SPONTANEOUS OSCILLATIONS IN HAIR CELLS OF THE BULLFROG SACCULUS

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It has long been observed that there is some form of amplification process in the inner ear, and one prominent sign of this process is the spontaneous oscillation of hair cells in the sacculus of the bullfrog, *Rana catesbeiana*. These oscillations have been previously observed and studied, however the frequency of said oscillations with regards to the innervation patterns of the bullfrog sacculus had not yet been mapped. Innervation patterns of other bullfrog hearing organs suggests that there is some correlation between location of the cell and the frequency of the vibration that is picked up acoustically, suggesting that there might perhaps be some link as well between the frequency of the spontaneous oscillations of the hair cells and the area of the nerve fiber that it is located. Unfortunately, results were very inconclusive and the correlation between oscillation frequency and location on the sacculus remains unknown.

Introduction

It has long been known that the inner ear is an active system. The human ear can detect sounds that are both huge and very small deflections of the inner ear hair cells, and such a range, 140dB of sound, must have an amplification system inside of it in order to function so well. This amplification system is best observed in the form of otoacoustic emissions, which are emissions of the inner ear that can be recorded by a microphone after a sound is emitted into the ear. These emissions are thought to be the response of the inner ear cells to the stimulus noise, where the hair cells themselves deflect, moving in turn the inner ear membrane, bones, and, finally, eardrum, which acts as a speaker. These otoacoustic emissions are a sign of an active process inside of the ear, where nonlinear amplification takes place.

Another effect of the active process of the system is the setting up of spontaneous oscillations due to the inherent nonlinearity of the hair cell system, where the stereocilia on the top of the hair cell will oscillate randomly for a relatively short period of time. While the amplification process is thought to be understood in mammals through membrane based electromotility (Dallos, 1992, and Nobili et.al., 1998), this function does not exist outside of mammals (Martin P and Bozovic D 2003), because other classes of animals do not possess electromotile cells. The spontaneous oscillations, therefore, are thought to be linked to the source of amplification in animals other than mammals.

A better view into the workings of spontaneous oscillations in the bullfrog would therefore contribute to the knowledge of how the amplification process in amphibians is set up, and mapping spatially the frequency distribution of the oscillations would give better insight into whether the amplification existed within the sacculus hair cell structure itself, or whether it was a product of a higher function in the body.

Materials and Methods

The inner ears of the bullfrog were first extracted from the bullfrog in an endolymph solution consisting of (in m_M) 110 Na^+, 2 K^+, 4 Ca^{2+}, 122 Cl^-, 3 $_D$-glucose, and 5 HEPES (as outlined in Martin and Bozovic, 2003). The area around the roots of the nerve of the sacculus was cut from the rest of the sacculus, leaving a relatively two dimensional circular surface that contained the hair cells connected to the otolithic membrane. The said surface was then subjected to a 30 minute exposure to 40 µg/ml protease type XXIV in N-methyl-$_D$-glucamine (NMDG) endolymph containing (in m_M): 2 Na^+, 3 K^+, .25 Ca^{2+}, 110 NMDG, 111 Cl^-, 3 $_D$-glucose, and 5 HEPES (Martin and Bozovic, 2003). An eyelash tool was then used to remove the otolithic membrane from the hair cells, and the sample was mounted in a two chamber compartment to view (Martin and Hudspeth, 1999). The cell side of the compartment was then covered with an artificial endolymph composed of (in m_M): 2 Na^+, 118 K^+, .25 Ca^{2+}, 118 Cl^-, 3 $_D$-glucose, and 5 HEPES (Martin and Bozovic, 2003). All solutions were kept with a pH of around 7.3 and an osmotic strength of about 230 mmol/kg, as done in Martin and Bozovic, 2003.

A standup microscope with a 20x water emersion lens, powered with a 100 W light source, was used to image the cells. Because the movement of the oscillations of hair cells can be very small the image then went through a series of lenses to send it into a CCD camera (where additional variable magnification was used as well), and this video signal was sent to a DVD video recording machine and a video monitor. The entire microscope, lens, and DVD recorder were all floated on a mechanical apparatus designed to eliminate extraneous signals, and the entire setup was run in a soundproof booth, and in this way significant error due to outside vibrations were eliminated. Each prepared sample was searched for spontaneous oscillations using lateral crossings of the innervated area of the sacculus, and any areas where cells were moving were recorded for longer lengths of time.

The obtained video footage was sent into a video conversion software program that converted the files to .mpeg files, which were then fed into a freeware tracking program (Tracker: Open Source Physics Java Video Analysis) obtained off of the Open Source Physics website (http://www.cabrillo.edu/%7edbrown/tracker/index.html). A line profile was taken of the moving cell, and, because only the frequency was needed, the profile was searched for a pixel that oscillated between bright and dark at a frequency close to that of the cell, with the idea that the transition from dark to bright to dark again would correspond with the stereocilia tip moving from a position over the pixel to one farther away, causing the pixel to change brightness.

