Mechano-Electrical Properties of the Lateral Line

by

David N. Chen

Submitted to the Department of Electrical Engineering and Computer Science
in partial fulfillment of the requirements for the degree of

Degrees of Bachelor of Science in Electrical Engineering and Masters of Engineering in Electrical Engineering and Computer Science

at the

MASSACHUSETTS INSTITUTE OF TECHNOLOGY

June 2001

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Abstract

A preparation was developed to mechanically stimulate the lateral line of *Xenopus laevis* while simultaneously measuring its neural responses. A piece of skin was dissected from *Xenopus laevis* and placed in an experimental apparatus that allowed the lateral line to be mechanical stimulation and its innervating nerve recorded. A glass micropipette driven by a piezoelectric crystal was used to stimulate a single stitch of the lateral line. To isolate the neural activity of the single stitch from the other stitches innervated by the same nerve, the other stitches were ablated. Neural responses were amplified and sampled for storage in a computer, and the voltage drive for the piezoelectric crystal was simultaneously sampled and stored. The resulting data were filtered and thresholded to determine neural firing times. These times were analyzed statistically to compute post-stimulus-time and interval histograms. Although spontaneous neural activity was detected as predicted, no correlation was found between mechanical stimulation of the stitch and its neural response to the stimulus.

Thesis Supervisor: Dennis M. Freeman
Title: W.M. Keck Career Development
Associate Professor in Biomedical Engineering

Acknowledgments

Thanks to my Mom, Dad, and Vincent for their continued support throughout the years of succeeding, failing, and letting me run after my dreams.

Thanks to my advisor, Denny Freeman, for his guidance and patience with the research conducted, for staying late, for showing me how wondering Postscript is, and for the endless supply of Cokes.

Thanks to A.J. Aranyosi, for his guidance and support, for his enthusiasm in the research, for helping me through linux and Latex, for being the hero when I cannot locate something in the lab, and for making stupid bets. And of course, being a fellow bassist.

Thanks to Dr. William Sewell, Rosie Dawkins, and Gerry Bailey of the Eaton-Peabody Laboratory of the Massachusetts Eye and Ear Infirmary for their assistance in developing this preparation.

Thanks to the National Institutes of Health for supporting this project through grant R01-DC00238.

Thanks to my band, Thomas Chan, Milton Shih, Robin Lai, and Liz Man for letting me disappear in the midst of getting a CD released.

Thanks to Enily Liu, Wendy Fan, Cornelia Tsang, and Anita Chung for always feeding as I'm always forgetting to eat or just too lazy to buy or cook food.

Thanks to Christ Jesus, for giving me peace throughout all these years, for fulfilling dreams beyond imagination, and for His continued blessings.

Chapter 1

Introduction

The inner ear is a remarkable signal processing system: 1) Its high sensitivity allows the ear to detect sounds that vibrate the eardrum less than 100pm. 2) It has the ability to distinguish between two frequencies differing by as little as one hertz. For decades, the research community has attempted to account for these properties of hearing in terms of a model for the cochlea but the biophysical basis for these properties remains unclear. No model has yet been able to account for the properties of the cochlea with physically plausible parameters.

Cochlear mechanics have long been split into two categories: macromechanics—motion of the cochlear partition in response to sound; and micromechanics—how hair cells within the cochlea are stimulated in response to macromechanical motion (Patuzzi, 1996). Although macromechanical motion has been studied extensively, relatively few micromechanical measurements have been reported, as studies within the cochlea are difficult due to its inaccessibility and its susceptibility to damage.

However, one way to improve our understanding is to study the mechanics of simpler systems. The lateral line is such a system—it has hair cells and an overlying gelatinous structure, is mechanically sensitive, and generates electrical responses. This thesis documents the development of a preparation for studying the mechanical responses of the lateral line.

1.1 Hair Cells

Hair cells are the sensory receptor cells in the inner ear. They are modified epithelial cells possessing stereocilia (figure 1-1) organized in bundles that are asymmetrically conical in shape (figure 1-2). Each hair bundle may consist of tens to hundreds of stereocilia that are graded in length and organized in a staircase fashion. The high sensitivity of the ear is attributed to the hair bundles as their whole dynamic range is realized when the tip of the hair bundle is moved by as little as 100 nm (Kros, 1996). Although there have been extensive studies of the individual hair cell, there is still much to be discovered on the hair cells operating at a system level.

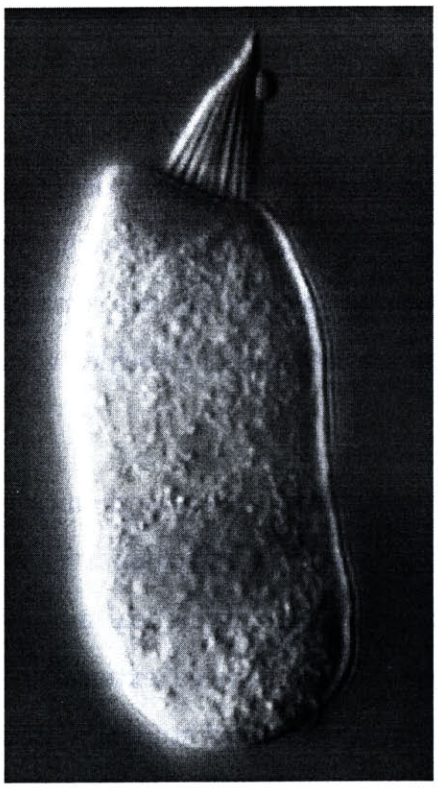

Figure 1-1: Image of hair cell (from (Hudspeth, 1985)). This image shows a hair cell of a frog that has been enzymatically isolated from the cells that would normally surround it. Atop the hair cell are the stereocilia making up the hair bundle. This image was taken using light microscopy (differential interference contrast optics).

Hair cells are found in a number of different systems. Within the cochlea, they are the basis of our ability to hear. In the vestibular system, they give us our sense of balance. In the lateral line, they give fish and amphibians their sense of navigation in water. Although the hair cells between these systems are not identical, they all share a role as mechanoelectrical transducers.

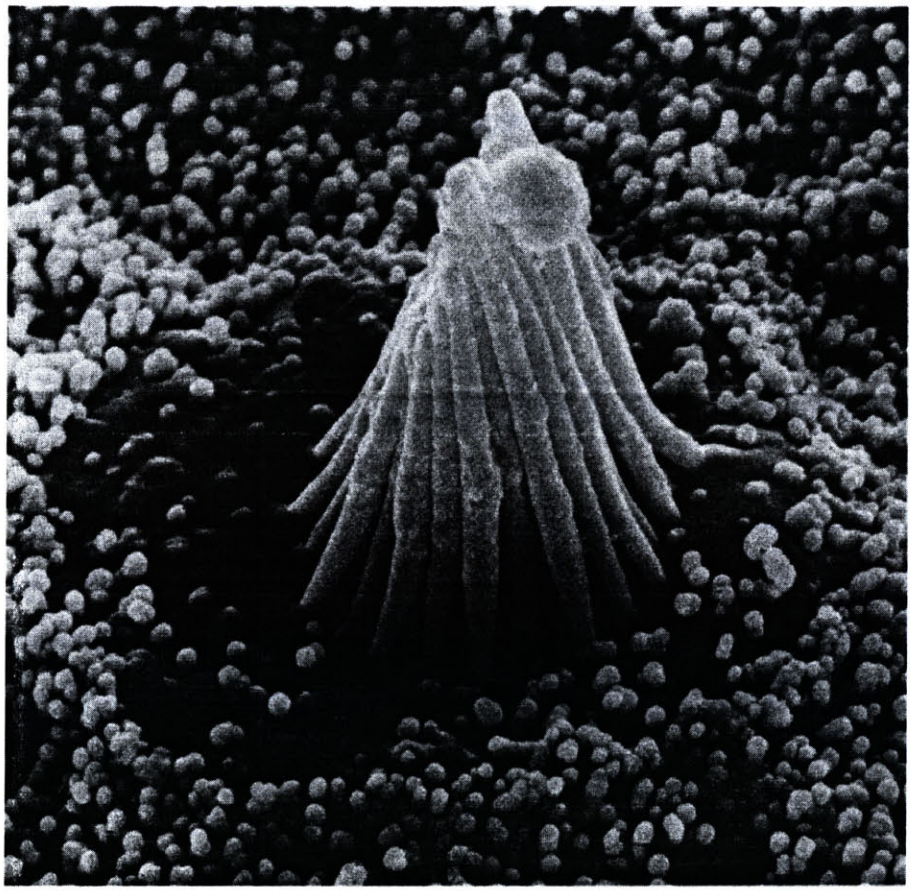

Figure 1-2: Surface view of a hair cell (from (Hudspeth, 1983). Atop the hair cell is the hair bundle with approximately 70 stereocilia. The image was taken using a scanning electron microscope.

With the lateral line, previous studies have shown that general response characteristics of the fibers innervating hair cells in the lateral line organ are similar to those described for auditory nerve fibers (Elepfandt, 1988) as they display a spontaneous discharge that is modulated by mechanical stimulation and respond most vigorously at a certain characteristic frequency (Bailey and Sewell, 2000). Studying the mechanics of the hair cells from a simple preparation like the lateral line organ can help develop a foundation for the biophysical basis for mechanics in the cochlea, a more complex tissue.

1.2 Lateral Line System

Found on amphibians and fish, the lateral line is a surface organ which allows the animal to sense the flow of water in its surroundings. It usually runs laterally down the length of the body, and an animal will usually have several lateral lines on each side. The lateral line is composed of several stitches aligned either horizontally or vertically. Each stitch contains several neuromasts that contain the hair cells. Each neuromast is covered by a a gelatinous structure, called the cupula, into which stereocilia from the hair cells stick.

