



# The Impact of Electromagnetic Field on Conditioned Reflex Memory

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## ABSTRACT

The aim of this study is to investigate whether household frequency electromagnetic field affect memory processes. Apparently in various studies researchers note that different frequencies electromagnetic field improve or impair memory. In memory process hormone ghrelin plays an important role, which participates in the neurogenesis processes of memory formation. We have studied effect of electromagnetic field (EMF) on ghrelin's concentration and memory changes under EMF. We exposed male Wistar rats under EMF. The household frequency electromagnetic field was generated with a GSM mobile phone. For memory tests we used the two feeder and five choice serial reaction time tests (5-CSRTT). With this latter test we studied attention and concentration and in serum we measured the concentration of ghrelin by immunoferminal analysis. In serum ghrelin concentration increased in electromagnetic field exposure group, after 30th day of the exposure level of ghrelin is higher than after 10 day of exposure and the sham control group. During training EMF exposure group rats task needed more trials (sessions) than in sham control group (in 5-CSRTT in sham control and EMF exposure groups, the values were  $92,9 \pm 2,084$  and  $101,8 \pm 2,764$ , respectively.  $n=10$ ;  $p < 0.05$ , in two feeder tests in sham control and EMF exposure groups, the values were  $82,7 \pm 2,989$  and  $99,1 \pm 3,903$ , respectively.  $n=10$ ;  $p < 0.05$ ). As for memory consolidation, for performing 5-CSRTT after 30 day of EMF action we received significant differences in the number of correctly performed tasks (sham control and EMF exposure groups, the values were  $0,7 \pm 0,1528$  and  $0,2 \pm 0,1333$ , respectively;  $p < 0.05$ ) and results in prematurity activities (in sham control and EMF exposure groups  $0,5 \pm 0,2236$  and  $1,5 \pm 0,3416$ ,  $p < 0.05$ ) and in accuracy (in sham control and EMF exposure groups  $0,5 \pm 0,1667$  and  $1,4 \pm 0,2667$ ,  $p < 0.05$ ). In our study It was found, that EMF affected as well as on learning and memory recall process, but with more advanced research reason, that clinically gave a picture of memory loss mainly related to decreased attention and concentration, which is also confirmed by the increase in concentration of hormone ghrelin, participating in memory processes, under influence of the household frequency EMF.

**Key Words:** Ghrelin, Memory, Electromagnetic Field, Behavior

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## Introduction

The modern living environment is unimaginable without the electromagnetic field. Electric appliances, which are used for daily activities, generate electromagnetic fields (EMF) with different frequencies. Changes caused by the effects of EMF on a living organism are not always clear. However, increased stress, nervous diseases, reproductive system disorders, irritability and

depression, frequent cases of cancer, especially childhood leukemia, are directly and indirectly linked to electromagnetic field impact on the body. Nervous tissue is particularly sensitive to EMF. (Van Wijngaarden *et al.*, 2000; Lewczuk *et al.*, 2014).

The impact of the electromagnetic field has an effect on the functioning of the neurotransmitter (Grassi *et al.*, 2004) and therefore it reflects on memory and cognitive process.

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The electromagnetic field is considered as an important physical factor capable changing the cognitive and non-cognitive behavior (Gray and McNaughton, 2000). It should be noted that different authors' vision on EMF, as a factor that affects the biological system, varies regarding memory changes. In particular, some researchers note that the mobile frequency EMF, which is usually generated from a mobile phone, causes memory loss; while other studies confirmed that the EMF leads to improving memory. However, various tests and different frequency EMF are being used for the memory evaluation. It is also noteworthy that the EMF does not always violate all forms of memory at the same level. The spatial, short-term and psycho-emotional memory type is especially sensitive to the EMF; meanwhile, the low-frequency EMF can even improve the short-term memory in rats (McGregor, 2002; Vázquez-García *et al.*, 2004; Reyes-Guerrero *et al.*, 2006).

While using mobile phone generated EMF, spatial memory and learning process is being reduced in rodents (Fragopoulou *et al.*, 2004). Studies on adolescents found that the frequency of using wireless connection had an impact on memory performance (Schoeni *et al.*, 2015). Studies on children who use mobile phones, revealed the difference in conjunctive process, namely the noticeable decrease in response (while operating with the response inhibition task) (Bhatt *et al.*, 2017).

In 1996 scientists discovered hormone ghrelin, which happens to participate in memory formation, neurogenesis process: proliferation and differentiation and acts on hippocampus progenitor cells (Li *et al.*, 2013). Ghrelin has an impact on the central nervous system; it is involved in different functions of a nervous system regulation (Gherzi *et al.*, 2015). In addition to neuroendocrine action, it strengthens the cognitive functions (Andrews, 2011), reduces anxiety and depression, participates in reward action, motivation process and controls the functions of neurons.

