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INSIGHT INTO THE BIOLOGICAL EFFECTS OF NON-IONIZING RADIATION THROUGH THE PROPERTIES OF THE ELECTROMAGNETIC WAVES

A. S. Skouroliakou[1*], N. E. Sagioglou[2], A. F. Fragopoulou[2], I. K. Giannarakis[2], A. K. Manta[2], M. P. Ntzouni[2], L. H. Margaritis[2]

[1]Department of Physics and Chemistry, TEI of Athens, Aigaleo 12210, Athens

[2]Department of Cell Biology and Biophysics, Faculty of Biology, Athens University, Panepistimiopolis 15784, Athens, Greece

[*]email: kskourol@teiath.gr, web page: http://kyttariki.biol.uoa.gr/emr_group.htm

Keywords:

Abstract. *The widespread wireless technology initiated several decades ago, has been gradually occupying nearly all daily activities of the modern society. The major sources of this non-ionizing radiation (NIR) include cell phones (6 billion users worldwide), mobile phone base stations (thousands in a crowded city), FM and TV broadcast stations, wireless phones, Wi-Fi routers and units in i-phones, i-pads, notebooks, laptops. All these sources comprise a frequency spectrum from 87 MHz (FM stations) to 2.5 GHz (Wi-Fi, blue-tooth, Microwave oven). We consider that in order to study the biological/human effects of NIR, it is necessary to know exactly the radiation source properties (single frequency or multiple frequencies, repetitive or discontinuous emission and precise knowledge of the peak and average values of the electromagnetic wave in each case). Given the controversy of the existing so far published data, we intend to explore the issue of different biological effectiveness from simple exposure (CW emission) to the more complex pulsed radiation using two major model systems; mice and insects. So far, we have shown, in mice, memory impairment, stress induction and brain protein expression changes and in insects, fecundity decrease and apoptotic cell death increase following microwave (MW) radiation. In addition, by using the NARDA SRM 3000 spectrum analyzer we have performed a mapping of frequencies and E field intensities near base stations and other radiation sources. In this study, we initially attempted to study biological effects on flies from environmental exposure, in Athens University campus region (Dept. of Biology building), to non ionizing radiation in the range of 87 MHz to 2.5 GHz containing FM stations, TV broadcast stations, GSM 900, GSM 1800 MHz and UMTS 2100 MHz. A control group of flies was present in the same area inside a custom made Faraday type cage.Our results under these conditions indicate: a) decrease on Drosophila melanogaster reproduction and b) increase in apoptotic cell death during oogenesis, although at a lower degree compared to mobile phone signals. The work shall be continued with more frequencies and modulation schemes in insects and afterwards in mice where other parameters shall be examined.*

1 INTRODUCTION

Before introduction of radars and microwave ovens in the 50s there was no significant radiofrequency (RF) exposure of the population. The present generations though, are being chronically exposed to RF due to different types and doses of emissions ranging from FM and TV broadcast stations up to mobile communication technologies including GSM and UMTS phones/base stations, WLAN networks, WPAN networks and DECT wireless phones. Further development of devices emitting in the RF frequency region provide rapidly increasing input to the exposure of general population, such as the smart meters monitoring power consumption. Concerns about possible effects of exposure from radiofrequency operating equipment have been discussed in many countries and attracted major scientific interest. Research up to now suggest that bioeffects of non ionizing radiation depend not only on the total energy available, i.e radiation quantity, but also on radiation quality, with the latter being the most important factor.

In an attempt to further examine the mechanisms and dependence of bioeffects induction, our research team has planned a set of experiments. The initial approach was to check the possible effects that can be induced on living organisms from non ionizing environmental radiation of the 35 MHz – 3 GHz range, utilizing either signal generator with selectable frequency and modulation or environmental sources as described above. The insect *Drosophila melanogaster* was selected as model system because

it has been proven to be extremely useful in studies of EMF effects, due to the wealth of available genetic information in combination with the thoroughly investigated developmental biology of oogenesis (Margaritis, 1986).

