Heliyon 11 (2025) e41626



Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article



Compound exposure of 2.8 GHz and 9.3 GHz microwave causes learning and memory impairment in rats

Liu Sun [b,1], Xiaoya Wang [a,b,1], Ke Ren [b], Chuanfu Yao [b], Haoyu Wang [b], Xinping Xu [b], Hui Wang [b], Ji Dong [b], Jing Zhang [b], Binwei Yao [b], Xiaohui Wei [a], Ruiyun Peng [b,**], Li Zhao [a,b,*]

[a] *Anhui Medical University School of Basic Medicine, Hefei, 230032, China*
[b] *Beijing Institute of Radiation Medicine, Beijing, 100850, China*

ARTICLE INFO

Keywords:
Compound exposure
Microwave
Learning
Memory function
Hippocampus
Rats

ABSTRACT

The anxieties and concerns about health hazards caused by microwave has been growing recently. Previous studies have reported microwave induced structural and functional injuries to brain. However, the biological effects caused by compound microwave were largely unexplored. In this study, we investigated the biological effects on rat's hippocampus after sequentially exposure to 2.8 GHz and 9.3 GHz at average power density of 10 mW/cm^2. Morris water maze (MWM) navigation tests showed that microwave exposure significantly extended the average escape latency (AEL) at 1d and 3d after exposure, suggesting reduced learning and memory ability. Importantly, compound microwave produced strongest responses at 3 d after exposure. Moreover, microwave also could induce abnormal electroencephalogram (EEG), such as increasing the power of θ wave and δ wave, suggesting brain damage or dysfunction. Histopathological analysis suggested that microwave resulted in obvious structural injuries in hippocampus at 7 d after exposure, and most serious injuries were observed in compound microwave exposed rats. Moreover, disorder of mitochondria and reduced Nissl bodies in hippocampus might contribute to the decrease of cognitive function. However, both the cognitive function and hippocampal structure restored to normal at 28 d after exposure, which might be attributed to self-recovery mechanisms. Gene ontology (GO) and Protein-protein interaction (PPI) analyses of differential expressed genes (DEGs) in peripheral blood suggested that Htt and Bdnf might be potential indicators to predict microwave. In conclusion, compound microwave of 2.8 GHz and 9.3 GHz could elicit reversible structural injuries to hippocampus, which could decrease the cognitive function of rats.

1. Introduction

Microwave is a kind of electromagnetic wave with frequency ranging from 300 MHz to 300 GHz, and wavelength between 1 m and 1 mm [1]. With the application of microwave in communication, transportation, medicine, military and industry, the biological effects

* Corresponding author. Anhui Medical University School of Basic Medicine, Hefei, 230032, China.
** Corresponding author.
E-mail addresses: ruiyunpeng18@126.com (R. Peng), lillyliz@163.com (L. Zhao).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.heliyon.2025.e41626
Received 20 August 2024; Received in revised form 27 December 2024; Accepted 1 January 2025
Available online 3 January 2025
2405-8440/© 2025 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

of microwave have been attracting more and more attentions. Numerous studies have demonstrated that microwave can damage multiple tissues and organs [2,3]. Compound exposure to microwave frequently occurred in our daily life. It has been demonstrated that compound exposure to multi-frequencies microwave always produces much more serious injuries than single frequency microwave [4–6].

Brain is one of the most sensitive organs to microwave radiation, and the learning and memory ability is susceptible to be injured [7–14]. Epidemiological studies have found that compound microwave exposure can impair the learning and memory ability, as well as affect cognition and emotion [15,16]. The alteration of neurotransmitters metabolism and energy metabolism might contribute to the impairment of learning and memory ability after microwave exposure. Nissl bodies, which can produce proteins and enzymes for supporting generation of organelles and synthesis of neurotransmitters, is an sensitive indicator for neuronal activity [17]. Moreover, mitochondria are the critical organelles for energy metabolism. Studies have reported that the structure and functions of mitochondria could be injured by microwaves with different frequencies [4,6,18].Microwave with 2.8 GHz and 9.3 GHz frequencies have been widely used, but the effects of their combined exposure on cognition have not been reported. In this study, we investigate the effects of 2.8 GHz and 9.3 GHz on cognition of rats. The structural damage of hippocampal tissue, brain electrical activity, and metabolic activity were detected to analyze microwave caused injuries of rats' learning and memory abilities. Studies have shown that microwave radiation can cause electrons or ions to vibrate through resonance, which in turn collides with other molecules in biological tissues, resulting in interactions with biological systems and causing a series of changes [19]. To explore the potential mechanism underlying microwave induced injuries on cognition, the alterations of gene expression or protein conformation were analyzed, which might in turn alter signaling pathways, ultimately resulting in structural damage and cognitive impairments in rats.

