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EFFECT OF 60 MINUTES EXPOSURE TO ELECTROMAG- NETIC FIELD ON FECUNDITY, LEARNING AND MEMORY, SPEED OF MOVEMENT AND WHOLE BODY PROTEIN OF THE FRUIT FLY *DROSOPHILA MEL-ANOGASTER*

By

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Abstract

This study investigated the effect of four different electrical devices as source of electromagnetic field on fecundity, learning and memory function, speed of movement, in addition to the whole body proteins of the fruit fly *Drosophila melanogaster*.

The results showed that exposure to EMF has no significant effect on adult fecundity (ANOVA and Duncan's test) but alters learning and memory function in Drosophila larvae, especially those exposed to mobile phone. Highly significant differences occurred in the larval speed of movement after exposure to EMF, with maximal effect occurred for larvae exposed to mobile phone (their speed of movement increased 2.5 times of wild type). Some protein bands serve as characters for exposure to certain electrical devices which suggest that exposure to EMF may affect the whole body proteins.

Keywords: *Drosophila melanogaster,* Electromagnetic field, Fecu-ndity, Learning and Memory, Movement speed, Proteins.

Introduction

An electromagnetic field is a force field generated around an electric current, equivalent to an electric field and a magnetic field at right angles to each other. Common sources of electromagnetic fields include household electrical wiring, motor driven instruments, computer screens, telecommunications and broadcasting facilities, and mobile telephones. Their biological effects vary considerably depending on the model of device and distance from it (Cakmak *et al,* 2012).

The electricity that comes out of every power socket has associated low frequency electromagnetic fields. Various kinds of higher frequency radio waves (30 kHz and 300 GHz) transmit information whether via TV antennae, radio stations or mobile phone base stations (Lahham and Hammash, 2012).

In spite of huge amount of data dealing with electromagnetic fields, some gaps about biological effects exist and need further research. Even the lowest limit of EMF flux densities will pro-

duce a biological reaction (Gutzeit, 2001).

There is great evolutionary conservation of genes affecting the common biological processes and molecular functions across a diverse array of taxa. In *Drosophila*, less than 20% of the 13,600 genes and predicted genes have been characterized by classic genetic and molecular methods (Adams *et al*, 2000). Furthermore, there is direct homology between *Drosophila* genes and genes that affect human disease. Of all the genes known to affect human disease, more than 60% have *Drosophila* orthologs, and more than half of all *Drosophila* protein sequences are similar to those of mammals (Rubin, 2000). Thus, lessons learned from studies of *Drosophila* would provide guidance for experimental design of human studies. Determining the effects of electromagnetic field affecting some traits in *Drosophila* will suggest the same effects in human. Further, *Drosophila* exhibit a rich repertoire of complex traits, some of which have clear human homologs; e.g., circadian rhythm, sleep, drug responses, locomotion, learning and memory, aggressive behavior, and longevity. Natural populations of *Drosophila* harbor substantial genetic variation and short duration of life cycle for practically any trait to be defined and measured (Falconer and Mackay, 1996).

The present study was conducted to investigate whether fecundity, learning and memory processes, speed of movement and protein banding pattern in *Drosophila* would be altered after exposure for 60 min to electrical devices normally used in every day's life.

Materials and Methods

Drosophila were of the Canton-S wild type strain kept in mass culture and maintained in the laboratory at 20-25ºC and 12⁄12 light ⁄ dark cycle, relative humidity 60-70%.

The main goal of this experiment was to evaluate the effect of EMF on adult fecundity, to achieve this; flies were divided into five groups, each containing 5 virgin females and 7 males in a fresh food vial. Four groups were exposed separately to 4 devices (computer, TV, mobile phone, radio) for an hour at distance 5cm from the device under normal light in a closed room (3m×4m). The room was devoid of any source of EMF except the device under estimation. The fifth group (control) was kept without any EMF exposure. The experiment was repeated 3 times (total no of groups=15). Flies were passed daily for 1-2 weeks. The flies were transferred to fresh food vial every day and the numbers of laid eggs were counted from the fifth day after mating. The fecundity of adults was calculated on the basis of total number of eggs laid everyday for seven days.

Learning and memory: This was performed at 2L stage larvae just before wandering stage (Sawin-McCormack *et al*, 1995). For experiments, five groups of larvae were established, each of 10 individuals. Each group was separately exposed to a device from 4 devices (computer, TV, mobile phone, radio) for one hr. at distance 5cm from the device under the same conditions as described before. The last group (control) was kept without EMF source. The experi-

ment was repeated 3 times. After exposure larvae were transferred immediately to the assay plates to start the experiments.

