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ARTICLE

Two-Segment Electophysiological Model of Excitable Membrane of *Paramecium*

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Abstract: Our goal in this paper is to design a two-segment electrophysiological model for *Paramecium* based on the work of Hook and Hildebrand (1979, 1980). This model considers *Paramecium* divided into two segments. Both segments are assumed to obey Hook and Hildebrand equations. The aim of constructing this model is to consider theoretically how the free- swimming *Paramecium* responses to the external electric stimulation, involving whether the organism is facing the anode or the cathode during the application of the electric pulse. We assume that there is an irregular distribution of Ca^{2+} and K^{+} channels on both segments.

We have designed a computer program to illustrate the relationship between the distribution channels and swimming reversal time of the *Paramecium.* It is found that the reversal time depends on the precise distribution of Ca^{2+} and K^{+} channels. The relevant ratio of ionic channels distribution is found to be 74.8% and 25.2% for K^{+} and Ca^{2+}, respectively over the cell anterior and *vice versa* for posterior.

Keywords: *Paramecium;* Potassium and calcium currents; Membrane potential; Ionic conductance; Ca^{2+} and K^{+} channels; Reversal time.

Introduction

Paramecium is covered with cilia which form an integral part of the cell and are enclosed within the cell membrane. *Paramecium* ordinarily swims in a forward left-handed spiral path. It has an ability to turn smoothly and swim towards the cathode when an electric current is passed through the cell suspension (galvanotaxis) [1-6].

The electric properties of *Paramecium* have become of interest because of the emerging role of membrane potential and conductance in the control of ciliary activity [7-9]. Experiments indicate that ciliary responses are coupled to a membrane potential by membrane regulated calcium fluxes, and ciliary reversal is associated with depolarization of the membrane [10-14].

The experimental results described in previous work [15] need to be interpreted in terms of a physiological model of the cell membrane. The model outlined by Hook and Hildebrand [16, 17] is ideally suited for this purpose. It describes ion flow across the ciliary membrane of *Paramecium* in terms of potassium and calcium channels whose conductivities depend upon the instantaneous membrane potential, and also takes into account the effects of changes in the surface charge on the ciliary membrane as external cation concentrations (e.g. of K^{+}) are varied; this is excluded in the following analysis, however, since the ionic concentrations in the electric field experiments were always kept constant.

One immediate deduction, which may be made from experimental results on *Paramecium,* is that the membrane is not functionally the same over the whole cell surface. Since the response depends on whether the cell is facing the cathode or the anode during the pulse, it follows that the front and rear halves of the cell are

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physiologically different. It is therefore desirable to modify Hook and Hildebrand's model to incorporate, if possible, the functional differences between the cell membrane at the front and rear sides of the cell.

In this paper, the modifications of Hook and Hildebrand's model will be discussed. Account is given of how a membrane asymmetry can be incorporated into the model leaving the whole body responses unchanged whilst permitting different responses to asymmetric stimulation to occur.

Outlines of Hook and Hildebrand's Model

In this section, we review some equations of Hook and Hildebrand's model [16, 17] because of their importance as a basis for our two-segment model.

The Hook and Hildebrand's model is based on Ca^{2+} influx mechanism. It assumed that Ca^{2+} channels are built up by subunits extending through the lipid phase of the membrane. Both subunit ends contain a twice negatively charged binding site. The degree of cation binding (of Ca^{2+} and K^{+}) to these negative sites is supposed to be high. There are four possible states of cation binding which may be denoted X_1 to X_4.

The subunit X_1 can undergo a conformational change to an active state, X_1^*, at a rate which depends exponentially on the transmembrane potential. A single Ca^{++} channel is assumed to require some number of subunits N in an active state X_1^* in order to open.

The conservation of subunit molecule gives:

$$\sum_{i=1}^{4} X_i + X_1^* = X_0 = \text{ constant} \qquad (1)$$

This may be normalized with respect to the overall concentration X_0.

$$\sum_{i=1}^{4} x_i + x_1^* , \qquad (2)$$

where $x_i \equiv X_i / X_0 \qquad i = 1, 2, 3, 4.$

The quantity x_1^* may be considered to be the probability of a subunit being in the active state, X_1^*. Because each subunit is independent of its neighbors, the probability that N such units will come together to form an open Ca^{2+} - channel is therefore $(X_1^*)^N$.

