Vestibular Responses Induced by Injection of Black Ink into the Facial Canal in Rabbit

—Electrophysiological and Morphological Studies—

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Vestibular manifestation accompanied with idiopathic facial palsy, Bell's palsy, was clinically observed by Philipszoon[1], Pfaltz[2], Mielke[3] and Morimoto, et al[4]. However, there is still no definite interpretation about the mechanism of the manifestation of these pathologenesis.

Fisch[5] described clearly about some anastomotic fibrous construction between the facial nerve sheath and the auditory nerve sheath in the internal auditory canal (meatus).

Kumagami[6,7] reported various manifestation of vestibular disturbance induced after instillation of various kinds of substance into the facial canal in the rabbits.

In this paper, we investigated the nystagmus induced by instillation of chinese black ink into the facial canal of the rabbits in order to obtain the clue to solve the mechanism of the vestibular disturbances and the facial nerve disorder. Authers observed also the morphological changes in the inner ears macroscopically and microscopically by light microscopy and by scanning electron microscopy.

MATERIALS AND METHODS

Thirty healthy rabbits, weighing from 2 to 3 Kg, were used.

All animals were examined preoperatively and had no notable abnormality on their ear canals and eardrums, showing normal hearing response (Preyer auditory reflex) and body equilibrium, i.e., posture, spontaneous nystagmus, head position nystagmus and rotatory nystagmic responses.

The rabbit was fixed in the rabbit holder. Local infiltration anesthesia with 0.5% xylocaine solution was made. Preauricular incision approach to the facial canal at the stylomastoid foramen was done.

More detail description about the operation technique to reach and

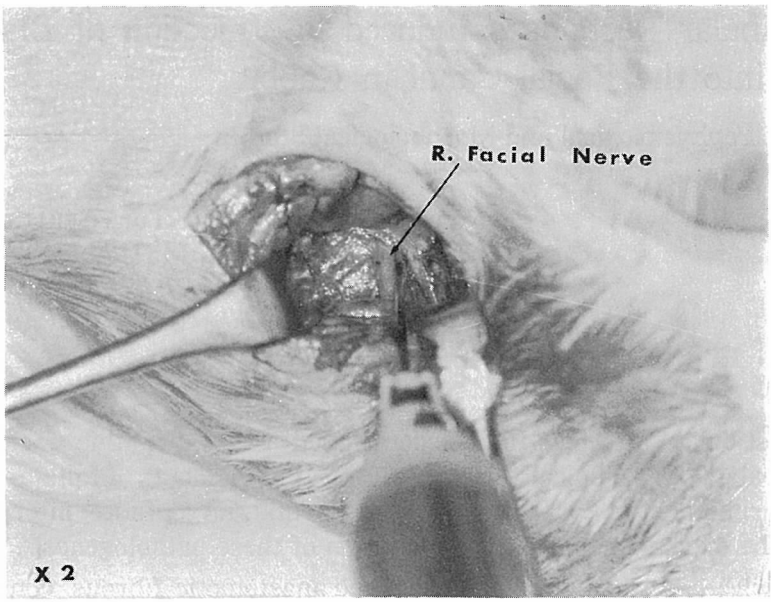

Fig. 1. Exposing the stylomastoid foramen after preauricular incision and retraction, a chinese black ink was injected into the facial canal.

open the facial canal at the stylomastoid foramen was available in previous paper[8].

The diluted chinese black ink was obtained by 10 times dilution of the commercial black ink, kept in room temperature.

A 0.3 ml. of the diluted chinese black ink was injected into the facial canal (Fig. 1).

Recording of induced nystagmus was done by 6 channel nystagmograph W-144 (San-ei Sokki K.K.). Needle electrodes were used, inserting in the skin, at both external canthi as horizontal lead for recording the horizontal eye movement and other 2 needle electrodes at the upper and lower eyelid skin as vertical lead recording. The last electrodes, at the parietal skin for grounding. Paper speed was 0.5 cm/sec. Time constant were 1.5 sec for eye movement and eye position, and 0.03 sec for eye speed recording (differentiated eye movement).

An upward deflection of the ENG tracing by horizontal lead recording represents the eye movement toward the right and downward deflection of the tracing represents the leftward eye movement.

In vertical recording, an upward deflection of the tracing represents the upward eye movement and the downward deflection of the tracing represents the downward eye movement.

Histological study on the temporal bone including the facial canal and nerve, the vestibular organs and cochlea was done in every rabbits.

Specimens were prepared by means of a modified Yamakawa method[9], which was deseribed by Ogata[10]. According to his preparation method, the rabbit was sacrificed on the 10th day after each experiment and was fixed by vital fixation procedure, described in detail elsewhere.

Specimens for scanning electron microscopic survey on the crista ampullaris were obtained from some of the rabbits when sacrified on the 10th day, under surgical microscope.

Specimens obtained were thoroughly washed out in isotonic saline at room temperature.

