Bioelectromagnetics 27:528–534 (2006)

Effects of a 50 Hz Magnetic Field on *Dictyostelium discoideum* (Protista)

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Some studies have demonstrated that a few biological systems are affected by weak, extremely low frequency (ELF) electromagnetic fields (EMFs), lower than 10 mT. However, to date there is scanty evidence of this effect on Protists in the literature. Due to their peculiarity as single-cell eukaryotic organisms, Protists respond directly to environmental stimuli, thus appearing as very suitable experimental systems. Recently, we showed the presence of propionylcholinesterase (PrChE) activity in single-cell amoebae of *Dictyostelium discoideum*. This enzyme activity was assumed to be involved in cell–cell and cell-environment interactions, as its inhibition affects cell aggregation and differentiation. In this work, we have exposed single-cell amoebae of *D. discoideum* to an ELF-EMF of about 200 μT, 50 Hz, for 3 h or 24 h at 21 °C. A delay in the early phase of the differentiation was observed in 3 h exposed cells, and a significant decrease in the fission rate appeared in 24 h exposed cells. The PrChE activity was significantly lower in 3 h exposed cells than in the controls, whereas 24 h exposed cells exhibited an increase in this enzyme activity. However, such effects appeared to be transient, as the fission rate and PrChE activity values returned to the respective control values after a 24 h stay under standard conditions. Bioelectromagnetics 27:528–534, 2006.

Key words: ELF magnetic exposure; cholinesterase activity; developmental cycle; single-cell amoebae

INTRODUCTION

Amongst the studies concerning the possible biological effects of extremely low frequency (ELF) electromagnetic fields (EMFs), particular attention should be paid to experiments on Protists. These organisms exhibit highly sophisticated behavior patterns in such biological functions as reproduction, nutrition, motility, excitability, and cell–cell communication (see the next section). Interest in experiments on Protists is based, on the one hand, that such tests can be considered as in vitro, as cells in culture medium are exposed to an EMF, and, on the other hand, the tests are typical of in vivo, because of the fundamental features of Protists as single-cell eukaryotic organisms with a relatively simple organization and a high degree of specialization. In addition, Protists can be cultured in the laboratory under seemingly natural conditions, so their biological responses are reliable.

Despite the very large number of experimental findings on Protists reported in the literature, the number of results available on the effects of ELF-EMFs on their cell activities is scanty. However, it has been observed that magnetic fields affect swimming behavior of different species of Ciliates. The movement pattern appears to be altered both by ELF-EMFs (50 Hz, 0.5–2 mT; 60 Hz, 0.6 T) [Hemmersbach et al., 1997; Nakaoka et al., 2000] and static magnetic fields (0.126 T, 0.68 T) [Rosen and Rosen, 1990; Nakaoka et al., 2002]. Studies on cell population growth have revealed significant decreases in the growth rate of *Amoeba hatchetti, A. castellanii*, and *A. polyphaga* exposed to a uniform static magnetic field (71 or 106.5 mT) [Berk et al., 1997], as well as of *Physarum polycephalum* exposed to a 75 Hz magnetic field [Marron et al., 1975; Greenebaum et al., 1982]; conversely, a 72 Hz EMF increased cell fission rate in *Paramecium* [Dihel et al.,

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Received for review 28 April 2005; Final revision received 6 February 2006

DOI 10.1002/bem.20240
Published online 19 May 2006 in Wiley InterScience (www.interscience.wiley.com).

1985]. Finally, a significant damping effect of a .4 mT pulsed magnetic field on natural nucleotide adenine oscillation has been found during the early aggregation phase of *D. discoideum* [Devies et al., 1999].

In this article, we present the results of experiments on *D. discoideum.* This amoeba, often referred to as a slime mould, exhibits a very simple organization with a very complex developmental cycle. The life cycle of *D. discoideum* (Fig. 1) starts from the reproduction phase by binary fission of single-cell amoebae feeding on bacteria. Depletion of the food source triggers the developmental phase. By chemotaxis, the amoebae aggregate in streams and migrate towards an extracellular cyclic AMP signaling source, giving rise to the first multicellular stage, the mound. The mound elongates upwards and becomes the first finger that can form a slug, migrating toward favorable conditions for culmination, or immediately develops the "mexican hat." This multicellular stage undergoes the process of culmination leading to the formation of the fruiting body anchored to the substratum by the basal stalk and containing two differentiated cell types: the spores localized to the apex and able to reproduce, and the stalk cells unable to multiply [for reviews see Loomis, 1975; Escalante and Vicente, 2000].

