Chapter 1. Introduction

The vestibular sense enables vertebrates to perceive motion of the skull, as well as the force of gravity. This sense is essential to balance, posture, and visual fixation. The vestibular system is responsible for the vestibular sense. The vestibular system relies upon a complex cascade of events to transform the input signal (motion or gravity) into a nerve signal received and interpreted by the brain (figure 1-1). This dissertation is concerned with a small portion of this process: the deflection of the ciliary bundles of hair cells.

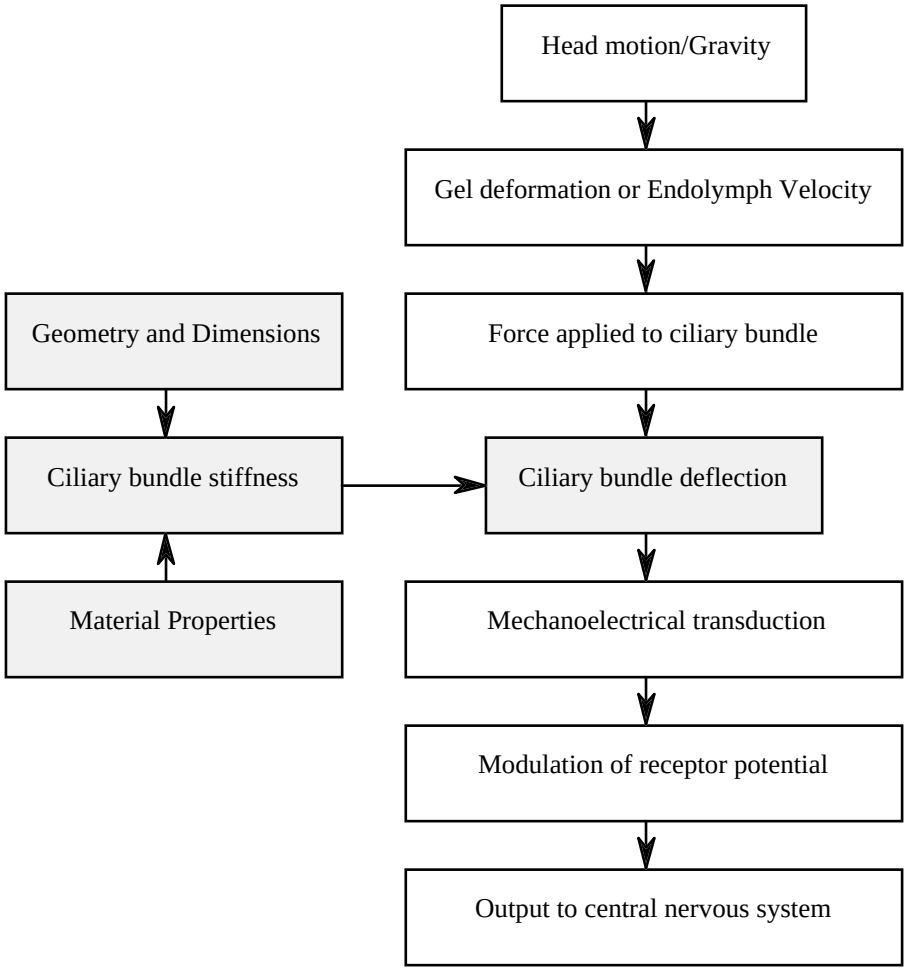

Figure 1-1. Diagram showing the cascade of events needed to detect motion in the vestibular system. The scope of this dissertation is shaded in gray. Where relevant, the processes in the white boxes shown above will be discussed in detail later in this chapter.

Hair cells form the final mechanical link in the vestibular system. They are generic displacement transducers that are also found in hearing organs and on the lateral line of fishes, for vibration sensing. Although this work is primarily concerned with the response of vestibular hair cells, its methods and conclusions can be applied to hair cell research in other systems.

This dissertation looks at modeling mechanical aspects of the hair cells' ciliary bundles. It will establish several new techniques which greatly expand current models of hair cell bundles. It will use these methods to better understand the bundle's myriad structural variations, as well as to decipher some of the finer structures that have been elusive to experimental techniques. Finally, it will provide insight into the actual transduction mechanisms of the bundle.

This work is motivated by a need to understand the normal function of hair cell bundles–which are an integral part of the vestibular system. There are many situations where the vestibular sense fails. Such failures carry a high price. In the weightlessness of space, disorientations have severe effects on the productivity of astronauts. The high accelerations of fighter aircraft cause misleading sensations which have resulted in dramatic accidents and loss of life.