The specimens were fixed in 2.5% phosphate-buffered (pH. 7.3) glutaraldehyde and 1% osmic acid solution. This specimens were dehydrated in ethanol and amyl acetate of ascending concentrations from 50 to 100 percent, and then were dried in critical point drying apparatus. In the vacum the specimens were coated with approximately 200A° of carbon and gold.

The specimens were examined with JSM S-I scanning electron microscope operating at from 8 to 25 Kv.

RESULTS

1) Nystagmus

Observation of eye movement or nystagmus induced by injection of a chinese black ink into the facial canl was done in 30 rabbits. Induced nystagmus was jerking type, shown their individual results on Table 1, 2, and 3.

The parameters of observation on this induced nystagmus were as follows: i.e., a) direction of quick component of nystagmus, b) latency and c) duration of the nystagmus.

a) Direction of nystagmus:

Direction of the nystagmus induced after chinese black ink injection in the facial nerve at the stylomastoid foramen was observed in various modes, such as there were some difference in the direction of initial nystagmus or other direction-changing in character time after time, and so on.

All animals were divided to 3 main groups, depending upon the character of these nystagmus direction.

Group A, in which the direction of nystagmus is toward the right

Table. 1. Data of Nystagmus (Group A)

No. (Animal No.)		Induced Nystagmus				Total Duration of Nystagmus (min. & sec.)	Total Hour (min. & sec.)
		Latency (sec.)	Direction & Duration (min. & sec.)				
1	(23)	35	←0.04	→ 0.52		0.56	1.31
2	(5)	5	↩0.15	→20.00		20.15	20.20
3	(27)	2	←0.11	↗ 0.35		0.46	0.48
4	(13)	13	←0.16	↘ 1.20	↗ 1.55	3.31	3.44
5	(14)	26	←0.05	↖ 0.20	↘ 6.11	6.36	7.02
6	(26)	7	←0.03	pause 0.30	↑ 2.11	2.14	2.51
7	(4)	5	←0.05	↑ 0.20	↗12.30	12.55	13.00
8	(3)	120	←0.10	↑ 0.20	→ 1.30	2.00	4.00
9	(20)	51	←1.16			1.16	2.07
10	(8)	18	←0.45			0.45	1.03
11	(18)	7	←0.12	↖ 0.22		0.35	0.41

Table. 2. Data of Nystagmus (Group B)

No. (Animal No.)		Induced Nystagmus			Total Duration of Nystagmus (min. & sec.)	Total Hour (min. & sec.)
		Latency (sec.)	Direction & Duration (min. & sec.)			
12	(22)	4	→0.40		0.40	0.44
13	(1)	17	→1.06		1.06	1.23
14	(7)	8	→2.14		2.14	2.22
15	(30)	20	→4.32		4.32	4.52
16	(35)	10	→6.11		6.11	6.21
17	(33)	5	→7.25		7.25	7.30
18	(21)	11	↗1.52		1.52	2.03
19	(10)	34	↗4.35		4.35	5.09
20	(19)	10	↗6.05		6.05	6.15
21	(34)	18	↗7.45		7.45	8.03
22	(9)	18	↘5.15		5.15	5.33
23	(15)	22	↘6.00		6.00	6.22
24	(11)	26	↘0.26	↗1.47	2.13	2.39
25	(17)	22	↘0.05	↗4.08	4.13	4.35

Table. 3. Data of Nystagmus (Group C)

No. (Animal No.)		Induced Nystagmus				Total Duration of Nystagmus (min. & sec.)	Total Hour (min. & sec.)
		Latency (sec.)	Direction & Duration (min. & sec.)				
26	(2)	10	↑0.30	→1.00		1.30	1.40
27	(28)	30	↑1.30	↗4.15		5.45	6.15
28	(24)	20	↑1.35	↗5.19	↑0.30	6.54	7.14
29	(31)	10	↑0.15	↗8.00		8.45	8.55
30	(25)	25	↑0.55			0.55	1.20