1.1 Environmental levels of non ionizing electromagnetic radiation

The WHO International EMF Project's RF Research Agenda identified as a research topic a need for measurement surveys to characterize population exposures from all radio frequency (RF) sources with a particular emphasis on new wireless technologies (WHO, 2010). Procedures for measurements in the vicinity of GSM (Global System for Mobile Communications) and UMTS (Universal Mobile Telecommunications System) base stations have been developed in Kim et al. (2008), Lehmann et al. (2002), Joseph et al. (2006), Neubauer et al. (2002), and Olivier and Martens (2007). Bornkessel et al. (2007) provided results of temporal and spatial measurements of GSM and UMTS signals. Measurements in the neighbourhood of WiMAX base stations are investigated in Joseph et al. (2008). Foster (2007) investigated exposure to Wi-Fi access points and checked compliance with international guidelines (ICNIRP 1998; IEEE 2005; FCC 2001). Also Kuhn et al. (2007), Myhr (2004), Schmid et al. (2007), and Verloock et al. (2010) investigated short-period exposures caused by Wi-Fi access points. Exposures from TV and radio transmitters have been studied in Joseph et al. (2006) and Sirav and Seyhan (2009), among others. Tomitsch et al. (2010) measured exposures in bedrooms of residences, where the highest values were caused by DECT telephone base stations (3.31 V/m) and mobile phone base stations (1.36 V/m). Finally, exposures from LTE have recently been investigated in Joseph et al. (2010), with the results for RF environmental exposure ranging between 0.023 and 3.9 V/m in terms of electric field strength values. Highest average electric fields (Eavg) were obtained for GSM 900 (0.5 V/m) and GSM 1800 (0.2 V/m). Average total exposures were equal to Eavg 0.7 V/m.

1.2 Effects of non ionizing radiation

Literature on the effects of non ionizing radiation is vast and often controversial.

A number of studies have reported DNA or cell damage, such as DNA breaks, cell malformations, cell death, changes in chromatin conformation and micronucleus formation in different cell types or organisms (Lai and Singh, 1996; Lixia et al., 2006; Zhao et al., 2007). In other studies, no genotoxic effects of exposure to EMF were observed (Belyaev et al., 2006).

According to others, there is evidence that molecular damage caused by Radio frequency (RF)-microwave fields activates the stress response of the cells, which then acts as a natural defense mechanism against this kind of stimuli. Such a response may be detected through an increase in reactive oxygen species (ROS) or as increased levels of stress proteins (Kwee et al., 2001; Friedman et al., 2007; Blank and Goodman, 2009). Moreover, mobile phone and DECT exposure conditions have produced reliable bioeffects in memory and brain proteome response in mice (Fragopoulou and Margaritis, 2010; Fragopoulou et al., 2010b, 2012; Ntzouni et al., 2011, 2012).

1.3 *Drosophila melanogaster*

The *Drosophila* ovary is divided into discrete units called ovarioles. Each adult ovary consists of 15 to 20 parallel ovarioles, which contain a series of developing follicles or egg chambers (King, 1970; Margaritis, 1986; Spradling, 1993). Egg chambers originate at the anterior end of each ovariole in a specialized region termed the germarium. Each egg chamber consists of 1 oocyte, 15 nurse cells and approximately 1.000 follicle cells surrounding the oocyte. Egg chambers leave the germarium as stage-1 egg and the entire process of oogenesis involves 14 stages where a stage-14 oocyte is mature to be fertilized and oviposited. The different stages have been fully characterized using morphological, biochemical and molecular criteria. Egg chambers develop sequentially within the ovarioles, and thus a single ovary contains egg chambers of all stages of oogenesis.

Programmed cell death in the *Drosophila* ovary has been extensively studied and occurs in response to both developmental and environmental stimuli, at distinct stages during oogenesis (Mc Gall, 2005), following two different patterns, playing an integral role in the normal development of every oocyte (Cavaliere, 1998). Stages 7–8 and the region 2 within the germarium are the two checkpoints that provide a protective mechanism in the process of oogenesis: they remove defective egg chambers that are unable to develop into fertile eggs, thus preventing the waste of precious nutrients (Nezis et al., 2001, 2002).

In addition to the developmental programmed cell death of nurse cells that occurs late in oogenesis entire egg chambers can be induced to die in response to poor nutrition or other insults. A diet lacking in protein leads to the degeneration of egg chambers (Drummond-Barbosa, 2001). It has been

suggested that these dying egg chambers are responding to a checkpoint, where the status of egg chambers and/or the environment is monitored before investing energy into egg production.

