

Short-term memory in mice is affected by mobile phone radiation

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Abstract

The effects of mobile phone electromagnetic fields (EMFs) were studied on a non-spatial memory task (Object Recognition Task – ORT) that requires entorhinal cortex function. The task was applied to three groups of mice *Mus musculus* C57BL/6 (exposed, sham-exposed and control) combined with 3 different radiation exposure protocols. In the first protocol designated “acute exposure”, mice 45 days old (PND45 – postnatal day 45) were exposed to mobile phone (MP) radiation (SAR value 0.22 W/kg) during the habituation, the training and the test sessions of the ORT, but not during the 10 min inter-trial interval (ITI) where consolidation of stored object information takes place. On the second protocol designated “chronic exposure-I”, the same mice were exposed for 17 days for 90 min/per day starting at PND55 to the same MP radiation. ORT recognition memory was performed at PND72 with radiation present only during the ITI phase. In the third protocol designated “chronic exposure-II”, mice continued to be exposed daily under the same conditions up to PND86 having received radiation for 31 days. One day later the ORT test was performed without irradiation present in any of the sessions. The ORT-derived discrimination indices in all three exposure protocols revealed a major effect on the “chronic exposure-I” suggesting a possible severe interaction of EMF with the consolidation phase of recognition memory processes. This may imply that the primary EMF target may be the information transfer pathway connecting the entorhinal–parahippocampal regions which participate in the ORT memory task.

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1. Introduction

Due to the worldwide concern on the possible health hazards induced by EMFs (electromagnetic fields), current research is extended using a variety of approaches: epidemiological, clinical, experimental exposure on laboratory animals and on cell lines or even on individual biomolecules (preferably enzymes).

A considerable amount of research has been focused on the behavioral impairments in rodents and in particular memory and learning deficits, following exposure to EMFs using a variety of approaches. These include spatial memory

paradigms such as the Morris Water Maze (MWM) in rats [1] and in mice [2], the Radial-arm Maze (RAM) [3] and recognition memory test i.e. the Object Recognition Task (ORT) [4]. Two of the earliest studies on this issue have shown, using the RAM and the MWM tasks, a reduced performance by rats following EMF 2450 MHz exposure [5,6]. That observation was however not confirmed by later similar studies, which examined the possibility of changes in “working” memory of rats being whole body exposed to microwave (MW) radiation for 10 days at 0.6 W/kg SAR level, 2450 MHz, 45 min/day. The investigators found no effects on spatial working memory as detected by Radial-arm Maze [7,8]. In addition recognition memory by ORT performance was tested using head-only exposed rats at 900 MHz GSM (1 and 3.5 W/kg SAR values) [9]. No effects were found as well at low SAR level but only some effects on exploratory activity at a high SAR level (3.5 W/kg). In these studies a radiofrequency genera-

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tor was used, instead of a mobile phone, pulsed at 217 Hz (a modulation used in GSM protocols).

There is only one report on mice so far that has studied the effect of repeated, acute exposure in strain C57BL/6J to a low intensity 900 MHz radiofrequency (RF) field (SAR of 0.05 W/kg) pulsed at 217 Hz using an appetitive-motivated spatial learning and working memory task [10]. Adult males were exposed under far field conditions to a GTEM (Giga-hertz Transverse Electromagnetic) cell for 45 min daily for 10 days. No effects were found something that can be attributed to the very low level of radiation exposure used. However, recent work of our research group has revealed spatial memory deficits in mice, using the Morris Water Maze task after just 4 days of exposure to a conventional mobile phone [2].

In addition, results derived from episodic-like memory task, suggested that cognitive memory functions in rats after GSM microwave exposure at different very low SAR levels (0.6 and 60 mW/kg) were significantly disturbed [4].

Despite numerous studies, there is no definitive conclusion up to now that high-frequency EMF exposure is a risk to memory function. On the contrary, just recently the first evidence has been reported that exposure directly associated with cell phone use (GSM 918 MHz, SAR value, 0.25 W/kg) provides cognitive benefits i.e. improvement in transgenic Alzheimer's mice performance after long term (8 months) EMF exposure [11].

