VESTIBULAR ENDORGAN OF THE FROG AFTER THE SPACE FLIGHT
AND POSTURAL ALTERATION OF THE NEURECTOMIZED FROG—
ITS MORPHOLOGICAL AND FUNCTIONAL RESILIENCE

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Abstract — The vestibular organ of frogs returned from space were observed by SEM. Morphology of the sensory epithelia was normal, including ultrastructure, such as tip links and side links. The frogs’ behavior and vestibular morphology after various types of vestibular neurectomy were studied. Vestibular neurectomy resulted in tilting posture toward that side. This tilting gradually decreased to zero and the nerve regenerated. When the same nerve was cut again after postural recovery, the tilting angle was smaller and recovery period was shorter than after the first neurectomy. When the bilateral nerves were cut and neural regeneration was inhibited on one side, tilting slowly developed toward that side. These results show that frogs’ postural change is modified by both central compensation and peripheral vestibular function.

Keywords — tilting posture; vestibular neurectomy; compensation; sensory epithelia.

Introduction

The vestibular system is one of the major research targets in space science. Change of vestibulo-ocular reflex and motion sickness in weightlessness has been studied by a number of clinical experiments. Yet, animal study in space is still needed to elaborate biological effects of weightlessness at ultrastructural and electrophysiological levels. The authors had studied the frog’s vestibular system, combin-
Behavioral Change and Vestibular Morphology Due to Vestibular Neurectomy

Effect of unilateral vestibular neurectomy. Under ether anesthesia, the labyrinth of the frog was opened through a ventral approach. The superior division of the right vestibular nerve, which includes anterior and horizontal semicircular canal nerves, utricular nerve, and saccular nerve, was sectioned peripherally to the vestibular ganglion. The effects of vestibular neurectomy were reported elsewhere (1). From the day following the surgery, the angle of the head tilt was measured periodically.

For morphological study, the utricular macula and the anterior and horizontal semicircular canal cristae were immersed in 2.5% glutaraldehyde and postfixed in 2% osmium. They were dehydrated in graded ethanol and in isoamyl acetate, critical-point dried, and then coated for observation of SEM.

Effect of repeated neurectomy. In this experiment, the right vestibular nerve was sectioned first. After the tilted posture returned to normal, the right nerve was cut again. Postural change and recovery period were compared between the 1st and 2nd neurectomy.

Effect of inhibited neural regeneration. In this experiment, the bilateral vestibular nerves were sectioned. At the same time, a small piece of the palatal bone was inserted between the cut ends of the right nerve in order to inhibit neural regeneration. Postural change was likewise observed.

Behavioral Change and Vestibular Morphology Due to SM Intoxication

Five bullfrogs were used. Streptomycin sulfate (SM) 6 mg diluted in 30 µL of saline was injected into the right perilymphatic space through the palatal bone for 2 days. Saline 30 µL was injected into the left side. This is the same method as gentamicin injection reported previously (2). Postural change and vestibular morphology were observed.

Results

Morphology of the Space Frogs

The sensory epithelia of the semicircular canals and the utricle showed normal structure when observed by SEM (Figure 1). The sensory cells appeared intact without loss or fusion of cilia. The individual cilium is arranged regularly, and ultrastructure of the ciliary surface, such as tip links and side links, are also clearly seen.

Behavioral Change and Vestibular Morphology Due to Vestibular Neurectomy

Effect of unilateral vestibular neurectomy. When the right superior division of the vestibular nerve was sectioned, the frogs sustained a forced position and tilting toward that side. The average angle of the head tilt was 28.3° in the bullfrogs, and 29.0° in the tree frogs. Tilting gradually decreased to zero, and the frogs returned to normal posture (Figure 2). The average recovery period after neurectomy was 7.3 weeks in the bullfrogs and 15.5 days in the tree frogs.

Effect of repeated neurectomy. Five tree frogs and 2 bullfrogs underwent neurectomy twice. Immediately after the second neurectomy, the frogs showed a tilting posture in the same manner as after the 1st neurectomy. However, the tilting angle of the tree frogs averaged 18.0°, which is smaller than that of the 1st neurectomy (26.0° average) (Figure 3). The same was observed in the bullfrogs. The periods of postural recovery after the 1st and the 2nd neurectomies were also compared. The average recovery period after the 2nd neurectomy was 9.6 days and was shorter than that for the 1st neurectomy (13.8 days) (Figure 3). The sensory epithelia of the semicircular canals and the utricle were normal when observed by SEM.

