Mechano- and electrophysiological studies on cochlear hair cells and lateral line cupulae

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Chapter *1*

General introduction

Mechano-detection

Mechanosensory systems enable an organism to perceive mechanical signals from its environment. These systems are optimised for the specific physical properties of the medium surrounding the organism, as well as to the frequency and amplitude ranges of the type of mechanical signal relevant to the organism.

The basis of these mechanosensory systems is formed by a highly specialised sensory cell, the mechanosensory hair cell. It serves as the primary mechano-electrical transducer in several mechanosensory systems found throughout the vertebrate kingdom, like the ear (hearing), the vestibular system (linear and angular acceleration detection) and the fish lateral line system (detection of water motion). The mechanical signals detected by these sensory cells are converted into a graded receptor potential, which controls the release of neurotransmitter substance at the neural innervated sites on the basolateral membrane of the hair cell. From here the information about the mechanical signal is coded in action potentials and conveyed to processing areas in the central nervous system.

The mechanosensory hair cell

The mechanosensory hair cell is an epithelial cell, derived from the embryonic ectoderm, and is morphologically characterised by the hair bundle that extrudes from its apical side. The bundle consists of actin-filled (Flock and Cheung, 1977; Tilney *et al.*, 1980) villi, called stereocilia, and are graded in height giving the hair bundle a staircase-like appearance. At the side of the tallest stereocilia resides a true cilium, called the kinocilium. If the bundle is mechanically displaced, it moves as a whole due to numerous interconnections between the stereocilia (Pickles *et al.*, 1984; Neugebauer and Thurm, 1985). The tapering of the stereocilia's stiff actin core near the insertion in the apical plate causes the stereocilia to pivot around their base, giving rise to a relative shearing motion between neighbouring stereocilia. One type of stereociliar interconnection, called the tip link (Pickles *et al.*, 1984), extends from the tip of a stereocilium to its neighbouring higher stereocilium and is thought to be stretched by this shearing motion. This tip link is assumed to be connected to a mechanically-gated channel (Howard and Hudspeth, 1988). Displacement of the bundle towards the highest stereocilia, the excitatory direction (Fig. 1), therefore favours the open conformation of the transducer channels whereas a displacement in the opposite direction favours the closure of the channels (Corey and Hudspeth, 1983). The influx of cations caused by the opening of the transducer channels leads to a membrane depolarisation, which subsequently

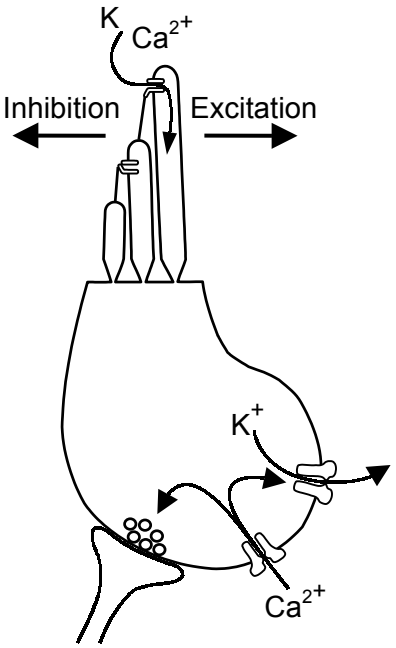

Figure 1: A schematic representation of a mechanosensory hair cell. A hair bundle represented by three stereocilia protrudes from the apical side of the cell body. Deflection of the bundle in the excitatory direction stretches the tip link connections between the tips of neighbouring stereocilia, favouring the open state of the transducer channel and allowing the influx of cations. The subsequent membrane depolarisation leads to opening of voltage-gated calcium channels in the basolateral membrane. The influx of Ca^{2+} triggers the fusion of vesicles causing the release of neurotransmitter substance at the site of neural innervation by an afferent nerve. The Ca^{2+} also facilitates the opening of Ca^{2+}-dependent potassium channels causing repolarisation of the membrane potential.

opens voltage-gated Ca^{2+} channels at the basolateral side of the hair cell. The result of the basolateral Ca^{2+} influx is twofold. On the one hand the Ca^{2+} causes fusion of vesicles with the membrane, thereby releasing neurotransmitter leading to an excitatory post-synaptic response. On the other hand it triggers the opening of Ca^{2+}-dependent K^+ channels producing an outward K^+ current, which repolarises the cell membrane.

