Mechano- and electrophysiological studies on cochlear hair cells and lateral line cupulae
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Chapter 2

Design and calibration of a fluid jet-producing stimulus device
ABSTRACT
When studying the micromechanical behaviour of such structures such as cupulae in the lateral line system, or hair bundles of sensory hair cells, a precise definition of the linearity and time- and frequency-dependent properties of the stimulus device is indispensable. Here we determine the frequency-dependent characteristics of a fluid jet as produced by a pressurised fluid-filled container combined with a glass pipette having a microscopically sized tip acting as an orifice. At small orifice diameter (< 10 µm), the fluid flow velocity of the jet is proportional to the displacement of the piezoelectric actuator imposing changes in the container’s volume. At larger pipette diameters and low frequencies, the jet velocity becomes gradually proportional to the actuator’s velocity. In addition a practical procedure is described, that can be applied during physiological experiments to obtain a fluid jet’s frequency response produced by individually differing pipettes.
INTRODUCTION

Sensory hair cells are the primary mechanodetectors in several sensory organs in vertebrates, such as the hearing- and vestibular organ and the fish lateral-line system. The hair bundle is the mechanosensitive organelle, a well-organised arrangement of stereocilia on the apical side of the hair cell. In most receptor organs the hair cells are covered by an overlying tectorial structure bathed in a fluid, often mechanically coupling many hair bundles (van Netten, 1997). These tectorial structures convey the mechanical signals to the hair bundles. Deflections of the bundle of stereocilia, which pivot at their base, induce changes in open probability of the mechanically gated ion channels in the tips of the hair bundle (Hudspeth, 1989). The combined mechanics and hydrodynamics of the hair bundles and tectorial structure determine an organ’s sensitivity to a specific aspect of motion.

To study the mechanics of the tectorial structures and individual hair bundles as well as the mechano-electrical transduction process, a well-defined mechanical stimulus is required. Due to the dimensions of most tectorial structures, stimulation at a microscopic spatial resolution is needed, especially when stimulating an individual hair bundle. A commonly used mechanical stimulus for individual hair bundles consists of a piezoelectric actuator driving a fibre (Strelioff and Flock, 1984; Crawford and Fettiplace, 1985; Howard and Ashmore, 1986; Howard and Hudspeth, 1988; Russell et al., 1992). The fibre adheres to the cell membrane of the kinocilium or tallest stereociliar row, allowing for force application to the bundle in both directions. A stiff fibre enables direct displacement of the bundle, overruling the intrinsic bundle mechanics, and can be used to study transducer channel kinetics (Howard and Hudspeth, 1988; Jaramillo and Hudspeth, 1993; Kennedy et al., 2003). Alternatively a fibre with a stiffness comparable to the hair bundle can be used. Although force application becomes less effective, the fibre stiffness does not dominate the intrinsic mechanics of the hair bundle and allows for estimating the bundle's mechanical characteristics (Benser et al., 1996). Viscous forces do, however, attenuate the higher frequency stimuli, limiting the use of these compliant fibres to low frequencies (Howard and Hudspeth, 1987; Crawford and Fettiplace, 1985).

Since most mechano-detecting structures in receptor organs are fluid-driven, a fluid coupling between stimulator and mechano-detector is a good alternative to fibre stimulation. Well-defined fluid displacements can be obtained using a stimulus sphere attached to a piezoelectric element (van Netten, 1991). It generates
a water displacement output as a function of applied voltage across the piezoelectric material that is constant for a range of frequencies of several hundred hertz, depending on its construction. It does, however, stimulate very ineffectively at larger distances, falling in stimulus power with the third power of the distance to the stimulus sphere centre. To compensate for this effect, large stimulus spheres can be used. As a consequence of the increased sphere diameter, the stimuli are produced in a larger volume. To obtain a more targeted mechanical stimulus a fluid jet emerging from a glass pipette with a microscopic opening at its tip can provide an alternative solution. Narrow pipettes producing a constant fluid velocity may deflect the target in a step-like fashion, or with alternating velocity can generate a vibrational response.