2. Materials and methods

2.1. Microwave exposure mode

The microwave exposure system was described previously [44,45]. Briefly, microwave energy is transmitted to an electromagnetic shield chamber, through rectangular waveguide and A16 dB standard gain horn antenna. The transmit power is measured with a semiconductor detector, which is connected to a directional coupler at one port of the circulator and displayed on an oscilloscope. Waveguide antenna, GX12M1CHP power meter (China Hefei Guanghua Microelectronics Instrument) and GX12M30A power head

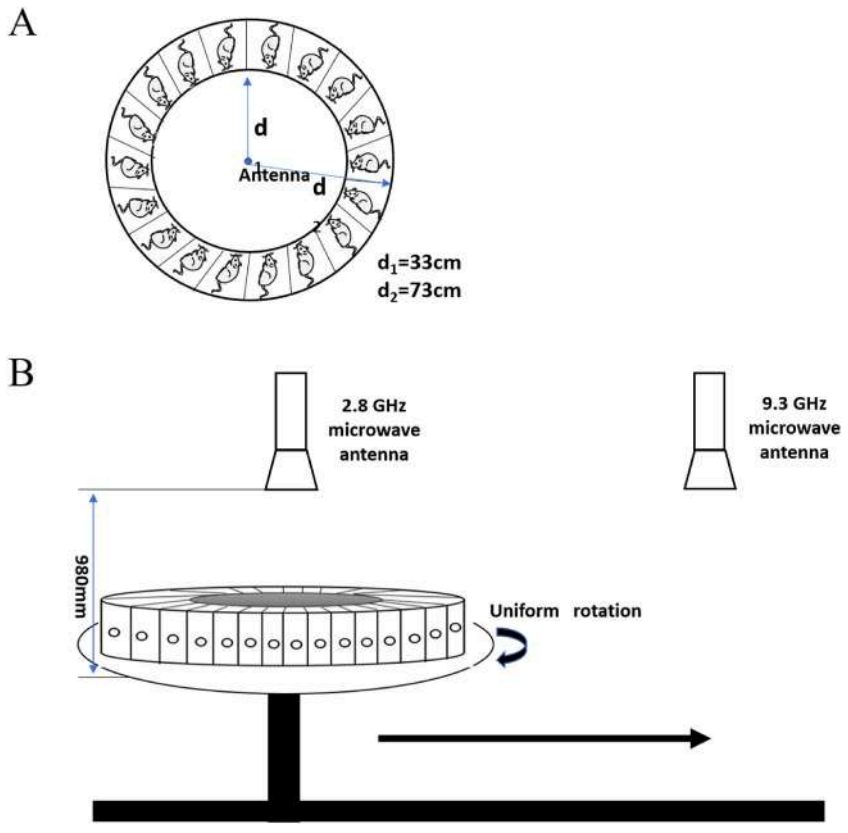


Fig. 1. Schematic diagram of the device for microwave exposure. A: Top view of microwave radiation device; B: Front view of microwave radiation device.

were used to measure the average power density. The schematic diagram of microwave exposure experimental device is shown in Fig. 1.

2.2. Animals and microwave exposure

A total of 100 male Wistar rats were randomly divided into four groups according to microwave exposure power density and exposure mode: Sham group, 2.8 GHz microwave exposed group (S10 group), 9.3 GHz microwave exposed group (X10 group), 2.8 GHz and 9.3 GHz compound microwave exposed group (XS10 group) (n = 25/group). Rats in S10 group and X10 groups were exposed to 2.8 GHz and 9.3 GHz microwave for 6 min, and then were kept in the cage for another 6 min with the microwave cut down. While the compound group was sequentially exposed to 2.8 GHz and 9.3 GHz microwave for 6 min. And, the Sham group was treated as that in microwave exposed groups, but without exposure.

The animal experiments were approved by Institutional Animal Care and Use Committee at Beijing Institute of Radiation Medicine (IACUC-DWZX-2020-781), and all the procedures were carried out in accordance with National Institute of Health Guide for the Care and Use of Laboratory Animals. Rats were intraperitoneally injected with 1 % pentobarbital sodium (0.5g/100g, IP). Here, we injected pentobarbital to anesthetize the rats in order to keep them stationary and make subsequent experiments more convenient.

