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Cell death induced by GSM 900-MHz and DCS 1800-MHz mobile telephony radiation

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Abstract

In the present study, the TUNEL (Terminal deoxynucleotide transferase dUTP Nick End Labeling) assay – a well known technique widely used for detecting fragmented DNA in various types of cells – was used to detect cell death (DNA fragmentation) in a biological model, the early and mid stages of oogenesis of the insect *Drosophila melanogaster*. The flies were exposed *in vivo* to either GSM 900-MHz (Global System for Mobile telecommunications) or DCS 1800-MHz (Digital Cellular System) radiation from a common digital mobile phone, for few minutes per day during the first 6 days of their adult life. The exposure conditions were similar to those to which a mobile phone user is exposed, and were determined according to previous studies of ours [D.J. Panagopoulos, A. Karabarounis, L.H. Margaritis, Effect of GSM 900-MHz mobile phone radiation on the reproductive capacity of *D. melanogaster*, *Electromagn. Biol. Med.* 23 (1) (2004) 29–43; D.J. Panagopoulos, N. Messini, A. Karabarounis, A.L. Philippetis, L.H. Margaritis, Radio frequency electromagnetic radiation within “safety levels” alters the physiological function of insects, in: P. Kostarakis, P. Stavroulakis (Eds.), *Proceedings of the Millennium International Workshop on Biological Effects of Electromagnetic Fields*, Heraklion, Crete, Greece, October 17–20, 2000, pp. 169–175, ISBN: 960-86733-0-5; D.J. Panagopoulos, L.H. Margaritis, Effects of electromagnetic fields on the reproductive capacity of *D. melanogaster*, in: P. Stavroulakis (Ed.), *Biological Effects of Electromagnetic Fields*, Springer, 2003, pp. 545–578], which had shown a large decrease in the oviposition of the same insect caused by GSM radiation. Our present results suggest that the decrease in oviposition previously reported, is due to degeneration of large numbers of egg chambers after DNA fragmentation of their constituent cells, induced by both types of mobile telephony radiation. Induced cell death is recorded for the first time, in all types of cells constituting an egg chamber (follicle cells, nurse cells and the oocyte) and in all stages of the early and mid-oogenesis, from germarium to stage 10, during which programmed cell death does not physiologically occur. Germarium and stages 7–8 were found to be the most sensitive developmental stages also in response to electromagnetic stress induced by the GSM and DCS fields and, moreover, germarium was found to be even more sensitive than stages 7–8.

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1. Introduction

There are three forms of cell death *viz.* apoptosis, autophagic cell death and necrosis [4,5]. Apoptosis is genetically controlled and plays a vital role in normal development. It is referred to as programmed cell death

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(PCD) when observed in certain types of cells during normal development, or as stress-induced apoptosis [6] when is induced by a variety of external insults like chemicals, temperature, poor nutrition, radiation, etc. 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 [4]. While apoptosis is mediated by activation of caspases, autophagic cell death is caspase-independent. Necrosis is characterized not only by DNA fragmentation, but also by ultrastructural changes in cytoplasm, loss of plasma membrane integrity and cell rupture, resulting in the cytosolic contents spilling into the surroundings [4,7–9]. Unlike apoptosis and autophagic cell death, which are genetically programmed, necrosis is an uncontrolled type of cell death that normally results from cellular injury [4,5].

Programmed cell death during *Drosophila* oogenesis is an intensively studied phenomenon during the last years [10–16]. It is an evolutionary conserved and genetically regulated process, where cells that are no longer needed undergo self-destruction by activation of a cell-suicide program [17].

Each *Drosophila* ovary consists of 16–20 ovarioles. Each ovariole is an individual egg assembly line, with new egg chambers in the anterior moving toward the posterior as they develop, through 14 successive stages until the mature egg reaches the oviduct. The most anterior region is called the germarium. Each egg chamber consists of a cluster of 16 germ cells surrounded by an epithelial monolayer of somatic follicle cells (FCs). In the germarium, the germline cyst originates from a single cell (cystoblast) that undergoes 4 mitotic divisions to form the 16-cell cluster. Among the 16 germ cells, one differentiates as the oocyte and the rest become nurse cells. The nurse cells enter a phase of endo-replication and become highly polyploid during the rest of oogenesis. Approximately 80 FCs surround the germline cyst at the time that an egg chamber buds from the germarium (stage 1). FCs divide mitotically until the end of stage 6, at which time they undergo three rounds of endo-replication and growth, amplifying chromosomal regions required for egg-shell production. The oocyte remains arrested in prophase I until late stage 13, when the nuclear envelope breaks down and meiosis progresses to metaphase I, where it remains arrested again during the final stage 14, before activation [18,19].