If the potential-dependent conformational changes are rapid compared with the binding rate of the cations, then $X_1 \rightarrow X_1^*$ can be treated as a quasi steady – state process:

$$X_i^* = c \cdot X_1 , \qquad (3)$$

where:

$$c = c_o \,.\, \exp(\varepsilon \,.\, V_m), \qquad (4)$$

$$\varepsilon = \varepsilon_o \,.\, F / RT , \qquad (5)$$

where c_o is a constant, ε_o is the effective valency of the gating charge; F is Faraday's constant = 96485.34 C/mol of electrons (i.e., electric charge carried on one mole of electrons); R is the gas constant = 8.314 $J.K^{-1}.mol^{-1}$; and T is the absolute temperature.

Eq. (2) can be written in the form:

$$x_1^* = \frac{c}{c+1}(1 - x_2 - x_3 - x_4) \qquad (6)$$

The calcium current can be introduced as:

$$i_{Ca} = \bar{g}_{Ca} \cdot \left(x_1^*(t)\right)^N \cdot (V_m - E_{Ca}) + P_{Ca} , \qquad (7)$$

where i_{Ca} is the Ca^{2+} current (ions / s); $\bar{g}_{Ca}$ is the maximum possible Ca^{2+} - conductivity per cilium (1 / mV.s); V_m is the membrane potential (mV); E_{Ca} is the Nernst potential of Ca^{2+} given by:

$$E_{Ca} = \frac{RT}{2F} \ln \frac{\left[Ca^{2+}\right]_0}{\left[Ca^{2+}\right]_i}$$

P_{Ca} is the rate of active Ca^{2+} - extrusion (Ca^{2+} - pump), ions / s.

The calcium current is taken to be positive outward; thus the calcium pump is positive.

Because Ca^{2+} – channels are known to be located in the cilia, it is suggested that the Ca^{2+} - pump is also located in the vicinity of the cilia [16]. The membrane is considered to be a potential barrier for Ca^{2+} - carrying proteins, with the potential peak placed halfway across the lipid matrix, and the following simplified substrate kinetic for active Ca^{2+} - transport:

$$P + Ca_i^{2+} \underset{\beta}{\overset{\alpha}{\rightleftarrows}} \overline{P}_{Ca} \xrightarrow{\gamma \exp FV_m / RT} P + Ca_o^{2+} \qquad (8)$$

So, Ca^{2+} - extrusion depends on the membrane potential. $\overline{P}_{Ca}$ is an intermediate complex formed by Ca^{2+} bound to the ligand *P* (an ion, a molecule or a molecular group that binds to another chemical entity to form a larger complex). The binding reaction of *P* and Ca_i^{2+} is treated as fast as compared with actual Ca^{2+} transport across the membrane, so the association of Ca^{2+} and *P* is a quasi steady – state reaction. The conservation law is given by:

$$P + P_{Ca}^{-} = P_0 = \text{constant} . \tag{9}$$

The overall electric current (in amperes) is then given by:

$$I_{Ca} = z\, n\, i_{Ca} , \tag{10}$$

where *z* is the cation valency (being 2 for calcium).

Turning next to the case of potassium ions, the transmembrane flux of K^+ (K^+ - current) is given by:

$$I_K = \overline{g}_K \cdot X_K \left(V_m - E_K\right) + P_K , \tag{11}$$

where $\overline{g}_K$ is the effective K^+ - conductance (1 / mV. *s*); X_K is the ratio of the open – channels; P_K is the pump rate of K^+ $P_K < 0$ (1 / *s*).

It is assumed that $[K^+]_i$ is independent of time, as it is highly compared with $[K^+]_o$.

($[K^+]_i$ = 20 mmol dm^{-3}). At steady – state, $I_K = 0$, $V_m = V_m$; thus from Eq. (11):

$$\frac{\overline{g}_K \cdot X_K}{P_K} = -\frac{1}{\overline{V}_m - E_K} . \tag{12}$$

The complete K^+ - current is:

$$I_K = \overline{g}_k \cdot \left(V_m - E_K\right) \cdot \sum_{i=1}^{3} X_{Ki} + P_K . \tag{13}$$

The total current across the *Paramecium* membrane is:

$$I = C \cdot \frac{dV}{dt} + I_{Ca} + I_K , \tag{14}$$

where $C \cdot \frac{dV}{dt}$ is the capacitive current and *C* is the overall capacitance of the membrane (in e / mV).

