A pulsed magnetic stress applied to *Drosophila melanogaster* flies

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Abstract. We report the development of a system to feed pulsed magnetic stress to biological samples. The device is based on a RLC circuit that transforms the energy stored in a high voltage capacitor into a magnetic field inside a coil. The field has been characterized and we found that charging the capacitor with 24 kV results in a peak field of 0.4 T. In order to test its effect, we applied such a stress to the *Drosophila melanogaster* model and we examined its bio-effects. We analysed, in the germ cells, the effects on the control of specific DNA repetitive sequences that are activated after different environmental stresses. The deregulation of these sequences causes genomic instability and chromosomes breaks leading to sterility. The magnetic field treatment did not produce effects on repetitive sequences in the germ cells of Drosophila. Hence, this field doesn’t produce deleterious effects linked to repetitive sequences derepression.

1. Introduction

Magnetic field effects on biological matter are fairly common among scientific literature. A wide range of reports dealt with the effects of static magnetic fields of various intensities. In this context, particular emphasis has been devoted to understand the mechanisms of action of such fields on biochemical reactions$^1$ and on cells$^{2,3}$. In particular, biochemical reactions could change their kinetics if they involve a stage in which radical pairs are formed$^4$. A magnetic field, interacting with radicals’ spin, could indeed remove the energy degeneration of the pairs, offering a preferred pathway for a specific energy level. This in turn could accelerate the corresponding reaction rate. On the other hand, cells could be affected by magnetic field due to diamagnetic anisotropy$^{5,6}$ of their membrane (lipid bilayers). When the anisotropy is big enough and the lipid bilayer molecules are sufficiently free in movements (for example in an external temperature greater than 20°C), the reorientation of the membrane become energetically favorable and this could influence the operation of embedded proteins, such as ion channels.

In the case of pulsed magnetic fields (PMFs) the situation is somewhat less explored. Despite of this, PMF are widely used, for example, in medical practice$^7$. Currently, no mechanism of action
could be considered as definitively accepted in order to explain the action of PMFs, although some models have been suggested[8].

In this work we examined the biological effects of an intense PMF on the Drosophila model. To test if the applied magnetic field mimics stress conditions, we analyzed the effects on the control of specific DNA sequences that are “repetitive sequences” and “transposons”. In physiological conditions, these sequences are maintained blocked in their chromosomal positions. Many environmental stress conditions cause their deregulation [9,10] and the “transposons” can move in the genome causing gene mutations [11,12]. We used a simple genetic system to test the deregulation of these sequences. Particularly, we searched for the presence of crystalline aggregates composed by Stellate protein in testes, since their presence is a symptom of transposon movement.

2. Materials and methods

2.1. The experimental apparatus

PMF was generated through an ad-hoc device consisting of a pulsed current generator and a solenoid (figure 1). The pulsed current generator, together with the solenoid, constitutes an RLC circuit. A high voltage generator charges the capacitor $C$ (150 nF, 100 kV). Through a fast switch (a spark-gap), the circuit could be closed, in order to transform the electric energy stored in the capacitor into magnetic energy inside the solenoid. The solenoid is made by a 0.8 cm round copper wire. It has a radius $a$ of 3.0 cm and an height $l$ of 12.0 cm for a total of 11 loops ($N$). By means of the formula

$$L = \frac{\mu_0 a^2 N^2}{l}, \quad (1)$$

its inductance has been estimated as 3.6 $\mu$H. In order to obtain an high current, no explicit resistor has been inserted within the circuit. Instead, we used as resistor the internal impedance of the whole circuit. From measurements through an high voltage probe, we estimated experimentally both $L$ as 3.8 $\mu$H (slightly increased by parasitic inductance) and $R$ as 0.5 $\Omega$. This resulted in a damping time $\tau = \frac{2L}{R}$ of 15.2 $\mu$s.

![Figure 1. Sketch of the experimental apparatus. Within the solenoid is represented also the container in which the Drosophila flies were kept during the treatment.](image)

The circuit of figure 1, for which $R < 2\sqrt{L/C}$, generates a current $I(t)$ that is described by the equation

$$I(t) = \frac{V}{\omega L} e^{-\frac{Rt}{2L}} \sin \omega t, \quad (2)$$
where $V$ is the voltage at which the capacitor is charged, while $\omega = \sqrt{\frac{1}{LC} - \frac{R^2}{4L^2}} \approx \frac{1}{\sqrt{LC}}$ is the angular frequency at which the circuit oscillates (figure 2). In our setup, it corresponds to 210 kHz. This value is the result of both geometrical and duration constraints. In effect, we had to realize a coil that could fit the samples (a constraint on the inductance). Moreover, we decided to obtain a main pulse that lasts for about 1 µs at least (constraint on the capacitor).