Single-cell amoebae of *D. discoideum* were exposed to a 50 Hz magnetic field of about 200 μT. It was found that such exposure affected both the developmental cycle and the activity of the enzyme propionylcholinesterase (PrChE). Here, we show the results of our experiments; but no attempt has been made to determine the possible biophysical mechanisms involved in the action of the EMF on the cell populations.

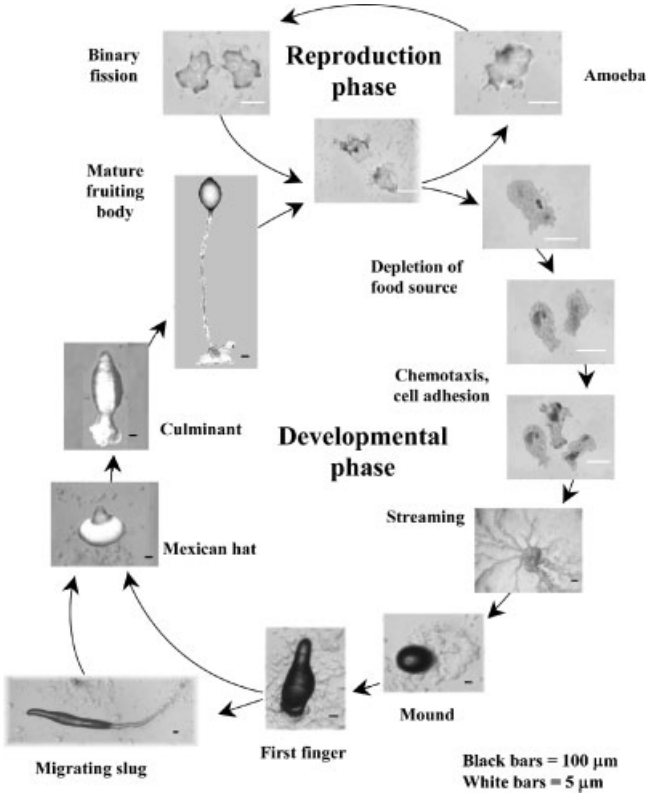

Fig. 1. Developmental cycle of *Dictyostelium discoideum.* The life cycle of *D. discoideum* includes two phases: the reproduction and the developmental phase. The reproduction phase consists of growth and multiplication by binary fission, of single-cell amoebae feeding on bacteria. Nutrient starvation triggers the developmental phase. The amoebae aggregate in streams and migrate, giving rise to the first multicellular stage, the mound, which becomes the first finger. This can form a slug, migrating toward favorable conditions for culmination, or immediately develops "the mexican hat" that undergoes culmination resulting in the formation of the fruiting body anchored to the substratum.

MATERIALS AND METHODS

Cell Reproduction and Development

The phases of reproduction and development were obtained in the laboratory, according to the following procedures. Cell growth and multiplication were induced by inoculating one-eighth of the fruiting bodies, grown on a Petri dish, 9 cm in diameter, into a 50 ml sterile Falcon flask containing 15 ml of Ax-2 axenic medium (14.3 g bacteriological peptone, 7.15 g yeast extract, 0.491 g Na_2HPO_4, 0.486 g KH_2PO_4, 18 g maltose, and distilled water up to 1000 ml, pH 6.7, submitted to 120 °C for 20 min), and supplemented with 30 μl tetracycline (5 mg tetracycline in 1 ml ethanol 96%) [Schwalb and Roth, 1970; Watts and Ashworth, 1970]. The exponentially growing Ax-2 culture was kept at 21 °C and maintained at a cell density of $2–3 \times 10^6$ cells/ml for about a fortnight by periodic dilutions.

The developmental phase was induced by transferring eight drops, each containing 10 μl of the Ax-2 culture, to a B2 *Escherichia coli* monolayer growing on nutrient agar-N plates (1000 ml of 17 mM Sorensen 1×, pH 6.0, 20 g Agar Difco, 1 g glucose, and 1 g peptone, submitted to 120 °C for 20 min). The plates were incubated in a moist chamber for 3 days at 25 °C [Swan et al., 1977]. When the fruiting bodies had developed, the plates were kept at 4 °C. In our experiments, the developmental phase was induced as described in the last subsection.