So far there have been no reliable reports on the effect of real GSM signals deriving from conventional mobile phone on the recognition memory in mice. Also, nearly all studies have used signal generators as a source of radiation exposure. In addition it is the first time that radiation exposure is administrated during the various phases of the information transfer pathways following a visual stimulus along the recognition memory function. Therefore our objective was to find out whether real mobile phone radiation affects the object recognition memory in mice *Mus musculus* C57BL/6. To approach this goal we applied the hippocampus-independent Object Recognition Task (ORT) which is used to assess this type of memory through the dissection of the acquisition, the consolidation and the retrieval phases [12].

2. Materials and methods

2.1. Animals

Male *M. musculus* C57BL/6 mice were obtained from the Biomedical Research Foundation of the Academy of Athens. They were left for 2 weeks to be acclimatized in the environment of our animal facilities before the initiation of experiments. The mice were 45 days old (PND45 – post-natal day 45) at the beginning of the experiment (Fig. 1) and they weighted approximately 20 g. Their weight was measured once a week. Animals were group housed (four animals per cage) in Techniplast, USA Plexiglas cages, 1264C Euro-standard Type II, 267 mm × 207 mm × 140 mm – floor area

370 cm², under standard laboratory conditions at 22 ± 1 °C room temperature, 40–60% relative humidity and light cycle 12 h:12 h light/dark. Food (standard rodent chow) and water were available *ad libidum*. Enrichment nesting material was used within their home cages i.e. soft paper and red plastic houses [13]. The bedding used was autoclaved, non allergenic, no dust, and NH₃ absorbent.

All experimental procedures were conducted out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and were approved by the Animal Research Ethics Committee of the Biology Faculty of the University of Athens. All efforts were made to fulfil the 3Rs principle (Replacement, Reduction and Refinement) during the preparation and the implementation of the experimental protocol which was approved by the vet in charge of our animal facilities.

2.2. Exposure conditions

Mice were divided into three groups (8 animals per group): the first group consisted of the exposed mice to electromagnetic fields, the second group consisted of the sham-exposed mice and the third group consisted of the control mice. An a priori power analysis was performed to estimate the sample size which demonstrated that 8 animals per group, $\alpha = 0.05$, effective size 0.54 (based on a pilot study), would yield a statistical power of more than 0.80. The power analysis was repeated a posteriori with this study's means and standard deviations and the power was at least 0.75. Exposed mice cages were placed within specially constructed Faraday cages half-opened in order to allow mobile phone operation and also to prevent radiation leakage towards the similar sham-exposed mice cages. Control mice were maintained in a separate room with all other conditions being the same besides radiation and sound (radio). Radiation exposure was performed with a conventional mobile phone operating at GSM 1800 MHz placed underneath the home cage, as previously described by our group for 90 min/day (see Fig. 1) [2,14].

In order to simulate the conditions of human voice during mobile phone use, a radio station was playing at a sound level of 70 db, throughout the exposure period, as a source of auditory stimulation.

2.3. EMF measurements – dosimetry

Careful dosimetry was performed by measuring the mean electrical field density averaged over 6 min according to ICNIRP's standards [15] with the NARDA SRM 3000 (Narda Safety Test Solutions, Inc., Germany). An average value of 17 V/m was recorded. The specific absorption rate (SAR) for the brain tissue of the exposed mice can thus be approximately calculated according to the equation:

