POSSIBLE INFLUENCE OF CENTRAL NERVOUS ACTIVITY OF MICE BY EXPOSURE TO UHF FIELDS

C. Goiceanu (1), F. Grădinaru (2), D.D. Sandu (3), R. Dănulescu (4), G. Bălăceanu (5), O.G. Avădanei (6)

(1) - Institute of Public Health, Department of Occupational Health, 14 Victor Babes Street, 6600 Iasi, Romania; E-mail: goiceanu@iasi.mednet.ro

(2) – As (1) above, but E-mail: FeliciaGradinariu@pub-health-iasi.ro

(3) - "Al. I. Cuza" University, Faculty of Physics, 11 Blvd. Copou, 6600 Iasi, Romania; E-mail: ddsandu@uaic.ro

(4) - As (1) above, but E-mail: razdan@iasi.mednet.ro

(5) - As (1) above, but E-mail: GheorgheBalaceanu@pub-health-iasi.ro

(6) - As (3) above, but E-mail: minu@uaic.ro

ABSTARCT

In this paper we present some results of an exposure experiment carried out on mice. Ten male Swiss mice were exposed to ultra high frequency field inside a transverse electromagnetic cell. The operating frequency was 400 MHz and the field level in terms of power density was about $1mW/cm^2$. The mice were exposed 8h/day, 5 days per week for 13 weeks. A control lot of 10 male Swiss mice was kept in similar environmental conditions. The behaviour of both lots was examined by a battery of three behavioural tests applied every 15 days.

INTRODUCTION

Electromagnetic waves influence in living bodies was emphasised by quite a variety of experimental and epidemiological studies. Low intensity radiation flows are susceptible of specific, non-thermal effects and in the last decades, the interest of many research groups was directed toward the study of such *in vitro* and *in vivo* effects. Although there is also available a substantial body of data describing biological responses to low-level fields from cellular [1], [2], animal [3], [4], [5] or human [6], [7], [8], [9] studies, knowledge of effects due to chronic exposure to weak fields is inadequate to determine whether subtle effects exist and to establish causal and dose-effect relationships. The aim of the present study is to investigate some effects of long-term low-level exposure to ultra high frequency (UHF) fields upon central nervous activity of mice.

MATERIALS AND METHODS

The experiment was carried out on 20 Swiss male mice, divided in two lots: exposed lot and sham exposed lot. All the animals were housed individually in separate glass jars (13 cm diameter, 20 cm height) and, during exposure or sham exposure time intervals, were placed in plexiglas cages (15 x 15 x 15 cm) in identical conditions of temperature (18-20 °C) and humidity (40-60 %). Inside a transverse electromagnetic (TEM) cell, ten Swiss male mice were exposed to low-level microwave fields for a relatively long period of time, comparing to their lifetime. Exposure lasted 8 hours a day, beginning every day at 9.00 PM, five days a week, for 13 weeks. The operating frequency was 400 MHz and the field level, in terms of power density, was about $1mW/cm^2$. The electric field strength inside the cell was about 60 V/m. The exposure field was unmodulated.

The behaviour of animals was assessed using three tests, which investigate the exploring behaviour and motor activity: *open field* test, *evasion* test and *perforated plate* test.

In the *evasion* test the animal is placed in a wood open box [10] of 50 x 25 x 10 cm on an inclined plane board of 55 x 25 cm. Animal performances consist of the routs number evaluation made in a period of 3 minutes.

The *open field* test was designed to evaluate the animal behaviour in a new environment characterised by large, lighted space. The mouse is placed in a 80x80 cm plate divided in 16 equal squares and bordered by a 30 cm height wooden wall. The number of squares explored by each animal was recorded. Due to rodent innate fear of openness and height the

mouse develops in such space a phobic reaction of fear and anxiety [11]. After the adaptation with the new milieu the animal begins to explore it.
In the *perforated plate* test mice are placed on a 40 x 40 cm wood plate with 16 holes of 2 cm diameter having the centres situated at 8 cm distance one of each other. The performance is assessed by counting the number of inspected holes during 3 minutes period.

RESULTS AND DISCUSSION

The *Evasion* Test

The *evasion* test did not reveal significant differences between exposed and controls. Despite the apparent increasing evolution of animal performances during the 90 days of exposure, the regression curves indicate a progressive (but nonsignificant) loss of mobility during the experiment, probably due to weight gain and aging. Consequently, the mobility seems to be not affected by exposure to UHF field.

The *Open Field* Test

In the *open field* test the mean number of inspected squares varied in a similar time-depending manner in both lots ($r = 0.82$, $p = 0.02$ for exposed and $r = 0.93$, $p = 0.002$ for control). The performances had an ascending profile, with a short decrease in the 75th day of the experiment, but with a recovery of the shape at the end of the exposure. (Fig. 1). The lack of statistical significance of the differences between the two lots suggests that these increasing performances in time could be attributed to learning capacity and habituation to the new environment, rather than to exposure to UHF field.

