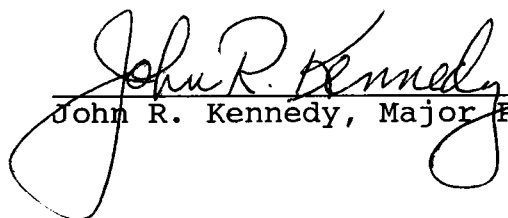


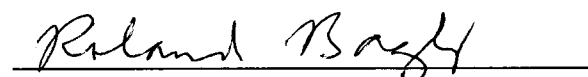
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EXTENDED TIME ANALYSIS
OF
CILIARY BEAT FREQUENCY

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Susan Austin
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ABSTRACT

Patterns of change in ciliary beat frequency have been investigated and characterized through the application of signal pattern recognition techniques. The frequency patterns of rabbit tracheal ciliated cells in culture were examined utilizing a photomultiplier system and the signals digitized and converted by Fast Fourier Transform analysis to a fundamental frequency. Frequencies were updated at one second intervals. Data was gathered continuously from individual cells for 8.5 minute periods and displayed graphically on the CRT screen of a DEC LSI 11/03 computer. Individual ciliated cells were found to fluctuate within a 1 Hz range along a frequency baseline. Recurrent rapid increases in frequency, ranging from 2-11 Hz above baseline have been observed. Subsequent rate of return to baseline occurred at a slower rate than increase. The duration of a single frequency fluctuation lasted from as short as 5.5 seconds to as long as 54 seconds from increase in frequency until return to baseline. These fluctuations in frequency create a signal which has both constant (baseline) and sinusoidal (peak) components. A mathematical model has been proposed to describe these components.

The frequency fluctuations in an individual cell in a 8.5 minute period has been observed to vary from 0-17 Hz. Peak heights & widths were relatively constant within

cells; variations between non-adjacent cells was substantial. Patterns of frequency fluctuation in adjacent cells also appear non-homogenous. Baseline levels of frequency were, in contrast, markedly similar for cells in close proximity. No doubt this constancy in the baseline frequency of beat for adjacent cells contributes to the integrity of metachronal waves.

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CHAPTER 1

INTRODUCTION

In the trachea, mammalian cilia function to clear mucus and trapped particulates from the epithelial surface of the respiratory conductive zones. One approach to assessment of the efficiency of this transport system involves quantitation of the rate of ciliary activity of the ciliated cells. Historically, determination of ciliary beat has been accomplished by various methods. One of the earliest ways to quantitate ciliary beat frequency utilized a stroboscope (Ballenger and Orr, 1963). When the frequency of the ciliary field was equivalent to the frequency of the flash of the stroboscope, the cilia were reported to appear static (Ballenger and Orr, 1963; Gosseblin et al., 1961). This technique allows for determination of a single frequency value for a region of ciliated cells. Estimated error at frequencies of 1400-1600 beats/minute or about 23-27 Hz is reported from +50 beats/minute or about +1 Hz (Mercke et al., 1974a) to +100 beats/minute or about +1.5 Hz (Ballenger and Orr, 1963). However, this method is unable to provide information about change in frequency through time or to resolve frequency differences between individual cells.

High-speed cinematography has also been used to measure ciliary frequency (Dalhamn, 1956; Irvani and Melville, 1974; Irvani and Melville, 1975; Toremalm, 1961). Although high-speed cinematography is more accurate in its determination of ciliary movement and could conceivably characterize fluctuations in frequencies through time, this method requires tedious study of raw data for time intervals of less than one second. This drawback practically eliminates this method of studying ciliary beat frequency through extended time intervals.

As techniques to quantitate ciliary beat frequency evolved, a photomultiplier combined with an oscilloscope was employed to study changes in the light transmitted through respiratory epithelium (Dalhamn and Rylander, 1962; Yager et al., 1978) or changes in light reflected from the beating cilia (Mercke et al., 1974). Lee and Verdugo (1977) further developed a technique using laser light-scattering spectroscopy to facilitate collection of frequency data.

An optical spectrum analysis system described by Kennedy and Duckett (1981) modified these earlier techniques. A limiting aperture phase contrast microscope was utilized so that only a 0.3mm area was studied. The microscope was combined with a photomultiplier system which converts the light signal produced by the beating cilia

into an electrical signal with both amplitude and frequency. This signal was then fed through an oscilloscope for monitoring. The signal was altered by Fast Fourier Transform analysis to produce a frequency spectrum. The primary harmonic was then extracted and could be used to study the changes in frequency of ciliary beat through time. With this experimental setup, it was realized early that ciliary tufts change their beat frequency through time (frequency shift phenomenon) in a cyclical fashion (Kennedy and Duckett, 1981).

Kennedy and Ranyard (1983) added a video camera to project the image from the phase contrast microscope onto a television monitor. This allowed for study of individual cells on the rabbit tracheal ciliated outgrowth cells. They further characterized the frequency shift phenomenon. They noted that most cells ($54.6\% \pm 3.1$ S.E.) shifted with a 0-2 Hz range. In other cells, the frequency shifts were as great as 8-17 Hz.

This system was further improved with the addition of a videocassette recorder (Duckett et al., 1986). This addition equipped the system to replay segments of tape which contained adjacent or nearby cells which could be studied during the same time frame. Modifications for this project included the addition of a Digital PDP 11-02 computer which enabled rapid storage and access of the

frequency spectra generated by the Fast Fourier Transform. Up to eight and one-half minutes of frequency spectra data could be stored for access and manipulation by the computer programs developed for this project by the author (Appendix A).

Eshel et al., (1985) used Fast Fourier Transform analysis to characterize frog palate ciliary beat frequency over short time intervals. Power spectra collected over one second intervals contained several primary frequencies of comparable power. Eshel et al., (1985) noted that these multiple frequencies changed through time. They further stated that they felt that frequency changes were not artifactually induced, but were an "intrinsic property of ciliary beat" (Eshel et al., 1985). They also presented a mathematical model which simulates the behavior of the ciliated cells under study.

Sanderson and Dirksen (1986) studied the effects of mechanically stimulating rabbit tracheal ciliated cells cultured from explants. They presented evidence which suggests that mechanical stimulation may help to regulate ciliary beat frequency. They stated that in response to stimulation by a microprobe, the cells increased their frequency in most cases by 20-60% after a lag phase lasting an average of 285 msec. They suggested that the transient frequency response to mechanical stimulation was

transmitted to adjacent and nearby cells. However, they presented no statistical parameters such as correlation coefficients which support this assertion.

This project attempts to clarify many of the questions regarding the behavior of rabbit tracheal cells in the absence of any extrinsically applied stimulus. Calculation of mean frequencies and standard deviations are clearly inadequate to characterize the behavior of ciliated cells through extended periods of time. This study describes patterns of change in ciliary beat through the use of statistical parameters and signal pattern recognition techniques. Cells are monitored for time periods as long as 8.5 minutes. Baseline frequencies of beat, shifts in frequency of beat and other parameters are described. Development of a mathematical expression which attempts to characterize ciliary activity is presented. Parameters are defined which may be used to quantitatively compare the behaviors of individual cells.

Ciliated cells are also evaluated at two separate sampling periods to assess whether any changes in patterns of beat are noted over even longer intervals than can be stored during one 8.5 minute period of data acquisition. During two separate data acquisition periods, baseline levels of ciliary beat frequency are compared, amplitudes

of frequency shifts are compared, and relationships between quantitatively descriptive parameters are explored.

Different tufts of cilia on the same cell are also monitored to assess whether there is any difference between the activities of spatially distinct tufts on the same cell. It has been shown that the calcium ion can affect ciliary beat frequency (Girard and Kennedy, 1986). It has been suggested that ciliary and somatic spaces of protozoans are separate spaces with respect to calcium (Machemer, 1986). If there exists a calcium pump at the transition between the somatic and ciliary membrane as proposed by Blum and Hines (1983), then different tufts of cilia may or may not exhibit similar behaviors. By studying different tufts of cilia on the same cell, we can acquire indirect evidence regarding the possibility that different tufts are electrically or otherwise coupled. Sanderson et al., (1988) similarly have studied two areas on the same cell which were micrometers apart. They report that when mechanically stimulated with a microprobe, "the increase in frequency occurred almost simultaneously in all cilia across a cell".

Studies have been conducted to examine the frequency of ciliated cells which are adjacent and/or in close proximity in culture (Sanderson, Chow, and Dirksen, 1988). They found that cells closest to a cell mechanically

stimulated with a microprobe responded with an increase in beat frequency before cells at a greater distance. The relationship between the time which elapsed before adjacent cells responded and the distance between cells was reported to be somewhat linear (Sanderson et al., 1988). However, they also note that not all cells behave as predicted by considering distance from the stimulated cell as the only independent variable controlling ciliary response. They label three cell types: "1) responding cells that display similar frequency responses, 2) boundary cells at the limits of propagation that display unusual frequency responses, and 3) nonresponding cells" (Sanderson et al., 1988).

Sanderson, Chow, and Dirksen (1988) also injected current into a cell and measured the voltage change in nearby cells. They classified cells as "receiving" if "a synchronous membrane potential change" could be detected when 1-3 nA of current was injected. By this method, fifty-seven of the cells which displayed similar frequency responses to mechanical stimulation of one cell were found to be electrically coupled by this method. Nine cells which displayed similar frequency responses to mechanical stimulation were found not to be electrically coupled by this method. Of the nine cells which did not display similar frequency responses when a nearby cell was

mechanically stimulated, none of these were found to change voltage when the cell mechanically stimulated was injected with 1-3 nA of current. The experimenters postulate that the electrical coupling is accomplished by means of gap junctions found between adjacent cell membranes (Sanderson et al., 1988).

Sanderson, Chow, and Dirksen (1988) do not characterize patterns of ciliary beat in the absence of a mechanical or electrical stimulus, however. Also, the time period for which data is reported is only 18 seconds. In this project, the patterns of frequency fluctuation for adjacent cells, clusters of cells, and cells in close proximity on the explant outgrowth are examined to discover whether the relationship of the cells as they reside on the outgrowth influences their behavior in the absence of any stimulus. For the respiratory epithelium to function as a coherent unit, the baseline frequency of beat for adjacent cells on the outgrowth should theoretically be within a more narrow range than cells that are spatially unrelated. The activity of ciliated cells in culture is assessed to study whether or not distance between cells influences baseline frequency of beat. A comparison of frequency patterns generated by ciliated cells through extended time periods is also attempted in order to examine whether in

the absence of stimuli, frequency beat patterns of cells in close proximity in culture are correlated.

CHAPTER 2

MATERIALS AND METHODS

Tissue preparation

Tracheas extending from just below the larynx to the tracheal bifurcation were obtained from mixed breed rabbits. The tracheas were cut in half longitudinally and the epithelial layer removed. The epithelial layer was then cut into squares approximately 2 mm by 2 mm and placed in small culture dishes with 10 ml of Waymouth's MB 752/1 tissue culture medium with 10% Nuserum, 50 units/ml penicillin and 50 micrograms/ml streptomycin added. The tissue was incubated at 37°C with water-saturated 5% carbon dioxide and 95% air according to the method described by Kennedy and Ranyard (1983). Culture medium was changed every other day and ciliated outgrowths were obtained and maintained for periods as long as three weeks. During this period, the change in morphology of the ciliated outgrowths as the cells migrated away from the explants was observed as previously reported (Kennedy and Ranyard, 1983). From these cultures, ciliated outgrowth cells were selected for analysis.

Quantitation of ciliary frequencies

Ciliary frequencies were obtained from individual cells through the use of a modification of methods previously described (Kennedy and Duckett, 1981; Duckett et al., 1986). Culture dishes were placed on the stage of a Nikon inverted, phase contrast microscope and kept at a constant temperature of 37°C by an airstream incubator.

Ciliary frequencies were monitored through the use of an Oriel photomultiplier (Model 7070) with small aperture. The photomultiplier was used to transduce the light signal generated by the beating cilia into an electrical signal, which was then fed to a Hewlett-Packard Spectrum Analyzer (Model 3582A). The spectrum analyzer was used to report the ciliary frequencies by Fast Fourier Transform. The Fast Fourier Transform converts the analog time-domain signal received from the photomultiplier to a digitized frequency-domain signal. The frequency data is displayed in graphical form on the oscilloscope screen of the spectrum analyzer. The major frequency was determined by noting the relative heights of the spikes observed on the screen display of the spectrum analyzer.

A DEC LSI/11-03 minicomputer was used to acquire the frequency spectra from the spectrum analyzer and to store a record of the Fast Fourier Transforms generated. Use of the minicomputer both to acquire the frequency data in

graphical form and to display it on the CRT screen of the terminal was made possible by software already available. New computer software has been subsequently developed to analyze the data generated over extended time periods. These programs utilize pattern recognition techniques implemented through subroutines packaged and marketed by Digital Equipment Corporation (1980). The parameters which characterize the data sets of ciliary frequency as monitored over long periods of time include statistical parameters usually associated with reporting frequencies of ciliary beat such as the mean and standard deviation. In order to more completely describe quantitatively the behavior of the cilia over time, the following experimental design was employed.

Experimental design

An RCA video camera attached to a video monitor was used to project the field to the TV screen. A time-date generator was used to record relative time elapsed during videotaping by a Panasonic video cassette recorder. Ciliary activity was monitored and stored on videotape for periods of up to thirty minutes, and data sets of frequencies were acquired for as long as 8.7 minutes. Individual cells were monitored in order to analyze ciliary beat patterns with regard to frequency range, through

extended time periods. For those cells which remain at a baseline frequency, the degree of variability that exists in the frequency data set was examined. The mean frequency as calculated from the data was examined as an appropriate measure of the tendency to remain at constant frequency. The standard deviation as it fluctuates around the mean was used to estimate the amount of variability within a data set. The range of frequencies spanned by a cell that remains at a constant baseline frequency was also investigated.

For cells which exhibited a sustained change in ciliary beat frequency, the following questions were posed: Is the mean value for the frequency of beat as monitored through time an appropriate measure of the activity of the cell in question? Is the standard deviation representative of the increase in variability of the data set created by a sustained change in the frequency of ciliary beat? Does the cell maintain a constant beat frequency between periods of frequency shift? Do the cells which exhibit shifts in ciliary frequency reach a maximum far above the baseline level of ciliary beat? Is the range of frequencies spanned correlated to the shifts in frequency?

For cells which exhibit more than one shift in frequency, the shifts in frequency were examined for their occurrence at regular intervals, maximum amplitude when they

increase in frequency, and duration. The relationship of the mean frequency of beat to the number of times a cell will exhibit a shift in frequency was examined as was the relationship of the maximum frequency to the baseline. The rate of decrement in frequency as the cell returns to baseline level was estimated.

For cells which were sampled at two different times, these questions were asked: Does a cell which does not exhibit a shift in frequency at one time of monitoring have the ability to shift in frequency at another time? Does the frequency distribution of the data set change with frequency shift? Does the baseline level of ciliary beat change in time? Does the amplitude of frequency shifts change in time?

When different tufts of cilia on the same cell were monitored during the same time frame by using the videotape system, experiments were designed to answer the following: Are the mean frequencies and standard deviations of the separate data sets similar? Is the range of frequencies spanned similar? Are the maximum amplitudes attained during shifts in frequency similar for distinct tufts? Are the durations of shift similar? Does cross-correlation analysis indicate that the signals generated by the frequency of ciliary beat change in a similar manner?

Questions were posed regarding cells in close proximity on the outgrowth or whose membranes are in contact to investigate the possibility that the relationship of the cells on the outgrowth could influence the frequency patterns of the cells: Are the baseline levels of ciliary beat within closer range than cells chosen at random? Is the probability that a cell will exhibit a frequency shift increased when other cells in close proximity are noted to exhibit a shift? Are frequency beat patterns correlated when cells reside in close proximity? Is the amplitude of increase in frequency similar for cells in close proximity?

CHAPTER 3

PROGRAMMING

A DEC LSI/11-03 minicomputer was used to store and to analyze the frequency data generated over extended time periods through the experimental set-up described in Chapter 2. The effective management of large volumes of data required the use of four computer programs. Two of the programs were available in our laboratory for use with the minicomputer; two of the programs were developed especially for use for this project.

Acquisition of frequency data

The first program used for data analysis, written by Greg Barker, acquires frequency data from the spectrum analyzer by copying the display from the screen of the spectrum analyzer. The program records on floppy disk the Fast Fourier Transform display of the spectrum analyzer. A variation in the amount of light is generated by the beating cilia as they move back and forth in front of the small aperture of the photomultiplier. The change in the amount of light as tracked through time produces a sine wave with frequency, amplitude, and period. Fast Fourier analysis detects the fundamental frequency present in a ten second window of data and reports a frequency spectrum in

the form of spikes with height proportional to the time a cell spends at the fundamental frequency.

The program used to acquire frequency data through extended time periods copies the Fourier transforms from the screen of the spectrum analyzer and records them in this form. They are written to the floppy disk in the order in which they were acquired, therefore generating a timed sequence of fundamental frequencies attained from the cell. The minicomputer uses disks which have enough storage space to accomodate up to 487 complete displays of the Fast Fourier Transforms. The amount of data which may be collected in a given time span is thus limited to the amount of storage space available to store sequentially acquired transforms. Usually a convenient number of transforms such as 200 or 450 is used during the aquisition of data from a cell. The rate of signal acquisition is approximately 1 transform per 1.1 seconds. This method thus provides a continuous update of the activity of single tufts of cilia as seen through the small aperture to the photomultiplier and can reflect real variations in frequency over time periods extending longer than 8 minutes.

Displaying the transforms

The second program used in analysis of the data, also written by Greg Barker, displays the sequentially acquired Fourier transforms stored on the floppy disk. It allows for the original Fourier transforms to be displayed in graphic form simulating the original transform displayed on the oscilloscope of the spectrum analyzer. It has the additional capability to generate a frequency histogram of the fundamental frequency from each transform as it varies through time. This program creates a more compact file containing the numerical values of the frequencies. These values may then be stored in a separate file apart from the graphical displays acquired from the spectrum analyzer. This arrangement decreases the bulk of the data and reduces the data to a more manageable form for access by other software. The values are stored as a timed record of the frequency of ciliary beat as monitored through periods of approximately eight minutes.

Processing the data

The third program (Appendix A) used in the analysis of the frequency data was designed to perform one task: to detect the presence of shifts in the ciliary frequency over time. The shifts create patterns or waveforms in the record of the ciliary frequency.

This program utilizes a subroutine (Digital Equipment Corporation, 1980) which is capable of calculating the area under the waveform generated by a change in ciliary frequency. The waveforms, created by changes in frequency, produce sawtooth waves in the data. The value of the maximum frequency obtained at the crest of the peak is reported by the subroutine along with its position in the data set or x-coordinate. The leading minimum height, defined as the lowest value recorded preceeding each sawtooth waveform, and the x-coordinate which corresponds to the leading minimum height is similarly reported. A true increase or decrease in the frequency data is set to be sensitive to 1 Hz. The subroutine also records the minimum frequency value which follows a peak and its time or x-coordinate which trails the positive deflection. The frequency data is interpreted to display an increase or decrease if a change of 1 Hz is noted. The program is designed to detect persistent changes, not random transient variations, in the trends of the frequency data (Digital Equipment Corporation, 1980).

Additional information, though not included in the output of the subroutine, is calculated by the program from parameters reported by the subroutine. This additional information includes a count of the number of transforms which are recorded between crests of the shifts in

frequency. This allows for a comparison of the amount of time which elapses between noted changes in ciliary frequency. The amount of change from the minimum frequency values to the crest of the waveform is also calculated from parameters reported by the subroutine. This additional data allows for a comparison of the amounts of increases and decreases noted for separate peaks in frequency.

To summarize, this program possesses the capability to call up a subroutine which calculates certain relevant parameters for extended time analysis of ciliary beat frequency. It reports whether or not a frequency shift is detectable in the data set, and the maximum value of the frequency attained during the shift if one is present. Also reported are the minimum heights recorded both before and after a shift in frequency. The x-coordinates of these values are reported in order to judge the relative time frame for each shift. The area under the waveform is reported as well. The number of transforms recorded between crests of the waveforms and the difference from the leading and trailing minimum frequencies are calculated from parameters drawn from subroutine output.

Spectral analysis of the data

Spectral analysis of the data is accomplished by accessing the array of frequency values stored previously

and analyzing them for correlations within and between data sets with software developed especially for this project (Appendix A). The program also creates a histogram of the frequencies present, without regard to the time sequence in which a frequency was reported, in order to study the relative number of times a frequency occurs within a given time frame.

This program was developed to handle one or two files of frequency data. When presented with a set of frequency data, the program reports the mean, the variance, the standard deviation, and the standard error for each file. These parameters are useful in examining the tendency of the values to cluster around a given value and the amount of variability within the data set.