2.3. Morris water maze (MWM)

2.3.1. Instrument setting

The Morris Water Maze testing system was sourced from Beijing Shuolinyuan Technology Company. And the water maze pool was divided into four quadrants, with the platform placed in the center of the first quadrant, 1–2 cm beneath the water surface. The camera was located directly above the pool and was covered with a light colored cloth curtain to ensure soft and uniform lighting. The surrounding scenery and furnishings remained unchanged and the environment remained quiet.

2.3.2. Water maze training

All rats were trained in water maze for 3 connective days before microwave exposure. The rats were placed into the water from four quadrants facing the wall of the pool at a fixed time point once a day, and the time was counted at the same time. If a rat found the platform within 60 s, the timer was stopped and the rat was allowed to stay on the platform for 15 s. If a rat did not find the platform within 60 s, it was guided to the platform after 60 s and allowed to stay there for 15 s. The average escape latency (AEL) of each rat in the water maze was obtained by averaging the time of finding the platform in the four quadrants.

2.3.3. Positioning cruise test

Positioning cruise test was performed on rats, at 6 h, 1 d, 2 d, 7 d, 14 d and 28 d after exposure. The rats were placed in water from four quadrants facing the wall of the pool and timed. If the rats find the platform within 60 s, the time was recorded. If the rats do not find the platform within 60 s, the time was recorded as 60 s. The average time of finding the platform for each term of rat was calculated, which was named AEL.

2.4. Electroencephalogram (EEG)

EEG recordings were collected from rats at 6 h, 7 d, 14 d, and 28 d after microwave exposure. Five rats were selected from each group and injected intraperitoneally with 0.5 ml/100 g of 1 % pentobarbital sodium. When the rats were in a state of mild anesthesia (with pain reflexes), a multi-channel physiological recorder (BIOPAC, USA) was used to detect EEG. Firstly, the hair on the top of the head was removed using a hair clipper. Then, the area was disinfected and degreased. An electrode was placed on both sides of the center of the head and fixed to the scalp with a needle electrode. The reference electrode was inserted at the edge of the ipsilateral earlobe. The electrodes were connected to an EEG amplifier and the EEG waveforms were classified into Alpha (α), Beta (β), Theta (θ), and Delta (δ). The sensitivity was set to 2000 Hz, and the EEG changes were continuously recorded. Finally, the power of α, β, θ, and δ brain waves was statistically analyzed.

2.5. Histopathological analysis

The rats in each group were euthanized at 6 h, 7 d, 14 d and 28 d after exposure (n = 6/group), and the brain was removed. The hippocampus in the right hemisphere of brain was fixed in 10 % formalin solution. Three weeks after fixation, it was taken out of formalin solution, cut into appropriate size and put into an embedding box. The cassette was rinsed with running water overnight and placed in an automatic dehydrator (Leica, Germany). Then the tissue was subjected to dehydration, transparency and wax immersion according to a preset program. Following wax immersion, paraffin embedding was performed and 3 μm sections were prepared. The sections were laid flat on glass slides and dried overnight in a 60 °C incubator. Subsequently, dewaxing was done using xylene and gradient ethanol, followed by staining with hematoxylin and eosin (HE). Next, we used gradient ethanol and xylene for dehydration and transparency. Finally, the sections were sealed. The morphological features and staining of hippocampal tissues of six rats in each group at different time points were observed under a light microscope (Leica, Germany) and 5 random visions were selected from each slide for photography. Moreover, the sections of hippocampal tissue in each group were stained with toluidine blue (ZSGB-BIO, China) for 10 min, and then de-stained in 95 % alcohol for 3 s. Following dehydration in gradient alcohol, xylene clearance and coverslipping,

the Nissl bodies were observed under a microscope. Five visual fields were randomly selected and photographed. The image Pro Plus software was used to detect the mean optical density (MOD) and integral optical density (IOD) of the positive areas of each visual field.