More commonly, according to the National Ambulatory Medical Care Survey of 1991, there are an estimated 5 million physician visits a year related to vertigo or dizziness in the United States. In extreme cases, the patient is unable to walk or even sit, and is troubled by a extreme nausea. In the elderly, vestibular malfunction is frequently the root cause of severe falls.

The remainder of this chapter will introduce the reader to the complexities of the vestibular system, especially those of the hair cell bundle. It will familiarize the reader with the structure and observed behavior of hair cells. It will also introduce vocabulary and predominant theories of hair cell physiology. Finally it will present some results of earlier modeling efforts.

Chapter two will discuss the two methods by which the analyses of hair cell bundles under a static load will be performed in this work. The methods are analytic solutions using simple strength of materials formulations, and a finite element analysis. The later method has been written into a computer program, *bmod*, capable of simulating the response to force of a user-defined full bundle.

Chapter three will establish the basic mechanical principles of hair cell bundles, starting with huge simplifications of structure and adding complexities throughout the chapter. Parametric studies are performed to identify material properties and geometric configurations that are compatible with observed results.

Chapter four will model three biologically observed hair cells from the turtle, *Trachemys (Pseudemys) scripta*–two from the semicircular canal and one from the utricle. These studies will indicate the breadth of information available from models.

Finally, chapter five presents a study of the dynamic effect of a fluid flow over a single kinocilium. It will derive the equations of motion and solve for the ciliary response to several initial conditions and forcing functions.

Anatomy of the Vestibular Labyrinth

The sensing apparatus of the vestibular system is located in a bony labyrinth within the temporal bone, immediately behind the ear, one on each side of the skull. Within this cavity lies fine tissues known collectively as the membranous labyrinth. The entire labyrinth in humans is about the size of a small marble.

The vestibular labyrinth of mammals (see Kelly, 1991, p. 502) contains five sensing organs: three semicircular canals, and the two otolith organs, individually known as the utricle and the saccule. All are interconnected and filled with a endolymphatic fluid. The vestibular labyrinth also connects to the cochlea, which is responsible for the final mechanical stage (and the beginning of nervous transduction) of hearing.

Semicircular Canals

The semicircular canal is filled with endolymphatic fluid and blocked at one end by the cupula, a deformable gelatinous membrane (see Hudspeth, 1983, p 60). The cupula sits within a bulged area of the canal, known as the ampula, and sits on a base, known as the crista, whose surface is covered with hair cells.

The semicircular canals sense angular motion. When the skull rotates, the fluid in the canals lags behind due to inertia. The relative fluid flow (compared to the surrounding tissue) causes the cupula to deform. The cupular deflection is precisely measured by thousands of hair cells in the crista (see Lewis, 1985, p. 38).

There are three semicircular canals in each ear of most vertebrates which are laid out in approximately orthogonal planes. An individual canal senses rotations perpendicular to its radial plane. Thus, by combining the output of all three canals, an animal can sense any arbitrary head rotation in three-dimensional space.

Otolith Organs

Each otolith organ consists of a cavity in the bony labyrinth (see Friedman and Ballantine, 1984). Within this cavity lies a flat mass of small dense bodies called *otoconia* or *otoliths* (Latin: ear stones). These are held together by an extracellular matrix of polysaccharides, making the *otoconial layer* (figure 1-2 a). This dense, mass is connected to the wall of the labyrinth by a viscoelastic gel, called the *gel layer*. The labyrinth wall where the gel layer attaches contains thousands of hair cells. The entire cavity is filled with the viscous endolymph fluid.

When the skull undergoes linear motion (figure 1-2 b), the dense otoconial mass lags behind due to inertia. This causes the gel layer to shear, which is sensed by the hair cells beneath. Furthermore, under the influence of gravity (figure 1-2c), the denser otoconial layer will deflect towards the earth, causing shear.