Nurse cells and follicle cells undergo programmed cell death during the late developmental stages 11–14 of oogenesis, exhibiting chromatin condensation, DNA fragmentation and phagocytosis of the cellular remnants

by the adjacent follicle and epithelial cells, events that are required for the normal maturation and ovulation of the egg chamber [11,15,16,20,21].

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, [10,11,15,22–24]. 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” [10,15]. Both checkpoints are found to be very sensitive to stress factors like poor nutrition [10,25] or exposure to cytotoxic chemicals like etoposide or staurosporine [11]. The mid-oogenesis check point was at first observed [11,23,24] in response to cytotoxic chemicals and triggering the death of entire egg chambers in mid-oogenesis. Shortly after this, the same checkpoint was found by other experimenters [10] in response to poor nutrition stress. Additionally, the same experimenters observed another checkpoint much earlier in oogenesis, in the region 2a/2b of the germarium, in response to poor nutrition stress. Apart from these two checkpoints, until now egg chambers were not observed to degenerate during other previtellogenic or vitellogenic stages (germarium to stage 10) [10,15].

A widely used method for identifying dying cells is the TUNEL assay. By use of this method, fluorescein dUTP is bound through the action of terminal transferase onto fragmented genomic DNA, which then becomes labelled by characteristic fluorescence. The label incorporated at the damaged sites of DNA is visualized by fluorescence microscopy [26].

The biological effects of man-made electromagnetic fields especially in the RF (radio-frequency) and ELF (extremely low frequency) regions of the spectrum, is a subject that has been of concern in the scientific community and the public during the last decades. The most powerful RF antennas in the proximate daily environment of modern man are handsets and base station antennas of cellular mobile telephony. In Europe the two systems of digital mobile telephony are GSM with a carrier frequency around 900 MHz and DCS referred also as GSM 1800 with a carrier frequency around 1800 MHz and same rest characteristics as GSM. Both systems use a pulse repetition frequency of 217 Hz, [27–30]. Thereby the signals of both systems combine RF and ELF frequencies.

RF and ELF electromagnetic fields have been reported to induce cell death in several *in vitro* studies [31–37]. Additionally, in several *in vivo* studies mostly on mice and rats, DNA damage or apoptosis were found

to be induced by ELF magnetic fields [38–41] and RF fields [42–44]. At the same time, several other studies do not find any connection between electromagnetic field exposure and DNA damage or apoptosis [45–51]. Thus the reported results are contradictory and studies examining cell death induced by electromagnetic fields in the model biological system of *Drosophila* oogenesis had not been conducted until now.

The aim of the present study was to investigate whether GSM and DCS radiation can induce cell death during the early and mid stages of *Drosophila* oogenesis, where programmed cell death does not physiologically occur.

2. Materials and methods

2.1. *Drosophila* culturing

Wild-type strain Oregon R *Drosophila melanogaster* flies were cultured according to standard methods and kept in glass vials with standard food [1]. Ovaries from exposed and sham exposed/control flies were dissected into individual ovarioles at the sixth day after eclosion and then treated for TUNEL assay.

