

Changes in Puffing Pattern of *Drosophila melanogaster* (Diptera: Drosophilidae) Polytene Chromosomes after Egg Exposure to Microwave Radiation and Magnetic Field¹

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J. Entomol. Sci. 53(3): 295–306 (July 2018)

Abstract We determined the effects of microwave radiation and static magnetic field on the gene activity in *Drosophila* by measuring the dimensions of puffing the salivary gland polytene chromosomes of *Drosophila melanogaster* Meigen following exposure of eggs to the radiation and magnetic field. *Drosophila* eggs were exposed to either microwave radiation of 36.64 GHz frequency and 1 W/m² intensity for 30 s, a static magnetic field of 25 mT for 5 min, or both. The diameter of puffs was measured in squashed salivary gland preparations extracted from larvae from those eggs as they entered the prepupal stage. The puffs measured were 50CD, 63F, 71CE, 72CD (chromosome 3L) and 82EF, 83E, 93D (chromosome 3R). Results demonstrated that (a) microwave radiation exposure decreased puffing activity in puffs 63F, 71CE, and 82 EF but had no effect on puffing at 72CD, 93D and 50CD; (b) exposure to magnetic fields only did not change puff activity, but magnetic field exposure appeared to enhance the impact of microwave radiation exposure at locus 83F by decreasing puff activity; (c) puffs 63F, 71CE, and 82EF were smaller when exposed to microwave radiation and magnetic field combined than with microwave radiation alone, and; (d) no apparent changes were observed at the 93D puff after exposure to microwave radiation and the combined treatments.

Key words electromagnetic field, microwave radiation, *Drosophila* salivary gland, chromosome puffs

The biological effects of electromagnetic fields (EMF) have been intensively investigated in connection with animal navigation (Kishkinev 2015; Marley et al. 2014), plant growth and development (Maffei 2014), possible health hazards (Berg et al. 2006; Vecchia et al. 2009), and medical uses (Raffin and Siebner 2014). The mode of action of EMF on living cells is likely related to regulation of gene activity (Blank and Goodman 2009; Goodman and Blank 1998, 2002; Goodman et al. 1987, 1992, 1993; Lin et al. 2001). EMF exposure has been shown to induce quantitative and qualitative changes in transcriptome and proteome (Ennamany et al. 2008; Nikolova et al. 2005; Remondini et al. 2006; Stock et al. 2012; Trivino et al. 2012; Zhao et al. 2007), but other studies indicate slight to no changes (Sakurai et al. 2011, 2012, 2013; Zeng et al. 2006). Proteomic analysis of ex vivo human tissues

¹Received 3 July 2017; accepted for publication 26 September 2017.

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and cell lines also revealed differences among cells exposed and not exposed to microwaves (Karinen et al. 2008; Nylund et al. 2009, 2010). Transcriptome changes were observed in osteocyte-like cells (MLO-Y4) exposed to static magnetic fields (12 and 16 T) for 48 h with expression of enzymes, peptide hormones, and G-protein receptors genes (Wang et al. 2015). The interactions of different types of electromagnetic radiation also have been studied. Lai and Singh (2005) showed that simultaneous exposure to a temporally incoherent magnetic field blocked microwave-induced DNA damage in brain cells of rats. Yao et al. (2008) also reported that magnetic fields often block other effects induced by microwaves. Some of these interaction effects are reviewed Manti and D'Arco (2010).

We selected the *Drosophila melanogaster* Meigen polytene chromosomes to further assess the impact of electromagnetic radiation on gene regulation. The system is well studied, and one can assess quantitatively the observed changes in chromosomal puffing patterns. Earlier, we reported the effect of low-intensity microwave radiation on the puffing pattern of *D. melanogaster* polytene chromosomes (Shakina et al. 2011). We also demonstrated the effects of magnetic field and microwave exposure, individually and combined, on the rate of development, fecundity, embryonic mortality, and pupal lethality of *D. melanogaster* (Gracheva et al. 2015). Herein, we examined the effects of successive exposures to these electromagnetic radiation sources on the puffing pattern in *Drosophila* polytene chromosomes. Our results are expected to have broad applicability.

Materials and Methods

Drosophila melanogaster wild type inbred line Canton-S used in this study was obtained from the *Drosophila* collection of the Department of Genetics and Cytology of the V.N. Karazin Kharkiv National University (Kharkiv, Ukraine). Flies were grown in a standard sugar-yeast medium at a temperature of $24 \pm 0.5^\circ\text{C}$. Five-day-old females were collected and used for oviposition. Eggs from these females were collected 2 h after oviposition began, immediately exposed to the respective radiation treatments, and placed on the rearing medium. Larvae were removed from the medium as they entered the prepupal stage (0-hr prepupae). Squash preparations of the polytene chromosomes in salivary glands of these larvae were stained with 2% orcein solution in acetic acid (45%) and studied at magnification (800 \times) as per methods of Sullivan et al. (2000). Puff size was assessed only in chromosomes with the degree of polyteny 1,024. Puffs were identified by the updated maps of gene location in *D. melanogaster* originally cited by Bridges (1921) and updated by Lindsley and Grell (1968).