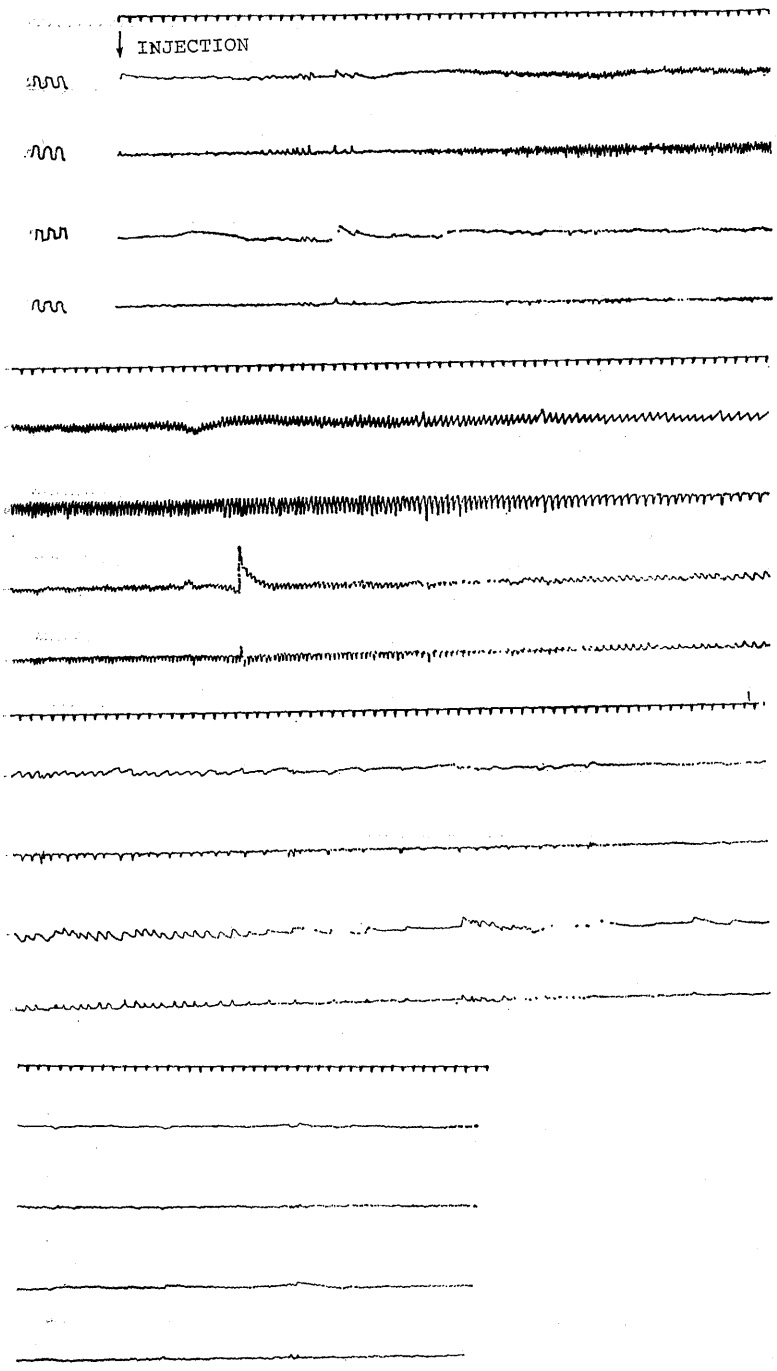

Fig. 2. ENG of representative case of Group A (No. 4, Animal No. 13). Latency (13 seconds) prior to elicitation of nystagmus after injection of black ink. Initial nystagmus directing rightward horizontal persisted for 16 seconds. And then, the direction was changing toward left-upward and oblique.