The model describes ciliary reversal on a short-time scale. In order to make the model describe long-lasting changes in the Ca^{2+} - influx system, the reaction scheme of Ca^{2+} - channel was extended by assuming an additional slow reaction coupled to the conformation X_4 [17].

$$X_4 \underset{f}{\overset{d}{\rightleftarrows}} X_5 \tag{15}$$

where *d* and *f* are the rates of reaction on a long time scale (>10 sec.). So, Eq. 1 is no longer valid, and it must be written in a new form:

$$\sum_{i=1}^{5} X_i + X_1^* \equiv \tilde{X_\circ} = \text{constant} \tag{16}$$

and then normalized with respect to $\tilde{X_0}$.

Hook and Hildebrand (1979) estimated the parameters of their model for *Paramecium* in the following way.

As a fixed point, the steady-state membrane potential V_m is -30 mV, and the ionic concentrations are as follows: The intracellular Ca^{2+} - concentration is 10^{-8} mol. dm^{-3}, $[K]_i$ is 20 x 10^{-3} mol.dm^{-3}, at given external Ca^{+2} and K^+ - concentrations $[Ca^{2+}]_o$ is 10^{-8} mol.dm^{-3} and $[K^+]_o$ is 2 x 10^{-3} mol.dm^{-3}.

The overall membrane capacitance of 1 μF/cm^2 together with an estimated total area of ciliary and intraciliary membrane of about 10^{-3} cm^2, yield a value of membrane capacitance, C, of 6.5 x 10^6 (e/mV).

For the probability of Ca - channel being open $(X_1^*)^N$, it was found that the steepness of the current-voltage relationship requires a value of N = 10. The effective valency of the gating charge is $\varepsilon_o = 2$.

The number of cilia is n = 10^4, with a respective volume of Δ_i = 3 x 10^{-13} cm^3 (area of cilia A_i = 3 x10^{-10} cm^2, length = 10 μm) and an effective volume of the *Paramecium* body Δ_c = 10^{-5} cm^3. The choice of effective K^+ and Ca^{2+} channel conductivity is as follow:

$$\overline{g}_K = 2.5 \times 10^8 \left(1/\text{mV.s}\right),$$

$$\overline{g}_{Ca} = 3.75 \times 10^5 \left(1/\text{mV.s}\right) \cdot C_\circ = N = 10 .$$

With respect to the extended reaction system (Eq. 15), *f* was chosen to be equal to 5 x 10^{-4} s^{-1}. The relaxation from a high adapted state (high X_5) to a normal excitability is:

$$\frac{d}{dt} X_5^{\sim} \cong -f \cdot X_5^{\sim} . \tag{17}$$

The relaxation time $t_r = 1 / f$, so that t_r lies in the range of 30 min. d was estimated to be 0.1 s^{-1}.

In Hook and Hildebrand's model, the simulated membrane responses to depolarizing and hyperpolarizing current pulses were in good agreement with the measured response [16-18].

So, the advantage of Hook and Hildebrand's model is that it has the ability to fit any cell response expression, because there are enough arbitrary constants from which values can be chosen to make the model to provide a satisfactory fit.

A Two–Segment Ionic Model

When a membrane covered cell, which is freely swimming, is exposed to an external electric field, that part of the cell facing the cathode becomes depolarized (resulting in ciliary reversal), while the part facing the anode becomes hyperpolarized (making the cilia increase their beating in the normal direction; i.e., toward the cell's rear side). A middle area between these two regions is unaffected and the cilia are partially inactivated [3].

In order to solve the Hook and Hildebrand's equations in these circumstances, it is necessary to divide the organism into several segments, the membrane potential perturbation within each segment being constant. Faced with the problem of inordinately long computation times, however, it was decided to utilize a two–segment model, the membrane perturbation in each segment being constant over its surface, and being equal and opposite in the two segments. Thus, no degree of inaccuracy is being tolerated in the interest of obtaining realistic execution times on the computer.

Both segments are assumed to obey the Hook and Hildebrand's equations, the linking conditions being (a) the algebraic sum of the membrane currents entering and leaving each segment is zero (Kirchhoff's first law), and (b) the electrical potentials inside each segment are always equal.

The total currents through each membrane segment, if leakage currents are ignored, are given by:

$$I_1 = I_{Ca1} + I_{K1} + C_1 \, . \, dV/dt \, , \qquad (18)$$

$$I_2 = I_{Ca2} + I_{K2} + C_2 \, . \, dV/dt \, , \qquad (19)$$

where I_{Ca} and I_K are outward calcium and potassium currents (in amperes) through each membrane segment, and C_1 and C_2 are the membrane capacitances (in Farad).

