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The Gravikinetic Response of *Paramecium* is Based on Orientation-Dependent Mechanotransduction

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Abstract *Paramecium* generates persistent shifts of the membrane potential of a few millivolts depending on its orientation with respect to the gravity vector. The resulting potential-induced modulation of the speed of propulsion is called gravikinesis because it acts to neutralize, fully or in part, sedimentation. Gravisensitivity is maximal at neutral orientation, i.e., in horizontally swimming cells, when the gravitational force per unit membrane area is at minimum. Stimulus-response relationships and energetic considerations show that sensing of the gravity vector by a nonspecialized, single-cell organism ranks among the most sensitive mechanoreceptors known in nature.

Introduction

Ciliates and other free swimming protists regulate their rate of locomotion while they pursue various sources of food, engage in mating, or escape from hazardous stimuli such as mechanical shocks (Machemer and Teunis 1996). These cells perceive mechanical disturbances and can generate phasic receptor potentials, the polarity and amplitude of which reflect the site of stimulation (Machemer and Deitmer 1985). Potential changes are sensed by Ca^{2+} channels of the ciliary membrane and are transformed to motor responses via modulation of ciliary activity (Machemer 1986). Gravity has long been known as guiding upward or downward orientation (=gravitaxis) in protists (Verworn 1896; Wager 1911) and metazoans (Schöne 1975). The common gravitactic orientation of cells away from the center of gravity (negative gravitaxis) has raised a continuing controversy on its mechanism, which may invoke sensory transduction or may be exclusively physical (Lebert and Häder 1996; Machemer and Bräucker 1996).

We started from established distributions of mechanoreceptor conductances in *Paramecium* to avoid possible ambiguities inherent in interpretations of gravitaxis. We postulated that the gravitational force, as derived from the apparent density of a swimming cell acts, in the first line, on mechanoreceptor channels of the "lower" membrane causing an orientation-dependent modulation of ciliary propulsion (Machemer 1989a). This hypothesis of gravikinesis has now been verified in seven different species of ciliates using computer-based tracking methods of horizontally and vertically swimming cells under normal gravity (Baba et al. 1989; Machemer-Röhnisch 1989; Machemer et al. 1991), artificially raised acceleration in a centrifuge (Ooya et al. 1992; Bräucker et al. 1994), and weightless conditions (see Machemer 1998). The theory of gravikinesis predicts, for instance, that cells at upward orientation have their posterior (=lower) membrane deformed by gravity. This stimulus opens K^+ mechanoreceptor channels which are most abundant in the posterior soma membrane. The resulting hyperpolarization induces the cilia to augment their beating rate (Machemer 1986). The *increment* in upward speed compensates sedimentation fully or in part (Nagel et al. 1997). In cells swimming downwards, a gravity-induced outward deformation of the anterior membrane activates Ca^{2+} mechanoreceptor conductances prevailing in the anterior soma membrane. The induced weak depolarization depresses ciliary beating rates. Here, it is a *decrement* in downward speed which offsets sedimentation effects. We call the motor response to gravity a *negative gravikinesis,* referring to the conventional positive sign of the gravity vector and direction of sedimentation. Gravikinesis differs from gravitaxis in that it starts from cell orientation but does not generate it. Gravikinesis and gravitaxis in conjunction fundamentally affect protistan ecology counteracting sedimentation and maintaining micro-organisms at favorable levels for food capture, as guided by chemical or light stimuli (Machemer-Röhnisch et al. 1993; Bräucker et al. 1994).