It is known that during stress the concentration of ghrelin increases (Bali and Jaggi, 2016). In our earlier research, under the high-frequency EMF activity, we found an increase in the concentration of ghrelin (Dondoladze *et al.*, 2016), while in other studies, under low-frequency EMF changes in the concentration of ghrelin did not occur (Hong *et al.*, 2012).

Attention and concentration can greatly influence memory (Templer and Hampton, 2013). In

the case, when for the sake of attention and concentration, mobilization amplifying willpower and the psychic force of the individual is reduced, memory encoding, storage, and recall are decreasing; but in case of attention and concentration mobilization, information coding and recall happens in a short time. In their research, Trimmel and Schweiger show that less than 50Hz EMF expose „indicates an immediate reduction of cognitive performance in attention, perception and memory performance” in humans (Trimmel and Schweiger, 1998). According to research, exposure to wireless technologies causes neurodevelopmental and neurobehavioral changes in children (Sage and Burgio, 2018).

Scientific studies of negative impacts of EMF often contradict studies conducted by mobile phone enterprises, new media communication specialists or magnate companies manufacturing household appliances. Our interest is to disclose the potential danger of EMF independently.

To study the effect of the household frequency (800 Hz) electromagnetic field on memory processes, we conducted a research on Wistar line male rats, in which we used GSM system mobile phone for EMF generation. In order to study the role of the ghrelin in these processes, we have determined the concentration of the hormone using the Enzyme-Linked Immunosorbent Assay (ELISA).

The study has shown that EMF affects both memory encoding and information recall, although the further research gave the clinical picture of memory loss; mainly related to the impact of electromagnetic fields on the difficulty of paying attention and decreased concentration, which was also confirmed by ghrelin's concentration increasing under EMF action.

## Methods

### Animals

In the study, we used 110-120g 8-week Wistar male rats ( $n = 40$ ). Meals were distributed 2 times in a day: at 10:00 am and 18:00 pm; access to water was not limited. We divided animals into 2 groups: EMF-exposure (experimental) group rats ( $n = 20$ ) that we placed under EMF, and sham control group rats ( $n = 20$ ) in identical conditions, but without EMF. In both groups, we studied memory associated with attention and concentration changes.



### *EMF Exposure System*

generation of EMF was operating with GSM system mobile phone, that was placed on the 5mm height from the center of experimental (EMF exposure) group rats' cage. With special tool – "CORNET Microsystems, Electrosmog Meter" we measured the generated electromagnetic field in the moment of the phone call and which has shown 800 Hz frequency. The phone's special program allows to receive a phone call in every 10 minutes (phone was on a silent mode, no vibration) and at the moment of the call household frequency electromagnetic field was generated.

### *Study design*

After the adaptive period (3 days) we had experimental (EMF exposure) group rats under the household frequency EMF, to be more specific in 80/80/30 plastic cages where animals were placed on the mobile phone. The call duration was 10 seconds. The electromagnetic field was generated only during the daytime - 10 hours per day for 30 days. On EMF exposure group animals EMF affected not permanently but only for a period of time. In the memory tests, as an irritant, we used food and have related to its neutral stimulus-light. These tests are conditioned-reflex memory tests and need to be learned. Preparation and learning for memory tests (Two Feeder test and 5-Choice Serial Reaction Time Test "5-CSRTT") in the EMF exposure and sham control groups have started on the same day, more precisely, after the 10<sup>th</sup> day of placing the EMF exposure group rats under EMF. To check memory storage, we tested animals from both groups after the 20<sup>th</sup> day. The latter was 30<sup>th</sup> day after being placed under EMF exposure group rats under EMF.

### *Memory tests*

*Two feeder tests:* We had 10 rats per group (EMF exposure and sham control group); we used 130X90X40 cm trapeze-shaped box, on one side of the box (130 cm side) the bulb was fixed at 10 cm height from the bottom. The lamps were switched on on one side using a special checker. In both corners, there was a 25x25 mm sized hole attached to the feeder. We were providing the food on the alighted area.

On the opposite side of the feeder was a transparent 15x30x20 cm sized box. We placed rats in the box and after lifting the box with the remote control rats were released into the box. We illuminated one or the other side of the box. After 20 seconds of one side being into the light, we

lifted the transparent box for rats to get to their food (sunflower). To eat the rat had to pick its nose into the feeder and get a sunflower. After getting the food the light was turned off immediately. We recorded test results and calculated them. This test was performed after the 20<sup>th</sup> day of starting the study.