The reaction of seven chromosomal puffs to exposure to microwaves and static magnetic fields were measured. Those puffs were 50CD (chromosome 2R), 63F, 71CE, 72CD (chromosome 3L), and 82EF, 83E, 93D (chromosome 3R) (Fig. 1). Diameters of puffs were measured using an ocular micrometer and compared with the width of the adjacent disc of the chromosome not involved in the puffing. Puff size is correlated with the level of transcriptional activity (Gruenbaum 2015); thus, the ratio of size of the puff to the size of the adjacent disk (puff:disk ratio) was used to assess puff activity (e.g., 50CD/51B, 63F/64B, 71CE/73A, 72CD/73A, 82EF/84A, 83E/84A, 93D/93F). All puffs studied are involved in development, except the puff

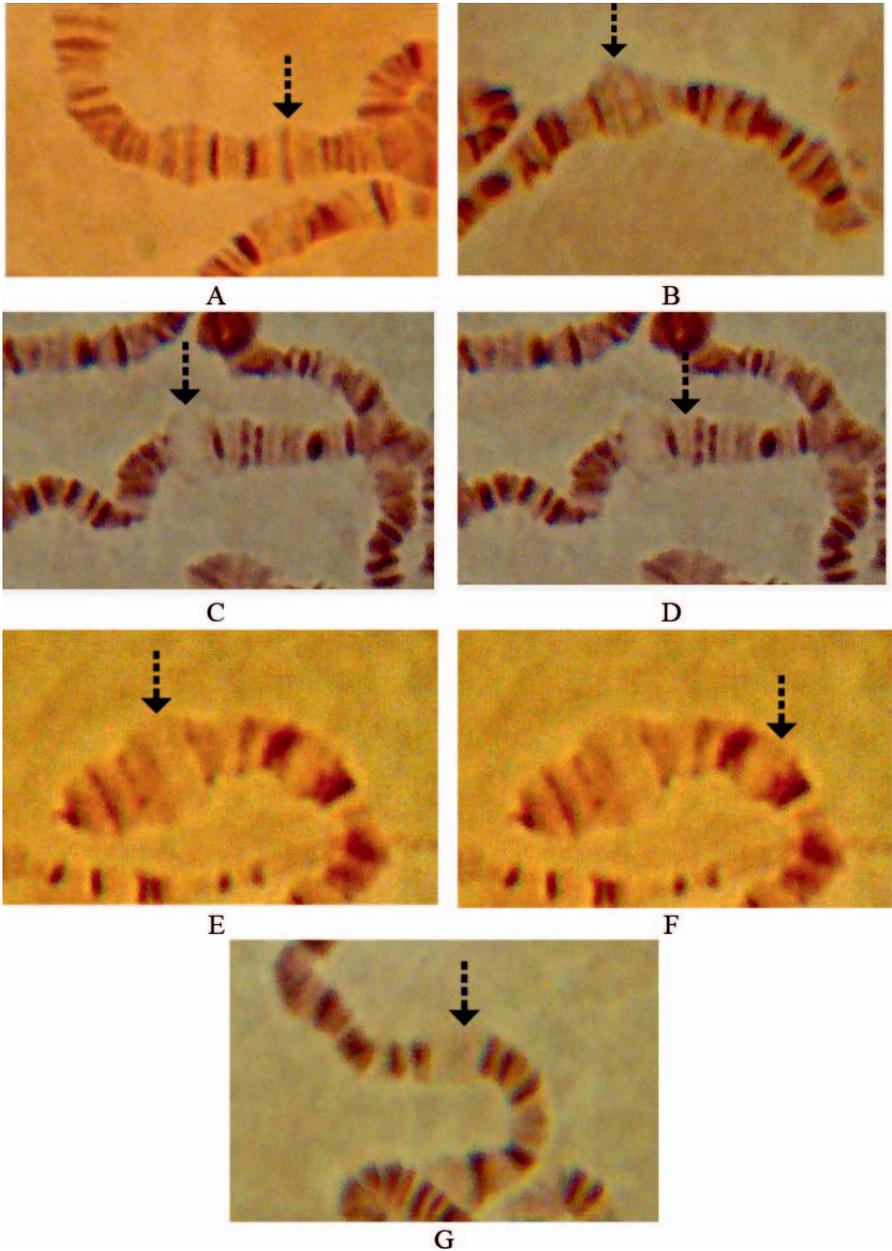


Fig. 1. Microphotographs of seven *Drosophila melanogaster* puffs taken into investigation in the present work. (A) 50CD (chromosome 2R); (B) 63F (chromosome 3L); (C) 71CE (chromosome 3L); (D) 72CD (chromosome 3L); (E) 82EF (chromosome 3R); (F) 83E (chromosome 3R); (G) 93D (chromosome 3R).

93D which is the heat-shock puff. The average size of one puff was determined by examining 25–65 nuclei in each variant of experiment in 5–17 larvae, with no more than five nuclei in each salivary gland preparation.

The source of microwave radiation was a semiconductor device, based on a Gunn diode, designed and manufactured by V.N. Bykov (Department of Theoretical Radiophysics, V.N. Karazin Kharkiv National Univ.). The device produced a frequency of 36.64 ± 0.05 GHz, which was delivered through the pyramidal horn antenna on the device (30×30 mm at the edge of the horn). *Drosophila melanogaster* eggs were exposed to microwaves at room temperature (24°C) with the eggs placed in an open area and at 15 cm from the edge of the antenna, thus ensuring uniformity of radiation exposure. The power density of the microwave radiation on the surface of the exposed eggs was 1 W/m^2 with an exposure time of 30 s. This intensity was approximately 10,000 times greater than the microwave radiation that normally occurs in urban areas (Hutter et al. 2006).