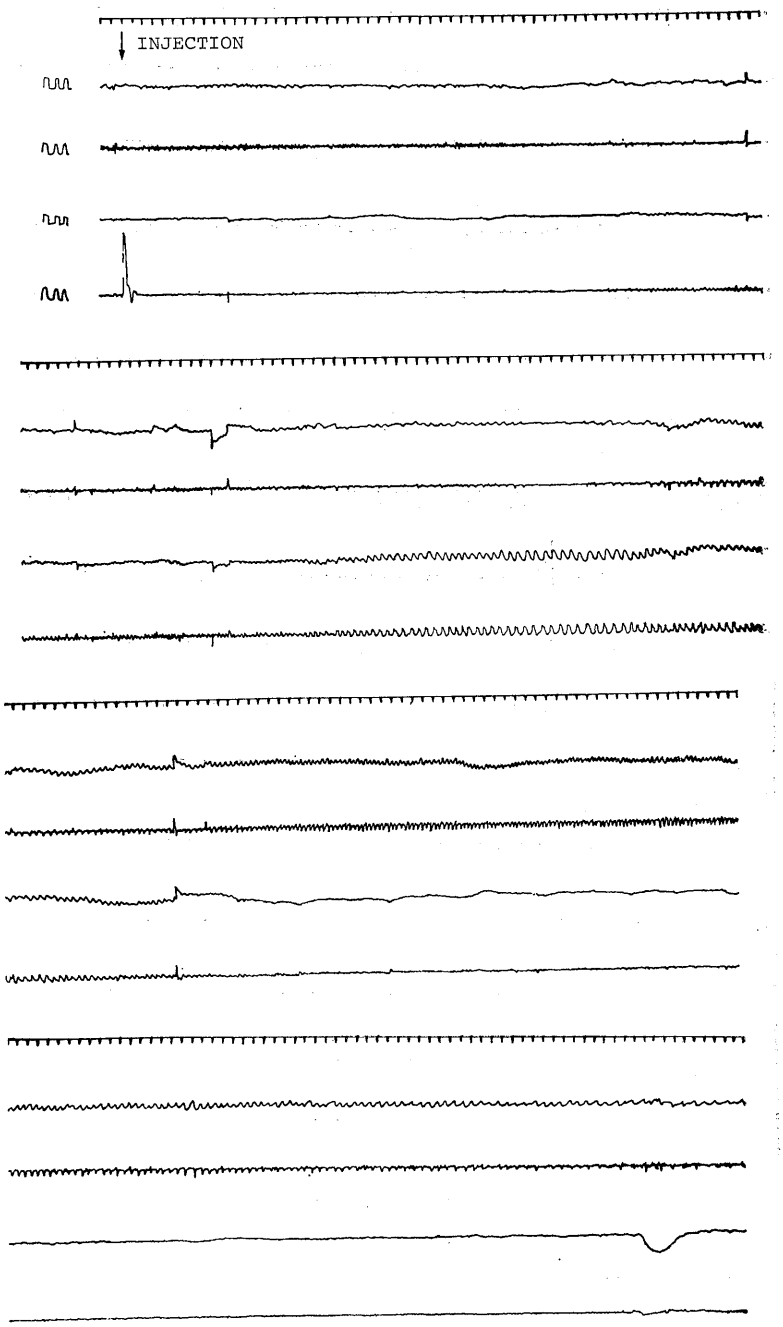

Fig. 3. ENG of representative case of Group B (No. 21). Left upward oblique nystagmus as the initial response after 18 seconds of latency. Nystagmus persisted for 7 min and 45 sec, and then subsided.

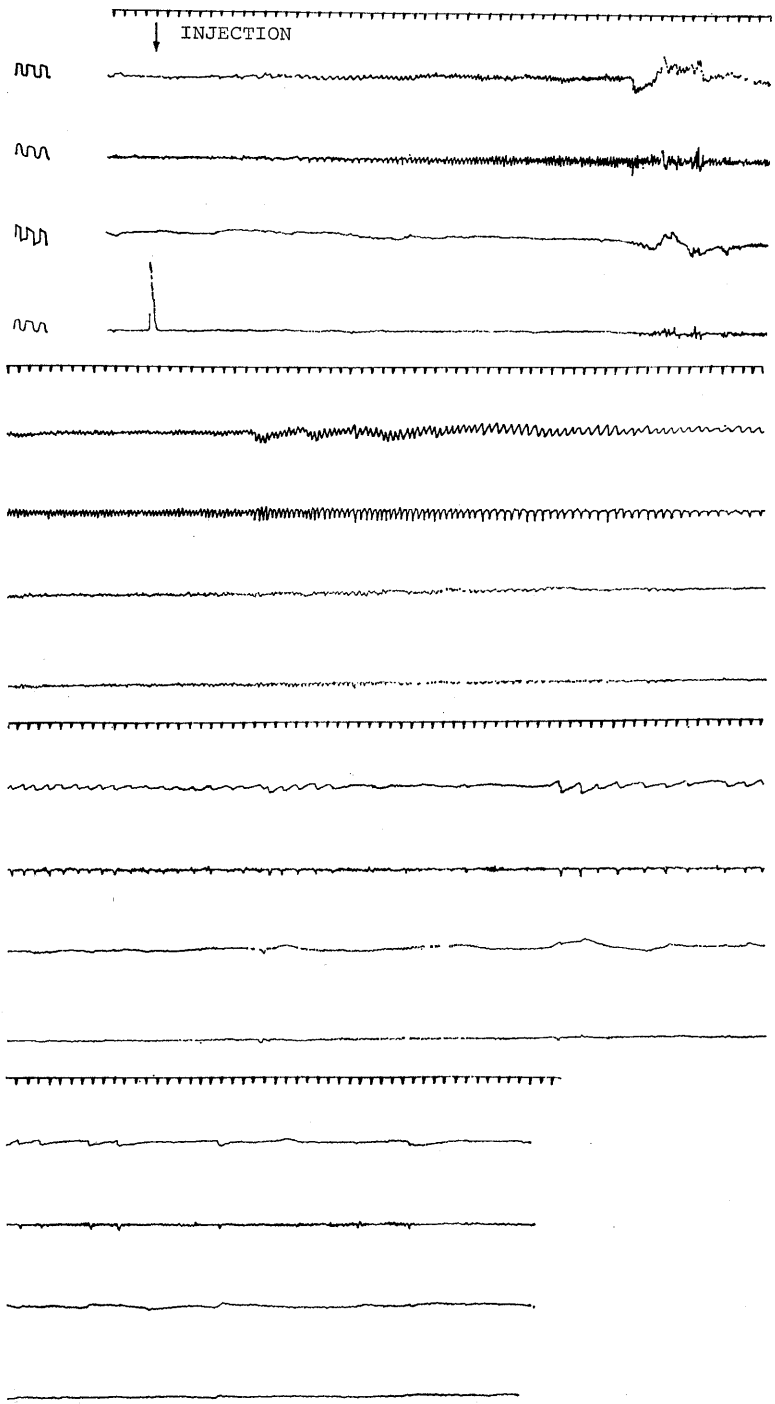

Fig. 4. ENG of representative case of Group C (No. 27, Animal No. 28). Upward vertical nystagmus was elicited in 30 sec of latency after a black ink injection. Duration was 1 min and 30 sec. Thenafter, to left upward oblique for 4 min and 15 sec.

