Summary

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The measurements in this thesis are all related to the mechanosensory hair cell, which can be found in several sensory systems sensitive to mechanical stimuli. The sensory hair cell owes its name to the tuft of stereocilia on its apical side. These actin-filled pillar-shaped stereocilia are nicely stacked in rows of increasing height, and when deflected they pivot around their base. The tips of these stereocilia contain interconnections, the so-called tip links, which are assumed to be connected to the mechano-transduction channel. Deflection of the bundle in the direction of the tallest stereocilium causes the tip links to be tensioned, which directly increases of the open probability of the transducer channel allowing the selective entry of cations. A deflection in the opposite direction leads to a decrease in the tension and closure of the channels. The mechanically-gated current induces membrane depolarisation, triggering voltage-gated calcium channels to open in the basolateral membrane. An influx of calcium subsequently induces neurotransmitter release, transmitting the signal to the innervating neurons, which convey and further process the information in the central nervous system. The entering calcium also increases the conductance via calcium-dependent potassium channels causing a repolarisation of the membrane due to the efflux of potassium.

This above description of the sensory hair cell is the minimal representation of the type of sensory hair cell that is found in several sensory systems. First of all, we find them in ears, where they enable perception of sounds. Secondarily, we find them in balance systems, where they are used for the detection of linear and circular acceleration. Thirdly they are present in the lateral line system of fish and amphibians enabling them to detect nearby water motion (velocity and acceleration). Although these sensory systems are sensitive to different types of motion, the relevant stimulus to the hair cell is the deflection of its bundle. The actual type of motion (displacement, velocity, acceleration) as well as the frequency range to which the sensory system is sensitive, is for a large part determined by the mechanics of the structure into which the hair cells are incorporated.

This thesis describes studies on these structures but also on individual hair cells. These measurements on mechanodetectors are experimentally demanding for several reasons. Firstly, mechano-detectors are often difficult to reach. The hair cells of the ear for instance are enclosed in a bony structure, called the cochlea, placed in four rows within the basilar membrane, a piece of tissue with a thickness of only a few cell layers. Isolating the hair cells without damaging them takes some

experience. The lateral line system, however, provides easier access to its sensory units, called neuromasts. These are located at the surface of the fish or just underneath, in small canals. This even allows for *in vivo* measurements on hair cells. Secondly, the sensory hair cells are small and sensitive to displacements orders of magnitude smaller than its dimensions. A microscope is needed to visualise the hair cells. The displacements, however, are so small that they are not even visible with a microscope. Therefore, the microscope was modified into a laser interferometer set-up. Based on the Doppler shift occurring when the laser light interacts with the moving object, the displacement can be obtained with nanometer accuracy (nm = 10^{-9} meters).

Besides the bundle displacement, the electrical response needs to be measured. The currents measured are in the order of tens to hundreds of pico-amperes (pA = 10^{-12} amperes) and the potential differences in the order of microvolts (μV = 10^{-6} volts). The emphasis being put on the units stresses the fact that very small signals had to be dealt with. The same goes for the mechanical stimuli that have to be applied. The stimulus device used to mechanically stimulate several mechanosensory systems is therefore described and studied in detail, as presented in **Chapter 2**.

The stimulus device consists of a fluid filled chamber with a rear side that can be displaced using piezoelectric material, producing a jet of fluid when activated. The chamber is connected to a glass capillary with a tip narrowed down to several (tens) of micrometers. A displacement of the rear side of the chamber produces a pressure difference causing fluid displacement at the tip of the capillary. This way, a microscopically small targeted fluid stimulus is obtained.

To access the stimulus characteristics as a function of time and frequency, a flexible probe was used. Sinusoidal displacements of the probe (frequencies ranging from 1 to 1000 Hz), induced by the stimulus device, were accurately detected using the laser interferometer. This way, information was obtained on the waveform of the water motion, as well as the water displacement and phase as a function of frequency (the frequency response).