Gravity Modulates Locomotion Speed

Gravikinesis is a change in active propulsion in a cell orientation-dependent manner. Data on gravikinesis result from geometric processing of four parameters of locomotion (Machemer and Bräucker 1992): the speed (V), the sedimentation rate (S), the gravity-independent rate of propulsion (P), and the inclination angle of the recorded track (θ) of the cell, the latter being inclined at a smaller angle (o) (Machemer et al. 1997). Figure 1 shows the distribution of gravikinesis as a function of cell orientation (0°=upward) using sectors of 15°. The median gravikinetic response was minimal or absent near horizontal orientations of the cell and rose to values between 60 and 70 μm/s at about vertical orientations. Maximal speeds in cells at upward orientation were documented at angles 15°–30° degree off from the vertical. Noteworthy is the steep change in the gravikinetic response of cells which turned away from neutral (=horizontal) orientations.

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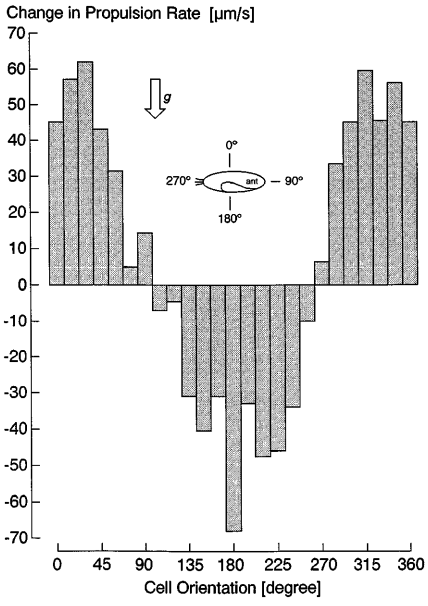

Fig. 1. Orientation-dependent changes in active propulsion of *Paramecium caudatum* as induced under normal gravity in a vertical plane. *Inset*, 0° = vertical up. Each sector of 15° includes at least 408, maximally 991 individual data. Medians were calculated from observed speed, track angle, sedimentation rate, and horizontal speed at 1 *g* (= reference speed, as unaffected by gravity; Machemer et al. 1993)

Stimulus-Response Relationship

Gravity accelerates the mass of a cell and can, by means of differences in density of the cytoplasm and the surrounding medium, induce sensory transduction in the membrane. *Paramecium caudatum*, at a typical length of 250 µm, 50 µm diameter, and a density of 1040 kg m^{-3} (40 kg m^{-3} apparent density in freshwater; Kuroda and Kamiya 1989) exerts a total *g*-force of 1.3×10^{-10} N to the lower membrane. Depending on cell orientation, the maximal effective force per membrane unit area (= pressure) is 20 mPa (2×10^{-2} N m^{-2}) in horizontal swimmers and five times that value in vertical cells. Correlation of the induced changes in propulsion rate with orientations gives a sigmoidal curve. In this curve, responses of a horizontally swimming cell exposed to 20 mPa take the central position in a steep stimulus-response relationship (Fig. 2). A minor upward turn of *Paramecium* from the horizontal position shifts the area affected by the gravity vector more posteriorly, raising pressure and inducing an increase in cell propulsion. Likewise, a minor downward turn of the cell relocates effects of the gravity vector more anteriorly to cause a decrease in propulsion of the cell. Between 80° and 100° inclination, the gain in propulsion was 46 µm/s (inset Fig. 2) corresponding to 3 µm/s per mPa change in local pressure (or per 10^{-15} N change in 1 μm^2). An exclusive action of pressure on the orientation-dependent gravikinetic response (Fig. 1) is nevertheless unlikely because maximal increments in gravikinesis (between 60° and 120°) were not associated with maximal changes in pressure, which occurred from 0° to 60°, and from 120° to 180°. Moreover, the specific gravikinetic responses seen during upward and downward orientation (Fig. 2) are not explained by pressure. Addition to the abscissa of the ratios of local conductances, $\Delta g_{Ca}/g_K$, as activated by gravity in the lower membrane, explains why *Paramecium* was maximally sensitive near horizontal orientations (large changes in conductance ratio), and why sensitivity saturated near vertical orientations (virtually no changes in conductance ratio). The somatic cilia of *Paramecium caudatum* beat at 17 Hz at resting potential and room temperature (Machemer 1976) corresponding to a swimming speed of approximately 1 mm/s (Machemer 1989b). Ciliary beating rates follow minor negative or positive shifts of the membrane potential in a linear fashion. From the voltage-dependent slope of ciliary frequency (Machemer 1976), it follows that a documented gravikinesis of 60 µm/s corresponds to a gravity-induced change in beating rate of 1 Hz or a mean change in