The two linking conditions then yield:

$$\frac{dV}{dt} = \left(I_{Ca1} + I_{Ca2} + I_{K1} + I_{K2}\right) \cdot \frac{1}{C} \, , \qquad (20)$$

where $C = C_1 + C_2$ is the total membrane capacitance.

In these calculations, the electric potentials in various regions are given relative to a point just outside the cell, in the external medium and lying in the mid-plane of the cell.

During a pulse, the potentials outside the two segments are taken to be V_p and $-V_p$, respectively, while before and after the pulse these potentials are taken to be zero. Eq. 20 permits the change in internal potential dV to be calculated during time dt once the various ionic currents are given.

The membrane potential V_m during the pulse is given by:

$$V_m = V_{in} - V_p \, , \qquad (21)$$

where V_{in} is the potential inside the cell. In a resting cell, V_{in} = -30 mV.

It is clear from the experimental results that the membranes at the front and rear sides of the organism are different. A two-segment asymmetric model is therefore required to interpret the experimental results. Such an asymmetry may be reduced in two different ways. First, the ionic channels (Ca^{2+} and K^+) may be non-uniformly distributed over the cell. Second, the characteristics of the ionic channels (e.g. ionic conductance, g_{Ca} and g_K or ionic pump rates) may be different in the two halves of the cell.

It is found that the mutant of *Paramecium* is defective in the stimulus response pathway [20]. The "pawn" mutant; i.e., the cell which cannot swim backward, does not deflect the passive electrical properties (resting potential and input impedance) of the membrane and it also shows no change in delayed rectifying active electrical properties. The important result is that the "Pawns" are defective in their inward current mechanism; i.e., affect Ca^{2+} - channels' mechanism, since they show a different degree of ciliary reversal [20-22].This means that the membrane is not depolarized enough to induce a

reversal. Such evidence of an existence of a difference in the ionic channels' mechanisms in any part of the wild type is not available. So, it is suggested that the only relevant reason so far to cause asymmetry in the two-segment model is to assume that the ionic channels are non-uniformly distributed over the cell's membrane.

One of the observations made in the present study is that ciliates never reverse when swimming towards the cathode, but usually do when swimming initially towards the anode. The present two-segment model was used to find out whether or not a non-uniform distribution of ion channels could account for this phenomenon. Cells were deemed to reverse if the intraciliary calcium concentration exceeded 5 x 10^{-7} mol. dm^{-3}. The table below (Table 1) shows the effect on reversal time of redistributing the Ca^{2+} and K^+ channels between the two segments such that the total number of channels remains constant.

TABLE 1. The distribution ratio of Ca^{2+} and K^+ channels and ciliary reversal time

Y_1 Ratio of Ca^2 channels	Y_2 Ratio of K^+ channels	T_{SR} ciliary reversal time (ms)
0.735	0.265	10
0.745	0.255	120
0.746	0.254	135
0.747	0.253	220
0.748	0.252	450
0.750	0.250	H > 20 s
0.850	0.150	H > 20 s

Y_1 and Y_2 are the distribution ratios of the Ca^{2+} and K^+ channels, respectively, over the posterior segment and *vice versa* for the anterior segment. The total number of Ca^{2+} and K^+ channels for the cell is assumed to be 10^4. T_{SR} is the ciliary reversal time in ms. H signifies a ciliary reversal time of more than 20s. The pulse duration is 0.6 ms and its voltage is 100 mV with the anterior segment towards the anode. No reversal occurs when the polarity of the pulse is reversed. X_5^- is 0.1449.

The reversal time is clearly very dependent on the precise channel distribution. For the purposes of this study, it was found that the value of X_5^- affects the period of ciliary reversal, if X_5^- increases or decreases, the ciliary reversal time decreases or increases, respectively. The optimum ionic channels' distribution was found to be 74.8% and 25.2% for K^+ and Ca^{2+} channels, respectively over the cell's anterior and *vice versa* for posterior. The reasons for this choice are: First, it is found that the longest relevant period of ciliary reversal is 450 ms, at X_5^- = 0.1449, 74.8% and 25.2% of ionic channels' distribution. This ciliary reversal time is followed by a predictable return to a normal ciliary beating. Second, the symbol H in the above table means that the reversal time produced by the model of 75% and 25% or that of 85% and 15% of ionic channels is more than 20 s. At this situation, the model predicts no return to normal beating, and the reason for this is unknown. So, for that reason, this ciliary reversal time, H, is considered as an irrelevant time and is excluded as a criterion for choosing the ratio of ionic channel distribution. Third, there were no significant differences in the values of these ratios; i.e., 74.8% and 25.2%, as X_5^- changed; for example, if X_5^- = 0.1842, the ratios of ionic channels' distribution, Y_1 and Y_2, are 71.6% and 28.4%, which also produced a ciliary reversal time of about 400 ms as the longest relevant time. This model exhibits reversal when the posterior segment is depolarized, but not when the anterior segment is depolarized. Thus, a non-uniform ion channel distribution can account for the asymmetry of the electric pulse response if the correct channel distribution is chosen. With this choice of channel distribution, the two- segment model can now be characterized by the following equations. The calcium and potassium currents through the posterior segment are given by:

