## Otoconial Mass of the Bullfrog Sacculus

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In order to study the effects of mechanical loads on hair-bundle function, one must consider the effects of accessory structures in the intact organ. In the sacculus, hair bundles protruding from the sensory epithelium are coupled to an overlying otolithic membrane. Sitting atop this membrane is an otoconial mass, divided in two along its striola. While we have estimates of the elastic load imposed by the otolithic membrane (1350  $\mu N \cdot m^{-1}$  [1]), the mass load imposed by the overlying otoconia has not been reported.

Here we measure the otoconial mass of the sacculus of the American bullfrog, Rana catesbeiana. Measurements were taken for two ears in two female frogs. Prior to dissection, we measured the mass of a small petri dish in three independent measurements, yielding  $1.74430\pm0.00002~g$ . Each sacculus was dissected and excess tissue removed, leaving only otolith in the petri dish after completion of this process. We let the contents dry, covered, over a period of five hours. We next measured the mass of the petri dish with added otoconia in three independent measurements, yielding  $1.75510\pm0.00008~g$ . This yielded a difference of  $0.0108\pm0.0001~g$ .

Given these data, we find a mass of approximately 0.0054 g for the otoconial mass of a single sacculus. Calcium carbonate, the primary component of this mass, has a density of 2.71  $g \cdot \text{cm}^3$ . With a mass of 5.4 mg, this yields a sphere with a radius of 781  $\mu m$ . This value is within the range of the sacculus of the bullfrog, which has a diameter of approximately 600-900  $\mu m$ .

The bullfrog sacculus is estimated to have roughly 3,000 hair cells [2]. Given this value, one might expect an individual hair bundle to feel the effect of approximately 3.6 ug of mass load. If we assume a stiffness of 1350  $\mu N \cdot m^{-1}$ , this yields a characteristic frequency of:

$$f = \frac{1}{2\pi} \sqrt{\frac{1350 \times 10^{-6} N \cdot m^{-1}}{3.6 \times 10^{-9} kg}} = 97.5 Hz,$$

a value within the range of best frequencies measured from saccular afferents in vivo [3]. Given a frequency range of of 35-90 Hz (Figures 2A-5A from [3]) and a stiffness of  $(100~\mu N \cdot m^{-1})$ , this yields an estimated mass range of 3-21ug.

This mass measurement is contrasted with the 35-100  $ng \cdot row^{-1}$  experienced by outer hair cells coupled to the tectorial membrane within the cochlea of the Mongolian gerbil [4]. Perhaps one might expect the mass load between an auditory and vestibular organ to differ by 40-100 times, and this result may thus be unsurprising. Nonetheless, it is important to note that a study of the effects of mass loading a vestibular hair bundle may require values much greater than those experienced by hair bundles of auditory and lateral-line organs.

## References

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