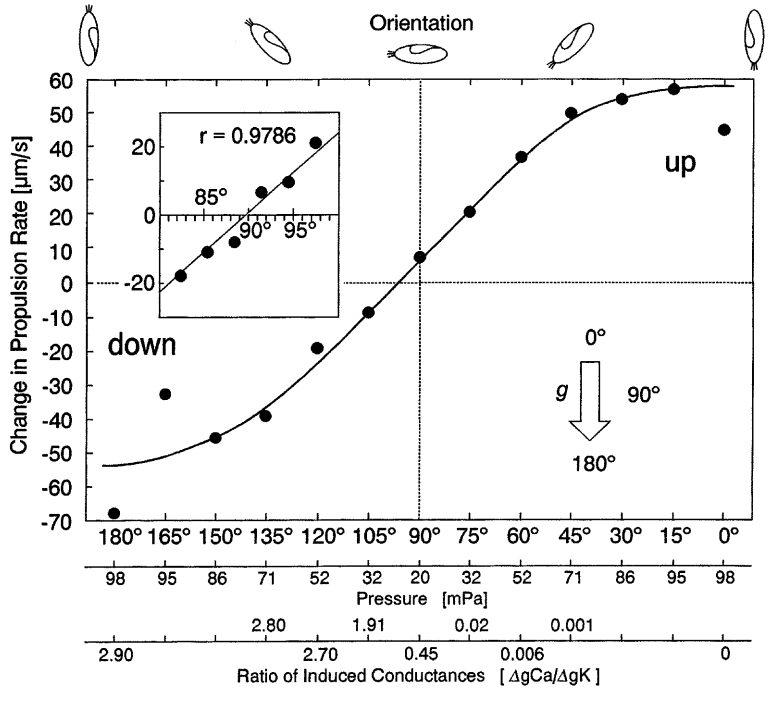

Fig. 2. Relationship of gravity-induced changes in propulsion rates (gravikinesis) as a function of cell inclination from the up orientation (0°). Orientation-dependent pressure to the horizontal segment of the lower membrane was calculated considering *Paramecium* as a rotational ellipsoid of 250 µm length, 50 µm diameter, with an apparent density of 40 kg m^{-3}, and using the *g*-force acting on the horizontal membrane. The orientation-dependent ratio of induced mechanoconductances is based on the quantitative distributions of these conductances in the *Paramecium* surface membrane (Ogura and Machemer 1980). *Inset*, refined orientational resolution of gravikinesis near the horizontal position using sectors of 3° shows perfect absence of gravikinesis at 90° (neutral orientation)

membrane potential of 1–2 mV. This potential shift is possible with minor shifts in the total conductance ratio of Ca^{2+}- and K^+-channels.

A gravikinetic response in *Paramecium* is therefore likely to result from three conditions in conjunction: (a) orientation, (b) gravity-induced pressure, and (c) specific distribution of mechanoreceptor channels. With the available low *g*-forces, gravitransduction in a ciliate appears to be crucially dependent on orientation, not pressure.

A sigmoidal stimulus-response relationship with the neutral point of the sensor located in the center is a characteristic of highly sensitive mechanoreceptors such as vertebrate hair cells (Howard et al. 1988) and insect sensilla (Thurm et al. 1983). These mechanoreceptors are considered as primarily displacement-sensitive. Open and closed states of these mechanically sensitive ion channels are thought to be at thermodynamic equilibrium using the stimulus energy of displacements for shifting open probabilities in one or the other direction.