Experimental Samples

Single-cell amoebae, growing in a 50 ml sterile Falcon flask containing 15 ml of Ax-2 axenic medium, were exposed to the ELF-EMF for 3 h (3 h exposed

samples), or 24 h (24 h exposed samples), or kept in a nonworking ELF-EMF generator for 24 h (24 h pseudo-exposed samples), at 21 °C. The respective controls, 3 h, and 24 h controls, were kept outside the ELF-EMF generator. Moreover, the 24 h exposed samples were transferred to standard conditions for 24 h (24 h nonexposed samples), and their respective control was 48 h control.

Exposure System

The sample holder containing the cell culture was exposed to an ELF-EMF of ∼200 microtesla (RMS), 50 Hz, generated by the exposure system described in the sequel.

A 10-turn coil carrying a suitable current generated the ELF field used in the experiments. This coil of copper wire was supplied with an AC voltage of 220 V_{rms} with a series resistance of 484 Ω, so that a current of 0.45 A (RMS) appeared in the circuit generating the above-stated magnetic field. Figure 2 reports a simplified scheme of the exposure system adopted in our experiments.

The coil was directly wound on the sample holder having a radius of 1.4 cm; the total height of the helix (measured between the first and the last turn) was 1.5 cm. Therefore, the helix pitch measured 1.5×10^{-3} m. Furthermore, since the radius of the sample holder was 1.4 cm, we have estimated the radius of the helix as approximately 1.45 cm.

During the experiments the small space of the sample holder where the cells were confined was completely contained in the volume inside the solenoid in order to expose all cells to similar conditions. The magnetic induction generated by the coil in the region containing the cell culture has been calculated according to the following technique.

Since all media involved in the experiments are either para- or dia-magnetic, then, from the point of view of magnetic polarization, they can be considered almost identical to vacuum, that is, their permeability is $\sim\mu_0 = 4\pi \times 10^{-7}$ H/m, within a negligible error. (Here we consider a macroscopic description, so that, for example, the anisotropic diamagnetism of cellular membranes is not accounted for.) So, the magnetic induction due to the coil in any point P can be calculated simply as [Stratton, 1952]

$$\mathbf{B} = \frac{\mu_0}{4\pi} I \int_l \frac{\mathrm{d}\mathbf{l} \times \mathbf{r}}{r^3} \qquad (1)$$

where d**l** is a vector element of the coil wire (which is considered an ideal line with zero thickness) and **r** is the vector which joins d**l** to P. The integral is extended over the whole coil, modeled as regular line l. In our case, **B** has been calculated through Eq. (1) using a very simple numerical integration procedure in MATLAB® based on the trapezoidal rule, using an integration step $\Delta l = 0.254$ mm.

We may observe that the wires that connect the coil to the rest of the circuit produce a magnetic field that should modify our predictions above described. We have estimated that the maximum contribution of this effect is equal to 5–10 μT in the region occupied by the cell, which lies at the sample holder bottom. This additional contribution of magnetic induction has been calculated using again Eq. (1), but now ***l*** refers to each of the two segments of straight lines which can be used to model the wires connecting the coil to the source. The additional **B** generated by these wires is almost parallel to the axis of symmetry of the sample holder and its RMS value is less than 6 μT; that is, in practice this contribution is very small in comparison to the main field due to the coil.

In the small region occupied by the amoebae, the magnetic induction B_z has an average value of 173 μT (RMS); here, z represents the axis of symmetry of the sample. As regards the transverse component, that is, the projection of **B** on any plane perpendicular to the z axis in the region of interest, this is two decades below B_z, so that it can be neglected. Accounting for that and for the small contribution due to the wires connecting the coil to the generator, it can be stated that **B** is spatially uniform and with a RMS value 173 μT within an error less than ∼4%.

The (static) geomagnetic field has been measured using a Bartington MAG-01 magnetometer (Bartington Instruments Ltd, Oxford, UK); its intensity was 42.3 μT at a 33° angle with respect to the coil axis.