In one of the first reports on a fluid jet in mechano-reception research (Jielof et al., 1952) it was applied to drive the cupula of a fish lateral line neuromast. The 4 mm pipette diameter used in that study was relatively wide compared to later, more miniaturised models (Flock and Orman, 1983), which were designed to displace individual hair bundles. A first step to calibrating the frequency...
dependence of a fluid jet was given by Saunders and Szymko (1989). They used glass micro-beads, with a specific gravity close to that of water, which were captured in the pressure field of the pipette while monitoring their motion by stroboscopic-illuminated microscopy.

In this chapter a simple piezoelectric-driven fluid jet device is presented. A compliant glass fibre with a resin sphere attached to its tip is used as a sense probe to calibrate the fluid displacement output of the fluid jet device. Sense probe displacements are monitored using a heterodyne laser interferometer coupled to a transmitted-light microscope. The procedure, which can be easily applied in an experimental situation, enables the determination of the frequency dependence of the fluid jet, which is essential to correct experimental data obtained using the same fluid jet as a stimulus. To improve the understanding of the physical factors of the fluid jet device that govern the frequency response of the produced fluid jet, a model of its output has been developed and compared to the measured data. The resistance of the fluid jet pipette tip will be shown to be the key parameter influencing the amplitude and phase characteristics of the fluid jet.

MATERIALS AND METHODS

Fluid jet-producing device
The device used for the generation of a fluid jet is shown in Fig. 1A and its individual parts in Fig. 1B. The main parts are a Perspex body part (B) and a brass rear end (R). Three screws (S) pull both parts together, sandwiching a piezoelectric-driven brass disc (P), a configuration that is only clamping the piezoelectric disc at its rim. The Perspex body contains a cone-shaped fluid-filled chamber, which is closed by the piezoelectric disc. A rubber o-ring (O) between the Perspex and the piezoelectric disc provides a fluid-tight seal. When displaced by the voltage-driven piezoelectric material, the piezoelectric disc induces volume changes of the fluid chamber.

At the front end of the Perspex body part a glass pipette (Pi, outer diameter 1.5 mm, inner diameter 1.17 mm) is inserted. The screw cap (Sc) applies pressure on two cone washers (C) separated by a Perspex spacer (Sp) to produce a fluid tight seal and fix the glass pipette in a mechanically stable way. The pipette is narrowed at its tip using an electrode puller. Tip diameters were varied between 6 and 62 µm and tapering lengths were kept at approximately 3-4 mm. The total length of the glass pipette was about 5 cm of which about 1.5 cm was clamped
inside the fluid jet device. To facilitate horizontal application of the fluid jet in the experimental chamber, the glass was bent one centimetre from the tip over an angle of about 20°, so that it could be aligned with the horizontal plane (Fig. 1A).

To obtain an air-free filling of the fluid chamber in the Perspex body part, it was filled with a low viscosity silicone fluid (200 Fluid 5 CS, Dow Corning), which has low surface tension properties. The silicone fluid was degassed under vacuum conditions for several hours before use to prevent dissolved gas from creating bubbles inside the fluid jet. The glass pipette was filled with demineralised water. The last centimetre of the pipette, which was to be inserted into the Perspex body part, was filled with the silicone fluid. Inserting the glass pipette generated a pressure increase inside the fluid chamber, displacing a small volume of the water out of the pipette. During this temporary overpressure the fluid jet device was transferred to its position in the set-up and the pipette tip was submerged in the bath solution (water) preventing air to enter the tip.

The instrument also benefits from the high resistivity \((1.0 \times 10^{15} \text{ Ohm-cm})\) of the silicon fluid creating an electrical resistance between the brass disc and the bath solution, thereby preventing electrical cross talk of the piezoelectric control signal and electrophysiological recordings obtained from the preparation (relevant for chapters 3, 5-6). Additional electrical shielding was obtained by connecting the brass disc, which is facing the preparation, to the electrical ground. The rear end of the fluid jet device, which together with the grounded brass disc enclosed the piezoelectric material, was also grounded, thereby shielding off the applied voltages.