$$\text{SAR} = \frac{\sigma E^2}{\rho}$$

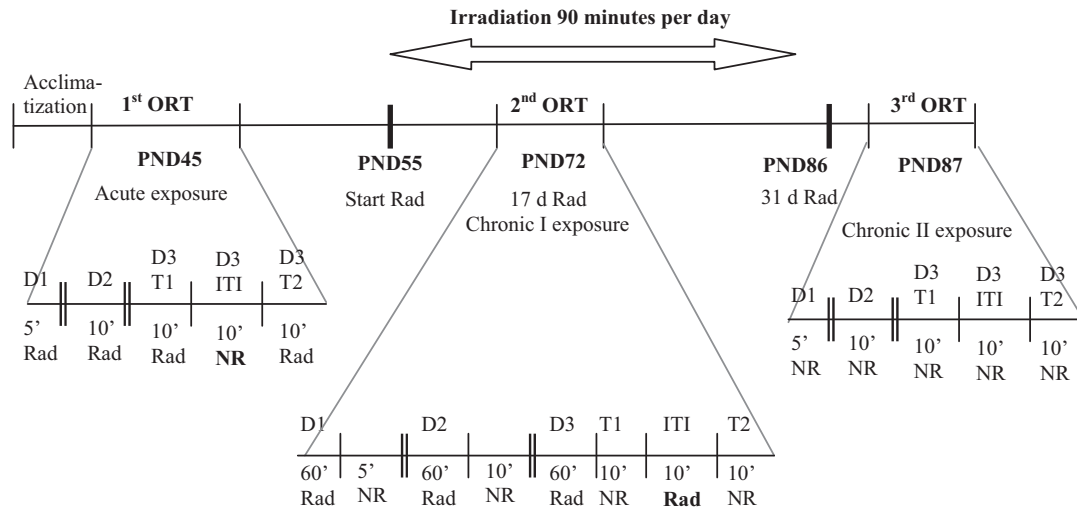


Fig. 1. Timetable of the EMF exposure – ORT combinations showing details about the acute, chronic I and chronic II exposure protocols. The ORT sessions are designated as follows: D1 (day one)=habituation session, D2 (day two)=training session, D3 (day three)=test session, T1 (Test1)=acquisition phase, ITI (inter-trial interval)=consolidation phase, T2 (Test2)=retrieval phase, Rad=exposure to mobile phone radiation, NR=no radiation exposure, PND=post natal day. Radiation started on 55 days old animals (PND55) and finished on 86 days old animals (PND86).

where E is the value of the electrical field measured within the cages in V/m, σ is the mean electrical conductivity of the tissues in S/m and ρ is the mass density in kg/m^3 [15,16].

The dielectric properties of the mouse brain were estimated according to the published parameters [17,18], $\sigma=0.8$ S/m (average brain value) and mass density $\rho=1040$ kg/m^3 .

Therefore the created SAR level within the each cage by the mobile phone was $\text{SAR}=0.8(17)^2/1040=0.22$ W/kg which is below the value considered by ICNIRP as a safety level for human brain tissue exposure [15].

The irradiation procedure was conducted in specially designed installations of the Electromagnetic Biology Lab of the Department of Cell Biology and Biophysics at the Faculty of Biology in the University of Athens.

All experimental manipulations, both irradiation and ORT protocols, were performed during the light cycle, between 10:00 am and 16:00 pm (lights on at 7 am) in a specially designed sound proof, air-conditioned room. The experimenter was the only person handling the animals daily for food/water supply, for weekly cleaning and for performing ORT experiments throughout the experimental period in order to avoid stress induction.

2.4. Object Recognition Task

2.4.1. The apparatus

The ORT apparatus consisted of an open box made of Plexiglas (40 cm long \times 60 cm high \times 40 cm wide), illuminated by diffuse white light at 32 lux uniformly on the arena surface. Before each trial the arena was covered by a thin layer of bedding material, portion of this was taken from the corresponding animal cage so that the olfactory cues of the environment were familiar. The arena was thoroughly cleaned

after each trial with H_2O_2 which is odourless. The bedding was renewed when animals from another cage were to be used for the ORT trials.

The objects to be presented were in four different shapes and colors: cubes, pyramids and two different in texture cylinders 5 cm high, which could not be displaced by mice. The cubes were made of black metal, the pyramids were made of yellow plastic and the cylinders were made of silver metal and transparent glass with white cap. In addition, these objects had no genuine significance for mice and had never been associated with reinforcement [19]. Every possible combination and location of the objects on the arena was used in a balanced manner to reduce potential biases due to preferences for particular locations or objects. To avoid the presence of olfactory trails the apparatus and the objects were thoroughly cleaned as described above.

2.4.2. Object Recognition Task procedure

Each Object Recognition Task lasted for three days (Fig. 1) and was performed as has been described before [19,20].

- *Day one, habituation session*: mice were placed in the box and were allowed to freely explore the apparatus for 5 min each animal.
- *Day two, training session (acquisition phase)*: mice were allowed to explore the apparatus and two identical objects (e.g., two plastic pyramids) which were placed in two opposite corners of the apparatus 10 cm from the sidewall. Each mouse was placed in the middle of the apparatus and was left to explore the two identical objects.
- *Day three, test session*: it consisted of two trials and an inter-trial interval.
 - A single 10 min acquisition phase (Test1 – T1 trial) was given. During T1, two identical objects (F, F') which were