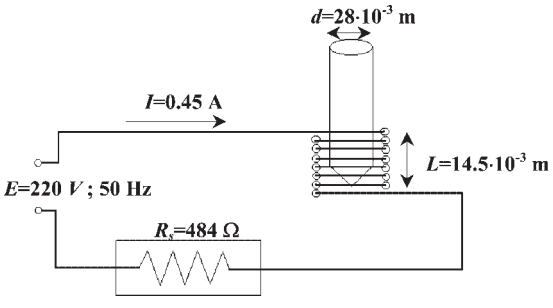

Fig. 2. Sketch of the experimental circuit for magnetic field exposure (detailed description in the text).

Electric Field

In our experiments the electromagnetic exposure has been declared as being purely magnetic. This is a typical assumption in ELF exposure which, however, in the present case can be easily quantified. Indeed,

according to the previous results, the magnetic induction can be considered, within a good degree of approximation, parallel to the axis z, the axis of symmetry of the sample holder. In this approximation, the electric field in any given point P of the sample is [Bianco et al., 1995]

$$\mathbf{E} = \frac{1}{2}\mathbf{r} \times \frac{\mathrm{d}\mathbf{B}}{\mathrm{d}t}$$

where $\mathbf{r} = P_0P$, P_0 being the projection of P on the z axis. In our actual case of time-harmonic fields, the previous equation yields, as regards RMS values,

$$E_{\mathrm{rms}} = \frac{1}{2} r\omega B_{\mathrm{rms}}.$$

As regards numerical values of the electric field, to an order of magnitude, one can assume $B_{\mathrm{rms}} \approx 200 \times 10^{-6}$ T, $\omega = 2\pi \times 50 = 314.16$ rad/s and $r = .014$ m. This latter value corresponds to the maximum distance between the sample axis and any point in the cell culture, that is, to the inner radius of the cylinder of the sample holder. This yields a maximum E_{rms} around 0.5 mV/m. As regards power dissipation, the power dissipated for unit volume is [Stratton, 1952]

$$\sigma E_{\mathrm{rms}}^2 = \approx 2 \times 10^{-7} \mathrm{J/m^3}$$

where σ is the conductivity of the sample. We assume $\sigma \approx 1$ S/m, which is a typical value, as an order of magnitude, for a cell culture medium. Notice that we do not consider here the possible power dissipation due to retarded polarization [Von Hippel, 1954], since its effect is negligible for ELF fields.

The power dissipated per unit mass is referred to as "Specific Absorption Rate" (SAR). It is obviously given by the power dissipated per unit volume divided by the cell culture mass density δ,

$$\mathrm{SAR} = \frac{\sigma E_{\mathrm{rms}}^2}{\delta}$$

A good value for δ is that of water, $\delta = 1000$ kg/m^3.

As regards the possible temperature rise in the sample, the volume of the latter is some cubic centimeters, say around 15 cm$^3 = 15 \times 10^{-6}$ m^3. In 1 s, the power dissipated in the sample is then given by $(15 \times 10^{-6}) \times (2 \times 10^{-7})$ (i.e., volume times power dissipated per unit volume), which amount to 3×10^{-12} J/s. In an experiment lasting as much as 24 h (=86400 s), the overall dissipated power is lower than 3×10^{-7} J. This corresponds to about 2×10^{-6} J dissipated, as a maximum value, in one cubic centimeter of the sample volume; and such a result guarantees that the heat production in the sample is so low as to render highly improbable the existence of biological effects related to a temperature increase.

Evaluation of the Fission Rate

The cell density of the single-cell amoebae was evaluated by counting the number of cells, using a hemocytometer (Bürker Chamber). The binary fission rate was computed as $\log_2 n_x/n_0$, where n_x and n_0 were the cell densities checked after and before exposure to the ELF-EMF, respectively. Seven experiments were carried out. We used Student's t-test and the nonparametric Mann–Whitney U-test (Wilcoxson) to compare the fission rate values of experimental samples to that of the controls.

Spectrophotometric Evaluation of PrChE Activity

The samples were centrifuged at 3000 rpm for 10 min and resuspended in 400 µl of .2 M phosphate buffer, pH 8.0. PrChE activity was recorded at $\lambda = 412$ nm by means of a Uvikon 930 spectrophotometer (Kontron Instruments, Milan, Italy), during the first 10 min after exposing to propionyl thiocholine iodide, used as a substrate, according to Ellman's modified method [Ellman et al., 1961]. The total protein content was evaluated spectrophotometrically using the BioRad system (BioRad Microscience, Cambridge, MA, USA), according to the manufacturer's instructions. The PrChE activity was expressed as PrChE units (UPrChE). Experiments were carried out at least in triplicate. We used Student's t-test and the nonparametric Mann–Whitney U-test (Wilcoxson) to compare the PrChE activity values of experimental samples to that of the controls.