All stitches of the lateral line are innervated by branches of the same two nerves: the afferent—used for messages sent from the lateral line to the brain, and the efferent—used for messages sent from the brain to the lateral line. With this structure, nerve activity from a particular stitch can be recorded by recording from the one afferent nerve for the lateral line containing the stitch.

Since the lateral line can be accessed with little or no surgery and contains large hair bundles that are easily viewed (Sewell, 1990), we have developed a lateral line preparation to allow the neural activity of hair bundles to be recorded and to simultaneously allow motion of hair bundles to be observed and recorded using computer microvision (Davis, 1997).

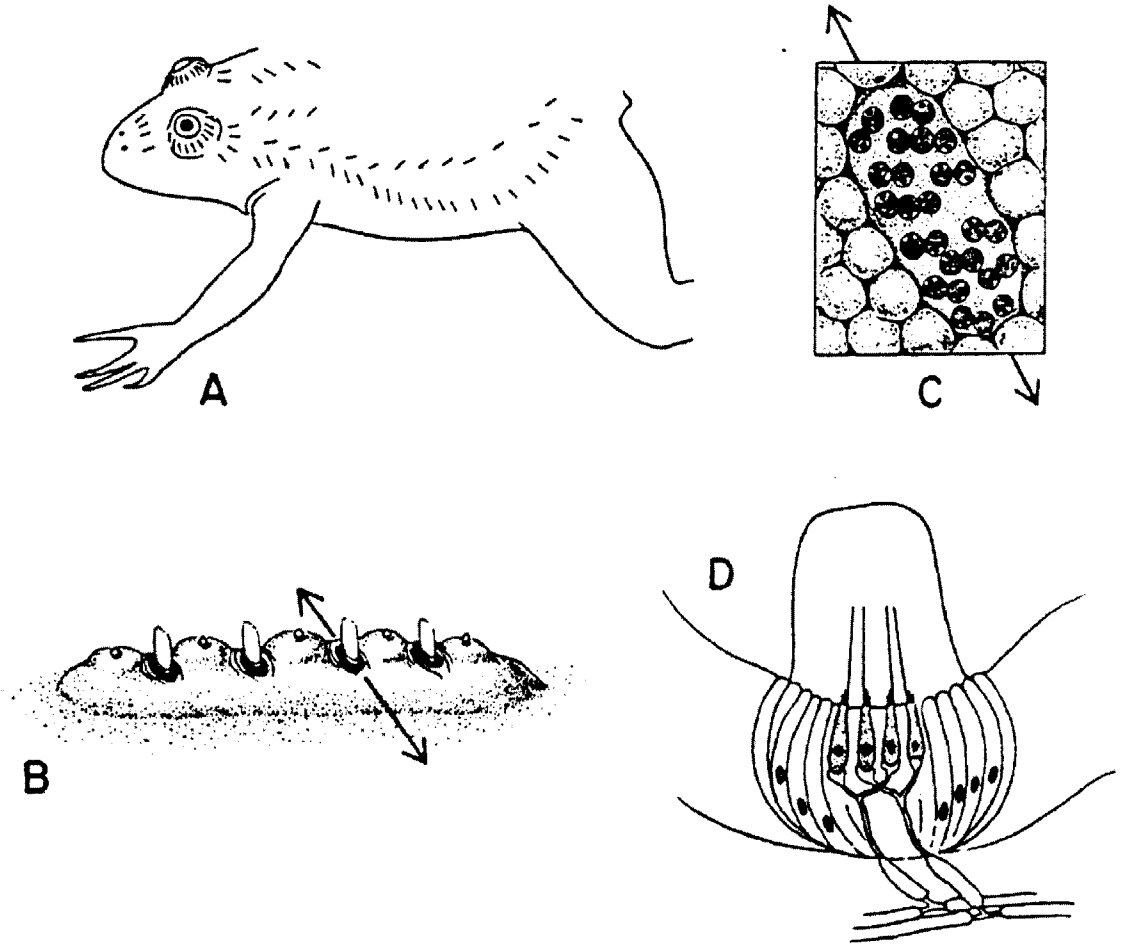

Figure 1-3: Lateral Line of *Xenopus laevis* (from (Strelioff and Honrubia, 1978)). A shows the different lateral lines and their stitches running the length of the body. B shows an individual stitch and its four neuromasts. The arrow refers to the direction in which the stitch is mechanically most sensitive. C shows a surface view of a neuromast and the stereocilia bundles within. The arrow refers the direction of most sensitivity. D is a side view of the neuromast and shows the cupula encapsulating the stereocilia as well as the innervating nerves.

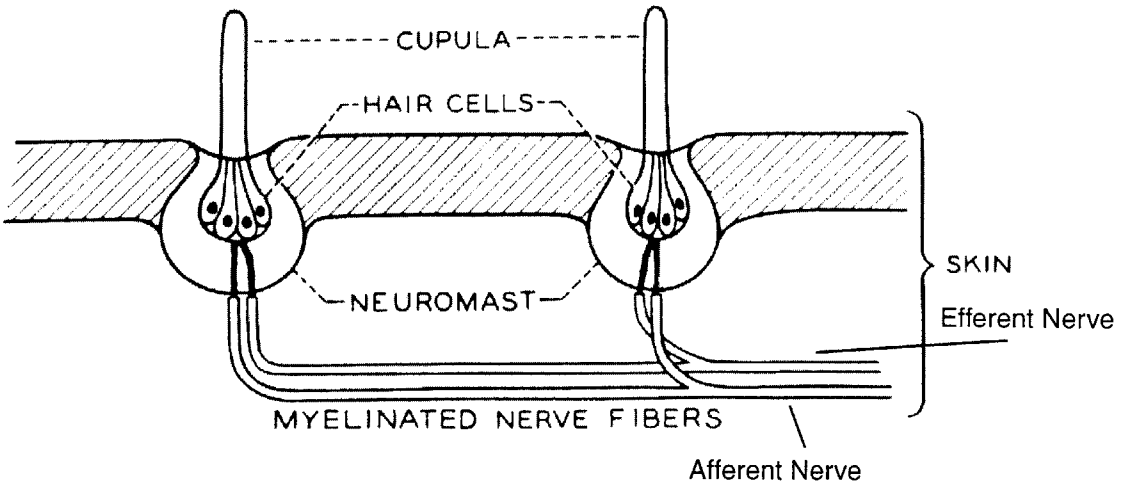

Figure 1-4: One efferent and one afferent nerve innervate all of the neuromasts of a stitch. (from (Harris and Flock, 1967))

1.3 Importance of Electrical Measurements

The micromechanics in living cochleae have been shown to differ from those of dead cochleae. In basilar membrane motion studies, measured electrical responses of the cochlea have played a key role in separating physiologically relevant measurements from those of "dead" cochleae. By recording the electrical responses from the nerve fibers innervating the hair cells, the presence or absence of nerve activity in the lateral line can establish whether the preparation is alive or dead.

Chapter 2

Methods

2.1 Dissection

To measure electrical responses from the lateral line, it is necessary to surgically dissect the skin containing the lateral line of interest from the rest of the body. Once the skin is removed, the afferent nerve innervating the lateral line is prepared for recording. Postmetamorphosis *Xenopus laevis*, approximately 3 cm from nose to vent, were used[1] in this preparation.

Prior to surgery, the frog was anesthetized in MS222 for twenty minutes and then washed in artificial perilymph. The frog was then promptly decapitated and pithed. Surgical cuts down the back, abdomen, leg, and shoulder separated the lateral line from the rest of the body. The lateral line used for this preparation is depicted in figure 2-1. At the shoulder, the plexus there was cut to ensure the nerve innervating the lateral line is separated from the rest of the body. Once separated, the skin is carefully pulled off of the body and soaked in artificial perilymph. Since the lateral line of interest runs along the side of the frog, a second preparation was prepared

[1]Ideally, for electrical responses from the lateral line alone, *Xenopus laevis* larger in size (approximately 8-10 cm from nose to vent) would be used. Due to size, the lateral line is longer and there is more nerve length making the dissection easier. However, with the larger *Xenopus laevis*, the skin is thicker and a "slime" layer coats the skin. Since we wish to be able to view hair bundles using light microscopy, the thicker skin would provide for poor optics. With *Xenopus laevis* approximately 3-5 cm from nose to vent in size, the skin is almost transparent and there is very little slime coating the skin.

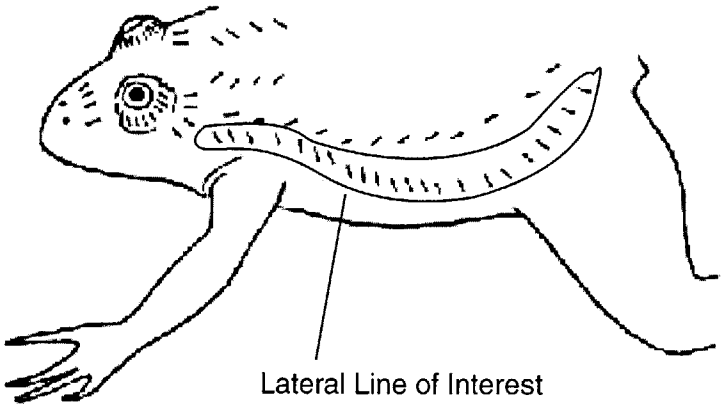

Figure 2-1: *Xenopus laevis* has several different lateral line structures. The lateral line section used for this preparation is circled.

with the lateral line on the other side[2].

