torn apart.

reported, in particular the effect of the direct current on the vestibular organ still remains unknown. When electrical stimulation is applied to the inner ear, the vestibular organs may well be affected as well as the cochlea. It is reported that some patients experience dizziness during electrical stimulation [2,13] and, in fact severe degenerative changes of the vestibule and the semicircular canal were observed in human temporal bone sections [8].

When the electrode is inserted into the inner ear, damage to the sensory cells and the nerve may occur not only from the electrical current, but from material reaction and operative trauma. In this study an extracochlear electrode was used in an attempt to limit the damage to

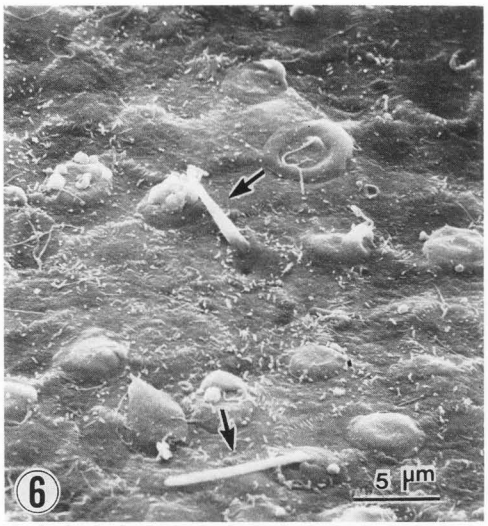

Figure 6 The utricular macula of the same animal as Figure 4. Most sensory hairs are missing throughout the macula and a few remaining hairs show severe changes (arrows).

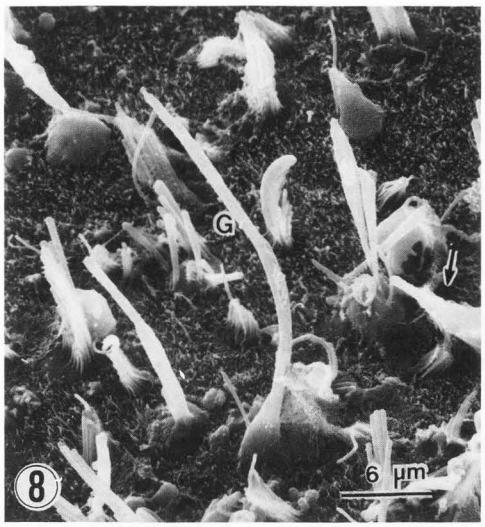

Figure 8 The utricular macula from animal No.14 stimulated with positive wave (300 μA). Giant hair (G) and protruding of the cuticular plate (arrow) are observed.

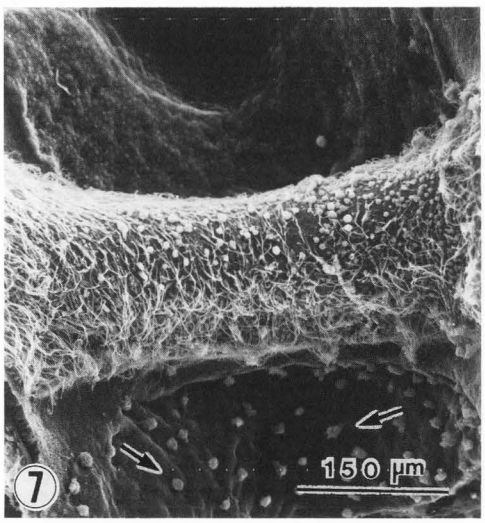

Figure 7 The l.s.c.c. from animal No.20. Loss of the hairs and globular substances are observed (arrows).

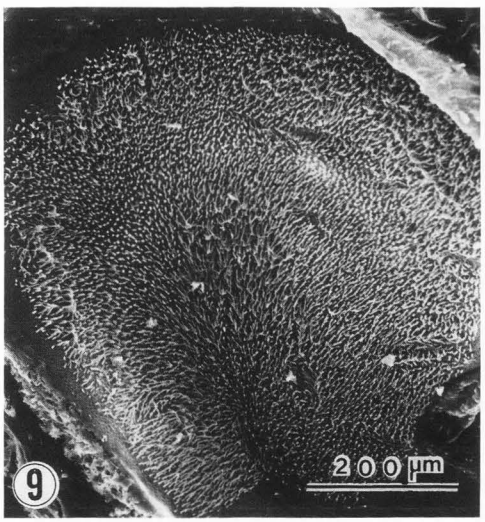

Figure 9 The utricular macula from the control ear of animal No.19. Normal anatomy is preserved.

that of the electrical stimulation. In the present experiment, 1 h direct current stimulation at intensities of 100 or 200 μA did not cause change in the vestibular organ. However, 300 μA current resulted in degenerative changes in some animals. The degree of damage varied considerably. Variety in the damage may be due to different current distribution within the inner ear or different vulnerability among the individuals.