The pixel brightness over time was then mapped into Microsoft Excel. Because of the inherent irregularity of frequencies of spontaneous oscillations of hair cells a Fourier transform was not useful, however, it was possible to view the movement and get an approximation of the frequency of the movement of the cell. This frequency was then compared to an eyeballed approximation of the frequency and, if the two were found to correlate, the approximate frequency was verified for the cell. The cell location was then mapped onto a picture of a sacculus in order to see if there was any correlation between frequency of the spontaneous oscillation and of the location on the sacculus.

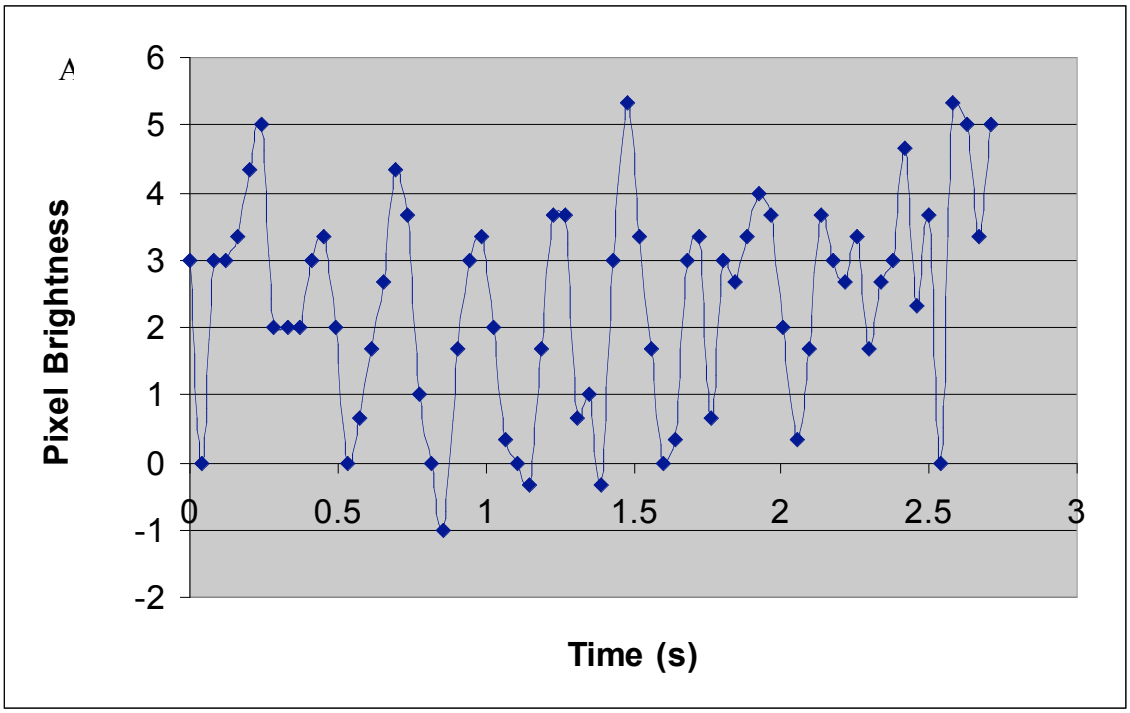
Results

Analysis of the Video

During the capture of the video of the sacculus, there were several hair cells that were observed to be spontaneously oscillating. They were observed initially to be at various frequencies between 1 and 10 Hz, and they were found in multiple preparations of the sacculus, for both ears. These were recorded for a period of about 10 seconds, and then the position of the hair cell in the sacculus itself was determined by zooming out and marking down the location of the cell.

While many of the hair cells that were oscillating produced movement that was either too small of a deflection or too fast to analyze accurately with the motion analysis method that was being used, there were some cells that moved both widely and slowly enough to be accurately analyzed (FIG. 1). The range of frequency for this motion was anywhere from 3 to 6 Hz, and the typical deflection was much smaller than the length of the cell bundle itself (approximately 3µm), moving anywhere from 25 to 80 nm.

It could be seen that though the cell exhibited a typical frequency range for oscillation there were definite changes in the frequency of oscillations in one cell's spontaneous oscillation period. The cells could be seen on the video to first oscillate rapidly with a wide range, then to slow down with shallower movements, and then to proceed back to wide and rapid oscillations. This behavior has been more extensively looked at in Martin and Bozovic, 2003, where an electrical signal was used to map the exact oscillation pattern of the cells' movements.