2.2. Electromagnetic field exposure system

As an exposure device we used a commercial cellular mobile phone itself, in order to analyze effects of real exposure conditions to which a mobile phone user is subjected. Real GSM or DCS signals are never constant. There are continuous changes in their intensity and frequency. Electromagnetic fields with changing parameters are found to be more bioactive than fields with constant parameters [31,52] probably because it is more difficult for living organisms to get adapted. Experiments with constant GSM or DCS signals can be performed, but they do not represent actual conditions. Since our early experiments [2,3] we have been using cellular mobile phones as exposure devices and we have been consistently detecting effects on reproduction [1–3]. Other experimenters have also used cellular phones as exposure devices, obviously for the same reasons [31,53,54]. In our present experiments we used a dual band cellular mobile phone that could be connected to either GSM 900 or DCS 1800 networks simply by changing SIM (“Subscriber Identity Module”) cards on the same handset. The highest specific absorption rate (SAR) given by the manufacturer for the human head is 0.89 W/kg. The exposure procedure was the same as in our earlier experiments [1–3]. The handset was fully charged before each set of exposures. The experimenter spoke on the mobile phone’s microphone during the exposures. The GSM and DCS fields were thus “modulated” by the human voice (“speaking emissions” or “GSM basic”), as described previously [1]. The intensity of the emitted radiation is considerably higher when the user speaks while being connected than when he is not speaking

(“non-modulated” or “non-speaking” emission, or discontinuous transmission mode-DTX) [2,30,31].

GSM 900-MHz mobile phones and base-station antennas operate with double power output than the corresponding DCS 1800-MHz ones [27–30]. The measured power density of the mobile phone antenna is usually higher when the phone operates in GSM mode than the corresponding one at the same distance when the same handset operates in DCS mode.

Exposures and measurements of mobile phone emissions were always conducted at the same place where the mobile phone had full perception of both GSM and DCS signals. Measurements of the mobile phone emissions were performed as described before [1]. The measured mean power densities in contact with the mobile phone antenna for six min of modulated emission were 0.402 ± 0.054 mW/cm² for GSM 900-MHz and 0.288 ± 0.038 mW/cm² for DCS 1800-MHz. As was expected, the GSM 900-MHz intensity at the same distance from the antenna and with the same handset was higher than the corresponding DCS 1800-MHz. For better comparison between the two systems of radiation we measured the GSM signal at different distances from the antenna and found that at 1-cm distance the GSM 900-MHz intensity was 0.292 ± 0.042 mW/cm², almost equal to DCS 1800-MHz at zero distance. Measurements at 900 and 1800 MHz were made with a RF Radiation Survey Meter, NARDA 8718. Since both GSM and DCS signals have a pulse repetition frequency at 217 Hz, we measured electric and magnetic field intensities in the extremely low frequency (ELF) range, with a Holaday HI-3604 ELF Survey Meter. The measured values for the modulated field, excluding the ambient electric and magnetic fields of 50 Hz, were 23.7 ± 1.8 V/m electric field intensity and 0.53 ± 0.06 mG magnetic field intensity for GSM at zero distance, 15.7 ± 1.2 V/m and 0.35 ± 0.05 mG, respectively, for GSM at 1-cm distance, and 15.5 ± 1.3 V/m and 0.36 ± 0.05 mG, respectively, for DCS at zero distance. All the above-measured values, which are averaged over 10 separate measurements of each kind \pm standard deviation (S.D.), are typical for digital mobile telephony handsets and they are all within the current exposure criteria [55].

2.3. Exposure procedure

In each experiment we separated the collected insects into five groups: the first group named “900” was exposed to GSM 900-MHz field with the mobile phone antenna in contact with the glass vial containing the flies. The second, named “900A”, was exposed to GSM 900 MHz also, but at 1 cm distance from the mobile phone antenna. The third group (named “1800”) was exposed to the DCS 1800-MHz field with the mobile phone antenna in contact with the glass vial. The comparison between the first and third group represents comparison with the usual exposure conditions between GSM 900 and DCS 1800 users, while comparison between the second and third group represents comparison between possible effects of the RF frequencies of the two systems under equal radiation intensities. Therefore the second group (900A) was introduced for better comparison of possible effects between the two sources of radi-

ation. The fourth group (named “SE”) was sham-exposed and the fifth (named “C”) was the control. Sham-exposed animals were treated exactly as the exposed ones except that the mobile phone was turned off during the “exposures”. In contrast, control animals were never exposed in any way or taken out of the culture room. Each group consisted of 10 male and 10 female insects.

In each experiment, we collected newly eclosed adult flies from the stock early in the afternoon, and separated them into the five different groups following the same methodology as in previous experiments [1].