To examine another measure of central tendency not as commonly reported in ciliary frequency studies, the program utilizes a subroutine packaged by DEC which formulates a bin histogram of the frequency data (Digital Equipment Corporation, 1980). This histogram reports the number of times a frequency is recorded in the data set in 1 hertz intervals. A relative frequency distribution of the ciliary frequencies present in the data set may then be calculated from the values returned by the histogram subroutine. The relative frequency histogram is a better measure of the tendency of the cells to maintain a constant

frequency through time than is the mean frequency value. In addition, it may be used to judge whether cells that are in close proximity on the explant are beating at similar frequencies. The relative frequency histogram may be used to compare the frequency distributions of the same cell measured at two separate time periods as well.

The program can calculate the autocorrelation function and coefficients. These parameters reveal the degree of similarity of the waveforms which are created by changes in the frequency of ciliary beat as the frequency changes through time. For example, if there is a frequency shift occurring at regular intervals, the autocorrelation function reveals these regular shifts by increasing in value when calculated at an overlap corresponding to the separation of the repeating waveforms. In other words, the autocorrelation is a mathematical description of how well the signal correlates with itself as it shifts through time. The time difference when the waveform is compared to itself is referred to as the lag. The program can calculate the autocorrelation function and coefficients for lag times up to one less than the number of transforms stored in the frequency data file. The sample autocorrelation function is calculated by an equation which averages the sample products separated by a lag of k , viz.

$$R_{XX}(k) = \frac{1}{N-k} \sum_{i=1}^{(N-k)} \{ x_i * x_{(i+k)} \}$$

where k = lag (number of samples),

$R_{XX}(k)$ = the sample autocorrelation function at lag k ,

x_i = the i th frequency value recorded,

$x_{(i+k)}$ = the $(i+k)$ th frequency value recorded,

and N = the number of sampled frequencies

(Schwartz and Shaw, 1975). This equation calculates the unbiased estimate of the sample autocorrelation function. At lag $k=0$, the autocorrelation function is equal to the variance of the data set for a zero mean function, so that

$$s_x^2 = R_{XX}(0) = \frac{1}{N} \sum_{i=1}^N \{ x_i^2 \}$$

where s_x^2 = the variance,

$R_{XX}(0)$ = the sample autocorrelation coefficient at lag $k=0$,

x_i^2 = the i th sampled frequency squared,

and N = the number of sampled frequencies

(Schwartz and Shaw, 1975). Therefore, the autocorrelation

function at a lag of zero gives an estimate of variability within the sample without regard to the mean. This allows for characterization of the activity of the ciliary tufts as having a higher or lower level of activity considering only the variable portion of the data. The power of the fluctuating portion of the data is thus quantitatively assessed (Schwartz and Shaw, 1975).

For all parameters calculated, it has been assumed that the observations made are independent of the time of reference, $t=0$, so that the data set is sampled from a stationary signal (Schwartz and Shaw, 1975). Also, since the variable portion of the data set is the portion which is fluctuating with time, the mean frequency has been subtracted out for all mathematical operations.

The sample autocorrelation coefficient is calculated from the autocorrelation function through the equation

$$r_{xx}(k) = \frac{\{R_{xx}(k) - (\text{mean})^2\}}{s_x^2}$$

where $r_{xx}(k)$ = the sample autocorrelation coefficient at lag k ,

$R_{xx}(k)$ = the sample autocorrelation function value at lag k ,

$(\text{mean})^2$ = the square of the mean,

$$s_x^2 = \text{the variance}$$

(Schwartz and Shaw, 1975). For a zero mean function, this equation simplifies to

$$r_{xx}(k) = \frac{R_{xx}(k)}{s_x^2}$$

(Schwartz and Shaw, 1975). Therefore at a lag of $k=0$,

$$r_{xx}(0) = \frac{s_x^2}{s_x^2} = 1.0$$

and the signal is seen to be perfectly correlated with itself (Schwartz and Shaw, 1975). The autocorrelation coefficient varies between -1.0 and +1.0, which are the extremes for perfect inverse and perfect direct correlation, respectively. The program reports the autocorrelation coefficient between these limits according to these specifications. For the purposes of examining fluctuations in ciliary beat, as the frequency changes in time, calculation of the autocorrelation coefficients allow for inspection of how closely the pattern of fluctuation is preserved during the period of observation.

The program has the additional capability of handling two files of frequency data to calculate an estimate of the sample cross-correlation function and coefficients. The sample cross-correlation function is estimated by

$$R_{xy}(k) = \frac{1}{N - k} \sum_{i=1}^{(N-k)} \{ x_i * Y(i+k) \}$$

where $R_{xy}(k)$ = the sample cross-correlation function for x and y at lag k,

x_i = the ith value of first function x,

$Y(i+k)$ = the (i+k)th value of second function y,

k = the lag (number of samples),

and N = the number of frequencies sampled

(Schwartz and Shaw, 1975). The sample cross-correlation function serves much the same purpose as the autocorrelation function. The cross-correlation function increases when the patterns of fluctuation of frequency for two data sets are similar. The cross-correlation function can also detect patterns of similar fluctuation in the presence of noise or random interference (Schwartz and Shaw, 1975).

The cross-correlation coefficient is calculated in a manner similar to that of the autocorrelation coefficient. The cross-correlation coefficient reflects the degree of correlation, as the autocorrelation coefficient does,

with values varying between -1.0 and +1.0. A cross-correlation coefficient is judged to be significant at levels of +0.50 for these purposes. This indicates that the pattern of frequency shift shows significant similarity for the two cases under consideration. The data sets compared can arise from two different cells, from the same cell monitored later in time, or from different tufts of cilia on the same cell while monitored during the same time frame.

CHAPTER 4

RESULTS

Extended time analysis of ciliary beat frequency revealed patterns in the fluctuation of beat that can be described with statistical parameters and signal pattern recognition techniques. Over one hundred total cells were studied and the following was revealed.

Baseline beat frequencies

Cells which display no frequency shift as monitored through time produce a flat, unremarkable graphical display when computer plotted with respect to time (Figure 1). The plot of these cells can be contrasted with those cells which show obvious, transient increases in frequency (Figures 2, 3). Eighteen cells which displayed no obvious change in frequency of beat (frequency shift) over time periods as long as 8.25 minutes were analyzed (Table 1, Appendix B). These cells displayed mean frequencies which ranged from 6.88 Hz to 17.24 Hz.

Ciliary beat frequency for each cell was found to vary within a span of 2-3 Hz. Ten cells (56%) varied within a 2 Hz span; eight cells (44%) varied within a 3 Hz span. The average interval spanned by the frequencies was 2.44 Hz. Since the frequency of beat remains within such

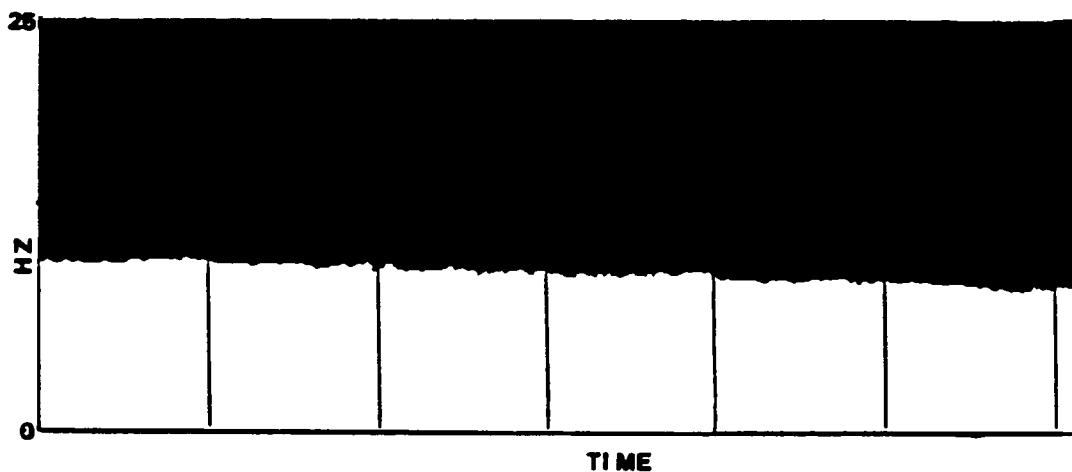


Figure 1. Computer plot of frequency with respect to time showing no sustained changes in frequency. (N = 450).

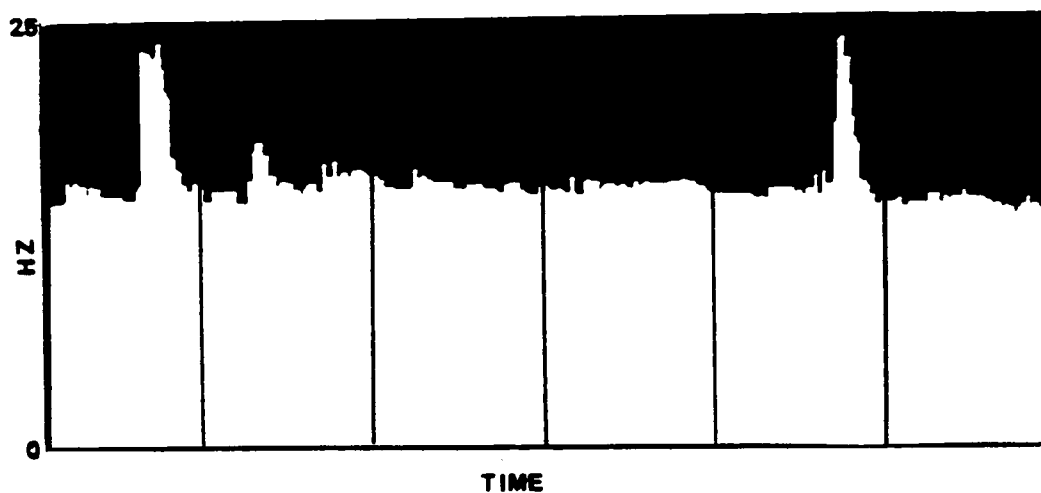


Figure 2. Computer plot of frequency with respect to time showing several large transient increases in frequency. Note abrupt increase in frequency followed by slow, regular decline back to baseline levels of ciliary beat frequency.

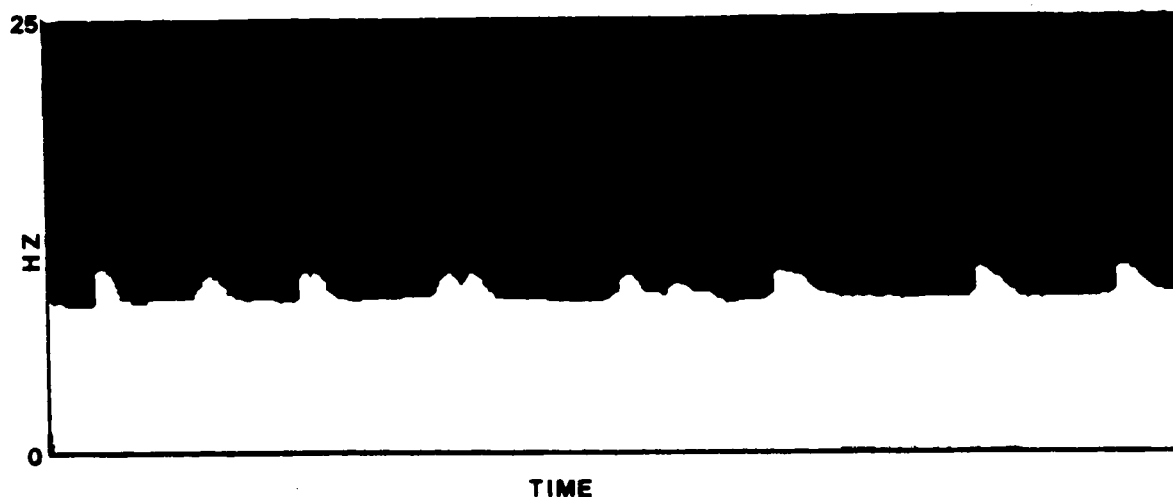


Figure 3. Computer plot of frequency with respect to time showing many small, sustained increases in frequency. Compare these small increases with the large ones seen in Figure 2.

a narrow range for time periods extending from 3.3 minutes to 8.25 minutes, it was inferred that there exists a baseline frequency of beat intrinsic to each cell.

The standard deviations of the frequencies ranged from values of 0.13 to 0.46, the average value of the standard deviation for these non-fluctuating periods being 0.28. Assuming a normal distribution for the frequency of beat in the absence of frequency shift, about 95% of the frequency measurements will lie within 2 standard deviations around the mean frequency according to the empirical rule (Agresti and Agresti, 1979). Since a value of 1 Hz is a quantity greater than two standard deviations from the mean frequency for cells observed which exhibit no obvious change in frequency, an increase or decrease of more than 1 Hz is interpreted as a real change in the frequency of beat. This is the value used by the computer program to detect any sustained, notable changes in ciliary beat frequency. For these 18 cells, computer assessment of the data sets revealed no real shift in frequency at a sensitivity of 1 Hz.

Relative frequency distributions for the cells reveal that the cells tended to remain within a frequency span of 1 Hz for as long as 98% of the sample time span (Table 1, Appendix B). The relative frequency distribution for a

cell may therefore be employed to determine the baseline beat frequency for a ciliary tuft within a 1 Hz interval.

Cells which display a shift in frequency

A transient increase in frequency (frequency shift) creates a waveform in the data set which may be analyzed for trends using the software especially developed for this purpose. Forty-two separate cells displaying shifts in frequency have been observed and analyzed. Ciliary tufts as monitored through extended time periods have been noted to exhibit transient frequency increases as many as twenty-one times in approximately 8 minutes. It is notable that in no case has a frequency pattern been recorded that exhibits transient decreases in ciliary beat frequency analogous to the transient increases.

The cells which exhibit a shift in frequency have been observed to increase in frequency, then decrease back to the baseline frequency at a rate slower than their rate of increase (Figures 2, 3). The increase in frequency is a sudden, quick event which appears in the frequency data set as an abrupt rise from the baseline. The ciliary frequency attains its maximum level usually just after the abrupt increase, and then slowly and regularly declines back to the baseline attained before the increase.

The waveform created by the frequency changes can be characterized by a mathematical expression which approximates the transient increases in frequency as a portion of a rectified sine wave. Since the increases arrive at irregular intervals in time, a rectangular pulse is added to the mathematical expression to introduce each increase in frequency. For cells which show a shift in frequency (Figure 4), the expression may then be written as:

$$y(t) = B_0 + \sum_{i=1}^N \{ [A_i \cos w_i(t-T_i)] * [u(t-T_i) - u(t-(T_i+D_i))] \}$$

where $y(t)$ = the frequency of ciliary beat at time t ,

B_0 = the baseline frequency of beat,

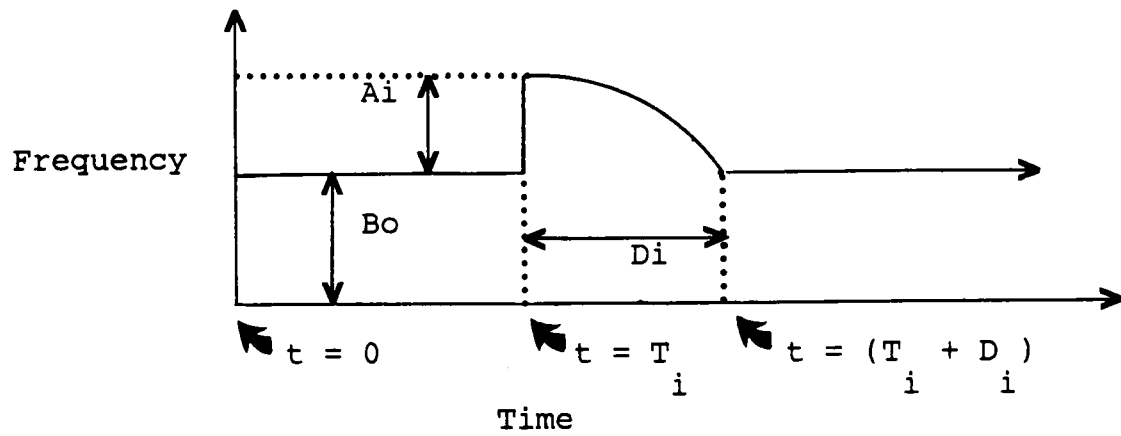
N = the number of shifts present in the data set,

A_i = the amplitude of the i th frequency shift over baseline,

T_i = the time of onset of frequency shift,

D_i = the duration of the frequency shift,

$w_i = 2\pi / D_i$, and

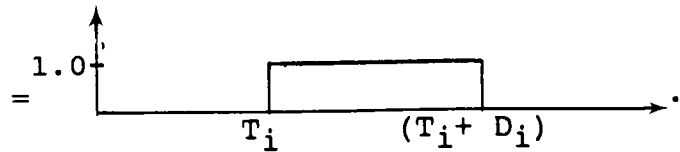


$$y(t) = B_0 + \sum_{i=1}^N \{ [A_i \cos w_i (t - T_i)]$$

$$* [u(t - T_i) - u(t - (T_i + D_i))] \}$$

Figure 4. Diagram of mathematical model of fluctuations in ciliary beat frequency.

$u(t-T_i)-u(t-(T_i + D_i))$ = a rectangular pulse of amplitude 1.0 beginning at time T_i and ending at $(T_i + D_i)$



The development of a mathematical model has several useful applications. A mathematical model can be used to describe the behavior of cells under changing conditions. By analyzing the behavior of cells under the conditions of this experimental set up, variables can be introduced to study their quantitative effects on ciliary beat frequency. The characterization of ciliary beat through a mathematical expression allows us to quantitate the parameters given in the equation and systematically compare the activity of a cell during one time frame with its activity during a later time frame. The activities of cells in close proximity may also be characterized in the same manner, as can different tufts of cilia on the same cell. Additionally, ciliated cells from individuals with genetic defects such as cystic fibrosis can be studied and their behavior quantitatively compared to unaffected cells. Similarly cells infected with viral or bacterial pathogens can be studied.

Cells which display one frequency shift

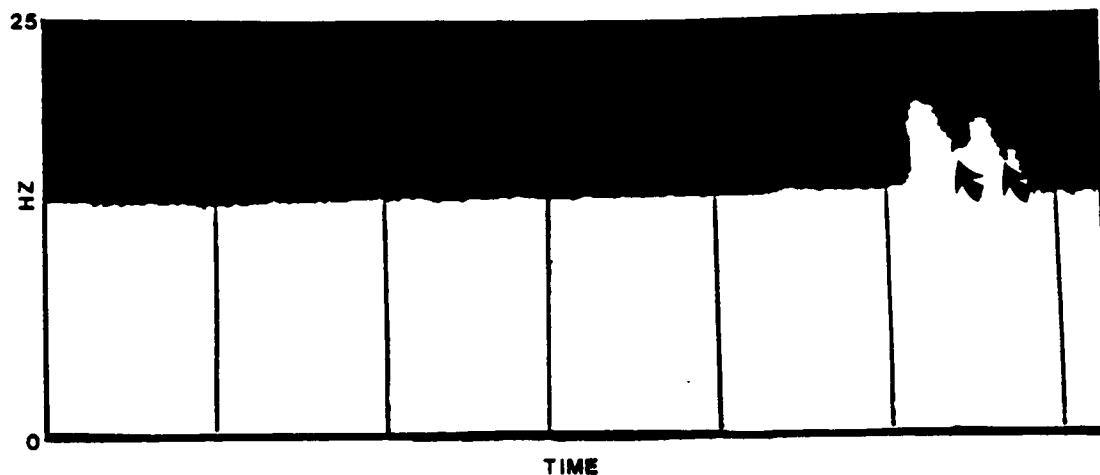
Some cells demonstrated only one frequency shift during the time in which they were monitored (Tables 2, 3, Appendix B). Six cells of this type were analyzed. The mean frequencies of the cells ranged from 8.32 Hz to 23.87 Hz.

The baseline frequency of these cells was determined from relative frequency histograms. The use of relative frequency histogram allows for determination of the frequency within a 1 Hz interval which occurs most often in the data set. Statistical parameters such as the mean can be altered by the presence of multiple shifts in frequency. Therefore the measure of the tendency for some cells to maintain a constant frequency of beat when the cell maintains its baseline is not appropriately judged by calculation of the mean with corresponding standard deviation. The standard deviation does not reflect spread of the data around the baseline frequency but reflects variability introduced by frequency shifts. The frequency of beat maintained for the longest periods during monitoring should be considered to be the baseline frequency of ciliary beat, and the relative frequency histogram allows for detection of such. For example, one cell that maintained its baseline frequency over 375 samples (6.9 minutes) and then displayed large fluctuations

in frequency showed an increase in the mean value reported of 0.48 Hz when the frequency shift was included in the data set (Figure 5). The standard deviation was increased by a factor of 3 when a large frequency shift was introduced into the data set. The baseline as determined by relative frequency histogram was the same regardless of whether the frequency shift was included in the data set, and the percentage of time spent at the baseline was only slightly affected by the inclusion of the frequency shift even though the range of frequencies spanned increased by 6 Hz.