5-Choice Serial Reaction Time Test (5-CSRTT) was used to study rodents memory, attention and concentration, it was developed by Robin and his colleagues (Humby *et al.*, 2005). We conducted the test by respecting its protocol. We studied attention mobilization, concentration, and impulsiveness associated with it. The video cameras were connected to a computer with special software studying animal translocation, locomotor activities and counted the number of correctly and or incorrectly performed tasks.

### *Laboratory examination*

to study the effect of EMF on ghrelin concentration and its role in memory processes, we measured ghrelin in serum. The blood was taken from the lateral tail vein on the first day of the study and the 20<sup>th</sup> day, which corresponds to day 10<sup>th</sup> and 20<sup>th</sup> after placing EMF exposure group rats under EMF. We have determined the concentration of the hormone using the Enzyme-Linked Immunosorbent Assay (ELISA), (GHRL ELISA Kit (Rat)-OKEH03462).

### *Data analysis*

For statistical analysis of obtained data from sham control and EMF exposure groups, we used Single Variable Data Analysis (one-way ANOVA). The results are presented (mean +/- standard error (SE), in statistical significance of evidence  $p < 0.05$ . In case of nonsignificant effect, through t-test, we have identified the possible differences between the groups. Program used: Prism - GraphPad.

Living conditions of animals for the experimental research were agreed by Beritashvili Center for Experimental Biomedicine committee.

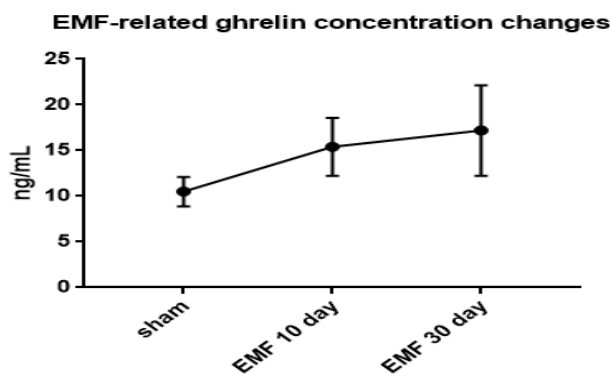
## **Results**

### *Effect of EMF on Serum ghrelin levels*

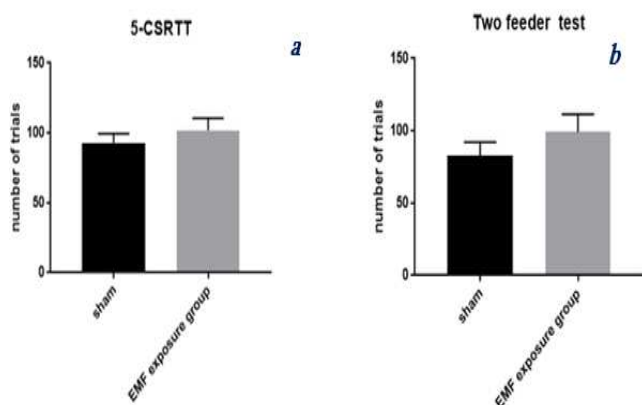
We measured the level of ghrelin in serum to evaluate the effect of EMF on memory processes. In fasting serum, taken from rats tail vein, the ghrelin concentration in the sham control group was  $10.5 \pm 1.62$  mg/mL; in EMF exposure group rats after 10<sup>th</sup> and 30<sup>th</sup> day EMF action induced an increase in ghrelin concentration by  $15.4 \pm 3.175$



and  $17.19 \pm 4.953$  mg/mL respectively  $n=10$ .  $p < 0.05$  (Fig. 1).



**Figure 1.** EMF-related ghrelin concentration changes on start and after 20 days from start learning animals. In serum, taken from fasting blood we measured ghrelin concentration. In EMF exposure group learning start day was 10<sup>th</sup> day after EMF exposure and after 20 day from this date (30<sup>th</sup> day under EMF-exposure) we repeat the research



**Figure 2.** EMF effect on memory encoding. In EMF exposure group learning training starter after 10<sup>th</sup> day of exposure. The number of trials (sessions) to generate a conditioned -reflex reaction (learning) is given according to a) 5-CSRTT b) Two-feeder test