Once removed from the frog, the skin is placed upon a 1x3" glass slide in preparation for electrical recording of the lateral line. The afferent nerve innervating the lateral line is carefully pulled out from underneath the skin and cut without disconnecting it from the stitches of interest.

2.2 Perfusion

To maintain the health of the preparation for several hours, it is necessary to perfuse the surface of the skin. The surface was perfused with artificial perilymph at a rate of 72 μL/min. The artificial perilymph was prepared by mixing together the compounds in the table below into 1 L of deionized water. The solution is then titrated with NaOH until the pH is 7.5 and then finally filtered before use. The osmolarity of the artificial perilymph is approximately 250 mmol/kg.

[2]Soaked in artificial perilymph, the second lateral line remained viable for several hours.

Artificial Perilymph	
Compound	Weight(g)
HEPES	4.766
NaCl	7.013
NaOH	0.400
KCL	0.261
Glucose	1.000
$CaCl_2 \cdot 2H_2O$	0.221

2.3 Experimental Chamber

A chamber was developed to allow the nerve to be easily recorded while simultaneously mechanically stimulating the lateral line which it innervates. The chamber is a plexiglass plate containing a slot to hold the glass slide in place, steel plates surrounding the slot, and a nerve holder mounted on a single axis micromanipulator.

The perfusion system consists of two lengths of tubing with one delivering fresh artificial perilymph and the other removing the old. The artificial perilymph is delivered through hollow glass rods acting as spouts for the tubing. Both tubes are magnetically mounted to the steel plates for ease of placement.

The nerve holder, shown in figure 2-3, is a micropipette tip fused to a sealed chamber. On the other end, the sealed chamber has a gold pin connector and on the side is a port which connects the chamber to a syringe with tubing. The syringe allows the nerve to be easily held in place by simply "sucking up" the end of the nerve. A chlorided silver wire, the positive electrode, resides inside the pipette tip and is attached to the gold pin. The ground electrode is a silver wire with a silver chloride pellet at one end which is submerged in the perfusion during experimental runs. The ground electrode is held in place by an alligator clip mounted on a stand.

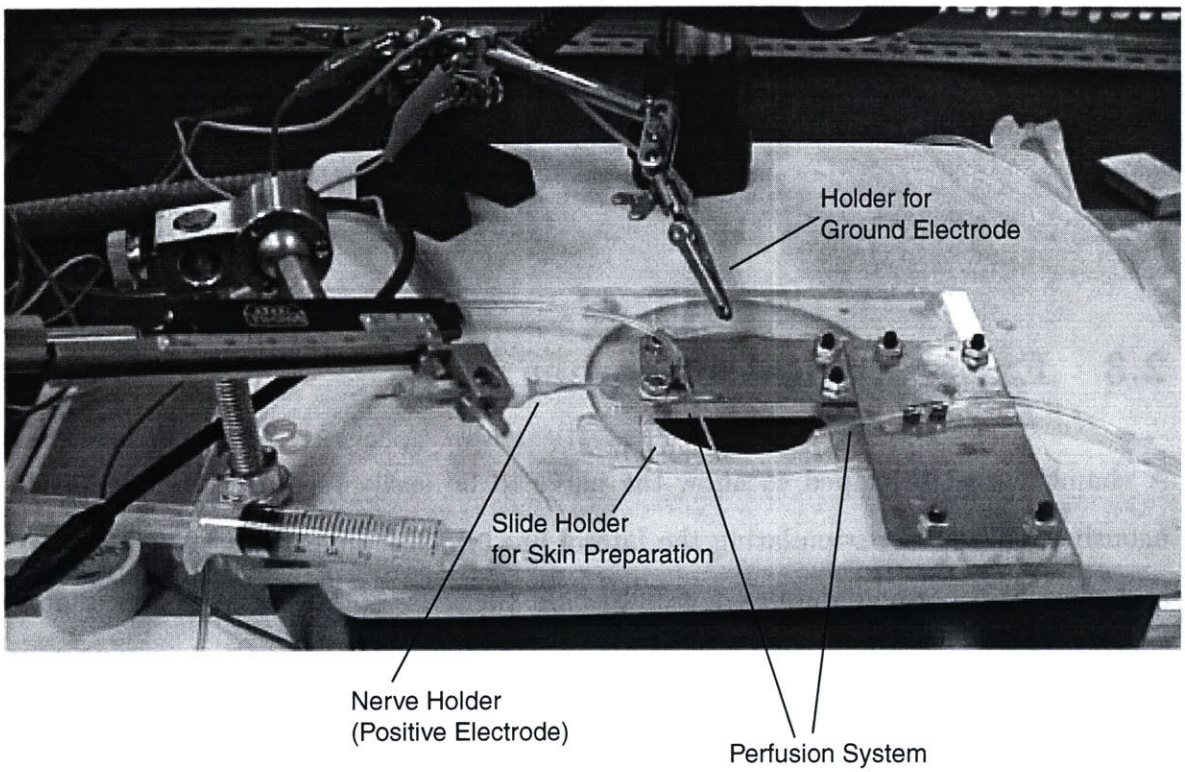

Figure 2-2: Experimental Chamber. The chamber has a slot where the preparation can simultaneously be perfused, stimulated, and have its nerve activity monitored.

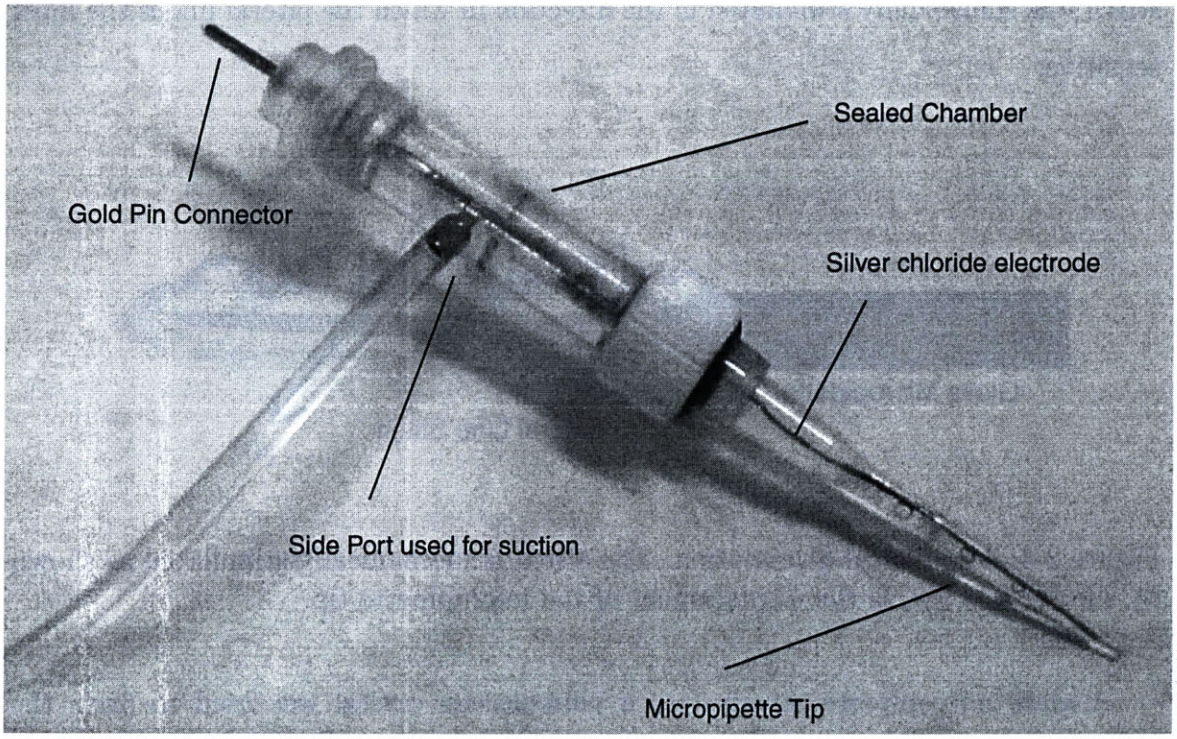

Figure 2-3: The nerve holder was used to record neural activity from the lateral line. The silver chloride wire inside connected to the gold pin on the outside form the positive electrode. The port on the side is attached to syringe to gather the nerve end as well as saturating the nerve in artificial perilymph.

2.4 Mechanical Stimulation

To study the mechanics in the lateral line, the hair bundles are externally stimulated in a controlled fashion. By stimulating a single stitch of the lateral line, we hope to see how the neural activity is affected. To achieve the greatest neural response, the stitch was sinusoidally stimulated in the direction in which the lateral line is the most sensitive.

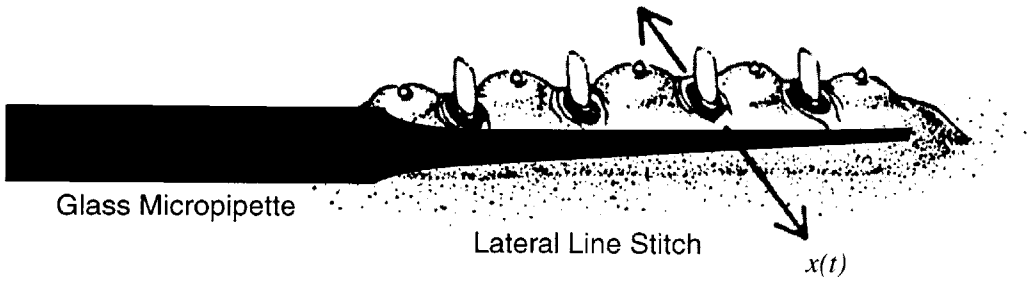

Figure 2-4: Mechanical Stimulation. The stitch is mechanically stimulated as shown by the arrows. $x(t)$ is the displacement of the micropipette tip.