We exposed the flies within the glass vials by placing the antenna of the mobile phone outside the vials, parallel to the vial axis. The total duration of exposure was 6 min/day in one dose and exposures were started on the first day of each experiment (day of eclosion). The exposures took place for 5 days in each experiment, as previously described [1]. Then there was an additional 6-min exposure in the morning of the sixth day and 1 h later, female insects from each group were dissected and prepared for the TUNEL assay. The only difference in the exposure procedure from previous experiments [1] was this additional exposure time. Since we were studying the effect on early and mid oogenesis during which the egg chambers develop from one stage to the next within few hours [18], we considered that an additional exposure, 1 h before dissection and fixation of the ovarioles, might be important in recording any possible immediate effect of cell death. The daily exposure duration of 6 min was chosen in order to have exposure conditions that can be compared with the established exposure criteria [55] and because our earlier experiments had shown that only a few minutes of daily exposure were enough to produce a significant effect on the insect’s reproductive capacity [1–3].

In each experiment we kept the 10 males and the 10 females of each group in separate vials for the first 48 h. As explained before [1,2] keeping males separate from females for the first 48 h of the experiment ensures that the flies are in complete sexual maturity and ready for immediate mating and laying of fertilized eggs. This part of the procedure is not necessary in TUNEL experiments, but we kept it as in previous experiments in order to be able to compare the results.

After the first 48 h of each experiment, males and females of each group were put together (10 pairs) in another glass vial with fresh food. They were allowed to mate and lay eggs for the next 72 h, during which the daily egg production of *Drosophila* is at its maximum [1].

After the last exposure in the morning of the sixth day from the beginning of each experiment, the flies were removed from the glass vials and the ovaries of females were dissected and fixed for TUNEL assay. (The vials can be maintained in the culture room for six additional days without further exposure, in order to count the F₁ pupae as in previous experiments [1]. This part of the procedure is not required for the TUNEL experiments, but it is necessary if the two kinds of experiments are running simultaneously so that a direct comparison of the results can be made.)

The temperature during the exposures was monitored within the vials with a mercury thermometer with an accuracy of 0.05 °C [1].

2.4. TUNEL assay

To determine the ability of GSM and DCS radiation to act as possible stress factors able to induce cell death during early and mid oogenesis, we used the TUNEL assay as follows: ovaries were dissected in Ringer’s solution and separated into individual ovarioles from which we took away egg chambers of stages 11–14. In egg chambers of stages 11–14 programmed cell death takes place normally in the nurse cells and follicle cells. Thereby we kept and treated ovarioles and individual egg chambers from germarium up to stage 10. Samples were fixed in PBS solution containing 4% formaldehyde plus 0.1% Triton X-100 (Sigma Chemical Co., Germany) for 30 min and then rinsed three times and washed twice in PBS for 5 min each. Then samples were incubated with PBS containing 20 µg/ml proteinase K for 10 min and washed three times in PBS for 5 min each. *In situ* detection of fragmented genomic DNA was performed with a Boehringer Mannheim kit containing fluorescein dUTP, for 3 h at 37 °C in the dark. Samples were then washed six times in PBS for 1 h and 30 min in the dark and finally mounted in anti-fading mounting medium (90% glycerol containing 1,4-diazabicyclo(2.2.2)octane (Sigma Chemical Co., Germany) to prevent fading, and viewed under a Nikon Eclipse TE 2000-S fluorescence microscope. The samples from different experimental groups were blindly observed under the fluorescence microscope (i.e. the observer did not know the origin of the sample) and the percentage of egg chambers with TUNEL-positive signal was scored in each sample. Statistical analysis was made by single factor Analysis of Variance test.

3. Results

In Table 1 the summarised data from eight separate experiments are listed. The data reveal that both GSM 900 and DCS 1800 mobile telephony radiations strongly induce cell death (DNA fragmentation) in ovarian egg chambers of the exposed groups, (63.01% in 900, 45.08% in 900A and 39.43% in 1800), while in the SE and C groups the corresponding percentage of cell death was only 7.78% and 7.75%, respectively.

Ovarian cell death between the control group and the sham-exposed group did not differ significantly (differences were within standard deviation). The data from the C group are omitted in Table 1.