The mechano-electrical transducer channel

Up to now the protein sequence of the transducer channel has not been elucidated. One of the difficulties is its limited expression level of only several tens of channels per hair cell and the limited time window during which it is expressed. A second complication is that the channel operates in a molecular complex consisting of the channel and a gating spring and several more components necessary for anchoring these elements, making it difficult to express and functionally characterise it in a heterologous expression system. Thus far several gene alleles have been identified to be related to mechanotransduction in humans, mice and zebrafish (Nicolson *et al.*, 1998; Sidi *et al.*, 2003; Steel and Kros, 2001; Gillespie and Walker, 2001) and also non-vertebrates such as *Caenorhabditis elegans* (Ernstrom and Chalfie, 2002; Hill and Gillespie, 2003) and *Drosophila melanogaster* (Walker, 1967; Kim *et al.*, 2003). Just recently the TRPA1 channel

has been shown to be a likely candidate for the mechanosensative transducer channel (Corey, 2004).

Although the amino acid sequence of the transducer channel is not known, functional aspects of the transducer channel have been studied in detail in acutely prepared hair cells and mechanosensory structures (Jaramillo and Hudspeth, 1991; Denk *et al.*, 1995). The extremely fast activation of the mechano-electrical transducer channel, with an experimentally determined activation time constant in the order of less than tens of microseconds, suggests that the channels are instantaneously opened via an elastic coupling to the gate of the mechanical forces resulting from the hair bundle deflection (Corey and Hudspeth, 1979). The location of the transducer channel in the tips of the stereocilia (Hudspeth, 1982) makes the tip link (Pickles *et al.*, 1984) the most likely candidate to transfer the bundle motion to the transducer channel. When opened the transducer channel is permeant to cations with a high permeability for Ca^{2+} (Jorgensen and Kroese, 1995; Lumpkin *et al.*, 1997). Even at the low concentration of extracellular Ca^{2+} found in the cochlea (Bosher and Warren, 1978) a substantial part of the current is carried by Ca^{2+} ions. Besides its role in the depolarisation of the membrane, Ca^{2+} plays an important role in the adaptation of transducer current (Eatock *et al.*, 1987; Assad *et al.*, 1989), a process which continuously resets the operational point of the transducer channel. Experimentally, adaptation is often shown as a decrease in the transducer conductance during a static hair bundle deflection (see Fig. 2). The time course of adaptation has been shown to be altered by changing the extracellular Ca^{2+} concentration or the intracellular application of Ca^{2+} chelators (Ricci *et al.*, 1998). Also keeping the membrane potential positive to the reversal potential of the transducer current, effectively causing an efflux of calcium instead of an influx, abolishes adaptation (Crawford *et al.*, 1989), suggesting an intracellular site of action. Two underlying mechanisms driving the transducer current adaptation are thought to co-exist in the hair cell (Wu *et al.*, 1999; Holt and Corey, 2000). Firstly a fast adaptation process, with a time constant in the order of 1 ms, which is related to a decrease in the open probability of the transducer channel upon binding of Ca^{2+} (Kennedy *et al.*, 2003). Secondly, a myosin adaptation motor running along the stereocilia actin core is thought to tension the gating spring (Howard and Hudspeth, 1987; Yamoah and Gillespie, 1996; Hacohen *et al.*, 1989; Assad and Corey, 1992). This process is slower with a time constant in the order of tens of ms. Recently the most likely candidate for this adaptation motor has been shown to be Myosin-IC (Holt *et al.*, 2002), but other myosins, like Myosin-VIIA (Kros *et al.*, 2002) might

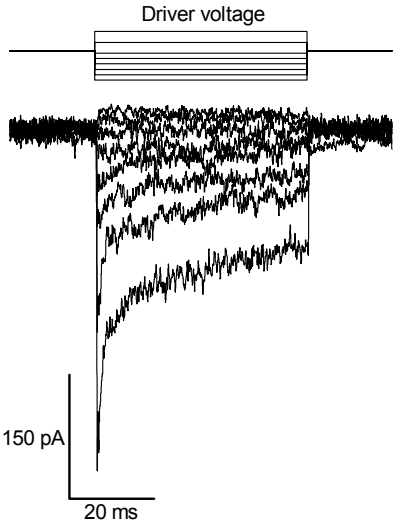

Figure 2: Transducer current of a neonatal mouse outer hair cell. Transducer current responses elicited by step-like displacements of the hair bundle brought about by a fluid jet stimulus. The driver voltage applied to the fluid jet-producing device is shown on top of the traces with upward steps corresponding with a deflection of the hair bundle in the negative direction. The deflections in the negative direction close the channels that are open in the bundles resting position. Deflections in the positive direction induce a fast activation (time to peak ~ 0.3 ms) of the transducer current followed by partial adaptation. The adaptation shows a fast component with a decay time constant of 1.4 ms and a slow component with a time constant of 30 ms.

play a role in pre-tensioning the transducer channel gating spring complex.