A glass micropipette attached to a piezoelectric crystal was used to drive the mechanical stimulation. The tip of the glass micropipette is small enough for accurate placement while the piezoelectric crystal is used to move the tip back and forth when the crystal is driven with a sinusoidal voltage.

Together, they are mounted on a three-axis micromanipulator to allow precise placement of the glass micropipette tip. The base of the micromanipulator is magnetic so that it stays in place during experimental runs.

2.5 Stitch Ablation

Without mechanical stimulation, the lateral line will have some neural activity that is termed spontaneous activity. Since the afferent nerve innervates all of the stitches of the lateral line, the response to mechanically stimulating one stitch will be masked by the spontaneous activity of other stitches in the lateral line. To reduce spontaneous

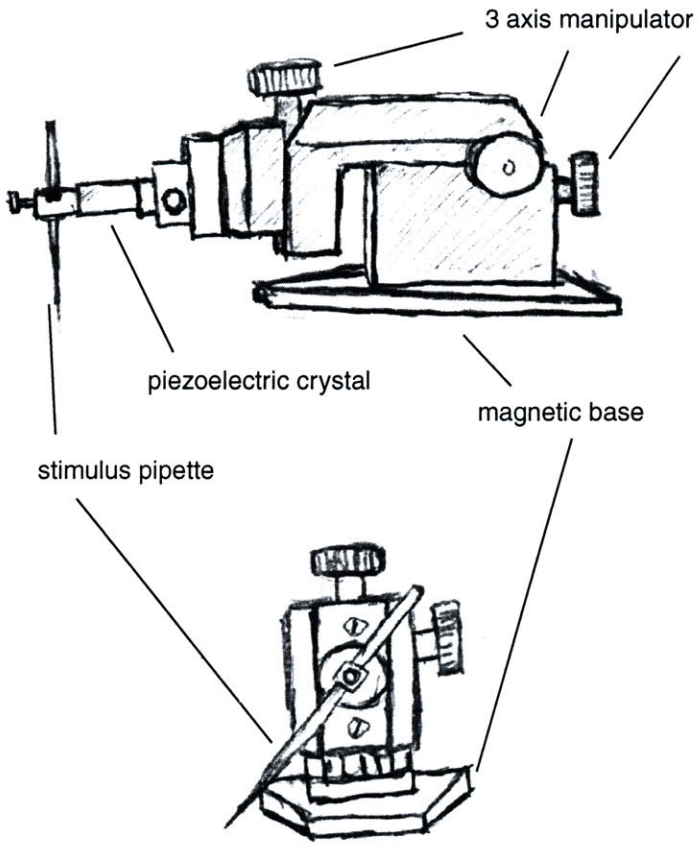

Figure 2-5: Mechanical Stimulator. A micromanipulator holds the glass micropipette via a the piezoelectric crystal which is used to stimulate a single stitch of the lateral line.

activity from the stitches of no interest, those stitches were ablated by crushing them. Once ablated, the spontaneous activity of the stitch ceases. With only neural activity of a single stitch responding, any effects of mechanical stimulation to the stitch can be seen without interference from other stitches.

2.6 Data Processing

To analyze effects of mechanical stimulation, it is necessary to be able to analyze the rate and time of the nerve spikes. To gather data, a low-noise 80dB amplifier[3] was used to amplify the neural responses and a two-channel A/D was used to sample the data at 10kHz. One channel gathered the neural activity from the amplifier and the other gathered data from the voltage waveform driving the piezoelectric crystal.

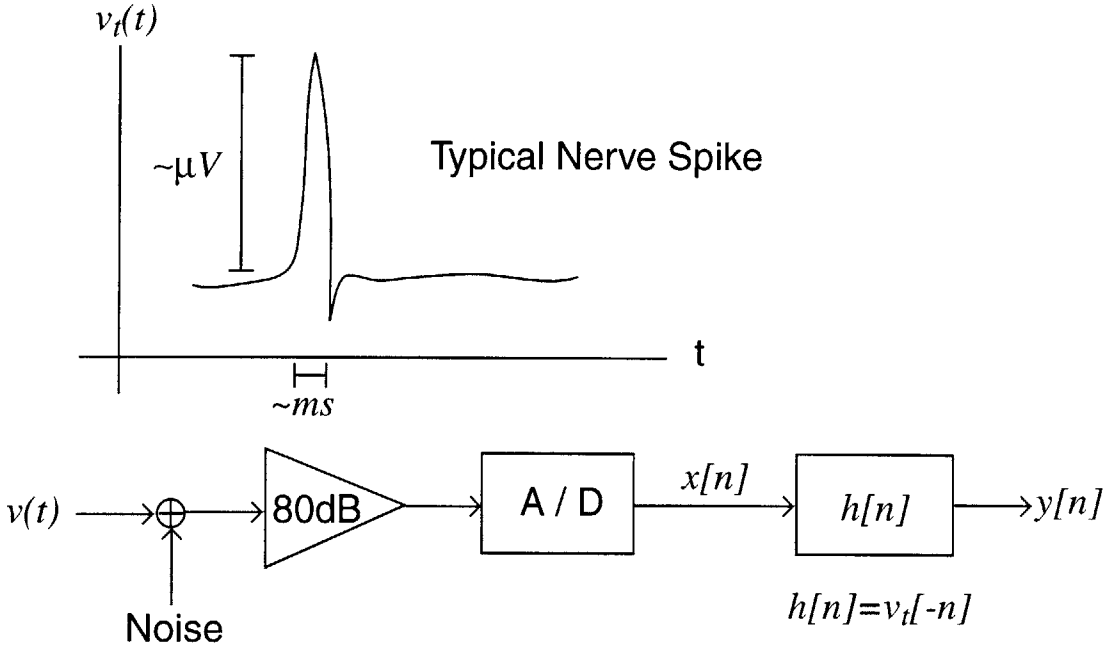

Figure 2-6: Spike Train Filtering. Above is a typical nerve spike. The nerve spikes *v(t)* along with ambient noise are amplified by 80dB, then sampled at 10kHz by a A/D unit. Once sampled, the nerve spikes are isolated with a matched filter.

The measured neural activity was contaminated by noise from several sources, especially from 60 Hz noise. Although, the preparation was placed within a Faraday

[3]Designed by Dennis M. Freeman and constructed by A.J. Aranyosi.

cage, some noise remained. In addition to amplifier noise, the mechanical stimulator added crosstalk interference. As the frequency and amplitude of the voltage driving the piezoelectric increased, the crosstalk noise increased. With filtering, the nerve spikes were able to be isolated for data analysis.

A discrete matched filter was used to filter the data. The filter was constructed in Matlab using a single nerve spike from a spontaneous data set. Filtering allowed the nerve spikes to be determined by a simple voltage threshold which is discussed later in section 3.2.

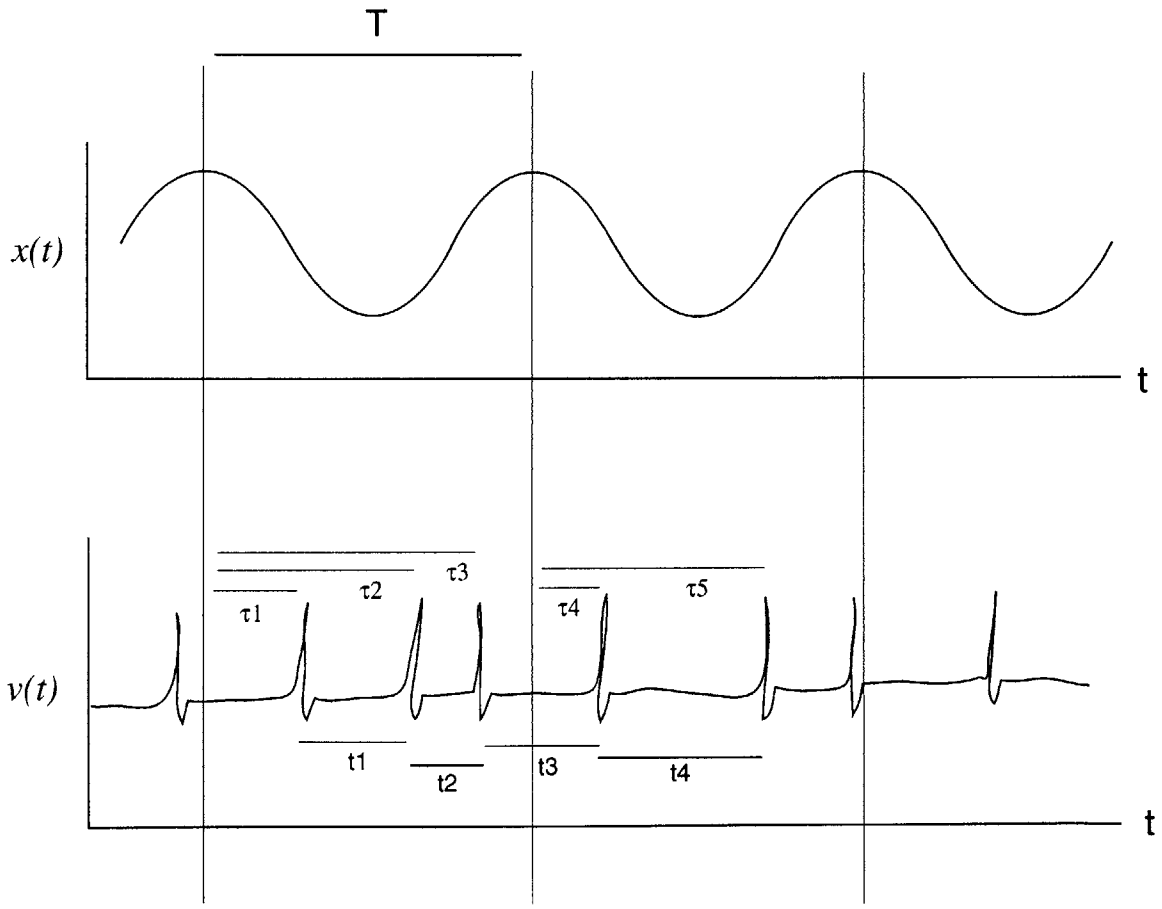

Figure 2-7: Data Analysis. The top plot shows the displacement of the mechanical stimulation. The bottom plot shows the nerve spikes on the same time axis used for the mechanical stimulus. The τ's represent the post stimulus time interval and the t's represent the inter-arrival times.

