Temporal pattern of *Drosophila subobscura* locomotor activity after exposure to extremely low frequency magnetic field (50 Hz, 0.5 mT).

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Summary

The aim of this study was to determine whether exposure for 48 h to extremely low frequency magnetic field – ELF-MF (50 Hz, 0.5 mT) at different developmental stages of *Drosophila subobscura* (egg-first instar larvae and just eclosed adult) changes locomotor activity recorded for 30 min. Linear regression analysis, performed with values of successive 10-min time intervals, showed significant differences in travelled distance and mobility in flies from all experimental groups. No matter which developmental stage was exposed, ELF-MF decreased locomotor activity of *D. subobscura* adults.

Introduction

All biological systems are constantly exposed to natural magnetic fields, but with intensive technological progress during the last 30 years, there are additional sources of electromagnetic fields. Many studies deal with magnetic field effects on different biological levels (Balcavage *et al.*, 1996; Santini *et al.*, 2009; Ivancsits *et al.*, 2003; Neumann, 2000; Rollwitz *et al.*, 2004). Effects of extremely low frequency magnetic field (ELF-MF, < 300 Hz) on nervous system are still undetermined, but many studies are researching it on different behavioral levels. Locomotor activity of animals is implicated in their everyday activities and as a complex type of behavior it is important to be analyzed through different parameters (distance travelled, mobility, speed, and so forth). Central neurotransmitter systems which play important roles in motor behavior expression might be modulated with applied ELF-MF. Therefore, these modulations might induce alternated motor behavior (Osborne, 1996). It has already been shown that in *Drosophila* larvae after exposure to ELF-MF there is decreased ability in digging of substrate (Ho *et al.*, 1992). Mostly in everyday life at different developmental stages humans are exposed to ELF-MF derived from power lines and almost all household electrical appliances. Therefore, ELF-MF presents an important ecological factor. The aim of this study was to detect changes in locomotor activity of *Drosophila subobscura* adults, after 48 h exposure of different developmental stages to ELF-MF (50 Hz, 0.5 mT).

Materials and Methods

Drosophila stock

D. subobscura were collected from beech a forest on Serbian mountain Goč and formed into isofemale (IF) lines. They were maintained in five full-sib inbreeding generations in controlled laboratory conditions at temperature 19°C, with humidity 60%, on standard cornmeal medium, which consists of 9% sugar, 10% cornmeal, 2% agar, 2% yeast, and nipagin dissolved in 96% ethanol, in a 12 h:12 h light:dark cycle - lights turned on at 6:00 AM, at 300 lux illumination.

Experimental procedure

Two types of experiments were performed. The first one was exposure of *D. subobscura* egg-first instar larvae developmental stage for 48 h to ELF-MF (50 Hz, 0.5 mT). After that their development was completed out of the ELF-MF apparatus. The second one was exposure of just eclosed *D. subobscura* adults for 48 h to ELF-MF (50 Hz, 0.5 mT). In both experiments when eclosing started males and females were separated under CO_2 anesthesia and placed in vials with standard medium for *Drosophila*.

Figure 1. ELF-MF apparatus (left), camera (middle) above Petri dishes, and computer (right) for locomotor activity recording.

Locomotor activity monitoring

There were three experimental groups:

- intact (egg-first instar larvae and just eclosed flies were out of ELF-MF apparatus),
- *sham* (egg-first instar larvae and just eclosed flies were in turned off ELF-MF apparatus) and
- ELF-MF (egg-first instar larvae and just eclosed flies were in turned on ELF-MF apparatus).