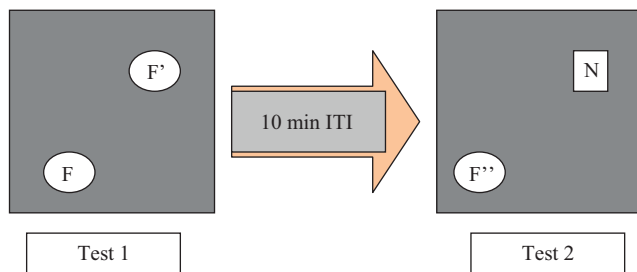


Fig. 2. Schematic representation of the Test1 and Test2 trials showing the location of the identical familiar ($F - F' - F''$) and novel (N) objects (see text for details). Test1 = acquisition phase, ITI (intertrial interval) = consolidation phase, Test2 = retrieval phase.

also presented on the previous day (day two) during the acquisition trial (e.g. two plastic pyramids) were placed in two opposite corners of the apparatus 10 cm from the sidewall (Fig. 2). Each mouse was placed in the middle of the apparatus and was left to explore the two identical objects.

- After T1, the mice were put back in their home cage for an inter-trial interval (ITI) of 10 min. This interval is considered as the memory consolidation phase.
- After ITI, a single 10 min retrieval phase (Test2 – T2 trial) was performed. During T2, a new object (N) different from the familiar objects both in texture and shape (i.e. a black metallic cube) replaced one of the identical objects (F, F') presented in T1 trial (i.e. one plastic pyramid); hence, the mice were re-exposed to two objects: a copy of the familiar (F'') and the new object (N) (Fig. 2).

At the end of each trial mice were gently put back in their home cages.

Since behavior of lab animals within the ORT box can be perturbed no other person except the experimenter was allowed to be entering within the room where the ORT experiment was taking place. In addition, behavior of animals was video-recorded via a web-camera, USB-connected to a computer in a next room. At the end of the appropriate time period (5 min or 10 min according to the protocol) the experimenter entered the ORT room, removing gently and quietly the animal back to the cage and placing the next mouse before leaving the room.

Exploration was defined as follows: directing the nose toward the object at a distance of no more than 2 cm and/or touching the object with the nose. Moving around the apparatus or sitting on the object was not considered as exploratory behavior. The time spent by mice in exploring the familiar (F'') and the novel (N) object during T2 was recorded separately using digital stopwatches and the difference between the two exploration times was calculated [21]. Thus the discrimination between F'' and N during T2 trial was defined by comparing the time spent in exploring the F'' object with that spent in exploring the N object. As this time may be biased by differences in overall levels of exploration, a discrimination index (DI) was then calculated, $DI = (N - F'') / (N + F'')$. This

index is a discrimination ratio and represents the difference in exploration time expressed as a proportion of the total time spent exploring the two objects in T2 [19,22].

2.4.3. EMF exposure – ORT combinations

The first exposure protocol (acute exposure) was performed as follows (Fig. 1):

Mice, 45 days old (PND45 – postnatal day 45), were put in the ORT apparatus on day one of the task having a mobile phone in operation under the box during the habituation session. Similar conditions were applied on day two in the presence of the two identical objects during the acquisition session with concomitant irradiation. On day 3 of the ORT, irradiation was present during T1 and T2 trial, but not during the consolidation phases (ITI).

The second exposure protocol (chronic exposure-I) was performed as follows (Fig. 1):

The same mice were exposed to the mobile phone radiation for 17 days at 90 min/day starting at PND55 and the ORT recognition memory task was performed at PND72 during the 90 min exposure. After the first 60 min of exposure to EMFs each animal was transferred into the ORT apparatus without irradiation, on day one (habituation session), on day two (training session) and on day three (T1 and T2 trial). Between T1 and T2 trial (ITI) of day 3, mice were returned to their home cages, where they received exposure by the mobile phone. At the end of each trial mice continued to be irradiated within their home cages till the end of the 90 min period of total daily exposure. Therefore mice at this ORT procedure were irradiated only during the consolidation phase (see Fig. 1).

The third exposure protocol (chronic exposure-II) was performed as follows (Fig. 1):

After the previous ORT, mice continued to be exposed daily under the same conditions up to PND86 and one day later the ORT was performed without any irradiation present.

2.5. Statistical analyses

All data were analyzed by SPSS v.18.0 software. Differences in mean scores were analyzed using one-way analysis of variance (ANOVA) followed by the LSD posthoc test.

3. Results

3.1. Body weight

Mice were weighted once a week during the irradiation period. All animals gained weight normally (data not shown). No significant difference was noted between the three experimental groups.