Induction of Cell Aggregation and Differentiation

Both 3 h exposed and 3 h control samples were washed twice in 20 mM cold potassium phosphate buffer (KK2), pH 6.8, and resuspended at a concentration of 10^7 cells/ml in the same medium [Siegert et al., 1994]. Afterwards, 1 ml of these cultures was transferred to Petri dishes containing KK2 agar 1% and kept in a moist chamber at 21 °C. The Petri dishes were kept under observation for 24 h until the formation of the fruiting bodies had occurred. Experiments were carried out in triplicate.

RESULTS

Effect of Exposure on the Fission Rate

The effects of a 24 h exposure to the ELF-EMF on the fission rate of *D. discoideum* are shown in Figure 3,

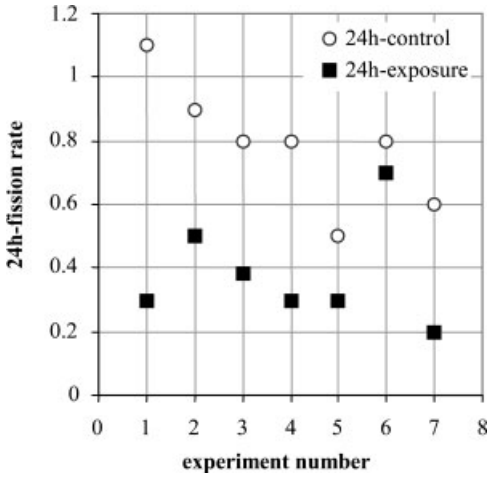

Fig. 3. Effect of a 24 h exposure to the ELF-EMF on the fission rate. The fission rate is plotted for each of seven experiments carried out on exposed samples and controls.

where the fission rate is plotted for each of seven experiments, carried out on exposed samples and controls. We wish to stress the strong difference in the averages of the exposed samples and controls. In Figure 4, we show that the mean fission rate (averaged on all experiments) of 24 h exposed samples was significantly lower (about 50%) than that of the 24 h controls ($P < .001$, Student's t-test; $P = .007$, Mann–Whitney U-test). Conversely, the fission rate of 24 h pseudo-exposed samples, kept for 24 h in a nonworking EMF generator, did not differ from that of the 24 h controls ($P > .05$). If the 24 h exposed samples were transferred to standard conditions for 24 h (24 h nonexposed samples), their fission rate returned to the control value (48 h control) ($P > .05$).

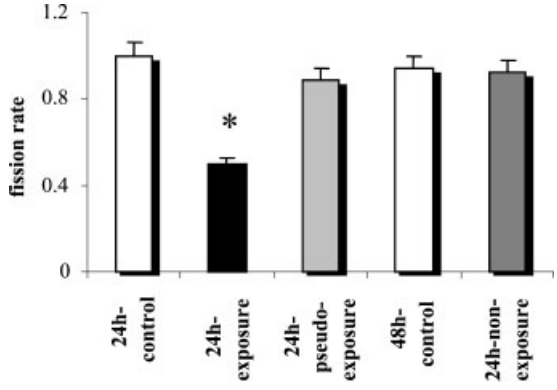

Fig. 4. Effect of a 24 h exposure to the ELF-EMF on the fission rate. Means of seven experiments and standard deviation. The fission rates, computed as described in the text, of 24 h exposed and 24 h pseudo-exposed samples were compared to that of the 24 h controls, whereas the fission rate of 24 h exposed samples transferred to standard conditions for 24 h (24 h nonexposed samples) was compared to that of the 48 h control. *Indicates a significant difference ($P < .001$, Student's t-test).

The cell morphology did not appear to be affected by a 24 h exposure to the ELF-EMF, as the cells maintained their amoeboid appearance and motility. The ELF-EMF-exposure experiments were performed in two different laboratories, in the Department for the Study of the Territory and its Resources and in the Department of Biophysical and Electronic Engineering.