The variability within the data set of ciliary beat frequency values is thus increased due to presence of transient shifts in frequency. The increase in variability is reflected by a corresponding increase in the value of the standard deviations of the cells exhibiting only one shift as compared to those cells which remain at their baseline level (Tables 1, 2, Appendix B). The average value of the standard deviation in the absence of frequency shift was noted as 0.28 Hz, while the average value of the standard deviation was noted as 0.59 Hz for cells displaying one frequency shift.

For cells with frequency shift absent while monitored, the frequency range spanned intervals of 2 Hz for 56% of cells, and 3 Hz for 44% of cells (Table 1, Appendix B).

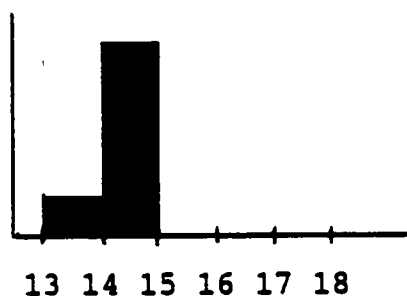


Without frequency shift

N = 375
 Mean = 14.25 Hz
 Standard deviation = 0.31
 Standard error = 0.02

With frequency shift

N = 470
 Mean = 14.63 Hz
 Standard deviation = 1.20
 Standard error = 0.06



Frequency Intervals

Range = 13 - 15



Frequency Intervals

Range = 13 - 21

Figure 5. Example to show changes in descriptive quantitative parameters introduced by including a shift in frequency in the data set. The mean of the data set is increased by approximately 0.5 Hz, the standard deviation increases from a value of 0.31 to a value of 1.20, and the range increases by 6 Hz. The standard deviation does not reflect the degree of fluctuation around the baseline frequency of beat, but reflects the added variability in the data set due to frequency shift. Arrows indicate point where the second frequency shift begins before complete return to baseline of the first frequency shift.

For cells exhibiting one frequency shift, the frequency varied over an interval spanning 4 Hz for three of the six cells, and an interval spanning 5 Hz for the remainder (Table 3, Appendix B). The range of frequencies for cells which shift only one time (Figure 5) was therefore noted to span intervals notably wider than the range of frequencies spanned by those cells which fluctuate around a baseline frequency without evidence of frequency shift (Table 3, Appendix B).

Cells which display multiple shifts in frequency

As has been previously mentioned, cells from rabbit tracheal epithelial outgrowths have been noted to display a shift in frequency as many as twenty-one times during a monitoring period of 8.25 minutes (Figure 6). Mean frequencies of cells exhibiting multiple frequency shifts range from 8.88 Hz to 20.39 Hz (Table 4, Appendix B). The baseline frequencies of these cells range from approximately 8 Hz to 20 Hz.

The number of frequency shifts per minute for cells which display shifts ranges from 1 shift in 8.62 minutes to 21 shifts in 8.25 minutes. A linear regression model was constructed to investigate the degree of linear relationship between the mean frequency and the number of shifts recorded per minute. This model was fitted to the

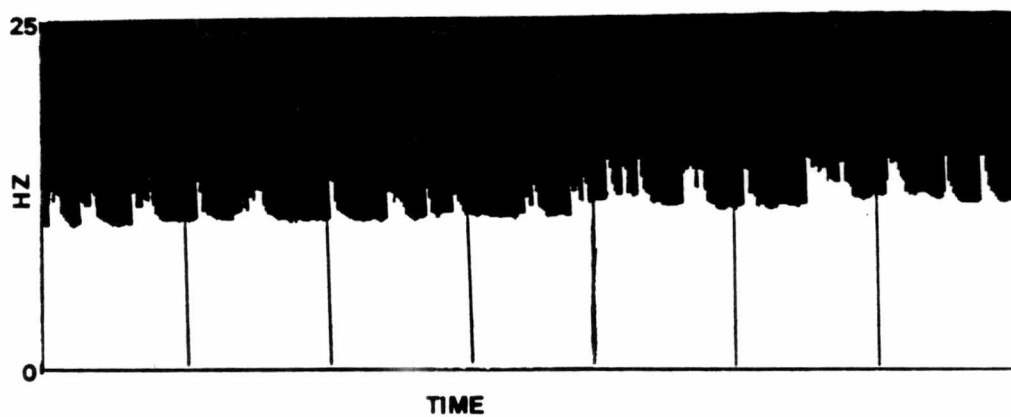


Figure 6. This cell exhibited 21 separate shifts in frequency. Return back to baseline levels following each shift is apparent.

data points in least squares analysis and a slope of 0.02 was obtained. Therefore, no important linear relationship was found between the mean frequency and the number of frequency shifts.

Cells which display frequency shifts seem to increase their frequency to a given amplitude unique for each cell (Figures 2, 3, pages 30, 31; Table 5, Appendix B). The maximum value attained during a shift in frequency is constant within a one hertz span for each shift in frequency for 22 of 35 cells examined which exhibit multiple frequency shifts. Five cells increased their frequencies to amplitudes which remained within a 2 Hz span; four cells increased their frequencies to amplitudes which remained within a 3 Hz span. In addition to possessing a characteristic baseline frequency level, it appears that the cells may also possess a given frequency to which they will rise during a frequency shift.

Smaller shifts in the frequency record are sometimes also present (Figure 7). These smaller increases in frequency are not as regularly seen within the data sets, but are nonetheless notable increases in the frequency record. These shifts shall be referred to as minor shifts in frequency. The minor shifts in frequency do not seem to attain a regular amplitude as do the larger, major shifts. These minor shifts were seen in 5 of 35 cells exhibiting

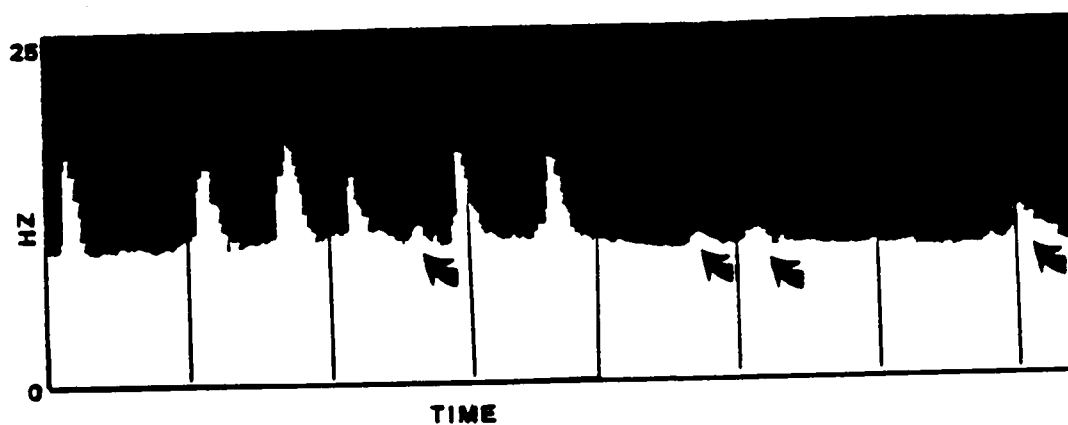


Figure 7. Minor shifts in frequency are sometimes interspersed with more prominent increases. Arrows show minor shifts.

multiple frequency shifts. These cells have maximum frequency shift amplitude which span a 4 - 5 Hz range.

Occasionally cells reveal the presence of overlap of frequency shifts (Figure 5; page 39). It appears that before the cell can re-establish its baseline frequency, another shift in frequency is begun. The waveforms in the graphical display appear to ride "piggy-back" due to the overlap of shifts. The maximum amplitudes attained by the two shifts which overlap are usually close in value.

The duration of each frequency shift is variable for cells which display multiple shifts in frequency (Table 6, Appendix B). Therefore, the period of the cosine wave in the mathematical model of ciliary beat frequency, which is approximated as four times the duration of the frequency shift, is also variable (Figure 4, page 36). Frequency shifts have been noted to last as long as 49 samples. At an average of 1.1 seconds/sample, this represents a time span of almost one minute. The average length of time which elapsed between an increase in frequency and the return to baseline is 18.92 seconds ($N = 226$, standard deviation = 8.42). The duration of the frequency shift appears extremely variable. Thus, for a cell exhibiting multiple frequency shifts, the duration of a shift is not predictable from analysis of shifts which occur at previous times.

The increase in frequency during a shift in frequency usually occurs so quickly that the spectrum analyzer reports the baseline frequency to the computer followed by the maximum value of ciliary beat attained with very few samples taken at intermediate frequencies. Calculation of the rate of frequency increase is thus outside the constraints of this system. The cilia do not usually beat at this maximal frequency for a period of time longer than a few seconds. The decline in frequency occurs at a rate that is much slower than the frequency increase. This rate may be approximated by noting the time of the maximum frequency last recorded and the decrease in frequency back to baseline. The ratio of these two parameters gives an estimate of the rate of decrement in the frequency after the crest has been reached (Table 7, Appendix B).

The rate of decrement in frequency is variable between shifts for cells which exhibit multiple shifts. Cells are noted to decrease their frequency as fast as 1 Hz per second. Even though the amplitude of the frequency obtained is constant and the decrease in frequency of the cell is also often constant, the rate at which the cell resumes its baseline ciliary beat frequency is non-uniform for each shift in frequency (Table 7, Appendix B).

The number of shifts which occur in a given period of time is non-uniform. The number of shifts per minute is

not proportional to the mean frequency of the cell (Table 4, Appendix B). Cells with a mean frequency of 8-9 Hz showed from 0.1-0.9 shifts/min. while cells with mean frequencies of 10-13 Hz showed from 0.2-2.5 shifts per minute. The maximum frequency attained during a frequency shift also is not proportional to the baseline frequency nor is the maximum frequency proportional to the number of shifts recorded in a given time period as judged by linear regression estimates in both cases (Table 8, Appendix B).

The time which elapses between crests of frequency shifts as measured by the number of samples between crests is also not constant (Table 9, Appendix B). Cells exhibit a shift in frequency at seemingly random times. This creates a stochastic signal with regard to the regularity of increases. The time of onset of a frequency shift for cells monitored for periods extending approximately 8 minutes is unpredictable. Therefore, it is judged that, unlike the baseline level of ciliary beat frequency, and the amplitude to which a cell will rise when increasing in frequency, the time of occurrence of frequency increase is not regular. There does not seem to exist an intrinsic rhythm for ciliary beat fluctuation. This would seem to suggest: (1) that the baseline and the amplitude reached during a frequency shift are properties of the cell, and (2) that a frequency shift is caused by administration of a

pulsatile stimulus to the apparatus of the cell responsible for controlling ciliary beat frequency.

The waveforms generated by a shift in frequency may be very similar or very dissimilar as judged by the autocorrelation function and coefficients. The autocorrelation function and coefficients were calculated for cells exhibiting only two shifts in frequency at lag times corresponding to the time between the two shifts (Table 10, Appendix B). Values of the autocorrelation coefficient ranged from -0.367 , which indicates a weakly inverse relationship between the two data sets, to $+0.927$, indicating a very strong direct relationship between the two data sets. It may be useful to characterize the power of the signal generated by the frequency as it changes in time (Table 11, Appendix B). The average power of the signal can be estimated by the autocorrelation function at zero lag (Schwartz and Shaw, 1975). The mean of the estimates of the average power for cells exhibiting no shift in frequency was calculated to be a value nine times less than that calculated for shifting cells when monitored for the same length of time. Any hypothesis which attempts to explain the mechanism responsible for controlling the frequency of ciliary beat must also account for such fluctuations in power.

Same cell sampled later in time

Cells were sampled at two different time periods at least three minutes apart to examine their baselines, relative frequency distribution, amplitudes of frequency shifts if present, and other relevant parameters (Table 12, Appendix B). Two cells were noted which did not exhibit any shift in frequency during the first sampling period, but did exhibit a shift in frequency during the second sampling period. Therefore, the ability to exhibit a shift in frequency does not seem to be affected by long time maintenance of baseline levels of ciliary beat. It is thus inferred that all cells have the ability to display a notable change in their frequency of beat. This lends further credence to the concept that shifts in ciliary beat frequency are caused by some pulsatile change in intracellular homeostasis which affects the apparatus responsible for regulating ciliary beat. This change could be a spontaneous shift of intracellular ions between compartments within the cell or a spontaneous transient change in electrical potential across the cell membrane. It is as yet unclear what type of physiological process acts to drive the system.

The baseline frequency for cells monitored later in time may increase, decrease, or remain the same (Table 12, Appendix B). One cell in ten exhibited a decrease in

baseline frequency; two cells showed no change in the level of baseline frequency; seven cells showed an increase of 1 to 5 Hz in baseline levels of ciliary beat (Figure 8).

For ten cells monitored at two different times, the range of frequencies spanned during the sampling periods changed very little for 3 cells and changed appreciably for the remaining 7 (Table 13, Appendix B). The standard deviations do not change appreciably according to chi-square analysis of the separate sample times. Therefore, it may be concluded that the baseline frequency and range of frequencies spanned change through time for an individual cell. In contrast the amount of random variability in ciliary beat frequency remains relatively the same through time.

These findings suggest that the apparatus responsible for controlling ciliary beat frequency may be tuned up or tuned down to increase or decrease the baseline level of ciliary beat. The sensitivity of the apparatus to whatever stimuli which cause variations in ciliary beat frequency is constant through time as reflected in the sameness of the standard deviations of the two different sample sets.

For cells demonstrating a shift in frequency during both sampling periods, the amplitude of frequency increase during a shift remained relatively constant from sample time to sample time (Table 14, Appendix B). The maximum

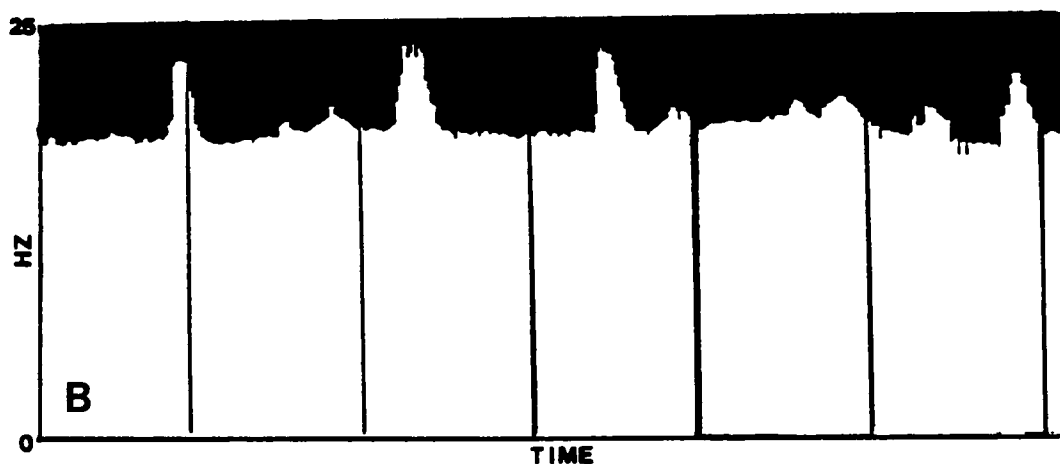
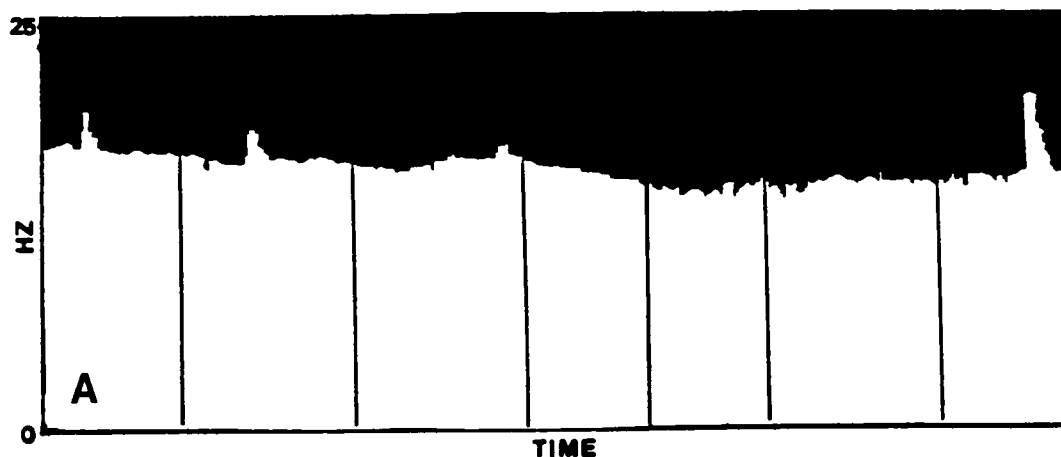


Figure 8. Computer plots of data sets of ciliary beat frequency from the same cell at two separate times. Baseline levels of beat at time "A" are in the interval between 16 - 17 Hz. Baseline levels at time "B" are in the interval between 18 - 19 Hz, reflecting an increase of 2 Hz. The maximum frequency recorded at time "A" is 20 Hz. The maximum frequency recorded at time "B" is 22 Hz. A linear relationship is noted between the increase in baseline and the increase in maximum frequency reported. (N = 450.)

frequency attained during a shift when compared to the change in baseline frequency exhibits a linear relationship with slope 1.00 (Table 15, Appendix B).

Thus, when the baseline frequency is increased or decreased, it is expected that the maximum value reported during a shift in frequency will increase to maintain a constant amplitude of increase. This suggests that the cell is capable of increasing its frequency of beat to a given level above baseline for any baseline the cell maintains. One could hypothesize that the apparatus responsible for controlling ciliary beat frequency has certain constraints. It can change its baseline frequency and can increase its beat frequency at random intervals and for variable durations.

The cross-correlation coefficients for the data sets acquired from the same cell at two different time periods revealed no consistent long term regular repeating pattern (Table 16, Appendix B). The cross-correlation coefficients varied from 0.819 to 0.127. The signals generated by changes in frequency over time, by a cell monitored at two different times, were, therefore, not well correlated.

Cells which change baseline

From the previous it is readily apparent that cells change baselines more often than they maintain them. Two

types of change in baseline may occur: a gradual drift from one level of beat to another, and an abrupt change or jump in baseline. Both types of change have been observed (Figure 9). Cells which exhibit an abrupt change in baseline frequency have been noted to display a frequency shift immediately preceding the change, and it may be inferred that the frequency shift may influence such abrupt changes.

For those cells which change frequency, the mathematical model describing ciliary beat frequency patterns may be modified to include terms describing the rate of change if the change is gradual, or the new baseline after a shift if the change is abrupt. The model for a gradual change in baseline frequency then becomes

$$y(t) = B_0 + \sum_{i=1}^N \{ [A_i \cos w_i(t-T_i)] * [u(t-T_i) - u(t-(T_i+D_i))] \} + dB/dt (t_b - t_a)$$

where $y(t)$ = the frequency of ciliary beat at time t ,

B_0 = the baseline frequency of beat,

N = the number of shifts present in the data set,

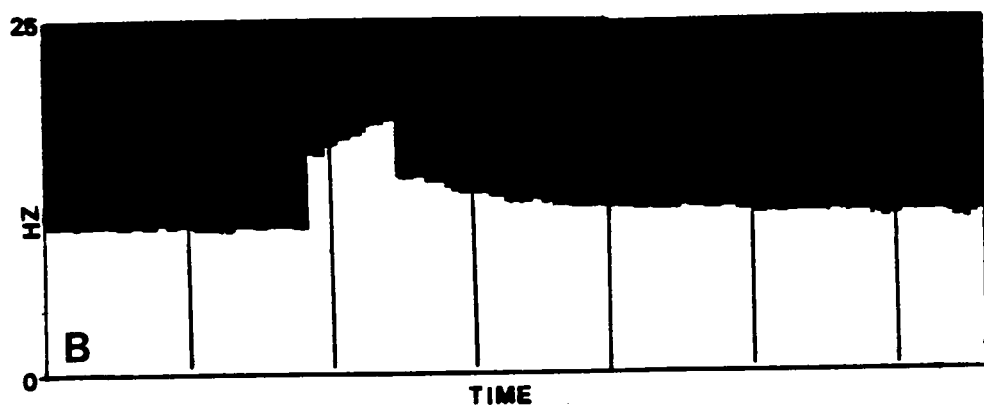
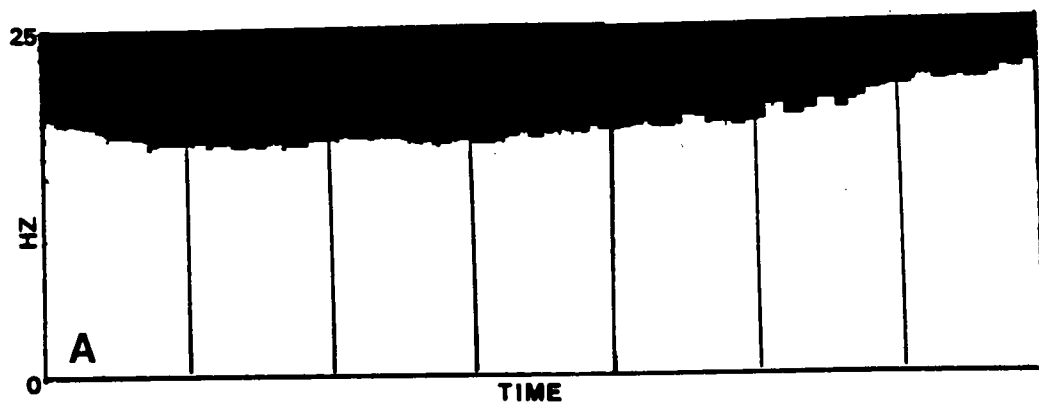


Figure 9. Computer plotted data set displaying gradual changes in the baseline level of beat frequency, "A", and abrupt changes in the baseline level following a frequency shift, "B".