Abnormal egg chamber development or exposure to cytotoxic chemicals also triggers the death of entire egg chambers in mid-oogenesis (Nezis et al., 2001). Proper egg production requires appropriate day length and temperature, as well as mating, specifically the transfer of the male sex peptide to females (Chapman et al., 2003).

Apoptotic cell death in general is defined by morphological criteria and it is mainly characterized by nuclear condensation and DNA fragmentation, without major ultrastructural changes of cytoplasmic organelles (Lockshin and Zakeri, 2004). In addition to PCD during the late stages of *Drosophila* oogenesis, stress-induced cell death takes place during the early and mid stages in response to starvation or other stress factors. The most sensitive developmental stages during oogenesis for stress-induced apoptosis are region 2 within the germarium, referred to as "germarium checkpoint", and stages 7–8 just before the onset of vitellogenesis, referred to as "mid-oogenesis checkpoint". Both checkpoints are found to be very sensitive to stress factors like poor nutrition or exposure to cytotoxic chemicals like etoposide or staurosporine as demonstrated by Nezis et al (Nezis et al, 2006).

2 MATERIALS AND METHODS

All experiments were performed with *Drosophila melanogaster* Oregon R **(**Figure 1**)**, wild type flies maintained under standard conditions, as described in Chavdoula et al. (Chavdoula et al.,2010).

Figure 1: *Drosophila melanogaster* Oregon R.

2.1 Exposure setup

The experiment involved assessment of the effect of exposure to environmental levels of non ionizing radiation in the frequency range of 40 MHz to 3 GHz.

a **b** **c**

Figure 2: a. *Drosophila melanogaster* Oregon R flies within their culture vial as used for exposing to electromagnetic radiation b. Exposed group setup, c. Custom made Faraday – type ca**ge**

Three exposed groups were used for each set of experiments; each group consisting of 20 flies. The control group was kept in a controlled environment in a fly room, the sham exposed group was

positioned in the exposure area in a Faraday type cage and the exposed group was positioned in the exposure area in a cage constructed from non conducting semi transparent fabric (Figures 2, 3). Thus,

light conditions were similar for the exposed and sham exposed groups. Humidity and temperature at the exposure areas were continuously recorded**.**

Figure 3: Exposure setup.

Varying values of exposure duration were used. The 6 min daily duration exposure was selected as it has been shown to produce an effect on insect reproduction. In addition, 30 min, 1h and 24h duration exposures were selected as the exposure was due to environmental radiation levels. The flies were not anesthetized during exposure.

2.2 Dosimetry

The NARDA SRM 3000 was used to acquire measurements of maximum as well as 6 min (as proposed by the ICNIRP) averaged electric field strength measurements (Figures 4, 4a). The NARDA

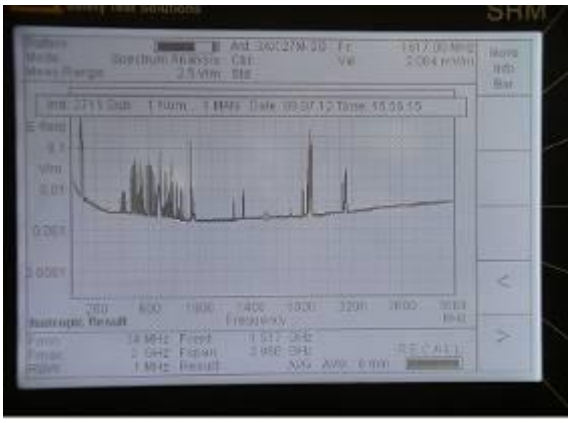

Figure 4: Narda SRM 3000 spectrum analyser - Environmental background. The spectrum shows the FM stations band (far left) followed by a gap, a rich TV station band extending up to 850 MHz, a sharp peak of the GSM 900MHz base station emission, two short peaks at 1350 MHz. The predominant GSM 1800 MHz and the UMTS 2100 MHz emission peaks are apparent. No Wi-Fi-signal is detected at the location of the experiment

EFA 300 was used to monitor environmental ELF magnetic field strength values due to presence of power lines. Measurements were acquired in different time points (morning, midday, evening) in order to evaluate any daily variability.

