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The effects of gravity on the development of otolith organs in the Japanese quail

Melissa Lowe

THE EFFECTS OF GRAVITY ON THE DEVELOPMENT OF OTOLITH ORGANS IN THE JAPANESE QUAIL

by

Melissa Lowe

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Approved by: David Dickman, Ph.D., Independent Study Advisor

Introduction

Imagine waking up and not being able to get out of bed because of this overpowering feeling of dizziness or constantly experiencing motion sickness because you can not seem to focus on anything. Many people with vestibular problems experience such problems as vertigo, dizziness, and nausea.

The vestibular system located within the inner ear is responsible for maintaining balance. The structures that are most important within this system are fluid filled semi-circular canals and otolith organs. Each of these structures reacts differently in order to maintain our sense of balance during movement. The semicircular canals detect rotational movement, resulting from turns of the head and/or body, whereas the otolith organs have tiny crystals, otoconia, which are sensitive to gravity. In humans, the otolith organs are found within the utricle and saccule (Dickman, 2002). Birds have a third structure used in maintaining balance known as the lagena, but this structure is virtually unexplored and little is known about its function (Rosenhall, 1970).

NASA is particularly interested in how the vestibular system works since many of their astronauts become ill during space flight. In order to obtain basic information about vestibular system development, the use of animal models was necessary . Japanese quail were chosen since these animals have a short gestational period (16 days), and are self-contained during development (Padgett & Ivey, 1960). The latter was extremely important as a part of this project, since eggs were sent up into space in order to determine how gravity effects the development of the otolith organs. Quail eggs were flown on Endeavor, STS-108 in December, 2001 in a specially designed incubator. During the development period, several eggs were injected with a fixative to stop the development at day 4 and day 7. Other animals were allowed

to develop to 12 days when the shuttle returned to Earth. In order to see how development progresses, it is necessary to examine the structures at different time periods.

This particular study is unique in that it is the first to examine the detailed embryologic development of the otolith organs in quail. Several studies have been performed to examine the development of the otolith organs in zebrafish (Riley & Moorman, 2000), as well as chick embryos (Kido et al, 1993, Ballarino and Howland, 1982). Further studies have been done to examine the adult sensory vestibular structures of other animals including monkeys (Fernandez et al, 1995), chinchillas (Lysakowski & Goldberg, 1997), snails (Gao & Wiederhold, 1997), and pigeons (Si et al, 2002; Zakir et al, 2002). Nevertheless, no studies have previously been done to examine the otolith organs of the developing quail embryo.

All the information collected during this study will be used as baseline data to compare with the data collected from the space flight tissue. This will allow for a direct comparison between the tissue measurements to determine if gravity had an effect on the developmental process. Some studies have been done to determine the effects of gravity on the developing chick embryo (Hara et al, 1995). In the study by Hara et al, the developing eggs were exposed to hypergravity (2G) using a centrifuge. Results indicated a change in otoconial development to compensate for the increase in gravity. Exposure to hypergravity resulted in smaller otoconia stones. On the other hand, exposure to microgravity seems to result in larger otoconia stones (Wiederhold et al, 2000). Similar results are expected for quail exposed to microgravity.

METHODS

All experiments were conducted on Japanese quail embryos from embryonic day 6 (E6) through embryonic day 15 (E15). All methods and procedures were approved and in accordance with the rules of the Institutional Animal Care and Use Committee. The eggs were allowed to

develop in a forced air incubator (Humidaire, Inc.) in the laboratory. The incubator maintained the ambient temperature at 37°C with 60% relative humidity. In order to thoroughly examine the otolith organs, the structures were removed from the embryo once they had reached the appropriate developmental stage. The shell was cut open and the embryo was removed. The embryo was anesthetized in ice-cold saline and decapitated. The head was then bisected along the midline and the brain was removed under a dissecting microscope. The vestibular organs were preserved by injecting fixative directly into the vestibular labyrinth using a tuberculin syringe. The fixative consisted of 2.5% glutaraldehyde in 0.1M phosphate buffer with a pH of 7.4 and allowed to set overnight. The temporal bone was removed and immersed in the same fixative for 16hrs at 4°C. Afterwards, the individual vestibular end organs were dissected free from the cartilaginous temporal bone. The fused otoconia stones were then separated from the sensory epithelium of the saccule and utricle. The fused otoconia stones and the vestibular end organs were stored in 0.1M phosphate buffer, with a pH of 7.4 at 4°C.