Since between intact and *sham* group there was not detected statistically significant differences, those groups were pooled in one control group. Single 3 days old *D. subobscura* naive males and virgin females were separately released to move in the empty plastic Petri dish – “open field” arena (35 mm diameter, 10 mm high to maximally limit vertical and fly movement) immediately before recording (Figure 1, middle). In our previous research we found that locomotor activity of *D. subobscura* flies was the highest in the morning time interval from 8:00 AM to 9:00 AM (Dimitrijević *et al.*, 2013). In this study was used this morning time interval for monitoring locomotor activity for 30 min. Seven *Drosophila* flies were simultaneously videotaped by camera (Microsoft LifeCam VX600) positioned above the dishes (Figure 1, middle). All experiments were performed at optimal laboratory conditions for *D. subobscura* (temperature 19°C, humidity 60%, and 300 lux illumination). ANY-maze software (v.4.73, Stoelting Co., Wood Dale, Illinois, USA) was used to analyze two parameters:

- distance travelled (the total distance in meters that fly travelled during the recording time) and
- mobility (the amount of time in seconds that fly was mobile during the recording time).

ELF-MF apparatus

The ELF-MF was obtained by an electromagnet that consisted of three circular coils of insulated copper wire (0.75 mm in diameter). The coils are 37 cm in diameter and are set at 23 cm distance from each other (Figure 1, left). The 50 Hz current was taken from local 220 V power network via an adjustable transformer. The electromagnet was supplied by a current of 2.8 A, producing uniform 50 Hz magnetic field without any observable temperature fluctuation or vibrations. Within the coils where the samples were placed, magnetic field was 0.5 ± 0.01 mT, measured using a Hirst GM05 Gaussmeter (probe PT 2837, Hirst Magnetic Instruments LTD, Cornwall, UK).

Statistical analysis

Linear regression analysis was used to detect changes in locomotor activity parameters during 30 min, in 10-min intervals. Since data did not show normal distribution (Shapiro–Wilk test), non-parametric Spearman Rank Order Correlations test was used for testing differences between regression slopes of locomotor activity parameters of control and ELF-MF exposed *Drosophila*. All analyses were conducted using Statistica 5.0 for Windows (StatSoft Inc., Aurora, CO, USA).

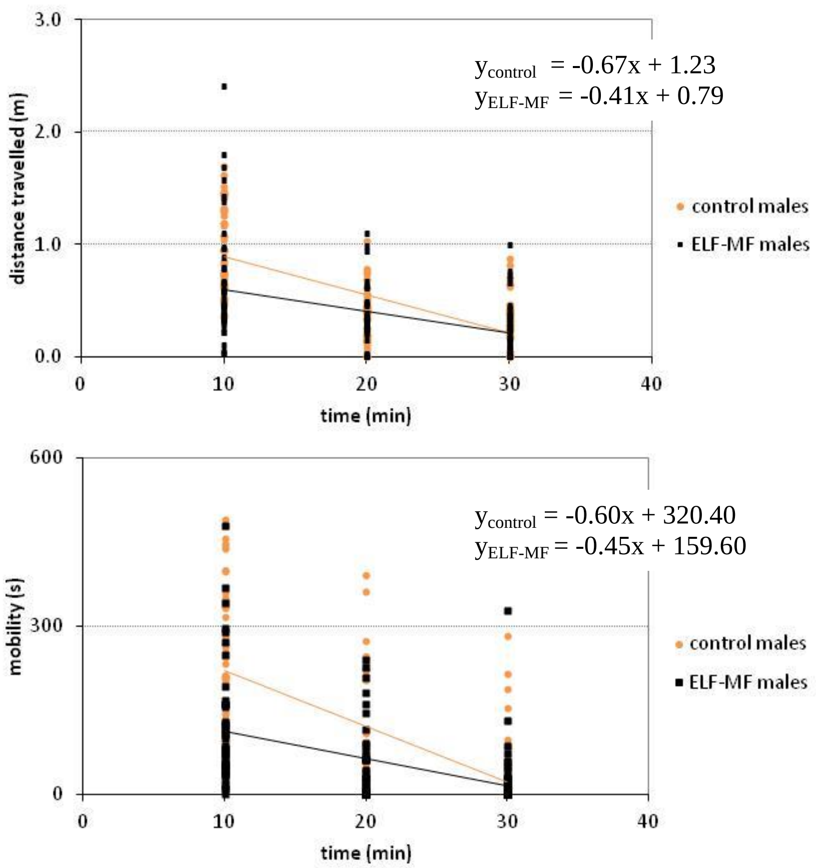


Figure 2. Linear regression lines and equations of both parameters of locomotor activity (distance travelled and mobility) for 3 days old *D. subobscura* males exposed to ELF-MF (50 Hz, 0.5 mT) at egg-first instar larvae developmental stage; $n_{control} = n_{ELF\text{-}MF} = 45$.