(Fig. 2), consisted of 11 rabbits.

Two of these 11 rabbits showed no direction changing through whole experiment (Group A. case No. 9 (Animal no. 20) and Case No. 10 (Animal no. 18)).

Two of 11 rabbits showed relatively short time period of the initial nystagmus directing toward right. After these short period, the nystagmus directed to the opposite side, i.e., leftward directioning nystagmus for longer period.

Two of 11 rabbits in Group A showed oblique upward rightward nystagmus follwing the initial horizontal rightward nystagmus. Three of 11 rabbits showed upward vertical nystagmns follwoing after the initial rightward directing nystagmus. (one of them had a short pause before the secondary nystagmus).

Other 2 cases showed oblique leftward nystagmus (one of them, upward leftward, and another was oblique downward leftward).

Group B, in which the direction of nystagmus following some latency after injection of chinese black ink into the facial nerve is toward the opposite side from the iniected side, consisted of 14 animals (Fig. 3).

Six cases in the 14 rabbits in this group showed the horizontal nystagmus toward the left. Four of the 14 animals showed the upward and left oblique nystagmus. Two of the 14 animals showed the downward and left oblique nystagmus. Remaining 2 had some change of the direction of nystagmus, showing the left-downward oblique nystagmus at the beginning and then the direction of nystagmus was the oblique left-upward.

Group C, in which the direction of nystagmus in the initial stage was toward upward vertical, consisted of 5 animals (Fig. 4). Four of them showed that the changing of direction of nystagmus was seen the upward vertical at the initial stage, but to the oblique downward in the latter stage.

Only one case showed the upward vertical through the whole observation period.

b) Latency Period

There is some latency period in each animal between the time of injection and the time of elicitation of initial nystagmus.

The shortest latency which was observed in Case No. 3 in Group A was 2 seconds. The longest latency was 120 seconds of Case No. 8 in Group A (Animal no. 3). By 10 seconds grading, latency period of 2-9 seconds was seen in 8 of total 30 cases. Ten of 30 cases showed the latency of 10-19 seconds. Seven of 30 cases showed the latency of 20-29 seconds.

Three of 30 cases showed the latency of 30-39 seconds. Two remainder showed 51 sec. and 120 seconds of latency, respectively.

Generally, 25 of 30 cases showed the initial nystagmus within 30 sec. of latency (83.3%). And there is no definite relationship between the direction of the nystagmus and the length of the latency period.

c) Duration of the nystagmus elicited.

Duration of the nystagmus induced after injection of a chinese black ink into the facial canal was estimated.

As shown in Table 1. and 2, the shortest duration of the induced nystagmus was 34 sec. of Case No. 11. (Animal no. 18) and the longest duration of the nystagmus was 20 min and 15 sec of Case No. 2 (Animal no. 5) in Group A.

By a minute grading, 6 in 30 cases showed the duration graded of 1-59 sec; 4 cases showed 60-119 sec, 4 cases was in the period of 2 min to 2 min and 59 sec.

One was in 3 min to 3 min and 59 sec. Three, in 4 min to 4 min and 59 sec. Two, in 5 min to 5 min 59 sec. Five, in 6 min to 6 min 59 sec. Two, in 7 min to 7 min 59 sec. Three were in 8 min and 45 sec, 12 min and 55 sec, and 20 min and 15 sec, respectively.

A half of total 30 cases showed their duration of nystagmus was within 3 min and 59 sec. One thirds of total cases showed 5 min and 59 sec of the duration. There was no significant relationship between the length of the nystagmus duration and each groups; Group A, B and C, which were devided by the nystagmus directions.

2. Localization of the injected chinese black ink.

a. Macroscopic findings (See Figure 5).

In order to inspect macroscopically the localization of the chinese black ink, which was injected into the facial canal at the stylomastoid foramen (Fig. 5), the rabbit was sacrificed and the facial canal was opened from the stylomastoid foramen to the internal auditory canal, and from the vestibular endorgans to the meningeal space.

The findings are as follows;

The chinese black ink was observed in the mastoid segment of the facial canal, in the internal auditory canal and in the space between the pia matar and the dura mater.

In more details, the N. chorda tympani and the great superficial petrosal nerve were stained in black. The facial nerve and auditory nerve and their anastomotic fibers in the internal auditory canal were stained in black.