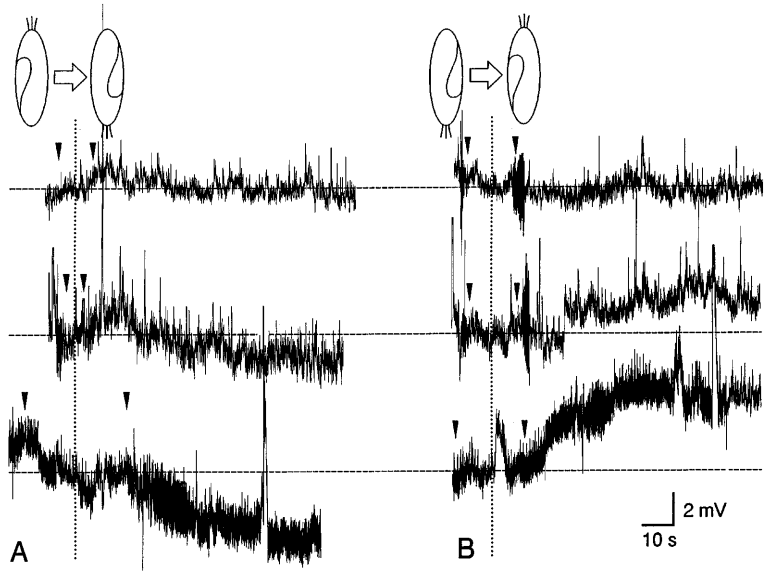

Fig. 3A,B. Examples of steady-state potential shifts during and after active 180° turns of *Paramecium* impaled centrally by horizontal microelectrodes. *Arrowheads*, start and end of turning period (reference lines centered at potentials at the middle of a turn). A) Three representative traces of downward to upward orientation to illustrate different sensitivities (from nothing to maximum) of the sample cells. B) Representative traces from upward to downward orientation. The turning was executed by the experimental cell itself. An external microneedle leaning near the bottom half of the cell, prevented further turning (leaning does not generate a receptor response). Spikelike deflections of the membrane potential (extremes cut for visibility reasons) are graded depolarizations common in *Paramecium*

Orientation-Dependent Potentials Mediate Gravikinesis

We equilibrated *Paramecium* cells from the early stationary phase for at least 3 h in the experimental solution (1 mM $CaCl_2$ + 1 mM KCl) at pH 7.2 and later supplemented 10 mM tetraethylammonium chloride (TEA) to depress the background membrane conductance by blocking voltage-sensitive and leakage conductances for K^+ (Naitoh and Eckert 1973). Inhibition of a major proportion, or even all, hyperpolarizing mechanoreceptor channels (Deitmer 1982) reduces antagonizing effects of mechanically activated Ca^{2+} and K^+ conductances (Ogura and Machemer 1980) on the membrane potential. We impaled *Paramecium* by horizontally held microelectrodes and extended potential recordings to 1 min following a 180° active reorientation of the specimen from upward to downward orientation, and vice versa (Fig. 3). In all recordings an observed persistent potential shift (a) was orientation dependent, (b) unidirectional, (c) not fully established immediately at the end of the turn, (d) grew in a quasi-logarithmic fashion at rates not exceeding 0.3 mV s^{-1}, and (e) tended to saturate 1 min following reorientation. Averaging over at least ten records for each type of 180° reorientation showed a mean potential shift of 1.5 mV after 1 min (Fig. 4).

How Many Channels Sense Gravity?

