BEHAVIORAL STUDY OF HAMSTERS EXPOSED TO LOW INTENSITY MICROWAVES

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Abstract. A significant number of studies deal with the impact of low intensity microwaves on animal behavior. The present work is as a novel, complementary approach, which aims to scrutinize the behavior of golden hamsters (*Mesocricetus auratus*) in order to reveal possible modifications elicited by chronic exposure to low intensity electromagnetic fields: microwaves from the frequency range of 2400 – 2484 MHz generated by a wireless Access Point appliance with an emission power lower than 100 mW. The spectrum of usual activities was compared with the well-known ethograms of this species. The behavior of the hamsters was evaluated before and after exposure by means of three distinct tests: arena, labyrinth and shelter test. The statistical analysis of the data, performed on a relevant amount of data, did not reveal major changes in the behavior of the investigated animals.

Key words: microwaves, behavior effects, behavioral tests, wireless appliance, Access Point.

INTRODUCTION

Along with the benefits brought by microwave technologies, concerns have been raised regarding possible health hazards associated with the exposure to the emitted electromagnetic waves. The population uses and depends to an increasing extent on devices that generate microwaves, such as mobile phones, civil and military radars, kitchen appliances, industrial equipments used for welding or drying as well as medical instruments used in diagnosis and treatment.

Heat dissipation in the exposed system is one of the major effects of electromagnetic waves. However, an increasing attention has been attracted by athermal effects, which are observed in the absence of a significant temperature increase. More precisely an observed effect is termed athermal if it is accompanied by a change of less than 0.1 ºC in the temperature of the organism or of other physical system studied [19].

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The nervous system is very sensitive to environmental disturbance. Disturbance to the nervous system leads to behavioral changes. Effects induced in biological systems by low intensity electromagnetic radiations from the microwave domain raised interest from the perspective of potential modifications in the behavior of laboratory animals as a result of the action of such electromagnetic waves [1, 2, 5, 6, 7, 8, 10, 11, 12, 13].

The most studied behavior is locomotor activity. A decrease in motor activity in rats after 7 hrs of exposure to continuous-wave 2450 MHz microwaves (10 $mW \cdot cm^{-2}$, average SAR 2.7 $W \cdot kg^{-1}$) was observed [12].

A decreased motor activity on a platform and no significant change in running wheel activity was found [6, 8] in rats exposed to 2450 MHz microwaves (continuous-wave, 0.5 $mW \cdot cm^{-2}$, SAR 0.1 $W \cdot kg^{-1}$, exposed 5 days/week with a total exposure time of 640 hrs, activity was measured every 2 weeks). However, they reported no significant effect in both behaviors in rats similarly exposed to 915 MHz microwaves even at a higher energy absorption rate (5 $mW \cdot cm^{-2}$, SAR 2.5 $W \cdot kg^{-1}$) [7].

In another study [7], microwaves of different frequencies and intensities were studied on their effects on bar-pressing rate on a variable-interval schedule. It was found that the latency time of stoppage to respond after the radiation was turned on correlated with the rate of rise in body temperature of the animal.

The results from another study [10] indicate some changes in mice exploratory activity due to a long-term low-level microwave exposure (400 MHz, 1 mW/cm^2, 8 h/day, 5 days per week for 13 weeks). The authors speculated that the microwave exposure can conduce in time to a chronicle tiredness that reduces the performances of animals.

The aim of the present study is to investigate possible behavioral changes brought about in hamsters by long-term exposure to low intensity microwave radiations.

MATERIALS AND METHODS

Two distinct golden hamster (*Mesocricetus auratus*) lots were subject to the action of microwaves, each of them being composed of 8 male individuals. A third group of the same size and gender was not exposed to microwaves, serving as a control. All the animals considered for the study were healthy. At the beginning of the experiment, the hamsters of lot 1 were 5 to 6 months old; those from lot 2 and the control group, lot 0, were older, with ages between 10 and 11 months. The average body mass of the animals was 102 ± 6 g for lot 0 and lot 2 and 98 ± 4 g for lot 1.