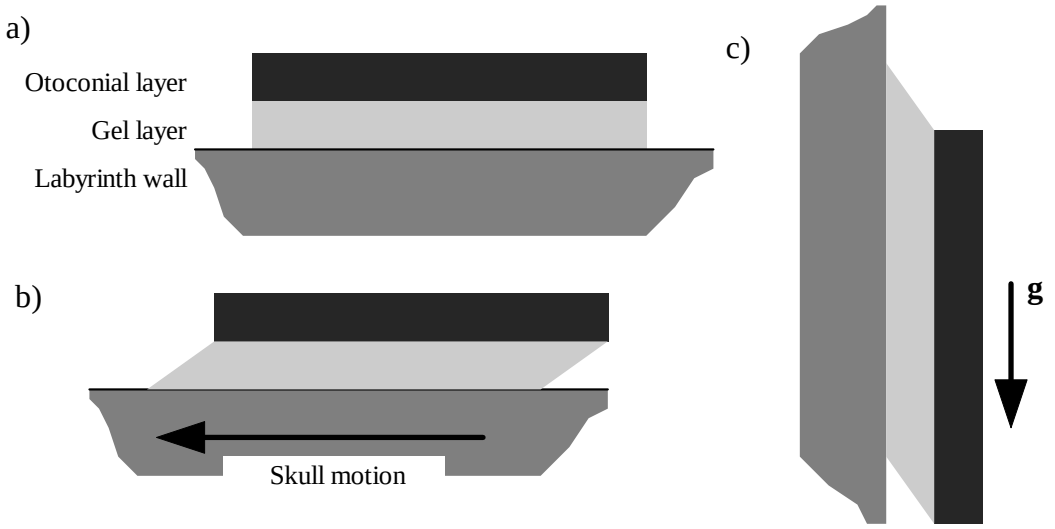

Figure 1-2. Schematic of an otolith organ showing the dense otoconial layer attached to the wall of the vestibular labyrinth (and ultimately the skull) by a deformable gel layer. In b) the skull undergoes motion which, due to inertia, causes relative motion of the otoconial layer, resulting in shearing of the gel layer. In c) the acceleration due to gravity results in a body force on the otoconial layer, deflecting it downwards.

There are two otolith organs in each ear, oriented orthogonal to each other. Each can sense two-dimensional motion in the plane of its otoconial layer. The saccule senses motion in a nearly vertical plane while the utricle senses motion in a nearly horizontal plane. By combining the results of these two organs, linear motion and accelerations can be detected in any arbitrary direction in three dimensional space.

Hair Cells

Hair cells are the final mechanical, and the first cellular, link in the vestibular system. They transform a force into an electrochemical signal which is received by a postsynaptic neuron.

Hair cells (see Freidmann and Ballantyne, 1984) consist of a *cell body* and a *ciliary bundle* or *hair bundle*, which extends out of the cell's apical (top) surface into a space in either the gel layer or the cupula. The ciliary bundle consists of many interconnected *stereocilia* and no more than one *kinocilium*.

There may be anywhere from ten to more than one hundred stereocilia in a bundle. The cilia are hexagonally packed such that each cilium has six neighbors an equal distance apart. Each stereocilium is composed of thousands of longitudinally oriented actin fibers covered by a thin membrane [Tilney, *et. al.* 1983]. As each stereocilium joins the apical surface of the hair cell, its diameter narrows and the number of actin fibers decreases drastically. This gives the stereocilium the appearance of a sharpened pencil, standing on its point. The stereocilium joins the cell body, deeply extending ten to twenty actin fibers into a dense, amorphous mass, called the cuticular plate, lying just beneath the surface.

In the vestibular hair cells of mammals, there is a kinocilium which is usually thicker and taller than the stereocilia. The internal structure of the kinocilium is not like the stereocilia. It has a small number of microtubules that extend up its length. In almost all hair cells, the top of the kinocilium is attached to either the gel layer or the cupula.

Stereocilia decrease in height as their distance from the kinocilium increases. They also exhibit a somewhat, if not perfect, bilateral symmetry of the cell bundle (dotted line in figure 1-3a). Columns of cilia (figure 1-3b) are defined as the cilia which lie on an imaginary line parallel to this line of symmetry. Similarly, rows (figure 1-3c) are defined as cilia which lie on a line perpendicular to the line of symmetry of the bundle.

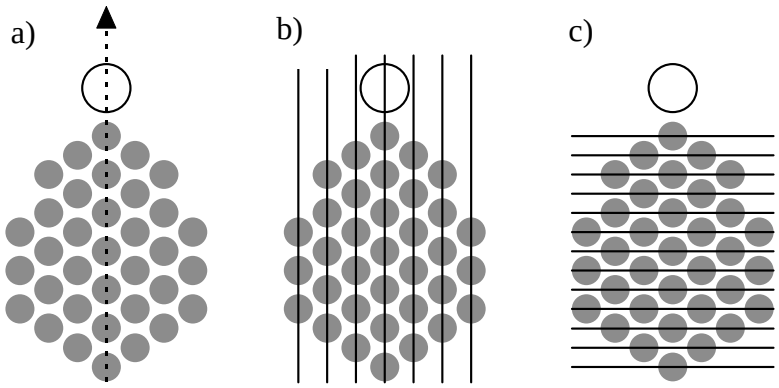

Figure 1-3. Top views of hair bundles showing ciliary distribution. The kinocilium is represented by the white circle, while stereocilia are represented by the gray circles. In a) the excitatory direction is illustrated by the arrow, while the dotted line along the line of bilateral symmetry is the E-I axis. In b) the lines represent the definition of columns and c) shows the definition of rows.