The tissue was prepared for scanning electron microscopy (SEM) using a procedure similar to that used to examine the inner ear organs of adult pigeons (Frank et al, 1999; Dye et al, 1999). First, the tissue went through six serial washings of distilled water. In order for the scanning electron microscopy to work, the tissue must be dehydrated, therefore the tissue was dried using a graded series of acetones (70%, 90%, 95%, and 100%). The tissue was washed for 10 minutes each in the 70%, 90%, and 95% acetone followed by three washes for 15 minutes in 100% acetone. The tissue was then transferred to a 100% tetramethylsilane (TMS) solution and washed for 10 minutes. The tissue was placed in fresh TMS solution and the liquid was allowed to evaporate by leaving it in a 60 degree centigrade oven for thirty minutes. Finally, the tissue

E13 UTRICLE OTOCONIA STONE

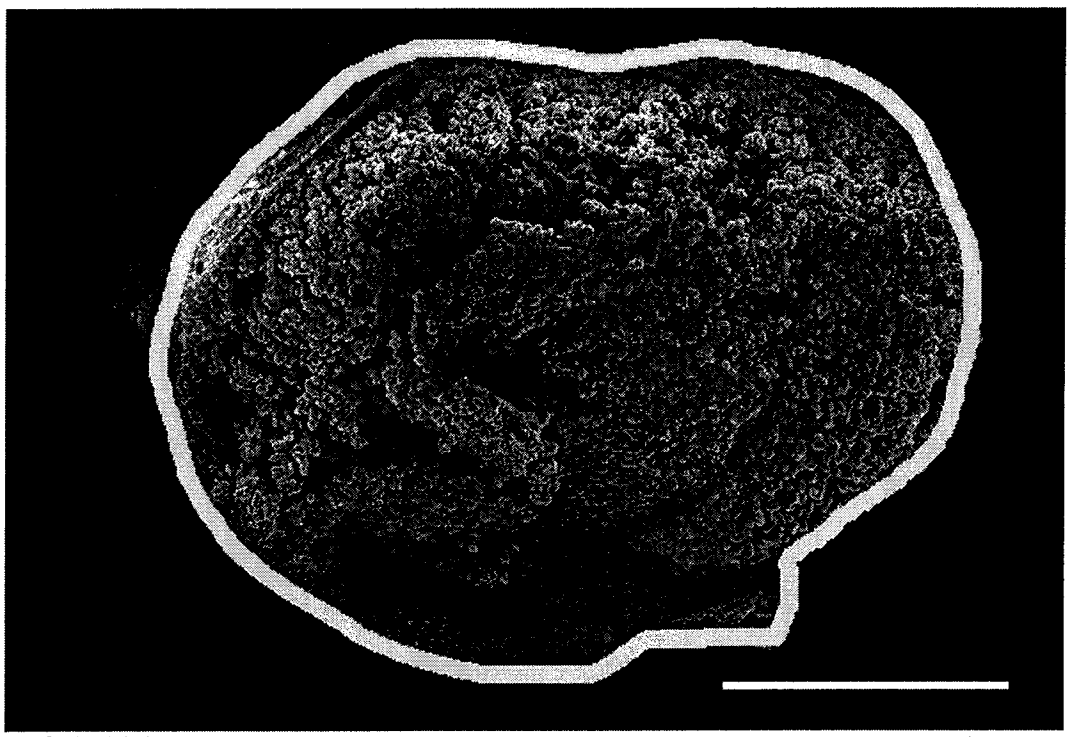
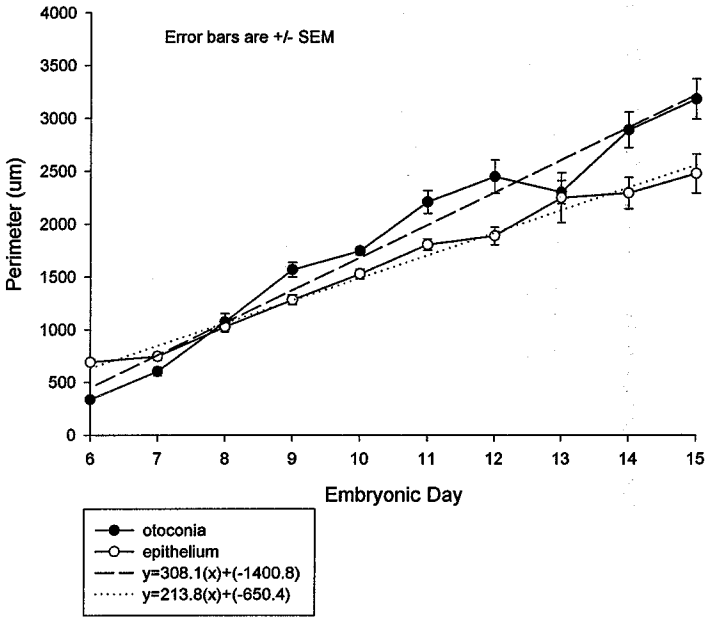


FIGURE 1

was mounted on aluminum studs with carbon tape and coated with palladium (Tousimis, Samsputter 2a). The tissue was stored in a dessiccator at room temperature.

