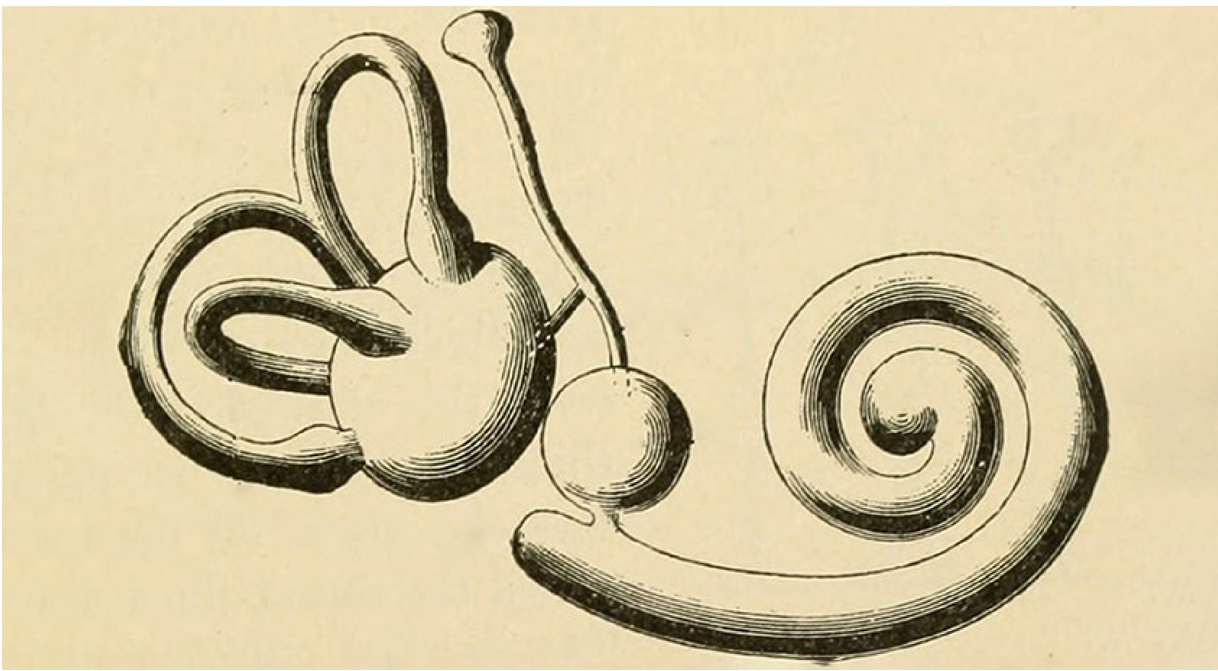
New study reveals the function of a mysterious component of the inner ear

June 28 2018, by Kevin Jiang



The human inner ear illustrated in a textbook circa 1897. The endolymphatic sac (top center) its connected to the rest of the inner ear via a long thin duct. Credit: Harvard Medical School

A few years ago, Ian Swinburne, HMS research fellow in systems biology, noticed something odd while conducting a time-lapse microscopy study of the inner ear of zebrafish. A tiny structure in the inner ear was pulsing like clockwork, inflating and deflating over and over.

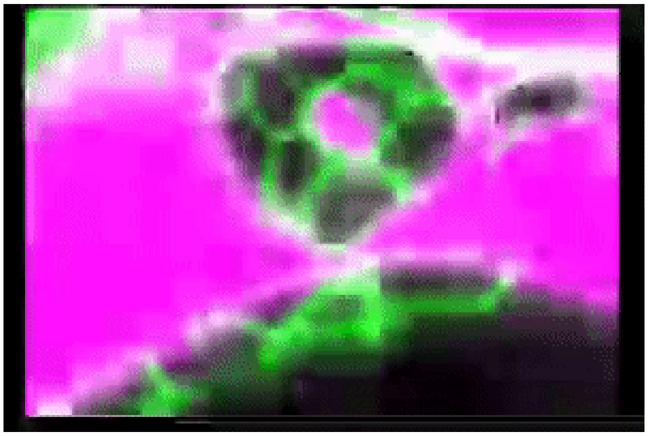
As Swinburne and his postdoctoral advisor Sean Megason, HMS associate professor of systems biology, probed further, they discovered that the structure was the endolymphatic sac, a fluid-filled pocket connected to the rest of the inner ear by a long, thin duct.

But neither could explain why it was pulsing.

"Scientists have known about the existence of the endolymphatic sac for maybe 300 years, but it wasn't understood exactly what it does," Megason said. "It's even often missing in models or textbook cartoons of the inner ear. We didn't set out to study it, but we became interested once we saw its striking behavior."