The first two lots were exposed to a microwave field emitted by a wireless Access Point device having an omnidirectional antenna working in the 2400 MHz – 2484 MHz frequency range, satisfying the 802.11b standard [18]. The used model has a typical indoor covering area of 50 m for an 11 Mbit/s transfer rate, respectively 90 m in case of 1 Mbit/s. This wireless device was placed at a distance of about 1 m below the plastic cages of the animals. Starting from the maximum value of 100 mW for the emission power of this Access Point, we estimated that the energetic flux of microwave radiations was at most 8×10^{-4} mW/cm^2. Previous to the present study we recorded the temperature variations on samples of distilled water and egg yolk, placed in a thermo-insulating holder in the microwave field of the Access Point device. The temperature increase of the samples was of maximum 0.2 °C. According to the used theoretical model, this value is correlated with a SAR value of 1,097 W/kg. For the whole duration of the experiment, the Access Point appliance maintained a data transfer between two computers connected in a wireless network.

The exposure of the first two groups of hamsters had a duration of 10 weeks. During the first half of this time period the animals were kept in microwave field during daytime, six hours per day, for six days per week, whereas during the last five weeks the exposure was done for nine hours during the night, five sessions per week.

This choice of experimental protocol is justified by the characteristics of the species under study, the activity of hamsters being much more intense during the night in comparison to daytime.

Given the relatively short lifetime of golden hamsters, of about 1.5–2 years, it is justified to consider the above described exposure, with a total duration of 405 hours, as being chronic.

The activity of the animals was surveyed during test periods by a digital video camera (AVT/AVC 780 4 channel DVR) connected to a computer. By repeatedly reviewing the saved videos, standard gestures were quantified and statistically analyzed.

The evaluation of the behavior of the animals from all three groups was done at the beginning of the experiment (week 0), as well as in weeks 2, 5, 7 and 10, by performing two standard behavioral tests known in ethology under the names open field and shelter test. In week 10, the evaluation procedure was supplemented with a third test, that of passing through a labyrinth.

These are standard behavioral tests, widely used in laboratories throughout the world in pharmacological and psychobiological studies [14].

The open field or *arena* test was initially proposed by C. S. Hall in the period 1934 – 1936 as a modality of quantitative estimation of the emotional reactivity of rats placed in an open territory. Today the arena test is successfully used for the evaluation of locomotive activity, hyperactivity, as well as emotional and exploratory behavior of rodents. This test may also be used to derive an index, which is a quantitative measure of the degree of anxiety of hamsters. These animals have the natural tendency of avoiding unknown, illuminated, open spaces; in this respect the arena may be considered as an anxiogenic factor [3, 4, 16]. The arena test consists in placing each individual hamster on a plane, checkered surface (arena) of 0.8 m × 0.8 m in size, divided into 64 identical squares of 10 cm × 10 cm and equipped with a detachable peripheral transparent wall made of polycarbonate. The latter restricts the territory of exploration and prevents the hamsters from leaving the surveyed area. The set of squares includes 28 peripheral and 36 central ones. The camera recorded the activity of each animal during five minutes spent in the arena. At the end of each movie the experimenter counted the peripheral and central squares traversed by the animal. As a supplementary emotional index, we also considered the frequency of another three specific manifestations – grooming, freezing and rearing.

The *labyrinth* test was introduced by Thorndyke as a means for quantifying learning abilities of rats. The labyrinth is a set of walls delimiting a number of alternative ways; only one of them connects the entrance to the exit. The animals are placed individually in the entrance region of the labyrinth and are recorded using a digital video camera while passing through it. What is measured during such an experiment is the time needed to traverse the labyrinth and the number of errors made by the animal during this time. The decrease of both quantities along with the repetitions of the experiment proves the learning skills of the animals. Hamsters are preferred in such studies due to the fact that their natural environment is a labyrinth, which requires memorizing and learning abilities.

The usual expectations during a labyrinth test are that (i) the latency period, which precedes the start of the exploration of the labyrinth by the animal, decreases as a result of repetitions, (ii) the time needed to pass through the labyrinth shortens significantly, and (iii) some mistakes made during earlier runs are not repeated. In the present study, at the end of chronic exposure period, we performed on the same day 10 successive measurements for each animal. The ten passages through the labyrinth are chronologically numbered from the first trial. At the end of each run, the animals were rewarded by food products. The scheme of the labyrinth is presented in Figure 1.