The stitch was mechanically stimulated with sinusoidal stimuli with frequencies

from 19Hz to 99Hz in 5Hz increments. From the data gathered, the neural activity of the stitch was analyzed and correlated with the mechanical stimulus. By calculating the time between each nerve spike (t_i in figure 2-7), a time-interval time histogram was constructed and used to provide an analysis of at what interval the nerves are firing most prominently.

To better correlate neural activity to its mechanical stimulus, a post-stimulus time (PST) histogram can be constructed. In figure 2-7, τ_i represents the time of a nerve spike from the last peak amplitude of the stimulus—the post stimulus time. With the post stimulus times, the PST histogram shows any correlation of the neural activity with mechanical stimulation. If the PST histogram showed an increased response at a particular phase of the mechanical stimulation, then that suggests the stitch is responding to the mechanical stimulus at the corresponding phase of the stimulus.

2.7 Animal Care

The care and use of animals reported in this study were approved by the Massachusetts Institute of Technology Committee on Animal Care through CAC Protocol #01-001.

Chapter 3

Results

3.1 Mechanical Stimulator

Computer microvision (Davis, 1997) was used to characterize the motion of the mechanical stimulator. Figure 3-1 shows that the motion of the mechanical stimulator is flat up to 400Hz. It resonates and lags out of phase at approximately 2000 Hz. The phase jump at 2000 Hz is explained by the usage of the *atan* function used to analyze the data since the *atan* function does not account for phase wrapping. The phase should continue to fall to -360 degrees.

The lateral line is most sensitive to frequencies below 100 Hz, therefore it will only be be mechanically stimulated at frequencies less than 100 Hz. Since the resonant frequency of the mechanical stimulator is well above 100 Hz and the response of the glass micropipette is flat and in phase at frequencies lower than 100 Hz, the mechanical stimulation will move in phase with the stimulus voltage of the piezoelectric crystal. This calibration allows motion of the micropipette to be accurately predicted from the stimulus voltage alone, making it unnecessary to measure the glass micropipette tip during experimental runs.

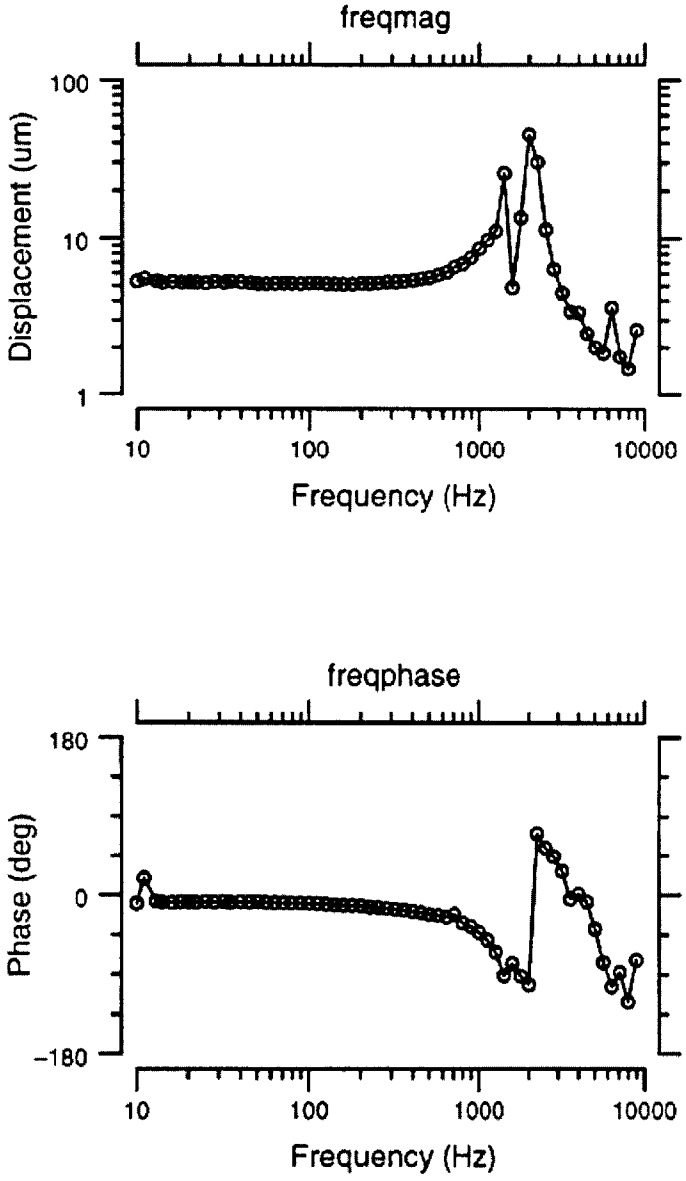

Figure 3-1: Above is the magnitude response of the motion of the mechanical stimulator as a function of frequency. Below is the phase response as a function of frequency. Motion of the mechanical stimulator resonates and moves out of phase at a frequency of approximately 2 kHz.

3.2 Matched Filtering

As depicted in figure 2-6, it is necessary to amplify the neural responses since they are on the order of microvolts. However, amplification also increases the level of any interference and noise. These degradations are reduced with a matched filter as described in section 2.6.

Figure 3-2 shows the neural data before and after matched filtering. Before filtering, spike detection in the spontaneous neural response and neural response under 19Hz mechanical stimulation can be reasonably determined with a simple voltage threshold. However, for the neural response under 99Hz mechanical stimulation, it is not possible to use a threshold for spike detection.

However, after filtering, as the right column shows, much of the noise has been eliminated. In all of the plots showing filtered data, a threshold of 1.5V provided for good spike detection. Even at 99Hz, the interference introduced from mechanical stimulation was significantly reduced.

3.3 Spontaneous Nerve Activity

Even in the absence of mechanical stimulation, the lateral line still exhibits spontaneous neural activity. Figure 3-3 shows the results of spontaneous activity. The inter-arrival time histogram suggests that neural activity is roughly linear on a log scale for intervals smaller than 0.02 seconds. Linearity on a log scale implies that the neural activity is roughly exponential e^{-x} in nature. However, at intervals below 0.002 seconds, the neural response does not support the exponential notion. This is due to refractoriness since neurons must have time to recover between firings. Otherwise, in general, the spontaneous neural activity can be reasonably fit by a Poisson process.

Another observation is that not all spikes share the same amplitude. This suggests that the nerve innervating each stitch of the lateral line consists of several neurons.