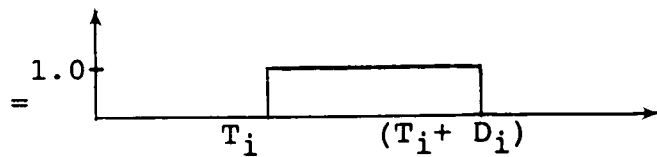
A_i = the amplitude of the i th frequency shift over baseline,

T_i = the time of onset of frequency shift,

D_i = the duration of the frequency shift,

$$w_i = 2 \pi f / D_i ,$$

$u(t-T_i)-u(t-(T_i + D_i))$ = a rectangular pulse of amplitude 1.0 beginning at time T_i and ending at $(T_i + D_i)$



and $dB/dt (t_b - t_a)$ = the gradual change in frequency through time.

For an abrupt change in frequency, the mathematical model becomes

$$y(t) = B_0 + \sum_{i=1}^N \{ [A_i \cos w_i(t-T_i)] \\ * [u(t-T_i) - u(t-(T_i + D_i))] \} \\ + (B_i - B_0)$$

where $y(t)$ = the frequency of ciliary beat at time t ,

B_0 = the baseline frequency of beat,

N = the number of shifts present in the data set,

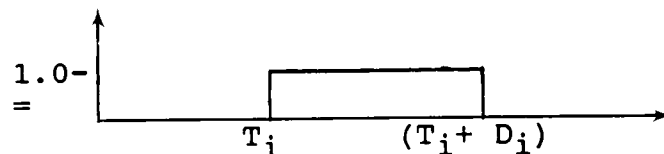
A_i = the amplitude of the i th frequency shift over baseline,

T_i = the time of onset of frequency shift,

D_i = the duration of the frequency shift,

$w_i = 2\pi / D_i$,

$u(t-T_i) - u(t-(T_i + D_i))$ = a rectangular pulse of amplitude 1.0 beginning at time T_i and ending at $(T_i + D_i)$



and $(B_i - B_0)$ = the abrupt change in frequency to new baseline B_i after a frequency shift.

Different tufts of cilia on the same cell

Using the videotaped record of the activity of ciliated outgrowths from tracheal explants, data from different tufts of cilia on the same cell may be collected. The tufts of cilia monitored were separated spatially and may or may not have been beating in phase. The signal generated by these separate tufts is obviously very similar for every cell monitored (Figures 10-12). The sample means for each tuft are statistically significantly different for all but 2 cells (Table 17, Appendix B). The z-scores for comparison of the sample means ranged from 0.11 to 34.74 as calculated according to the method of Miller (1965). The baseline frequency of the different tufts as determined from relative frequency histograms is reported as within the same one hertz interval for every tuft monitored for 5 of 10 cells. The baseline frequency for different tufts on the remaining 5 cells falls within a one hertz interval above or below the baseline reported for other tufts one each of these cells (Table 17, Appendix B). The range of frequencies spanned by the individual tufts is similar for each tuft but not identical.

The number of times a shift is noted is consistent for each tuft in 8 out of 10 cells (Table 18, Appendix B). In the remaining 2 cells, one shift is omitted from one of the three tufts monitored. The crests of corresponding shifts

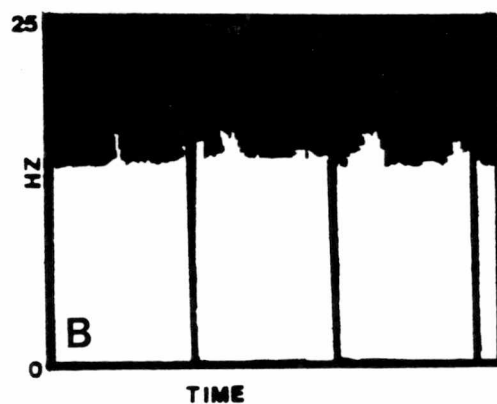
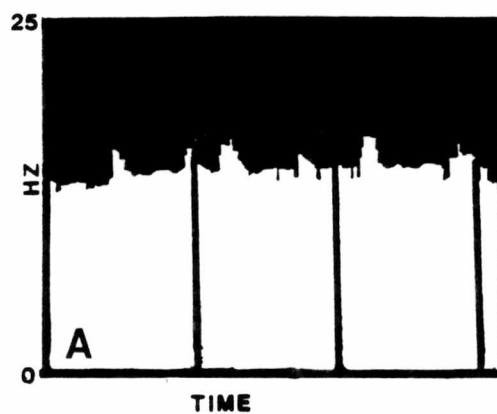


Figure 10. Computer plot of ciliary beat frequency as collected from two different tufts of cilia, "A" and "B", on the same cell.

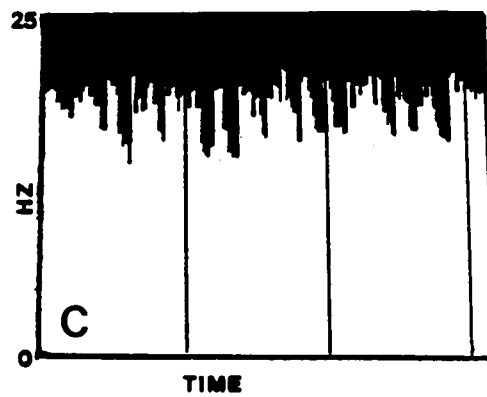
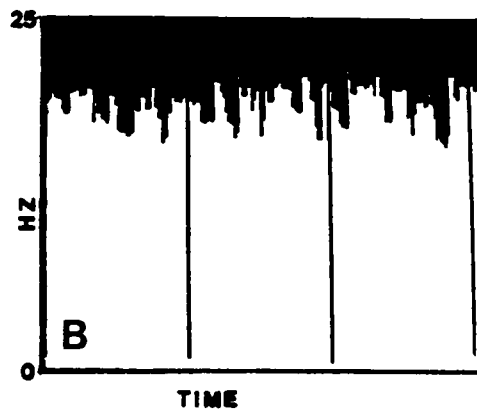
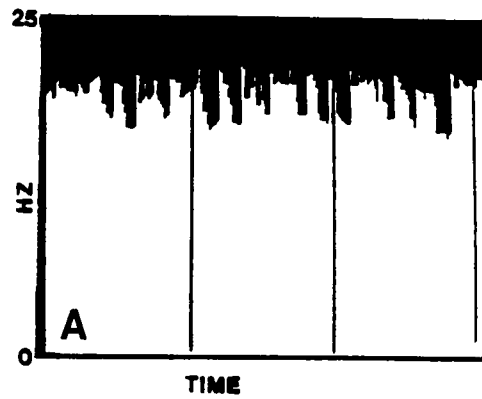


Figure 11. Computer plot of ciliary beat frequency as collected from three different tufts of cilia, "A", "B", and "C", on the same cell.

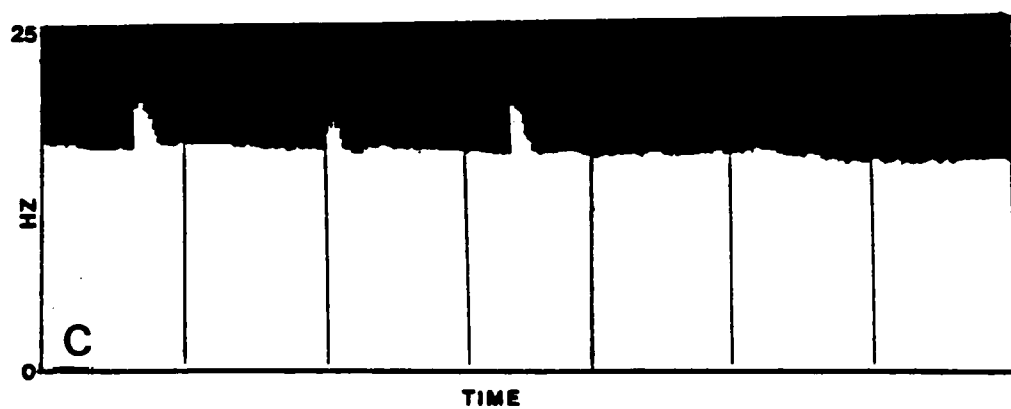
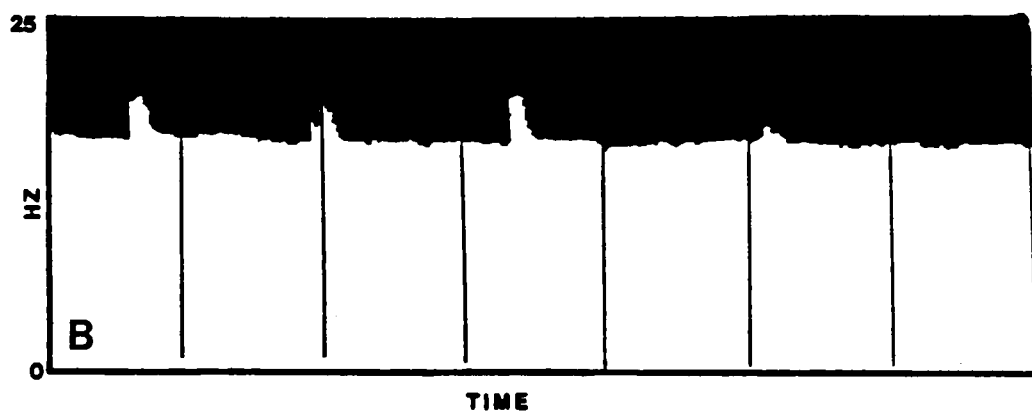
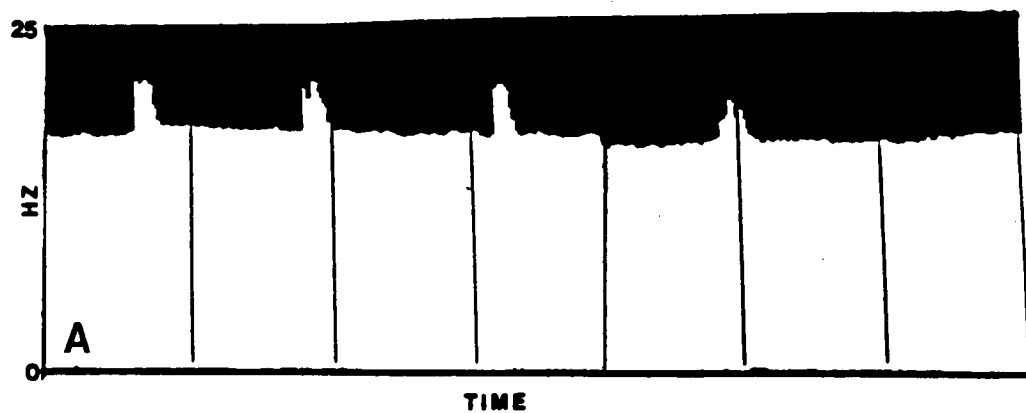


Figure 12. Another computer plot of ciliary beat frequency as collected from three different tufts of cilia, "A", "B", and "C", on the same cell. Note that the last shift is omitted from the record obtained from tuft "C".

reach amplitudes which do not differ significantly by chi-square analysis. The durations of the shifts in frequency also do not significantly differ statistically in all but one case (Table 19, Appendix B). Quantitation of the number of samples which are collected between crests of the frequency shifts allow for crude inspection of how well correlated the shifts are for the different tufts of cilia on the same cell (Table 20, Appendix B). The cross-correlation coefficients reveal more exactly the high degree of homology and coordination of signals generated by different tufts (Table 21, Appendix B).

The high degree of correlation suggests several hypotheses about the activities of different tufts of cilia on the same cell: (1) the ciliary tufts act similarly even when spatially separated on the surface of the outgrowth; (2) the baseline level of ciliary beat is maintained within about 1 Hz for different tufts of cilia on the same cell; (3) if a shift in frequency is noted, the maximum value attained during a shift is approximately the same for each tuft; (4) the duration of each shift is approximately the same for each tuft; and (5) the activity of the ciliary tufts are well correlated. This suggests that a shift in ciliary frequency may be due to the pulsatile administration of a stimulus, either extrinsic or intrinsic which affects each tuft of cilia present on the outgrowth

to increase ciliary frequency at the cellular level in the same fashion.

Cells in close proximity on the surface of the outgrowth

Data was collected from cells adjacent to each other on the surface of the explant, clusters of cells, and cells separated but still in close proximity in the microscopic field (Figures 13, 14). The baseline frequency of beat was again determined by relative frequency histograms. The baselines for each cell in 6 of 12 of the microscopic fields was within a range of 3 Hz (Table 22, Appendix B). For the remainder of the microscopic fields under study, at least three of the cells in the field maintained base line frequencies also within a 3 Hz range.

Cells that are randomly selected have approximately an equal probability of maintaining baseline frequencies of beat from 6 - 7 Hz to 23 - 24 Hz as judged from the values reported previously for randomly selected cells (Tables 1, 4, Appendix B). By chi-square analysis the baseline frequencies of ciliary beat for cells that reside close to each other on the outgrowth are, in fact, not assuming random values. The baseline frequencies for cells in close proximity on the outgrowth seem to assume values within a much narrower frequency range than would be expected from chance alone.

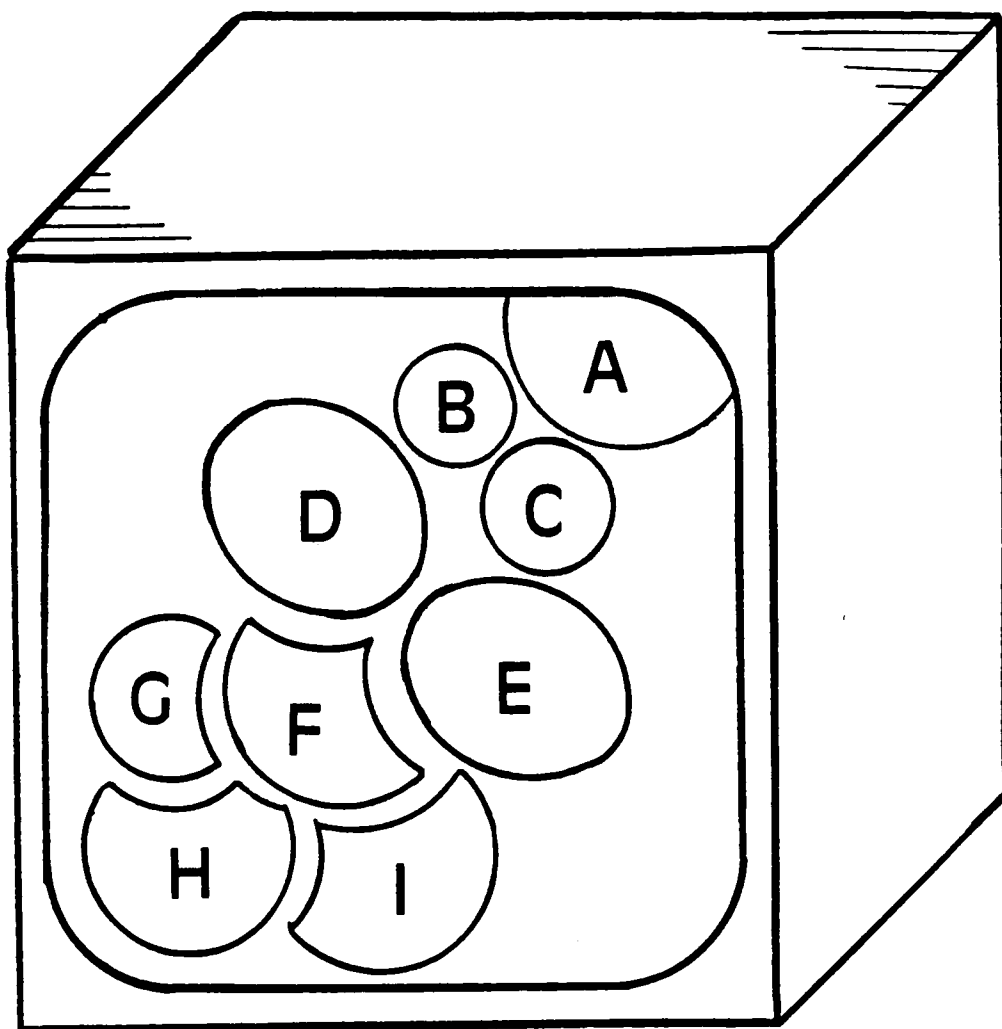


Figure 13. Diagram of example showing how cells were arranged on surface of the outgrowth. Frequency data was collected from these cells as shown in Figure 14. Data was analyzed according to the methods described in the text.

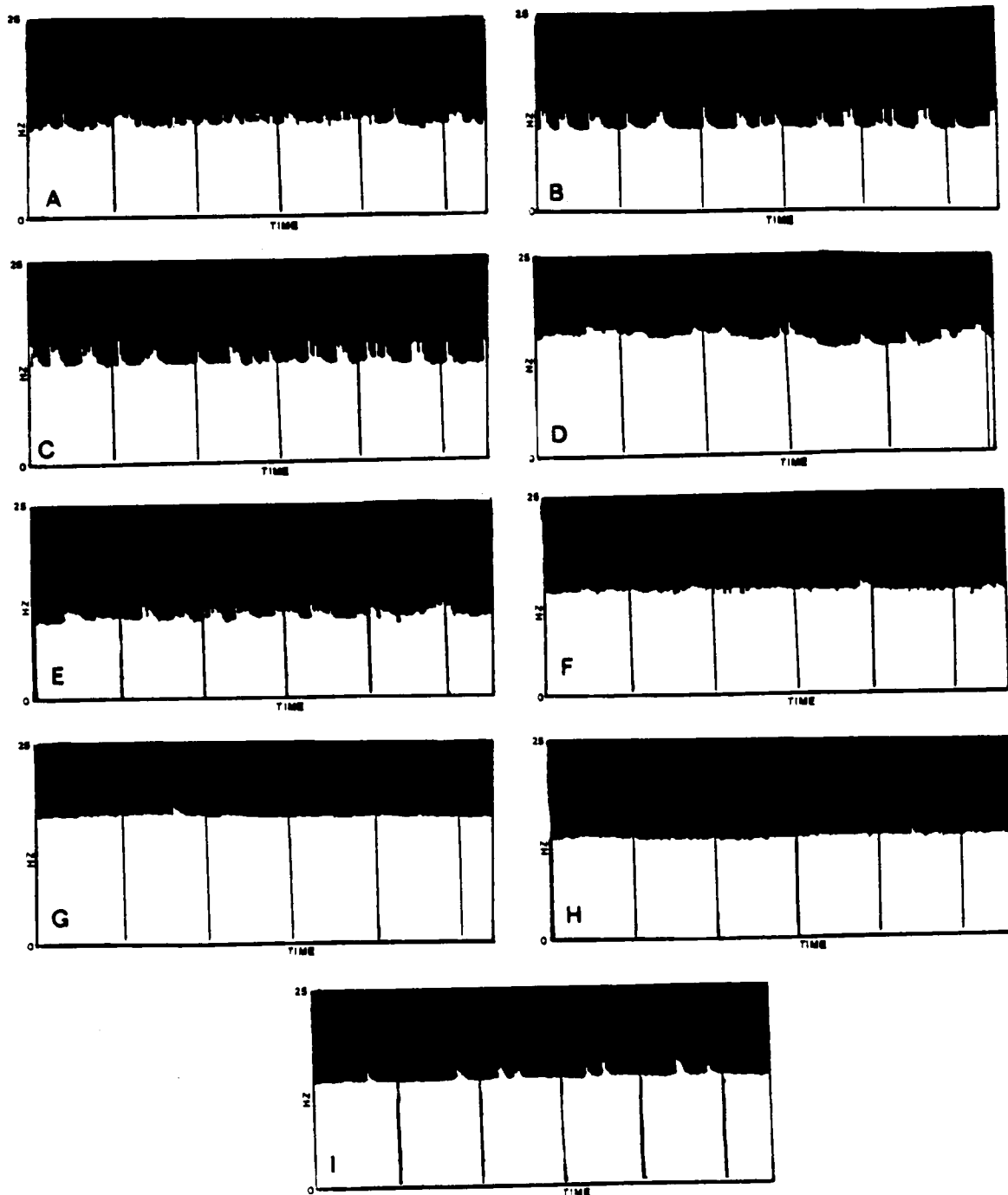


Figure 14. Computer plots of frequency with respect to time for cells diagrammed in Figure 13. Note that the shifting pattern of ciliary beat frequency is obviously not coordinated for adjacent cells or cells in close proximity on the outgrowth.