The measurements show that the size of the tip greatly influences the characteristics of the frequency response. A large tip gives rise to a constant water-displacement amplitude as a function of frequency of the voltages applied to the piezoelectric material over a limited range of frequencies (< 100 Hz). Narrowing the tip diameter to only a few micrometers changes the output characteristics into a velocity stimulus (a constant velocity amplitude as a function of frequency) within

the measured complete frequency range.

The probes, together with a described calibration method, can also be used to correct the measurements on mechanodetection systems for frequency-dependent characteristics of the fluid motion.

In **Chapter 3,** the fluid stimulus is used to study the cupular hydrodynamics in the neuromasts of the superficial lateral-line system in early stage zebrafish (*Danio rerio*). These neuromasts consist of about 10 hair cells, each with a hair bundle and a long kinocilium, covered by a pillar-shaped cupula, that extrudes in the surrounding water. Because the height of the cupula is limited to several tens of micrometers, it stays fully within the boundary layer of the fish's skin for the frequencies it is most sensitive to (several tens of Hz). The frequency response of the cupula of these superficial neuromasts is not only determined by the mechanical coupling of the cupula to the underlying tissue, but also by the frequency-dependent attenuation of the fluid motion, as a result of this boundary layer. Measured at tens of micrometers above the epithelium, the cupular motion as a function of fluid velocity is nearly constant at low frequencies (< 20 Hz), with a sensitivity ranging from 1-5 nanometers of cupular displacement per micrometer/second fluid velocity. Measurements at different heights show that the cupula bends during stimulation, such that the base region of the cupula driving the sensory hair bundles is estimated to possess only a fraction (20-30%) of this mechanosensitivity. Up to about 70 Hz the displacement as a function of stimulus frequency of the driving fluid velocity gradually decreases with a slope of approximately 10 dB/dec. At higher frequencies cupular displacement declines at 20 dB/dec.

A previous model, describing the cupular mechanics of canal neuromasts, could not adequately describe the cupular behaviour found in superficial neuromasts. Extending that model with a frequency-dependent boundary layer, however, improves the description considerably. The results suggest that the stiffness coupling to the hair cells of the sensory epithelium is governed by the compliance of the cupula.

Based of the measured cupular mechanics, a comparison was made between the superficial neuromasts of the zebrafish and the canal neuromasts from the ruffe (*Gymnocephalus cernuus*). As a result of their low stiffness and their small number of hair cells, the signal-to-noise ratio of the zebrafish superficial neuromasts was found to be inferior throughout the complete frequency range.

Chapter 4 focuses on the sensory hair cells from the mammalian ear, more

specifically, the mouse. The hair cells are enclosed in a coiled structure called the cochlea. Enclosed is the basilar membrane, which contains three rows of outer hair cells and one row of inner hair cells. Already at birth, the hair cells are present and equipped with a hair bundle and mechano-electrical transducer channels. This chapter, however, focuses on the kinetics of the potassium current, $I_{,K,neo}$, which is the major current in the basolateral membrane during the first week after birth. $I_{,K,neo}$ is characterised by a slow voltage-dependent activation, similar to the *delayed rectifier* type of potassium channel. The current is activated by membrane potentials higher than -60 mV, and the underlying channels determine the resting membrane potential in these cells.

To study the kinetics of $I_{K,neo}$ in more detail, acute cochlear preparations were used to isolate the basilar membrane. Using a suction pipette, neighbouring cells and debris were removed from the basolateral membrane of individual outer hair cells. Subsequently, a glass electrode was placed on the clean membrane, which was used to clamp the membrane potential of the individual hair cell at a desired value. The same electrode was used to record the total current flowing over the membrane as a result of the membrane potential changes applied.

A depolarising step in membrane potential elicits an activation of the current, followed by an inactivation (decrease in conductance, despite a continued depolarisation). The inactivation is characterised by a fast and a slow component and a non-inactivating part. The voltage-dependent extent of inactivation displays two components as well. Although the voltage-dependence of activation is not characterised by two clear components, the current activation cannot accurately be described based on a model incorporating only one channel. The data therefore suggest that $I_{K,neo}$ is brought about by two potassium channels, with comparable voltage-dependence of activation, but different activation kinetics and inactivation characteristics.