A shaft connected to the rear end of the fluid jet device was used to mount the device on a xyz-micromanipulator. The tip of the jet pipette was lowered in the bath and positioned under visual guidance. All calibration measurements were done in free field conditions, i.e. approximately 5 mm above the bottom and below the surface.

**Signal generation**

Sine wave stimuli at exponentially distributed frequencies ranging from 1 to 1000 Hz were generated at 32 points per period using the full amplitude range of a 16-bit D/A converter (Ariel, DSP-16). They were subsequently attenuated to the desired amplitude and filtered (8-pole Bessel) at 8 times the stimulus frequency. The first 4 seconds of a stimulus were not recorded to prevent effects of onset transients.
Motion detection
Object motion was measured with a heterodyne laser interferometer coupled to a fixed-stage transmitted light microscope (Fig. 2) mounted on a vibration isolated table. The objective lens focuses two parallel laser beams so that their beam waists intersect in the plane of focus. A frequency difference of 400 kHz between the two laser beams, produced by two Bragg cells driven at 40.0 and 40.4 MHz, creates a moving fringe pattern in the measuring volume where the beams intersect. Light scattered by a stationary object, therefore, fluctuates in intensity at a carrier frequency of 400 kHz ($f_{\text{carr}}$). Additional motion of the object induces a velocity-dependent frequency modulation (Doppler shift) of the 400 kHz carrier signal. The back-scattered laser light from an irregularity on the surface of the object is detected by a photo-multiplier (Hamamatsu, model H6780-02) coupled to an I/V converter. The output, containing the carrier frequency ($f_{\text{carr}} = 400$ kHz) modulated by the velocity-dependent frequency shift produced by object motion, is band-pass filtered with a centre frequency at $f_{\text{carr}}$ and electronically demodulated using a modified frequency demodulator (Polytec, OFV 3000). The output signal of the demodulator is a calibrated linear measure of the velocity of the object within the range of $10^{-1}$ to $10^{3}$ µm/s. For low frequency stimuli ($< 10$ Hz) a digital phase demodulator was used, which has an output proportional to the displacement of the object with a 32 nm resolution. The demodulator signal was low-passed filtered (8-pole Bessel) at 8 times the frequency of stimulation and was amplified before being digitised using a 16-bit A/D converter (Ariel, DSP-16) with a sample frequency at 32 times the frequency of stimulation. Displacement responses consisting of usually 10 consecutive stretches each 16 periods in length were averaged on-line by the data acquisition board. The averaged waveform was stored on hard disc. A fast-Fourier transform (FFT) was used to extract the amplitude and phase at the frequency of stimulation.

Sense probes
Sense probes for the detection of fluid displacement consisted of compliant glass fibres with a resin sphere attached to their tip. Sphere diameters ranged from 30-60 µm. The fibres were about 1 cm in length with a gradually decreasing diameter of about 100 µm to approximately 2 µm at their tips. They were glued to a stiff glass capillary, with which they could be mounted on a manipulator. The mechanical frequency response of the sense probes to fluid displacement was obtained by stimulating the sense probe with a glass stimulus sphere (Ø 1.1 mm) attached to a piezoelectric element. The frequency response of this stimulus sphere...
Figure 2: Schematic drawing of the heterodyne laser interferometer microscope.