Each stereocilium has a single small fiber connecting it to its next tallest neighbor, which is called a tip link. Tip links emanate from the top of the stereocilium, rising vertically towards the side of their tallest neighbor (see Goodyear and Richardson, 1994). The stereocilia are also interconnected by various types of fine fibers called (among other things) side links, lateral links, and/or subapical bands. Side links are found along the length of the stereocilium, run parallel to the apical surface, and connect the stereocilium to each of its neighbors. Observations of the distribution and density of these links vary depending on species, organ, location, and experimental technique [Goodyear and Richardson, 1994; Nagel, et al, 1991]. The nature and composition of these links are, as yet, undetermined.

Hair cell morphologies vary greatly from species to species, organ to organ, and even location within the same organ. A very small subset of observed hair cell shapes and sizes are presented in Lewis, *et al*, (1985). The variations include number of cilia, overall ciliary height, height drop-off, ciliary diameter, interciliary spacing, length of columns, number of rows. It also includes the density and distribution of the interciliary side links.

Hair Cell Physiology

The hair cell maintains a negative electrical potential compared to the endolymphatic fluid which surrounds the bundle. When the bundle is deflected towards the kinocilium, ionic channels open on the upper regions of the stereocilia. The open channels carry positive ions into the hair cell causing a drop in its potential. The drop in electrical potential within the body of the cell leads to a release of a chemical neurotransmitter into the synaptic space between the hair cell and adjacent neuron.

A prevalent theory supposes that the tip links are mechanically linked to the ionic channels. The increase in tip link tension caused by the bundle being deflected leads to the opening of the channels.

Saturation

An important hair cell feature is saturation (see Hudspeth and Corey, 1977). The cell's response over a range of deflections is not linear. Very small deflections may cause little drop in potential. The cell is very sensitive to medium size deflections. Finally, above a certain deflection, the cell's response is constant. The exact definition of deflections as "small", "medium", and "large" depends on the individual cell bundle. However, in general, saturation occurs with deflections at least an order of magnitude smaller than the overall bundle height. Saturation, along with the large population of ciliary bundles, is essential in filtering the input signal. It results in each individual hair cell being sensitive to a narrow range of mechanical input.

Directional Sensitivity

Another important aspect of hair cells is their directional sensitivity (see Fernandez and Goldberg, 1976). The hair cell response is strongly dependent on the direction of deflection. The strongest response is found along the axis of symmetry with deflection moving the kinocilium away from the rest of the bundle. This is called the excitatory direction (figure 1-3a). A negative response (inhibition) is seen if the hair cell is forced along the line of symmetry with the kinocilium being forced towards the bundle. Hence the line of bilateral symmetry is called the excitatory-inhibitory axis, or E-I axis.

Observed Stereociliary Response

Observations of several researchers describe the hair cell bundle as bending as a single unit. The cilia are tightly bound together and do not splay. Other observations describe the cilia as pivoting about their base when deformed rather than bending along their length.

Tilney points out a mechanically relevant feature of stereocilia [Tilney, et. al. 1983]. Micrographs of deformed stereocilia show a near linear deflection profile over a long distance. Furthermore, plane sections of the stereocilia, as visualized by crosslinks between the actin

fibers, do not remain perpendicular to the long axis of the cilia. Rather, they remain nearly parallel to the apical surface. This indicates the stereocilia undergo significant deformation due to shear.

Classical beam theory assumes that long, thin structures (such as stereocilia) experience negligible shear, deforming due to bending. This seeming contradiction is resolved by the realization that stereocilia are not isotropic, but merely radially isotropic (transversely anisotropic). When deformed, the individual actin fibers are resistant to the axial strain needed for bending, but are compliant to sliding past each other resulting in shear.

Mechanical Tests of bundles

A number of researchers have tested the stiffness (resistance to deflection) of hair cell bundles. Such tests involved deflecting a bundle with a compliant glass whisker or a forced jet of fluid. The applied force was deduced and divided by the deflection of the tip of the tallest stereocilium or kinocilium to determine bundle stiffness. Szymko [1992] presented as a summary the table below.