The static magnetic field of 25 mT intensity was produced by a magnet (permalloy, $26 \times 9 \times 1.8$ cm). Its intensity was determined using the IMI-3 Hall effect magnetic sensor (International Magna products Inc., Russia). The eggs were placed on the N-pole of the magnet for 5 min at room temperature. The strength of the magnetic field in our tests was approximately 1,000 times greater than the terrestrial, or background, magnetic field strength (Hulot et al. 2010).

Treatments were exposure to (a) microwave radiation only, (b) magnetic field only, (c) microwave followed by magnetic field, and (d) magnetic field followed by microwave. The control consisted of exposure to neither microwave nor magnetic field. Mean values and associated standard errors were calculated for at least 25 measurements for each treatment (Figs. 2–8). Paired means of puff dimensions were compared with a Student's *t*-test (Student [W.S. Gosset] 1908). An analysis of variance (ANOVA; Steel and Torrie 1960) was conducted to compare puffing patterns among all treatment means.

Results

The ANOVA revealed a significant impact by electromagnetic factors on the puffing pattern of the 63F ($F=8.924$; $df=4$; $P<0.05$), 71CE ($F=7.4505$; $df=4$; $P<0.05$), 83E ($F=2.454$; $df=4$; $P<0.05$), and 93D ($F=3.362$; $df=4$; $P<0.05$) loci of the polytene chromosomes of the salivary glands of *D. melanogaster* prepupae; however, the impact at the 50CD ($F=1.2126$; $df=4$; $P>0.05$), 72CD ($F=0.700$; $df=4$; $P>0.05$), and 82EF ($F=2.0033$; $df=4$; $P>0.05$) was not significant. In comparison to the untreated control, exposure to the microwave radiation decreased the puff size by 25% ($t=4.44$; $df=137$; $P<0.001$) at the 63F loci (Fig. 2), 37% ($t=4.69$; $df=88$; $P<0.001$) at the 71CE loci (Fig. 3), and 28% ($t=2.21$; $df=66$; $P<0.001$) at the 82EF loci (Fig. 5). Puff size at the 83E loci was not significantly impacted by exposure to either microwave or magnetic field alone, but the puff size was reduced by exposure to the microwave radiation followed by the magnetic field exposure (Fig. 6).

Likewise, exposure to microwave radiation followed by exposure to the magnetic field induced significant reductions in puff size in comparison to the controls at 63F (17% reduction; $t=3.11$; $df=140$; $P<0.05$ [Fig. 2]), 71CE (25% reduction; $t=$

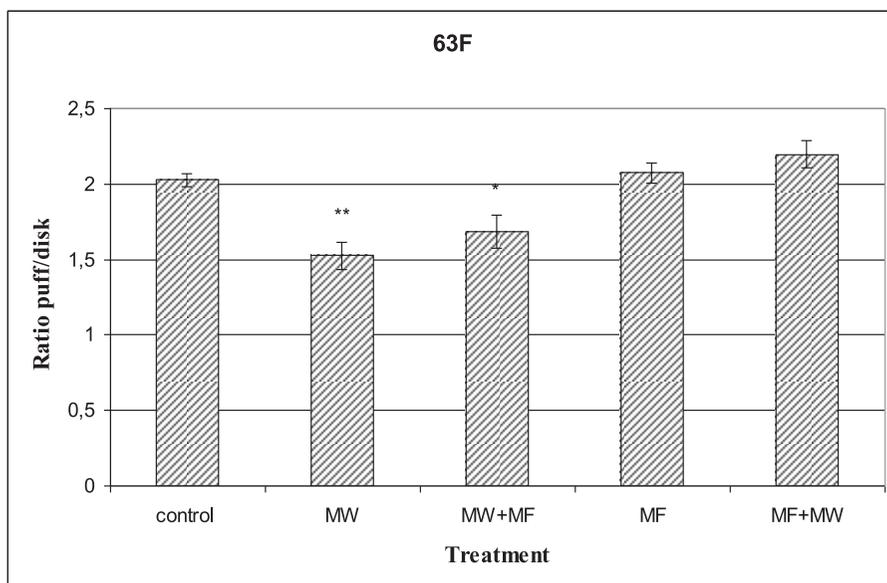


Fig. 2. The 63F/64B puff:disk ratio in *Drosophila melanogaster* larvae developed from the exposed eggs. Here and below: the bars in Figures 2–8 are marked by two asterisks (**) if probability level of difference from control was $P < 0.01$ and by one asterisk (*) if $P < 0.05$.

3.04; $df = 88$; $P < 0.05$ [Fig. 3]), and 83E (16% reduction; $t = 3.87$; $df = 65$; $P < 0.05$ [Fig. 6]). And, compared to the puff dimensions following exposure to microwave radiation only, the successive exposure to magnetic field followed by microwave decreased puff dimensions at 63F, 71CE, 82EF, and 93D (Figs. 2, 3, 5, 7). Puff dimensions at 72CD, 93D, and 50CD did not react to microwave exposure (Figs. 4, 7, 8).

Discussion

All loci examined in this study are related to development, with their diameter changing as development of the larvae progresses. These changes are attributed to ecdysone concentration (Ashburner 1967; Thummel 1990). The puff 93D, one of the largest heat-shock puffs (Ashburner 1970; Lakhotia 2011; Lindquist 1986), is also an early-late ecdysone-stimulated puff (Ashburner 1967; Thummel 1990). The lack of observed impact of electromagnetic radiation on the 93D puff is of special interest because its activity has been connected with the biological effects of microwave exposure and its accompanying thermal effects. Furthermore, the biological effect of microwave radiation has been assessed by specific absorption rate (SAR) (Vecchia et al. 2009).