Learning and memory experiments were done after Gerber and Stocker (2007). Briefly, as olfactory stimuli, amyl acetate and benzaldehyde were used. As negative gustatory reinforcer, NaCl was used and Fructose was used as a potentially positive reinforcer. These reinforcers were added to 1% agarose plate 10 min after boiling to reach final concentrations of 2M NaCl, or 1M fructose in the plates. Larvae underwent either of two treatment conditions: under one treatment condition amyl acetate was paired with fructose plates and benzaldehyde with NaCl plates (AM+/Benz -), where the other was treated reciprocally (AM-/Benz+). This cycle of two differential training was repeated 10 times (Fig. 1). After this training, larvae were tested five min. for their odor preferences (either AM or BENZ) on 1% agarose plate without reinforce. Larvae on each side of the test plate were counted, and a preference for AM and for BENZ was calculated. Both preference values were used to calculate the performance index (learning index).

$$PREF_{AM} = \frac{(\#\ AM - \#\ BA)}{\#\ Total}$$

$$PI = \frac{(PREF_{AM+/BA} - PREF_{AM/BA+})}{\#\ Total}$$

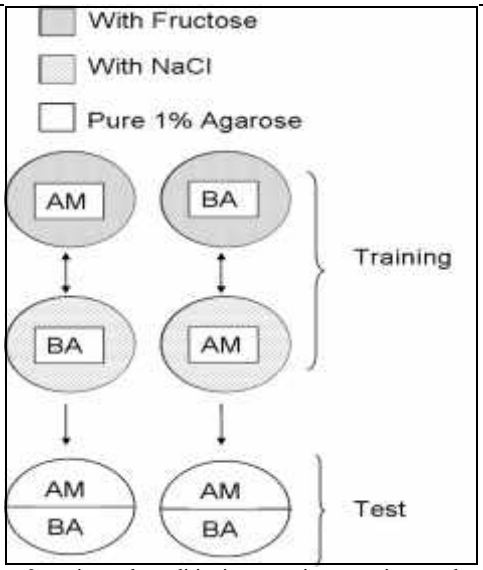

Fig. 1: Procedure diagram for a 2- reciprocal conditioning experi-ment using amylacetate (AM) and benzaldehyde (BA) reinforced either positively by Fructose or negatively by NaCl. Larvae of bothgroups, (BA+/AM-) right and (BA-/AM+) left, tested on pure 1% agarose plate for choosing AM or BA after 10 training cycles.

Larval speed of crawling: The set was exactly as described in the previous experiment. After exposure to EMF, larvae were allowed to move freely on 1% agarose plate. This movement was video recorded for one min. and videos were transferred to one sec. frames using Aimer software and then analyzed frame-by-frame to calculate the speed of movement using Image J software.

Protein electrophoresis: Sodium dodecyl sulphate- polyacrylamide gel electrophoresis (SDS-PAGE) was performed (Laemmli 1970), with modified for Drosophila larvae given by Tissiéres *et al.* (1974) and Arking (1978), using 12% polyacrylamide gel. The proteins were prepared in non-reduced (intact) and reduced forms. For the preparation of non-reduced protein samples, larvae were homoge-nized in in Ephrssi-Beadle Ringer′s (EBR), centrifuged and the supernatant and then mixed with sample buffer (10% of the total volume of the sample). The mixture was vortexed and then boiled at 100°C for 5 minutes. Electrophoresis was performed at a constant voltage of 173 volts when samples were in the stacking gel. When the dye front reached the resolving gel, voltage was increased to 245 volts. The run was stopped when the dye front was 2 to 3 mm away from the bottom edge of the gel, which was silver stained using the commercial silver staining kit (Bio-Rad) after the manufactures' instructions.

Statistical analysis: Statistical significance of differences in mean fecundity, mean were calculated among groups using ANOVA and further analyzed using Duncan's multiple comparison. The level of significance was set at $p<0.05$. Data were expressed as (mean ± standard deviation). SAS was used to perform all calculations.

Results

The results are shown in table (1) and figures (1, 2, 3, 4 & 5).

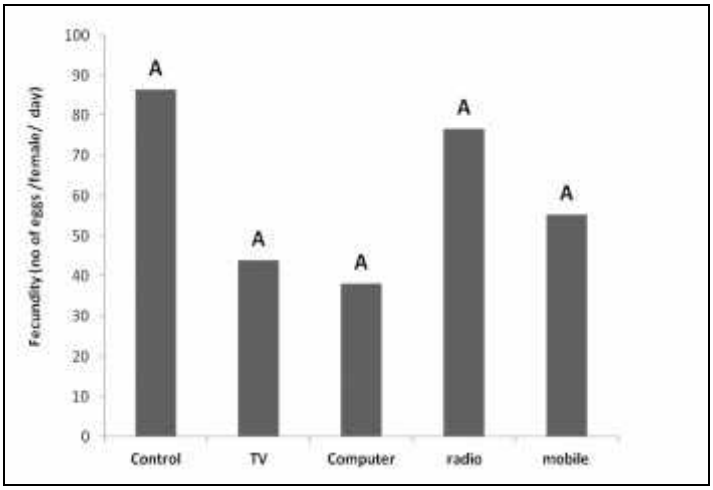

Fig. 2: Mean fecundity of *Drosophila* control and TV-, computer, radio- and mobile-exposed larvae respectively; means with same letter not significant.