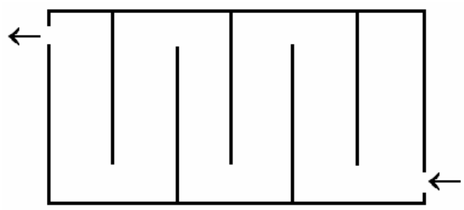

Fig. 1. The labyrinth through which each hamster performed ten consecutive passages in the eleventh week, at the end of chronic exposure to microwaves.

The third test, so called *shelter* test, is performed to estimate the behavior changes of the animals due to external factors applied while they live in their usual neighborhood. In our study, the perturbative factor is the chronic exposure to microwaves according to the irradiation protocol described above. The ethogram of an animal species is the inventory of specific gestures constructed on the basis of long-lasting observations of a numerous population living under unchanged conditions. The number of behavioral elements included in the ethogram of a species is correlated with the place occupied by the given species on the scale of evolution. A detailed knowledge of its ethogram is crucial for the use of the given animal species in the laboratory. Without this knowledge the baseline conditions of the experiment are not well defined [13]. A classical choice in this respect is the golden hamster, with a well defined ethogram [9], which offers a solid starting point for data interpretation in studies of behavioral changes elicited by external factors. During this test we followed a set of 10 specific activities: I – raising on hind limbs, C – cleaning simultaneously using fore limbs, V – cleaning alternatively with fore limbs, B – cleaning with hind limbs, P – combing, L – licking, S – rummaging, M – throwing with hind limbs, E – trying to escape, A – inactivity. A change in behavior is defined as a modification of the fractions of time dedicated to each of these activities. We recorded for five minutes the activity of each animal placed in its own plastic cage.

RESULTS AND DISCUSSION

RESULTS OF THE ARENA TEST

By applying this test we looked for possible changes in the level of anxiety of the animals as a result of chronic exposure to low intensity electromagnetic waves from the microwave region.

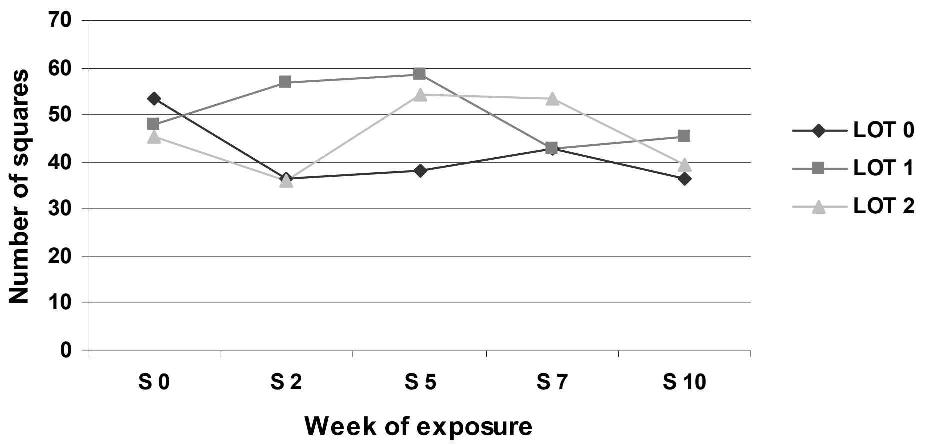

Fig. 2. The exploratory behavior of the animals reflected in the arena test: the total number of traversed squares during five minutes vs. duration of exposure to microwaves (at the beginning of the experiment (S0), after two (S2), five (S5), seven (S7) and ten (S10) weeks. Lot 1 refers to young individuals, lot 2 contains animals from a preceding generation while lot 0 is the control group.

The results of the arena test, which quantitatively reflect the exploratory behavior of the animals vs. the duration of exposure, are displayed in Figs. 2 and 3.

Figure 2 plots the evolution of the average number of traversed squares during five minutes recorded at the beginning of the experiment (S0), after two and five weeks of daytime exposure (S2 and S5, respectively), followed by two and five weeks of nighttime application of the microwave field (S7 and S10, respectively).

