Analysis of high speed imaging of auditory hair bundle motion

NBIO 228 Final Project
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Background

The sense of hearing begins at the auditory hair cell, which is the primary sensory cell of the inner ear, and is named for a bundle of hair-like microvilli that are specialized to detect nanoscale displacements caused by sound pressure waves. Hair cells and hair cell bundles vary in size, shape, and function depending on the sensory modality they transduce (hearing or balance), and the species in which they are found. How hair bundles are stimulated is unclear. Some hair bundles appear embedded in membranes, some are covered in a gelatinous cupula, and others seem to be stand free. Though the mechanical and electrical properties of hair cells has been investigated through biophysical experimentation, it is currently unknown how bundles move in vivo, or how various stimulation techniques (namely stiff probe vs fluid jet) affect hair cell response properties.

Our lab is investigating how the mammalian auditory outer hair cell bundle moves using high speed imaging (10,000 frames per second) of fluid jet stimulation of a single bundle in an acutely dissected rat cochlea. For this project, I have analyzed one 1ms episode of stimulation in order to characterize how the bundle moves over time.

Methods & Results

First I cropped the image stack to include only the region of interest (the bundle motion) and then I applied a bandpass filter (passing objects between 7 and 10 pixels) in order to decrease noise and enhance contrast.

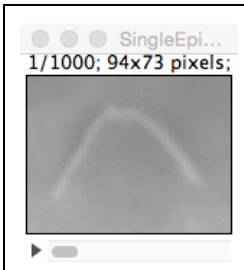	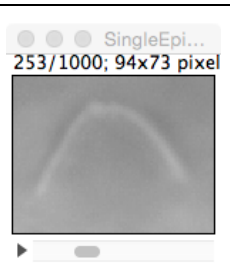	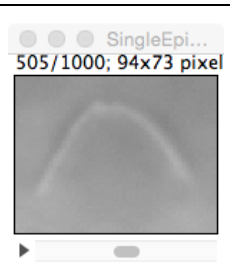	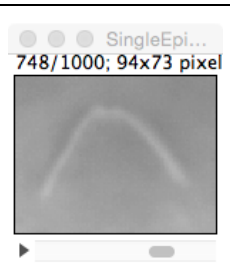	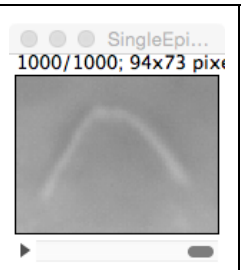
time = 0.100 ms	0.253 ms	0.505 ms	0.748 ms	1.000 ms

Fig 1. Raw data of bundle movement

				
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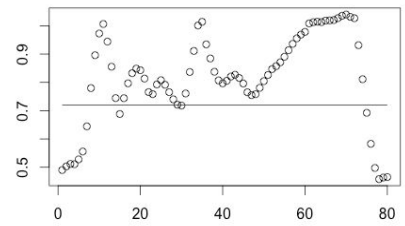
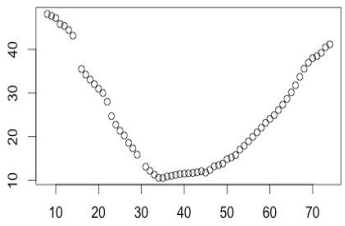
Fig 1. Bundle movement data after cropping and bandpass filter.

Next I treated each column of pixels as a vector of intensity values, selected the highest intensity point and the 10 surrounding points, and fit a third order polynomial to the intensities. I used the predicted maximum of the polynomial to predict the location in that column of that the bundle at sub pixel resolution.

To exclude the edges I thresholded intensity at the mean minus one standard deviation of the maximum intensity values of the middle columns (2nd and 3rd quartiles).



a.) Calculated intensity at polynomial maximum of one time point. Line indicates threshold.

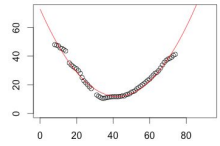


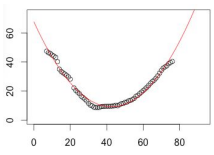
b.) x - column
y - location of maximum of polynomial

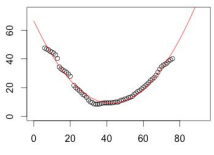
c.) Original frame used to create vector

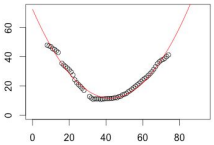
Fig 3. Example of thresholding and calculation of bundle location

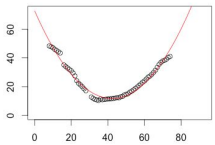
To characterize bundle motion over time, I fit the location of the bundle to a second order polynomial.