Although the morphological findings, such as fusion and loss of the sensory hairs, observed in this experiment were similar to those caused by the ototoxic drugs [5], localization of the damage is different, particularly in the utricle. The most severely damaged area in the utricle did not include the striola. In contrast, the ototoxic drugs severely affected the striola [5]. This could be due to a difference in the cytotoxic mechanism between the ototoxic drugs and the electrical current. Metabolic exhaustion of

the hair cell and change of its ionic environment may be the mechanism for cell damage due to electrical stimulation. The electrode material used in this study was silver, which is highly polarizable and may change pH of endolymph and blood flow. Direct thermal agitation also remains a potential destructive factor.

We calculated that surface charge density (charge transferred per unit area of electrode surface per half cycle of applied current) was 318 $\mu C/cm^2$/phase at 300 μA, supposed that one-third of the geometric surface area of the ball electrode was in contact with the round window membrane. The current level frequently used by Portmann et al. [12] was reportedly 100 μA or less with various frequencies. Since no information is available about the charge density in the study of Portmann et al., direct comparison is not possible. However, as far as the current level is concerned, our value is very high as compared to the stimulation level used for tinnitus suppression.

It has been reported [10] that direct current stimulation to the central nervous system causes greater damage than that observed in the vestibular organs. The difference in the degree of damage may be due to the different location of the electrode and the different susceptibility among the organs.

The degree of damage may be influenced by factors such as duration and current level. The stimulus period used in this study was short, however longer stimulation, even at lower intensity than 300 μA, may cause a more adverse effect on the vestibular organs. To elucidate these matters, further study is needed. This includes varying an electrode material, position and stimulus duration.

As Portmann et al. stated [13], the direct current should not be applied for tinnitus suppression when the patient has residual hearing. Even in case of the total deaf, direct current should be applied with utmost care, since the vestibular organ may well be affected by electricity. Special attention should be paid to the vestibular organ as well as to the cochlea when direct current is applied to the inner ear for relief of tinnitus.

Acknowledgement

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Discussion with Reviewers

R.K. Shepherd: Your choice of monophasic current pulses and silver stimulating electrodes would be expected to result in possible adverse electrochemical reactions. Did you observe any evidence of electrochemical damage either at the round window or within the vestibular organ? Did you

use any techniques such as energy disperse x-ray analysis to examine the tissues for silver content?
Authors: No, silver content was not evaluated both in the round window membrane and in the vestibular organ. We agree that electrochemical contamination with silver ion may be a destructive factor.

P.A. Leake: Three out of 5 animals subjected to positive square wave pulses at 300 μA showed morphological changes while 2 out of 3 guinea pigs stimulated with negative pulses (at the same current, duration and frequency) were observed to have damage to the vestibular sensory epithelia. Was there any systematic difference in the nature or degree observed in the two experimental groups?
Authors: No, there was no systematic difference in the nature or degree of damage between the two groups.

R.K. Shepherd: Monophasic D.C. current pulses are significantly different to the charge balanced current pulses generally used in cochlear prostheses. Was your choice of stimulus parameters based on those used in clinically available tinnitus suppressors?
Authors: Yes. As stated in the text, it is reported by Portmann et al. [12] that positive D.C. current suppresses tinnitus.

P.A. Leake: The finding that the vestibular sensory cells are damaged by D.C. electrical stimulation applied through stimulation at the round window for a 1 hour period is extremely interesting and may have important implications for those who propose a therapeutic role for electrical stimulation in tinnitus treatment. However, the probable damage to neural tissue from this type of electrical stimulation is an even greater concern in the cochlea since it is presumably closest to and maximally excited by the stimulating electrode. Did you evaluate cochlear pathology in your experimental series?
Authors: Yes, the changes of the organ of Corti were also observed. Degenerative changes similar to those observed in the vestibular organs were found in some of the cochleas stimulated at intensities of 200 and 300 μA. Morphological changes were limited to the basal turn.

J.-M. Aran: It is true that effects of electrical stimulation on the vestibular sensory epithelium have not been as extensively documented, but this is due in part to the fact that there is no actual need for such demonstration. That for the organ of Corti is sufficiently explicit to forbid the use of such a stimulation on a permanent basis, particularly in patients with residual hearing, as those suffering from tinnitus. Please comment.
Authors: Although the totally deaf patients may have severely damaged peripheral vestibular organs and peripheral vestibular dysfunction induced by D.C. current is compensated by the central nervous system in time, the possibility of the hazard of long lasting balance disorder should be kept in mind. In this respect, effects of electrical stimulation (including both A.C. and D.C.) on the vestibular organs are important subjects to be examined.

J.-M. Aran: Although the actual changes to the vestibular epithelium are nicely documented with SEM they do not bring any new information on the pathophysiological processes involved, but for the localization of the damage to the utricle at the periphery instead of the striola as with other toxic agents. Please comment.
Authors: As mentioned in the text, morphological changes themselves observed in this study were similar to those reportedly caused by other toxic agents, but we think the localization of the damage in the utricle has an important implication for speculating the cause of damage induced by D.C. stimulation.