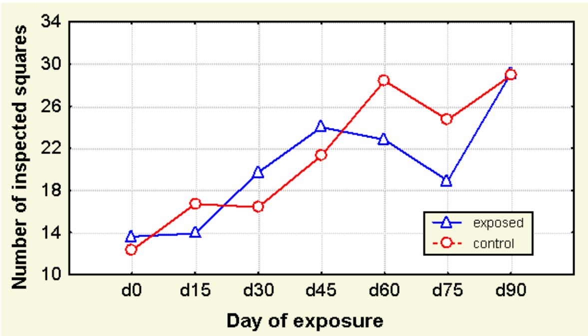

Figure 1: Mean values of squares inspected in the *open field* test

In the *open field* test, both lots showed a significant tendency of increasing the performances during the experiment period, which can be explained by the alleviation of the initial fear and anxiety of mice placed in a new open milieu.
The correlation coefficient for the exposed lot ($r = 0.82$) is nonsignificant but lower than the control one ($r = 0.93$). However, the lack of statistical significance of the difference between the two correlation coefficients is probably due to the small number of cases. This difference would have been significant if there had been investigated more than 20 cases. Nevertheless, for the exposed lot, the rate of increasing the performance is lower than the one for the controls and this difference could be attributed to the UHF exposure. Despite this lack of statistical significance, an analysis of the performance evolution in time, made by comparing the results obtained in a test session with the previous one, emphasised some features as shown in Table 1.

Table 1: Evolution in time of mice performances recorded in *open field* test.

LOT	**TEST SESSION**						
	Session 1	**Session 2**	**Session 3**	**Session 4**	**Session 5**	**Session 6**	**Session 7**
Exposed	13.6	13.9	19.7 ↑	24.0 ↑	22.8 ↓	18.9 ↓	29.1 ↑
Control	12.3	16.7 ↑	16.4	21.3 ↑	28.4 ↑	24.7 ↓	28.9 ↑

↑↓ - indicate the increase or decrease of the mean performance compared with the previous test session.

In the second test session, the controls showed an increase of the performances by 4.4 points, while the performances of the exposed ones are approximately the same as in the first test session. For the exposed lot, the first increase of the performances is recorded in the third test session. The fourth test session revealed increased performances for both lots. In the fifth session, the performances of the exposed mice decreased (from 24 to 22.8), while for the controls the performances increased (from 21.3 to 28.4). In the last two sessions, the two lots recorded similar tendencies: in the sixth a decrease, one and in the seventh one, an increase of performances.
Naturally, in the second test session, we expected to record increased performances for both lots as an effect of the decrease of the inhibition induced by a new milieu. For the exposed, the performances increase just in the third test session. This fact could be explained by the prolonged time of hesitation until the decision to smell the objects. A similar prolonged hesitation time is also noticed after the third and fourth session: the exposed mice performances decrease again for the two consecutive sessions (fifth and sixth) as good as they had needed a period of time for relaxation before recovering their abilities in the seventh session. The prolonged hesitation until mouse specific action of smelling could be an effect of a state of indecision or an effect of the reduction of the exploratory motivation.
The performance evolution of the exposed mice indicates an apparent phasic behaviour. How could this apparent cyclic evolution of exposed mice performances be explained: two stages of increasing performances, followed by two stages of decreasing performances and in the last test session a new increase one? Initially there is a prolonged inhibition (probably due to both the effect of UHF exposure and the naturally inhibition caused by the new milieu), followed by a period of exploratory interest recovery, and finally, a decreasing performances period. This apparent phasic behaviour could be explained by the presence of motivating visual stimuli, which activates the investigating behaviour, even in the case of general exploratory interest decrease due to UHF exposure. Thus, although it was observed a greater correlation coefficient in exposed lot, the statistical significance of the difference between the two correlation coefficients could be affected by the presence of motivating stimuli.

The *Perforated Plate* Test

The curves describing the evolution of mice performances in *perforated plate* test (showed in Fig. 2) indicated a slight different evolution of the two lots during the entire experiment. The initial differences between the two lots lasted during the first half of the experiment (until the 45th day) without any statistical significance. But this difference diminished in the second half of the experiment. In spite of the apparent oscillating shape of curves, their regression analysis showed a decreasing tendency in both lots, but more evident and significant in the exposed lot ($r = -0.81$, $p = 0.02$) compared to a non-significant one in the control lot ($r = -0.74$, $p = 0.06$) which suggests a relation with the UHF exposure. The progressive decreasing performances in the exposed lot indicate a loss of exploratory activity of these animals. One can postulate that nervous activity has been affected by the exposure, consisting in the reduction of the investigative potential of the animal and by inducing a certain psychological stress causing a state of indifference, apathy, which could be associated with an incipient syndrome similar to neurasthenia in human subjects.