The young animal group (lot 1) displayed an enhanced locomotive activity right from the second week and finally receded to the level of the control group.

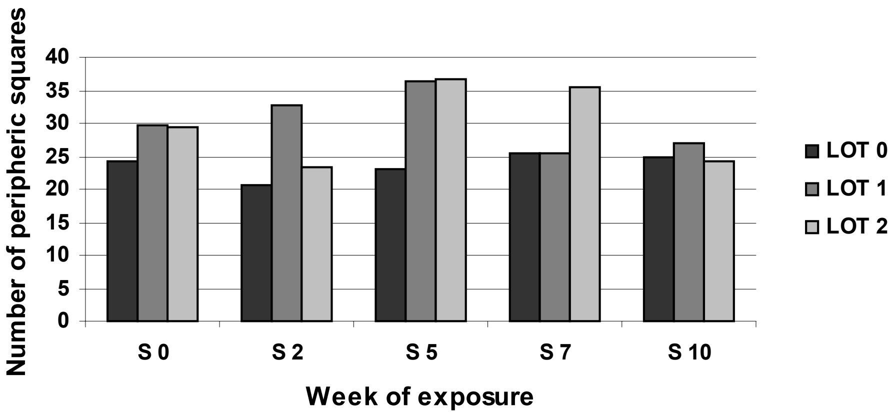

Fig. 3. The exploration of the peripheral domain of the arena: time course of the number of peripheral squares investigated during five minutes by the members of the three groups (notations are explained in the caption of Fig. 2).

The mature group (lot 2) containing individuals from an earlier generation reacted by intensified exploratory behavior; however this effect appeared later than in lot 1. By week 10 also lot 2 showed the same level of activity as the control lot.

A comparison of the three groups in what concerns the exploration of the peripheral domain of the arena is depicted in Fig. 3. By analyzing this plot one may be tempted to conclude that the applied microwaves enhance the exploratory movement focused on the periphery of the arena, as a sign of increased anxiety of the golden hamsters. However, such subjective estimations must be supported by a rigorous statistical analysis of the data. To this end, we calculated the correlation coefficients of the obtained data. The square of the correlation coefficient, r^2, of two equally numerous data sets gives the fraction of the total variation in the values of one set that can be explained by a linear relationship with the other set [15].

For example, the numbers of total and peripheral squares explored by the animals are strongly correlated in the case of the irradiated groups ($r_{\text{lot1}} = 0.7$; $r_{\text{lot2}} = 0.9$), whereas in the case of the control group the correlation coefficient is smaller

(r_{lot0} = 0,59). The values of correlation coefficient between the numbers of total and central squares traversed by the animals are smaller in the case of the exposed groups (r_{lot1} = 0,5; r_{lot2} = 0,6), which is again at variance with the control one (r_{lot0} = 0,8). These results are consistent with the interpretation that chronic exposure to low intensity microwaves may result in elevated anxiety levels, which prevents the hamsters from exploring open fields such as the central region of the arena. Differences between lot 1 and lot 2 suggest that age is also an important factor in modulating this response.

Concerning the three specific manifestations mentioned above – grooming, rearing, freezing: the first two of them did not come out in our recorded tests, whereas the third one had a statistically insignificant frequency of appearance.

RESULTS OF THE LABYRINTH TEST

During this test, performed after the completion of the experimental protocol of chronic exposure of lot 1 and lot 2, we recorded 10 times the latency period, the time needed by each individual animal for finding its way through the labyrinth, and also counted the mistakes made during this endeavor. In these experiments a mistake is defined as turning back to explore an already traversed portion of the labyrinth. Individuals belonging to lot 1 were tested in the first day of week 11, lot 2 in the second day of the same week and lot 0 in the third day after the end of chronic exposure to microwaves.