Our results presented herein agree with results of our previous study showing puff decrease in *Drosophila* larvae from radiation-exposed eggs. Shakina et al.

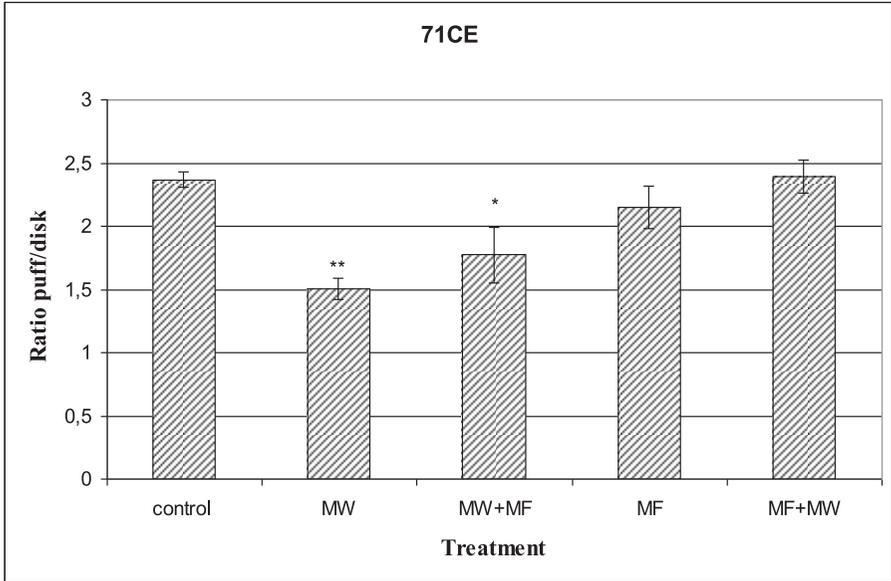


Fig. 3. The 71CE/73A puff:disk ratio in *Drosophila melanogaster* larvae developed from the exposed eggs.

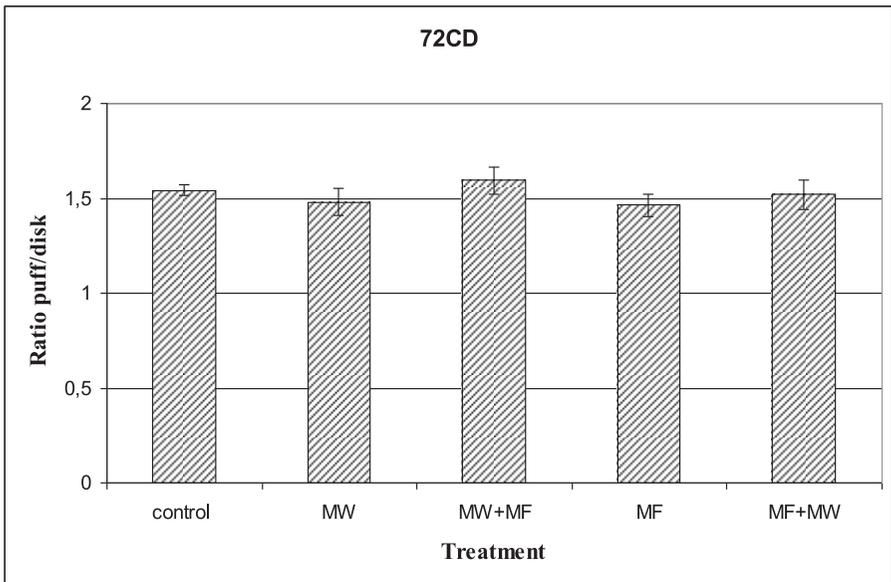


Fig. 4. The 72CD/73A puff:disk ratio in *Drosophila melanogaster* larvae developed from the exposed eggs.

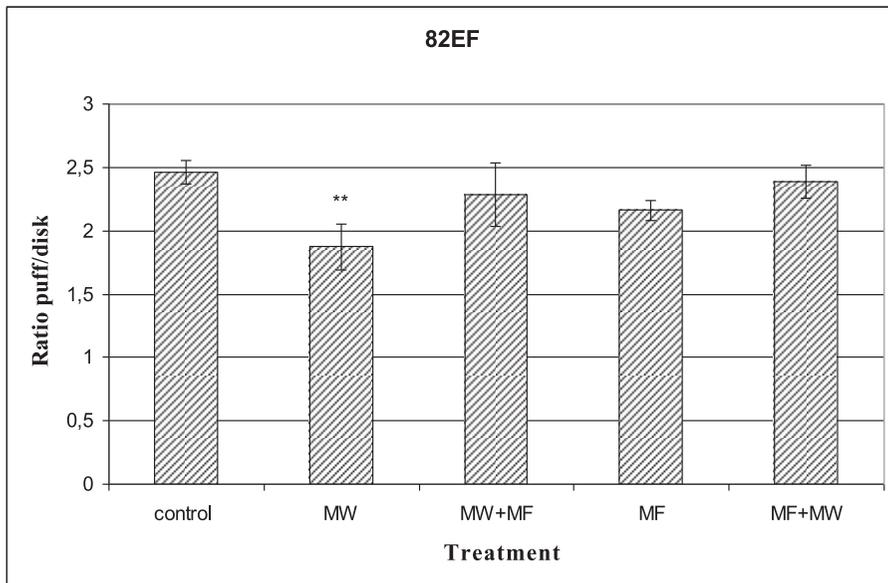


Fig. 5. The 82EF/84A puff:disk ratio in *Drosophila melanogaster* larvae developed from the exposed eggs.