The sensitivities of the measurement system for the various signals were varying from 0.002 V/m to 0.013 V/m depending upon the frequency, due to the varying antenna factor (sensitivity) of the triaxial measurement probe in the considered frequency range. The optimal RBW was automatically selected for each measurement procedure.

Figure 4a: Narda SRM 3000 spectrum analyser - Environmental background; Narrowband measurements in the FM range, TV range, GSM 900, GSM 1800 and UMTS range.

Measured electric field strength values were compared to corresponding values of environmental non ionizing radiation levels that have been measured by our group (Figure 5).

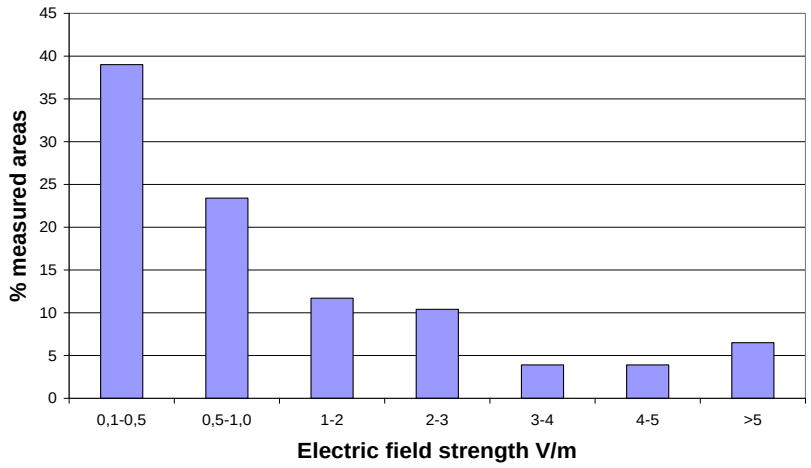

Figure 5: Electric field strength values due to environmental non ionising electromagnetic radiation levels.

2.3 Number of F1 pupae as a measure of reproductive capacity

Newly emerged flies (10 male and 10 female) were placed in plastic vials containing standard fly food. The exposed and sham exposed groups were transferred to the exposure area in a self constructed Faraday cage. On the sixth day of exposure parent flies were removed from the vial 5 hours after the exposure session. The vials with the developing embryos were kept in the culture room for another 20 days and the number of F1 pupae developed was measured.

2.4 Acridine orange staining

Newly emerged flies (5 males and 15 females) were placed in plastic vials containing standard fly food. The exposed and sham exposed groups were transferred to the exposure area in a self constructed Faraday cage. On the fourth day of exposure the maternal flies were removed from the vial 4 hours after the exposure session. The flies were anesthetized, dissected and their egg chambers from germarium to stage 10 were prepared for acridine – orange staining with the procedure described in Chavdoula et al. (Chavdoula et al., 2010).

Figure 6: Drosophila ovaries as seen in the stereoscope, Each fly contains a pair of ovaries and each ovary consists of 12-15 ovarioles

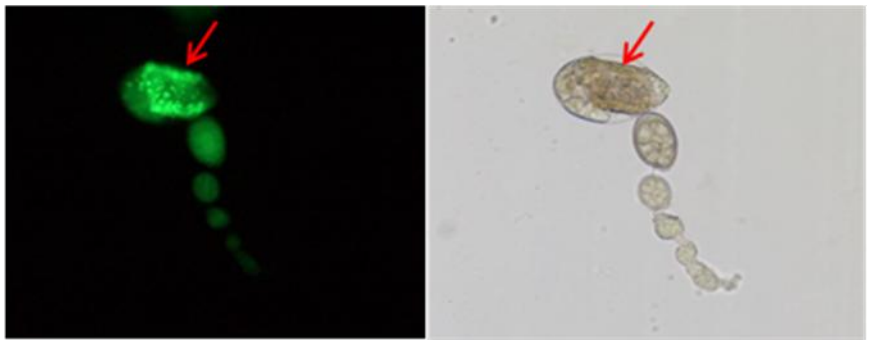

Figure 7: a. Fluorescent and b. Phase contrast micrographs of an acridine orange stained typical ovariole. Positive signal in stage 8 egg chamber indicates DNA fragmentation.