In **chapter 5,** the first step is taken to define the accuracy with which the transducer channel processes incoming signals, by determining the signal-to-noise ratio of the current through the transducer channel. The signal was defined as the change in current in response to a small displacement at a fixed hair bundle displacement *X*. The intrinsic stochastic fluctuations of the transducer channel were assumed to be the primary source of noise, since the channel is continuously fluctuating between open and closed conformations as a result of thermal noise ..

A dynamic stimulus protocol, consisting of a low frequency but large amplitude sinusoidal bundle displacement (dynamic *X* displacement) and a high-

frequency small-amplitude displacement (locally probing the sensitivity), was used to define the signal as a function of hair bundle displacement. To assess the channel noise, a static protocol was used in which the noise was measured over a 50 ms period, for several static displacements of the bundle. To describe the signal, as well as the noise, as a function of hair bundle position, a recently proposed model was used which assumes the gating spring to differentially engage the different conformational states of the channel. The state energies of the different conformations fully determine the open probability of the channel. Specifically, in this model, the gating spring is assumed to only contribute to the energetic state of the channel-gating spring complex when the gating spring is engaged. At negative hair bundle displacements (< -50 nm), where the gating spring is fully disengaged, the state energy of the different conformations does not further change, resulting in a finite minimal open probability. As a result, the channel noise does not go to zero, but to a fixed value, which was confirmed by experiment. Because changes in hair bundle position around these negative displacements do not produce any signal, the signal-to-noise ratio reduces to zero.

At the bundle's resting position the gating spring is slightly tensioned, causing a so-called *silent current* of approximately 10-15% of the maximal current. At this resting position a 5.3 nm bundle displacement is needed to equal the noise. The signal-to-noise ratio is maximal at a bundle displacement of about 40 nm, where the open probability is 0.5. A 2.7 nm displacement (a deflection of just ~0.03°) is needed to get a signal-to-noise ratio of one. Given that the maximal response is reached at a displacement of 150 nm, the dynamic range of the transducer apparatus is about 30 dB.

In **Chapter 6** the lower bound on signal transduction by the transducer channel is determined in more detail. The transduction apparatus (the system of the gating spring and the transducer channel) is being regarded as the estimator of hair bundle displacement containing the information on incoming sounds. Based on the transducer current, the hair bundle displacement will be estimated with a variance σ^2. In this chapter the lower bound of this variance, $\sigma^2_{\min}$, is determined. The optimal accuracy with which a single transducer channel, based on its transducer current, can estimate the hair bundle position is 47 nm and this is obtained at a hair bundle position where the open probability of the channel is 0.5. The accuracy of estimation scales down in proportion to the square root of the total number of channels (on average 80 per outer hair cell), leading to an accuracy of about 5.3 nm per cell. So far we have only taken into account the intrinsic stochastic noise of the

transducer channel. However, noise attacks the mechano-transducer system on multiple fronts. Also, the gating spring is susceptible to Brownian noise fluctuations, which are transduced by the transducer channel and which independently add to the total mechano-transducer noise. The gating spring noise adds an additional 24 nm to the maximal accuracy per transducer channel leading to a total accuracy of 6.5 nm per cell.

Theoretically, it is shown that the molecular gating force determines the accuracy of the mechano-transducer process. Increasing the gating force would therefore allow the transducer process to improve its accuracy. However, increasing the gating force will be at the expense of the effective operational range. Based on data from the literature, most hair cells show a channel noise that closely matches the noise of their gating springs. Reducing the channel noise considerably would, then, not pay off since the gating spring noise would continue to dominate while the operational range would, unfavourably, decrease.

Estimations of the hair bundle displacements at the threshold of hearing vary from a few nanometers to fractions of nanometers. That the ear is capable of detecting these displacements, which are smaller than the accuracy of an individual hair cell, can be understood by the fact that the ear has more than one hair cell, allowing for ensemble averaging across several hair cells. Moreover, in order to reach the observed detection thresholds, the effective bandwidth could be limited using passive electrical and mechanical filtering, as well as active electro-mechanical feedback.