Effect of Exposure on PrChE Activity

The spectrophotometric evaluation of PrChE activity in *D. discoideum* was carried out on 3 h exposed, 24 h exposed, and 24 h nonexposed samples and compared with that of their respective controls. As shown in Figure 5A, a 3 h exposure caused a significant

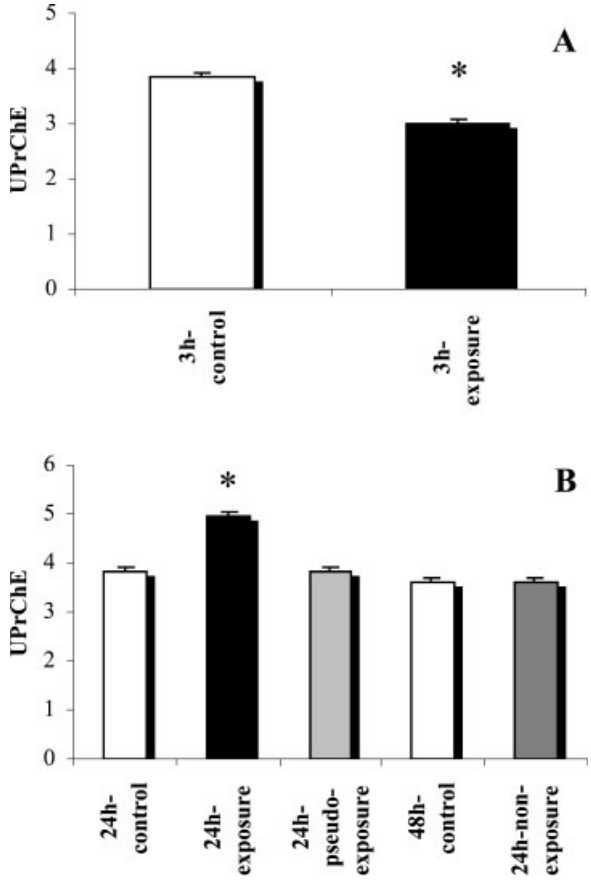

Fig. 5. Spectrophotometric evaluation of propionylcholinesterase activity (PrChE), expressed as UPrChE, after a 3 h (A) or 24 h (B) exposure to the ELF-EMF. Means of at least three experiments and standard deviation. The PrChE activity of 3 h exposed samples, measured as described in the text, was compared to that of the 3 h control, whereas PrChE activities of 24 h exposed and 24 h pseudo-exposed samples were compared to that of the 24 h control; the PrChE activity of 24 h exposed samples transferred to standard conditions for 24 h (24 h nonexposed samples) was compared to that of the 48 h control. *indicates a significant difference ($P < .001$, Student's t-test).

decrease (about 24%) in the enzyme activity ($P < .001$, Student's *t*-test. When Mann–Whitney *U*-test was used, we found $P = .16$. However, as all the six data related to the controls were higher than that of the 3 h exposed samples, this corresponds to the a priori value $P = .03$). Conversely, a 24 h exposure caused a significant increase of about 25% in the enzyme activity ($P < .001$, Student's *t*-test; $P < .002$, Mann–Whitney *U*-test) (Fig. 5B). In pseudo-exposed samples, kept for 24 h in a nonworking EMF generator, the enzyme activity did not appear to differ from that of the controls ($P > .05$). Likewise, in the 24 h exposed samples observed 24 h after their transfer to standard conditions (24 h nonexposed samples), the enzyme activity value was similar to that of their controls (48 h control) ($P > .05$).

Effect of Exposure to the ELF-EMF on Cell Aggregation and Differentiation

Single-cell amoebae of *D. discoideum* were also exposed to the ELF-EMF described above for 3 h, to evaluate its effects on cell aggregation and differentiation. In Figure 6, the exposed cells show a lag in the aggregation phase compared with that of the controls. Actually, 5 h after transferring the amoebae to agar plates, streaming was more evident in the controls than in the exposed cells, and likewise, the mound formation was more evident after 8 h. However, when the cell differentiation phase culminated after 24 h and the fruiting bodies developed, there was no difference between the control and exposed samples.

DISCUSSION

Our results demonstrate that exposure of single-cell amoebae of *D. discoideum* to the ELF-EMF described above can affect their developmental cycle, that is, reproduction and aggregation phases, and PrChE activity as well.