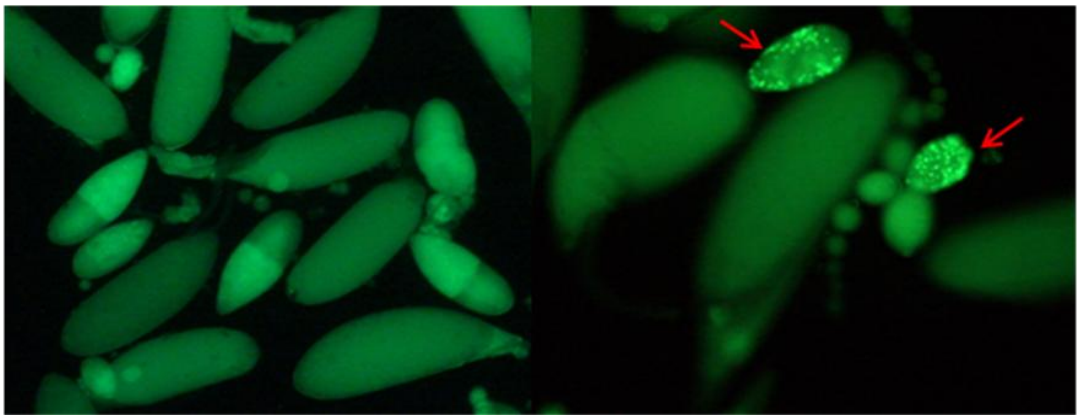

Figure 8: Fluorescent micrograph of acridine orange stained follicles a. negative signal, b.acridine stained follicles with positive signals as indicated by the arrows

3 RESULTS AND DISCUSSION

Values of total 6 minutes averaged electric field strength, dominant frequency and spectral distribution of electric field strength are presented in Table 1.

Frequency range (MHz)	**36-150 (FM)**	**300-880 (TV)**	**885-980 (GSM 900)**	**1720-1940 (GSM**	**2090 - 2200 (UMTS)**

				1800)	
6 min averaged electric field strength (V/m)	1,287	0,302	0,220	0,473	0,158

- **Dominant frequency: 89, 41MHz**
- **Total 6 minutes averaged electric field strength: 1,436 V/m**

Table 1: Average electric field strength, dominant frequency and spectral distribution of electric field strength, as measured by the spectrum analyser at the exposure area.

Comparing figure 5 and table 1 it can be stated that electric field strength levels measured at the exposure area are close to the average environmental levels that have been measured by our group, thus representative of the levels in everyday life. The predominant frequency as well as the major contribution to electric field strength are due to FM stations emission Measured levels are well below the reference levels set by ICNIRP (ICNIRP, 1998), but above the suggested safety levels suggested by Fragopoulou et al (Fragopoulou et al, 2010b)

The mean number of F1 pupae per maternal fly is presented in Table 2, Figure 9 and the mean ratio of ovarian cell death after acridine orange staining in Table 3, Figure 10.

Daily exposure duration	**Mean number of F1 pupae per maternal fly**		
	control	**exposed**	**sham exposed**
6 min	15,21± 0,67	13,22± 0,78	14,55± 2,0
30 min	15,21± 0,67	13,10 ± 0,68	15,44± 0,81
1 hour	15,21± 0,67	14,3± 0,53	12,9± 0,45
24 hours	15,21± 0,67	12,95± 0,65	10,6± 0,48

Table 2: Mean number and standard error of the mean of the number of F1 pupae per maternal fly