A laser beam with a Gaussian intensity distribution, generated by a He-Ne laser (632.8 nm) oscillating in the fundamental TEM₀₀ mode (Spectra physics) is aimed via an attenuator (A) through a telescope (L₁ and L₂), which controls the location and size of the beam waist at the location of the object. The beam is split using a translatable 50/50 beam splitter (BS₁) directing each beam through a separate Bragg-cell (B₁ and B₂) driven at 40 and 40.4 MHz respectively. From each Bragg cell one of the first order beams is optimised for intensity by changing the angle of incidence. Translatable mirrors M₁, M₂ and M₃ and beamsplitter BS₁ are used to parallel the first order bundles reflected from prisms P₁ and P₂. The dotted lines indicate a change of view from top view to front view of the set-up. The parallel bundles are then guided into the microscope via translatable mirror M₄ and M₅, onto an internally fixed mirror M₆ towards fixed 50/50 mirror M₇ (M₆ and M₇ are behind each other and at a 45 degree angle with the plane of the drawing). Mirror M₇ combines the laser beams with the microscope's light path, reflecting them down to the objective lens (OB). M₄ and M₅ can also be used to align the bundles parallel to the optical axis of the OB and allow for precision positioning of the interference spot in the visual field during an experiment. Translation of P₁ and P₂ changes the distance between the parallel bundles, thereby changing the angle (α) between the optical axis and each of the beams leading to a change in the fringe distance. The beam waists are focussed by OB and intersect at the focal plane, where they produce a moving fringe pattern.

The back-scattered laser light (light grey) reflected by the object (S = sense probe, Pᵢ = fluid jet pipette), falling within the aperture of OB, travels via the Wollaston prism (P₃) and mirror M₇ to a polarising beam splitter (BS₂). Using the λ/2 retardation plate the polarisation of the laser beam is rotated such that most of the laser light reflected by BS₂ is directed towards the photo multiplier detector (PMT). A translatable pinhole...
was separately determined using the laser interferometer.

When stimulating the sense probe with the fluid jet, the centre of the fluid jet was aimed at the centre of the resin sphere. The distance between the jet producing pipette tip and the sphere was at least one diameter of the resin sphere. Based on the frequency responses of the sense probes their effective stiffness coupling to the stiff glass tube held in a fixed manipulator was estimated to be of the order of $10^{-4}$ N/m.

**Modelling of the jet output**

A mechanical model containing the principal physical factors that determine the frequency response of the fluid jet is shown in Fig. 3. The fluid jet device is described by two coupled masses, representing the fluid mass in the Perspex body part, $M_1 = \rho L_1 \pi R_1^2$, and the fluid mass in the glass pipette, $M_2 = \rho L_2 \pi R_2^2$, where $L_1$ and $R_1$ are the effective length and radius of the wider Perspex body, $L_2$ and $R_2$ the effective length and radius of the narrower glass pipette and $\rho$ denotes the fluid density. A vibrational displacement amplitude, $X_0$, generated by the piezoelectric-driven brass disc is coupled to the fluid mass, $M_1$, in the Perspex body part by a spring, $S_1$, representing the rubber o-ring (Fig. 1B, O) and results in a fluid displacement amplitude, $X_1$. A resistive element in series, $R_1$, is included to describe friction forces in the fluid chamber. The displacement of the fluid mass in the Perspex body is coupled to displacement of the fluid mass in the glass pipette,
The displacement amplitude of the fluid emerging from the jet pipette, $X_2$, by a lever, $L$. The force ratio of this transfer is equal to the inverse of the ratio of the cross-section surfaces in the two compartments $F_i = 1/l = R_2^2/R_1^2$, reflecting the conservation of fluid mass. The displacement transferred to the glass pipette compartment is, therefore, equal to $lX_1$, which is the input displacement of the fluid in the glass pipette. The spring, $S_2$, resistive element, $R_2$, and mass $M_2$ determine the displacement response, $X_2$, of the fluid mass in the pipette, which is a measure of the displacement of the fluid of the jet.

The displacement amplitude of the fluid emerging from the jet pipette, $X_2$, as a function of frequency is then described by:

$$X_2(\omega) = \frac{-S_1}{-S_1T(\omega) + iS_2 - S_2l^2T(\omega) + \omega^2M_1T(\omega) + ioR_1T(\omega)}X_0(\omega),$$

(1)

with

$$T(\omega) = \frac{S_2 + ioR_2 - \omega^2M_2}{iS_2}.$$  

(2)