This could have valuable physiological significance for the efficient functioning of ciliated cells as a transport system. The effective transport of the protective mucus layer which resides atop the ciliated surface depends on the coordinated action of the carpet of cilia in metachronal waves. The formation of metachronal waves by ciliated cells would necessitate that ciliated cells in close proximity beat at approximately the same frequency. From the above, it seems as if levels of ciliary beat are maintained within relatively close ranges even in the outgrowth.

The number of shifts exhibited by cells which are adjacent or near each other on the outgrowth is not predictable from the analysis of an adjacent cell (Table 22, Appendix B). Cells may or may not exhibit a frequency shift if their neighbors are increasing their frequencies transiently. Therefore, it may be concluded that the agent responsible for inducing the increase in frequency is derived from an intracellular rather than extracellular sources. Furthermore, since the presence of gap junctions has been well-established in ciliated cells, it may be speculated that a stimulus responsible for the shift in ciliary frequency may be transferred through these gap junctions.

The maximum frequency attained during a frequency shift by different cells closely oriented on the outgrowth is not similar (Table 23, Appendix B). Some cells exhibited large shifts in frequency; some exhibited small shifts, and some cells did not exhibit a shift in frequency during the time monitored.

Physiologically, it may not be as important to the cells for maintenance of the metachronal wave to be able to achieve consistent maximal amplitudes. In fact, it might be speculated that these frequency increases are a mechanism adopted by ciliated cells to rid their surfaces of excess mucus or particulate matter which might hamper coherent, coordinated beat over the surface of the epithelium. As stated, the maximum amplitudes of increase in frequency for cells near each other on the surface of the explant are indeed variable (Table 23, Appendix B). This observation supports the hypothesis that a stimulus, exogenous or endogenous, is responsible for frequency increases, and that the amplitude of these increases in frequency to a maximum frequency level is of fairly constant value for an individual cell.

Since the waveforms produced by the timed records of frequency data were notably different from cell to cell, cross-correlation coefficients as a measure of comparison of cell-to-cell activities were expectedly low, and thus of

little value in examining the behaviors of adjacent cells. In some cases, the ciliary tufts of one cell were in contact with the ciliary tufts of adjacent cells. In these instances, the cross-correlation coefficients proved to be much higher and significant, reflecting similar activities of the two tufts monitored. These events are currently under study.

CHAPTER 5

DISCUSSION

Many methods have been employed in the effort to quantitatively assess the activity of ciliated cells. These methods have progressed with advances in technology and availability of sophisticated equipment from the stroboscopic methods used by Ballenger and Orr (1963) and Gosselin (1961) to the currently used photoelectronic techniques used by Sanderson and Dirksen (1985) and Kennedy and Ranyard (1983). The more recent techniques have several great advantages over methods employed previously. These include the capability to analyze single cells instead of broad areas of epithelium and to reduce human-introduced experimental error by including computer analysis of data generated.

The use of outgrowths instead of close-knit epithelium has also made the analysis of single, separate cells possible. The outline of the cell boundaries are easily discernible and therefore eliminates any question as to which cell is being monitored during a given period of observation. Sanderson and Dirksen (1985) have further advocated the use of cultured outgrowths for study of the activity of ciliated cells by noting that beat patterns of

the cilia on the outgrowths is not appreciably different from that of cilia on the original explants.

Data acquired by various investigators usually includes calculation of a mean frequency of beat from oscilloscope records or from Fourier analysis of beat patterns. Dulfano et al., (1981) calculated ciliary beat frequency by recording the mean of 10 sequential observations with as much as 8% experimental error between individual determinations. Teichtahl, Wright, and Kirsner (1986) determined ciliary beat frequency by recording activity from ten different areas of epithelium for each specimen and reporting mean frequency and standard deviations. This type of analysis does not permit quantitation of the activity of individual cells, and thus does not provide information which examines the function of the mucus transport system at the cellular level. The assessment of mean ciliary beat frequency from 10 areas of the epithelium can offer only a gross overview of the efficiency of ciliary beat function and gives no clues as to the mechanism which is responsible for maintaining ciliary beat.

Mercke, Hakansson, and Toremalm (1974a) outline several criteria for standardizing studies on mucociliary activity. These criteria for the experimental set-up include instantaneous recording ability, sensitivity to

rapid changes in frequency, uncomplicated analysis of recordings, simultaneous recording and regulation of experimental parameters including temperature and humidity, and incorporation of routine laboratory apparatus (Mercke et al. 1974a). The experimental set-up used in this project fulfills these criteria, with the exception of the addition of the spectrum analyzer to the laboratory apparatus.

To date, no other study has been made centering on rapid changes in frequency that are not the result of experimental manipulation, although references to frequency changes have been made. Dalhamn (1956) described a "very rapid flaring up and fading of points of light" over the tracheal epithelial surface of the rat. He raised the question as to whether the rapid movement of light was due to the cilia themselves or due to pulsations in the mucus layer. Toremalm et al., (1975) also described changes in ciliary beat frequency with a "rather regular pattern, indicating the presence of least two mixed frequencies." This group of experiments also posed questions attempted to be answered with this project: "Is there possibly a lack of coordination between adjacent ciliary cells or groups of cells with different frequencies?".

Kennedy and Duckett (1981) reported stable and unstable periods of activity over 60 second intervals.

Coherency of beat was noted for a 40 - 50 second time span followed by an upward shift during the final 10 second period. The range of frequencies reported for one cell ranged over a 4 Hz span. "Frequency fluctuations within a range (with interspersed) segments of constant activity" were also suggested (Kennedy and Duckett, 1981). They also reported that most cells in a localized region of tracheal epithelium are within the same frequency range.

Kennedy and Ranyard (1983) further explored the fluctuating nature of ciliary beat. They reported that 54.6% $\pm$ 3.1 of 800 cells exhibited frequency shifts within 0 - 2 Hz; 13.3% $\pm$ 2.5 of cells fluctuated within 2 - 4 Hz; 13.3 $\pm$ 1.5 of cells fluctuated 6 - 8 Hz. The remainder of the cells were noted to fluctuate at even higher frequencies. Also noted was the observation that outgrowth cells showed less fluctuation than intact epithelium. Implicit in these observations were the assumptions that these fluctuations represented activity around a mean frequency of beat and that the pattern of shift in frequency was an irregular one.

Extended time analysis of ciliary beat frequency in rabbit tracheal epithelial outgrowths has revealed regular patterns of frequency as monitored through time. Also, shifts in ciliary frequency do not represent fluctuation

around a mean frequency of beat. Mercke et al., (1974b) have promoted the idea of a "regular basic frequency produced by cilia." Extended time analysis has shown that cells do maintain baseline frequencies of beat for periods sometimes exceeding 8 minutes in duration. Cells do deviate from this baseline frequency, though. At intermittent periods, notable increases in frequency occur. These changes in frequency are variable in duration, but usually reach a constant amplitude. These increases do not occur in a regular rhythmic fashion. The rate of decline in frequency back to baseline levels is not constant as well.

Cells change their baseline level of beat in time. Thus, any argument for an intrinsic level of ciliary beat should incorporate both the concept of change in this intrinsic level, plus a mechanism to account for the variability of this level. In addition, assessment of ciliary beat frequency should recognize that baseline levels of beat are interrupted by shifts in frequency, and that the statistical parameters used to report ciliary beat frequency may be altered due to the presence or absence of shifts in frequency.

Gordon, Lane, and Miller (1980) have suggested that the basal bodies, which traditionally have been viewed as anchors for the cilia, play an active role in ciliary

function. Localization of ATPase activity to basal bodies in the oviduct has been accomplished by Anderson (1977). Localization of contractile proteins at the same sites of ATPase activity also has been demonstrated (Gordon *et al.*, 1980). Through morphological studies, Gordon (1982) has investigated the interconnections of microfilaments and microtubules between basal bodies, and suggests that "intermediate filament linkages make it possible for all basal bodies to react simultaneously in each cell." The data collected from different tufts of cilia on the same cell indicate that these different tufts operate at statistically significantly different frequencies which are very close in value. Since these values are so close, the basal bodies indeed may be cooperating to modulate the frequency of beat at or around the same frequency. The cells do react simultaneously in their frequency shift patterns as seen by the high values of the cross-correlation coefficients and other parameters which indicate similarity of action. Since the frequency of beat is statistically significantly different for each tuft, it can be assumed that the basal bodies operate somewhat independently as well.

The occasional incomplete coordination of the activities of different tufts of cilia on the same cell during a frequency shift may be explained in several ways.

The agent responsible for causing a frequency shift may be localized in one area of the cell. The increase in frequency may thus be seen differentially across spatially separate tufts due to differential dispersion of the agent responsible for causing the frequency shift. Another possible explanation for incomplete coordination of the activities of different tufts of cilia on the same cell could incorporate a "domino effect" for frequency increases. The agent responsible for eliciting a frequency shift may activate the frequency increase in one area of the cell. The frequency increase may then be transmitted to other tufts of cilia through the network of microtubules and microfilaments connecting the basal bodies.

In agreement with Toremalm et al., (1975) and Kennedy and Duckett (1981), analysis reveals that cells in close proximity beat at approximately the same frequency. Toremalm et al., (1975) simultaneously recorded frequencies from areas 20 μm apart and found that the same mean frequency was present, but with amplitude variations, indicating a phase shift. Kennedy and Duckett (1981) found that "(w)hereas the frequency of beat may vary substantially from region to region in the trachea, most cells in a local region of a normal trachea are within the same frequency range."

The pattern of frequency shift is not, however, shared by cells whose membranes are adjacent or that reside near each other on the outgrowth. The number of frequency shifts exhibited, the amplitudes of the shifts, and the timing of separate shifts are not consistent between or among cells sharing common boundaries or residing close to each other on the outgrowth. This prevents any speculation about the coordination of the activities of adjacent cells through gap junctions via transfer of electric potential, small ions, or other factors which might be exchanged through the gap junctions.

The regulation of ciliary activity by calcium has been the subject of much research. It has been found that calmodulin confers calcium sensitivity on ciliary dynein ATPase in Tetrahymena (Blum et al., 1980). It has also been found that calmodulin is present in tracheal epithelial cells (Gordon et al., 1982). Also, calcium is noted to regulate ciliary activity in rabbit tracheal explants and outgrowth (Girard and Kennedy, 1986; Bartusch, 1986). In this light, it would then be reasonable to hypothesize that calcium is the agent responsible for eliciting these transient shifts in frequency. A change in the intracellular levels of calcium in irregularly timed bursts could be responsible for the abrupt increases in frequency.

Murakami and Eckert (1972) have noted transient increases in ciliary frequency in response to mechanical stimulation of oviduct membrane bathed in a solution containing excess calcium ions. Due to the mechanical stimulation, calcium ions were assumed to enter the cell and elicit the frequency increase. A similar response has recently been demonstrated by Sanderson and Dirksen (1988) for ciliated rabbit tracheal outgrowth cells.

Calcium has been noted to be sequestered in such storage compartments as the mitochondria and the endoplasmic reticulum (Somlyo, 1984). The attainment of a constant amplitude during a shift in frequency may be due to the release of a quantum or set level of calcium ion from an intracellular storage compartment, or may be due to the release of a variable amount of calcium which could saturate all calcium binding sites available which are involved in ciliary beat frequency modulation.

Trifluoperazine, an inhibitor of calmodulin-sensitive calcium-dependent enzymes and phospholipid-sensitive calcium-dependent protein kinase, has been shown to reduce ciliary frequencies (Girard and Kennedy, 1986). Therefore, a cascade-type mechanism may be involved in modulation of ciliary beat frequency. Saturation of the binding sites in this cascade may involve very few ions to elicit changes in

beat frequency such as the ones seen during the frequency shifts described in the preceding.

The variability of the durations of each shift and the variability of the rate of decline back to baseline levels also support the latter hypothesized mechanism. The presence of minor shifts in frequency may be the result of fewer ions being bound by the frequency modulating apparatus. Variable calcium ion concentrations have been shown to produce a graded increase in ciliary beat frequency (Bartusch, 1986). The variability in the duration of frequency increase may be reflective of the variability of the amount of calcium available to sustain a maximal increase in frequency through such a cascade. The slow decline in frequency as the cell re-attains its baseline levels may be due to intracellular pumping of the ion back into its storage compartment. This type of activation/inactivation cycle has been suggested as the mechanism responsible for the regulation of ciliary arrest response in lamellibranch gill lateral cilia (Stommel and Stephens, 1985). Also, the presence of calcium ATPases have been demonstrated in Paramecium cilia (Doughty and Kaneshiro, 1982). Irregular rates of decrement in frequency may be related to variable levels of calcium ion which purportedly activate the frequency apparatus. Additionally, since repetitive frequency shifts are noted,

it would be reasonable to assume that the frequency modulating apparatus is repetitively activated with the agent responsible for initiating the shift in frequency, and subsequently inactivated by removal of this agent for each cycle.

The mechanism responsible for change in baseline frequency of beat is most likely not be related to the agent responsible for frequency shifts. Kakuta et al., (1985) have reported that skinned dog tracheal epithelium displays continued ciliary beat in virtual absence of calcium. Cells which are changing baseline may or may not exhibit transient shifts in frequency. This indicates that the agent responsible for initiating a shift in frequency is probably distinct from the factors governing baseline frequencies of beat, at least in terms of long term effects. The cause and mechanism underlying the presence of frequency shifts is currently under investigation.

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APPENDICES

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0021      TYPE 3,INFILE
0022      3      FORMAT(/,ZX,'Enter parameters for file -- ',I3)
0023      5      TYPE 10
0024      10     FORMAT(/,ZX,'The name of the file is -- ',I6)
0025      DO 11 I=1,7
0026      FNAME(I,INFILE)=SPACE
0027      DNAME(I)=SPACE
0028      11     CONTINUE
0029      ACCEPT 13,(FNAME(IG,INFILE),IG=1,6)
0030      13     FORMAT(SA1)
C
C
C
C      OPEN PEAKHZ FILE AND BEGIN PROCESSING
C
C
C
0031      IF (INFILE.NE.1) GO TO 17
0033      14     DO 15 JU=1,7
0034      DNAME(JU)=FNAME(JU,1)
0035      15     CONTINUE
0036      GO TO 18
0037      17     DO 18 JF=1,7
0038      DNAME(JF)=FNAME(JF,2)
0039      18     CONTINUE
0040      18     OPEN (UNIT=DY1,NAME=DNAME,TYPE='OLD',REASONLY,
1      ACCESS='SEQUENTIAL',DISP='SAVE',RECORDSIZE=8,MAXREC=451,
1      FORM='FORMATTED')
0041      READ(DY1,1050)KREC
0042      CLOSE(UNIT=DY1)
0043      TYPE 40,KREC
0044      40     FORMAT(/,XX,'The number of values stored in this file is -- ',
1      I6)
0045      TYPE 20
0046      20     FORMAT(/,ZX,'Enter the ID number of the FIRST transform to be',
1      ' processed -- ',I6)
0047      ACCEPT *,I6EC
0048      TYPE 30
0049      30     FORMAT(/,XX,'Enter the total number of values to be processed -- ',
1      X,I6)
0050      ACCEPT *,INLAST
0051      LAST=FLOAT(INLAST)
C
C NOW INPUT PEAK FREQ VALS INTO CORRECT PKVAL COLUMN IN ARRAY
C
0052      IF (INFILE.NE.1) GO TO 43
0054      DO 42 IK=1,7
0055      DNAME(IK)=FNAME(IK,1)
0056      42     CONTINUE
0057      GO TO 45
0059      43     DO 44 IE=1,7
0059      DNAME(IE)=FNAME(IE,2)
0060      44     CONTINUE

```



```

C
0127 270 TYPE 280
0128 280 FORMAT(//,2X,'Do you wish to create a bin histogram of the ',
1 'data ? ',6)
0129 ACCEPT 1050,RSP
0130 IF (RSP.NE.'Y') GO TO 401
0132 DO 400 M=1,NFILES
0133 ITABLE(1)=1
0134 ITABLE(2)=1
0135 ITABLE(3)=30
0136 ITABLE(4)=INLAST
0137 DO 290 KX=5,10
0138 ITABLE(KX)=0
0139 290 CONTINUE
0140 DO 300 N=1,INLAST
0141 INPUT(N)=PRVAL(N,M)
0142 300 CONTINUE
C
0143 CALL HISTC(ITABLE,INPUT,IHERAM)
C
C OUTPUT
C
0144 TYPE 310,M
0145 310 FORMAT(//,2X,'Do you wish to save the histogram data ',
1 //,2X,'for file ',I3,' displayed on the terminal screen ? ',6)
0146 ACCEPT 1050,REP
0147 IF (REP.NE.'Y') GO TO 350
0148 TYPE 320,(FNAME(N,M),K0=1,6)
0150 320 FORMAT(//,2X,'BIN HISTOGRAM FOR -- ',6A1,//,2X,
1 4(' INTERVAL COUNT'),//)
0151 TYPE 330,(NB,(NB+1),IHERAM(NB),NB=5,25)
0152 330 FORMAT(4(I7,'-',I4,I6))
0153 TYPE 340,(ITABLE(LI),LI=6,8)
0154 340 FORMAT(//,2X,'UNDERFLOW COUNT = ',I3,//,2X,
1 'OVERFLOW COUNT = ',I3,//,2X,
1 'NO. OF COUNTERS WHICH OVERFLOWED = ',I2,//)
C
C PRINTING OUTPUT
C
0155 350 TYPE 350,M
0156 350 FORMAT(2X,'Do you wish to print out the histogram ',
1 'for file ',I3,' ? ',6)
0157 ACCEPT 1050,RSP
0158 IF (RSP.NE.'Y') GO TO 400
0159 WRITE(5,320)(FNAME(N,M),N=1,6)
0161 WRITE(5,330)(NN,(NN+1),IHERAM(NN),NN=5,25)
0162 WRITE(5,340)(ITABLE(LX),LX=6,8)
0163 400 CONTINUE
C
CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
C
C PART (3) AUTOCORRELATION FUNCTION AND COEFFICIENTS
C
CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC

```

```

      C
      C PAD REST OF PKSUB FILE WITH NOISE
0164 401 DO 407 LH=1,NFILES
0165      DO 405 ID=INLAST+1,512
0166      PKSUB(ID,LH)=RAN(SEED,SEED2)-0.5
0167 405 CONTINUE
0168      SEED=4
0169      SEED2=5
0170 407 CONTINUE
      C
      C WHITE NOISE PRODUCES A FLAT POWER SPECTRUM
      C
0171 410 TYPE 420
0172 420 FORMAT(//,2X,'AUTOCORRELATION CALCULATIONS ',//,2X,
      1 'Do you wish to calculate the autocorrelation ',
      1 'function ? ',9)
0173      ACCEPT 1050,RSP
0174      IF (RSP.NE.'Y') GO TO 2000
0175      DO 550 LL=1,NFILES
0176      TYPE 425,LL
0177 425 FORMAT(/,2X,'FILE -- ',13,/,2X,
      1 'Autocorrelation is calculated for lags of zero ',
      1 'to LAG')
0178      TYPE 430
0179 430 FORMAT(//,2X,'What is the lag time that you wish to use ?',
      1 //,2X,' -- must be less than the number of values -- ',9)
0180      ACCEPT *,LAG
      C
      C AUTOCORRELATION IN DOUBLE PRECISION
      C
      C N-K
      C FORMULA USED : R(K) = 1/(N-K) SUM X(I) * X(I+K)
      C
      C I=1
      C
      C FLOAT FXN -- INTEGER TO REAL CONVERSION
      C IFIX FXN -- REAL TO INTEGER CONVERSION
      C
      C AUTOCORRELATION CALCULATED FOR LAGS OF JJ=0,LAG
0182      DO 447 JJ=1,(LAG+1)
0183      SUMAC=0.0
0184      XLAG=FLOAT(JJ)-1.0
0185      ILAG=IFIX(XLAG)
      C SUMMATION LOOP
0186      DO 446 JJX=1,(INLAST-ILAG)
0187      SUMAC=PKSUB(JJX,LL)+PKSUB((JJX+ILAG),LL) + SUMAC
0188 446 CONTINUE
      C DIVIDE WHOLE SUM TO GET CORRELATION FUNCTION
0189      AUTOCD(JJ,LL)=(1.0/(LAST-XLAG))*SUMAC
0190 447 CONTINUE
      C
      C OUTPUT
      C
0191      WRITE(6,450)(FNAME(LK,LL),LK=1,6)
0192 450 FORMAT(/,'AUTOCORRELATION FUNCTION FOR -- ',6A1,/)
0193      WRITE(6,450)

```



