Directed Cell Movement in Pulsed Electric Fields

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Human granulocytes exposed to pulsed electric guiding fields were investigated. The trajectories were determined from digitized pictures (phase contrast). The basic results are: (i) No directed response was induced by pulsed electric guiding fields having a zero averaged field. (ii) A directed response was induced by pulsed electric guiding fields having a non-zero averaged field. (iii) The directed response was enhanced for pulse sequencies having a repetition time of 8 s. (iv) The lag-time between signal recognition and cellular response was 8–10 s. The results are discussed in the framework of a self-ignition model.

Introduction

Cells like leukocytes, fibroblasts, neural crest cells, etc., can adhere on a substrate in order to move and they have the ability to respond to extracellular chemical and physical signals resulting in directed and non-directed movements.

Cybernetics is an important concept to understand biological phenomena also known as the theory of automatic control (Wiener, 1961). It was already shown (Gruler, 1990; Gruler and Franke, 1990; Gruler, 1991) that chemotaxis, galvanotaxis, galvanotropism, contact guidance are functions of cells having a goal-seeking system which is an automatic controller having a closed-loop feedback system.

The translational migration of cells in an external guiding field requires two components for cellular response: the speed, υ_c, and the angle of migration, φ. One might expect that these two parameters would depend on each other. But they are independent of each other since (i) the temporal variations of $\upsilon_c(t)$ and $\varphi(t)$ are independent of each other, and (ii) the mean track velocity is independent of the angle of migration. This holds at least for human granulocytes and monocytes (Gruler, 1984; Rapp *et al.,* 1988; de Boisfleury-Chevance *et al.,* 1989; Franke and Gruler, 1990), somitic fibroblasts (Gruler and Nuccitelli, 1984), and neural crest cells (Gruler and Nuccitelli, 1991). Thus the speed and the angle of migration are two independent variables. A model on a molecular scale for the directed and non-directed movement will be presented. The predictions of this model are compared with experimental results.

Speed controller and automatic steering mechanism

In the chemokinetic response, the cell receives an exogenous signal. By means of the signal transduction/response system of the cell, the product – here the speed – corresponding to the received signal, is released. To our knowledge a cellular speed controller has no device which controls whether the product really corresponds to the received signal or not. The speed controller works linearly since the mean speed is proportional to the mean concentration of membrane-bound receptors loaded with chemokinetic molecules (Gruler, 1990; Gruler and Franke, 1990).

Let us now consider the steering mechanism for the directed movement: The directed movement can be modelled by assuming an automatic steering mechanism (Gruler, 1990; Gruler and Franke, 1990). The basic elements of an automatic controller are: first, an element which measures the output of the biological system; second, a means of comparing that output with the desired one. Third, a means of feeding back this information into the input in such a way as to minimize the deviation of the output from the desired level. This means in the case of directed movement, the cell must have

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the ability to measure its actual angle of migration with respect to the guiding field. It is assumed in a simplified model of the chemotactic response that the cell compares the number of loaded receptors between the left and right side (Gruler, 1988; Tranquillo *et al.*, 1988). An intracellular signal is built up by the receptors so that the cell rotates during its migration towards the desired angle. At the end of this article a model will be presented how this comparing mechanism could work.

The rate equation of the angle to migration is composed of two terms (Gruler, 1990; Gruler and Franke, 1990):

$$\frac{d\varphi}{dt} = -\Gamma_D(Signal,\ \varphi) + \Gamma_N(t). \qquad (1)$$

(i) The deterministic torque, $\Gamma_D(Signal,\ \varphi)$, tries to render the movement in the desired direction and (ii) the stochastic torque, $\Gamma_N(t)$, tries to render the movement in an arbitrary direction. The stochastic torque exists since random walk is observed in case of the absence of a signal.