Additional evidence for a direct mechanical coupling between bundle displacement and opening of the transducer channel is the existence of gating compliance. When the bundle is deflected in the excitatory direction, the opening of transducer channels causes a decrease in the tension of the actuating gating springs. In hair cells in which the stiffness of the gating springs determine a considerable part of the total hair bundle stiffness, like in the hair bundles of the sacculus (Howard and Hudspeth, 1988) or the fish lateral line (van Netten, 1997), the gating compliance causes a detectable decrease in the total bundle stiffness. In bundles with a high passive stiffness, like for instance outer hair cells, it is less apparent (van Netten and Kros, 2000). The gating compliance obviously is maximal when half the channels is open, and in extreme cases might cause the total hair bundle stiffness to become negative (Martin *et al.*, 2000).

A second non-linearity in the hair bundle mechanics, related to the channel gating-spring complex, occurs when it is pushed in the inhibitory direction. Tension in the gating spring reduces so that the probability for the channels to close increases. At some point the gating spring becomes slack, loosing its direct interaction with the channel just leaving the bundle with its passive stiffness. It has therefore been suggested that *gating string* is a more appropriate term for an element that becomes slack, since a spring can also exert compression forces on the channel (van Netten and Kros, 2000). Also recent structural analysis of the tip link

supports the idea of a string rather than a spring showing that it is either a helical polymer or a braided pair of filamentous macromolecules and thus likely to be inextensible (Kachar *et al.*, 2000) Deflections more negative than the engaging position of the gating spring do not alter the open probability of the channel. Unlike the membrane potential in voltage-gated ion channels, which always exerts a force on the channel's gating charge, the gating spring only exerts a force when engaged to the channel. In the case of a voltage-gated channel the open probability will thus become infinitely small with increasing hyperpolarisation due to the increasing force on the gating charge, whereas the transducer channel has a finite minimum open probability of about 1% (van Netten and Kros, 2000).

The mechanosensory lateral line system

Physical properties of water, as well as particles floating in the water, limit the penetration depth of light. Eyesight is therefore of limited use to aquatic animals. As an alternative sensory system, fish and amphibians make use of a highly specialised mechanoreceptive organ called the mechanosensory lateral line system. The function of the lateral line system as a mechanosensory system was first acknowledged by Jakobson in 1813 (Walker, 1967) who suspected it to be a system for touch. Hofer, in 1908, for the first time, demonstrated that the lateral line system detects local water motion (Dijkgraaf, 1963). Dijkgraaf termed this function "*Ferntastsinn*", which means as much as "touch at distance". The lateral line system is related to several functions, such as the detection of prey (Bleckmann, 1980; Janssen *et al.*, 1994; Dijkgraaf, 1963; Enger *et al.*, 1989; Montgomery and MacDonald, 1987) and stationary objects (Weissert and von Campenhausen, 1981; von Campenhausen *et al.*, 1981; Hassan *et al.*, 1992) as well as rheotaxis (orientaion with respect to water current; Montgomery *et al.*, 1997) and schooling (Pitcher *et al.*, 1976; Partridge and Pitcher, 1980; for review: Bleckmann, 1993).

The mechanosensory lateral line system consists of multiple small detection units, called neuromasts, which are distributed on the head and along the mid-body line, the latter giving name to the sensory system. Each neuromast consists of a sensory epithelium, the macula, surrounded by mantle cells and an overlying accessory structure, the cupula. The macula contains supporting cells and mechanosensory hair cells, which on their apical side contain several rows of stereocilia organised in a hexagonal array. Also in these hair cells the stereocilia are graded in height forming a staircase-like bundle with a long kinocilium at the side of the tallest stereocilium. The hair bundle and kinocilium penetrate the cupula (Flock, 1967) so that the hair bundles within a neuromast are mechanically

coupled. Displacements of the cupula are passed on to the hair bundle, leading to a change in open probability of the transducer channel.

Due to their superficial location, neuromasts can be used to study mechanophysiology *in vivo*. Making use of the mechanical coupling of hundreds to thousands of hair bundles by one cupula, non-linearities in hair bundle stiffness due to gating of the transducer channel, have previously been investigated in neuromasts form the ruffe (*Gymnocephalus cernuus*) (van Netten and Khanna, 1994; van Netten, 1997).