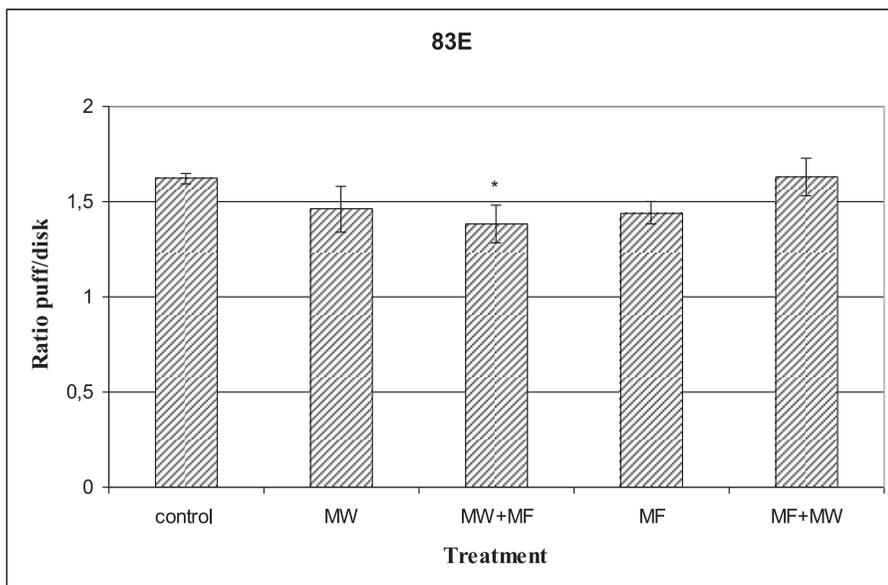


Fig. 6. The 83E/84A puff:disk ratio in *Drosophila melanogaster* larvae developed from the exposed eggs.

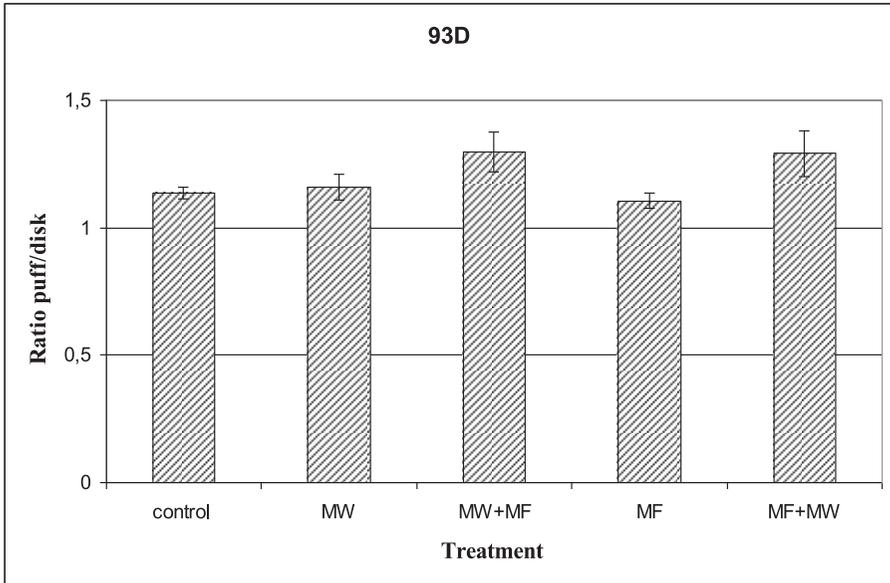


Fig. 7. The 93D/93F puff:disk ratio in *Drosophila melanogaster* larvae developed from the exposed eggs.

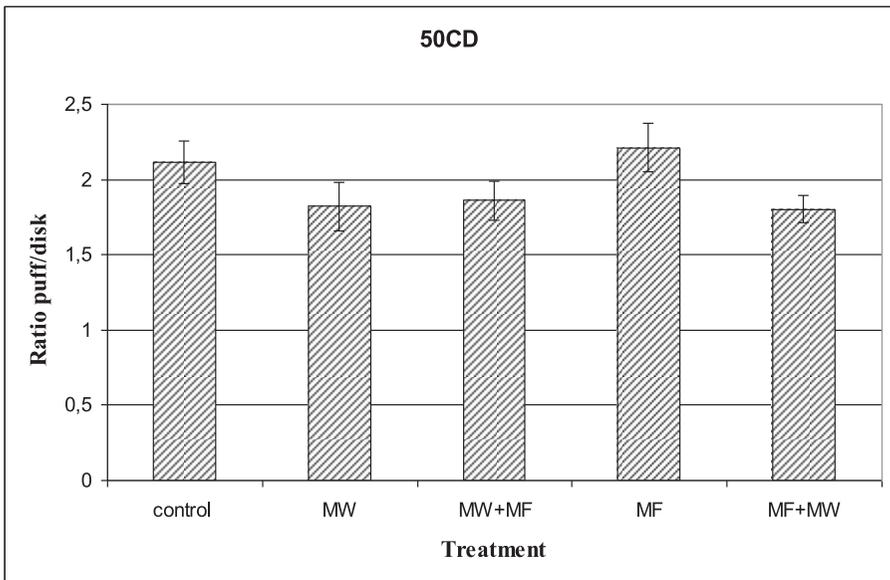


Fig. 8. The 50CD/51B puff:disk ratio in *Drosophila melanogaster* larvae developed from the exposed eggs.

