The purpose of this investigation was to study the effect of microwave irradiation, static magnetic field and their combined action on the violation of homologous pairing of polytene chromosomes in Drosophila melanogaster Meig. Inbred line Canton-S was used as an experimental object. Microwaves with frequency 36.64 GHz and power density 1 W/m² for 30 sec and static magnetic field of 25 mT intensity for 5 min were used. Exposure to electromagnetic fields was applied at the egg stage after 2-hour oviposition. Polytene chromosomes were examined in squashed salivary glands preparations stained with acetoorcein. For preparations, females at the 0-hour prepupa stage were used. Microwaves decreased the asynapsis frequency of homologous chromosomes by 18.1%. The constant magnetic field had no significant effect. Combined action of these factors decreased the asynapsis frequency in Drosophila giant chromosomes by 37.1%. Obtained results testify to the influence of electromagnetic factors on the homologous pairing of chromosomes in the nuclei of somatic cells. Thus, the possibility of their effect on the trans-interactions of the loci of homologous chromosomes is shown.

Key words: giant chromosomes, Drosophila, homologous conjugation, microwaves, static magnetic field, trans-interactions.

The purpose of the work was to study the effect of microwaves and constant magnetic field on the homologous conjugation of polytene chromosomes after exposure to microwaves and static magnetic field in Drosophila melanogaster Meig.

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The purpose of the work was to study the effect of microwaves on the homologous conjugation of polytene chromosomes after exposure to microwaves and static magnetic field in Drosophila melanogaster Meig. Microwaves with frequency 36.64 GHz and power density 1 W/m² for 30 sec and static magnetic field of 25 mT intensity for 5 min were used. Exposure to electromagnetic fields was applied at the egg stage after 2-hour oviposition. Polytene chromosomes were examined in squashed salivary glands preparations stained with acetoorcein. For preparations, females at the 0-hour prepupa stage were used. Microwaves decreased the asynapsis frequency of homologous chromosomes by 18.1%. The constant magnetic field had no significant effect. Combined action of these factors decreased the asynapsis frequency in Drosophila giant chromosomes by 37.1%. Obtained results testify to the influence of electromagnetic factors on the homologous pairing of chromosomes in the nuclei of somatic cells. Thus, the possibility of their effect on the trans-interactions of the loci of homologous chromosomes is shown.

Key words: Giant chromosomes, Drosophila, homologous conjugation, microwaves, static magnetic field, trans-interactions.
Introduction

In recent decades a new important environmental factor has emerged and developed – electromagnetic fields (EMFs) of anthropogenic origin (Shchkorbatov, 2014). Technological equipments for various purposes using microwave radiation are widely introduced. These are variable and pulsed magnetic fields, medical therapeutic and diagnostic facilities, visual information displays on cathode ray tubes (monitors, computers, televisions, etc.), industrial equipment with power supply, household appliances, personal communications (mobile phones) and the like. For today, we can state an increase in the effect of electromagnetic radiation on living organisms. The World Health Organization has included this issue in the list of the most pressing for humanity (WHO, 1997). International Agency for Research on Cancer (IARC) considers radiofrequency EMFs as potentially carcinogenic to humans (WHO/IARC, 2011).

The biological effects of EMFs are intensively studied at various objects and at different levels of the organization. In view of genetics, research on the chromosome level is of particular interest. A convenient object for this is the giant chromosomes of Diptera, which represent a modification of the actively functioning interphase chromosomes.

The capacity for homologous pairing is a fundamental property of eukaryotic chromosomes. Homologous and non-homologous (ectopic) chromosome associations play an important role in the spatial organization of the cell nucleus, providing functional connections of the different elements of genome. This issue is being considered currently in the light of the epigenetic control of gene expression (Duncan, 2002; Stegni, 2006; Schneider, Grosschedl, 2007).

In most cases, homologous chromosomes in *Drosophila* polytene nuclei tightly conjugated to each other. But often the conjugation is broken, there is an asynapsis phenomenon.

Asynapsis was investigated in homo- and heterozygous organisms. The interline differences in the frequency of violations of homologous chromosomes pairing were shown. The increase of spontaneous asynapsis was observed due to hybridization in the study of heterosis phenomenon (Lapta, Shakhbazov, 1976; Shakina et al., 2005; Taglina, 2006), as well as in interspecific hybrids of *Drosophila* (Evgen’ev, Polianskaya, 1976).