Table 1: Duncan's Multiple Range Test for variation between groups, each exposed separately to 4 devices (computer, TV, mobile phone, radio).

Variable	Control	T.V.	Computer	Radio	Mobile
Fecundity*	86.33 A	43.89 A	38.21 A	76.67 A	55.33 A
Pref AM+BA-**	0.6333 A	0.3000 B	0.2833 B	0.4333 AB	0.2667 B
Pref AM-BA+***	0.6000 C	0.1333 B	0.3000 AB	0.1333 B	0.5000 A
Learning index***	0.61667 A	0.08333 B	0.11667 B	0.15000 B	0.11667 C
Movement speed***	0.08420 BC	0.04180 C	0.13020 B	0.07920 BC	0.21180 A

Means with same letter not significant, *=significant, **=moderately significant & ***=highly significant.

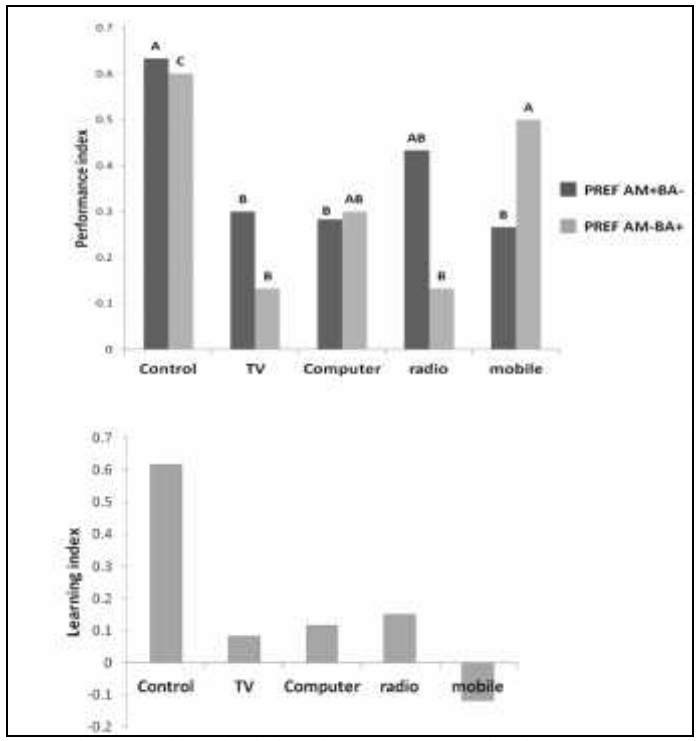

Fig. 3: Preference and learning index of *Drosophila* control and TV, computer, radio- & mobile- exposed larvae respectively; means with same letter not significant.

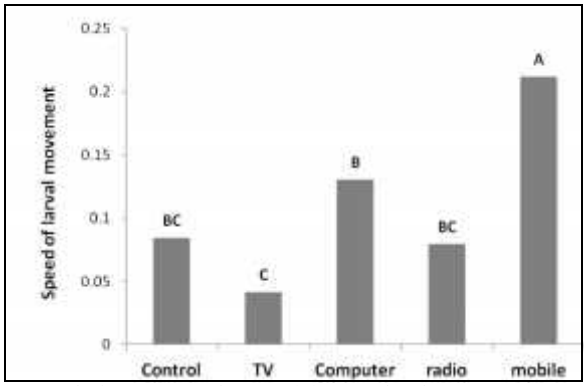

Fig. 4: Speed of movement of *Drosophila* control and TV-, computer, radio- & mobile- exposed larvae respectively, means with same letter not significant.

Band no	Apparent molecular weight, KDs				
	Control	TV	Computer	Radio	Mobile
1					58
2	51	51	51	51	
3			46	46	
4		42			
5			39	39	39
6		37			
7			34	34	
8	32		32	32	
9	30				
10	26	26	26	26	26
11	23	23	23	23	23
12	21	21	21		
13	19	19	19	19	19
14	10	10	10	10	10

Fig. 5: Schematic diagram of the electrophoresis SDS-page showing protein bands *Drosophila* control & T.V-, computer-, radio-, & mobile- exposed larvae respectively. Apparent molecular weight of bands shown.