Over the course of the next several years, Swinburne and Megason worked to better understand the function of this mysterious structure. To do so, they had to visualize it in action. Collaborating with some of the world's leading microscopy laboratories, they pieced together different views of the endolymphatic sac until a clear picture emerged.

In a study published June 19 in *eLife*, the team reported the results of their investigation: the endolymphatic sac acts as a pressure-relief valve and is formed by a thin barrier of cellular projections that opens and closes to regulate the release of fluid from inside the inner ear.



Endolymphatic sac inflating and deflating. Credit: Swinburne/Megason

Their findings reveal a unique biological mechanism for maintaining fluid pressure and composition and may inform the study and treatment of disorders involving defects in inner ear pressure such as Meniere's disease, a condition marked by vertigo, hearing loss and ringing in the ears. The results could also help researchers study pressure control in other organs such as the eyes and kidneys, which also have liquid-filled cavities.

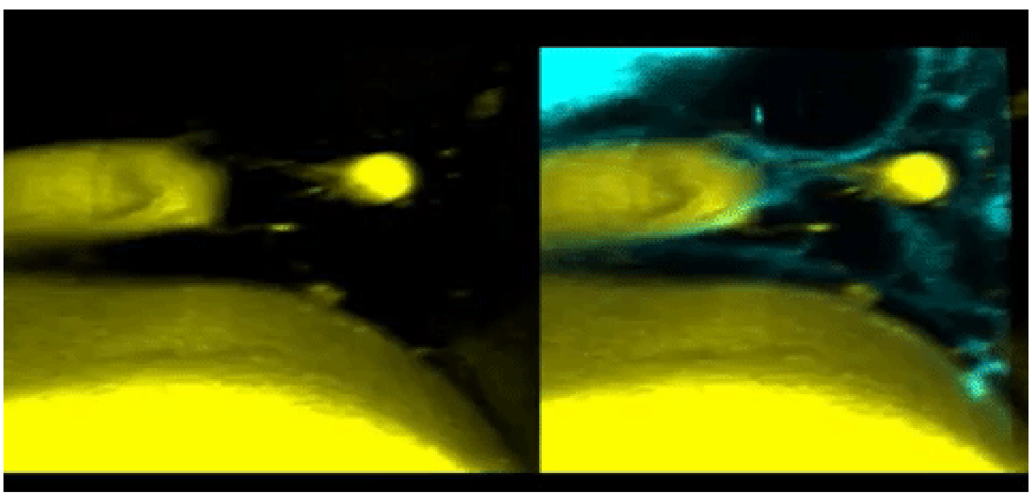
"Every once in a while, you hear about a house being destroyed by a water heater because its pressure release valve was defective," Swinburne said. "It's important to have these safety control systems in our organs as well."

Something Weird

The inner ear is the sensory organ responsible for hearing and balance and is composed of several complex structures. In mammals, sound is detected by the snail shell-shaped cochlea, and head movement is detected by three hollow loops of bone called the semicircular canals.

All the structures of the inner ear are interconnected and filled with specialized fluid, which moves in response to sound waves or head movement. These subtle fluid movements are detected by sensory cells and converted into neural signals for the brain to process. Both the pressure and chemical composition of inner ear fluid must be carefully maintained, and certain disorders such as Ménière's disease are thought to stem from abnormal pressure fluctuations.

Scientists have long hypothesized that the endolymphatic sac plays a role in regulating the pressure of this fluid, but the mammalian inner ear is small and encased by extremely dense bone, which makes it difficult to access and study.



Dye injection reveals inner ear fluid (yellow) escaping through the endolymphatic sac. Credit: Swinburne/Megason.

The inner ears of zebrafish embryos, which Swinburne and Megason study, are much more visible. When the team first observed the pulsing behavior of the endolymphatic sac, they suspected a connection with pressure control. Proving it, however, was another matter.