The distribution of the migration angles, $f(\varphi)$, can be predicted from Eqn. (1) if the random term $\Gamma(t)$ is known. In case of a white noise source ($\langle\Gamma(t)\rangle = 0$ and $\langle\Gamma(t)\Gamma(t-t')\rangle = Q\cdot\delta(t-t')$ where Q quantitates the white noise strength) the predicted steady state angle distribution is (for details see Schienbein and Gruler, 1993))

$$f(\varphi) = e^{V(\varphi)} \qquad (2)$$

where $V(\varphi)$ is the generating function (Haken, 1983)

$$V(\varphi) = \frac{2}{Q}\int_0^{\varphi} \Gamma_D(Signal,\ \varphi')\,d\varphi'. \qquad (3)$$

The still unknown deterministic torque, $\Gamma_D(Signal,\ \varphi)$, was approached by a Fourier Series

$$\Gamma_D(Signal,\ \varphi) = c_0 + c_1\cdot\sin\varphi + c_2\cdot\sin(2\varphi) + \ldots \qquad (4)$$

where the coefficients c_i are independent of the angle of migration but functions of the cellular machinery and the cellular environment.

The following results were obtained:

- If only the first term in Eqn. (4) is non-zero, the deterministic part of Eqn. (1) reads then $\frac{d\varphi}{dt} = c_0$: The object of interest moves with a constant angle velocity. A constant track velocity in connection with a constant angle velocity yields circles as trajectories. Helical motion is common for free-swimming organisms: spirochaete bacteria (Goldstein and Charon, 1988; Jennings and Crosby, 1901), flagellates (Jennings, 1904; Mast, 1911), ciliates (Bullington, 1925; Jennings, 1904), the motile spores of fungi (see Hickmann and Ho, 1966) and of plants (Brokaw, 1958a and 1958b; Buller, 1900; Müller, 1978), the spermatozoa of many invertebrates (see Miller, 1985) and vertebrates (Philips, 1972), and many micrometazoa (Jennings, 1904) all move along helical paths.
- The orientation of movement (or growth) is directed (= polar) if the coefficient of the second term is non-zero. Different directed cellular responses are investigated as galvanotaxis where an electric field guides the cells, and as chemotaxis where a concentration gradient acts as a guiding field. The coefficient $a_1\left(=\frac{2c_1}{Q}\right)$ which is related with the second coefficient of Eqn. (4), is a function of the strength of the guiding field. The coefficient a_1 can be determined from the steady state angle distribution function. In the case of galvanotaxis, the coefficient, a_1, is found to be proportional to the electric field strength E, $a_1 = K_G\cdot E$, where the galvanotaxis coefficient, $K_G = 2k_{PG}/Q$, quantifies the cellular response. k_{PG} and Q are machine coefficients which quantify the deterministic and the stochastic part of the cellular automatic controller, respectively. This linear response was found for human granulocytes (Rapp *et al.*, 1988; Franke and Gruler, 1990), for neutral crest cells (Gruler and Nuccitelli, 1991), for bracken spermatozoids (Brokaw, 1958a and 1958b; Gruler 1988) and for the directed growth of spores in an electric field (Gruler and Gow, 1990).
- The orientation of moving (or growing) cells is bidirected (= apolar) if the third coefficient of Eqn. (4) is non-zero. Different bidirected cellular responses are investigated as contact guidance, apolar orientation in an electric field, bidirectional growth in an electric field. Again the coefficient $a_2\left(=\frac{2c_2}{Q}\right)$ which is related with the third term of Eqn. (4) is a function of the strength of the guiding field. The coefficient a_2 can be determined from the steady state angle distribution function. Granulocytes are mainly elongated in the direction of migration. But other cells like fibroblasts (Erickson and Nucci-

telli, 1984), neural crest cells (Cooper and Keller, 1984), etc., in addition have the ability to orient their long axis perpendicularly to the applied electric field. A similar situation holds for hyphae growing in an electric field (McGillivray and Gow, 1986; Gruler and Gow, 1990). The coefficient c_2 is found to be proportional to the square of the electric field strength, $c_2 = (k_2 \cdot E)^2$. The quadratic response was found for fibroblasts (Erickson and Nuccitelli, 1984; Ross *et al.,* 1989), amoebas (Friend *et al.,* 1975) and growing hyphae tips (Gruler and Gow, 1990).