Results

Linear regression analysis of locomotor activity of 3 day old *D. subobscura* flies exposed to ELF-MF at egg-first instar larvae developmental stage showed significant reduction during recording (Figures 2 and 3, Table 1A).

The highest values of the examined parameters for males and females from both experimental groups were observed in the first 10 min. The slopes of linear regressions from control and ELF-MF exposed groups indicated that the mean travelled distance by males significantly decreased by 0.67 m and 0.41 m, respectively, in each 10-min time interval. The mean mobility by males from control and ELF-MF exposed group significantly decreased by 0.60 s and 0.45 s, respectively, in each successive 10-min time interval. In females from control and ELF-MF exposed group the mean travelled distance and mobility significantly decreased in each successive 10-min time interval by 0.41 m and 0.45 m, and 0.49 s and 0.46 s, respectively.

Table 1. Linear regression analysis for both parameters of locomotor activity (distance travelled and mobility) of 3 days old *D. subobscura* males and females, previously exposed to ELF-MF (50 Hz, 0.5 mT) at egg-first instar larvae developmental stage (A) and at just eclosed adult developmental stage (B), during 30 min of recording.

A	egg-fist instar larvae	df	R^2	F	
distance travelled	control males	133	0.442	107.14	***
	ELF-MF males	133	0.168	26.78	***
	control females	133	0.366	76.87	***
	ELF-MF females	133	0.203	33.83	***
mobility	control males	133	0.359	74.47	***
	ELF-MF males	133	0.199	33.23	***
	control females	133	0.236	41.15	***
	ELF-MF females	133	0.213	35.97	***

B	just eclosed adults	df	R^2	F	
distance travelled	control males	223	0.619	361.92	***
	ELF-MF males	223	0.389	141.95	***
	control females	223	0.624	369.70	***
	ELF-MF females	223	0.424	163.87	***
mobility	control males	223	0.622	367.63	***
	ELF-MF males	223	0.414	157.45	***
	control females	223	0.580	308.41	***
	ELF-MF females	223	0.435	171.6	***