The tissue was scanned and photographed using a Hitachi 2600 scanning electron microscope (20kV). Once the pictures were captured on the SEM scope, they were transferred to another computer equipped with a three-dimensional reconstruction program, Neurolucida (MicroBrightField, Inc.). Images of the otoconia and epithelial surface were traced and measurements of the area and perimeter of the otolith structures were calculated. The measurements were entered into a spreadsheet where the average, standard deviation, and standard error of the mean were calculated. Graphs were plotted, indicating the growth pattern of the area and perimeter of both the otoconia stones and the utricle and saccule epithelium.

FIGURE 2



RESULTS

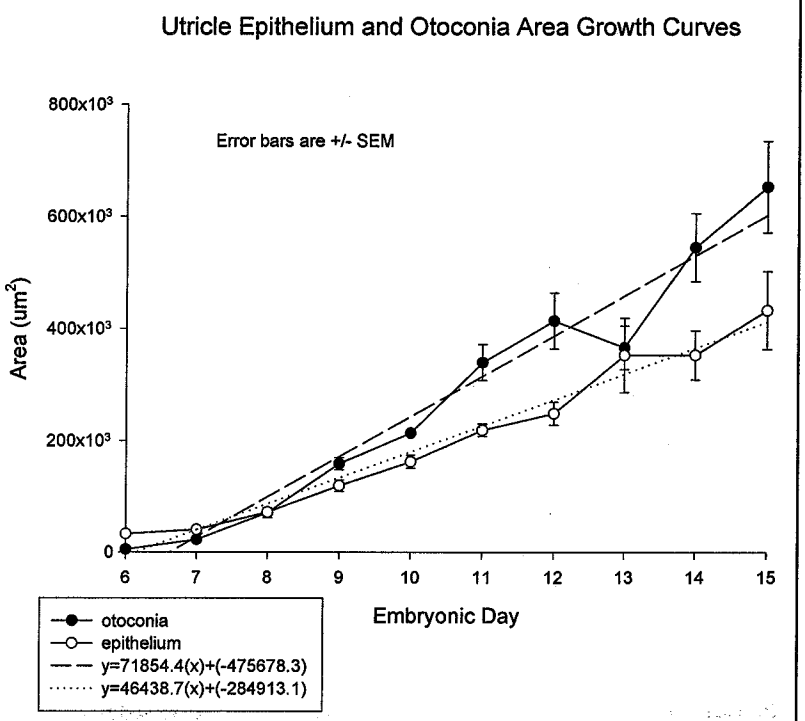
No previous studies have been conducted in which the embryonic development of quail otoconia were examined. Therefore, the results of this study are relatively unique. Data reported here will be used as a direct comparison to that obtained from the space flight tissue.

As shown in Figure 1, the utricle otoconial stone is oval shaped. The perimeter and area were easily measured using the Neurolucida software. The white line along the perimeter indicates the epithelial edge traced using the software and video image. The scale bar for this particular image equals 250μm.

Approximately three or more utricle and saccule epithelium tissue and otoconia stones from each embryonic day were measured. The resulting means for each embryonic day are plotted on the following graphs.

The mean perimeter measures of both the otoconia stones and the sensory epithelium of the utricle were observed to increase in size from E6-E15. It was noted that the sensory epithelium tissue seems to start out larger than the respective otoconia stone. However, around the eighth day of development, the otoconia stone seems to bypass the epithelium tissue and increase in size at a faster rate than the epithelium tissue. Both of these growth rates appeared to

FIGURE 3



be linear, as shown by the total regressions in Figure 2. The slope of the regressions also shows that the growth rates were different for the stones and the epithelium. As shown in Figure 3, similar results were observed for the area measures of the utricle. However, the area increased over development as an exponential function.