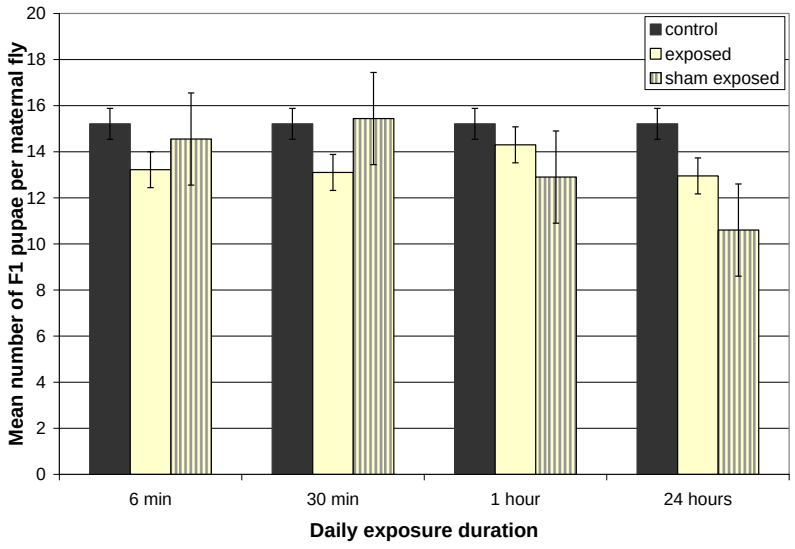

Figure 9: Bar graph presentation of the mean number of F1 pupae per maternal fly (±standard error of the mean) from a total of 5 experiments from each exposure protocol under environmental conditions of electromagnetic radiation..

In previous experiments it has been shown that a short daily exposure of newly emerged adult flies of *D. melanogaster* to mobile phone radiation, decreases significantly and non-thermally their

reproductive capacity, as defined by the number of F_1 pupae (Panagopoulos and Margaritis, 2008; Chavdoula et al., 2010). Fragmented DNA in all the developmental stages of early- and mid-oogenesis (*i.e.* from the germarium stage up to stage 10) and in all three different types of egg-chamber cell, has also been detected in response to stress induction from the exposure of the insects to this radiation. The above-mentioned checkpoints, germarium and stage 7–8, were shown to be the most sensitive developmental stages also to this kind of electromagnetic insult. The observed DNA fragmentation and the induced cell death explain the above-mentioned decrease in the reproductive capacity. The findings have also indicated that the effect on the reproductive capacity increases almost linearly with increasing daily exposure duration from 1 to 21 min to GSM-900 and 1800-MHz EMF and that the effect is non-thermal.

The results of this experiment demonstrate that reproductive capacity in the control group has an average value of 15,21 with a very low standard error. For the groups of 6 and 30 min daily exposure, reproductive capacity decreases in agreement with our previous results. Nevertheless, when daily exposure duration increases, reproductive capacity does not decrease significantly, whilst a significant decrease is demonstrated in the sham exposed group. As mentioned in the introduction section, the environmental conditions play a major role in the reproductive capacity of drosophila. Although temperature and humidity were continuously monitored in the exposure areas, light conditions differed from the corresponding group in the control room. The sham exposed group was positioned in a Faraday cage constructed from galvanised iron grid. In order to achieve similar light conditions the exposed group was positioned in a wooden frame covered with layers of transparent fabric. Therefore, while the 6 and 30 min exposed groups demonstrate a clear decrease in reproductive capacity compared to the control and sham exposed groups, there is a need to further investigate longer exposure duration, in order to check whether a repair mechanism develops as it has been observed by our group in mice memory impairements (Ntzouni et al., 2012).

daily exposure duration	**Mean percent ratio of ovarian cell death detected with acridine orange staining**		
	control	**exposed**	**sham exposed**
1 hour	2,69 ± 0,48	4,41 ± 0,77	1,99 ± 0,57
24 hours	1,64 ± 0,51	2,58 ± 0,56	1,35 ± 0,51

Table 3: Mean percent ratio and standard error of the mean of apoptotic follicles as detected with acridine orange staining.