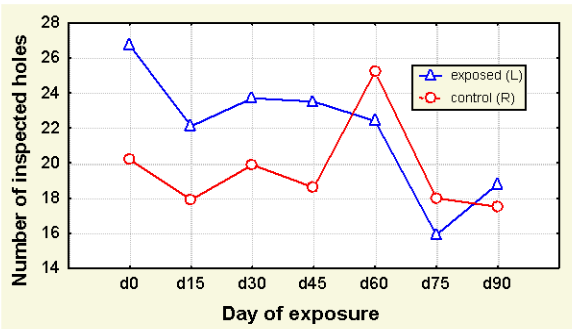

Figure 2: Mean values of holes inspected in the *perforated plate* test

The results of the *open field* test and *perforated plate* test show different performance evolutions for the exposed mice compared to control ones. This difference is a result of characteristic features of the two tests. In the *open field* test there are motivating stimulus - objects that activates the need for the object identification. In the *perforated plate* test there are no specific stimuli, but only a number of holes, which could release the natural exploring behaviour in the absence of a specific stimulus.

CONCLUSIONS

Motor activity of exposed animals does not seem to be affected by exposure to ultra high frequency field, in spite of moderate loss in time of motor activity in both lots, as long as a quite similar evolution was recorded. This moderate loss of motor activity for both exposed and controls is probably due to increased weight and ageing.

Regarding exploratory activity, the results of the *open field* test and *perforated plate* test seem to emphasise an effect of exposure consisting in a reduction of natural exploratory activity. This effect is directly indicated by the progressively decrease of the exposed mice performances in the *perforated plate* test, while controls performances are relatively constant. Perhaps we could have obtained a similar statistic significant difference between exposed and controls in the *open field* test, but the presence of the specific stimulus acted as an additional activation of exploratory behaviour, reducing the significance of differences.

The presence of other factors, which act either like motivating agents or like agents that decrease the motivation, can influence the intensity of effects due to exposure to microwave fields. We suppose that in human subjects, the association of ultra high frequency field exposure with a motivation diminution (i. e. a reduced professional satisfaction or different motives generated by the psychosocial climate at the workplace), could intensify the effects on human organism. Anyhow, there is very difficult to quantify the manner and the extent to which different factors may influence the central nervous activity, even when the effects seem to be quite similar. In this context, our study put in evidence some possible non-thermal effects consisting of influences of low-level ultra high frequency fields on central nervous activity, consisting in a reduction of the investigative potential of the exposed animals. Nevertheless, there is still necessary to carry out animal studies in order to accumulate new data on biological effects of long-term long-level exposure to electromagnetic fields.

REFERENCES

[1] R. P. Blackwell, R. D. Saunders, “The effects of low-level radiofrequency and microwave radiation on brain tissue and animal behaviour”, *Int. J. Radiat. Biol.,* vol. 50, 5, pp. 761-787, 1986.
[2] C.F. Blackman, S.G. Benane, D.E. House, W.T. Joines, “Effects of ELF (1-120 Hz) and modulated (50 Hz) RF fields on the efflux of calcium ions from brain tissue in vitro”, *Bioelectromagnetics*, vol. 6, pp. 1-11, 1985.
[3] C.K. Chou, A.W. Guy, L.L. Kunz, R.B. Johnson, J.J. Crowley, J.H. Krupp, “Long Term, Low-Level Microwave Irradiation of Rats”, *Bioelectromagnetics,* vol. 13, pp. 469-496, 1992.
[4] H. Lai, “Research on the Neurological Effects of Nonionizing Radiation at the University of Washington” *Bioelectromagnetics,* vol. 13, pp. 513-526, 1992.
[5] M. A. Navakatikian, L.A. Tomashevskaya, “Phasic Behavioural and Endocrine Effects of Microwaves of Nonthermal Intensity”, in *Biological Effects of Electric and Magnetic Fields*, D. O. Carpenter and S. Ayrapetyan Eds. San Diego, London: Academic, pp. 333-343, 1984.
[6] J.A. Dennis, C.R. Muirhead, J.R. Ennis, *Human Health and Exposure to Electromagnetic Radiation*, London: NRPB, pp. 3-17, 22-24, 49-53, 1992.
[7] R. Nilsson, Y. Hamnerius, H.K. Mild, H.A. Hansson, E. Hjelmqvist, S. Olanders, L.I. Persson, “Microwave Effects on the Central Nervous System - A Study of Radar Mechanics”, *Health Physics*, vol. 56, 5, pp. 777-779, 1989.
[8] L.I. Kolosova, G.N. Akoev, V.D. Avelev, O.V. Riabchikova, K.S. Babu, “Effect of low-intensity millimeter wave electromagnetic radiation on regeneration of the sciatic nerve in rats”, *Bioelectromagnetics*, vol. 17, pp. 44-47, 1996.
[9] R. Dănulescu, V. Borza, E. Dănulescu, C. Goiceanu, “Peripheral nervous system impairment in occupational exposure to pulsed microwaves”, *J. of Prev. Med.*, vol. 8, 3, pp. 60-69, 2000.
[10] A. Turner, - *Screening methods in pharmacology*, London: Academic, pp. 69-99, 1965.
[11] F.G. Graeff, , M.B. Viana, C. Tomaz, - “The elevated T-maze, a new experimental model of anxiety and memory: effect of diazepam”, *Brazilian J. Med. Biol. Res.*, vol. 26, 1, pp. 67-70, 1993.