```

      C DIVIDE WHOLE SUM TO GET CROSSCORRELATION FUNCTION
0230      CRCORR(KP)=(1.0/(LAST-XLAG2))*SUMCR
0231      S10  CONTINUE
      C
      C OUTPUT
      C
0232      WRITE(S,620)(FNAME(LC,1),LC=1,6),(FNAME(LY,2),LY=1,6)
0233      620  FORMAT(//,'CROSSCORRELATION FUNCTION FOR -- ',6A1,
1          ' ' AND ',6A1,/)
0234      WRITE(S,490)
0235      WRITE(S,470)((IW-1),CRCORR(IW),IW=1,(LAG2+1))
0236      TYPE 490
0237      ACCEPT 1050,RSP
0238      IF(RES.NE.'Y') GO TO 2000
      C
      C FORMULA USED :

$$RHO(K) = \frac{R(K) - MEAN * MEAN}{\sqrt{(R(0) - MEAN * MEAN) * (R(0) - MEAN * MEAN)}}$$


$$RHO(K) = \frac{XY}{\sqrt{XX * YY}}$$

      C
      C REMEMBER FOR A ZERO MEAN FUNCTION R(0)=VARIANCE
      C CAN ALSO DROP MEAN CALCULATIONS SINCE MEAN =0
      C
      C FORMULA SIMPLIFIES TO  $RHO(K) = R(K) / \sqrt{VAR(X) * VAR(Y)}$ 

$$RHO(K) = \frac{XY}{\sqrt{XX * YY}}$$

      C PP 48 BENDAT AND PIERSON
      C
0240      DO 640 JT=1,(LAG2+1)
0241      RHOCR(JT)=CRCORR(JT)/(SQRT(VAR(1)*VAR(2)))
0242      S40  CONTINUE
      C
      C OUTPUT
      C
0243      WRITE(S,650)(FNAME(KF,1),KF=1,6),(FNAME(KS,2),KS=1,6)
0244      650  FORMAT(//,'CROSSCORRELATION COEFFICIENTS FOR -- ',6A1,
1          ' ' AND ',6A1,/)
      C SHOULD BE A VALUE BETWEEN -1 AND 1
0245      WRITE(S,530)
0246      WRITE(S,540)((IC-1),RHOCR(IC),IC=1,(LAG2+1))
0247      2000  STOP
0248      END

```

```

CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
C
C
C          PEAK PROCESSING PROGRAM
C
C                      S AUSTIN
CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
C
C  THIS PROGRAM ACCESSES THE PEAKHZ ARRAY TO ANALYZE THE DATA SET OF
C  MAXIMUM FREQUENCIES TO DETECT TRENDS. INCLUDES OUTPUT OF THE
C  (1) AREA OF THE PEAKS, (2) HEIGHT OF THE PEAK, (3) CREST TIME OR
C  TIME OF CREST OF PEAK, (4) LEADING MINIMUM HEIGHT OR BASELINE BEFORE
C  PEAK, (5) LEADING MINIMUM TIME OR X COORDINATE OF BASELINE
C  BEFORE PEAK, (6) WIDTH OF PEAK, (7) TRAILING MINIMUM HEIGHT OR
C  X COORDINATE OF NEW BASELINE FOR EACH PEAK, (8) TRAILING MINIMUM
C  TIME OR X COORDINATE OF NEW BASELINE FOR EACH PEAK, (9) ENDING
C  INDICATOR OR PLACE WHERE NEW PEAKS BEGUN AND (10) CURRENT
C  NUMBER OF INPUT POINTS AVERAGED.
C
CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
C
C          INITIALIZATION ROUTINE
C
CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
C
0001      DIMENSION INPUT(512),OUTPUT(10,10),ITABLE(63),VTYPE(2,2)
0002      DIMENSION PKVAL(512),TIMELG(9),XINCR(10,2)
0003      REAL*4 ADVANC
0004      INTEGER*2 DY1,IREC,NREC,ENDREC,ILAST,INREC,I,JJ,NPEAKS
0005      BYTE SPACE
0006      DATA DY1/2/, SPACE/' '/
0007      DATA ITABLE/1,2,2,1,1,63*0/
0008      DATA INPTR,IDIMO,NPEAKS/0,10,0/
0009      DATA VTYPE/' VA','LLEY','BASE','LINE'/
0010      BYTE FNAME(7),RSP
0011      COMMON/RSPRNT/FNAME,RSP
C
C
0012 1050  FORMAT(A1)
0013 5      TYPE 10
0014 10     FORMAT(///,2X,'Enter the name of the file to be processed --',
0015          1 $)
0015      DO 12 I=1,7
0016          FNAME(I)=SPACE
0017 12      CONTINUE
0018          ACCEPT 15,(FNAME(I),I=1,6)
0019 15      FORMAT (6A1)
C
CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
C
C          OPEN PEAKHZ FILE AND BEGIN PROCESSING
C
CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC

```

```

      C
0020      OPEN (UNIT=DY1,NAME=FNAME,TYPE='OLD',READONLY,
           1  ACCESS='SEQUENTIAL',DISP='SAVE',RECORDSIZE=6,MAXREC=451,
           1  FORM='FORMATTED')
0021      READ(DY1,18)KREC
0022  18    FORMAT(I6)
0023      CLOSE(UNIT=DY1)
0024      TYPE 40,KREC
0025  40    FORMAT(//,XX,'THE NUMBER OF VALUES STORED IN THIS FILE IS -- ',
           1  I6)
0026      TYPE 20
0027  20    FORMAT(//,XX,'ENTER THE ID NUMBER OF THE FIRST TRANSFORM TO BE',
           1  ' PROCESSED -- ',*)
0028      ACCEPT *,IREC
0029      IREC=IREC+1
      C THIS IS TO ACCESS ONLY THE VALUES STORED AND NOT THE #OF VALS STORED
0030      TYPE 30
0031  30    FORMAT(//,XX,'ENTER THE TOTAL NUMBER OF VALUES TO BE PROCESSED --',
           1  X,*)
0032      ACCEPT *,INLAST
      C
      C NOW INPUT PEAK FREQ VALS INTO INPUT ARRAY WHICH IS ACCESSED BY THE
      C PEAK ALGORITHM. FIRST OPEN AND CLOSE ARRAY, THEN MANIPULATE.
      C
0033      OPEN (UNIT=DY1,NAME=FNAME,TYPE='OLD',READONLY,
           1  ACCESS='SEQUENTIAL',DISP='SAVE',RECORDSIZE=6,MAXREC=451,
           1  FORM='FORMATTED')
      C NOW ADVANCE TO CORRECT RECORD NUMBER TO BEGIN READING SINCE SEQUENTIAL
      C
0034      READ(DY1,18)KREC
0035      IF(IREC.EQ.1)GO TO 70
0037      DO 60 KL=1,IREC
0038      READ(DY1,50)ADVANC
0039  50    FORMAT(F6.2)
0040  60    CONTINUE
      C
      C NOW READ OFF FIRST VALUE AND THEN TRUNCATE TO AN INTEGER VALUE FOR
      C THE PEAK PROCESSING SUBROUTINE TO UTILIZE
      C PKVAL IS A DUMMY VARIABLE WHICH CONTAINS EACH OF THE READ-OFF VALS
      C
0041  70    DO 90 J=1,INLAST
0042      READ(DY1,80)PKVAL(J)
0043  80    FORMAT(F6.2)
0044  90    CONTINUE
0045      CLOSE (UNIT=DY1,DISP='SAVE')
0046      DO 100 IJ=1,INLAST
0047      IFAKE=PKVAL(IJ)
0048      TRUN=PKVAL(IJ)-IFAKE
0049      IF(TRUN.LT.0.5) INPUT(IJ)=PKVAL(IJ)
0051      IPK=PKVAL(IJ)+1.0
0052      IF(TRUN.GE.0.5) INPUT(IJ)=IPK
0054  95    FORMAT(2X,F6.0,5X,I6)
0055  100   CONTINUE

```

```

      C NOW VALUES SHOULD BE PUT INTO INPUT ARRAY WITH FIRST VALUE IN INPUT(1)
      C OPTION TO WRITE VALUES TO PRINTER
      C
0056      TYPE 105
0057 105  FORMAT(//,' DO YOU WISH TO PRINT OUT THE INPUT VALUES ? ','$)
0058      ACCEPT 1050,RSP
0059      IF (RSP.NE.'Y') GO TO 175
0061      WRITE(6,110)(FNAME(KJ),KJ=1,6)
0062 110  FORMAT(//,6A1,X,'INPUT',//)
0063      DO 150 JJ=1,INLAST
0064      JK=JJ+1
0065      JL=JK+1
0066      JN=JL+1
0067      WRITE(6,140)JJ,INPUT(JJ),PKVAL(JJ),JK,INPUT(JK),PKVAL(JK),
1      JL,INPUT(JL),PKVAL(JL),JN,INPUT(JN),PKVAL(JN)
0068 140  FORMAT(I3,3X,I6,5X,F6.2,5X,I3,3X,I6,5X,F6.2,5X,I3,3X,I6,5X,
1      F6.2,5X,I3,3X,I6,5X,F6.2)
0069 150  CONTINUE
      C
      C NOW USE PEAK PROCESSING ALGORITHM
      C
0070 175  TYPE 200
0071 200  FORMAT(//,2X,'MAX # OF PEAKS WHICH MAY BE FOUND IS 10')
      C
      C ZERO PAD THE REST OF THE ARRAY
0072      DO 225 LX=INLAST+1,512
0073      INPUT(LX)=0
0074 225  CONTINUE
      C
0075      CALL PEAK(ITABLE,INPUT,INLAST,INPTR,OUTPUT,IDIMO,NPEAKS)
      C
      C PRINTOUT
      C
0076      TYPE 450,NPEAKS
0077 450  FORMAT(//,2X,'THE NUMBER OF PEAKS FOUND IN THIS DATA IS -- ','I2)
      C IF(INPTR.LT.0.AND.NPEAKS.EQ.0) GO TO 2000
0078      TYPE 500
0079 500  FORMAT(//,2X,'DO YOU WISH TO HAVE THE DATA DISPLAYED ON',//,2X,
1      'THE TERMINAL SCREEN ?','$)
0080      ACCEPT 1050,RSP
0081      IF (RSP.NE.'Y') GO TO 1400
0083      TYPE 600,(FNAME(K0),K0=1,6)
0084 600  FORMAT(//,XX,'DATA FOR -- ','6A1)
0085      TYPE 800
0086 800  FORMAT(///,2X,' PEAK NO.',8X,'AREA',4X,'CR HEIGHT',5X,'CR TIME',
1      4X,'L HEIGHT',6X,'L TIME',//,11X,'HALF WIDTH',4X,'T HEIGHT',6X,
1      'T TIME',8X,'TYPE',8X,'RATE',//)
0087      DO 900 L=1,NPEAKS
0088      KK=OUTPUT(9,L)+1
0089      TYPE 850,(L,(OUTPUT(I,L),I=1,8),(VTYPE(K,XX),K=1,2),
1      OUTPUT(10,L))
0090 850  FORMAT(2X,I9,5F12.2,/,8X,3F12.2,4X,2A4,F12.2)
0091 900  CONTINUE
      C NOW ADD LOOP TO FIGURE DIFFERENCES IN PARAMETERS, IE LAG TIMES BETWEEN

```

C PEAKS AND AMOUNT OF INCREASE FROM BASELINE

C