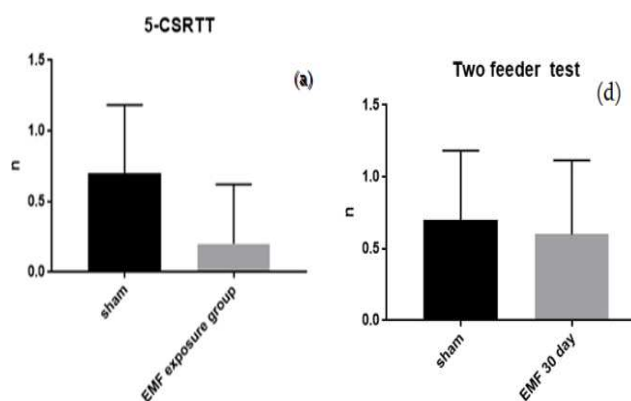
### Effect of EMF on learning processes and memory encoding

To evaluate the effect of EMF on memory encoding we analyzed the learning processes, in particular, the number of trials (sessions) to perform 80% of tasks correctly in the sense of formation conditioned reflex. In **5-CSRTT** task-performing learning sham-control group, rats needed fewer trials than in EMF-expose group rats (Fig. 2-a, in sham control and EMF exposure groups, the values were  $92.9 \pm 2.084$  and  $101.8 \pm 2.764$ , respectively.  $n=10$ ;  $p < 0.05$ ). In EMF-exposure group rats, we started to study learning sessions after the 10<sup>th</sup> day of EMF exposure. In Two Feeder test too, for learning task performance sham control group rats were needing fewer sessions than EMF-expose group rats (Fig. 2-b, in sham control and EMF

exposure groups, the values were  $82.7 \pm 2.989$  and  $99.1 \pm 3.903$ , respectively.  $n=10$ ;  $p < 0.05$ ).

### Effect of EMF on conditioned reflex memory tracks consolidation

On the 20<sup>th</sup> day of the study, we checked the number of correctly performed tasks with 5-CSRTT and Two Feeder test, researching memory formation and retention. In the survey we received the significant difference results in sham control and EMF exposure groups for performing the 5-CSRTT correctly (number of correct responses after the trigger action) (Fig. 3-a, in sham control and EMF exposure groups, the values were  $0.7 \pm 0.1528$  and  $0.2 \pm 0.1333$ , respectively.  $n=10$ ;  $p < 0.05$ ), however, credible difference was not received in the results of two feed test (fig. 3-b: sham control and EMF exposure group Mean  $0.7 \pm 0.483$  and  $0.5 \pm 0.527$  respectively,  $P=0.6601$ ).

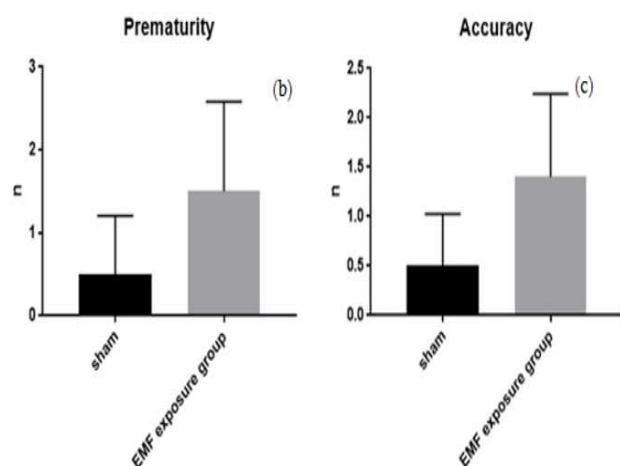


**Figure 3.** Memory retention after 20<sup>th</sup> day start learning (in EMF exposure group after 30<sup>th</sup> day under EMF). a) In 5-CSRTT we calculated number of correct responses (memory recall). EMF exposure group rat make more mistakes and cannot perform task correctly, than sham control group rats. b) Number of correct responses (memory recall) in Two-feeder test

While performing the 5-CSRTT, the behavior of animals was different, in particular in EMF-exposure group rats, it increased prematurity (the number of activities before the trigger action) and its performance was less accurate (the number of activities after the trigger action) than in sham control group rats (Fig. 4-a, prematurity activities in sham control and EMF exposure groups, the values were  $0.5 \pm 0.2236$  and  $1.5 \pm 0.3416$ , respectively.  $n=10$ ;  $p < 0.05$  and Fig. 4-b, accuracy in sham control and EMF exposure groups, the values were  $0.5 \pm 0.1667$  and  $1.4 \pm 0.2667$ , respectively.  $n=10$ ;  $p < 0.05$ ), which means that attention and concentration are reduced in EMF exposure group than sham control group.