*** $p < 0.001$

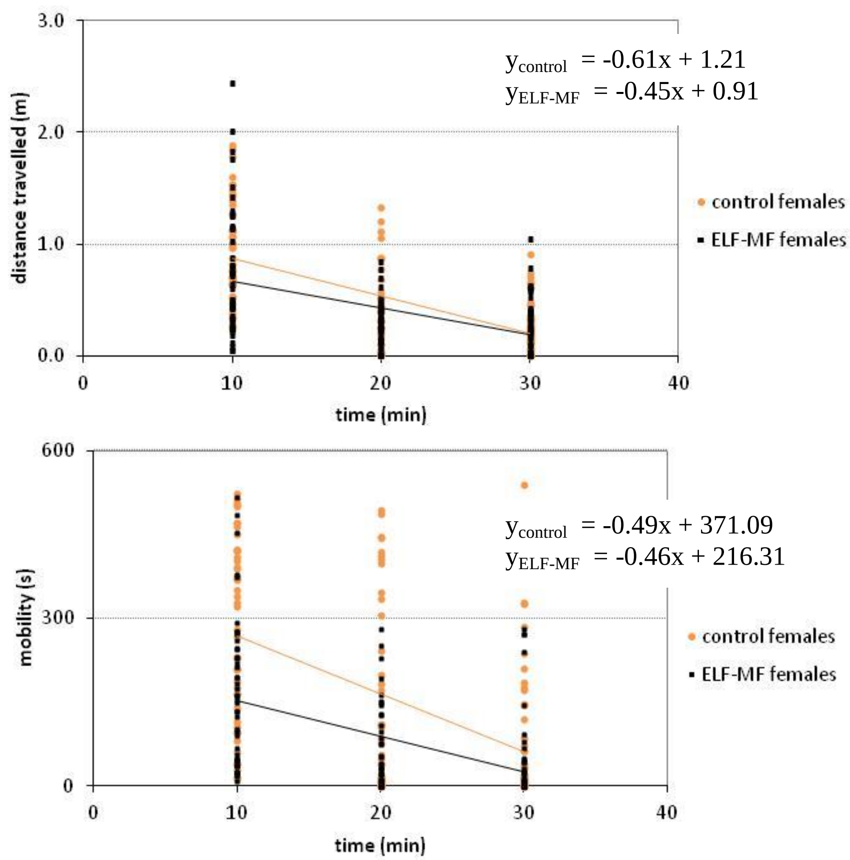


Figure 3. Linear regression lines and equations of both parameters of locomotor activity (distance travelled and mobility) for 3 days old *D. subobscura* females exposed to ELF-MF (50 Hz, 0.5 mT) at egg-first instar larvae developmental stage; $n_{control} = n_{ELF\text{-}MF} = 45$.

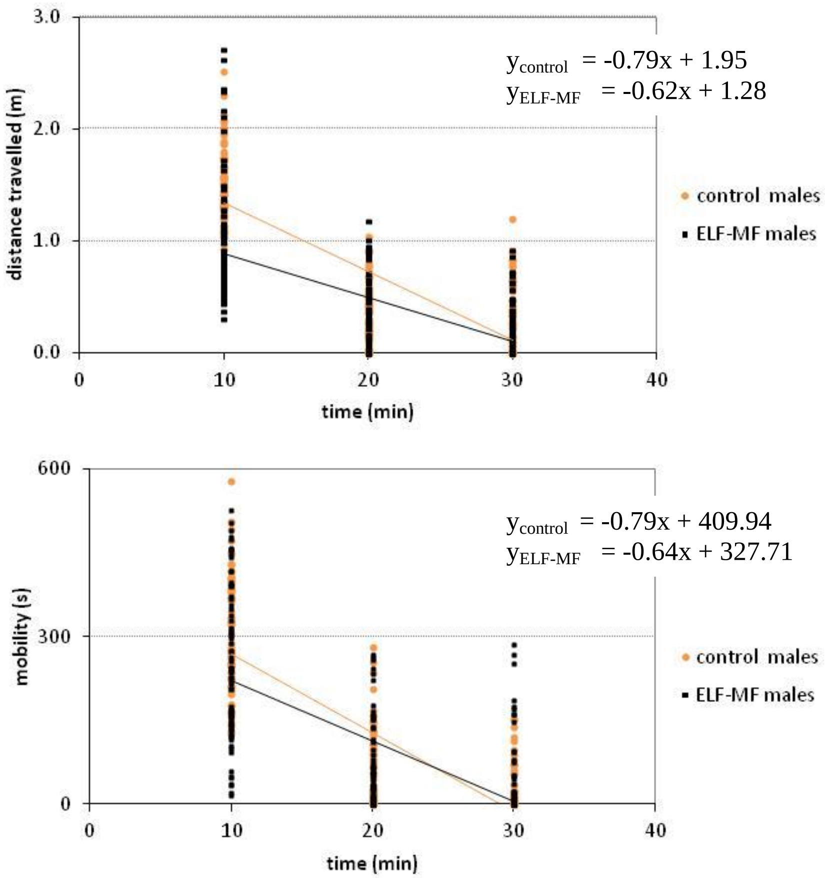


Figure 4. Linear regression lines and equations of both parameters of locomotor activity (distance travelled and mobility) for 3 days old *D. subobscura* males exposed to ELF-MF (50 Hz, 0.5 mT) for 48 h at just-eclosed adult developmental stage; $n_{control} = n_{ELF\text{-}MF} = 75$.