Figure 2. Screenshot of a current pulse diagnosed with the Rogowski coil on a digital oscilloscope. The signal has been taken using a 15x voltage attenuator. The charging voltage used was 24 kV.

The measurement of the current flowing in the circuit has been performed by means of a self-integrating Rogowski coil[13]. This device could be used to obtain a reliable measure of voltage that is roughly proportional to the current flowing within the circuit[14]. Since the magnetic field inside the solenoid depends linearly with respect to the applied current[14], it has been mapped with a Hall probe at low constant current (10 A). Consequently, fixing at 24 kV the charging voltage, we determined a peak current of 4.3 kA, that in turn corresponds to a peak field of about 0.4 T. The map of the axial magnetic field inside the solenoid at the peak current is presented in figure 3.

Figure 3. Axial magnetic field at the peak current within the solenoid as a function of the position; $z = 0$ coincides with the symmetry center of the solenoid. The figure shows also the height of the container in which the samples were maintained during experiments

2.2. Biological samples and analytical procedures

The experiments have been conducted using a Drosophila Oregon-R wild type strain. The flies were housed within a cylindrical plastic container of height 4.0 cm and diameter 1.5 cm. One of the extremity of the cylinder was filled with nutrient (1 cm height) for sustaining the flies.

2.2.1. Immunofluorescence on spermatocytes

Testes were dissected in Ringer’s solution (182 mM KCl, 46 mM NaCl, 3 mM CaCl$_2$, 10 mM Tris-HCl, pH 7.5) and immediately visualized under phase contrast optics in a Zeiss photomicroscope. Testes were fixed with methanol, washed with Phosphate buffered saline (PBS) 1X and 0.5% Triton

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for 15min, washed with PBS1x for 5min and incubated in primary antibody (polyclonal mouse anti-Stellate 1:50) over night at 4°C. Testes were washed with PBS 1X and 0,5% Triton and incubated with Fluorescein isothiocyanate (FITC)-conjugated anti-mouse (1:100, Jackson Immunoresearch). After washes testes were stained with DAPI or 4,6-diamidino-2-phenilindole and mounted.

2.3. Experimental procedure
In order to test if the magnetic stress can have a reliable effect on the repetitive sequences control, we enclosed within the plastic cylinder 70 samples of Drosophila adult males. The container was placed within the solenoid such that the symmetry center of the space reserved for the flies coincides with that of the solenoid (figure 3). The flies underwent a magnetic stress (0.4 T peak) at a repetition rate of 1 Hz. We left the flies under magnetic field for 2 hours, for a total of 7200 pulses.

3. Results and discussion
We analyzed the spermatocytes of flies subjected to magnetic field looking for the presence of crystalline aggregates. Indeed, the presence of such aggregates is a symptom of the deregulation of repetitive sequences. The spermatocytes did not exhibit the presence of crystalline aggregates (figure 4). We can hypothesize that the treatment does not produce effects on the deregulation of repetitive sequences in the germ cells of Drosophila.

![Figure 4](image)

**Figure 4.** In blue the nuclei of spermatocytes from testes: a) control; b) stressed males. The spermatocytes of stressed males do not present crystalline aggregates.

4. Conclusions
We presented the characteristic of an intense pulsed magnetic fields used to induce stress in biological samples. Magnetic field pulses of 0.4 mT (peak) and repetition rate of 1 Hz were applied to a set of Drosophila melanogaster adult males. To test if the applied magnetic field mimics stress conditions, we analyzed the effects on the control of specific DNA repetitive sequences that are activated after different environmental stresses. We also looked at the structural state of the chromosomes in individuals subjected to stress. The magnetic field treatment did not produce effects on the deregulation of repetitive sequences in the germ cells of Drosophila. This field doesn’t produce deleterious effects normally linked to repetitive sequences derepression, such as gene mutations, DNA damage and genomic instability that lead to sterility.

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