There are too few works, dealing with violations of conjugation of polytene chromosomes due to exogenous influences (Navrotskaya, 2006; Vasserlauf et al., 2008). Researches in this direction are very promising, given the association of terminal points of asynapsis with heterochromatic regions (Lapta, 1977), which are known to have a high polymorphism and dynamic structure (Grewal, Jia, 2007).

It should be also noted a lack of researches on the effects of EMFs on the chromosomal level (Tonomura et al., 1990, 1992; Goodman, Blank, 1998). In our previous works, we studied the effect of microwaves irradiation and a constant magnetic field on the puffing activity (Shakina et al., 2011) and endoreduplication (Dyka et al., 2016) in polytene chromosomes. An additional reason for our study were modifications of trans-inactivation effect under the influence of microwave irradiation, depending on the presence or absence of synopsis of homologous chromosomes, found in experiments on *Drosophila* (Shchkorbatov et al., 2004).

The purpose of this investigation was to study the effects of microwave radiation, a constant magnetic field and their combined action on violations of the homologous pairing of polytene chromosomes in *Drosophila melanogaster* Meig.

Objects and methods of research

Studies were carried out on inbred strain Canton-S. This strain was obtained from *Drosophila* collection of the Department of Genetics and Cytology of the Kharkiv National University through inbred breeding by mating siblings. The degree of inbreeding before experiments was 77 generations.

Flies were grown on standard sugar-yeast medium at 24.0\(\pm\)0.5°C. *Drosophila* culture developed in vials of 60 ml. The volume of culture medium in each vial was 10 ml.

EMF exposures were done by using an microwave emitter, which generates linearly polarized EMF with a frequency of 36.64\(\pm\)0.05 GHz. This semi-conductor device was designed and constructed at the
Department of Theoretical Radiophysics of V.N.Karazin Kharkiv National University based Gunn diode (the author is V.N.Bykov). Microwaves of this frequency refer to the Ka band (27–40 GHz). Such radiation is used in various radar systems (Richards, 2014). The power density in our experiments was 1 W/m², exposure time – 30 sec. The distance between the emitting antenna horn edge and the eggs was 15 cm. The SAR (Specific Absorption Rate) was 4 W/kg. In the literature, the level of microwave radiation below 100 W/m² assumed as not thermal (Michaelson, 1980).

In addition, exposure to static magnetic field with 25 mT intensity for 5 min was applied. We studied separately the effects of a single exposure to microwaves or magnetic field as well as the sequential action of microwaves and magnetic fields.

Exposure to EMFs was applied in early embryogenesis. We used newly-laid Drosophila eggs obtained from the five-day-old flies after a short 2-hour lay. Eggs were laid on the fresh culture medium surface and then were exposed. Examinations of the effects of EMFs were performed at the 0-hour prepupa stage. It is known that asynapsis pattern is formed in the early stages of development and doesn’t vary in larvae of different age (Lapta, Shakhbazov, 1976). In the control experiments Drosophila “sham eggs” were not exposed to microwave irradiation and magnetic field.

Fig. 1. Giant chromosomes of Drosophila melanogaster stained by acetoorcein with different number of asynapsis regions: (a) asynapsis is absent; (b) a single asynapsis; (c) two asynapsis segments; (d) three asynapsis segments

Polytene chromosomes were examined on squashed salivary glands preparations, stained with acetoorcein: 2% orcein (Merck KGaA, Darmstadt, Germany) solution in 45% acetic acid (Reahimtrans, Kyiv, Ukraine). For preparations, females at the 0 hours prepupae stage were used. The preparations were analyzed by light microscopy (MBI-6 microscope, “LOMO”, St. Petersburg, Russia) at magnification ×200. We assessed the percentage of nuclei with different number of asynapsis regions in the preparations: no asynapsis; a single asynapsis segment; two asynapsis segments; three and more asynapsis segments (Fig. 1). The asynapsis frequency was defined as the ratio of non-conjugating chromosomal segments to the total number of investigated nuclei.
Experimental data were statistically analysed. Data are presented as the mean ± standart error. For each experiment 10 larvae were used. The asynapsis regions were determined in 25–50 nuclei per preparation. We examined from 297 to 392 nuclei in each variant of experiment.

The significance of differences between the study groups was assessed using Student's t-test with the Bonferroni amendment for multiple comparisons. Results considered valid when \( p<0.017 \).