Investigator	Stiffness ($\times 10^{-4}$ N/m)	Hair Cell Organ
Ashmore (1984)	1.32	frog sacculus
Flock and Strelioff (1984)	7.8 to 34.7	guinea pig cochlea
Strelioff and Flock (1984)	1 to 97.2	guinea pig cochlea
Crawford and Fettiplace (1985)	6	turtle cochlea
Howard and Ashmore (1986)	2.56	frog sacculus
Howard and Hudspeth (1987)	6.3	bullfrog sacculus
Denk, Webb, and Hudspeth (1989)	3.41	frog sacculus
Russell, Richardson, and Kossl (1989)	16 to 35	mouse cochlea
Szymko, Dimitri, and Saunders (1992)	5.04	chick cochlea

Over a variety of animals and organs, bundle stiffnesses vary from 10^{-2} to 10^{-4} N/m. These studies have concentrated on stiffer hair cells, as more compliant ones have been extremely difficult to measure experimentally. Notably absent are hair cells from the semicircular canal. These cells tend to be taller than saccular and cochlear hair cells, and consequently, less stiff. We expect biologically realistic hair cell bundle stiffness to range from 10^{-2} to 10^{-5} N/m, or even lower for tall hair cells.

Unfortunately, the above studies included only cursory descriptions of bundle structure. So much data is missing, that an attempt to model the same hair cell bundles as above would yield inconclusive results.

Motivation for modeling

It is reasonably assumed that the stiffness of a hair cell bundle correlates to its range of sensitivity. A map of stiffness values across an epithelial surface will reveal much about the organ, similar to the map of directional sensitivities (see Kelly, *1991*, p. 508). However, there

are thousands to tens of thousands of hair cell bundles per organ, each with its own morphology–and this says nothing of variation across species. In contrast, stiffness estimates are available for only a small number of hair cell bundles.

Presently, experimental stiffness measurements are the accepted technique for generating stiffness values. Unfortunately, due to limits of light microscopy and stiffness measurement techniques, these experimental methods are unable to test stiffnesses below 10^{-4} N/m, excluding large populations of bundles. These methods are also unable to accurately measure individual bundles in areas where hair cell bundles densely populate the epithelial surface. Some researchers express doubt whether their glass fiber is forcing 1, 2, or even 5 hair cell bundles. Other limitations make stiffness measurements inaccurate, not to mention expensive and time consuming. The modeling techniques presented here will allow estimation of bundle stiffnesses from structural data alone. While gathering such data is difficult itself, it represents a less expensive, faster, and more universal technique.

We have other questions dealing with the general hair cell structure. Because of their small size (on the order of 10^{-7} to 10^{-9} meters) stereocilia interconnections have been difficult to characterize. By mechanical modeling, certain structural combinations will be rejected as incompatible with the observed hair cell deflections and/or response. Identifying the mechanical characteristics will help in the deduction of the link material, which is still unknown.

Modeling may also allow us to gain insight into the transduction mechanism. This mechanism is still debated by well regarded researchers. While the tip link transduction paradigm presented earlier is prevalent, a few researchers [Hackney, et. al. 1997] believe the sliding of stereocilia past each other is a possible the mechanism of transduction.

Previous Models

As biologic data has been obtained on actual bundle stiffnesses, several researchers have proposed models for predicting the stiffness. Howard [Howard, et. al. 1988] estimated stereocilium stiffness based upon bending in the tapered section (which was modeled as having a small constant diameter) and assuming the remaining ciliary shaft to be rigid. Bundle stiffness was then calculated by multiplying this single ciliary stiffness by the number of cilia in the bundle.

Pickles [1993] created a lumped parameter model. It assumed the cilia were rigid shafts, connected to the cuticular plate by rotational springs. The effect of all lateral links were combined into a single linear spring at the tip of the cilia. Tip links were also modeled as linear springs. Single columns of cilia were modeled. Many parametric combinations were eliminated based upon biological observations. The relative stiffnesses of a cilium to tip link to lateral link were concluded to be 1:100:400.

An earlier finite element analysis was performed marking the first distributed parameter modeling [Duncan, 1993. Duncan and Grant, 1997]. The study was limited to a single column

of up to 4 stereocilia. Tests were performed to determine feasible values of several parameters, and the influence of several structures.

A major discovery of this work was the realization that an isotropic stereocilia results in a mechanical paradox. For a ciliary Young's modulus that gave biologically realistic bundle stiffness values, the bundle would buckle in a most unbiologic way. To obtain biologically shaped deformations, the bundle assembly was well below any observed values for bundle stiffness. This further supports the observations of shear deformation described earlier.

There were two other important conclusions from Duncan's work that will be used in this dissertation. The first is that for reasonable values, cuticular plate stiffness has negligible effect on bundle mechanics. This will further justify modeling all of the cilia as rigidly attached at their base. The second was that the presence of lateral links below the top portion of the stereocilia has little effect. This effect will be studied in more detail later.