It is worth noting that these results were obtained from ELF-EMF-exposure experiments performed in two different laboratories. This means that the environmental parameters of the laboratories did not account for the differences observed between exposed and control samples. Moreover, no significant differences in fission rate and PrChE activity were found between pseudo-exposed samples and their controls, thus indicating that the results were not affected by exposure to the nonworking EMF generator.

The decrease in the fission rate observed after a 24 h exposure of the cell population to ELF-EMF could be interpreted as a cell response to an environmental stress, as reported for *D. discoideum* exposed to hydrogen peroxide or sodium nitroprusside [Taminato et al., 2002]. Both nitrosoative and oxidative stresses act in a manner similar to nutrient starvation, inhibiting cell reproduction and triggering the signaling pathway leading to cell aggregation and differentiation.

PrChE activity in *D. discoideum* was found to be sensitive to exposure to the ELF-EMF. This result is in line with outcomes for other biological systems, such as the frontal cortex and hippocampus of rats, where the cholinergic activity is sensitive to exposure to a 60 Hz magnetic field [Lai et al., 1993; Lai and Carino, 1998, 1999]. In addition, the sensitivity of cholinesterase (ChE) activity to exposure to a pulsed EMF (80 kA/m) and to a static magnetic field (0.008 T and 0.15 T) has been reported for rat cardiac structures [Abramov and Merkulova, 1980] and serum [Gorczyńska and Wegrzynowicz, 1989], respectively. In *D. discoideum* the PrChE activity appears to be differently affected by the ELF-EMF depending on the exposure time, inhibited by a 3 h exposure or even significantly increased by a 24 h exposure. The latter datum is in line with the results obtained by other authors who have reported that environmental stress conditions, such as thermal shocks, can induce an increase in the ChE

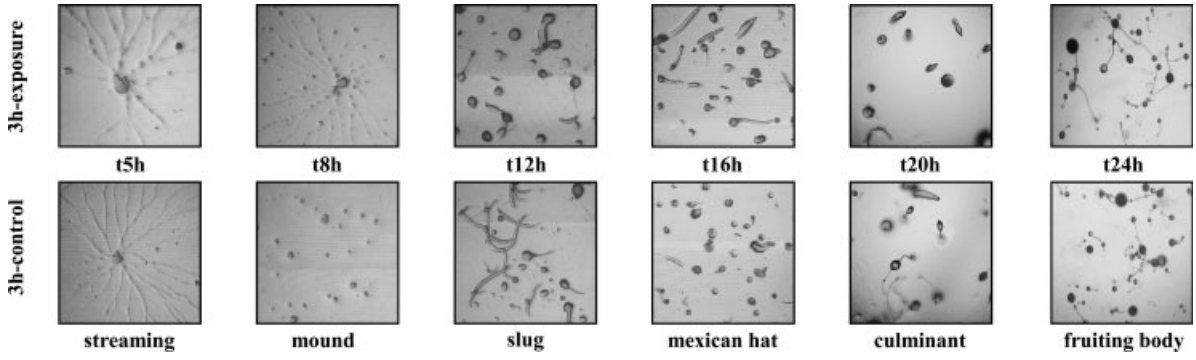

Fig. 6. Effect of a 3 h exposure to the ELF-EMF on cell aggregation and differentiation. The time of induction of the subsequent stages of the developmental phase (see Fig.1) is shown.

activity in the sea urchin coelomocytes [Angelini et al., 2003].

The lag in the aggregation phase observed in 3 h exposed samples could be related to the decrease in their PrChE activity. Actually, it has been suggested that this enzyme activity detected in single-cell amoebae of *D. discoideum* is involved in cell–cell and cell-environment interactions, as inhibition of PrChE activity affects cell aggregation and differentiation [Falugi et al., 2002; Amaroli et al., 2003; Delmonte Corrado et al., 2005].

In conclusion, the results indicate that the short exposure times of single-cell amoebae of *D. discoideum* to the ELF-EMF previously described can affect the developmental cycle and PrChE activity. However, such effects seem to be transient, as the fission rate and the enzyme activity values return to their respective control values after a 24 h stay under standard conditions, and the lag observed during the early aggregation phase is recovered when the formation of the fruiting bodies occurs.

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