If a cell exhibits both a polar and an apolar response than one can define a transition: The response is mainly a directed one if the maximum of the angle distribution function is in the direction of the applied electric field. This holds for $|a_1| > 4|a_2|$. The bidirectional response becomes dominant if the maximum of the angle distribution is off the direction of the applied electric field. This holds for $|a_1| < 4|a_2|$. Growing hyphae tips showed the expected behaviour (McGillivray and Gow, 1986; Gruler and Gow, 1990).

Up to now we have a detailed knowledge of the deterministic torque, $\Gamma_D(Signal, \varphi)$. For example, the directed response is proportional to the applied polar guiding field ($c_1 = k_{PG} \cdot E$). This knowledge originates from steady state angle distribution functions. For the stochastic torque we assumed a white noise source where all frequencies have the same amplitude. The white noise source was introduced in order to decrease the mathematical problems with the stochastic differential equation. To test the white noise assumption the frequency dependent cellular signal transfer function has to be investigated.

The dynamic behaviour of the cellular signal transduction/response mechanism can be investigated if the extracellular signal is varied in time. The electric field as the extracellular signal is the most suitable candidate for this type of experiment since the signal is fast and easy to apply to many cells simultaneously.

Materials and Methods

The experiments were performed as previously described (Franke and Gruler, 1990). In short, the cell preparation – granulocytes were separated from heparinized venous blood of healthy human blood donors on a histopaque density gradient: Equal volumes of blood and of Hanks Balanced Salt Solution (HBSS) were mixed. The centrifuge tube contained layers of 3 ml Histopaque-1119, and 3 ml Histopaque-1077, and 6 ml blood-HBSS solution. The cells were centrifuged for 20 min at 300 × *g*. The granulocytes were found enriched between the two Histopaque regions. The cells were washed 3 times in plasma-HBSS of the same donor (first wash: 300 × *g* for 10 min, second and third wash: 200 × *g* for 10 min). All solutions were adjusted to pH 7.4 (stabilization with sodiumhydrogencarbonate (0.35 g/l) and 28 mM HEPES (N-2-hydroxythylpiperazin-N′-2-ethanesulfonic acid). The air in the test tube was enriched with CO_2 before it was closed (Franke and Gruler, 1990).

An electric field is created in a galvanotaxis chamber by the electric current driven through the aqueous solution. In short the preparation of the galvanotaxis chamber – a drop of the cell suspension (4×10^6 cells/ml) was transferred to a glass slide and then covered with a coverslip (20 × 30 mm^2). Capillarity makes a homogeneous thin film (10–30 μm). The long sides of the coverslip were attached with paraffin to the glass slide. On the small sides two assemblies consisting of two filter papers (20 × 20 mm^2), an enamel block (20 × 20 × 0.5 mm^3) containing two parallel ribbons of metal (Ag) foil as power- (7 × 25 mm^2) and measuring (4 × 25 mm^2) electrode, were attached with paraffin to the glass slide. The electrodes were chlorinated before every use. The damp layer of filter paper beneath the enamel block must be in direct contact with the cell suspension. The power electrodes were connected with the output of an amplifier. The measuring electrodes were connected to a AD-converter of a computer (AT). The output of the DA-converter of the computer was connected with the input of the amplifier. The current through the sample was regulated to get a desired voltage drop across the measuring electrodes.

The galvanotaxis chamber was placed on the heating stage of a phase contrast microscope (25x) where the objective was kept on a constant temperature and had its own control unit. The temperature in the galvanotaxis chamber was calibrated (37 °C) with a temperature-sensitive cholesteric liquid crystal as previously described (Matthes and Gruler, 1988).

The cell movement was observed with a video camera and recorded on VHS video tape (real time). The video signal was also digitized for the computerized data analysis (Atari 1040 ST). Before this procedure the inhomogeneous illumination of the sample was eliminated by sending the video signal through a high pass filter ($f > 15$ kHz). The center of gravity of the migrating cells is approximated by the center of area of the cell contour line.