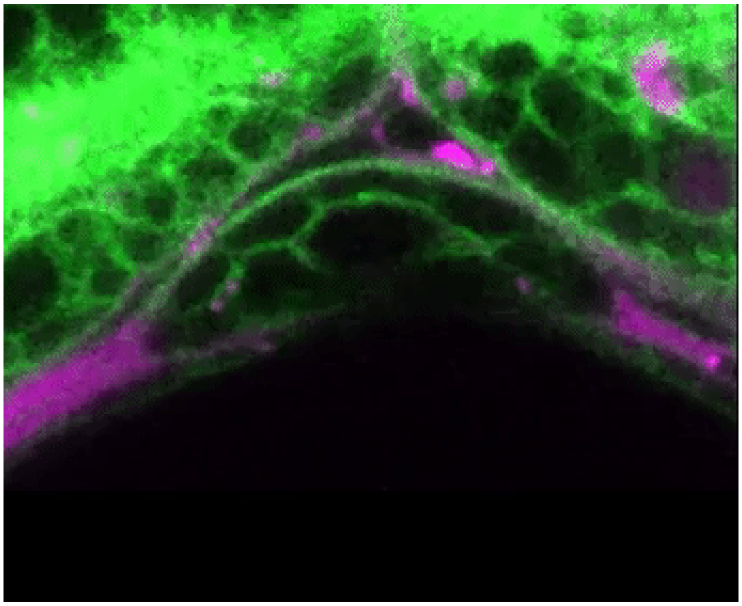
"We had all these movies where you could see the whole structure pulsing, and when Ian injected dye into the sac we could see fluid flowing out," Megason said. "But it wasn't clear how that fluid was getting out. It seemed like something weird was going on."

Eureka Moment

At that time, Swinburne was also engaged in a side project reviewing previously published studies of various zebrafish with mutated genes. One of the mutants he stumbled upon, with an abnormal form of the transcription factor lmx1bb, had endolymphatic sacs that were far larger than normal.

Through dye injection experiments, they discovered that in lmx1bb mutants, inner ear fluid was not flowing out of the endolymphatic sac as it should, and the buildup of fluid caused the structure to balloon. The team noted that normally, a little bit of fluid also leaks back into the sac when it deflates. No such leakage occurred in lmx1bb mutants, however, suggesting that the structure was somehow closed.

They were stumped until they connected with Jeff Lichtman, the Jeremy R. Knowles Professor of Molecular and Cellular Biology and the Santiago Ramón y Cajal Professor of Arts and Sciences at Harvard University. The Lichtman lab specializes in imaging the brain, and among the data they've collected happened to be high-resolution electron micrographs of the inner ear.



Mutations to lmx1bb cause abnormal ballooning of the endolymphatic sac. Credit: Swinburne/Megason

When Swinburne and Megason analyzed these images, they observed flap-like membrane projections called lamella extending from cells that make up the endolymphatic sac. These flaps overlapped with each other, forming a barrier.

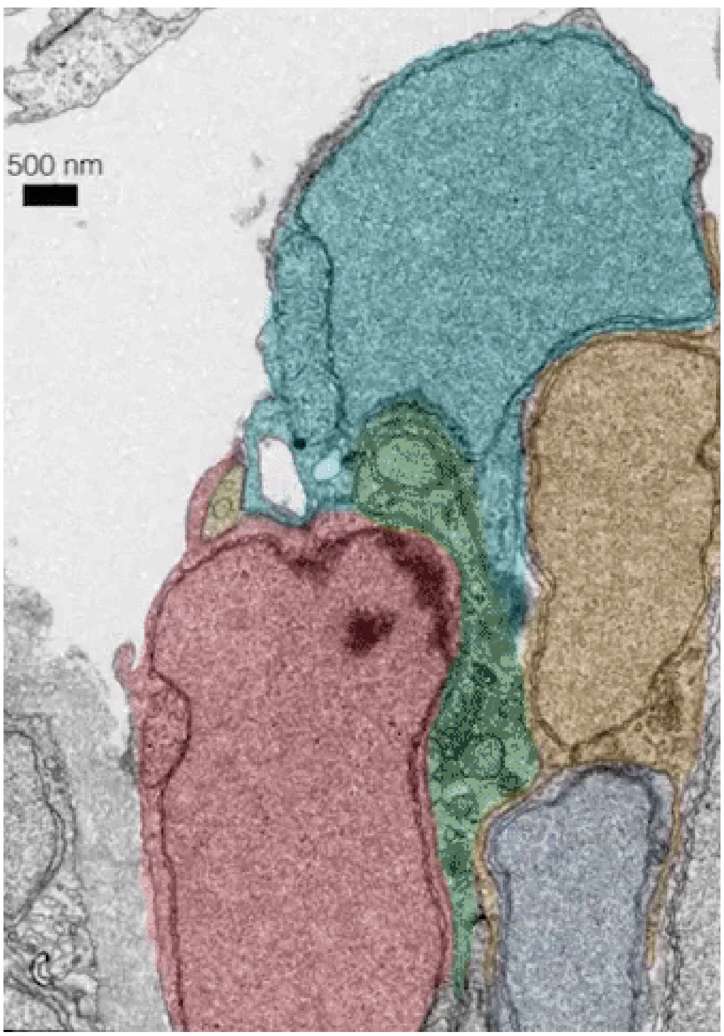
"Biologists like to say that structure determines function. When we saw the lamella for the first time, it all clicked," Swinburne said.