(2011) reported that the microwave-induced (frequency 36.64 GHz; power density 0.40 W/m^2) decreased puff diameter at the 71CE, 82EF, and 83E loci, that puff dimensions at loci 21F, 22C, 23E, 63F, and 72CD were not changed significantly, and some tendency for decreasing puff size was observed at loci 21F, 23E, 63F, and 72CD. In our present study, we detected a decrease of activity at 71CE and 82EF, but also at 63F, which may be due to the increase of microwave radiation surface power (1 W/m^2) in the present study.

Exposure to microwave followed by magnetic field exposure appeared to reduce the impact of exposure to microwave radiation alone at the 63F, 71CE, 82EF, and 93D loci (Figs. 2, 3, 5, 7). If, after exposing eggs to the magnetic field they were subsequently exposed to microwave radiation, an increase in puff size was sometimes observed, namely at 93ED (*t*-test: $t = 2.36$; $df = 52$; $P = 0.026$) (Fig. 7). Therefore, the reactions of puffing activity to sequential treatment of *Drosophila* eggs with microwave followed by magnetic field exposure is apparently subtle and, thus, reveals no distinct protective or restorative effect by the magnetic field following microwave-induced impacts.

Initially, the observed decrease of activity of puffs after exposure to microwaves appears to contradict results obtained in previous studies showing increases in transcription activity in the polytene chromosomes induced by electromagnetic radiation (72 Hz frequency) measured by ^3H -uridine radioautography (Goodman et al. 1987, 1992, 1993); however, the apparent contradictions might be attributed to the differing methodologies employed. Goodman et al. (1987, 1992, 1993) used low-frequency electromagnetic radiation while we used microwave radiation. Also, the former studies assessed impact immediately after exposure of the cells to the radiation while we exposed eggs to the radiation and assessed impact later in the development of the larvae.

Regulation of chromosomal puff activity as shown in our work presented herein agrees with the differential gene activity reaction to electromagnetic radiation reported by others (Karinen et al. 2008; Nylund et al. 2009; Remondini et al. 2006; Stock et al. 2012) as well as the conclusion of the occurrence of gene promoters in response to electromagnetic exposure (Blank and Goodman 2009; Goodman and Blank 1998, 2002; Lin et al. 2001). We further add that this postulation of positive regulation of gene promoters might be supported by two points. One, the electromagnetic-induced genome activity may not be connected with activation of gene activity but with depression of genes. Repression of gene activity was shown in our results herein and were also supported by previous studies with *Drosophila* following microwave exposure (Shakina et al. 2011), the phenomenon of chromatin condensation (heterochromatinization) in human cells induced by exposure to microwaves and magnetic fields (Shckorbatov 2012, 2014; Shckorbatov et al. 1998), and in several studies of transcriptome changes in cells after electromagnetic exposures (Ennamany et al. 2008; Fedrowitz and Löscher 2012; Feng et al. 2013; Nikolova et al. 2005; Remondini et al. 2006, Stock et al. 2012; Trivino et al. 2012; Wang et al. 2015). Second, the phenomenon of electromagnetic-induced gene regulation may embrace not only gene promoters but also other regulatory elements (enhancers, LCRs) and by the other mechanisms of regulation of gene activity, namely by chromatin condensation (heterochromatinization).

In conclusion, *D. melanogaster* larvae that developed from eggs exposed to monochromatic microwave irradiation (36.64 GHz; 1 W/m^2 intensity) and also

exposed to a static magnetic field (25 mT intensity) exhibit changes in puffing pattern in the polytene chromosomes. Exposure to the low-level microwave irradiation usually reduces puff diameter with puffs related to insect development. However, successive exposure to microwave radiation and magnetic fields does not modify those effects.

Acknowledgments

The authors are grateful to the Ministry of Education and Science of Ukraine (Project State registration No: 0112U008334) for financial support. We also thank Professor V.N. Bykov (Department of Theoretical Radiophysics, V.N. Karazin Kharkiv National Univ.) for constructing and providing the semiconductor device that served as our source of microwave radiation.