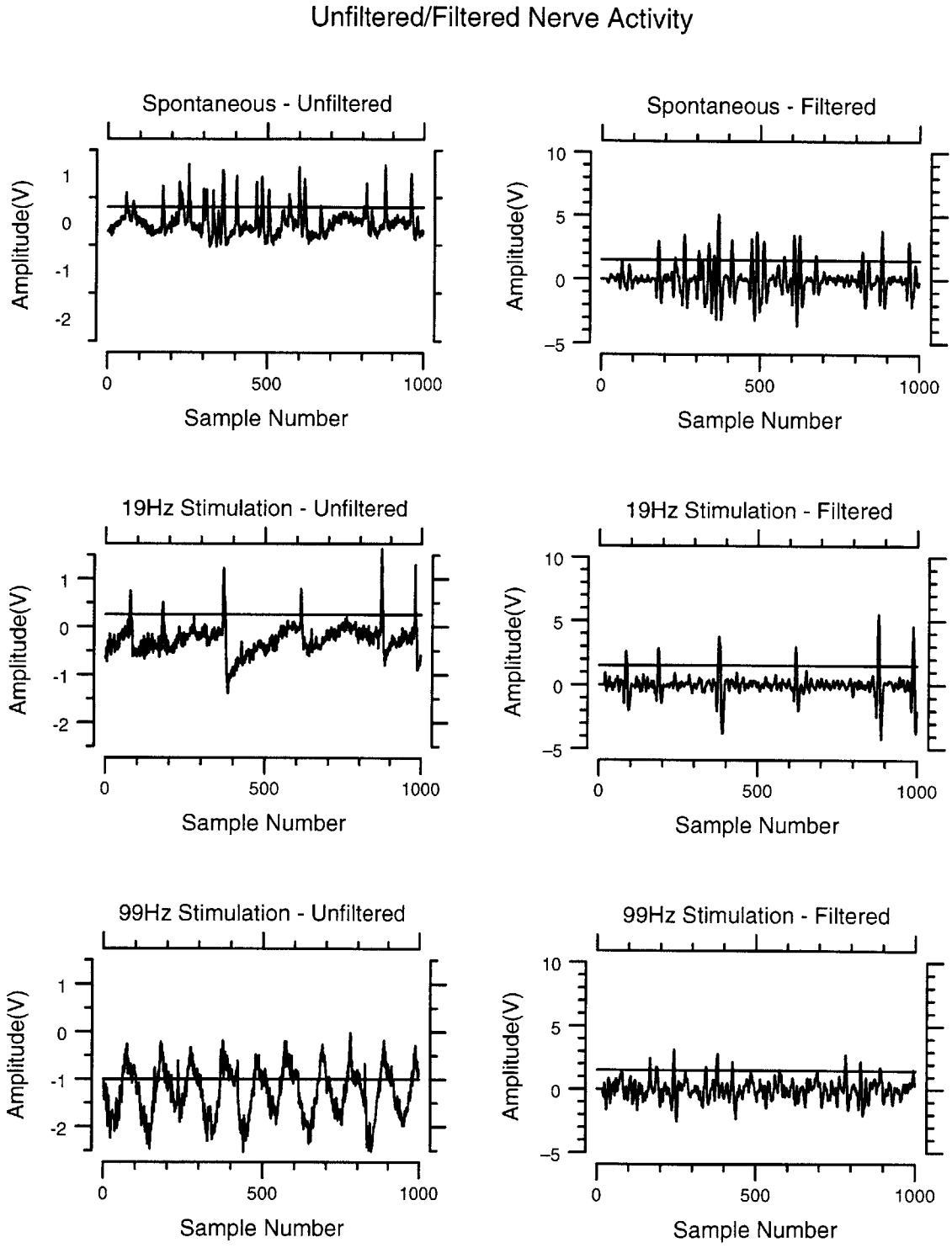

Figure 3-2: Effects of Matched Filtering. The left column shows the unfiltered spontaneous neural response and neural responses under mechanical stimulation at different frequencies. The right column shows the filtered data for the respective plots in the left column. The horizontal line within each plot shows the threshold used to determine nerve spikes in the filtered data.

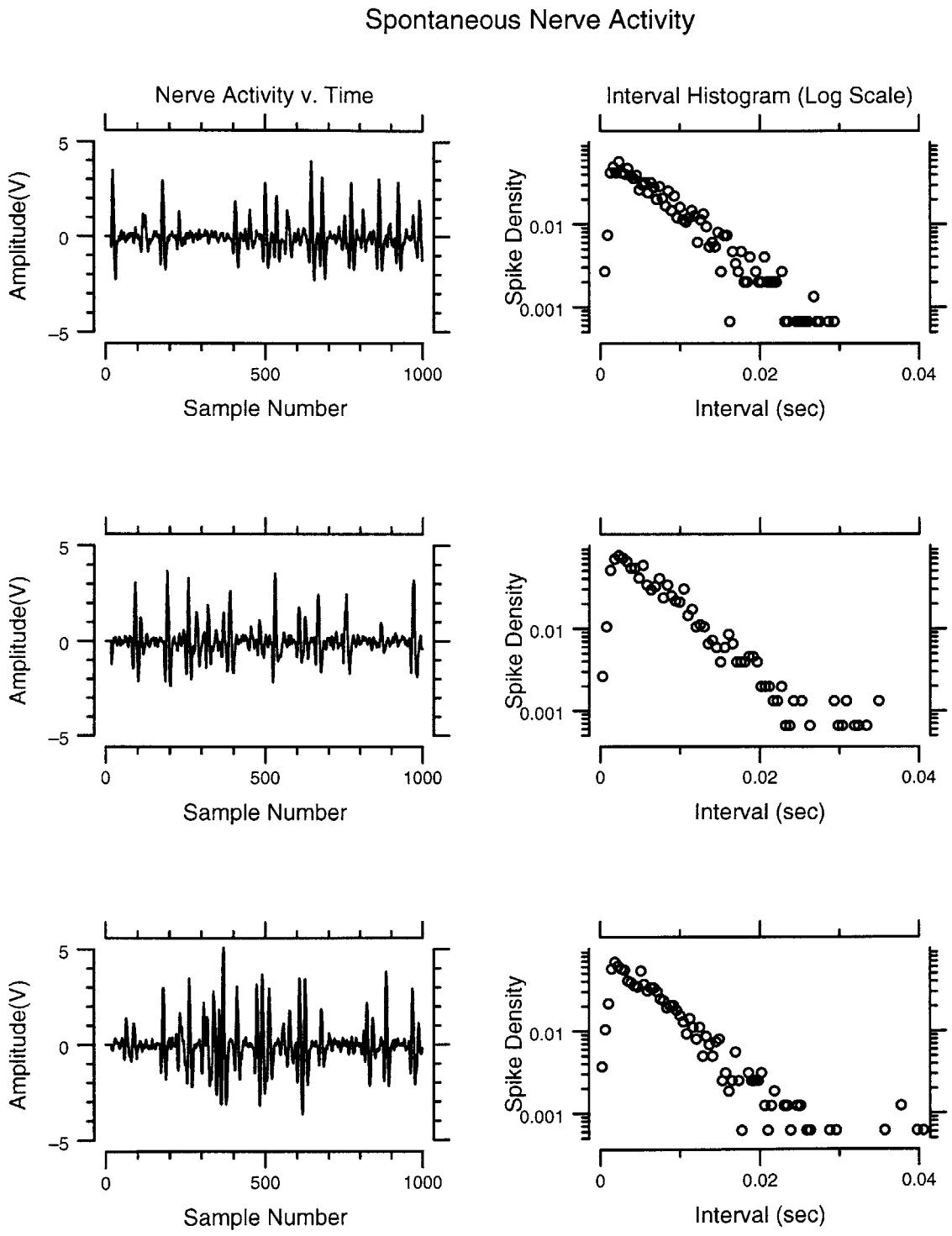

Figure 3-3: Spontaneous Nerve Activity. The left column shows spontaneous neural activity from three separate recordings. On the right are their respective inter-arrival time histograms

3.4 Stitch Ablation

Since the nerve recorded innervates all of the stitches for the lateral line, the neural activity recorded is from all stitches. Due to the aggregated neural activity, the rate of activity is in the range of 150-200 spikes/second. Although this is normal, mechanically stimulating one stitch may not result in a significant change in activity. Stitch ablation was implemented to isolate the neural activity of a single stitch.

Figure 3-4 shows the effects of stitch ablation. As the ablation of stitches increases, the rate of spontaneous activity decreases. This is also reflected in the inter-arrival time histograms. Between no ablation and some ablation, the histograms show no change and show that there are roughly 50-100 spikes/second. However, between some ablation and most ablation, the peak shifts slightly to the right as the rate decreases to approximately 20 spikes/second.

3.5 Mechanical Stimulation

To correlate between the stimulus and neural activity, figure 3-5 shows plots of the neural activity and stimulation as a function of time. In addition, post-stimulus time (PST) histograms reflect at what phase of the mechanical stimulation neural activity occurs. An increased response at a particular phase of the stimulation is a correlation—it suggests that the mechanical stimulation is modulating the firing rate.

However, figure 3-5 shows that there is no apparent correlation between stimulation and neural activity. In the plots on the left, there is no obvious pattern of nerve spikes in respect to the stimulation. On the right, the response of the nerves is not particularly prominent at any particular phase of the stimulation, further suggesting no correlation between neural activity and the stimulus.

In figure 3-6, we see the effects of mechanical stimulus on neural response over a span of frequencies. It shows tiny peaks at the fundamental frequency and its harmonics. For 19 Hz stimulation, there are small peaks at 0.026, 0.017, and 0.013 seconds, which correspond to the 2nd, 3rd, and 4th harmonics of 19 Hz respectively.