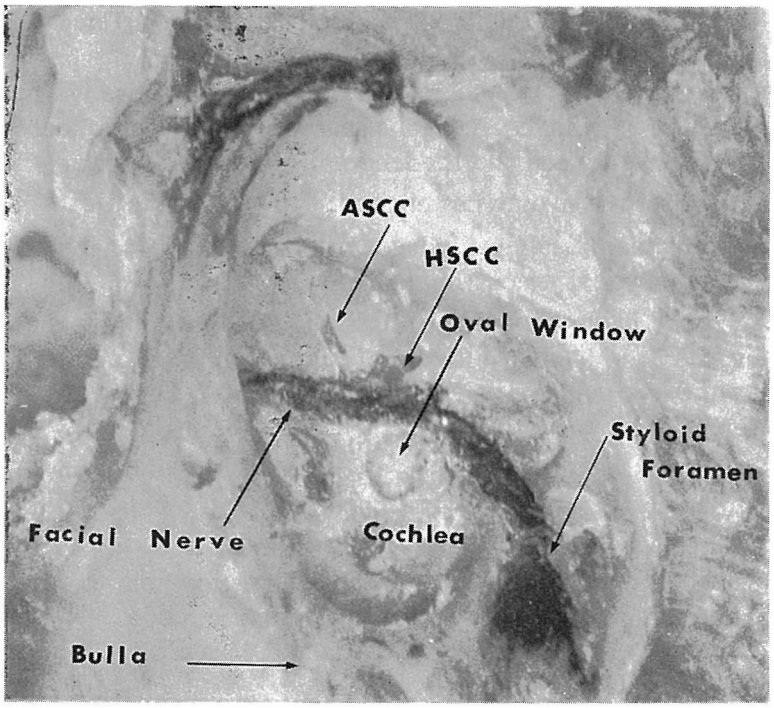

Fig. 5. Dissected temporal bone (Right) showing the black ink injected in the stylomastoid foramen. Particle of black ink was seen in the facial canal, but not in the semicircular canal or in the cochlea. ASCC: Anterior semicircular canal. HSCC: Horizontal semicircular canal.

However, there is no observable particle of the chinese black ink in the perilymphatic spaces of the anterior and posterior semicircular canals, the vestibule and the cochlea. Even by the exposure of each parts after removal of the bony capsule.

And also, there was no detectable particle of the chinese black ink in the membranous labyrinth of the anterior and lateral semicircular canals, where were opened under surgical microscope.

b. Light microscopic findings.

Serial section specimen of the temporal bone for microscopic study were made, obtaining after vital fixation of the rabbit on the 10th post-injection day (Fig. 6).

Microscopic survery was done at the following parts of each organs, i.e., i) endolymphatic space, ii) perilymphatic space, and iii) cristae ampullaris of the semicircular canals; iv) endolymphatic space, v)

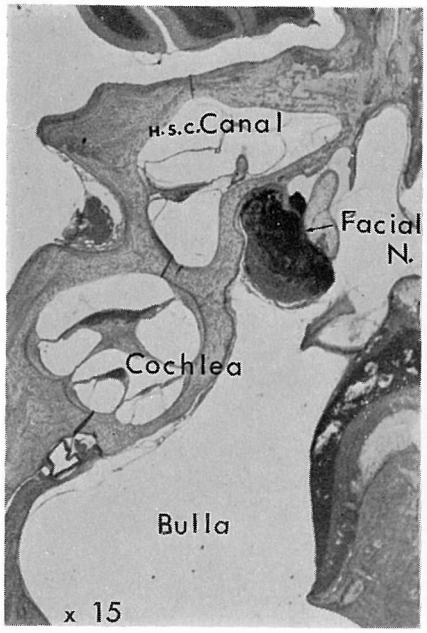

Fig. 6. Histological specimen of the temporal bone of the rabbit (Group C, No. 12). Obtained on the 10th day after black ink injection into the stylomastoid foramen on the right side. The black ink particle was not found in the labyrinth, but in the facial canal.

perilymphatic space and vi) maculae of the vestibule; and vii) endolymphatic space and perilymphatic space of the cochlea.

Those various portion of the organs in the temporal bone were observed as normal in the structure and there is no black ink particle, even any hemorrhagic or degenerative finding observed.

Facial nerve at every segments and the auditory nerve in the internal auditory canal were observed as well stained in black with microscopy.

c. Scanning electron microscopical findings (Fig. 7 and 8).

The rabbits on the 10th post-injection day was sacrificed and fixed by the vital fixation technique as usual procedure.

Their membraneous labyrinths of each semicircular canals were dissected out under surgical microscope and prepared for specimen of the scanning electron microscopical study, by ordinary procedures mentioned already.

Crista ampullaris were thoroughly investigated in reference to the basic description of the fine structure of these organs.

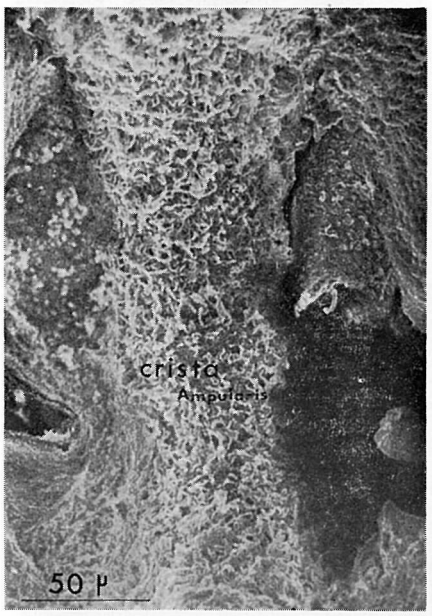

Fig. 7. Surface of the crista ampullaris of the rabbit. By scanning electron microscopic view, there are large number of sensory hair cells observed.