**Figure 4.** Attention and concentration after 20th day start learning (in EMF exposure group after 30th day under EMF) in 5-CSRTT we calculated: a) prematurity - the number of activities (nose-poke in holes and in feeder) before the trigger action, b) accuracy - the number of activities (go to feeder, turning around, standing, touch nose on the lamp) after the trigger action

## Discussion

In this study, we described a novel approach, which can be one of the reasons for the memory loss under the action of a household frequency electromagnetic field. Considering the results, it seems that under EMF action conditioned-reflex memory encoding, memory tracks consolidation and recall occurs with delay. According to our study to obtain learning criterion (generation conditioned-reflex reaction) after the 10<sup>th</sup> day of EMF action EMF exposure group needed more trials than the sham control group.

As for memory-related neurogenesis and ghrelin concentration changes, in our study ghrelin concentration was significantly high after EMF action in EMF expose group, concentration increased much more after 30<sup>th</sup> day than the 10<sup>th</sup> day on EMF action; but as for neurogenesis, there was not a case of memory improvement. Meanwhile, ghrelin plays an important role in neurogenesis via proliferation and in the differentiation of neuronal progenitor cells of certain brain structures (hypothalamus, hippocampus-amygdala) which participate in memory processes (Bruehl-Jungerman *et al.*, 2007).

Conducted studies show that newly formed neurons are involved in brain system integrations before maturity and for a short period of time they get involved in memory coding processes. Ghrelin participates in memory consolidation (Gherzi *et al.*, 2015), hippocampus receptor expression, its artificial introduction leads to the strengthening of learning and improving memory processes; as well

as peripheral infusions of ghrelin, improve learning and memory processes, increase the viability of cells, reduce the death of cells caused by ischemia (Müller *et al.*, 2015). The injection of ghrelin in intracerebroventricular and/or intrahippocampal area increases memory retention in rodents (Carlini *et al.*, 2010). Studies conducted on rats shows that hippocampus participates in creating memory-stimulating responses when the action of rewarding or punishing occurs (Gray and McNaughton, 2000), ex. Light/dark box tests in rats; during which an animal make a decision for potentially dangerous or pleasant results (Adhikari *et al.*, 2010). In our study, despite ghrelin's high concentration under EMF action, learning (encoding) or recall of memory is impaired.

The possible reduction of the pyramidal neuron may be one of the reasons for the learning difficulties because it is known that the effect of the mobile phone's magnetic field on rats reduces the pyramidal neuron (involved in memory processes) up to 50%. (**Error! Reference source not found.**) Based on the results obtained from the in vitro experiments, the scientists found that the ghrelin stimulates adrenocorticotrophic hormone (ACTH) and corticosteroids secretion. (**Error! Reference source not found.**) The secretion and cortisol prevent the recall of episodic memories (Ackermann *et al.*, 2013), whose regulatory centers are located in the hippocamp (Templer VL, Hampton RR. 2013), which is responsible for the creation of new episodic memories using the identification of information about events, stimulus and places and after their recall.

After training, when we checked the results of conditioned reflex recall on the 20<sup>th</sup> day of starting learning, in 5-CSRTT we received a significant difference between sham control and EMF exposure group rats. In this test the most rats from EMF exposure group did not perform task correctly; in deeper analyze we found that on 30<sup>th</sup> day EMF action rats reacted to a neutral stimulus (light), but accuracy to perform the task was different than in the group outside of EMF attention and concentration was more reduced, failure of inhibitory control and increased number of premature responses involve increased motor impulsivity, which gives the picture of the memory recall impairment.

The reason for this may be EMF's neurostimulating effect. Studies have shown that the EMF can act as a stressor. It changes excitation and inhibition amino acid ratio in the brain, the latter

causes the impairment of passive avoidance reactions in development and decision-making processes in rats, increases the animals' anxiety, neuroticism, and hesitation (Zurabashvili *et al.*, 2010). The 950 MHz mobile phones have caused a significant increase of stress hormone cortisol in female hamsters (Seyednour and Chekaniazar, 2011), which also obstructs episodic memory recall (Ackermann *et al.*, 2013).

In our study rats under EMF became unpredictable, they acted at the reflex-level, which also gave a picture of memory loss, causing difficulties in coding and recall processes. This appears to be the reason why the attention and concentration were decreasing. The role and action mechanism of EMF requires more enhanced surveys, which is our future work plan.

### Conclusion

Our study shows that EMF affect the learning as well as the memory recall process, but the scientific cause of it is much deeper and advanced. Our research gave a clinical picture of a memory loss - mainly related to decreased attention and concentration. The latter was also confirmed by the increase in the concentration of hormone ghrelin (participating in memory process), under influence of household frequency EMF.

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