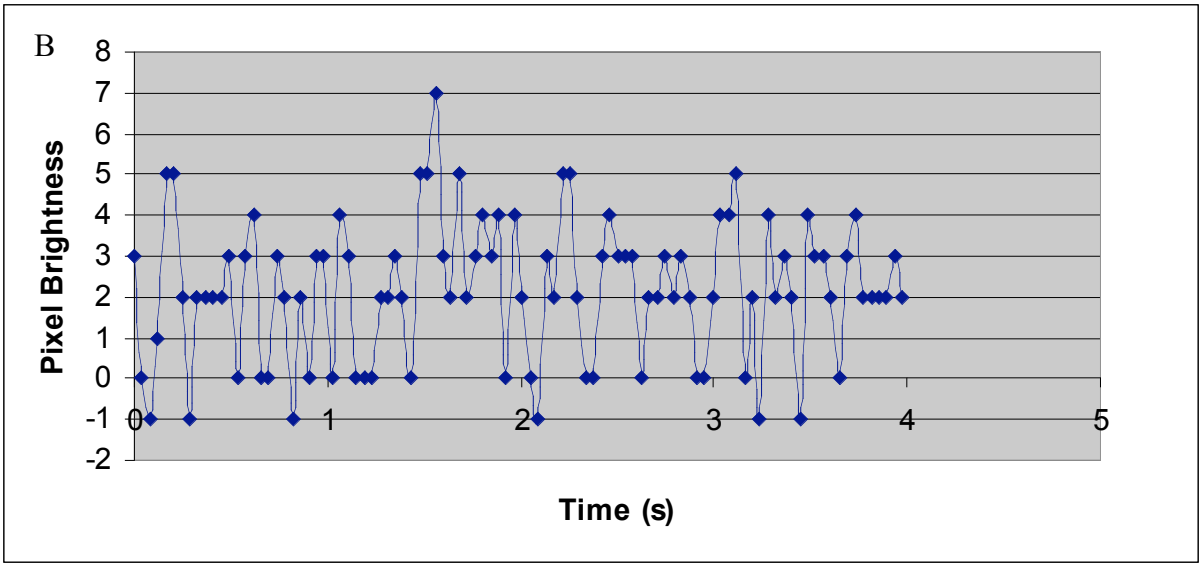


FIG 1. The brightness of a pixel as it varies over time. The frequency of the brightness of this pixel is correlated to the frequency of the cell that is oscillating spontaneously. Both (a) and (b) showed signs of a frequency that matched that of what was seen on the video. The frequency of (a) can be seen to be around 4 Hz, whereas (b) begins faster, around 6Hz, and slows down to around 3-4 Hz later on in its oscillation.

Mapping of Frequencies

Each of the cells had the brightness analysis done, and a frequency was determined from the resulting graph of brightness versus time. Each of these frequencies was then matched up to the location on the sacculus where the cell was found to have been on the preparation. This range of frequencies can be seen in FIG. 2, and consists of both the left ear data and the right ear data.

The expectation of the data was for there to be a clear pattern to the location of the spontaneous oscillations of the sacculus. Innervation patterns in the amphibian papillae of the frog suggest that there is a very high correlation between location and frequencies that are picked up by hair cells (Simmons DD 1992), and the innervation pattern of hair cells to nerves in general points to a pattern where groups of 5 to 10 cells are linked to a single nerve fiber, suggesting that these cells should all be oscillating at similar frequencies (Simmons DD 1992).

When the frequencies were mapped onto the sacculus ear, however, there was no pattern that could be found. The distribution did not point to any one spot being the location of lower frequency oscillations, nor did it point towards a lack of pattern in frequency distribution. Results were simply inconclusive due to too few data points.