Results and Discussion

Electric field jump studies

Granulocytes cannot react immediately to variations in the extracellular signal as it was already demonstrated by electric field jump studies (Franke and Gruler, 1990) and by concentration jump experiments (Gerisch and Keller, 1981). In the electric field jump study, the electric field was altered from $-E(t<0)$ to $+E(t>0)$ and the mean galvanotactic response was determined. The two remarkable results are: (i) The measured lag-time between signal recognition and cellular response was 8.3 s. (ii) The cellular relaxation process can be described by a single exponential function with a characteristic time of 32 s. These measurements were performed at an electric field of $|E| = 1.0$ V/mm (Franke and Gruler, 1990). But unfortunately above 0.8 V/mm the galvanotactic response is inhibited and therefore we repeated the field jump experiment with $|E| = 0.8$ V/mm. The result is shown in Fig. 1. The delay-time was 10 s and the characteristic time of the single exponential was 42 s. The results are in accordance with the previously measured ones. The measured delay-time in the cellular signal transduction line is one of the key-stones in the development of a new model for the directed and non-directed movement.

The time-dependent polar order parameter can be predicted from the stochastic differential equation (Eqn. (1) in connection with Eqn. (4)) by solving the corresponding Fokker-Planck equation. For more details to the calculation see Schienbein and Gruler, 1993. A systematic deviation exists between the measured characteristic time ($\tau_{exp} = 42$ s) and the predicted one ($\tau_{calc} = 31$ s). The calculation is based on the galvanotactic coefficient, $K_G = (2\,k_{PG})/Q = -4.5$ mm/V, obtained from the galvanotactic dose-response curve, and the white noise strength, $Q = 0.034\ \mathrm{s}^{-1}$, obtained from the mean-squared displacement. The systematic deviation between prediction obtained from the automatic controller having a white noise spectra, and the actual measured data is a hint that the mechanism responsible for directed movement has to be altered slightly.

In a second series of experiments, the directed response was investigated when the pulsed electric guiding field was switched-on. For ($t<0$), no electric guiding field was applied. The migrating cells showed a random walk and the directed cellular response, ⟨*cos* φ⟩, was zero. At $t = 0$, the pulsed electric guiding field was swiched-on. The measured polar order parameter as a function of time is shown in Fig. 2. The cellular response would be a

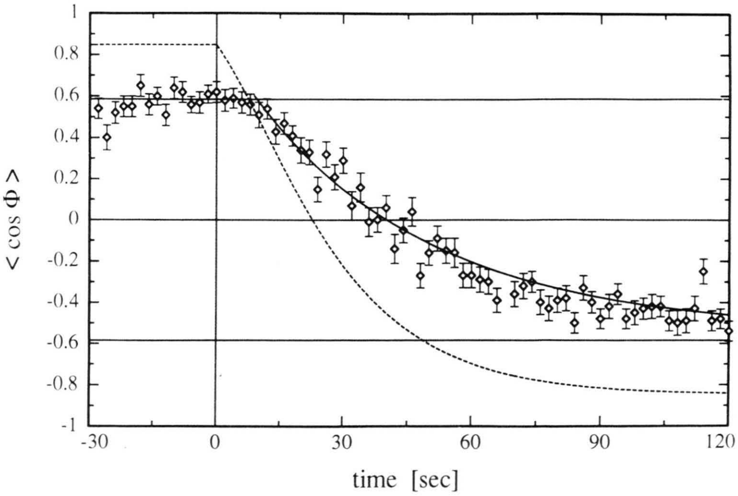

Fig. 1. Electric field-jump experiment: The polar order parameter, ⟨*cos* φ⟩, as a function of time, *t*, is shown (0.8 V/mm, 80 cells). The electric field changed sign at $t = 0$. The cell pictures were measured every 2 s. The dots are the measured polar order parameter, ⟨*cos* φ⟩. The horizontal lines indicate the two steady state values, respectively. The line fitted to the experimental data points, is an exponential decay function with a characteristic time of 42 s and a lag-time of 10 s. The dashed line is a theoretical prediction (Schienbein and Gruler, 1993). Notice, the polar order parameter measured every 2 s, is systematically too small due to the error in the determination of the cell position.