Fig. 1a shows an ovariole from a sham-exposed female insect, containing egg chambers from germarium to stage 8, all TUNEL-negative. This was the typical picture in the vast majority of ovarioles and separate egg chambers from female insects of the sham-exposed and control groups. In the SE groups, only 154 egg chambers

Table 1
Effect of GSM and DCS fields on ovarian cell death

Groups	Developmental stages	Ratio of TUNEL-positive to total number of egg-chambers of each developmental stage	Sum ratio of TUNEL-positive to total number of egg-chambers of all stages	Percentage of TUNEL-positive egg chambers (%)	Deviation from sham-exposed groups (%)
SE	Germarium	37/186	154/1980	7.78	0
	1–6	32/1148			
	7–8	78/364			
	9–10	7/282			
900	Germarium	165/189	1315/2087	63.01	+55.23
	1–6	675/1252			
	7–8	310/384			
	9–10	165/262			
900A	Germarium	116/184	930/2063	45.08	+37.30
	1–6	484/1248			
	7–8	213/374			
	9–10	117/257			
1800	Germarium	101/169	776/1968	39.43	+31.65
	1–6	388/1202			
	7–8	196/358			
	9–10	91/239			

(including germaria) out of a total of 1980 in 8 replicate experiments (7.78%), were TUNEL-positive (Table 1), a result that is in full agreement with the rate of spontaneously degenerated egg chambers normally observed during *Drosophila* oogenesis [11,16].

Fig. 1b shows an ovariole of an exposed female insect (group 900A), which is TUNEL-positive only in the region 2a/2b of the germarium (nuclei of the nurse cells) and TUNEL-negative at all other stages. Corresponding pictures from all three exposed groups (data not shown) had identical characteristics. A sum ratio of 165/189 germaria in 900, 116/184 in 900A and 101/169 in 1800, respectively, were TUNEL-positive, while the corresponding sum ratio in SE was only 37/186 (Table 1).

Fig. 1c shows an ovariole from an exposed female insect (group 1800), with TUNEL-positive signals only in the stage 8 egg chamber, while all other stages were TUNEL-negative. In this specific picture the TUNEL-positive signal can be seen in the nurse cells but in many others (Fig. 1e and f), the TUNEL-positive signal could also be seen in the follicle cells and the oocyte. Corresponding pictures from 900 and 900A (data not shown) had identical characteristics. At the “mid oogenesis checkpoint” (stages 7–8), there was a significant sum ratio of TUNEL-positive egg chambers in all exposed groups (310/384 in 900, 213/374 in 900A and 196/358 in 1800), while in the SE groups the corresponding sum ratio was much smaller (78/364) (Table 1).

Fig. 1d shows an ovariole of an exposed female insect (group 900A) with a TUNEL-positive signal in the

nurse cells at both checkpoints, germarium and stage 8, while egg chambers of intermediate stages are TUNEL-negative. Corresponding pictures from groups 900 and 1800 (data not shown) had identical characteristics. The two checkpoints in all groups (exposed and SE/C) had the highest percentages of cell death compared with the other developmental stages 1–6 and 9–10 (Table 1). While in the SE groups the sum ratio of TUNEL-positive to total number of egg chambers was slightly higher in stages 7–8 (78/364) than in the germarium (37/186), in all three exposed groups this ratio was higher in the germarium than in stages 7–8 (Table 1).

Fig. 1e and f, show ovarioles of exposed female insects (groups 900A and 900, respectively) with a TUNEL-positive signal at all developmental stages from germarium to 7–8 and in all the cell types of the egg chamber (nurse cells, follicle cells and the oocyte). In Fig. 1f, a characteristic TUNEL-positive signal in the follicle cells of a stage-7 egg chamber is presented.

Although in most pictures the TUNEL-positive signal was most evident in the nurse cells, in the majority of the egg chambers in all the exposed groups a TUNEL-positive signal was detected in all three kinds of egg chamber cells (Fig. 1e and f).