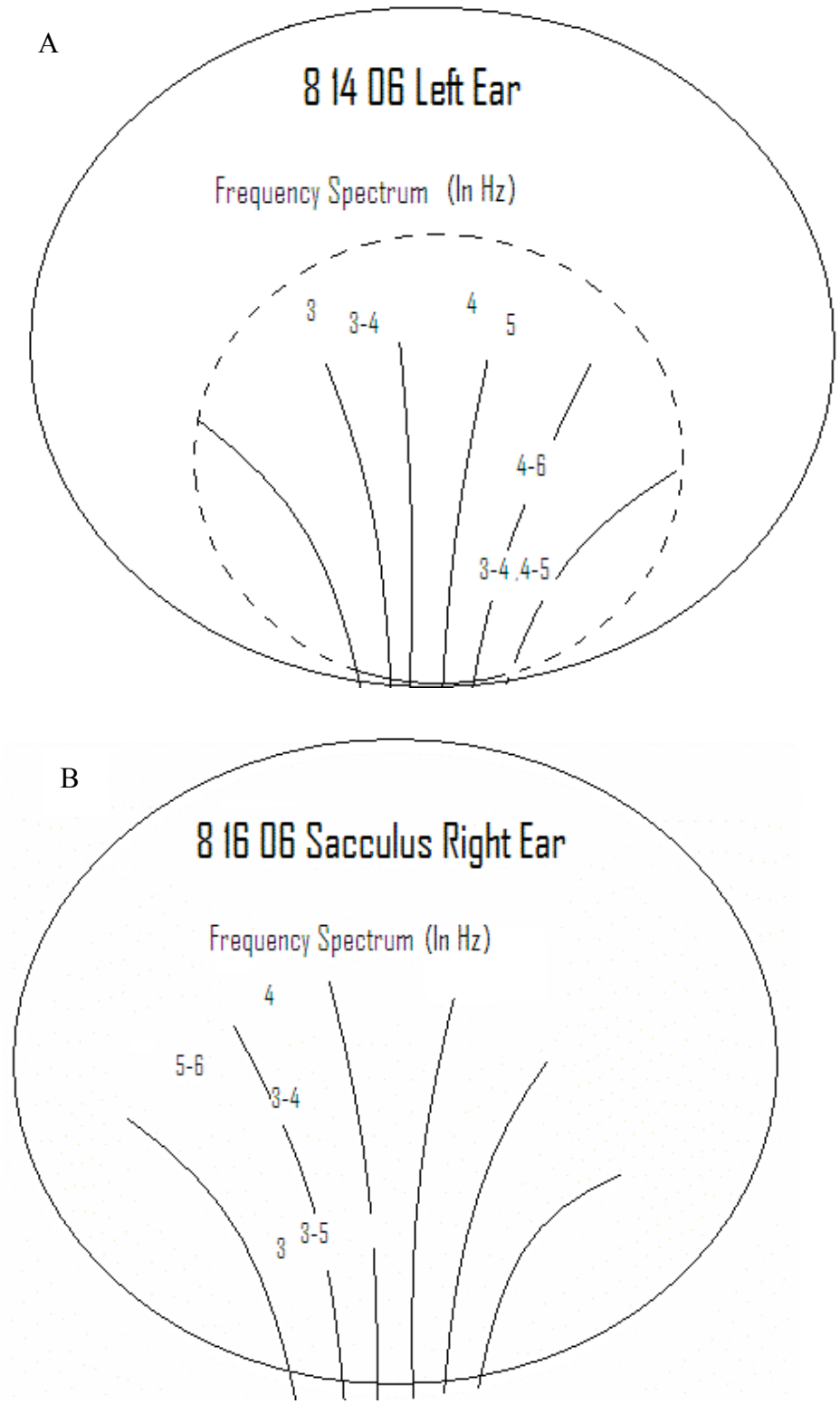


FIG. 2 The frequencies of the spontaneous oscillations at different spots in the sacculus ears. Each number represents the Hz value for the frequency of the oscillation found in (a) the left ear, and (b) the right ear.

Discussion

All of the viable cell data were collected, which consisted of that of twelve cells. Unfortunately, because half of the data were gathered from the left ear and half from the right, all of the data could not be mapped onto the same spectrum, which left large areas of the ear where no spontaneous oscillations could be found. Because of time the data came from two different ears, one left, one right, so results were inconclusive on whether the areas without spontaneous oscillations were due to human error (somewhere that is frequently damaged during dissection), or whether there was some other factor, such as the spontaneous oscillations all occurring out of the range of vision of the camera.

It is planned, then, to continue this project by adding more data points as a whole using both more CCD camera recordings of more ears as well as utilizing a faster camera that Professor Katsushi Arisaka at the University of California at Los Angeles is developing. This camera will reach frame rates of over 200 Hz, which will be over the Nyquist frequency to prevent aliasing of information for the higher frequency spontaneous oscillations.

Another complication in the mapping of frequencies is the short period of time that the sample does actually survive. Because the system is highly sensitive, as well as fragile, the maximum life span for cells to continue to oscillate is around 30 minutes. Because the cells die so quickly, it is quite possible that some time dependence on the frequency of the oscillations is being missed, such as some kind of lowering of frequency as the cells die. The camera has to be quite zoomed in to see the small deflection of individual cells, and because of that moving from cell to cell is quite slow, so the actual area that is covered in a preparation is quite small, and any effect sensitive to that time dependence would be easily missed.

The results were hindered as well by the problems surrounding the actual analysis of the data because, although the pixel brightness analysis is a good rough test, it fails in general at more sensitive systems that are visible by eye but not currently by analysis. A more sensitive movement analysis program such as LabVIEW, which is currently being set up, will allow for much more delicate observations on the system. An electrical monitoring of spontaneous oscillations, if the location of the cell can be monitored, can also assist in this goal.

References

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