**Results**

The percentage of nuclei with different number of asynapsis segments in *Drosophila melanogaster* in the control and after exposure to electromagnetic factors are presented in Fig. 2.

In the control group violations of conjugation of homologous chromosomes weren’t found in 67.3% of the examined nuclei. The single asynapsis was revealed in 21.7% of the nuclei, 9.2% of nuclei had two violations of conjugation, in 1.8% cases three or more asynapsis segments were found.

After the exposure to microwave radiation, as well as after the action of a constant magnetic field, the percentage of nuclei without asynapsis didn’t differ from control values. After the microwave irradiation the portion of nuclei with a single asynapsis section increased by 22.6%, the number of nuclei with two violations of synopsis decreased by 55.4%, while the percentage of nuclei with three or more asynapsis segments didn’t differ from the control.

After the action of a constant magnetic field the content of nuclei with a single, as well as three or more violations of synopsis was the same as in the control, the proportion of nuclei with two asynapsis decreased by 39.1%.

The most significant changes were found after the combined effects of microwaves and static magnetic field. The portion of nuclei without violations of chromosomes’ pairing increased by 11.4%. The number of nuclei with a single asynapsis segment wasn’t changed, the portion of nuclei with two asynapsis regions decreased by 62.0%, while the nuclei with three or more violations of synopsis weren’t found.

![Graph showing the ratio of nuclei with different numbers of asynapsis segments](image)

* – \( p<0.017 \); ** – \( p<0.01 \); *** – \( p<0.00 \): versus to control group

**Fig. 2.** The ratio of nuclei with different numbers of asynapsis segments in polytene chromosomes after microwave irradiation, the action of a constant magnetic field and their combined influence in *Drosophila melanogaster*: 1 – asynapsis is absent; 2 – a single asynapsis; 3 – two asynapsis segments; 4 – three asynapsis segments

The data on the portion of nuclei with different numbers of asynapsis segments were used for calculation of average frequency of violations of homologous conjugation in giant chromosomes in *Drosophila melanogaster* in norm and after experimental influences. The results are presented in Fig. 3.
Fig. 3. The frequency of violations of homologous conjugation in polytene chromosomes of _Drosophila melanogaster_ after the microwave irradiation, the action of a constant magnetic field and their combined effect

The average frequency of asynapsis in the control group was 45.2%. After the microwave irradiation decrease of the asynapsis frequency by 18.1% was observed. The influence of constant magnetic field wasn’t statistically significant. The largest effect occurred when combined action of microwave radiation and magnetic field was applied: frequency of asynapsis decreased by 37.4% compared with the control. This index was also lower than after exposure to microwaves – by 23.5%, or a constant magnetic field – by 30.6%.

Thus, the 36.64 GHz microwave irradiation on the stage of embryogenesis with power density 1 W/m² and 30 sec of exposure led to reduction in of the homologous chromosomes asynapsis frequency in the salivary glands cells in _Drosophila melanogaster_. The constant magnetic field with 25 mT intensity for 5 min hadn’t significant effect on the asynapsis frequency of itself, but significantly increased the effect of microwave radiation.

**Discussion**

The violations of conjugation of homologous chromosomes are of two types: specific and non-specific, or spontaneous. The first type is a regular asynapsis and associated with various pattern of chromosomal discs. This occurs, for example, as a result of interspecific hybridization (Evgen’ev, Polianskaya, 1976). The second type occurs when the picture of disks is identical and its nature is not fully understood (Lapta, 1977; Taglina, 2006).

There is ample evidence, which indicates that the principles of somatic and meiotic conjugation are the same (Martinez-Perez et al., 2001). In the literature, the synopsis of homologous chromosomes is explained by preferential attraction of certain chromosomal regions, which localization usually correlates with position of heterochromatin (Tiang, Pawlowski, 2012). It has been also shown that zones of violation of conjugation begin or end in the centromeric, telomeric and intercalary heterochromatin (Lapta, 1977).

The character of homologes interaction affects in a certain way on gene expression. There are examples of puffs heterozygosity in sites of asynapsis in interlinear hybrids of _Drosophila_: sizes of puffs derived from different parental lines differ in the asinapsys segments of homologous chromosomes (Ashburner, 1970; Strashnyuk et al., 2009). Such examples are also characteristic for interspecific hybrids in which the regularly asynapsis occurs apart from the spontaneous one (Ashburner, 1969).