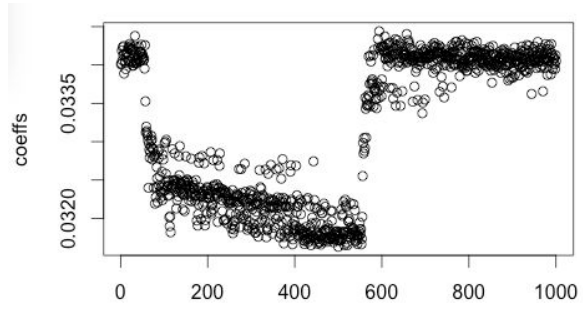




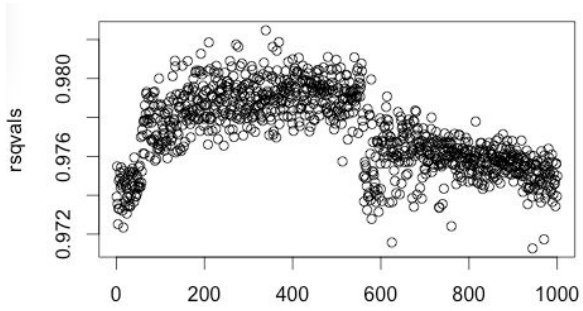
time = 0.100 ms	0.253 ms	0.505 ms	0.748 ms	1.000 ms

Fig 4. Second order polynomial fit over calculated bundle locations.

I then plotted the x^2 coefficients and R^2 values over time to track both the convexity of the bundle and how closely the bundle fit a second order polynomial before, during, and after stimulation.



a. Second order coefficients over time



b. R^2 values over time

Fig 5. Second order coefficients and R^2 values over time

From these results, I see that the bundle rapidly becomes more convex with the onset of stimulation and then even more convex over time during the stimulation. When stimulation ends, the bundle returns to the baseline level of convexity. The R^2 values show that the bundle better fits a second order polynomial during stimulation. All of this indicates that the bundle changes shape during stimulation, and does not simply displace uniformly in one direction.

To investigate this difference in movement, I tracked individual parts of the bundle over time. To do this, I broke up each bundle location vector into five parts: the beginning, ¼, ½, ¾, and the end. I then

plotted the displacement in the y direction over time, and plotted them together on one graph (Fig 6.).

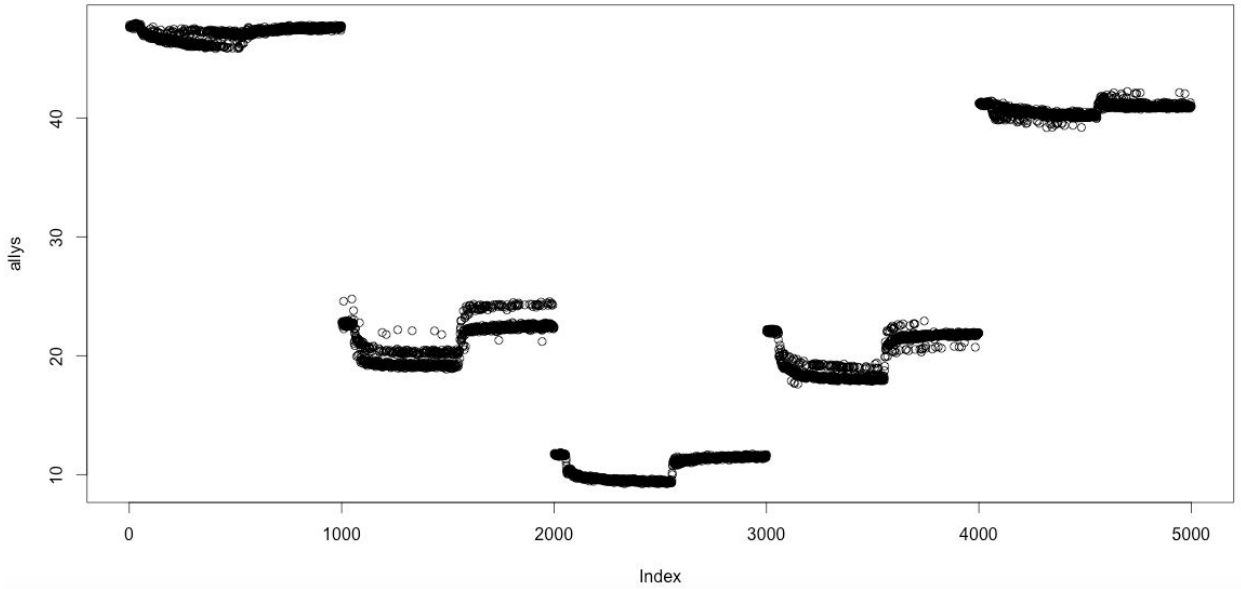


Fig 6. Displacement of different parts of the bundle in the y direction over time, plotted sequentially.

By eye, one can see that the bundle does not move uniformly. The ends of the bundle do not move as much as the middle, but the edges seem to move more than the apex. This corresponds with the observation that the fitted convexity of the bundle increases during stimulation, and adds the detail that this increase generally comes from the middle sides of the bundle and not the very ends.

Discussion

This preliminary analysis is interesting in that it shows that the mammalian auditory outer hair cell bundle does not move uniformly when stimulated with a fluid jet. This is different from previous reports using frog saccular hair cells that indicated that that bundle moves as a unit. It is possible that this difference is due to a difference in function and in-vivo stimulation. Saccular hair cells are used for vestibular sensation, which is of a much lower frequency that typical rat auditory stimulation. It will be most interesting to try and correlate these measurements with the current and voltage information obtained from patch clamping hair cells during stimulation. It is possible that certain shapes or motions correspond with certain electrochemical responses.