```

0092     IF(NPEAKS.EQ.1) GO TO 1275
0094     TYPE 1125
0095 1125  FORMAT(/,2X,'TIMES BETWEEN SUCCESSIVE PEAKS IN SECS',/)
0096     DO 1200 LX=1,NPEAKS-1
0097     LXX=LX+1
0098     TIMELG(LX)=OUTPUT(3,LXX)-OUTPUT(3,LX)
0099     TYPE 1250,LX,LXX,TIMELG(LX)
0100 1250  FORMAT(2X,'BETWN PEAKS ',I2,' AND ',I2,' -- ',F10.0)
0101 1200  CONTINUE
0102 1275  TYPE 1300
0103 1300  FORMAT(/,2X,'AMOUNTS OF INCREASE FROM BASELINES TO CRESTS',/,
        3X,'PEAK NO.',3X,'LEADING',11X,'TRAILING')
0104     DO 1350 IZ=1,NPEAKS
0105     XINCR(IZ,1)=OUTPUT(2,IZ)-OUTPUT(4,IZ)
0106     XINCR(IZ,2)=OUTPUT(2,IZ)-OUTPUT(7,IZ)
0107     TYPE 1375,IZ,XINCR(IZ,1),XINCR(IZ,2)
0108 1375  FORMAT(16,6X,F6.0,12X,F6.0)
0109 1350  CONTINUE
        C
        C NOW ASK IF WANT TO TYPE TO PRINTER
        C
0110 1400  TYPE 1450
0111 1450  FORMAT(/,2X,'DO YOU WISH TO HAVE THE DATA PRINTED ON THE',
        1 /,2X,'PRINTER ?',9)
0112     ACCEPT 1050,RSP
0113     IF(RSP.NE.'Y') GO TO 2000
0115     WRITE(6,600)(FNAME(KD),KD=1,5)
0116     WRITE(6,1000)
0117 1000  FORMAT(/,3X,'PEAK',5X,'AREA',4X,'CR HEIGHT',4X,'CR TIME',
        1 4X,'L HEIGHT',4X,'L TIME',4X,'1/2 WOTH',4X,'T HEIGHT',
        1 4X,'T TIME',6X,'TYPE',6X,'RATE')
0118     DO 1500 LL=1,NPEAKS
0119     II=OUTPUT(9,LL)+1
0120     WRITE(6,1100)(LL,(OUTPUT(I,LL),I=1,8),(VTYPE(K,II),K=1,2),
        1 OUTPUT(10,LL))
0121 1100  FORMAT(13,8F11.0,4X,2A4,F9.0)
0122 1500  CONTINUE
        C
0123     WRITE(6,1125)
0124     DO 1550 JZ=1,NPEAKS-1
0125     JZZ=JZ+1
0126     WRITE(6,1250)JZ,JZZ,TIMELG(JZ)
0127 1550  CONTINUE
0128     WRITE(6,1300)
0129     DO 1600 KZ=1,NPEAKS
0130     WRITE(6,1375)KZ,XINCR(KZ,1),XINCR(KZ,2)
0131 1600  CONTINUE
0132 2000  STOP
0133     END

```

APPENDIX B

Table 1. Statistical parameters characterizing ciliary beat when no frequency shift is present.

CELL	N	MEAN (Hz)	STD DEV (STD ERR)	RANGE(Hz) (SPAN)	FREQUENCY DISTRIBUTION (Percentages)
1	450	6.88	0.18 (0.01)	6-8 (2)	6-7 Hz = 91.33 ** 7-8 Hz = 8.67
2	200	8.96	0.22 (0.02)	8-10 (2)	8-9 Hz = 46.50 9-10Hz = 53.50 **
3	450	9.33	0.30 (0.03)	8-11 (3)	8-9 Hz = 30.89 9-10Hz = 53.11 ** 10-11Hz = 16.00
4	200	9.67	0.13 (0.01)	9-11 (2)	9-10Hz = 98.00 ** 10-11Hz = 2.00
5	450	9.94	0.42 (0.02)	8-11 (3)	8-9 Hz = 2.89 9-10Hz = 38.89 10-11Hz = 58.22 **
6	200	10.43	0.29 (0.02)	9-12 (3)	9-10Hz = 10.50 10-11Hz = 87.50 ** 11-12Hz = 2.00
7	200	10.90	0.18 (0.01)	10-12 (2)	10-11Hz = 76.00 ** 11-12Hz = 24.00
8	200	12.21	0.25 (0.02)	11-13 (2)	11-12Hz = 26.00 12-13Hz = 74.00 **
9	450	12.79	0.27 (0.01)	11-14 (2)	11-12Hz = 0.67 12-13Hz = 83.78 ** 13-14Hz = 15.56
10	450	13.01	0.46 (0.02)	11-15 (2)	11-12Hz = 0.67 12-13Hz = 50.22 ** 13-14Hz = 48.67 14-15Hz = 0.44
11	200	13.04	0.27 (0.02)	12-14 (2)	12-13Hz = 52.50 ** 13-14Hz = 47.50
12	200	13.75	0.42 (0.03)	12-15 (3)	12-13Hz = 10.00 13-14Hz = 57.50 ** 14-15Hz = 32.50

Table 1. (Continued)

CELL	N	MEAN (Hz)	STD DEV STD ERR	RANGE(Hz) (SPAN)	FREQUENCY DISTRIBUTION (Percentages)
13	200	13.86	0.17 (0.01)	13-15 (2)	13-14Hz = 83.00 ** 14-15Hz = 17.00
14	200	14.28	0.30 (0.02)	13-15 (2)	13-14Hz = 12.00 14-15Hz = 88.00 **
15	450	15.93	0.21 (0.01)	15-18 (3)	15-16Hz = 53.56 ** 16-17Hz = 46.22 ** 17-18Hz = 0.22
16	200	16.14	0.32 (0.02)	15-18 (3)	15-16Hz = 36.00 16-17Hz = 62.50 ** 17-18Hz = 1.50
17	200	16.67	0.22 (0.02)	15-18 (3)	15-16Hz = 1.50 16-17Hz = 92.00 ** 17-18Hz = 6.50
18	180	17.24	0.43 (0.03)	16-19 (3)	16-17Hz = 40.00 17-18Hz = 51.67 ** 18-19Hz = 8.33
AVE. VALS.	X	X	0.28 (0.02)	X (2.44)	AVE. PERCENTAGE OF FREQUENCY DISTRIBUTION SPENT AT BASELINE = 70.36 (s = 17.11, std. error = 4.03)

** indicates the baseline frequency of beat.

Table 2. Statistical parameters characterizing ciliary beat when one frequency shift is present.

CELL (N)	MEAN (Hz)	STD DEV (STD ERR) (Hz)	BASELINE BEFORE/AFTER INCREASE (Hz)	CREST (Hz) (1 sample= 1.1 sec	BASELINE BY REL. FREQUENCY DISTRIB'N
1 (450)	8.32	0.50 (0.02)	9/9	13 9 samples	8-9 Hz (76.44%)
2 (450)	8.83	0.45 (0.02)	8/8	12 13 samples	8-9 Hz (72.22%)
3 (180)	11.45	0.47 (0.03)	11/11	13 26 samples	11-12 Hz (81.11%)
4 (180)	14.89	0.67 (0.05)	16/15	18 18 samples	14-15 Hz (68.89%)
5 (180)	16.55	0.55 (0.04)	17/17	19 13 samples	16-17 Hz (78.33%)
6 (450)	23.87	0.89 (0.04)	23/24	27 40 samples	23-24 Hz (49.11%)
AVE.	X	0.59	X	X	X (71.00, s = 11.58, std err = 4.73)

Table 3. Frequency range span of cells exhibiting one shift in ciliary beat frequency.

CELL	FREQUENCY RANGE	SPAN
1	7 - 12 Hz	5 Hz
2	8 - 13 Hz	5 Hz
3	10 - 14 Hz	4 Hz
4	14 - 18 Hz	4 Hz
5	15 - 19 Hz	4 Hz
6	21 - 26 Hz	5 Hz

Table 4. Mean, standard deviation, number of frequency shifts, and number of frequency shifts per minute.

CELL	MEAN (Hz)	STD DEV (Hz)	NUMBER OF SHIFTS	TIME MONITORED (MINUTES)	NUMBER OF SHIFTS/MIN
1	8.32	0.50	1	8.25	0.121
2	8.67	0.58	8	8.25	0.970
3	8.83	0.45	1	8.25	0.121
4	8.88	0.12	8	8.25	0.970
5	9.11	0.73	4	8.25	0.485
6	9.36	1.01	8	8.25	0.970
7	9.37	1.09	3	8.25	0.364
8	9.38	1.02	7	8.25	0.848
9	10.49	1.69	7	8.70	0.805
10	10.75	0.58	9	8.25	1.091
11	10.82	0.78	17	8.25	2.061
12	10.96	0.81	19	8.25	2.303
13	11.18	0.79	2	3.30	0.606
14	11.45	0.47	1	3.30	0.303
15	11.48	1.36	4	8.62	0.464
16	11.84	0.93	7	8.25	0.848
17	11.85	1.37	3	8.25	0.364
18	12.73	0.95	21	8.25	2.545
19	12.77	0.66	2	8.25	0.242
20	13.57	0.77	2	8.25	0.242
21	13.64	1.85	8	8.25	0.967

Table 4. (Continued)

CELL	MEAN (Hz)	STD DEV (Hz)	NUMBER OF SHIFTS	TIME MONITORED (MINUTES)	NUMBER OF SHIFTS/MIN
22	14.28	1.32	8	8.16	0.980
23	14.63	1.20	2	8.62	0.232
24	14.79	1.20	3	8.07	0.372
25	14.89	0.67	1	3.30	0.303
26	14.95	0.68	4	3.67	1.090
27	15.71	1.46	2	3.30	0.606
28	16.24	1.12	4	3.67	1.090
29	16.36	0.70	5	8.01	0.624
30	16.41	1.70	11	8.25	1.333
31	16.45	2.10	3	8.25	0.364
32	16.55	0.55	1	3.30	0.303
33	17.07	0.91	4	8.25	0.485
34	17.81	0.61	3	8.25	0.364
35	18.23	1.67	7	8.25	0.848
36	18.79	1.39	8	8.25	0.970
37	18.91	1.37	3	8.25	0.364
38	18.92	1.31	11	8.25	1.333
39	19.18	1.31	4	3.67	1.090
40	20.39	2.18	5	8.25	0.606
41	23.24	1.44	2	8.25	0.242
42	23.87	0.89	1	8.25	0.121

Note: times monitored are calculated as (N samples) *
1.1 sec/sample * 60 sec/min.

Table 5. Baselines of ciliary beat frequency, number of shifts, increase during a shift and maximum frequency obtained during each frequency shift.

CELL (N)	BASELINE INTERVAL (Hz)	NUMBER OF SHIFTS RECORDED	INCREASE/DECREASE OF EACH SHIFT (Hz)	MAXIMUM FREQUENCY ATTAINED (Hz)
1 (450)	11-12 = 45.00%	2	2/3, 2/2	14, 13
2 (450)	12-13 = 50.89%	2	2/2, 2/2	14, 15
3 (180)	13-14 = 61.11%	2	3/3, 4/4	16, 17
4 (470)	14-15 = 72.89%	2	5/3, 2/4	20, 19
5 (180)	15-16 = 52.22%	2	9/8, 5/5	23, 20
6 (450)	22-23 = 34.89%	2	5/7, 3/4	29, 27
7 (450)	9-10 = 41.33%	3	5/5, 5/5, 4/4	15, 15, 14
8 (450)	11-12 = 71.78%	3	6/6, 2/2, 6/6	18, 13, 17
9 (440)	15-16 = 34.09%	3	3/4, 2/2, 3/2	18, 17, 18
10 (450)	16-17 = 35.56%	3	8/9, 7/7, 8/8	25, 24, 25
11 (450)	17-18 = 54.89%	3	3/3, 3/3, 1/1	20, 20, 19
12 (450)	18-19 = 50.22%	3	2/2, 2/1, 4/4	19, 18, 20
13 (450)	8-9 = 61.56%	4	2/2, 2/2, 2/2, 3/3	11, 11, 11, 13

Table 5. (Continued)

CELL (N)	BASELINE INTERVAL (Hz)	NUMBER OF SHIFTS RECORDED	INCREASE/DECREASE OF EACH SHIFT (Hz)	MAXIMUM FREQUENCY ATTAINED (Hz)
14 (470)	11-12 = 48.44%	4	4/3, 4/4, 7/2, 2/4	15, 15, 16, 16
15 (200)	14-15 = 63.50%	4	3/2, 2/2, 3/3, 2/2	17, 17, 17, 16
16 (200)	15-16 = 46.50%	4	3/3, 4/4, 4/4, 3/3	19, 19, 19, 19
17 (450)	16-17 = 69.33%	4	4/4, 4/4, 3/3, 4/4	21, 21, 20, 20
18 (200)	18-19 = 46.50%	4	5/5, 6/5, 5/5, 4/2	23, 24, 23, 21
19 (437)	16-17 = 49.11%	5	2/3, 6/4, 6/3, 2/3, 3/2	18, 19, 19, 18, 19
20 (450)	20-21 = 28.22%	5	7/7, 7/7, 4/5, 9/8, 5/6	26, 26, 24, 28, 25
21 (450)	9-10 = 50.59%	7	3/3, 5/4, 4/4, 2/2, 4/4, 5/5, 3/2	12, 13, 13, 11, 13, 14, 12
22 (475)	9-10 = 55.11%	7	8/8, 7/7, 7/7, 5/4, 7/7, 6/6, 2/3	17, 16, 17, 15, 17, 16, 12
23 (450)	11-12 = 46.44%	7	4/5, 3/3, 2/2, 3/1, 2/4, 3/3, 3/2	15, 14, 13, 13, 15, 14, 14
24 (450)	18-19 = 36.89%	7	6/7, 7/7, 7/7, 5/5, 6/5, 5/3, 2/5	23, 23, 23, 23, 23, 23, 22
25 (450)	8-9 = 71.11%	8	1/1, 1/1, 1/1, 1/1, 1/1, 1/1, 1/1, 1/1	10, 10, 10, 10, 10, 10, 10, 10
26 (450)	8-9 = 72.22%	8	2/2, 2/2, 2/2, 2/2, 2/2, 2/2, 2/2, 2/2	10, 10, 10, 10, 10, 10, 10, 10
27 (450)	9-10 = 50.22%	8	3/2, 1/2, 4/4, 3/4, 2/2, 3/2, 5/5, 3/2	12, 11, 13, 13, 11, 12, 14, 12

Table 5. (Continued)

CELL (N)	BASILINE INTERVAL (Hz)	NUMBER OF SHIFTS RECORDED	INCREASE/DECREASE OF EACH SHIFT (Hz)	MAXIMUM FREQUENCY ATTAINED (Hz)
28 (450)	12-13 = 25.78%	8	7/6,6/6,6/5,5/5, 5/7,7/4,3/3,5/5	18,18,18,18,18, 18,17,18
29 (445)	14-15 = 44.27%	8	6/6,4/5,6/7,5/5, 4/5,6/6,5/5,6/5	19,18,19,19,20, 20,19,19
30 (450)	18-19 = 30.89%	8	3/3,2/2,3/3,5/5, 2/2,3/2,2/3,2/2	20,19,21,23,20, 22,22,21
31 (450)	10-11 = 62.22%	9	2/2,2/2,2/2, 2/2,2/2,2/2, 2/2,2/2,2/3	12,12,12, 12,12,12, 12,12,12
32 (450)	15-16 = 23.56%	11	3/3,5/3,6/4,4/2, 6/7,5/4,5/5,5/6, 3/2,3/2,3/*	16,17,20,19, 21,20,20,20, 17,20,19
33 (450)	19-20 = 33.00%	13	* /3,3/4,4/3,3/4, 5/4,4/3,3/4,4/4, 3/3,4/4,4/4,3/4, 5/4	20,20,20,20, 21,21,21,21, 20,21,21,20, 21
33 (450)	10-11 = 62.56%	17	2/2,2/2,2/2,3/3, 2/3,2/2,3/3,2/2, 3/3,3/3,2/3,3/3, 2/2,3/3,3/3,3/2, 3/1	12,12,12,13, 13,12,13,12, 13,13,13,13, 12,13,13,13, 13
34 (450)	10-11 = 62.22%	19	3/3,3/3,3/3,3/3, 3/3,3/3,3/3,1/1, 3/3,3/3,2/3,3/3, 3/3,3/3,2/3,3/3, 2/3,3/3,2/2	13,13,13,13, 13,13,13,13, 13,13,13,13, 13,13,13,13, 13,13,13,13
35 (450)	12-13 = 57.78%	21	3/3,3/3,3/3,3/3, 2/2,3/3,2/2,3/3, 2/2,3/3,3/3,3/2, 2/3,3/3,3/3,3/3, 3/2,2/3,3/3,3/3, 3/3	15,15,15,15, 14,15,14,15, 14,15,15,15, 15,15,15,15, 15,15,15,15, 15

Note: * indicates that parameter could not be determined from data set.

Table 6. Duration of frequency shifts.

CELL	N (samples)	BASELINE INTERVAL (Hz)	NUMBER OF SHIFTS RECORDED	DURATION OF SHIFTS (samples)
1	450	11-12	2	18,10
2	450	12-13	2	23,13
3	180	13-14	2	6,37
4	470	14-15	2	21,22
5	180	15-16	2	19,9
6	450	22-23	2	34,65
7	450	9-10	3	16,11,17
8	450	11-12	3	39,26,25
9	440	15-16	3	20,20,16
10	450	16-17	3	12,15,15
11	450	17-18	3	*,21,24
12	450	18-19	3	7,6,9
13	450	8-9	4	7,13,11,*
14	470	11-12	4	15,21,15,*
15	200	14-15	4	10,16,19,*
16	200	15-16	4	21,33,18, *
17	450	16-17	4	23,17,14,26
18	200	18-19	4	20,26,23,17
19	437	16-17	5	24,33,16,18,24
20	450	20-21	5	18,18,37,18,32
21	450	9-10	7	30,12,15,11,8,11,12
22	475	9-10	7	12,25,23,19,17,16,12

Table 6. (Continued)

CELL	N (samples)	BASELINE INTERVAL (Hz)	NUMBER OF SHIFTS RECORDED	DURATION OF SHIFTS (samples)
23	450	11-12	7	20,14,24,14,15,31,20
24	450	18-19	7	20,17,14,15,25,15,11
25	450	8-9	8	15,10,12,14,19,30,11,22
26	450	8-9	8	10,15,17,31,41,27,18,24
27	450	9-10	8	10,18,7,11,11,6,13,18
28	450	12-13	8	14,18,21,16,21,49,41,19
29	445	14-15	8	11,8,10,16,9,11,11,12
30	450	18-19	8	14,32,42,17,33,22,25,18
31	450	10-11	9	19,15,20,18,34,16,10,23,30
32	450	15-16	11	10,13,18,11,19,26,26,35,26, 18,*
33	450	19-20	13	*,7,15,19,12,12,14,8,9, 18,8,10,19
34	450	10-11	17	10,16,14,14,21,11,12,8,14, 15,5,7,17,11,23,9,12
35	450	10-11	19	10,15,16,12,21,12,15,19,8, 15,15,6,10,17,7,21,16,13,11
36	450	12-13	21	13,16,22,11,20,9,13,6,7, 16,15,15,5,7,13,8,13,12, 12,9,10

Note: * indicates that parameter could not be determined from data set.

Table 7. Rate of decline in frequency following frequency shift.

CELL	TIMES SHIFT NOTED	DURATION OF DECLINE IN FREQUENCY (samples)	AMOUNT OF FREQUENCY DECREASE (Hz)	RATIO OF DURATION/DECREASE (samples/ 1 Hz decrease)
1	2	11,9	3,2	3.7,4.5
2	2	9,14	2,2	4.5,7.0
3	2	20,12	3,4	6.7,3.0
4	2	18,21	3,4	6.0,5.3
5	2	9,6	8,5	1.1,1.2
6	2	24,22	7,4	3.4,5.5
7	3	22,11,9	4,2,2	5.5,5.5,4.5
8	3	9,8,10	9,7,8	1.0,1.1,1.3
9	3	10,10,8	3,3,2	3.3,3.3,4.0
10	3	11,8,9	2,1,4	6.5,8.0,2.3
11	4	10,14,10,11	2,2,2,3	5.0,7.0,5.0,3.7
12	4	9,21,8,19	3,4,2,4	3.0,5.3,4.0,4.8
13	4	5,8,4,11	2,2,3,2	2.5,4.0,1.3,6.5
14	4	23,20,13,16	3,4,4,3	7.7,5.0,3.3,5.3
15	4	16,10,9,13	4,4,3,4	4.0,2.5,3.0,3.3
16	4	10,11,13,6	5,5,5,2	2.0,2.2,2.6,3.0
17	5	16,19,12,13, 19	3,4,3,2, 2	5.3,4.8,4.0,5.0,6.5
18	5	13,9,24,12, 11	7,7,5,8, 6	1.9,1.3,4.8,1.5,1.8
19	7	9,11,11,12, 7,10,11	2,4,4,2, 4,5,2	4.5,2.8,2.8,6.0,1.8, 2.0,5.5

Table 7. (Continued)

CELL	TIMES SHIFT NOTED	DURATION OF DECLINE IN FREQUENCY (samples)	AMOUNT OF FREQUENCY DECREASE (Hz)	RATIO OF DURATION/DECREASE (samples/ 1 Hz decrease)
20	7	10,11,13,17, 12,14,7	8,7,7,5, 7,6,3	1.3,1.6,1.9,3.4,1.7, 2.3,2.3
21	7	11,8,8,8, 10,17,9	5,3,2,1, 4,3,2	2.2,2.7,4.0,8.0,2.5, 5.7,4.5
22	7	21,13,11,12, 19,8,10	7,7,7,5, 5,3,5	3.0,1.9,1.6,2.4,3.8, 2.7,2.0
23	8	9,7,7,6, 6,3,6,13	1,1,1,1, 1,1,1,1	9.0,7.0,7.0,6.0,6.0, 3.0,6.0,13.0
24	8	7,9,9,9,6, 19,17,15	2,2,2,2, 2,2,2,1	3.5,4.5,4.5,4.5,3.0, 9.5,8.5,15.0
25	8	5,5,8,9,8, 7,10,9	2,2,4,4, 2,2,5,2	2.5,2.5,2.0,2.3,4.0, 3.5,5.0,4.5
26	8	11,13,14,10, 17,38,13,9	6,6,5,5, 7,4,3,5	1.8,2.2,2.8,2.0,2.4, 9.5,4.3,1.8
27	8	8,8,8,11,8, 10,8,10	6,5,7,5, 5,6,5,5	1.3,1.6,1.1,2.2,1.6, 1.6,1.6,2.0
28	8	7,12,14,12, 19,12,16,9	3,2,3,5, 2,2,3,2	2.3,6.0,4.7,2.4,9.5, 6.0,5.3,4.5
29	9	17,13,10,8, 12,13,11,8, 20	2,2,2,2, 2,2,2,2, 3	8.5,6.5,5.0,4.0,6.0, 6.5,5.5,4.0,6.7
30	11	6,6,6,4,16, 6,9,7,12, 12,*	3,3,4,2, 7,4,5,6, 2,2,*	2.0,2.0,1.5,2.0,2.3, 1.5,1.8,1.2,6.0,6.0, *
31	13	7,8,4,7,8,6, 8,7,9,16,8, 3,*	3,4,3,4,4, 3,4,4,3, 4,4,4,*	2.3,2.0,1.3,1.8,2.0, 2.0,2.0,1.8,5.0,4.0, 2.0,2.3,0.8,*
32	17	11,5,4,5,6, 5,7,5,6,4, 3,7,9,7,4, 4,7	2,2,2,3,3, 2,3,2,3, 3,3,3,2, 3,3,2,1	5.5,2.5,2.0,1.7,2.0, 2.5,2.3,2.5,2.0,1.3, 1.0,2.3,4.5,2.3,1.3, 2.0,7.0

Table 7. (Continued)

CELL	TIMES SHIFT NOTED	DURATION OF DECLINE IN FREQUENCY (samples)	AMOUNT OF FREQUENCY DECREASE (Hz)	RATIO OF DURATION/DECREASE (samples/ 1 Hz decrease)
33	19	11,9,7,8,9, 10,5,6,6,8, 3,8,5,5,6, 6,16,5,7	3,3,3,3, 3,3,3,3, 1,3,3,3, 3,3,3,3, 3,3,2	3.7,3.0,2.3,2.7,3.0, 3.3,1.7,2.0,6.0,2.7, 1.0,2.7,1.7,1.7,2.0, 2.0,5.3,1.7,3.5
34	21	9,6,11,8,10, 7,12,5,6,13, 9,5,4,6,9,9, 9,6,7	3,3,3,3, 2,3,2,3, 2,3,3,2, 3,3,3,3, 2,3,3,3,3	3.0,2.0,3.7,2.7,5.0,2.3, 3.0,4.3,3.0,2.5,1.3,2.0, 3.0,2.0,4.5,3.0,3.0,2.0, 2.3

Table 8. Baseline frequencies, mean frequencies, maximum frequencies, and maximum increases noted for frequency shifts.

CELL	TIMES SHIFT NOTED	BASELINE FREQUENCY (Hz)	MEAN FREQUENCY (Hz)	MAXIMUM FREQUENCY (Hz)	MAXIMUM INCREASE (Hz)
1	1	8-9	8.32	13	4
2	8	8-9	8.67	10	2
3	1	8-9	8.83	12	4
4	8	8-9	8.88	10	1
5	4	8-9	9.11	13	3
6	8	9-10	9.36	14	4
7	3	9-10	9.37	15	5
8	7	9-10	9.38	14	5
9	9	10-11	10.75	12	2
10	17	10-11	10.82	13	3
11	19	10-11	10.96	13	3
12	7	10-11	11.84	15	4
13	3	10-11	11.85	18	6
14	21	12-13	12.73	15	3
15	2	12-13	12.77	15	2
16	8	12-13	13.64	18	7
17	8	14-15	14.28	20	6
18	11	15-16	16.41	21	6
19	3	16-17	16.45	25	8

Table 8. (Continued)