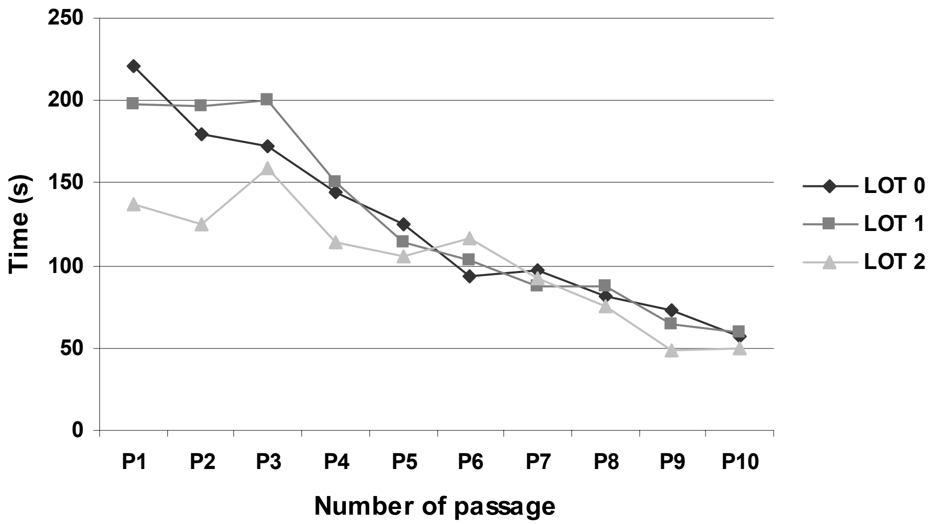

Fig. 4. The evolution of the hamsters as a result of training sessions in passing through the labyrinth: the average time needed for traversing the labyrinth vs. number of performed passages chronologically numbered from the first contact with the labyrinth (notations explained in caption of Fig. 2).

The evolution of the hamsters as a result of training sessions in passing through the labyrinth is illustrated by Figs. 4, 5 and 6, which plot the time needed to find the exit and the number of mistakes made during successive passages.

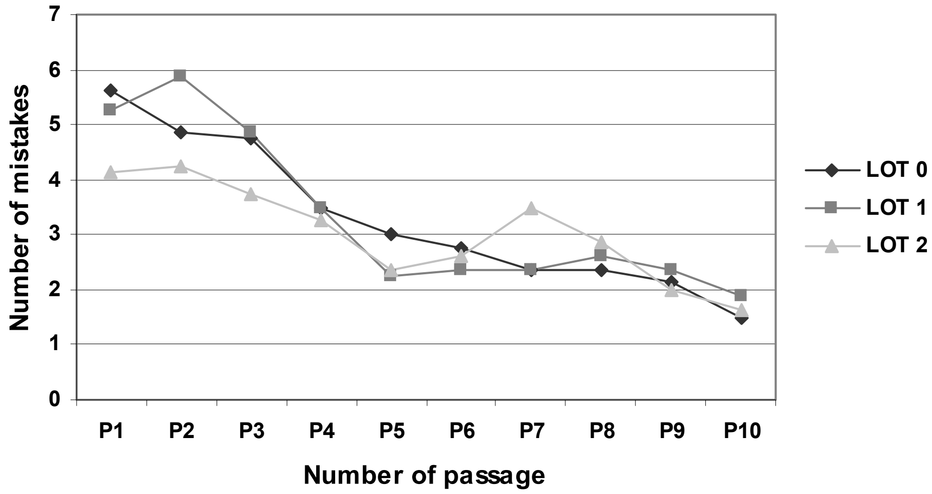

Fig. 5. The average number of mistakes in the labyrinth made by the members of each lot vs. the number of elapsed passages, chronologically numbered (see caption of Fig. 2 for notations).