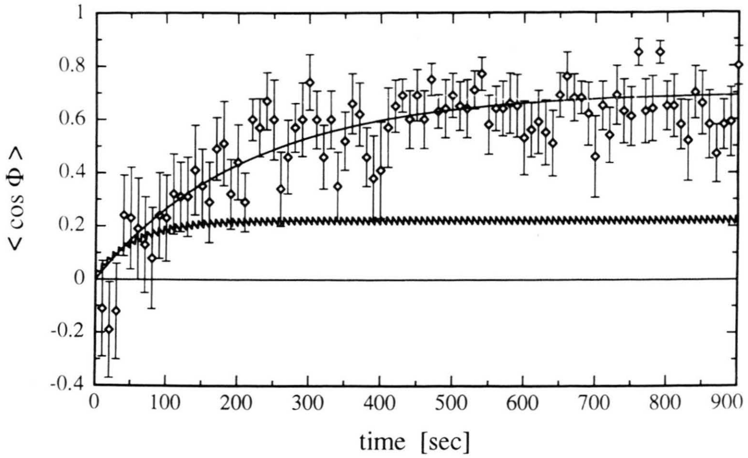

Fig. 2. Electric field-jump experiment with pulsed electric fields ($t_1 = 1$ s, $E_1 = 0.8$ V/mm and $t_2 = 7$ s, $E_2 = 0$ V/mm): Polar order parameter as a function of time is shown. For $t < 0$ s, no voltage was applied. At $t = 0$ s, the pulsed electric field was switched on. A single exponential function was fitted to the data ($\langle cos\ \varphi \rangle_{steady\ state} = 0.6$ and $\tau = 200$ s). The zig-zag line is a theoretical prediction obtained with $K_G = (2\ k_{PG})/Q = -4.5$ mm/V and $2/Q = 58$ s (Schienbein and Gruler, 1993).

step function if the cells could react immediately. However, the cells required some time to react to the new environmental condition. Again the relaxation process in the directed movement can be fitted by a single exponential function as in case of a constant electric guiding field (see Fig. 1). The fitting parameters are $\tau_{exp} = 200$ s and the steady state polar order parameter, $\langle cos\ \varphi \rangle_{ss} = 0.6$. The zig-zag line in Fig. 2 is the predicted time-dependent polar order parameter ($K_G = (2\ k_{PG})/Q = -4.5$ mm/V and $2/Q = 58$ s). Again there are systematic deviation between the model calculation and the actual data: First, the cells responded to the pulsed guiding field about three times more sensitive as expected. Second, the cells did not react so fast as predicted ($\tau_{exp} = 200$ s and $\tau_{calc} = 50$ s).

Next the cellular response to pulsed fields is investigated in details.

Pulsed field studies

Pulses having a zero averaged field

In the first series of experiments the pulses were chosen symmetric in respect to the field direction: The first electric pulse had a duration time of t_1, then the polarity of the electric field was changed. The duration time, t_2, of the second pulse was identical with that of the first pulse ($t_1 = t_2$). The mean electric field measured in the galvanotaxis chamber was zero as expected. The experiment was performed with a repetition time T ($= t_1 + t_2$) between 1.5 s and 10 s. Approximately 20 cells were observed over 5 to 10 min in each experiment. The polar order parameter (Gruler, 1990) as the average of $cos\ \varphi$ and as the McCutcheon index, $\langle \upsilon_{||} \rangle / \langle \upsilon_c \rangle$, were: 0.24 ± 0.075 and 0.25 ± 0.07 ($T = 1.5$ s), 0.01 ± 0.07 and 0.015 ± 0.07 ($T = 3$ s), 0.17 ± 0.05 and 0.18 ± 0.06 ($T = 5$ s), -0.13 ± 0.06 and $-0.14\ \pm\ =\ .08$ ($T\ =\ 6$ s), $0.02\ \pm\ 0.1$ and 0.04 ± 0.11 ($T = 9$ s), 0.05 ± 0.05 and 0.04 ± 0.06 ($T = 10$ s). The cells showed no directed movement if the mean electric guiding field is zero.