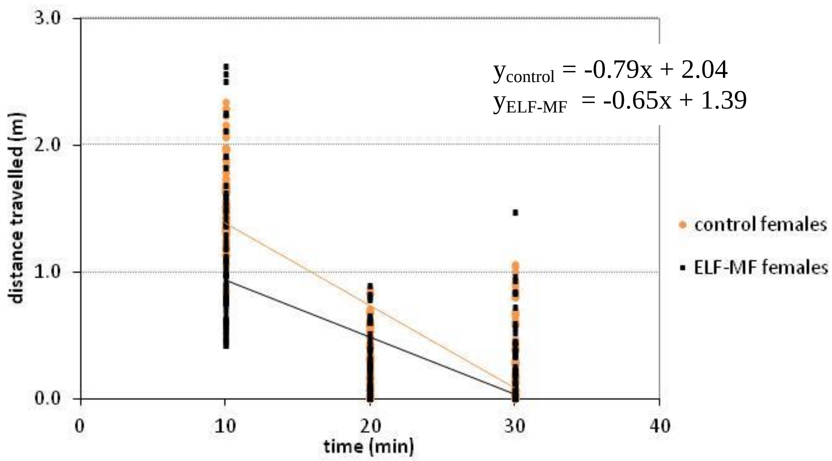
The percentage of the total variation in distance travelled and mobility, that is measured and shown by the fitted regression, was defined with a coefficient of determination (R^2), which represents a measure of the strength of the straight line relationship. Results in Table 1A for groups of egg-first instar larvae developmental stage show that total variation in travelled distance and mobility of males from control and ELF-MF group was 44% and 17%, and 36% and 20%, respectively. For females it was 37% and 20%, and 24% and 21%, respectively. These results showed that travelled distance and mobility of each sex from both experimental groups were more variable after exposure to ELF-MF.

Spearman Rank Order Correlations test showed that linear regression slopes of males and females distance travelled and mobility after exposure of egg-first instar larvae developmental stage to ELF-MF (50 Hz, 0.5 mT) were significantly lower compared to control groups (Spearman $Rho_{males,DT} = 0.21$; Spearman $Rho_{females,DT} = 0.13$; Spearman $Rho_{males,M} = 0.21$; Spearman $Rho_{females,M} = 0.18$; $p < 0.05$).

Analyzing locomotor activity of 3 day old *D. subobscura* males and females after exposure to ELF-MF (50 Hz, 0.5 mT) for 48 h of just eclosed adults, linear regression analysis showed significant reduction during recording (Figures 4 and 5, Table 1B). As it was seen from results of exposed egg-first instar larvae developmental stage, in this experiment the highest values of the examined parameters for males and females from both control and ELF-MF exposed group were observed in the first 10 min.

Results in Table 1B for just eclosed adult developmental stage showed that total variation in travelled distance and mobility of males from control and ELF-MF group was 62% and 39%, and 62% and 41%, respectively. For females it was 62% and 42%, and 58% and 43%, respectively. These results showed that travelled distance and mobility of each sex from both experimental groups were more variable after exposure to ELF-MF. It was shown that regression slopes of males and females distance travelled and mobility after exposure of just eclosed adults to ELF-MF (50 Hz, 0.5 mT) for 48 h were significantly lower compared to control groups. The slopes from control and ELF-MF exposed groups indicated that the mean travelled distance by males significantly decreased by 0.79 m and 0.62 m in each 10-min time interval. The mean mobility by males from control and ELF-MF exposed groups significantly decreased by 0.79 s and 0.64 s in each successive 10-min time interval. In females from control and ELF-MF exposed groups, the mean travelled distance and mobility significantly decreased in each successive 10-min time interval by 0.79 m and 0.65 m, and 0.76 s and 0.66 s, respectively.

Testing slopes of linear regressions for both parameters of locomotor activity using Spearman Rank Order Correlations test, a significant decrease was found in travelled distance (Spearman $Rho_{males,DT}$ = -0.18; Spearman $Rho_{females,DT}$ = -0.70; $p<0.05$) of males and females, which were exposed to ELF-MF (50 Hz, 0.5 mT) for 48 h at just eclosed adult developmental stage, compared to control ones. On the other hand, there were no significant differences in regression slopes of either males or females for mobility.