References Cited

- Ashburner M. 1967.** Patterns of puffing activity in the salivary gland chromosomes of *Drosophila*. I. Autosomal puffing patterns in a laboratory stock of *Drosophila melanogaster*. Chromosoma 21: 398–428.
- Ashburner M. 1970.** Patterns of puffing activity in the salivary gland chromosomes of *Drosophila*. V. Responses to environmental treatments. Chromosoma 31: 356–376.
- Berg, G., J. Spallek, J. Schüz, B. Schlehofer, E. Böhler, K. Schlaefer, I. Hettinger, K. Kunna-Grass, J. Wahrendorf and M. Blettner. 2006.** Occupational exposure to radio frequency/microwave radiation and the risk of brain tumors: Interphone Study Group, Germany. Am. J. Epidem. 164: 538–548.
- Blank, M. and R. Goodman. 2004.** Initial interactions in electromagnetic field-induced biosynthesis. J. Cell Physiol. 199: 359–363.
- Blank, M. and R. Goodman. 2009.** Electromagnetic fields stress living cells. Pathophysiology 16: 71–78.
- Bridges, C.B. 1921.** Current maps of the location of the mutant genes of *Drosophila melanogaster*. Nat. Acad. Sci. 7: 127–132.
- Ennamany, R., R. Fitoussi, K. Vie, J. Rambert, L.M. De Benetti and M.D. Mossalayi. 2008.** Exposure to electromagnetic radiation induces characteristic stress response in human epidermis. J. Invest. Dermat. 128: 743–746.
- Fedrowitz, M. and W. Löscher. 2012.** Gene expression in the mammary gland tissue of female Fischer 344 and Lewis rats after magnetic field exposure (50 Hz, 100 μ T) for 2 weeks. Int. J. Radiat. Biol. 88: 425–429.
- Feng, J., H. Sheng, C. Zhu, H.H. Jiang and S. Ma. 2013.** Effect of adjuvant magnetic fields in radiotherapy on non-small-cell lung cancer cells *in vitro*. BioMed Res. Intern. doi: org/10.1155/2013/657259.
- Goodman, R., J. Abbott and S. Henderson. 1987.** Transcriptional patterns in the X chromosome of *Sciara coprophila* following exposure to magnetic fields. Bioelectromagnetics 8: 1–7.
- Goodman, R. and M. Blank. 1998.** Magnetic field stress induces expression of hsp 70. Cell Stress Chaperones 3: 79–88.
- Goodman, R. and M. Blank. 2002.** Insights into electromagnetic interaction mechanisms. J. Cell Physiol. 192: 16–22.
- Goodman, R., D. Weisbrot, A. Uluc and A. Henderson. 1992.** Transcription in *Drosophila melanogaster* salivary gland cells is altered following exposure to low-frequency electromagnetic fields: Analysis of chromosome 3R. Bioelectromagnetics 13: 111–118.
- Goodman, R., D. Weisbrot, A. Uluc and A. Henderson. 1993.** Transcription in *Drosophila melanogaster* salivary gland cells is altered following exposure to low frequency

- electromagnetic fields: Analysis of chromosomes 3L and X. *Bioelectrochem. Bioenerg.* 28: 311–318.
- Gracheva, M.A., T.M. Cheshko and Y.G. Shckorbatov. 2015.** Some ontogenic aspects of the influence of physical factors on viability of *Drosophila melanogaster*. *Faktori Eksper. Evol. Organ.* 16: 37–41. (in Ukrainian.)
- Gruenbaum, Y. 2015.** Nuclear organization. *Annu. Rev. Biochem.* 84:61–64.
- Hulot, G., C.C. Finlay, C.G. Constable, N. Olsen and M. Manda. 2010.** The magnetic field of planet Earth. *Space Sci. Rev.* 152: 159–222.
- Hutter, H.-P., H. Moshhammer, P. Wallner and M. Kundi. 2006.** Subjective symptoms, sleeping problems, and cognitive performance in subjects living near mobile phone base stations. *Occup. Environ. Med.* 63: 307–313.
- Karinen, A., S. Heinävaara, R. Nylund and D. Leszczynski. 2008.** Mobile phone radiation might alter protein expression in human skin. *BMC Genomics* 9:77. doi: 10.1186/1471-2164-9-77.
- Kishkinev, D. 2015.** Sensory mechanisms of long-distance navigation in birds: A recent advance in the context of previous studies. *J. Ornithol.* doi: 10.1007/s10336-015-1215-4.
- Lai, H. and N.P. Singh. 2005.** Interaction of microwaves and a temporally incoherent magnetic field on single and double DNA strand breaks in rat brain cells. *Electromag. Biol. Med.* 24: 23–29.
- Lakhotia, S.C. 2011.** Forty years of the 93D puff of *Drosophila melanogaster*. *J. Biosci.* 36: 399–423.
- Lin, H., M. Blank, K. Rossoi-Haseroth and R. Goodman. 2001.** Regulating genes electromagnetic response elements. *J. Cell Biochem.* 81: 143–148.
- Lindquist, S. 1986.** The heat-shock response. *Annu. Rev. Biochem.* 55: 1151–1191.
- Lindsley, D.L. and E.H. Grell. 1968.** Genetic variations of *Drosophila melanogaster*. *Publ. No. 627.* Carnegie Institute of Washington, Washington, DC.
- Maffei, M.E. 2014.** Magnetic field effects on plant growth, development, and evolution. *Front. Plant. Sci.* 5: 445. doi: 10.3389/fpls.2014.00445.
- Manti, L. and A. D'Arco. 2010.** Cooperative biological effects between ionizing radiation and other physical and chemical agents. *Mutat. Res.* 704: 115–122.
- Marley, R., C.N.G. Giachello, N.S. Scrutton, R.A. Baines and A.R. Jones. 2014.** Cryptochrome-dependent magnetic field effect on seizure response in *Drosophila* larvae. *Sci. Reps.* 4:5799. doi: 10.1038/srep05799.
- Nikolova, T., J. Czyz, A. Rolletschek, P. Blyszczuk, J. Fuchs, G. Jovtchev, J. Schuderer, N. Kuster and A.M. Wobus. 2005.** Electromagnetic fields affect transcript levels of apoptosis-related genes in embryonic stem cell-derived neural progenitor cells. *FASEB J.* 19: 1686–1688.
- Nylund, R., N. Kuster and D. Leszczynski. 2010.** Analysis of proteome response to the mobile phone radiation in two types of human primary endothelial cells. *Proteome Sci.* 8: 52. doi: 10.1186/1477-5956-8-52.
- Nylund, R., H. Tammio, N. Kuster and D. Leszczynski. 2009.** Proteomic analysis of the response of human endothelial cell line EA.hy926 to 1800 GSM mobile phone radiation. *J. Proteomics Bioinform.* 2: 455–462.
- Raffin, E. and H.R. Siebner. 2014.** Transcranial brain stimulation to promote functional recovery after stroke. *Curr. Opin. Neurol.* 27: 54–60.
- Remondini, D., R. Nylund, J. Reivinen, F. Poulletier de Gannes, B. Veyret, I. Lagroye, E. Haro, M. Trillo, M. Capri, C. Franceschi, K. Schlatterer, R. Gminski, R. Fitzner, R. Tauber, J. Schuderer, N. Kuster, D. Leszczynski, F. Bersani and C. Maercker. 2006.** Gene expression changes in human cells after exposure to mobile phone microwaves. *Proteomics* 6: 4745–4754.
- Sakurai, T., T. Kiyokawa, E. Narita, Y. Suzuki, M. Taki and J. Miyakoshi. 2011.** Analysis of gene expression in a human-derived glial cell line exposed to 2.45 GHz continuous radiofrequency electromagnetic fields. *J. Rad. Res.* 52: 185–192.