Effect of inhibited neural regeneration. Three bullfrogs were used. Neural regeneration was
block on the right side. The time course of the head tilt is presented in Figure 4. No tilting was observed during the initial week, but tilting slowly developed toward the side with the bone piece. The tilting angle reached 20° to 40° in 4 to 5 weeks. The sensory epithelia from the side of the bone piece appeared normal even without neural regeneration.

Figure 1. Scanning electron micrograph of the posterior canal sensory epithelia of the tree frog returned from the space. The sensory cell and cilia appear normal.

Figure 2. Time course of the head tilt angle (in degrees) after unilateral neurectomy (tree frogs). The tilt angle gradually decreased to zero.
Behavioral Change and Vestibular Morphology Due to SM Intoxication

SM injection in the labyrinth resulted in severe damage to the sensory epithelia of the semicircular canals and the utricle (Figure 5). The pattern of damage was represented by loss of cilia, which involved most of the epithelial area. Behavioral change after SM injection was very different from that after neurectomy. All the frogs showed tilting toward the side of SM injection, but the head tilt remained unchanged throughout a period of 15 weeks or more in 2 frogs (Figure 6). In the other frogs, the head tilt decreased, but was not completely compensated.

Discussion

The effect of microgravity on the vestibular end organ is not fully known. As already mentioned, the sensory epithelia of the frogs that stayed in the space for 8 days remained basically intact. In humans, abnormality in VOR recovers in a few days after returning to the terrestrial condition (4). Therefore, it is hard to conceive that the weightless condition readily affects the vestibular sensory cell or cilia in a short period. Space sickness and vestibular dysfunction in weightlessness are most likely due to central confusion of the information carried from the vestibular end organ. Whether or not the vestibular end organ sustains damage after a longer stay in space will require future study.

The authors demonstrated that the tilting
postural change of the frog after vestibular neurectomy was primarily due to dysfunction of the utricle (1). The recovery pattern of the posture of the bullfrog was previously reported in detail (3). When the posture recovered normally, the sectioned nerves appeared well regenerated and united. The sensory epithelia also stayed intact and decent action potentials were recorded through the regenerated nerve (3). The tree frogs also showed a similar pattern of recovery, but the period of recovery was much shorter than that of the bullfrogs. Either central compensation or neural regeneration which develops fast may result in a fast postural recovery in tree frogs.

When the nerve was sectioned after initial postural recovery, the frogs sustained a similar forced position. Yet, the degree of tilting was smaller than that after the 1st neurectomy. Also, recovery time after the 2nd neurectomy was shorter. Flohr and colleagues (5) reported that the activity of the vestibular nucleus markedly increases following labyrinthectomy. Therefore, central compensation had already developed after the 1st neurectomy and had possibly reduced the vestibular imbalance caused by the 2nd neurectomy.

When neural regeneration was prevented by a bone piece incarcerated in the nerve, tilting slowly developed toward the side without neural regeneration. The authors already reported that the end organ–nerve unit remains viable both physiologically and morphologically after vestibular neurectomy (3). Therefore, the left nerve underwent regeneration, thus resulting in relative vestibular hypofunction in the right side.

SM injection induced marked damage to the sensory epithelia. The frogs tilted toward the side of SM injection. This is a change comparable to gentamicin injection previously reported (2). A noticeable finding in SM injection is that the recovery was incomplete and, furthermore, the recovery period was much longer than the neurectomy. This is possibly because the damaged sensory cells did not regenerate and only central compensation contributed to postural recovery.

Figure 5. Scanning electron micrograph of the utricular sensory epithelia induced by SM intoxication (bullfrog). Number of sensory cells markedly decreased.
Figure 6. Time course of the head tilt angle after SM intoxication (bullfrog). Note that tilting slowly decreased and recovery was not complete.

The present study suggests that development of postural recovery is modified by a functional balance between the central compensation and the end organ-nerve unit activity as far as the vestibular nerve has a potential for regeneration. Frogs seem to serve as a convenient model to study how the compensatory process is affected and how compensation can be accelerated under weightlessness.

REFERENCES