Fig. 1g presents a stage-9 egg chamber of an exposed insect (group 900A) with a TUNEL-positive signal in the nurse cells and follicle cells. Fig. 1h shows a stage-10 egg chamber of an exposed insect (group 900) with a TUNEL-positive signal in the nurse cells. Pictures corresponding to Fig. 1g and h from all three exposed groups

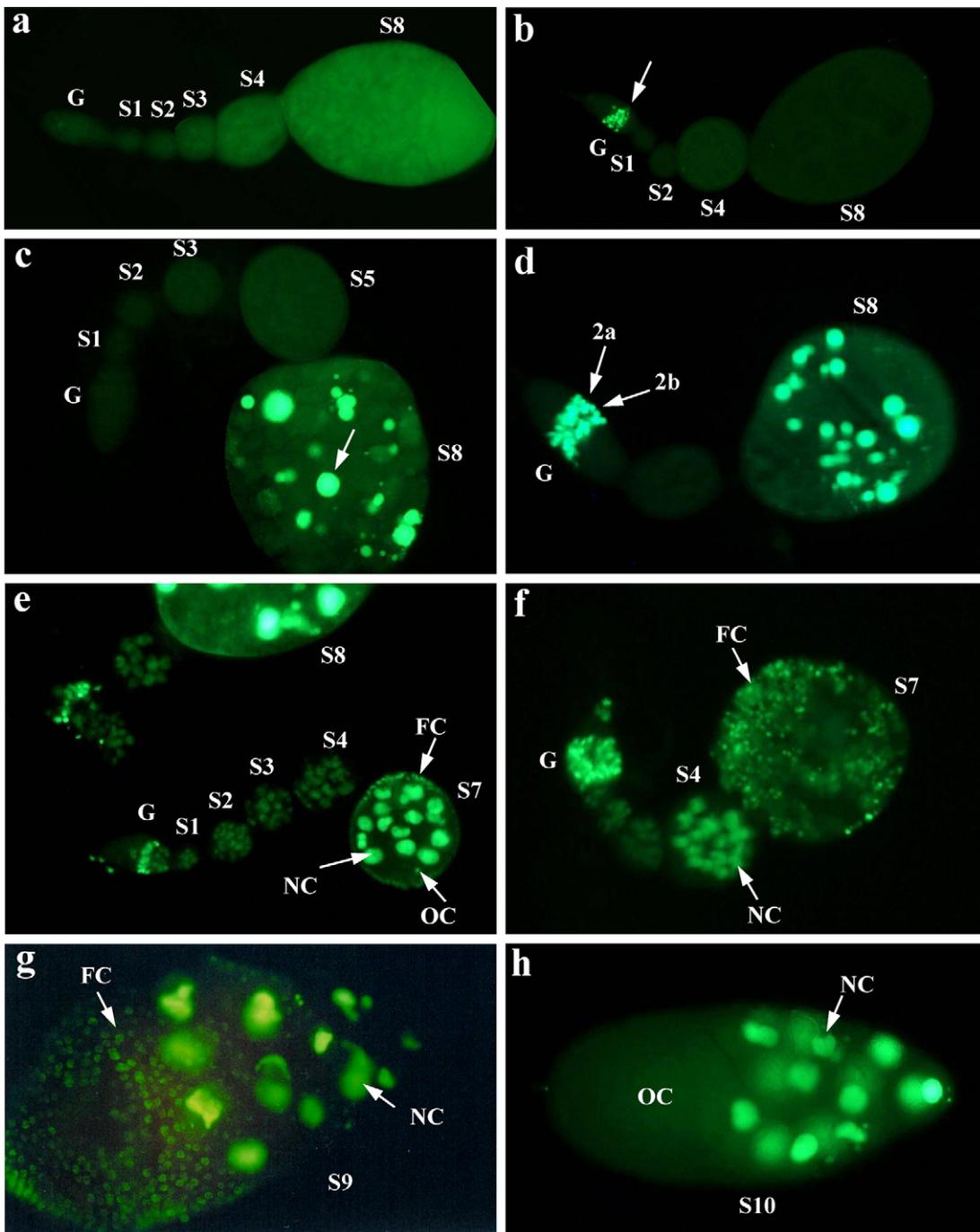


Fig. 1. (a) Typical TUNEL-negative fluorescent picture of an ovariole of a sham-exposed/control female insect, containing egg chambers from germarium up to stage 8. (b) Ovariole of an exposed insect with fragmented DNA only on cells of the germarium 2a/2b region (arrow). (c) Ovariole of an exposed insect with TUNEL-positive signal only at the nurse cells of a stage 8 egg chamber (arrow) and TUNEL-negative at all other stages. (d) Ovariole of an exposed insect with TUNEL-positive signal only at the two checkpoints, regions 2a and 2b of the germarium plus stage 8 egg chamber and TUNEL-negative intermediate stages. (e) Ovarioles of exposed female insects with fragmented DNA at all stages from germarium to stages 7 and 8 and in all kinds of egg chamber cells, (NC: nurse cells, FC: follicle cells, OC: oocyte). (f) Characteristic picture of TUNEL-positive signal in the follicle cells (FC) of a stage-7 egg chamber in an ovariole of an exposed female insect. At the stage-4 egg chamber, cell death appears in the nurse cells (NC). (g) Characteristic picture of induced cell death in the nurse cells (NC) and follicle cells (FC) of a stage-9 egg chamber of an exposed female insect. (h) Stage 10, TUNEL-positive egg chamber of an exposed female insect.

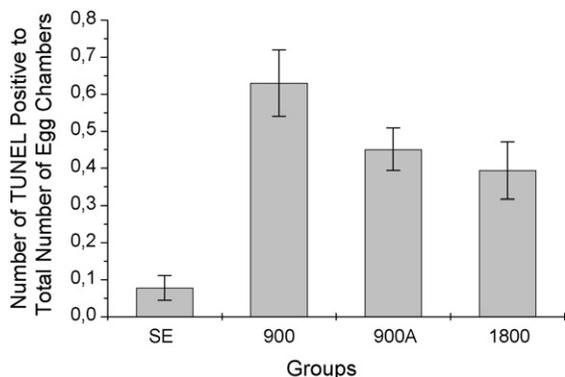


Fig. 2. Mean ratio of ovarian cell death (number of TUNEL-positive to total number of egg chambers), in each experimental group \pm S.D. (0.078 ± 0.0335 in SE, 0.630 ± 0.0898 in 900, 0.451 ± 0.0574 in 900A and 0.394 ± 0.0777 in 1800).

(data not shown) had identical characteristics. While in the SE groups the ratio of TUNEL-positive egg chambers of stages 9–10 was very small (7/282), the corresponding ratio was significantly higher in all three exposed groups: 165/262 in 900, 117/257 in 900A and 91/239 in 1800.