The electric properties of *Paramecium* are fairly well understood, thus allowing estimation of the membrane conductances as induced by gravity. A mean resting potential of –29 mV of a horizontally oriented cell (Fig. 4) in a bath solution of 1 mM $CaCl_2$ + 1 mM KCl + 10 mM TEA (E_{Ca} = +116 mV; E_K = -89 mV; Ogura and Machemer 1980) gives a conductance ratio of the resting membrane, g_{Ca}/g_K, of near 0.4. Addition of 10 mM TEA raised the mean input resistance from 70 MΩ to 100 MΩ. TEA (10 mM) also blocks K^+ mechanoreceptor channels (Machemer and Deitmer 1985), suggesting that the observed orientation-dependent mean (and maximal) voltage swings (Figs. 3, 4) are based on the closure and opening of Ca^{2+} mechanoreceptor channels only. An observed orientation-dependent shift in steady-state potential by 1.5 mV (maximally 5 mV) corresponds to a change in membrane conductance for Ca^{2+} of 124 pS (maximally 425 pS). Voltage-independent single channel conductances of *Paramecium* have been established from membrane vesicles incorporated in planar lipid bilayers (16 pS, cation-selective, Boheim et al. 1982; 30 pS, divalent-cation selective, Ehrlich et al. 1984) and from membrane blisters (40 pS; K^+-selective, Martinac et al. 1988) but so far not from viable cells. Comparison of conductance changes in *Paramecium* with unitary mechanoconductances of vertebrate hair cells (10–50 pS; Howard et al. 1988) suggests that reorientation in *Paramecium* in the gravity field involves the opening and closing of a few mechanochannels, which are sen-

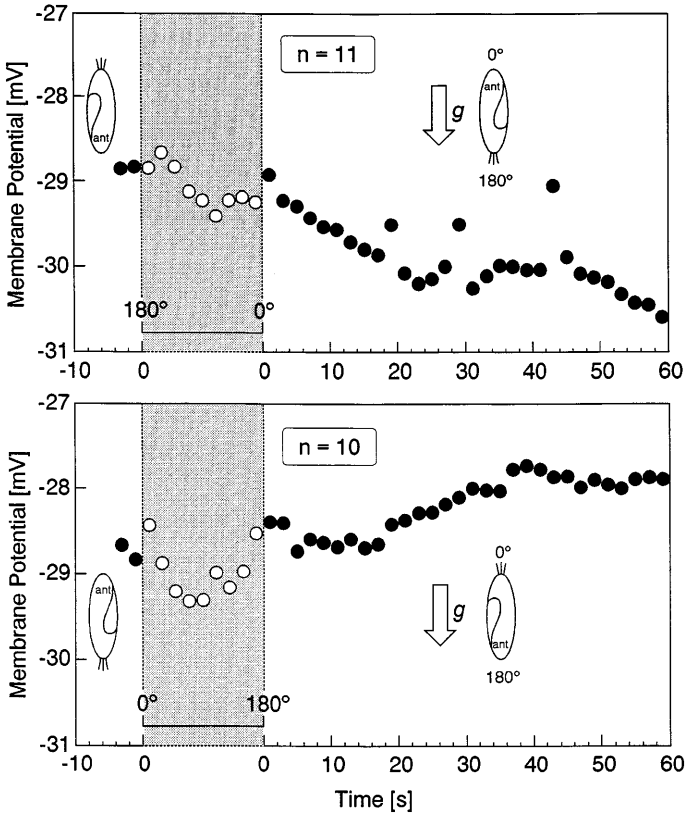

Fig. 4. Upward turning responses (11 records from 6 cells; *inset*: *ant*, anterior cell end) using intervals of 2 s for averaging (9 equal and uncalibrated time intervals chosen for the turning period). Corresponding averaging of downward turning responses (10 records from 5 cells). Note that upward turns hyperpolarized, and downward turns depolarized the membrane by 1.5 mV, and these potential shifts were not established during and immediately after fast turns

sitive to extremely weak *g*-forces. With most favorable assumptions: focusing the total *g*-force (1.3×10^{-10} N) to the area taken by the few activated gravireceptor channels, and assuming gating distances as reported for hair cells (3.5 nm; Howard et al. 1988), the energy for transduction is 4.6×10^{-19} N m. This high estimate of energy for gating exceeds the level of thermic noise by a factor of 200.

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