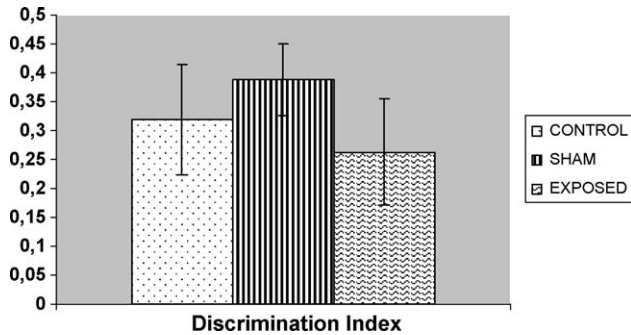


Fig. 3. Acute exposure protocol: bar graph showing the mean discrimination indices (DI) of the first ORT in the three experimental groups. A decrease in DI was seen in the exposed mice in comparison with sham-exposed and control animals, but this difference was not statistically significant. The mice were 45 days old (PND45) at the beginning of the ORT and they had not experienced any prior exposure to EMF.

3.2. Object Recognition Task (ORT)

Video analysis of the exploration times in all ORT experiments led to the calculation of the discrimination indices (DI) as follows.

3.3. Control and sham-exposed animals

The control and the sham-exposed animals in all three exposure protocols showed the anticipated preference for exploration of the novel object as reflected by the high mean discrimination index (DI) (Figs. 3–5) of the ORT experiments.

3.4. Acute exposure protocol

The first ORT applied in the mice on PND45 of the first exposure protocol was performed in the presence of EMF exposure during all ORT sessions apart from the inter-trial interval (ITI) on day three (Fig. 1). By doing so, we intended not to interfere with the consolidation of informa-

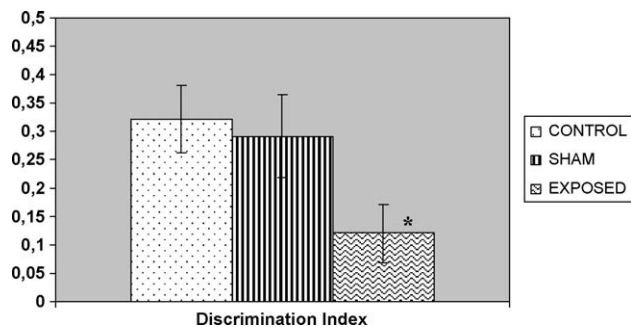


Fig. 4. Chronic I exposure protocol: bar graph showing the mean discrimination indices (DI) of the second ORT in the three experimental groups. After a period of 17 days of 90 min daily irradiation mice 72 days old (PND72) were subjected to the ORT with irradiation only during the ITI phase. The DI of exposed mice was statistically significantly lower ($p < 0.05$) compared to the DI of both the sham-exposed and the control mice.

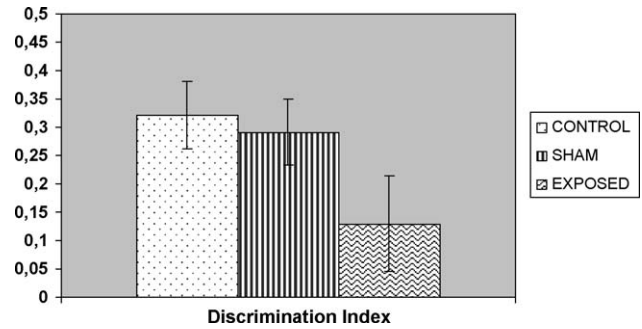


Fig. 5. Chronic II exposure protocol: bar graph showing the mean discrimination indices (DI) of the third ORT in the three experimental groups. Mice, 86 days old (PND86) had been irradiated for 90 min daily for 31 days. ORT sessions were applied 24 h later without any radiation present during all trials. The results revealed a decrease in DI in the exposed mice compared to the sham-exposed and the control mice, which is marginally statistically significant ($p = 0.063$).

tion. Although the exposed mice had a numerically lower mean DI (Fig. 3) compared to the sham-exposed or the control mice, this difference was not statistically significant as revealed by one way ANOVA.

3.5. Chronic-I exposure protocol

The second ORT (17 days EMF exposed group) was performed on the 17th day after daily 90 min-irradiation on PND72. In contrast to the first ORT, exposed-animals were irradiated only during the inter-trial intervals ITI (consolidation phase) of day 3 (Fig. 1).