RESULTS

Fig. 4A shows a typical sinusoidal displacement of the sense probe driven by a fluid jet with a jet pipette diameter of 62 µm. The average sense probe displacement of 10 consecutive traces is given, each containing 16 periods at a frequency of 106 Hz. The waveform clearly shows the 16 periods of the fundamental frequency with negligible distortion. The degree of the distortion can
be more clearly demonstrated by the Fast Fourier Transform (FFT) calculated from the response in Fig. 4A (Fig. 4B). It shows the frequency content of the waveform up to 500 Hz. The fundamental frequency is clearly visible at 106 Hz and rises about three orders of magnitude above the noise floor. The second harmonic is visible, but has an amplitude of more than 40 dB below the response at the fundamental frequency. Higher harmonics are even smaller. The total harmonic distortion (THD) had a value of -32.7 dB and was calculated according to:

\[
THD = 20 \log \left[ \sum_{a=2}^{n} \frac{|a_n|}{|a_1|} \right],
\]

where \(a_1\) is the amplitude of the fundamental frequency and \(a_n\) is the amplitude of the \(n\)th harmonic frequency.

**Fluid jet calibration**

To determine the frequency-dependent motion of the fluid jet, the displacement of the sense probe, induced by the fluid jet, was measured as a function of frequency. However, the frequency-dependent amplitude and phase characteristics obtained this way are contaminated with frequency-dependent characteristics of the sense probe and other possible frequency selective components of the equipment. To isolate the fluid jet frequency response, three additional frequency responses have to be measured, which are displayed in Fig. 5. The first step (Fig. 5A1) is to obtain the frequency dependent properties of the demodulator (D) including the additional signal conditioning equipment (E). To do so a voltage-controlled oscillator was
used to generate a frequency modulated 400 kHz signal, which was sent to the demodulator, instead of the photomultiplier output. The resulting response, $ED(f)$, is presented in Fig. 5B1, as a function of frequency, $f$. It shows a nearly constant amplitude characteristic and a clear phase rotation starting around 310 Hz as a result of the anti-alias filter, set at 8 times the stimulus frequency up to the 2.5 kHz $(8 \times 310 \text{ Hz})$ limit of the filter. The second step characterises the frequency response of a stimulus sphere, $S(f)$. The laser interferometer is used to directly measure its displacement amplitude as a function of frequency. This response, consisting of $ED(f) \times S(f)$ can now be divided by $ED(f)$, obtained in step 1, to isolate the pure stimulus sphere response, $S(f)$ (Fig. 5B2). In the third step the same stimulus sphere is used to displace a sense probe, with frequency characteristics $SP(f)$, that subsequently will be used to determine the fluid jet displacement. The sense probe displacement resulting from sphere stimulation, as measured by the laser interferometer, is still contaminated with equipment and stimulus sphere characteristics, $ED(f) \times S(f) \times SP(f)$, this response is divided by the previously (step 2) isolated stimulus sphere response, $S(f)$, resulting in the sense probe frequency response (Fig. 5B3), which is (intentionally) still contaminated with the equipment response characteristics, $ED(f)$. In the fourth step the remaining $ED(f) \times SP(f)$ is used to correct the fluid jet-driven sense probe response measured by the interferometer $ED(f) \times SP(f) \times FJ(f)$, thus eventually isolating the pure fluid jet amplitude and phase response, $FJ(f)$, as a function of the frequency of displacement (Fig. 5B4).

**Tip resistance**

The dimensions of the object that has to be stimulated largely determine the diameter of the tip of the glass-pipette to be used for producing the fluid jet. An individual hair bundle usually requires tip diameters smaller than 10 µm, whereas some tectorial structures may require tip openings of up to 0.5 mm. A narrow tip increases the outflow resistance and thus decreases the fluid flow of the jet, as evidenced by the much larger voltage amplitudes that have to be applied across the piezoelectric disc to obtain equal displacement amplitudes of the sense probe.