The summarised data of Table 1 are graphically represented in Fig. 2.

Statistical analysis (single factor analysis-of-variance test) shows that the probability that groups differ between them because of random variations is negligible, $P < 10^{-13}$.

We note that in the sham-exposed/control groups, induced DNA fragmentation was observed almost exclusively at the two developmental stages named checkpoints (37/186 in the germarium and 78/364 in stages 7–8), and only in few cases at the other provitellogenic and vitellogenic stages 1–6 (32/1148) and stages 9–10 (7/282), correspondingly. In contrast, ovarian egg chambers of animals from all three exposed groups, were found to be TUNEL-positive to a high degree at all developmental stages from germarium to stage 10 (Table 1).

In all cases (both in the sham-exposed/control and also in the exposed groups) the TUNEL-positive signal was observed predominantly at the two checkpoints, germarium and stages 7–8.

There was no detectable temperature increase within the vials during the exposures, as measured by the sensitive mercury thermometer.

4. Discussion

Although egg chambers during early and mid oogenesis in *Drosophila* were not reported until now to

exhibit either stress-induced or physiological degeneration at other stages except germarium and stages 7–8 [10–12,15], in the present experiments cell death was observed at all provitellogenic and vitellogenic stages 1–10 and the germarium. Additionally, it is the first time that cell death can be observed in all cell types of the egg chamber, i.e. not only in nurse cells and follicle cells – which was already known [15,10–12,20,21] – but also in the oocyte (Fig. 1e). A possible explanation for these effects is that the electromagnetic stress induced in the ovarian cells by the GSM and DCS fields is a new and probably more intense type of external stress, against which ovarian cells do not have adequate defence mechanisms like they do in the case of poor nutrition or chemical stress.