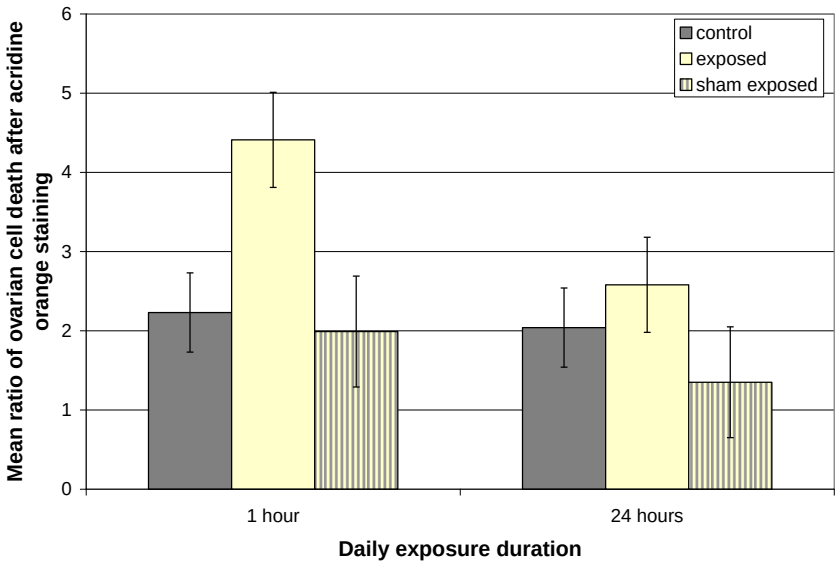

Figure 10: Bar graph presentation of the percent ratio of ovarian cell death (± standard error of the mean), detected with acridine orange staining, from 2 experiments from each exposure protocol under environmental conditions of electromagnetic radiation.

Results concerning ovarian cell death present a significant increase in apoptotic cells for the 1 hour daily exposure ($p<0,05$). Although apoptotic cells from the 24 hour exposed insects appear increased, the increase is not statistically significant.

It is clear by literature and by our experiments that the number of insects in a vial influences reproduction rate (Pearl and Parker, 1922). In fact, the number of pupae decreases as an inverse power of the number of female insects per vial (Figure 11). This could be attributed to evolutionary mechanisms where the status of egg chambers and/or the environment is monitored before investing energy into egg production.

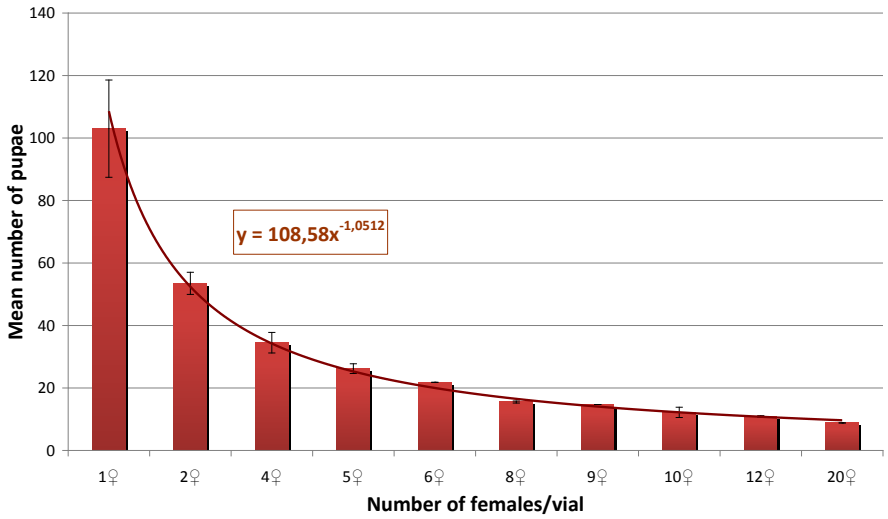

Figure 11: Reproductive rate dependence on number of females (±SEM).

Effort to assess and clarify the bioeffectiveness of non ionizing electromagnetic radiation is strong and difficult. The exposure of individuals is continuous in varying levels and field patterns. New wireless applications contribute to a complicated pattern of environmental electromagnetic background.

Model organisms such as insects and mice have been proven valuable for the quantitative and qualitative study of non ionizing radiation biological effects.

In this study we attempted to assess the impact of environmental levels of electromagnetic radiation on the reproduction of *Drosophila melanogaster*. Our results suggest a clear decrease on reproductive capacity after a 6 minute or a 30 minute daily exposure. Apoptotic cell death increases after 1 hour of daily exposure, while it still shows an increasing tendency after the 24 hours exposure.

Longer exposures seem to have no effect on reproductive capacity, though it should be further investigated whether this could be attributed to a repair mechanism or to the experimental setup.

Following experiments, already in progress, are designed aiming to investigate the bioeffectiveness of radiofrequency electromagnetic radiation depending on the exact electromagnetic wave pattern.

ACKNOWLEDGMENTS

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