Video analyses of the familiar vs. the novel object exploration times revealed that the mean DI of the exposed mice was statistically significantly lower than the mean DI of the sham-exposed and the control group mice (one way ANOVA main effect of group: $F_{2,21} = 4.023$, $p = 0.035$; posthoc tests: exposed vs. control $p = 0.015$, exposed vs. sham-exposed $p = 0.05$) (Fig. 4).

3.6. Chronic-II exposure protocol

The third ORT (31 days EMF exposed group) was performed on PND87 without irradiation one day after 31 days of daily 90 min-irradiation (Fig. 1). A lower mean DI was calculated for exposed mice compared to sham exposed and control mice, but this difference was marginally statistically significant ($F_{2,20} = 3.227$, $p = 0.063$) (Fig. 5).

4. Discussion

This study was conducted in order to investigate whether short-term memory is affected by ordinary mobile phone exposure. Achieving this goal we used mice as a model system under three types of irradiation protocols, acute, chronic-I and chronic-II in a sequenced manner combined with Object Recognition Task (ORT). The main finding of this

study concerns the impairment in object recognition memory. The major effect was noticed in chronic-I exposure protocol in which the animals were irradiated exactly before the trials as well as during the 10 min inter-trial interval of the task (consolidation phase). In addition, 24 h after the end of 31 days of daily irradiation and without any exposure during the trials (chronic-II exposure protocol), recognition memory deficit was marginally affected. This means that a partial reversal of the recall impairment in ORT was observed 24 h after the end of irradiation.

The one-trial Object Recognition Task used in this work was initially developed for rats [23] and later on was adapted for mice with only minor modifications [24–26]. This behavioral approach is based on the spontaneous exploration of rodents that spend more time with a novel object compared to a familiar one explored before.

Recognition memory refers to the ability to judge a previously encountered item as familiar and depends on the integrity of the medial temporal lobe [27]. Despite ORT's widespread applications mainly in rats and in some cases in mice, the findings regarding the anatomical structures involved are rather mixed. However it has been shown that perirhinal cortex along with the entorhinal cortex is involved in both object and spatial encoding [28–30]. In addition, protein synthesis in the entorhinal cortex seems to be necessary early after training for the consolidation of object recognition memory [31].

It is also well documented that ORT performance can be disturbed by a variety of hippocampal–entorhinal lesions as well as phenotypes of transgenic mice created for certain defective genes. For instance, TAG-1 deficient mice exhibit ORT related learning and memory impairment [22]. It is therefore possible to suggest that EMF in our experiments has disturbed the normal entorhinal–hippocampal functions.

Our data on the induced impairment of the mice to pass successfully the Object Recognition Task may indicate a malfunction of the entorhinal cortex possibly due to disturbance of ion channels particularly of Ca^{2+} as also suggested by the EMF effect on calcium binding protein [32,33].

It is hard to tell which molecule or structure within the related tissues is being affected by EMFs. It is highly possible, based on our knowledge so far that EMFs are a multi target entity capable of producing very diverse and unpredictable molecular alterations including as reported, ROS (reactive oxygen species) formation and oxidative stress induction [34]. These in turn may produce a cascade of short term or long term cellular defects. For instance exposure of rats to 900 MHz radiation 1 h/day for 28 days resulted in an almost complete loss of pyramidal cells in the CA1 hippocampal area [35].

To this point we need to emphasize the importance as well as the uniqueness of the EMF external factor since it can be ON and OFF at will, unlike drugs which are administered and there is no way to stop their presence unless they are removed by the physiological clearing processes. In this respect, exposure to RF fields may affect a specific function depending on

the timing of EMF “administration” in relation to the task applied and therefore reversible effects may be anticipated. The limitations of the study are focused on the fact we are dealing with a whole body exposure with real mobile phone conditions, difficult to replicate accurately. Nevertheless, this does not reduce the validity of the data.

It seems that the acute exposure did not affect mice memory as shown by the non-significant difference on the DI between the exposed animals and the two other groups (sham-exposed and control). On the contrary the chronic-I exposure had an impact on the recognition memory in a statistically significant manner. It seems that the critical phase is the consolidation of information. Moreover, the overall radiation effect on mice memory may be reversible (within the framework of our experimental setup) as shown by the not significant difference of the DI ($p > 0.05$), observed in the chronic-II exposure protocol where mice were left unexposed for one day and also during the ORT sessions and trials.

In translating our data into possible effects in humans, there is support by the finding that memory deficits occur following EMF exposure on volunteers during clinical studies [36].

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