Our experiments and the statistical analysis show that genomic DNA fragmentation of the egg chambers cells is induced by the mobile telephony radiation. Both types of radiation, GSM 900 MHz and DCS 1800 MHz induce cell death in a large number (up to 55% in relation to control) of ovarian egg chambers in the exposed insects with only 6 min exposure per day for a limited period of 6 days.

DNA fragmentation is induced in all cases predominantly at the two developmental stages named checkpoints, germarium and stages 7–8. Since the above checkpoints were already known to be the most sensitive stages in response to other stress factors [23,24,11,10,15] such an observation could be expected. Our results show that these two checkpoints are the most sensitive stages also in response to electromagnetic stress.

Our experiments show that in case of electromagnetic stress induced by the GSM and DCS fields, the germarium checkpoint appears to be even more sensitive than the mid-oogenesis checkpoint at stages 7–8. Thus, the two checkpoints are not equally responsive to distinct types of stress and may therefore also respond differentially to other types of stress stimuli. A possible explanation for the more sensitive germarium stage is that it may be more effective in evolutionary terms for the animal to block development of any defective egg chamber at the beginning rather than at later stages, in order to prevent the waste of precious nutrients.

In conclusion, cell death was detected during all the developmental stages of early and mid oogenesis in *Drosophila*, from germarium to stage 10 and in all types of egg chamber cells (nurse cells, follicle cells, oocyte). Germarium and stages 7–8 were found to be the most sensitive stages in response to electromagnetic stress. However, the germarium checkpoint was found to be even more sensitive than stages 7–8 in response to this particular stress.

It is important to emphasize that the recorded effect in the oocyte, which undergoes meiosis during the last stages of oogenesis, may result in heritable mutations upon DNA-damage induction and repair, if not in cell death.

In comparing the two types of mobile telephony radiation, GSM 900 MHz seems to be more drastic than DCS 1800 MHz, not only when it is emitted at a higher intensity as usually happens, but also even at almost the same intensity, although differences between “900A” and “1800” were within the standard deviation (Fig. 2). A possible explanation can be given by the biophysical mechanism that we proposed previously [56–58] for the action of electromagnetic fields on cells, according to which lower frequency fields appear to be more bioactive than higher frequency fields of the same rest characteristics. Accordingly, ELF electric fields of the order of several V/m, are able to disrupt cell function by irregular gating of electrosensitive ion channels on the cells plasma membranes. The ELF components of both GSM and DCS fields appear to possess sufficient intensity for this. Nevertheless, a full comparison of the bioactivity between the two types of mobile telephony radiation needs further experimentation and verification.

Our present results are in complete agreement with our earlier results [1–3], according to which GSM radiation with a similar exposure procedure was found to decrease oviposition by up to 60%. The present results not only confirm our earlier data, but they also reveal a different explanation: the large decrease of reproductive capacity found in our earlier experiments is not due to retardation of cellular processes as we assumed at the time, but it is due to elimination of large numbers of egg chambers during early and mid oogenesis, either via stress-induced apoptosis or necrosis of their constituent cells, caused by the mobile telephony radiation.

Our present results are also in agreement with results of other experimenters reporting DNA damage in other cell types, assessed by different methods than ours, after *in vivo* or *in vitro* exposure to GSM radiation [31,32,59].

Since there was no detectable temperature increase during the exposures, the recorded effects are considered as non-thermal.

We do not know if the ovarian cell death found in our present work is due to apoptosis, i.e. caused by the organism in response to the electromagnetic stress, or the result of necrosis caused directly by the electromagnetic radiation. This very important issue remains to be uncovered in a next series of experiments.

Although we cannot simply extrapolate, we consider that similar effects on humans are certainly possible for two reasons. First, insects are found to be more resistant

than mammals, at least to ionizing radiation [60,61]. Second, our results are in agreement with reported effects on mammals [42–44,59]. It is also possible that induced cell death on a number of brain cells can explain symptoms like headaches, fatigue, sleep disturbances, etc., reported as ‘microwave syndrome’ [62,63]. Therefore, we think that our results imply the cautious use of mobile phones and a reconsideration of the current exposure criteria.

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