An even more important consequence of the tip diameter is its influence on the fluid flow characteristics of the fluid jet. To illustrate this, three examples of different tip diameters are given in Fig. 6. Fig. 6A shows the amplitude and phase response of the resulting fluid displacement when using a pipette without a tip restriction. In this case, the glass pipette was cut at the same length as the pipettes used in Fig. 6B and 6C but was not tapered at its tip. Its outflow diameter then
Figure 5: Fluid jet correction procedure. (A) Schematic representation of the correction procedure. Each column (1-4) represents a type of measurement needed to complete the correction. These frequency responses contain properties of ED (equipment, demodulator), S (stimulus sphere), SP (sense probe) or FJ (fluid jet) as a function of frequency. Results in a column are used to correct the measured response in the next column, indicated by the long arrows, and produce results pointed at with a short arrow. (B) Displacement amplitude and phase for each measurement (1-4) described in A. Each solid line is the result of a correction (except for column 1) and is used in the next column to correct the measured frequency response (symbols), both for the amplitude as well as the phase response.
equals the inner diameter of the glass pipette (1.17 mm). The fluid jet generated when using such an unrestricted tip effectively behaves as a displacement stimulus up to a certain cut-off frequency. This is evident from the displacement response at low frequencies (<100 Hz), which is flat as a function of frequency. At higher frequencies the amplitude starts increasing and approaches a resonant frequency at about 150 Hz. Beyond this resonant frequency the displacement amplitude decreases at a rate of -40 dB/dec, reminiscent of a second order system. During the constant displacement output there is a 0 degree phase lag with the voltage input to the piezoelectric disc. Around the resonant frequency the phase rotates over an angle of -180 degrees, consistent with the second order characteristics of the amplitude.

The pipette with an intermediate tip restriction of 56 µm (Fig. 6B) shows an intermediate response. At low frequencies the displacement amplitude is constant as a function of frequency and in phase with the voltage control of the piezoelectric
disc. In this case the displacement amplitude, however, starts declining around 15 Hz, changing towards a slope of about -20 dB/dec with an according -90 degree phase rotation. Around 400 Hz the slope starts to decrease further and also the phase starts rotating towards a 180 degree phase lag. No resonance is evident, as in the larger diameter (1.17 mm) case.

In case of an extremely restricted tip with a diameter of 7 µm, the fluid jet device effectively produces a constant fluid velocity in proportion to the voltage applied. This is clear from Fig. 6C, which shows a displacement amplitude response of about -20 dB/dec over the measured frequency range (1-1000 Hz). Consistent with this characteristic is the almost constant phase lag of -90 degrees except at the higher frequencies where it starts rotating slowly. These frequency characteristics reflect an overdamped system. Therefore, in the case of a small tip diameter, the output jet in terms of its velocity is flat and in phase with the voltage applied to the piezoelectric disc within the measured frequency range.

To get a better understanding of underlying parameters influencing the output characteristic of the jet producing device, the data were also described by a mechanical model (see Methods). The fluid jet device is modelled as two fluid masses coupled by a lever and a spring (Fig. 3). One fluid mass is contained in the large diameter chamber in the body part of the Perspex, the other is the fluid mass in the restricting pipette. The displacement amplitude of the latter is proportional to the displacement of the fluid jet emerging from the pipette tip and is calculated as a function of frequency of the piezoelectric disc displacement. This model was then fit to the data (Fig. 6A-C, solid lines).

The model shows that the resistance of the pipette, \( R_2 \), greatly influences the output response. When fitting the model to the data, \( R_2 \) increases drastically (×1540) when compared to the pipette without tip restriction and it results in an intermediate resistance for the 56 µm tip. Besides an increased \( R_2 \), \( R_1 \) and \( S_2 \) are also modified. This is most likely due to the increased force, which had to be applied on the cone washers as a result of the higher pressure during the insertion of the pipette with smaller tip diameter. As a result the cone washer partially restricted the pipette entrance.

Unless the system is overdamped by a resistive element, each combination of a spring and mass leads to a resonance accompanied by a -180 degree phase rotation and an amplitude response slope change of -40 dB/dec. The present model, therefore, predicts two resonant frequencies damped by two friction elements in the system, resulting in a total phase rotation of 360 degrees. The occurrence of just
one resonant frequency with the accompanying -180 degrees phase rotation within the measured frequency range thus suggests that there is a second resonance at a higher frequency. The existence of a second resonance at higher frequencies was confirmed by using a signal generator to drive the fluid jet-producing device while monitoring the sense probe’s velocity, as measured by the laser interferometer. A second resonant frequency was observed at about 4.5 kHz. Since this was far beyond the range of frequencies of our interest, we did not perform further detailed measurements.