Ashburner (1970) showed the influence of presence or absence of physical contact of homologues chromosomes on gene expression. In his view, synopsis may correct damages leading to restoration of gene activity that affects the puff morphology.

At present, it has been proven possible to restore a normal phenotype by interaction of differently
damaged genes that are located in homologous chromosomes when they physically contact. This phenomenon is called transvection. Broadly, the transvection is such cys-trans interactions, which may be accompanied by either activation or inactivation of genes (Henikoff et al., 1995; Kennison, Southworth, 2002; Csink et al., 2002). This phenomenon is associated with subtle mechanisms of genes activity regulation observed in eukaryotic organisms of different taxa: fungi, higher plants, insects, mammals (Duncan, 2002).

An example is the effect of trans-inactivation (Kennison, Southworth, 2002; Csink et al., 2002). To study this effect, in particular, Drosophila line, comprising a mutation brownDominant (bwD), is used. bwD gene is able to inhibit the expression of the gene bw+ and shows the effect of dominance in the case, if the conjugation of chromosomes occurs in the area in which bwD and bw+ genes are localized (Henikoff et al., 1995). Thus, by examining the expression of these genes, it is possible to judge on the changes in the subtle processes of chromosome interaction inside the cell nucleus.

Earlier in the line containing the mutation bwD, the effect of microwave radiation on the manifestation of trans-inactivation effect in Drosophila was studied (Shckorbatov et al., 2004). The linearly polarized and the left polarized radiation with λ=8 mm, E=0.2 mW/cm² caused increased trans-inactivation effect, manifested in the reduction of pigment in the eyes of the flies. This indicated an increase in homologous chromosomes conjugation degree in the cell nucleus. Right polarized radiation, on the contrary, caused an increase in pigment content, i.e. trans-inactivation effect decreased. Thus, it was shown the effect of electromagnetic waves on gene expression, which is associated with the state of conjugation of chromosomes in the nucleus.

The data of this study are consistent with the effect of microwaves on the manifestation of trans-inactivation effect. In our study, we applied linearly polarized EMF. The frequency of violations of homologous chromosomes pairing reduced as in the study of trans-inactivation effect. In the case of trans-inactivation authors judged about homologous chromosomes interaction indirectly, based on the data on mutant trait expressivity changes. The study of polytene chromosomes allows us to evaluate it directly by visual observation.

The effect of electromagnetic fields on the interaction of homologous chromosomes can be explained by taking into account the data about their impact on chromatin structure. Earlier in the human cells, it has been shown an increase of heterochromatin granules content in the nuclei under the influence of microwaves (Shckorbatov et al., 2009). Given that heterochromatic regions are the sites of synopsis initiation, the data on the increase of genome heterochromatinization degree under the influence of electromagnetic fields is consistent with homologous chromosomes asynapsis decrease. The dependence of asynapsis frequency on the amount of heterochromatin was demonstrated earlier (Belyaeva, 1973; Lapta, 1977).

Heterochromatinization apparently is a manifestation of a more general cell response on the stress, as DNA in a tightly packed chromatin is better protected against damaging influences. The signal for the conformational changes in the chromatin may be due to the change of ionic composition in cytoplasm and karyoplasm associated with the changes in membrane permeability, which takes place under microwave irradiation of the cell (Skamrova et al., 2013).

The magnetic fields also can induce the stress response in Diptera (Goodman, Blank, 1998). Exposure to low frequency magnetic field induced transcription in specific regions of giant chromosomes in Drosophila melanogaster and Sciar a coprophila. The activation of heat shock transcriptional factor (HSF1), increase in the synthesis of stress protein hsp 70, and 3–4-fold increase of heat shock element binding activity were shown.

**Conclusions**

In the present study, exposure to microwave radiation (frequency – 36.64 GHz, power density – 1 W/m², exposure time – 30 sec) on the stage of embryogenesis decreased the asynapsis frequency of homologous chromosomes in the salivary glands cells of Drosophila melanogaster by 18.1%. The constant magnetic field (intensity 25 mT, exposure time – 5 min) had no significant effect. Combined action of microwaves and static magnetic field decreased the asynapsis frequency in Drosophila polytene chromosomes by 37.1%. Obtained results testify to the influence of electromagnetic factors on the homologous pairing of chromosomes in the nuclei of somatic cells. Thus, the possibility of their effect on the trans-interactions of the loci of homologous chromosomes is shown.
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