"It was a eureka moment," Megason added.

Relief at Last

The team's analyses revealed that normal endolymphatic sacs contain an extremely thin shell of these overlapping lamellae, which they termed "lamellar barriers." In most tissues, cells are tightly connected and water cannot pass between them. In the endolymphatic sac, however, cells appeared to have small gaps between them, which are covered by lamellar barriers.

When fluid pressure builds, the sac inflates and the barriers begin to separate. Once a certain point is reached, the barriers open, allowing fluid to flow out of the sac and relieve pressure.



Serial electron microscope images reveal flaps of cellular projections that form the lamellar barrier. Credit: Swinburne/Megason

To further investigate, the researchers teamed up with microscopy pioneer and Nobel laureate Eric Betzig of the Howard Hughes Medical Institute and Tomas Kirchhausen, HMS professor of cell biology and a professor of pediatrics at Boston Children's Hospital.

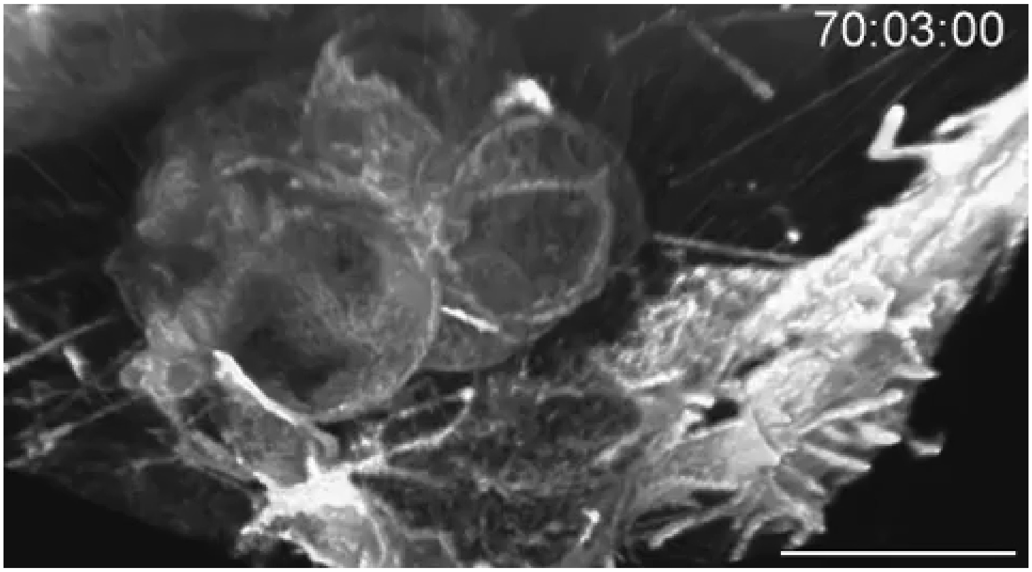
Earlier this year, Betzig, Kirchhausen, Megason and colleagues published a seminal paper describing a new technology called adaptive optics-lattice light sheet microscopy, which allows researchers to capture 3-D images and movies of cells inside living organisms in unprecedented detail.

The team applied this technology to the endolymphatic sac, and observed that the lamellar barrier actively and dynamically moves as the sac inflates and deflates.

"They're constantly crawling. It looks like a cell that's migrating, but they are part of the epithelium. It's really weird cell biology," Swinburne said.

Their results implicate the endolymphatic sac as a pressure-relief valve for the inner ear, but many mysteries remain for future study, such as how the lamellar barriers connect with each other, whether they are opened by physical pressure or some pressure-sensing protein and whether this same mechanism is present in other animals such as mice and humans.

The team also suspects that this mechanism may be present in other organs, such as the eye, brain and kidneys, which also contain pressurized fluid-filled cavities. Of particular interest is the role of genes related to lmx1bb, which, when mutated in mice, cause kidney and eye problems.



A 3-D movie of the endolymphatic sac reveals active, dynamic lamellar barrier behavior as the sac inflates and deflates. Credit: Swinburne/Megason.

Mutations to lmx1 genes in humans have been linked to glaucoma, a condition where fluid builds up in the front part of the eye. A better understanding of lamellar barriers and pressure- relief mechanisms could help inform the study and treatment of these diseases, the authors suggest.

"This study was definitely a case of seeing is believing," Megason said. "It was very important to have cutting-edge microscopy on many different fronts. Each of these different microscope techniques gave us a different piece of the puzzle and when put together, we get the whole picture."

More information: Ian A Swinburne et al, Lamellar projections in the endolymphatic sac act as a relief valve to regulate inner ear pressure, *eLife* (2018). DOI: 10.7554/eLife.37131