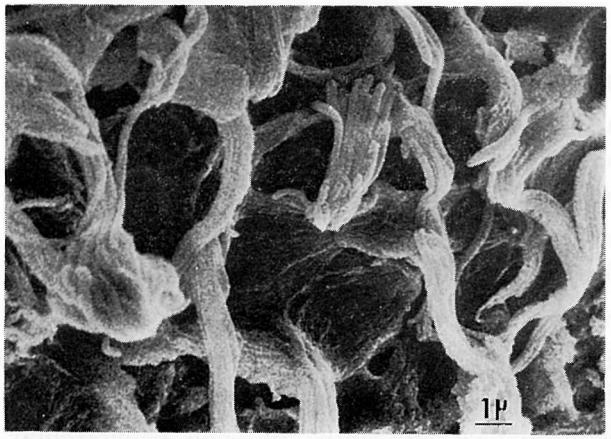

Fig. 8. Surface of the crista ampullaris (High power field of scanning electron microscopic view). Each structure of these hair cells are in normal.

The sensory hair cells showed a step-like arrangement consisting of 7-8 rows, each row containing 5-8 stereocilia. Near the longest stereocillia, a kinocillium was observed which was for longer in length and slightly larger in diameter than the stereocilium. Those findings were well consistent as normal structure mentioned by Harada[11].

Transitional epithelia was also observed as normally.

DISCUSSION

As described in the experimental results, the nystagmus was always induced by the injection of a chinese black ink into the facial canal. The auther will discuss about the followings problems, such as 1) direction of nystagmus induced by chinese black ink injection; 2) some comment on the mechanism of direction-changing in the nystagmus; and 3) comment on the mechanism of elicitation of nystagmus after injection of a chinese black ink.

1) Direction of nystagmus induced by the chinese black ink injection was somewhat varied in each individual cases.

As desribed already on the direction of nystagmus based on the direction of the initially observed nystagmus, 7 of all 30 cases showed the quick phase of nystagmus toward the injected side (Group A); 14 of 30 cases showed the inital nystagmus toward the opposite side from the initial nystagmus and to the end (Group B). Four of the 30 cases showed the initial nystagmus to the upward vertical direction. Above all, through the whole experimental course, the induced nystagmus in each animals showed once to direct to the opposite side (25 in all 30 cases). In other words, 83.3% of the cases examined showed that the induced nystagmus directed toward the opposite side at any period of the experiment.

It is suggested that the nystagmus directing to the opposite side after infecting a chinese black ink into the facial canal might be the principal form of the response. Meanwhile, the remaining 5 cases, which showed their direction of nystagmus were toward the ipsilateral side or the upward vertical and/or upward oblique, might be an exceptional form.

2) Comment on the mechanism of alteration of the direction of the nystagmus during the experimental course;

The nystagmus which was observed in the experiment following after injection of the chinese black ink into the facial canal was definitely noticed as jerking type, i.e., the similar type of nystagmus usually originating from labyrinthine stimulation.

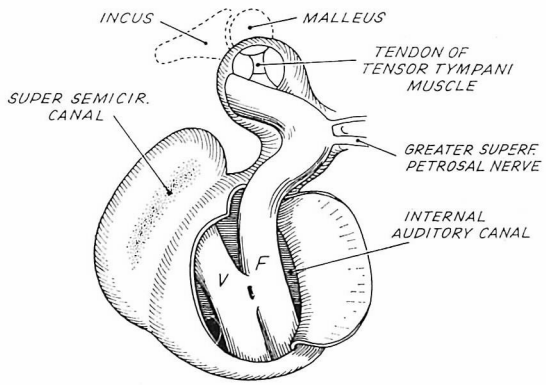

Fig. 9. Diagrammatic view of the internal auditory canal. (Transtemporal Surgery of the Internal Auditory Canal ,by Dr. Fisch[5]).

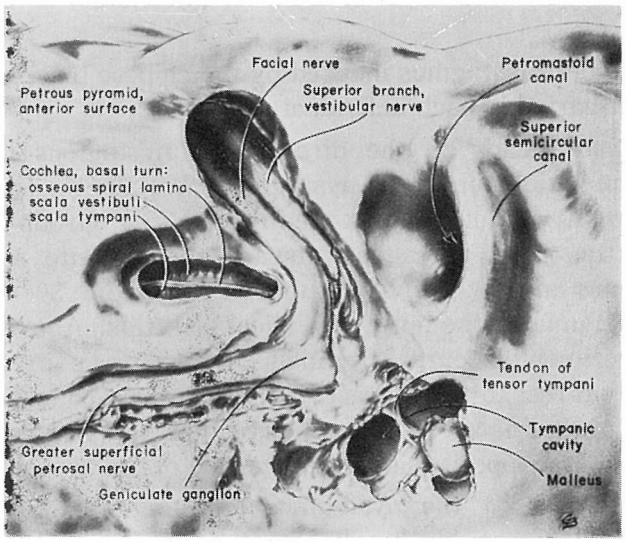

Fig. 10. Facial nerve canal and Internal auditory canal in the temporal bone. Facial nerve and inner ear organs. ("Surgical Anatomy of the Inner Ear," by Anson-Donaldson[12]).