DISCUSSION

The fluid flow of microscopic jets, produced by several types of devices, has been used in hair cell research for two decades (Flock and Orman, 1983; Saunders and Szymko, 1989; Kros et al., 1992; Rusch et al., 1994; Géléoc et al., 1997; van Netten et al., 2003). In almost all cases the mechanical characteristics are assumed to be constant in terms of displacement or velocity and generally lack detailed data on their actual output characteristics. In the present study a method was developed, using a flexible sense probe that was independently characterised by means of an additional stimulus device (stimulus sphere), to calibrate a microscopic fluid jet, providing its detailed frequency selectivity within a limited frequency range. This information is necessary to properly analyse any object displacements produced by this jet. Because slight changes in the tip diameter of the jet-producing pipette alter the frequency response, a calibration for each individual pipette is necessary. The proposed calibration using a sense probe has been shown to be useful within the frequency range tested. The frequency-dependent characteristics of a particular sense probe can be determined well in advance of the actual physiological experiment and the probes can be stored for later use. During an experiment a sense probe can be positioned in close proximity of the preparation to enable calibration of the fluid jet directly after the stimulation of the preparation (see chapter 3), without having to move the pipette tip out of the water. Essential for this procedure is that the fluid jet frequency response does not change over time. A potential danger is that the tip gets (partially) blocked by dirt floating in the bath solution. Such an additional restriction of the tip leads to a changed response profile, making it impossible to correct measured data for the jet response characteristics.
Comparison of measured fluid jet characteristics with model

A simple physical representation of the fluid jet-producing device as two fluid masses coupled via a lever and a spring, quite well describes the behaviour of the fluid jet emerging from the pipette’s tip. It follows from fitting the model to the data that the dynamics of the fluid in the pipette dominates the frequency response characteristics for the frequency range used in the experiments. The fluid in the body compartment of the device resonates at a much higher frequency (around 4.5 kHz). This implies that the first term in the denominator of Eq. 1, proportional to $S_1$, dominates. the other terms in the denominator so that Eq. 1 effectively reduces to $X_2 = T(\omega)^{-1} X_0$. In terms of the model (Fig. 3) this means that $X_1$ moves exactly the same as $X_0$ because of the very stiff element $S_1$ so that the dynamics of the fluid jet is governed by the elements describing the pipette. ($S_2, R_2, M_2$). This also implies that the parameters of only these elements can be meaningfully fitted (e.g. Fig. 6). The parameters of the Perspex body were fitted to have a (second) resonance above the frequencies used in this study, in line with the observations.

One of the most relevant features significantly affected by the tip resistance, which depends severely on the tip diameter, is the character of fluid motion that is produced as a function of frequency. A large tip diameter results in a displacement-producing device for a limited range of frequencies with accompanying resonance behaviour (Fig. 6A). A slight restriction of the pipette (critically) damps the system, preventing it from resonating. It produces a flat displacement output up to about 200 Hz (Fig. 6B). Above this frequency the displacement amplitude falls off with -40 dB/dec accompanied by a 180 degree phase rotation. The model shows that increasing the stiffness of the compliant components in the device shifts the roll-off frequency towards higher frequencies. Alternatively decreasing the length of the pipette increases the cut-off frequency, but this is in practice limited by the minimum length needed for positioning the device in the set-up. Highly restricted pipettes, with tip diameters in the order of several micrometers, strongly overdamp the system and produce a constant velocity output as a function of frequency (Fig. 6C).