$$I_{Ca1} = 0.748\, I_{Ca} \ , \qquad (22)$$

$$I_{K1} = 0.252\, I_{K} \ , \qquad (23)$$

where I_{Ca} and I_K are the total calcium and potassium currents, respectively, through the whole cell (Eq.18 and Eq. 19). Similarly, the currents through the anterior segment are given by:

$$I_{Ca2} = 0.252\, I_{Ca} \, , \qquad (24)$$

$$I_{K2} = 0.748\, I_{K} \, . \qquad (25)$$

The diffusion of cytoplasmic calcium within the two segments is given by:

$$\frac{d}{dt}\left[Ca^{2+}\right]_c = \upsilon' \cdot \left(\left[Ca^{2+}\right]_{i1} + \left[Ca^{2+}\right]_{i2} - 2\left[Ca^{2+}\right]_c\right), \quad (26)$$

where $[Ca^{2+}]_{i1}$ and $[Ca^{2+}]_{i2}$ are the intraciliary Ca^{2+} in cilia over posterior and anterior segments, respectively.

It is assumed that Ca^{2+} ions can diffuse freely from one segment to the other, so the intracellular calcium concentration is always equal in the two segments.

The one other parameter which needs adjustment in the two-segment model is the degree of long-term adaptation of the individual cell represented by the parameter X_5^-.

Hook and Hildebrand's (1980) estimates for the rate constants were d = 0.1 s^{-1} and f = 5 x 10^{-4} s^{-1}, corresponding to X_5^- = 0.2315. This value of X_5^- produces no reversal at all in the two-segment model, and presumably corresponds to cells in a state of high adaptation as voltage clamped specimens would be. By adapting the new values of f = 7.6 x 10^{-4} s^{-1} with d unchanged, the value of X_5^- becomes about 0.145 and corresponds more closely to cells which have not reversed for some time.

The computer program for the two-segment model is designed to solve, as a function of time, the change in *Paramecium* membrane parameters (e.g.; V_m, $[Ca^{2+}]_I$, I_{Ca}, I_K, … etc.), when the cell is exposed to an external electric field. The program consists of a subroutine loop, inside which are all the membrane parameters, which, apart from the membrane potential and cytoplasmic calcium concentration, are solved with respect to whether the segment is an anterior or a posterior segment.

Discussion

Hook and Hildebrand's model has been adapted to deal with asymmetrical excitation, due to external electric pulses, for the freely swimming paramecia, according to their swimming direction before the pulse. This adaption has been achieved by dividing the cell into two segments and by assuming that the ionic channels are non-uniformly distributed over the cell.

The two-segment model is based on the assumption that all the cells are identical, but this seems likely to be not true, because the experimental results show an opposite finding to this assumption. It is found for a given electric pulse, that some cells swimming in the direction of the cathode do reverse, while others do not. This asymmetrical response may be caused by one or more of the following suggestions: (1) Larger cells may have more ionic elements (especially Ca^{2+} - levels) than smaller cells, so the effect of a pulse will depend upon the cell size; thus the probability of reversing may depend upon cell size; (2) Cells of an identical size, but having differences in the lengths of cilia, may also have different numbers of ionic channels, lending to asymmetric responses; (3) The other possibility which may lead to an asymmetrical response is that the ionic conductance values are different among the cell population, so those cells which have a high ionic conductance, especially calcium conductance, before the pulse may reverse due to a given pulse are more readily than those which have low calcium conductance; (4) It is found that the steady–state value of X_5^- (the slow reaction) has affected the ciliary reversal time when the two-segment model is simulated. As X_5^- is low, the cell reverses for longer than one which has high X_5^-, and as X_5^- is higher, there is no reversal produced by the model. Since there is no evidence to support the suggestions in points 1, 2 and 3, I considered that the difference in the steady–state values of X_5^- among cell population is a reasonable cause to make the two–segment model explain the percentage of cell reversing. Not enough is known about the percentage reversal, but my analysis is a first attempt to address the problem.