It might be undoubted that some change of the electrical activity in the sensory cells in the vestibular endorgan and/or in the vestibular nerve cells will be induced by injection of chinese black ink into the facial canal from the stylomastoid foramen to the internal auditory canal, from the anatomical standpoint of view (Fig. 9 and 10).

Certain change of the electrical activity of the sensory cell in the

labyrinth are well known to cause the change of the polarization of the hair cells; i.e., for example, the hypopolarization of the hair cells increases the frequency of action potentials in the crista ampullaris of the horizontal semicircular canal. Increased action potentials in the vestibular nerve and in the vestibular nuclei, especially, in the medial vestibular nucleus, will trigger the vestibulo-ocular reflex to the eyeball moving slowly to the opposite side.

Following after this slow eyeball deviation to the opposite side, the quick return of the eyeball to the ipsilateral side. As the results from the repetition of the excitation in the labyrinthine nerve system, the nystagmus to the ipsilateral side was elicited.

However, it was easily understandable that the change in the electrical activity in the sensory cell and nerve will subside gradually and/or the change of the electrical activity further advance.

In the following stage of the vestibular nerve function, in the contrary to the preceeding stage, the hyperpolarization of the sensory cells will occur and it cause to decrease the action potential in the vestibular nerve on the ipsilateral side. Decrease action potential in the vestibular nuclei might trigger the vestibulo-ocular reflex to the eyeball moving slowly to the ipsilateral side and then the quick return of the eyeball to the opposite side of injection. It will be similar to be called as "paralyzed labyrinth".

CONCLUSION

The investigation of the nystagmus induced by injection of 0.3 ml of the chinese black ink into the facial canal of the rabbits was done, including histopathological study on the temporal bone specimen and also scanning electron microscopic study on the cristae ampullaris of the semicircular canals and membranous labyrinth.

Thirty adult healthy rabbits were used.

The following results were obtained:

1. Jerking nystagmus, with notable slow and quick phase in the eye movement, was elicited in all experimental animals.

2. All cases were divided into three main groups, based upon their nystagmus directions, i.e., Group A principally showed the nystagmus direction toward the ipsilateral side initially (11 of total 30 cases). Group B showed the nystagmus direction toward the opposite side from the beginning of nystagmus elicitation. Group C showed the nystagmus upward-vertically initially.

REFERENCES

1) Philipszoon, A. J.: Nystagmus and Bell's palsy. *Pract. O.R. L.*, 24 : 919-928, 1962.
2) Robert, F., and Pfaltz, C. R.: Vestibulare Funktionsstorungen bei idiopathischer Facialisparese. *Arch. Klin. Exph. Oren-Nasen Kehlkopfheilk.*, 197 : 183, 1970.
3) Miehlke,A.: *Surgery of the Facial Nerve*, (2nd Ed) W. B. Saunders Company, Philadelphia-London-Toronto, 1973.
4) Morimoto, M. et al: Hunt syndrome and Bell's palsy (Japanese). *Otolaryngology (Tokyo)*, 24 : 16-20, 1952.
5) Fisch, U.: Transtemporal Surgery of the Internal Auditory Canal. Report of 92 cases. Technique, Indication and Results. *Adv. ORL.*, 17 : 203-240, 1970.
6) Kumagami, H.: Inner Ear Disturbances due to injection of a substance-Comment on a route of influence—. (Japanese). Jap. *J. Otol. Tokyo*, 76 : 464-467, 1973.
7) Kumagami, H.: Experimental Facial Nerve Paralysis. *Arch. Otolaryng (Chicago)*, 95 : 305-312, 1972.
8) Mitani, N.: Nystagmus induced by insertion of Black Ink into the Stylomastoid Foramen in Rabbits (Japanese). *Jap. Jour.. Otol. Tokyo*, 78 : 44-55, 1975.
9) Yamakawa, K.: Gesichte und Technik der intravitalen Fixation. Anhang: Dekapitationsfixation (Japanieren); Kubo, I. (Ed) *Handbuch d. O. N. Kkhlk, Japan.* Vol. 2-3, 422-440 Kokuseido, 1935.
10) Ogata, M.: Experimental Study on drainage operation of perilymph in the labyrinth (Japanese). *Pract. Otokogica (Kyoto)*, 51 : 732-748, 1958.
11) Harada, Y.: Surface Structure of Semicircular canal ampullae. *Equilibrium Res.*, Suppl. 4 : 53-584 : 53-58, 1972.
12) Anson, B. J., and Donaldson, J. A.: *Surgical Anatomy of the Temporal Bone and Ear*, (2nd Ed) W.B. Saunders Company, Philadelphia-London-Toronto, 1973.