In the next series of experiments, the sequence of the electric pulses was altered: The duration, t_1, and the electric field strength, E_1, of the first pulse and the duration, t_2, and the electric field strength, E_2, of the second pulse was adjusted in such a way that the mean electric field was zero ($E_1 \cdot t_1 + E_2 \cdot t_2 = 0$). The polar order parameter, $cos\ \varphi$, and as the McCutcheon index, $\langle \upsilon_{||} \rangle / \langle \upsilon_c \rangle$, were: 0.12 ± 0.20 and 0.14 ± 0.23, respectively ($E_1 = 0.8\ V$/mm, $t_1 = 0.78$ s, $T = 5$ s), 0.03 ± 0.09 and 0.02 ± 0.10, respectively ($E_1 = 0.8\ V$/mm, $t_1 = 1$ s, $T = 8$ s), and 0.04 ± 0.13 and 0.02 ± 0.13, respectively ($E_1 = 0.8\ V$/mm, $t_1 = 1.57$ s, $T = 10$ s). Again, the cells showed no directed movement in case the mean electric guiding field is zero.

Next we will show how these results are in accordance with the automatic controller having a linear response (Eqn. (4) with $c_1 = k_{PG} \cdot E$).

The model assumptions inherent in the stochastic differential equation (Eqn. (1)) are that the migration angle changes stochastically without any time correlations (white noise assumption), but is driven deterministically towards the direction of the guiding field. The mean deterministic torque is in case of a linear response

$$\langle c_1 \rangle = k_{PG} \cdot \langle E \rangle. \tag{5}$$

The deterministic torque was zero for the above described experiments since $\langle E \rangle$ was zero. No directed movement is expected as actually observed. A more rigerous calculation where the Fokker-Planck equation is solved (see Schienbein and Gruler, 1993), leaded to the same result.

The next question: Can the applied alternating electric guiding field induce an apolar cellular response? The apolar order parameter can be determined by taking the average of $cos 2\varphi$ (Matthes and Gruler, 1988). The migration angle φ in respect to the guiding field direction was measured and the average of $cos 2\varphi$ was determined: 0.03 ± 0.015 ($T = 1.5$ s), 0.005 ± 0.015 ($T = 3$ s), 0.0 ± 0.03 ($T = 5$ s), -0.01 ± 0.02 ($T = 6$ s), 0.05 ± 0.03 ($T = 9$ s), 0.01 ± 0.02 ($T = 10$ s). No apolar response induced by an alternating electric guiding field was observed.

Pulses having a non-zero averaged field

In the next series of experiments, the sequence of the electric pulses was altered. In all the experiments, the duration, t_1, and the electric field strength, E_1, of the first pulse was unchanged ($t_1 = 1$ s, $E_1 = 0.8$ V/mm). A period, t_2 (between 0 and 31 s), followed with no voltage ($E_2 = 0$). A directed cellular response is expected since the mean electric field and the mean deterministic torque are non-zero. The experimental results are shown in Fig. 3 where the measured polar order parameter, $\langle cos\ \varphi \rangle$ as a function of the repetition time, T ($= t_1 + t_2$), is shown: The pulsed electric field with a non-zero averaged field induce a directed movement. The line is predicted from the automatic controller (Eqn. (1) and (4) ($K_G = (2k_{PG})/Q = -4.5$ mm/V and $2/Q = 58$ s)) and white noise assumption). The calculated line is systematically below the measured values. The pronounced 8 s period in the cellular transfer function reflects a characteristic time of the cellular machinery. The assumed white noise where every frequency has the same probability, is not correct. This failure is not surprising since white noise means that two events separated by a time increment, Δt, are uncorrelated. One expects a strong correlation of temporal events in a working machine. Thus the model of automatic controller has to be modified. The approximation of the cellular transduction/response system as described by Eqn. (1) has to be modified.