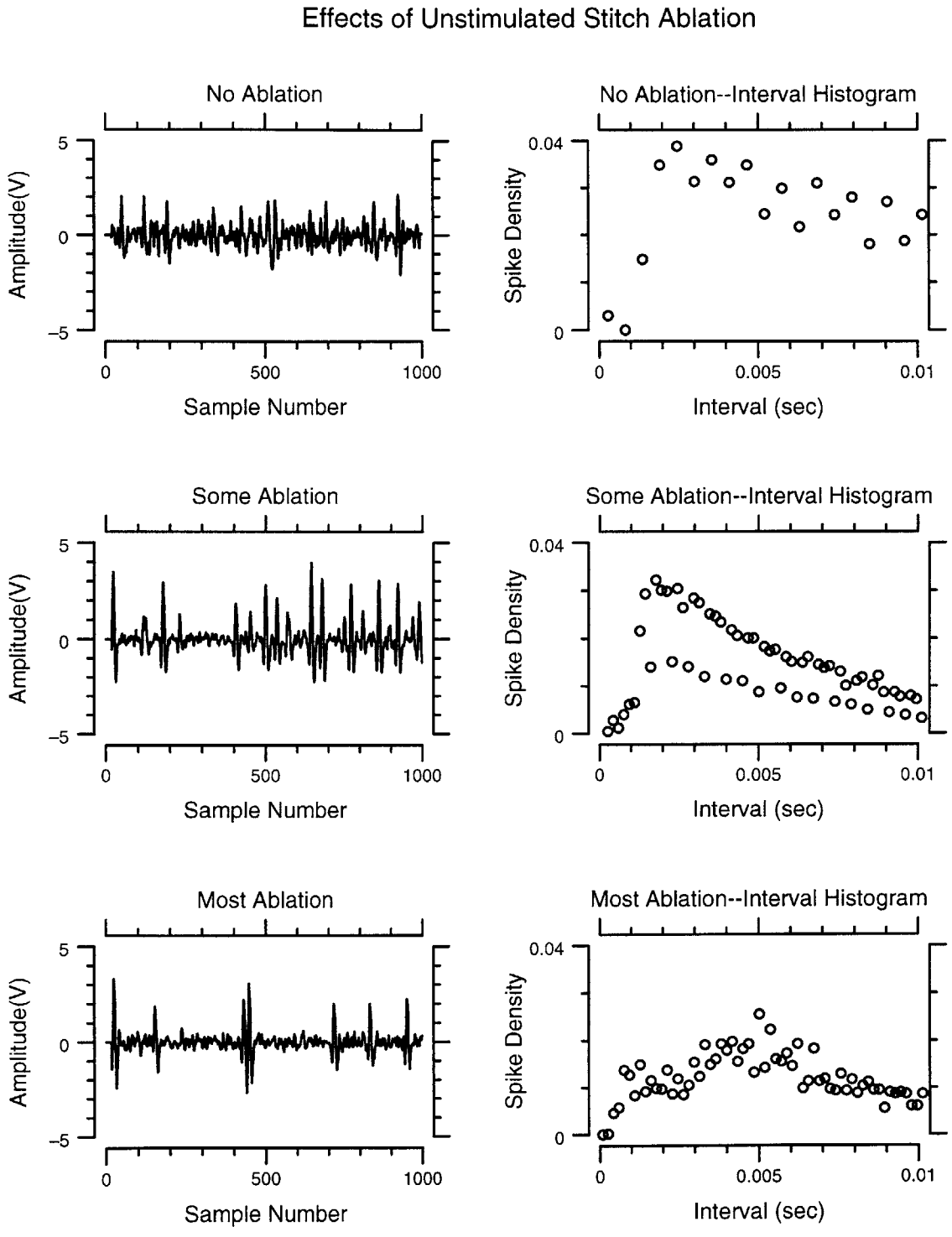

Figure 3-4: Stitch Ablation. Spontaneous activity for three different levels of stitch ablation are shown as well as their respective inter-arrival time histograms.

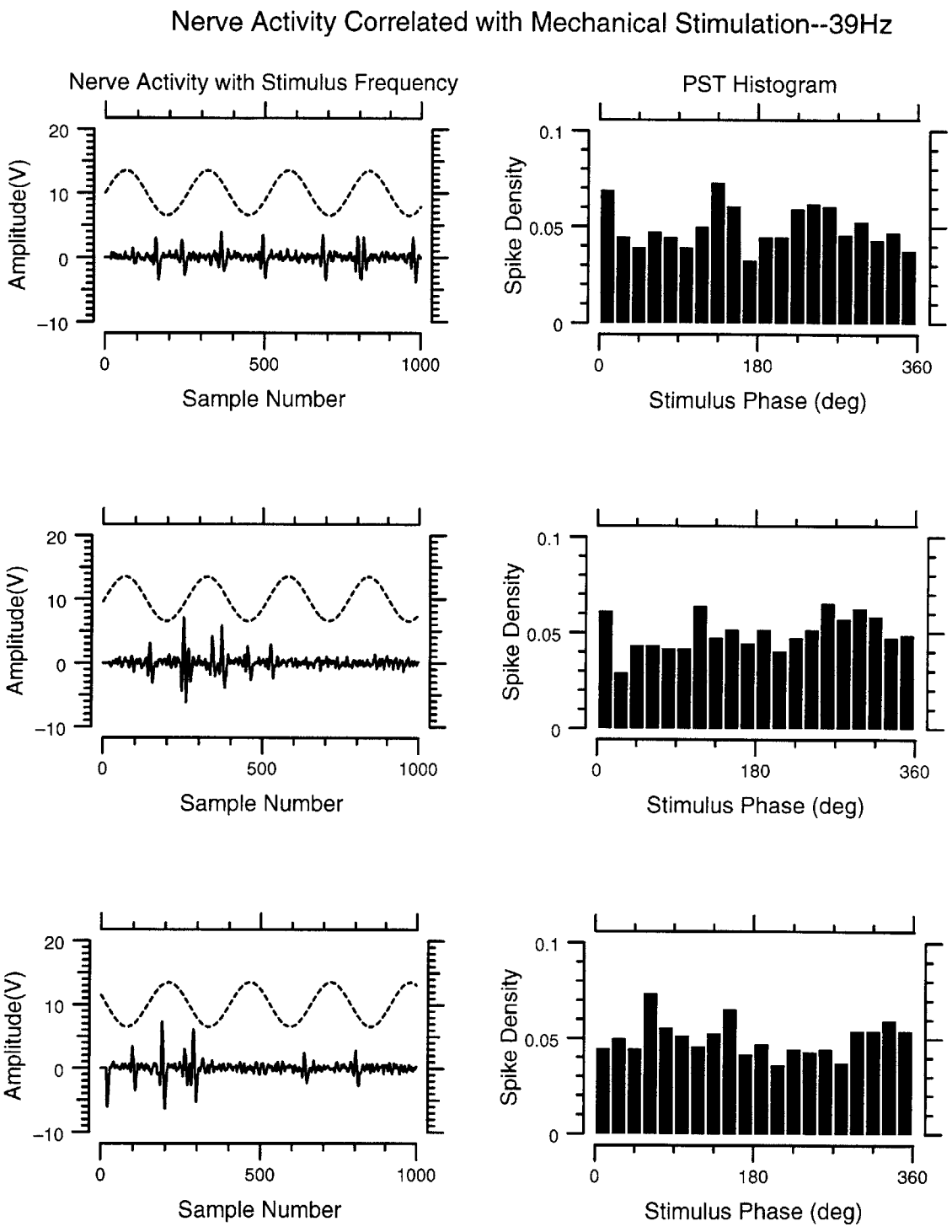

Figure 3-5: Nerve Activity Under Mechanical Stimulation. Three different runs with the same preparation and same stimulus frequency are shown. The left-hand plots show neural activity and mechanical stimulation vs. time. On the right are the respective PST histograms. There seems to be no obvious correlation between the neural responses and its mechanical stimulation.

For 44 Hz stimulation, there are small peaks at 0.023 and 0.011 seconds, which which correspond to the fundamental frequency and 2nd harmonic for 44 Hz. For 99Hz stimulation, there is a peak at .010 seconds, which corresponds to the fundamental frequency for 99 Hz. However, these peaks are not strong evidence of any correlation as the PST histograms in figure 3-7 shows a roughly flat response at all three frequencies.

Since some evidence of correlation is to be expected, there are several possibilities to explain the obtained results. These will be further discussed in the following chapter.

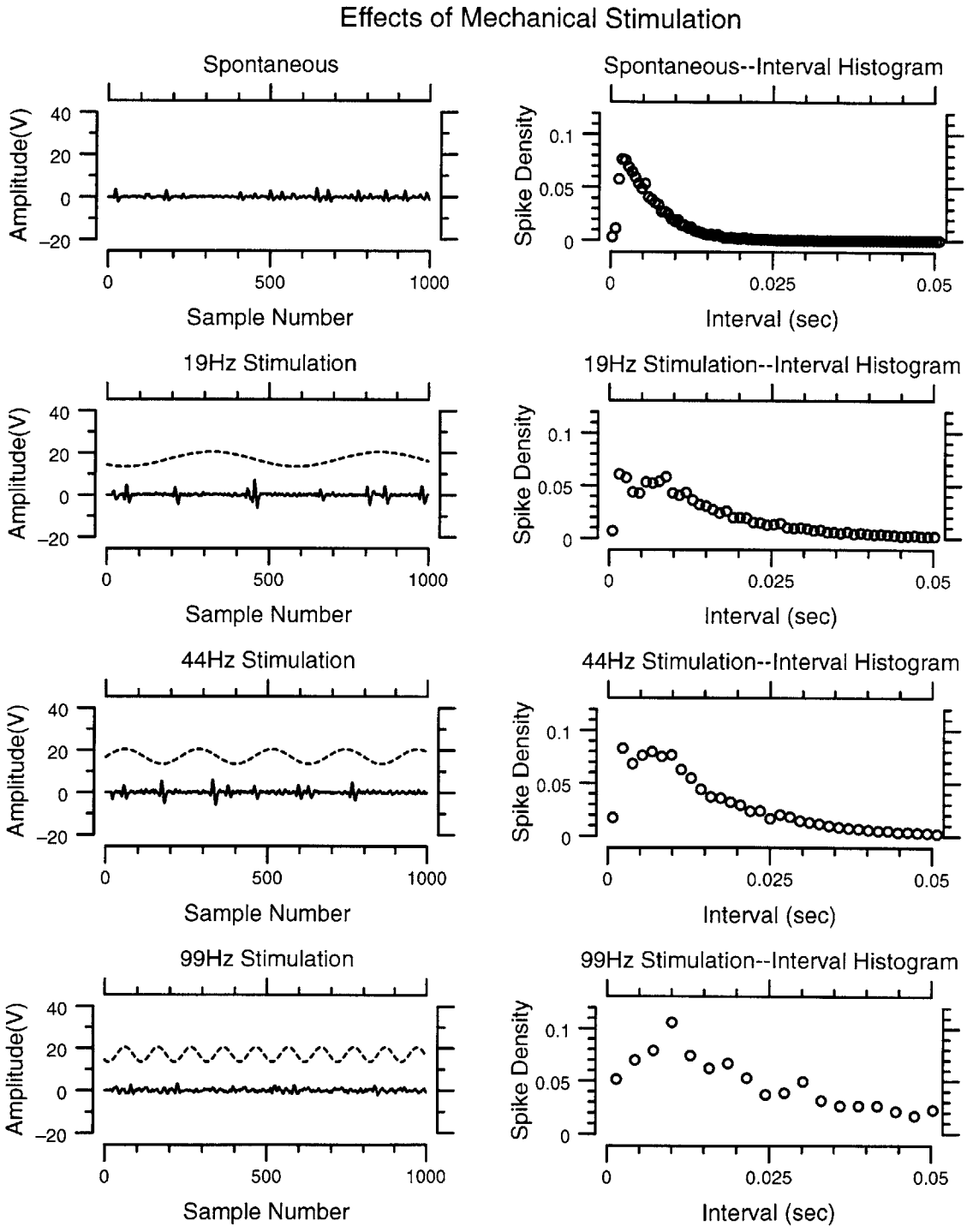

Figure 3-6: Mechanical Stimulation at Different Frequencies. For spontaneous response and response of stimulation at 19Hz, 44Hz, and 99Hz, neural activity and mechanical stimulation are plotted vs. time. In addition, respective inter-arrival time histograms are plotted on the right.