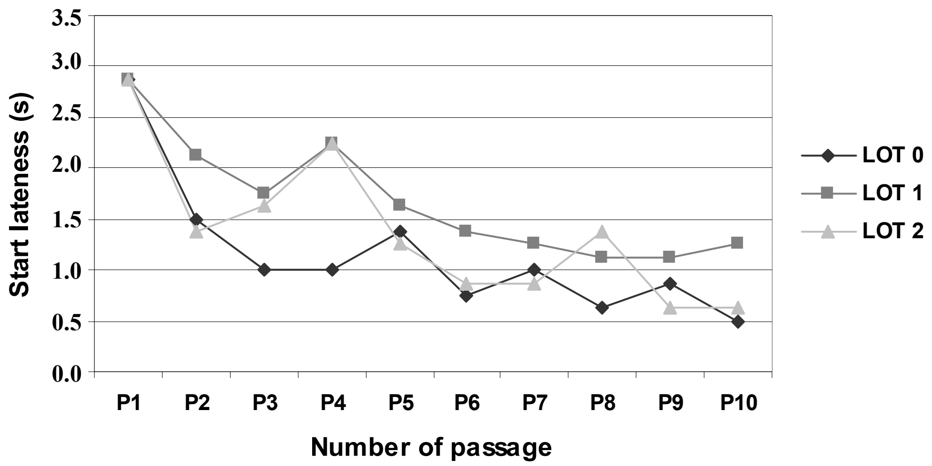

Fig. 6. Results of the labyrinth test concerning the decreasing of the latency period (the average time elapsed between the moment of entrance in the labyrinth and the moment of the first movement) *vs*. the number of elapsed passages, (see caption of Fig. 2 for notations).

It is clear from Figs. 4, 5 and 6 that all three groups of animals show a tendency of shortening of the latency period, of the time it takes to go across the labyrinth and a decline in the number of turnings back to go over an already traversed portion of the way, classified as mistakes. Statistical analysis of the data (for time, between lot 0 and 1, $p = 0.90$, between lot 0 and 2, respectively between lot 1 and 2, $p = 0.03$, $p = 0.02$) leads to the conclusion that the apparent differences between the three lots basically stem from the age difference rather than from microwave exposure.

RESULTS OF THE SHELTER TEST

During the shelter test each animal was monitored for 300 seconds by video recording in its normal habitation. The movies were scrutinized and 10 specific activities (I, C, V, B, P, L, S, M, E, A – see Materials and Methods) were counted and their duration was measured, using a computer program. Here we present the results of the shelter test obtained at the end of the experimental protocol, after 10 weeks of exposure to low intensity electromagnetic waves from the microwave domain (frequencies ranging from 2400 MHz to 2484 MHz).

Figures 7, 8 and 9 are pie charts, which depict the results of the shelter test performed after the completion of the irradiation protocol (in week 10). They show the percentage of time dedicated to each activity averaged over the members of each group (lot 0, lot 1 and lot 2, respectively).

Statistical calculations applied for the results data of this test did not reveal significant differences in the behavior of the animals living in their usual surroundings. For rummaging activity, which is an index of the hamster's degree of anxiety, no statistical differences were found (for time, between lot 0 and 1, $p_{1,0} = 0.79$, between lot 0 and 2, respectively between lot 1 and 2, $p_{2,0} = 0.89$, $p_{1,2} = 0.90$; for number of activities of this kind, $p_{1,0} = 0.98$, $p_{2,0} = 0.98$, $p_{1,2} = 1.00$).

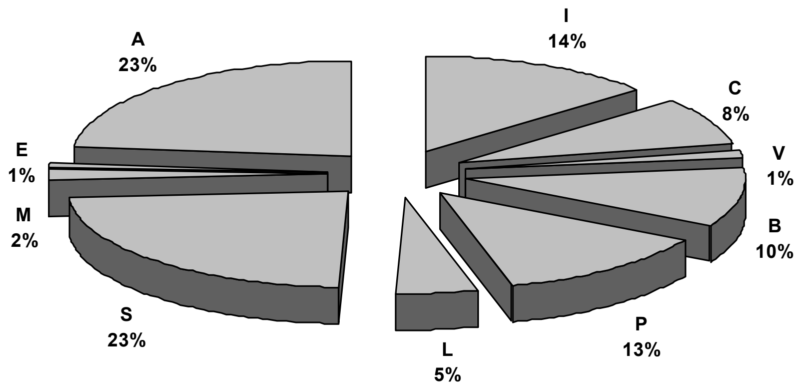

Fig. 7. The results of the shelter test obtained after 10 weeks of exposure to a low intensity microwave field: percentage of the duration for each specific activity in the control group (lot 0), from a total recorded time of five minutes.