Self-organized molecular machine

The important molecular events in the cellular machinery are: (1) Fresh receptors were exposed to the plasma membrane by a triggered vesicle-membrane fusion process. (ii) Specific molecules bind to the receptors and start the chemical amplification chain. (iii) One activated receptor activates many membrane-bound G-proteins. (iv) One activated G-protein activates many membrane-bound phospholipase C proteins. (v) One activated phospholipase C protein hydrolysis many polar head groups of an ATP activated phospholipid (phosphatidyl inositol 4,5-biphosphate) at the inner side of the plasma membrane. (vi) Two cellular messen-

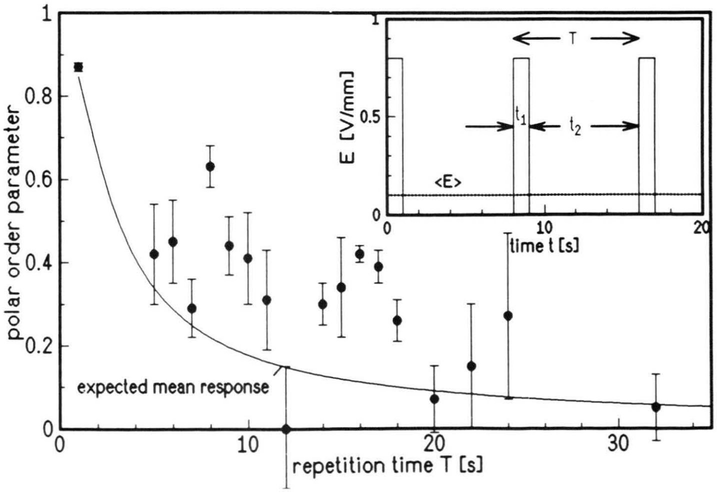

Fig. 3. The steady state polar order parameter as a function of repetition time, T, is shown where the granulocytes were exposed to pulsed electric fields ($t_1 = 1$ s, $E_1 = 0.8$ V/mm, $E_2 = 0$, repetition time T ($= t_1 + t_2$) see insert of this figure). The line is a theoretical prediction obtained with $K_G = (2\ k_{PG})/Q = -4.5$ mm/V and $2/Q = 58$ s (Schienbein and Gruler, 1993).

gers are formed by this process: (a) The inositol 1,4,5-triphosphate opens ion channels, triggers the release of intracellular Ca^{2+}, and the fusion of receptor-loaded vesicles with the plasma membrane and (b) diacylglycerol destabilizes locally the plasma membrane and activates the protein kinase C.

Our working hypothesis is that the increase in calcium concentration in connection with the second messenger inositol 1,4,5-triphosphate are the stimuli for the fusion of the vesicles with the plasma membrane. Thus we are dealing with a self-ignition machine like a Diesel engine where the lag-time between signal application and cellular response plays the important role.

The following experimental facts are in accordance with our working hypothesis. The measured lag-time between signal application and cellular response (= formation of a new leading front) has the expected value. It was approximately 10 sec in case of chemotaxis (micropipet) (Gerisch and Keller, 1981), 8–10 sec in case of necrotaxis where granulocytes were attracted by a lysed red blood cell (de Boisfleury-Chevance and Gruler, to be published) and 8–10 sec in case of glavanotaxis (see Fig. 1 and Franke and Gruler, 1990). The growth of a new leading front is correlated with an increase of intracellular free calcium and the lag-time was approximately 10 sec between signal application and the onset of a strong increase in the intracellular free calcium (de Boisfleury-Chevance and Gruler, to be published). In case of vesicle fusion the electrical capacitance of the membrane is altered and one expects an altered voltage. Jäger *et al.* (1988) measured the transmembrane potential difference of granulocytes with microelectrodes and registrated periodic fluctuations with a characteristic time of 7 sec.

A consequence of this model is that the cell cannot receive continously external signals. The cell can only receive extracellular signals in well defined time increments. This communication time of the cell with its surrounding must be one second or less. To show this we measured the cellular response with different electric pulses: $\langle cos\ \varphi\rangle = 0.62 \pm 0.05$ for $t_1 = 1$ s, $E_1 = 0.8$ V/mm, $T = 8$ s and $\langle cos\ \varphi\rangle = 0.58 \pm 0.05$ for $t_1 = 2$ s, $E_1 = 0.8$ V/mm, $T = 8$ s. No significant difference in the polar order parameter was observed.