CELL	TIMES SHIFT NOTED	BASELINE FREQUENCY (Hz)	MEAN FREQUENCY (Hz)	MAXIMUM FREQUENCY (Hz)	MAXIMUM INCREASE (Hz)
20	4	16-17	17.07	21	4
21	3	17-18	17.81	20	3
22	7	18-19	18.23	23	7
23	8	18-19	18.79	23	5
24	3	19-20	18.91	20	4
25	13	19-20	18.92	21	5
26	5	20-21	20.39	28	9
27	2	22-23	23.24	28	5
28	1	23-24	23.87	27	4

Note: All cells were monitored for 450 samples = 8.25 minutes.

Table 9. Times of shift and times between crests of shifts as estimated by sample numbers.

CELL	N (sam- ples)	TIMES SHIFT NOTED	TIMES OF CREST OF SHIFT NOTED (sample number)	TIME BETWEEN CRESTS OF SHIFTS (samples)
1	450	3	323,374,439	51,65
2	450	3	28,315,357	287,42
3	440	3	104,228,319	124,91
4	450	3	238,344,432	106,88
5	450	3	4,84,233	80,149
6	450	3	30,90,419	60,329
7	450	4	47,150,267,411	103,117,144
8	470	4	13,316,415,435	303,99,20
9	200	4	32,79,144,185	47,65,41
10	200	4	15,81,149,184	66,68,35
11	450	4	40,120,207,318	80,87,111
12	200	4	28,68,152,193	40,84,41
13	437	5	9,131,209,249,380	122,78,40,131
14	450	5	10,126,184,250,314	116,58,66,64
15	450	7	13,129,162,206,263, 363,401	116,33,44,57,100, 38
16	475	7	10,72,110,139,188, 231,451	62,38,29,49,43,220
17	450	7	29,74,154,257,297, 354,433	45,80,103,40,57,79
18	450	7	23,89,117,173,218, 284,293	66,28,56,45,66,9

Table 9. (Continued)

CELL	N (sam- ples)	TIMES SHIFT NOTED	TIMES OF CREST OF SHIFT NOTED (sample number)	TIME BETWEEN CRESTS OF SHIFTS (samples)
19	450	8	30,85,174,185,218, 315,394,426	55,89,11,33,97,79, 32
20	450	8	18,57,92,144,211, 277,348,400	39,35,52,67,66,71, 52
21	450	8	22,33,140,172,216, 271,371,406	11,107,32,44,99,100, 35
22	450	8	43,69,105,187,263, 290,361,440	26,36,82,76,27,71, 79
23	450	8	8,85,113,195,259, 310,148,407	77,28,82,64,51,38, 59
24	450	8	86,114,202,225,296, 360,382,406	28,88,23,71,64,22, 24
25	450	9	22,82,138,155,194, 215,249,314,387	60,56,17,39,21,34, 65,73
26	450	11	6,26,44,65,101,151, 234,318,367,409,442	20,18,21,36,50,83, 84,49,42,33
27	450	13	22,30,40,55,77,87, 100,119,127,137,157, 167,183	8,10,15,22,10,13,19, 8,10,20,10,16
28	450	17	7,19,44,71,98,134, 158,176,244,276,282, 302,330,358,395,421	12,25,27,36,24,18, 44,24,32,6,20,28, 18,37,26
29	450	19	5,24,50,71,97,131, 157,175,187,224,247, 273,280,307,328,362, 419,435	19,26,21,26,34,26, 18,12,37,23,26,7,27, 21,34,57,16
30	450	21	5,18,42,70,97,131, 156,174,187,218,243, 265,272,279,302,326, 354,369,391,417,434	13,24,28,27,34,25, 18,13,31,25,22,7,7, 23,24,28,15,22,26, 17

Table 10. Autocorrelation function and coefficients for cells displaying two frequency shifts.

CELL (N)	MEAN (VARIANCE)	TIME OF SHIFTS	TIME BETWEEN SHIFTS	R (t) xx	r (t) xx
1 (180)	11.18 (0.62)	14 97	83	R(83) = -0.022	r(83) = -0.036
2 (450)	12.77 (0.44)	118 435	317	R(317) = -0.162	r(317) = -0.367
3 (180)	13.57 (0.59)	4 81	77	R(77) = 0.020	r(77) = 0.033
4 (470)	14.63 (1.43)	395 418	23	R(23) = 0.738	r(23) = 0.515
5 (450)	15.04 (2.45)	36 304	268	R(268) = 2.280	r(268) = 0.927
6 (180)	15.71 (2.14)	62 128	66	R(66) = 0.641	r(66) = 0.299

Note: R (0) = variance, and r (0) = 1.00.
xx xx

Table 11. Average power of signals as estimated by $R(0)$.
xx

CELL	NUMBER OF SHIFTS	$R(0)$ xx	CELL	NUMBER OF SHIFTS	$R(0)$ xx
1	0	0.30	18	4	0.83
2	0	0.18	19	5	4.77
3	0	0.07	20	7	1.03
4	0	0.21	21	7	2.85
5	0	0.05	22	7	0.86
6	0	0.28	23	7	2.80
7	0	0.03	24	8	0.12
8	1	0.20	25	8	0.29
9	1	0.25	26	8	1.02
10	1	0.79	27	8	3.44
11	2	0.44	28	8	1.93
12	3	1.20	29	9	0.34
13	3	1.87	30	11	2.88
14	3	4.42	31	13	1.71
15	3	0.37	32	17	0.61
16	3	1.06	33	19	0.66
17	4	0.53	34	21	0.90
AVE.	0	0.16	AVE.	1 - 21	1.41

Note: $N = 450$, all cells.

Table 12. Frequency distributions and baselines for cells monitored at two separate times.

CELL (N)	TIMES SHIFT NOTED AT A, B	FREQUENCY DISTRIBUTION OF FREQUENCY DATA AT TIME A	FREQUENCY DISTRIBUTION OF FREQUENCY DATA AT TIME B	BASELINE AT TIME A (Hz)	BASELINE AT TIME B (Hz)
1 (450)	0, 1	8- 9 = 30.9% 9-10 = 53.1% 10-11 = 16.0%	8- 9 = 76.4% 9-10 = 22.2% 10-11 = 0.4% 11-12 = 0.2% 12-13 = 0.7%	9-10	8-9
2 (180)	1, 0	15-16 = 7.8% 16-17 = 78.3% 17-18 = 12.2% 18-19 = 1.7%	16-17 = 40.0% 17-18 = 51.7% 18-19 = 8.3%	16-17	17-18
3 (180)	1, 1	14-15 = 68.9% 15-16 = 26.1% 16-17 = 1.7% 17-18 = 3.3%	17-18 = 53.3% 18-19 = 41.7% 19-20 = 4.4% 20-21 = 0.6%	14-15	17-18
4 (180)	2, 1	9-10 = 1.7% 10-11 = 39.4% 11-12 = 45.0% 12-13 = 11.7% 13-14 = 2.2%	10-11 = 11.1% 11-12 = 81.1% 12-13 = 7.8%	11-12	11-12
5 (180)	2, 3	12-13 = 23.3% 13-14 = 61.1% 14-15 = 8.9% 15-16 = 4.4% 16-17 = 2.2%	13-14 = 3.9% 14-15 = 4.4% 15-16 = 5.6% 16-17 = 16.7% 17-18 = 28.9% 18-19 = 31.1% 19-20 = 3.9% 20-21 = 4.4% 21-22 = 0.0% 22-23 = 1.1%	13-14	18-19

Table 12. (Continued)

CELL (N)	TIMES SHIFT NOTED AT A, B	FREQUENCY DISTRIBUTION OF FREQUENCY DATA AT TIME A	FREQUENCY DISTRIBUTION OF FREQUENCY DATA AT TIME B	BASELINE AT TIME A (Hz)	BASELINE AT TIME B (Hz)
6 (450)	3, 3	13-14 = 6.9% 14-15 = 58.0% 15-16 = 28.4% 16-17 = 1.8% 17-18 = 0.9% 18-19 = 0.2% 19-20 = 0.4% 20-21 = 0.4% 21-22 = 0.2% 22-23 = 1.5% 23-24 = 1.1%	14-15 = 19.3% 15-16 = 26.7% 16-17 = 35.6% 17-18 = 9.8% 18-19 = 0.9% 19-20 = 1.3% 20-21 = 6.7% 21-22 = 6.7% 22-23 = 1.3% 23-24 = 1.1% 24-25 = 1.8% 25-26 = 0.9%	14-15	16-17
7 (450)	3, 6	13-14 = 0.9% 14-15 = 25.1% 15-16 = 27.1% 16-17 = 38.7% 17-18 = 5.8% 18-19 = 1.1% 19-20 = 0.4% 20-21 = 0.9%	16-17 = 0.4% 17-18 = 14.7% 18-19 = 50.2% 19-20 = 22.4% 20-21 = 5.8% 21-22 = 1.8% 22-23 = 2.2% 23-24 = 2.4%	16-17	18-19
8 (200)	2, 3	11-12 = 47.0% 12-13 = 43.0% 13-14 = 5.0% 14-15 = 1.0% 15-16 = 3.5% 16-17 = 0.5%	13-14 = 43.0% 14-15 = 53.0% 15-16 = 4.0%	11-12	14-15
9 (200)	0, 0	12-13 = 8.1% 13-14 = 67.2% 14-15 = 24.7%	12-13 = 6.0% 13-14 = 70.0% 14-15 = 18.5% 15-16 = 5.5%	13-14	13-14
10 (340)	1, 1	9-10 = 5.3% 10-11 = 42.9% 11-12 = 34.1% 12-13 = 2.4% 13-14 = 2.0% 14-15 = 6.5% 15-16 = 6.8%	9-10 = 1.5% 10-11 = 16.2% 11-12 = 61.8% 12-13 = 7.6% 13-14 = 0.0% 14-15 = 5.3% 15-16 = 7.6%	10-11	11-12

Table 13. Frequency range spanned by cells monitored at two separate times and standard deviations for those data sets.

CELL (N)	TIMES SHIFT NOTED AT A,B	FREQUENCY RANGE AT TIME A (Hz)	FREQUENCY RANGE AT TIME B (Hz)	STD DEV AT TIME A,B
1 (450)	0, 1	8 - 11	8 - 13	0.54, 0.45
2 (180)	1, 0	15 - 19	16 - 19	0.55, 0.43
3 (180)	1, 1	14 - 18	17 - 21	0.67, 0.66
4 (180)	2, 1	9 - 14	10 - 14	0.79, 0.47
5 (180)	2, 3	12 - 17	13 - 23	0.77, 1.55
6 (450)	3, 3	13 - 24	14 - 26	1.57, 2.10
7 (450)	3, 6	13 - 21	16 - 24	1.03, 1.20
8 (200)	2, 3	11 - 17	13 - 16	0.87, 0.53
9 (200)	0, 0	12 - 15	12 - 16	0.57, 0.65
10 (340)	4, 1	9 - 16	9 - 16	1.50, 1.30

Table 14. Amplitudes of frequency shifts of cells monitored at two separate times.

CELL	AMPLITUDES OF SHIFTS AT TIME A	AMPLITUDES OF SHIFTS AT TIME B
3	Increase 2 Hz to 18 Hz	Increase 3 Hz to 20 Hz
4	Increase 2 Hz to 14 Hz Increase 2 Hz to 13 Hz	Increase 2 Hz to 13 Hz
5	Increase 3 Hz to 16 Hz Increase 5 Hz to 17 Hz	Increase 7 Hz to 21 Hz Increase 3 Hz to 21 Hz Increase 5 Hz to 22 Hz
6	Increase 8 Hz to 23 Hz Increase 2 Hz to 17 Hz Increase 9 Hz to 24 Hz	Increase 9 Hz to 25 Hz Increase 7 Hz to 24 Hz Increase 8 Hz to 25 Hz
7	Increase 2 Hz to 19 Hz Increase 4 Hz to 18 Hz Increase 4 Hz to 20 Hz	Increase 4 Hz to 22 Hz Increase 2 Hz to 20 Hz Increase 5 Hz to 23 Hz Increase 4 Hz to 22 Hz Increase 2 Hz to 20 Hz Increase 5 Hz to 22 Hz
8	Increase 4 Hz to 16 Hz Increase 4 Hz to 17 Hz	Increase 2 Hz to 15 Hz Increase 2 Hz to 16 Hz Increase 2 Hz to 16 Hz
10	Increase 4 Hz to 15 Hz Increase 4 Hz to 15 Hz Increase 7 Hz to 16 Hz Increase 2 Hz to 16 Hz	Increase 6 Hz to 16 Hz

Note: Cells 1, 2, and 9 showed no frequency shift during one time of sampling. Therefore, a comparison is inappropriate.

Table 15. Change in baseline frequencies and maximum frequencies of cells monitored at two separate times.

CELL	BASELINE AT TIME A (Hz)	BASELINE AT TIME B (Hz)	MAX FREQ AT TIME A (Hz)	MAX FREQ AT TIME B (Hz)	CHANGE IN BASELINE	CHANGE IN MAX FREQ
3	14-15	17-18	18	20	+3	+2
4	11-12	11-12	14	13	0	-1
5	13-14	18-19	17	22	+5	+5
6	14-15	16-17	24	25	+2	+1
7	16-17	18-19	20	23	+2	+3
8	11-12	14-15	17	16	+3	-1
10	10-11	11-12	16	16	+1	0

Note: Cells 1, 2, and 9 showed no frequency shift during one time of sampling.

Note: Linear regression of values for change in baseline (x) and change in maximum frequency (y) produced a slope of 1.00 indicating a strong linear relationship between these values.

Table 16. Cross-correlation coefficients for data sets acquired from the same cell at two different time periods.

CELL	CROSS-CORRELATION COEFFICIENT OF DATA SETS FROM THE SAME CELL AT TWO DIFFERENT TIMES
1	$r(0) = 0.202$
2	$r(100) = 0.819$
3	$r(43) = 0.475$
4	$r(200) = 0.530$
5	$r(71) = 0.781$
6	$r(84) = 0.464$
7	$r(77) = 0.311$
8	$r(129) = 0.358$
9	$r(9) = 0.425$
10	$r(94) = 0.314$
11	$r(88) = 0.127$

Table 17. Mean frequencies and standard deviations of data sets collected from different tufts of cilia on the same cell.

TUFT (N)	MEAN (Hz)	STD DEV	BASELINE (Hz)	RANGE (Hz)
CELL 1 (450) A	9.36	1.01	9-10	7-14
B	9.62	0.95	9-10	8-14
C	10.00	1.01	9-10	8-15
CELL 2 (200) A	13.04	0.27	12-13	12-14
B	13.05	0.27	12-13	12-14
CELL 3 (200) A	14.95	0.68	14-15	14-17
B	14.18	0.82	14-15	12-17
CELL 4 (450) A	17.07	0.91	16-17	16-21
B	16.44	0.65	16-17	15-20
C	15.89	0.71	15-16	14-20
CELL 5 (200) A	16.24	1.12	15-16	14-19
B	16.46	1.24	15-16	14-19
C	17.14	1.12	16-17	15-20
CELL 6 (450) A	18.92	1.31	19-20	16-22
B	18.33	1.63	19-20	13-22
C	18.95	1.29	19-20	15-22
CELL 7 (200) A	13.66	0.90	13-14	12-19
B	13.65	0.77	13-14	12-19
CELL 8 (200) A	19.75	1.39	19-20	18-26
B	18.66	1.87	18-19	17-26
CELL 9 (200) A	10.65	0.27	10-11	10-12
B	9.94	0.39	9-10	9-10
CELL 10 (200) A	17.22	1.53	16-17	16-25
B	16.22	1.82	15-16	14-26

Table 18. Crests of shifts exhibited by different tufts of cilia on the same cell.

TUFT	TIMES SHIFT NOTED	CRESTS OF SHIFTS (Hz)
CELL 1		
A	8	12,11,13,13,11,13,14,12
B	9	13,11,14,13,11,13,14,12,13
C	9	13,12,14,13,12,14,14,12,14
CELL 2		
A	0	*
B	0	*
CELL 3		
A	4	17,17,17,16
B	4	16,16,16,16
CELL 4		
A	4	21,21,20,20
B	4	19,19,20,18
C	3	19,18,19
CELL 5		
A	4	19,19,19,19
B	4	19,19,19,19
CELL 6		
A	13	20,20,20,20,21,21,21,21,20,21,21,20,21
B	13	20,20,21,20,20,20,20,20,20,20,20,20,21
C	13	20,20,20,19,20,21,20,21,20,21,20,20,21
CELL 7		
A	1	18
B	1	18
CELL 8		
A	3	25,24,25
B	3	24,24,24
CELL 9		
A	0	*
B	0	*
CELL 10		
A	2	24,22
B	2	25,18

* indicates no crest noted.

Table 19. Durations of shifts noted from data collected from different tufts of cilia on the same cell.

TUFT	TIMES SHIFT NOTED	DURATIONS OF SHIFTS (samples)
CELL 1		
A	8	10,18,7,11,11,6,13,18
B	9	9,29,9,11,18,12,15,16,13
C	9	10,22,10,19,10,12,16,12,12
CELL 2		
A	0	*
B	0	*
CELL 3		
A	4	10,16,19,19
B	4	13,16,16,16
CELL 4		
A	4	23,17,14,26
B	4	22,16,16,16
C	3	13,8,13
CELL 5		
A	4	21,33,18,*
B	4	28,28,18,35
CELL 6		
A	13	28,7,15,19,12,12,14,8,9,18,8,10,19
B	13	28,6,13,18,7,14,15,8,6,23,8,10,*
C	13	28,9,15,17,13,8,17,8,11,24,10,15,*
CELL 7		
A	1	16
B	1	10
CELL 8		
A	3	9,21,11
B	3	15,10,15
CELL 9		
A	0	*
B	0	*
CELL 10		
A	2	15,6
B	2	13,5

* indicates no frequency shift present.

Table 20. Estimates of time elapsing between shifts of different tufts of cilia on the same cell.

TUFT	TIMES SHIFT NOTED	TIME BETWEEN SHIFTS (samples)
CELL 1		
A	8	11,107,32,44,99,100,35
B	9	17,99,33,43,56,104,37,39
C	9	10,106,33,44,55,102,35,41
CELL 2		
A	0	*
B	0	*
CELL 3		
A	4	47,65,41
B	4	33,15,64
CELL 4		
A	4	80,87,111
B	4	83,90,109
C	3	90,84
CELL 5		
A	4	66,68,35
B	4	62,80,30
CELL 6		
A	13	8,10,15,22,10,13,19,8,10,20,10,16
B	13	10,12,14,22,11,16,15,10,9,22,13,13
C	13	14,20,9,18,11,19,10,11,10,20,17,11,*
CELL 7		
A	1	*
B	1	*
CELL 8		
A	3	94,57
B	3	96,57
CELL 9		
A	0	*
B	0	*
CELL 10		
A	2	156
B	2	149

* indicates that only one or no frequency shifts noted.

Table 21. Cross-correlation coefficients for data sets collected from different tufts of cilia on the same cell.

DATA SETS CORRELATED	CROSS-CORRELATION COEFFICIENTS
CELL 1 - A with B B with C A with C	r(12)= 0.765 r(6) = 0.845 r(7) = 0.874
CELL 2 - A with B	r(1) = 0.988
CELL 3 - A with B	r(0) = 0.677
CELL 4 - A with B B with C A with C	r(11)= 0.574 r(0) = 0.718 r(10)= 0.603
CELL 5 - A with B B with C A with C	r(0) = 0.832 r(3) = 0.554 r(0) = 0.724
CELL 6 - A with B B with C A with C	r(2) = 0.644 r(0) = 0.548 r(1) = 0.761
CELL 7 - A with B	r(15)= 0.877
CELL 8 - A with B	r(0) = 0.465
CELL 9 - A with B	r(0) = 0.766
CELL 10- A with B	r(0) = 0.610

Table 22. Baseline frequencies and ranges of cells in close proximity.

CELL	BASELINE (Hz)	RANGE (Hz)	NUMBER OF SHIFTS
FIELD 1 - A	10-11	9-12	0
B	13-14	13-15	0
FIELD 2 - A	18-19	17-24	4
B	16-17	15-18	0
FIELD 3 - A	12-13	11-19	8
B	11-12	10-18	3
FIELD 4 - A	11-12	9-16	4
B	14-15	13-21	2
FIELD 5 - A	8-9	8-13	4
B	8-9	7-11	8
C	8-9	7-11	8
FIELD 6 - A	23-24	21-27	1
B	20-21	17-27	5
C	22-23	20-27	2
FIELD 7 - A	18-19	16-24	6
B	18-19	16-23	8
C	15-16	12-22	11
D	11-12	9-15	7
FIELD 8 - A	11-12	10-14	1
B	18-19	13-23	3
C	17-18	16-19	0
D	17-18	17-21	1
FIELD 9 - A	15-16	11-19	3
B	18-19	15-24	7
C	17-18	14-19	0
D	9-10	8-18	7
FIELD 10- A	9-10	8-14	1
B	8-9	7-12	1
C	9-10	7-14	7
D	12-13	10-16	2
E	14-15	11-21	8
F	9-10	6-15	3

Table 22. (Continued)

CELL	BASELINE (Hz)	RANGE (Hz)	NUMBER OF SHIFTS
FIELD 11- A	11-12	9-14	2
B	13-14	12-17	2
C	15-16	14-18	2
D	15-16	14-23	2
E	16-17	15-19	1
F	14-15	14-18	1
FIELD 12- A	11-12	10-14	2
B	14-15	13-17	20
C	10-11	9-14	9
D	10-11	9-14	19
E	12-13	11-16	21
F	12-13	11-15	0
G	15-16	15-18	0
H	12-13	11-15	0
I	*	*	8

* indicates that parameter was not discernible.

Note: Relationship of cells on outgrowth is diagrammed for Field 12 in Figure 13.

Table 23. Maximum frequency obtained and maximum amplitude of shift for cells in close proximity.

CELL	MAX FREQUENCY (Hz)	MAX AMPLITUDE OF INCREASE DURING SHIFTS
FIELD 1 - A	12	*
B	15	*
FIELD 2 - A	24	6
B	18	*
FIELD 3 - A	18	7
B	18	6
FIELD 4 - A	16	7
B	20	5
FIELD 5 - A	12	3
B	10	1
C	10	2
FIELD 6 - A	27	4
B	28	9
C	28	5
FIELD 7 - A	23	5
B	23	5
C	21	6
D	15	4
FIELD 8 - A	13	2
B	22	7
C	19	*
D	20	3
FIELD 9 - A	18	3
B	23	7
C	19	*
D	17	8
FIELD 10- A	13	3
B	11	3
C	14	5
D	15	2
E	20	6
F	15	5

Table 23. (Continued)

CELL	MAX FREQUENCY (Hz)	MAX AMPLITUDE OF INCREASE DURING SHIFTS
FIELD 11- A	13	2
B	16	5
C	18	3
D	23	9
E	19	2
F	18	2
FIELD 12- A	13	3
B	15	3
C	12	2
D	13	3
E	15	3
F	14	*
G	17	*
H	14	*
I	**	**

Note: * indicates that no frequency shift was present.

Note: ** indicates that the parameter could not be determined.

Note: Relationship of cells on outgrowth is diagrammed for Field 12 in Figure 13.

VITA

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