The model therefore predicts the change in fluid jet from a displacement stimulus in the situation of a small tip resistance (large tip diameter) to a velocity stimulus due to tip restriction. Fig. 7A compares the model results into more detail for increasing outflow resistance, $R_2$. At a low pipette resistance ($R_2 < 0.02$ Ns/m), a constant displacement amplitude of the jet is produced at frequencies below about 80 Hz, showing resonance around 150 Hz. Slightly increasing the resistance, $R_2 = 0.7$ Ns/m, (critically) damps the system making it a useful displacement
stimulus device. Increasing the resistance $R_2$ gradually changes the fluid jet apparatus into a velocity-producing device at expense of the jet flow. This velocity related input corresponds with a slope of -20 dB/dec in the amplitude response.

What type of fluid motion is needed to stimulate a mechanosensory structure? In other words how well does the output characteristic of the fluid jet-producing

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**Figure 7:** The effect of increasing pipette resistance (A) Series of modelled frequency responses showing fluid displacement output as a function of the frequency of the voltage signal put across the piezoelectric disc. Parameters values: $S_1 = 2.5 \cdot 10^5 \text{ N/m}$, $R_1 = 0.05 \text{ Ns/m}$, $S_2 = 40 \text{ N/m}$, values of $R_2$ are given next to each curve. (B) Calculated step response of the fluid jet-producing device, with low pipette resistance ($R_2 = 0.02 \text{ Ns/m}$). (C) The same as B but with a high pipette resistance ($R_2 = 200 \text{ Ns/m}$)
device match the fluid motion to which the mechano-sensory structure is physiologically sensitive? Based on the micromechanics and hydrodynamics of an individual hair bundle it was theoretically shown that a hair bundle’s displacement response is flat as a function of the frequency of a fluid velocity stimulus up to a cut-off frequency determined by its stiffness and its size (Géléoc et al., 1997; van Netten, 1997). The pipettes used to displace individual bundles are matched to the dimensions of the bundle with diameters below 10 µm. The accompanying high tip resistance results in a constant velocity output as a function of frequency, therefore, produces an adequate stimulus to displace individual bundles. Fluid velocity steps will thus induce step displacements of the bundle (Fig. 7C). The velocity output will, however, be contaminated with a ringing close to 4.5 kHz caused by resonance of the fluid in the body compartment. Adequate low-pass filtering of the applied voltage signal can prevent these oscillations, but will inevitably put an upper limit to the displacement rise time of a hair bundle that can be achieved.

A second consequence of tip restriction is the decreased output response. Much larger voltages have to be applied to the piezoelectric disc to produce similar fluid jet displacements. This consequence of tip restriction was previously recognised by Saunders and Szymko (1989) who showed a square relation between fluid displacement amplitude and tip diameter. Remarkably, they did not report differences in the frequency response of their fluid jet due to tip diameter. Their calibrating method did not give precise information about the phase of the response and, therefore, lacks this relevant information. Their displacement frequency responses reported have a slope of -10 dB/dec, which makes their apparatus neither a true displacement nor a true velocity-producing device, and additional phase information could perhaps help by the interpretation.

When a preparation is only stimulated at a single frequency, the detailed frequency response of the fluid jet is less relevant as long as the desired displacements of the object can be established. Harmonic distortion of the produced fluid jet might, however, cause a problem in the investigation of a system’s non-linearities. Although the harmonics produced by the described device (Fig. 1) are relatively small (Eq. 3, THD < -32 dB) and comparable to the fluid jet distortion reported by Saunders and Szymko (1989), it may nevertheless preclude the direct estimation of transducer related hair bundle non-linearities from harmonic analyses, as predicted for specific types of hair cells (e.g. van Netten and Kros, 2000).

This chapter accentuates the importance of the calibration of a jet-producing
stimulus device, demonstrating the importance of the outflow restriction as the prime parameter for the jet response characteristics. Not only the jet displacement output (power) is decreased with a higher tip resistance, but more importantly its output characteristic changes from a displacement to a velocity stimulus as a function of frequency (Fig. 7A) accompanied by a phase change. The simple model described in this chapter can help understanding the physical parameters underlying the output characteristics and can thus facilitate optimising the design of this stimulus device.