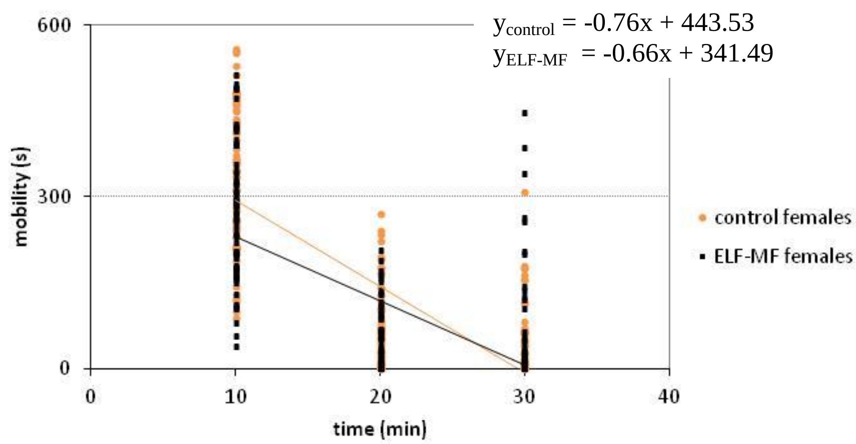


Figure 5. Linear regression lines and equations of both parameters of locomotor activity (distance travelled and mobility) for 3 days *D. subobscura* females exposed to ELF-MF (50 Hz, 0.5 mT) for 48 h at just-eclosed adult developmental stage; $n_{control} = n_{ELF-MF} = 75$.

Discussion

In changing environments organisms use sensory systems to respond appropriately to different cues and they also might develop mechanisms to adapt (Mayr, 1974; Seligman, 1970; Domjan, 2005). The adaptive advantage of organisms in that kind of environment depends on many functions, but one of the important ones is locomotor activity, because it facilitates animals to disperse, to find food, to mate, and to respond to different stress situations. It is shown that neurotransmitter serotonin has a role in response to stimuli from the environment (Water-house *et al.*, 2004). Moreover, serotonergic neurons are sensitive to changes in behavioral activation (Jacobs and Fornal, 1999; Portas *et al.*, 2000). This study demonstrated that changes in adults' locomotor activity after exposure to ELF-MF (50 Hz, 0.5 mT) at egg-first instar larvae developmental stage were detected even though other developmental stages (2nd, 3rd instar larvae, pupa, and adult) were not exposed to ELF-MF. Moreover, exposure of just eclosed adults to ELF-MF also alters locomotor activity. According to all these results we can propose that the effect of the applied magnetic field might be on the serotonergic transmission. In the case of exposure of egg-first larvae developmental stage to ELF-MF, changes expressed at adult stage indicate that egg and the first instar larvae developmental stage are very sensitive to ELF-MF.

In conclusion, observed significant reduction of locomotor activity during recording in males and females of *D. subobscura* which were exposed to ELF-MF for 48 h at egg-first instar larvae and just eclosed adult developmental stage could point out the consequences of exposure to ELF-MF (50 Hz, 0.5 mT) which might be extrapolated to humans.

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Mating frequency of *Drosophila subobscura* from two populations.

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Mating is a fundamental process for animals with sexual reproduction. Mating success and sexual selection are poorly investigated in *Drosophila subobscura*, a species with complex courtship behavior represented by: orientation, “wing dance”, jump (attempting copulation), forelegs posture, vibration, rowing, and copulation (Milani, 1956; Brown, 1965). The specific nature of behavior during courtship and mating, as well as mate choice, are genetically and environmentally determined (Terzić *et al*., 1996; Jennions and Petrie, 1997; O’Dell, 2003). Signals for mating, which are reciprocally exchanged between the sexes, allow identification of pairs. Mating choice largely depends on sexual traits and preferences for them (Servedio and Saetre, 2003). Adaptation to different environmental conditions can lead to modification of these signals and recognition systems (Coyne and Orr, 1998).