Discussion

Selected criteria of both adults (fecundity) and larvae (learning and memory process and speed of movement) in addition to the whole body larval protein electrophoresis of *Drosophila* were chosen to study and compare the effect of exposure to emissions from four different electrical devices (T.V., computer, radio and mobile phone) as a source of EMF (Tab. 1). There were no significant differences in fecundity (Fig. 2) between control and other groups which exposed to EMF emissions (p = 0.672). The present results agreed with results obtained by Alti and Hacer (2007) after exposure for 3 hours, and in mice (Elbetieha *et al*, 2002). But, they differed from results obtained by Lyubov *et al*. (2011), who found that no mi-

crowave-induced decrease in *Drosophila* fertility. This discrepancy can be explained by the fact that induced effects of EMF are depending on the time of exposure to microwave radiation. Also, Schwartz *et al.* (1985) reported that the reproductive performance of EMF-exposed adults (assessed by considering average egg laying, egg fertility and egg retention per female) was slightly less than that of the control group.

Drosophila larvae use some chemosensory cues in order to orient themselves in their environment, for example to locate their optimal food. Their behavioral response to odorants is not genetically fixed and can be modified by experience. In the present study, exposure to EMF emissions led to subsequent changes in their response to odorants. The measured response calculated the proportion of larvae moving towards or away from an odorant; the result were expressed as learning index (LI) ranging from -1 (total repulsion) to +1 (total attraction). As shown in figure (3), the mean Pref AM+ / BA- of control and EMF-exposed groups was moderately significantly different with *p*-value equal (0.243), while highly significant differences in the mean Pref AM-/BA+ occurred among these groups (p= 0.0005). Learning scores of larvae exposed to (T.V., computer and radio) reduced by (13%, 18% and 24%) respectively, to highly significantly different (p = 0.0001). Dworak *et al.* (2011) demonstrated that singular excessive exposure to TV and computer game affects children's sleep architecture, sleep continuity and verbal memory performance. Moreover, Fragopoulou *et al*. (2009), Narayanan *et al*. (2009) and Daniels *et al*. (2009) hypothesized that EMR could have a negative impact on the subject's mood and ability to learn. The present results showed very poor performance of mobile-exposed larvae (LI = - 0.11± 0.01), which was reduced by (0.07%) compared to wild-type performance (Fig. 3). These results agreed with Nittby *et al*. (2008) who provided evidence of alterations of memory functions in rats after long term exposure to mobile phones. On the other hand, using low frequency and various exposure protocols, Sienkiewicz *et al*. (2002) failed to reveal any effect of EMF on spatial memory, in mice or rats.

Highly significant differences occurred in the speed of movement of *Drosophila* larvae after exposure to EMF (p= 0.0004). The speed of crawling of larvae exposed to mobile phone emission increased by 2.5 times of wild-type. (Fig. 4) suggested that EMR exposure may load to abnormal brain functioning and behavioral abnormalities. These results agreed with Daniels *et al*. (2009) who found that in some exposed animals there was decreased locomotors activity. Decreased locomotor activity is usually indicative of disturbances in emotional states which may have been triggered by the stress of being placed in a novel environment (Prut and Belzung 2003).

Protein electrophoresis of the whole larval tissues of control group and EMF-exposed groups reveal certain diagnostic differences (Fig. 5). For ex-

ample, bands 4 & 6 appeared in TV-exposed larvae, while bands 3 & 7 appeared only in radio- and computer-exposed larvae. Band 5 appeared in groups exposed to computer, radio and mobile. Band 1 appeared uniquely in mobile-exposed group. Some protein bands disappeared after exposure to EMF emissions. For example, band 9 disappeared after exposure to EMF from any source, band 2 disappeared after exposure to mobile phone emission. Band 12 disappeared from samples exposed to radio or mobile emissions. These protein bands served as diagnostic characters as they appeared or disappeared after EMF exposure.

The present results agreed with Anu Karinen *et al*. (2008) who suggested that protein expression in human skin might be affected by the exposure to EMF, and El-Abiad *et al*. (2005) who showed changes in protein fractions in rats exposed to radiation generated from mobile base station 24 hours/day for 8 weeks. This may be due to the effect of EMF on the biological membranes (Salford *et al*, 1994).

Conclusion

The outcome data showed that EMR caused behavioral changes as evidenced by a reduction in locomotors activity, and behavioral response of *Drosophila* larvae to odorants (spatial learning and memory function), the electromagnetic radiation induced a decrease in *Drosophila* viability, but did not influence *Drosophila* fecundity. EMR influenced proteins in insect.

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