- Sakurai, T., E. Narita, N. Shinohara and J. Miyakoshi. 2013.** Alteration of gene expression by exposure to a magnetic field at 23 kHz is not detected in astroglia cells. *J. Rad. Res.* 54: 1005–1009.
- Sakurai, T., E. Narita, N. Shinohara and J. Miyakoshi. 2012.** Intermediate frequency magnetic field at 23 kHz does not modify gene expression in human fetus-derived astroglia cells. *Bioelectromagnetics* 33: 662–669.
- Shakina, L.A., V.N. Pasiuga, O.M. Dumin and Y.G. Shckorbatov. 2011.** Effects of microwaves on the puffing pattern of *D. melanogaster*. *Centr. Eur. J. Biol.* 6: 524–530.
- Shckorbatov, Y. 2012.** The state of chromatin as an integrative indicator of cell stress, Pp. 123–144. *In* Simpson, N.M. and Stewart V.J. (eds.). *New Developments in Chromatin Research*. Nova Science Publishers, New York.
- Shckorbatov, Y. 2014.** The main approaches of studying the mechanisms of action of artificial electromagnetic fields on cell. *J. Electr. Electron. Syst.* 3: 123. doi: 10.4172/2332-0796.1000123.
- Shckorbatov, Y.G., V.G. Shakhbazov, N.N. Grigoryeva and V.A. Grabina. 1998.** Microwave irradiation influences on the state of human cell nuclei. *Bioelectromagnetics* 19: 414–419.
- Steel, R.G.D. and J.H. Torrie. 1960.** *Principles and Procedures of Statistics*. McGraw-Hill Book Co., New York.
- Stock, M., B. Kirchner, D. Waibler, D.E. Cowley, M.W. Pfaffl and R. Kuehn. 2012.** Effect of magnetic stimulation on the gene expression profile of in vitro cultured neural cells. *Neurosci. Lett.* 526: 122–127.
- Student (W.S. Gosset). 1908.** The probable error of a mean. *Biometrika* 6: 1–25.
- Sullivan, W., M. Ashburner and R.S. Hawley. 2000.** *Drosophila* Protocols. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
- Thummel, C.S. 1990.** Puffs and gene regulation—Molecular insights into the *Drosophila* ecdysone regulatory hierarchy. *Bioessays* 12: 561–568.
- Trivino, P.J.C., S. Grimaldi, M. Taranta, I. Naldi and C. Cinti. 2012.** Microwave electromagnetic field regulates gene expression in T-lymphoblastoid leukemia CCRF-CEM cell line exposed to 900 MHz. *Electromagn. Biol. Med.* 31: 1–18.
- Vecchia, P., R. Matthes, G. Ziegelberger, J. Lin, R. Saunders and A. Swerdlow. 2009.** Exposure to high frequency electromagnetic fields, biological effects and health consequences (100 kHz–300 GHz). Tech. Rep. ICNIRP 16/2009, International Commission on Non-Ionizing Radiation Protection.
- Wang, Y., Z.H. Chen, C. Yin, J.H. Ma, D.J. Li, F. Zhao, Y.L. Sun, L.F. Hu, P. Shang and A.R. Qian. 2015.** Gene chip expression profiling reveals the alterations of energy metabolism related genes in osteocytes under large gradient high magnetic fields. *PLoS One* 10: e0116359. doi: 10.1371/journal.pone.0116359.
- Yao, K., W. Wu, K. Wang, S. Ni, P. Ye, Y. Yu, J. Ye and L. Sun. 2008.** Electromagnetic noise inhibits radiofrequency radiation-induced DNA damage and reactive oxygen species increase in human lens epithelial cells. *Mol. Vision* 14: 964–969.
- Zeng, Q., G. Chen, Y. Weng, L. Wang, H. Chiang, D. Lu and Z. Xu. 2006.** Effects of global system for mobile communications 1800 MHz radiofrequency electromagnetic fields on gene and protein expression in MCF-7 cells. *Proteomics* 6: 4732–4738.
- Zhao, R., S. Zhang, Z. Xu, L. Ju, D. Lu and G. Yao. 2007.** Studying gene expression profile of rat neuron exposed to 1800MHz radiofrequency electromagnetic fields with cDNA microassay. *Toxicology* 235: 167–175.