An experimental result is that the cellular speed and the angle of migration are statistically independent variables. This experimental fact can be explained with the self-ignition model. (i) The mean speed is induced by the total number of receptors: In case of a constant signal amplification (from the receptors to the activation of the microfilaments) the mean speed should be proportional to the total number of receptors activated by chemotaxis stimulating molecules as actually observed (Gruler, 1989). (ii) The direction of migration is induced by the spatial distribution of the activated receptors. Let us consider a simplified model: The cell has only two sensitive parts (separated by the distance l_0). Their physical and chemical state can be altered by the intra and extra cellular environment. These hypothetical patches are located at the front on both sides of the cell. Let us consider now the self-ignition model. Molecules bind to the receptors and after 8 s inositol 1,4,5-triphosphate and calcium are released. Due to the local biochemical amplification chain high concentrations of inositol 1,4,5-triphosphate and calcium (Marks and Maxfield, 1990) are expected at those positions with a high concentration of activated receptors. Thus the concentration of new receptor will be highest at the positions with high inositol 1,4,5-triphosphate and calcium concentrations since the vesicle fusion is more likely at the position with high inositol 1,4,5-triphosphate and calcium concentrations. In case of two patch model the probability of vesicle fusion $\left(\frac{1}{c}\frac{\partial c}{\partial x}\frac{l_0}{2} \ll 1\right)$ is

$$W_\pm = \frac{1}{2}\left(1 \pm n\cdot\frac{1}{c}\frac{\partial c}{\partial x}\frac{l_0}{2}\cdot sin\ \varphi \pm \ldots\right). \qquad (6)$$

The sum of the vesicle fusion probability must be one since the vesicle has to fuse somewhere. Thus one gets $W_+ + W_- = 1$. The induced cellular torque is then proportional to $n\cdot\frac{1}{c}\frac{\partial c}{\partial x}l_0\cdot sin\ \varphi$. The number, n, of cycles can be estimated from the ratio of the persistence time (≈ 58 s) and the cycle time (≈ 8 s) to $n \approx 7$. Galvanotaxis can be treated in analogy to chemotaxis: In this case the vesicle fusion is a function of the membrane potential difference ($U_\pm = U_0 \pm a\cdot E\cdot\frac{l_0}{2}\cdot sin\ \varphi$ with the intrinsic cellular potential difference, U_0, the applied electric field, E, and a geometrical factor a ($\approx 1\cdots 1.5$)). The galvanotactic response is obtained if $\frac{1}{c}\frac{\partial c}{\partial x}$ is replaced by aE/U_0. Now we have a model on a molecular scale which explains many types of directed movement (or growth). In addition the self-ignition is a model with a positive feedback in the signal chain and thus it can explain in parts the capping phenomenon.

The virus-disturbed directed response can be described by the vesicle fusion process (Bültmann and Gruler, 1983). One of the basic abilities of viruses is to fuse with cell membranes thus the proteins of their capsid have the physical ability to fuse with a membrane. Thus, it is not surprising that at sufficient virus concentrations the vesicle fusion process is dominated by the proteins of the viruses. The probability for vesicle fusion is highest at those parts of the membrane with highest virus protein concentrations. Thus one expects that granulocytes are blind in respect to concentration gradients as actually observed. Our model of the virus-disturbed self-ignition mechanism explains the puzzling results: (i) The virus disturbs chemotaxis but does not bind to the receptor and (ii) the virus disturbs chemotaxis but not the cellular speed and the cellular morphology.

Summary

The frequency-dependent signal transfer function of migrating cells can be investigated by using pulsed electric fields as guiding field. The white noise assumption for the stochastic torque in the angle rate equation is not correct and has to be replaced by a colored noise having a maximum at 1/8 Hz. The characteristic time of 8 sec is in accordance with the time delay in the cellular signal chain between loading the membrane-bound receptor and the release of intracellular Ca^{2+}. A self-ignition model is discussed as an essential mechanism involved in directed movement.

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