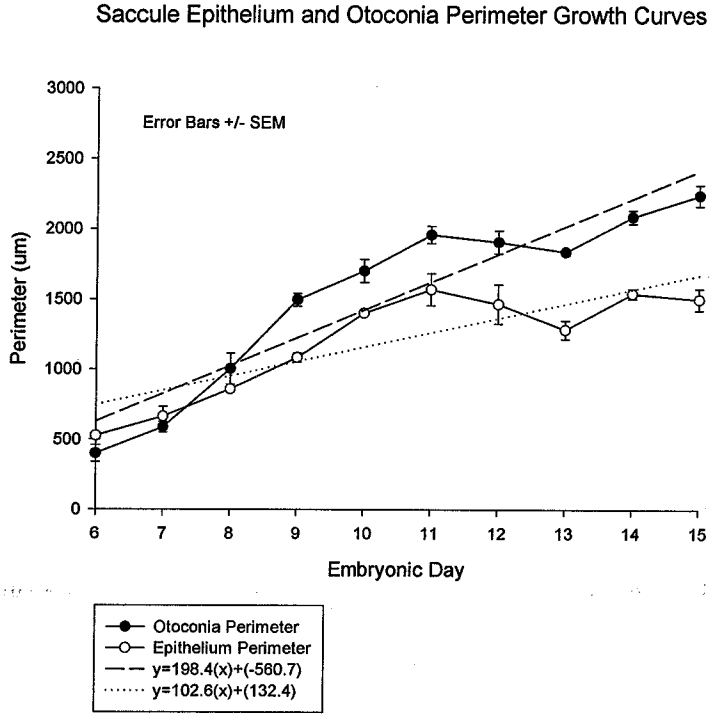
For the saccule, different growth curves were observed during embryonic development, as shown in Figures 4 and 5. The otoconia stones and the sensory epithelium tissue of the saccule increased rapidly during days E6-E11. During the last half of development (E11-E15), however, the rate of increase declined to an asymptotic level. These growth curves indicate that a decreasing exponential growth rate occurs in the saccule, as shown by the fitted functions on the following graphs.

The data also seemed to reveal a dip in development at E13. This drop was consistent across all the data and the possible implications will be discussed later on in the paper.

Discussion

In any scientific study, baseline data is essential to the overall project; without it, it is impossible to make any definitive decisions on the data collected during the study. In order to determine the effect gravity had on the development of the otolith organs, it was necessary to

FIGURE 4

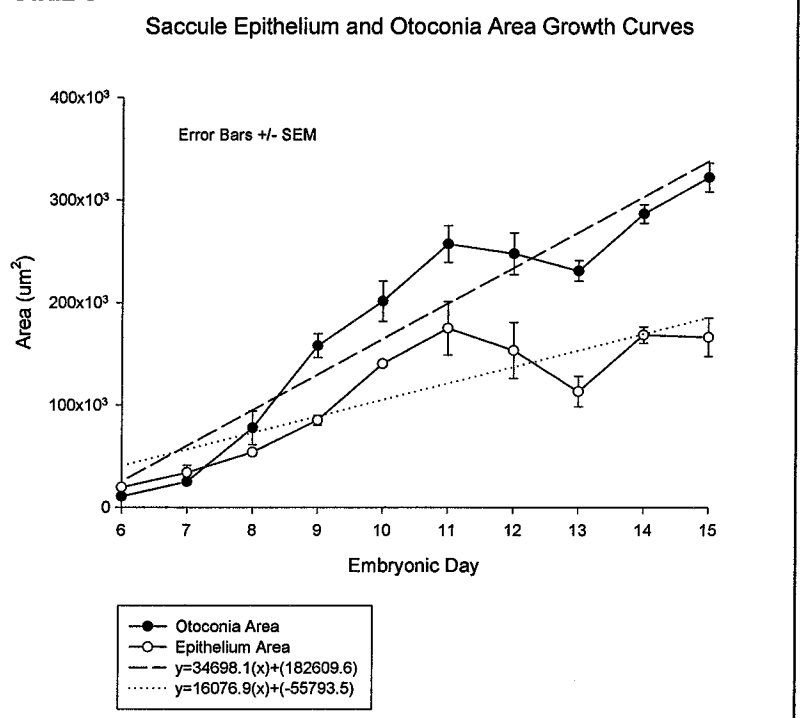


gather data on the development of those structures under normal conditions. Therefore, since no other data is available documenting the developmental growth of the otolith organs in quail, the results of this study will be used as baseline data. Once measurements are completed on the space flight tissue, that data will be compared with the results of this study to determine if gravity had an effect on the developing otolith organs.

The data in this study represents the growth of otoconia and epithelium tissue under normal conditions. The growth of otoconia appears to be related to exposure to gravity during development. For instance, Hara et al (1995) found that when chick embryos were exposed continuously to 2G gravity, the otoconia stones were much smaller. On the other hand, Wiederhold et al (2000) found that when snails were exposed to microgravity, their sensory organs were much larger in size.

The results of data obtained in this study indicate a relatively linear growth pattern for both the utricle otoconia and epithelium tissue. The growth rate of the otoconia stone versus the

FIGURE 5



sensory epithelium was found to be faster, with a gradual decrease in growth rate after day E11, particularly in the saccule epithelium. For the saccule, the otoconia stone and sensory epithelium appeared to develop at a rapid rate during days E6-E11. However, during the last half of development (E11-E15), the growth rate seemed to decrease dramatically.

In all graphs, a notch was noted around day E13. It is hypothesized that this notch may be due to the tissue changing shape and becoming more compact. However, there is no evidence to prove such a claim, and more research is needed.

More research is needed studying the normal development of otolith organs in quail. The data collected during this project is preliminary and should be compared with other normative data collected on quail.

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