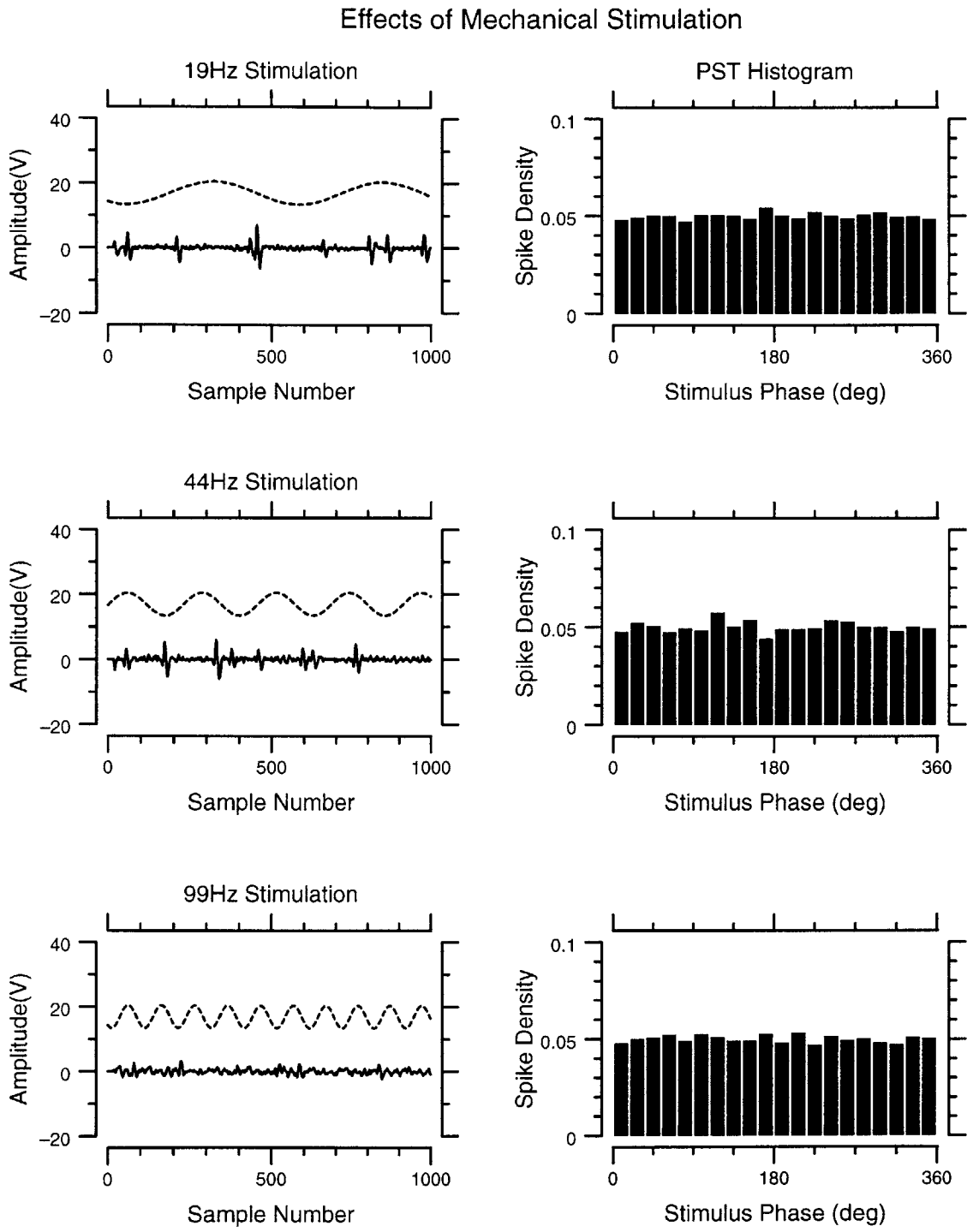

Figure 3-7: Mechanical Stimulation at Different Frequencies. For stimulation at 19Hz, 44Hz, and 99Hz, neural activity and mechanical stimulation are plotted vs. time. In addition, respective PST histograms are plotted on the right.

Chapter 4

Discussion

The goal of this thesis was to record nerve activity from the lateral line while simultaneously stimulating it mechanically. Even though we are able to mechanically stimulate the lateral line and at the same time record its nerve activity, it would be helpful to have the knowledge of which stitch of the lateral line the neural responses correspond to. However, this cannot be obtained from our results. A previous study on the lateral line have shown that the nerve fibers give a spontaneous discharge that is modulated by mechanical stimulation and respond most vigorously at a certain characteristic frequency (Bailey and Sewell, 2000). None of our results corresponded to the results of this study since no correlation was detected between neural activity and its mechanical stimulation in our experiments. There are several reasons why our results may not be accurate and will be discussed in this section.

4.1 Spontaneous Activity and Stitch Ablation

Before stitch ablation, the spike activity of the lateral line was between 150-200 spikes/second. After the crushing most of the stitches, the spike activity was reduced to usually between 50-100 spikes/second. The greatest reduction resulted in activity of about 20 spikes/sec. However, even at 20 spikes/sec, there is reason to believe that the stitch of interest is not the only stitch still contributing to spontaneous neural activity. Either not all of stitches, aside for the one of interest, were ablated or the

stitches ablated were not ablated properly. According to the previous study (Bailey and Sewell, 2000), results for spontaneous activity for a single stitch were shown to be approximately 1 spike/sec.

4.2 Different Lateral Line Sections

Another question that arises is confirmation that the correct nerve was being recorded. There are a few different sections of the lateral line contained in dissected skin. The nerves are easily distinguishable when the skin is bottom side up since they can be easily traced to the stitches they innervate. However, for our preparation, the skin must remain surface side up. In this configuration, the nerve innervating the stitches cannot be easily traced. Since the nerve must be pulled out from beneath the skin, there is a slight possibility that the wrong nerve was used for recording—a nerve innervating another lateral line on the skin.

Chapter 5

Conclusions

We have made significant progress in developing a preparation for studying mechano-electric transduction in hair cells based on the lateral line of *Xenopus laevis*. This preparation is simpler than the alligator lizard preparation previously used in this laboratory for a number of reasons. First, the dissection is easy and can be completed in less than 10 minutes. In addition, it is also less challenging as the precision required for the *Xenopus* preparation is on the order of millimeters as opposed to micrometers for the lizard preparation. Third, the experiment chamber is very simple compared to the experiment chamber for the lizard preparation. Fourth, only one solution must be perfused instead of the two required for the lizard preparation. Fifth, mechanical stimulation of the *Xenopus* preparation is simpler than acoustical stimulation of the lizard preparation. Finally, the *Xenopus* preparation is robust and remains functional for many hours. These simplifications allowed us to obtain a preparation with active electrical responses, a task that was not possible with the alligator lizard.

The most difficult aspect of this preparation is the isolation of the nerve for recording. This procedure is relatively simple when the preparation is mounted with the hair bundle side facing the chamber floor as the entire course of the nerve is readily visible. However, facing the chamber floor, the hair bundles cannot be mechanically stimulated. Therefore, we must mount the preparation with the nerve side facing the chamber floor. This orientation complicates isolation of the nerve. Nevertheless, the success rate was high: four of our last five experiments yielded responsive

preparations.

The neural signals that we recorded were small — on the order of microvolts. Successful recording required the development of a high-gain, low-noise amplifier and shielding of the preparation by a Faraday cage. Even with these improvements, the data were still noisy, and filtering was required to isolate action potentials.

Although small, the neural signals were surprisingly robust. Action potentials were recorded for several hours after dissecting preparation. The average size of these potentials did not change significantly over long time scales, although they often varied from one spike to the next. In addition, the response was present in multiple preparations, even after sucking up and losing the nerve multiple times or bathing the tissue in artificial perilymph for several hours. The strong responses of the *Xenopus* preparation make it ideally suited for *in vitro* studies.

We failed to demonstrate a correlation between neural activity and mechanical stimulation. We can identify several possible reasons for this. First, to reduce the spontaneous activity from unstimulated stitches that tended to mask mechanical responses from the stimulated stitch, we attempted to ablate all stitches except the one stimulated. This ablation may have been incomplete. Second, we may have inadvertently recorded from multiple fibers. This is especially plausible given the fact that we recorded action potentials with multiple amplitudes. It seems unlikely that a single neuron would produce action potentials with different amplitudes. Finally, further noise reduction, better filtering, and improved action potential identification algorithms would significantly improve the reliability of the data.

Visualization of the hair bundles was unexpectedly difficult. We anticipated that the layers of skin underlying the lateral line would degrade image quality since light passing through this layer would be significantly scattered. However, the degradation was significantly worse than anticipated. None of our images convincingly showed a hair bundle. Nevertheless, the presence of neural activity strongly suggests that hair cell bundles are present in this preparation.

A major advantage of the Xenopus preparation is that by measuring action potentials we directly assess the viability of the preparation. Similar recording is not

possible with the lizard preparation, which greatly complicates assessment of viability. We believe that this feature alone means that the *Xenopus* preparation is worthy of further study.

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