Two types of neuromasts exist. One type, the so-called canal neuromast (CN), resides in canals underneath the skin or in the scales. The other type has a cupula penetrating into the water surrounding the fish and is called superficial neuromast (SN). Neuromasts in both systems differ in morphology (Coombs *et al.*, 1987; Janssen *et al.*, 1987; Münz, 1989) and function. Cupulae of CNs are usually dome-shaped and mechanically couple large numbers (up to thousands) of hair cells. The hydrodynamics of these cupulae have been studied in detail, showing that the stiffness coupling of the cupula to the underlying epithelium can largely be attributed to the stiffness of the hair bundles (van Netten, 1991). The cupular-displacement frequency response of a canal neuromast is flat as a function of the frequency of the fluid velocity over a limited range of frequencies in which the cupula is driven by viscous forces. At higher frequencies inertial forces take over and the displacement decreases at 20 dB/dec as a function of frequency of fluid velocity. The mechanical cut-off frequency of CNs is in the range of one hundred to a few hundred Hertz depending on the species (Wiersinga-Post, 1997). SNs usually have more pillar-shaped cupulae with smaller amounts of hair cells and detect frequencies in the range of 10-70 Hz (Kroese *et al.*, 1980; Coombs and Montgomery, 1994; Kroese and Schellart, 1992). In SNs most of the cupular response characteristics have been obtained via electrophysiological measurements. Direct measurements of the cupular displacement response need to be done to get more insight in the relevant mechanical input for this mechanosenory system.

Outline of the thesis

Chapter 2 addresses the development of techniques and methods that are used to mechanically stimulate mechanosensory structures with high precision. It describes a fluid jet-producing device consisting of a pressurised fluid-filled container combined with a glass pipette having a microscopically sized tip acting as an orifice. Using a simple mechanical model describing experimental data the key

elements of the device are explored showing that the tip resistance is the prime parameter influencing the output response. In addition a calibration method is presented, which allows for the determination of the fluid jet's frequency dependent characteristics, which can subsequently be used to correct the measured frequency responses of mechanosensory structures.

In **Chapter 3** the stimulus device and correction procedure described in chapter 2 are used to obtain high-resolution measurements of the hydrodynamic behaviour of superficial neuromast cupulae in zebrafish (*Danio rerio*) larvae. The fluid jet stimulus is used to vibrate the cupula within a range of frequencies while a laser interferometer is used to measure the resulting displacements with sub-micrometer accuracy. This way frequency responses of the cupula have been obtained. A model previously used to describe the hydrodynamic behaviour of canal neuromasts, fails to provide an accurate description of the superficial neuromast frequency responses. Because of the limited height (~45 μm) with which these cupulae protrude in the surrounding water, they are mainly located within the boundary layer of the skin under the stimulus conditions used. The existing cupula model was therefore extended with frequency-dependent boundary layer properties and compared to the measured data. Further, the detection properties of the superficial neuromasts including the boundary layer effects are compared to canal neuromasts in terms of sensitivity and signal-to-noise ratio.

Chapter 4 focuses on the main outward potassium current found in the basolateral membrane of outer hair cells in neonatal mice. This current, called $I_{K,neo}$, is expressed in both inner and outer hair cells predominantly in the period before the onset of hearing. Detailed measurements on activation and inactivation properties of this current have been performed. The data obtained suggest that two channels underlie this current both with an approximately equal current density but markedly different inactivation kinetics.

In **Chapter 5** the signal processing performance of the mechanotransducer channel is investigated by estimating the signal-to-noise ratio of outer hair cell transducer channels.

In **Chapter 6** signal processing properties are taken a step further by applying information and estimation theory on measured characteristics of the transducer channel. Instead of determining the current response to a certain bundle deflection, we reversed the point of view and tried to define what the optimal accuracy is with which the bundle deflection can be estimated based on the transducer current through a single transducer channel. Besides the channel noise,

also the noise produced by the gating spring is taken into account. The optimal accuracy in estimating the hair bundle's position is found to be 47 nm per channel within the 5 kHz bandwidth over which was measured. A surprising finding, bearing in mind that at the threshold of hearing the estimated hair bundle displacement ranges from a fractions of a nanometer up to a few nanometer. Possible explanations as to how the hearing organ can reach its exquisite displacement sensitivity even though its individual detection instruments are poorer estimators are discussed.