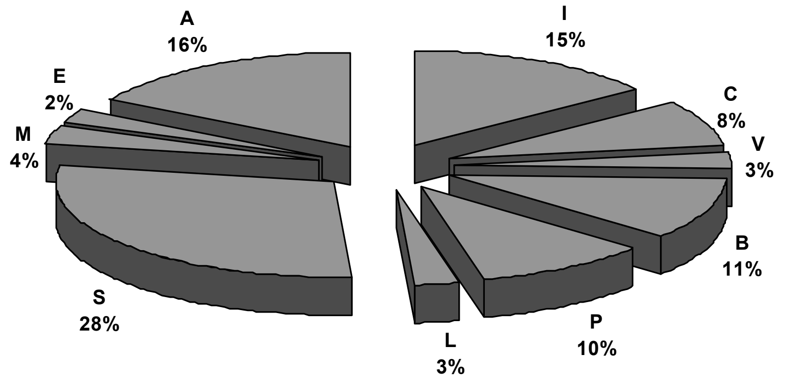

Fig. 8. Percentage of time dedicated to each activity, from a total testing time of five minutes, averaged over the members of the young animals group (lot 1) exposed for 10 weeks to low intensity microwaves.

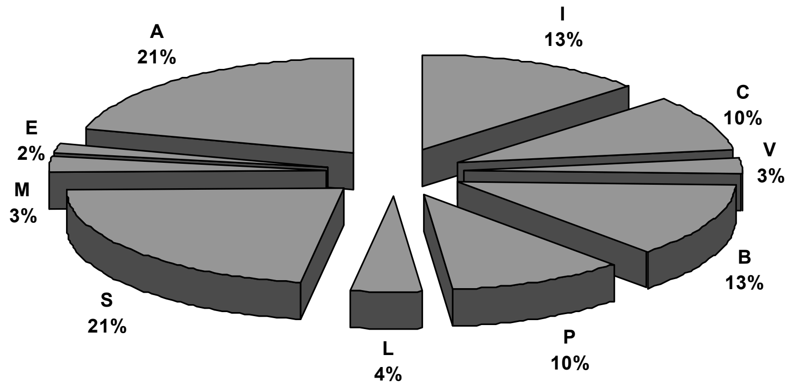

Fig. 9. The shelter test: percentage of the duration of each specific activity, from a total monitored time of five minutes, in the mature animals group (lot 2) exposed for 10 weeks to low intensity microwaves.

CONCLUSIONS

This article presents a complex animal behavior study of the impact of chronic exposure to low intensity microwaves. The choice of the animal model, the golden hamster (*Mesocricetus auratus*), is motivated by the fact that this species has a well-characterized ethogram [9].

The arena test revealed an intensification of the global exploratory behavior of the animals. Apparently this could be interpreted as a decline in anxiety since the number of explored squares in open fields increased slightly. However, statistical analysis shows that only the number of traversed peripheral squares correlates well with the total number of squares passed the animals, the number of central ones does not. This means that the overall increase of the exploratory activity is mostly concentrated at the periphery, leading to the opposite conclusion, that anxiety has a slightly elevated level. This effect seems to be transient, being attenuated by week 7 in the young animal group (lot 1) and by week 10 in the mature one (lot 2).

The labyrinth test shows that young animals (lot 1) improve their performance in passing through the labyrinth in the same way as the control group (lot 0) does. Lot 2, consisting of mature animals, perform better at the beginning than the other two groups, however, by the end of the 10 repetitions all the three lots show similar skills (Fig. 4). The average number of mistakes (Fig. 5) evolves analogously. When looking at the duration of each mistake (results not shown) we remarked that younger animals spend more time pursuing a wrong way. From Fig. 6 we can conclude that lot 1 has initially a greater average time comparatively to lot 2 and the control lot; this parameter also presents a decreasing tendency, however it levels off by the end of the experiment.

On the basis of the labyrinth test we conclude that the learning skills of the animals are not influenced by chronic exposure to low intensity microwave radiation. Performance discrepancies between the studied groups may be associated to differences in life experience rather than to the applied electromagnetic field.

Statistical analysis, through ANOVA test, performed on a large amount of data obtained by the shelter test did not reveal behavioral modifications in the case of animals subject to long-term exposure to low intensity microwaves while living in their normal habitat ($p > 0.05$ with one exception in week 5, when $p = 0.04$).

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