Many researchers proposed dynamic and physical models for *Paramecium* cells, to analyze their swimming behavior, geotaxis, thigmotaxis and gravikinesis, and their responses to different stimuli to utilize microorganisms as micro-robots in many applications [5, 6, 23, 24, 25, 26, 27, 28].

In a future work, I will try to make a comparison of experimental results and computer predictions of the two-segment model.

Summary

In this paper, I proposed a two-segment physical model of *Paramecium* based on Hook and Hildebrand's model (1979, 1980). This two-segment model is the first attempt to investigate the behavior of free-swimming *Paramecium* due to electrical stimulation.

References

[1]Machemer, R., Machemer, H. and Brauker, R. Journal of Comparative Physiology A, 179 (1996) 213.

[2]Eckert, R. and Naitoh, Y., J. Gen. Physiol. 55 (1970) 467.

[3]Machemer, H. and Peyer, J., "Swimming Sensory Cells: Electrical Membrane Parameters, Receptor Properties and Motor Control in Ciliate Protozoa", (Verhandlungen der deutschen, zoologischen Gesellschaft, G. Fischer Verlag, Stuttgart, 1977) 86-110.

[4]Clark, K.D. and Nelson, D.L., Cell Motile Cytoskeleton, 19(2) (1991) 91.

[5]Ogawa, N., Oku, H., Hashimoto, K. and Ishikawa, M., "Dynamics Modelling and Real –time Observation of Galvanotaxis in *Paramecium caudatum*". (Bio-Mechanisms of Swimming an Flying, Springer, Japan, 2008) 29-40.

[6]Ogawa, N., Oku, H., Hashimoto, K. and Ishikawa, M., Journal of Theoretical Biology, 242 (2006) 314.

[7]Yamaguchi, T., J. Fac. Sci., Tokyo Univ. IV, 8 (1960) 573.

[8]Okumura, H. and Yamaguchi, T., Zool. Mag. 71 (1962) 157.

[9]Naitoh, Y. and Eckert, Z. Vergl. Physiol. 161 (1968) 427.

[10]Doughty, J.M., Comp. Biochem. Physiol. 61c (1978) 369.

[11]Doughty, J.M., Comp. Biochem. Physiol. 61c (1978) 375.

[12]Doughty, J.M., Comp. Biochem. Physiol. 63c (1979) 183.

[13]Machemer, H., "Motor Control of Cilia", In: (Görtz, H.D., ed). (Paramecium, Springer Verlag, Berlin, Heidelberg, New York, Tokyo, 1988) 216-235.

[14]Machemer, H., J. Protozool. 3 (1989) 463.

[15]Al–Rudainy, A., Dirasat Journal, 36 (2) (2009) 105.

[16]Hook, C. and Hildebrand, E., J. Math. Biol. 8 (1979) 197.

[17]Hook, C. and Hildebrand, E., J. Math. Biol. 9 (1980) 347.

[18]Hill, T.L., J. Theor. Biol. 10 (1966) 442.

[19]Kung, C., Chang, SY., Satow, Y., Houten, J.V. and Hansma, H., Science, 188 (4191) (1975) 898.

[20]Stanley, J., Bennet, M.V. and Katz, G.M., J. Exp. Biol. 65 (1976) 699.

[21]Browning, J.L., Nelson, D.L. and Hansma, H., Nature, 259 (1976) 491.

[22]Satow, Y. and Kung, C., J. Exp. Biol. 84 (1980a) 57.

[23]Blake, J., Journal of Biomechanics, 6 (2) (1973) 133.

[24]Fukui, K. and Asai, H., Biophysical Journal, 47 (4) (1985) 479.

[25]Sikora, J., Braranowski, Z. and Zajaczkowska, M., Experientia, 48 (8) (1992) 789.

[26]Hasegawa, T., Ogawa, N., Oku, H. and Ishikawa, M., IEEE International Conference on Robotics and Automation, ICRA (2008).

[27]Jana, S., Bulletin of American Physics Society APs, 55 (2) (2010).

[28]Takeda, A., Mogami, Y. and Shoji, A.B., Biological Sciences in Space (BSS), 20 (2) (2006) 44.