Gerbil middle ear development: soft tissue and bones

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Background: The present study aims to better understand the changes in the middle ear's sound transmission during early development. Using X-ray micro-tomography, we quantified dimensions and ossification of the middle ear ossicles in gerbils aged 0-21 days. Measures include the stapes and oval window footplate areas, the distance between ossified sections of the ossicles at joint faces, and volume fractions of the bones and soft tissue structures. In contrast to previous studies of middle ear development which focused on the mineralized tissue, this study considers soft tissue and monitors the progression of ossification.

Methods: Gerbils of either sex, aged 0 to 21 days, were included in this study. After euthanizing the animals with a lethal dose of ≥100 mg/kg Euthasol (390 mg/ml sodium pentobarbital and 50 mg/ml sodium phenytoin), the temporal bones were harvested and placed immediately in a -40 degrees freezer and kept frozen until imaging at the I13L beamline at Diamond Light Source, Oxfordshire, UK. The central energy of the pink beam was 30 keV. The imaging setup consisted of a rotating sample stage and a PCO Edge 5.5 CCD camera with an objective lens providing a total magnification of either 2.5x or 4x. For the tomographic scans, the samples were secured in an upside-down Eppendorf tube. Saline solution was placed in the cap of the Eppendorf tube to reduce sample dehydration. Data was recorded in fly scan mode over 180 degrees and with 2160 projections. The exposure time was 0.1 sec. Twenty dark field and bright field projections were collected. The sample-detector distance was 323 mm for the ossified and 600 mm for the non-ossified or decalcified middle ears. Reconstruction procedures included flat-field correction, image distortion correction, phase-retrieval, image reconstruction, and ring artifact reduction. The resulting slices were rendered to a three-dimensional volume with customized Python code.

Results: Image rendering provides three-dimensional models of the ossified sections of the developing ossicles. Measurements from images of ossified middle ear ossicles showed that the footplate and oval window growth were similar to previously reported measures in gerbils (which is?). Volume fraction has not previously been measured in gerbils, but the growth pattern mirrors the pattern reported in humans, supporting gerbils as an animal model for middle ear development. Measurements from non-ossified or decalcified ossicles did not show size changes between 0 and 21 days. Due to the use of different animals for each of the 17-days, there is greater variability and random scatter in the results.

Conclusions: The decalcified tissue does not change size and shape over time; these findings may challenge results from previous studies using microtomography and middle ear development. Understanding the development process of decalcified tissue may give novel insight into the manifestation of diseases and the function of hearing.