2.6. Ultrastructural observation of hippocampus

After the rats were euthanized at 7d after exposure (n = 6/group), the hippocampal tissue of left hemisphere was removed and fixed overnight in 2.5 % glutaraldehyde phosphate buffer fixative solution. After washing with 0.1 mol/L phosphate buffer solution, the tissue was fixed with 1 % osmium acid and washed with double steaming water. The fixed tissue was dehydrated by gradient ethanol, soaked and embedded with acetone and embedding solution, and then were cut into semi-thin sections. And, the ultra-thin sections were made after located by light microscope. The ultra-thin slices were stained with uranium acetate and lead nitrate. And, the ultrastructure was observed by transmission electron microscope (TEM, Hitachi, Japan). We observed the ultrastructure of mitochondria and synapses in neurons in hippocampal tissue of each group of rats after microwave radiation, and at least seven images were taken for each group. Typical structures of mitochondria, mitochondrial cristae, synaptic gap and postsynaptic dense material were found in each picture, and quantitative analysis was performed using Image J. At least 20 data were quantified for each index in each group.

2.7. Transcriptomic analysis

The peripheral blood was collected at 7 d after exposure, and transcriptomic analysis was carried out [20]. Firstly, total RNA was extracted by Trizol (Invitrogen, CA, USA). The blood was mixed with chloroform upside down and left on ice for 15 min, then centrifuged for 15 min at 12000 rpm/min at 4 °C, carefully taking the supernatant and placing it in a new EP tube. It was added isopropyl alcohol to the supernatant and gently mixing. Next, the supernatant was centrifuged for 10 min at 12000 rpm/min. And the white precipitate was visible at the bottom of the tube. We added 80 % ethanol, 12000 rpm centrifugation for 5 min. Finally, we discard the supernatant and lay it flat on the paper to dry. When the white precipitate began to become transparent, we added RNase Free ddH_2O to dissolve the precipitate. Subsequently, the quality was confirmed by NanoDrop Spectrophotometer (Thermo Fisher Scientific, MA, USA). Lastly, RNA sequencing was performed on NovaSeq 6000 platform (Illumina). After data collection, DESeq2 was used to analyze gene expression, and DEGs were: $|\log2FoldChange| > 1$ and significant q value ≤ 0.05. GO enrichment analysis were applied to explore potential biological functions of screened DEGs.

2.8. Statistical analysis

All data were expressed as the mean ± standard deviation, and analyzed by GraphPad Prism software version 6.0 (GraphPad software, CA, USA). Longitudinal data were analyzed by a one-way ANOVA, followed by post-hoc test using S-N-K method. The

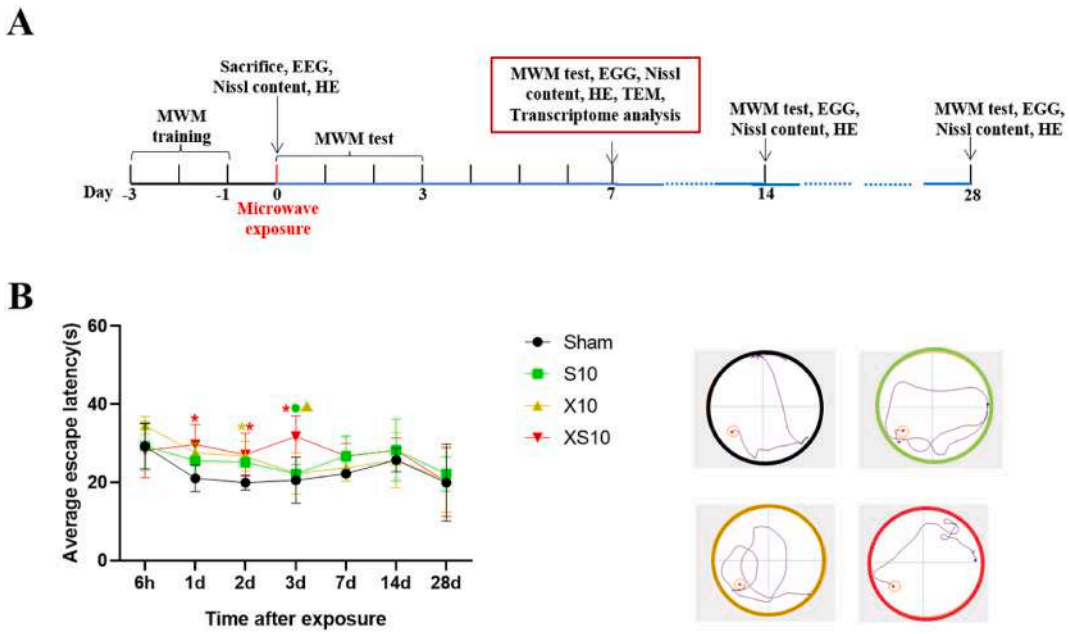


Fig. 2. The learning curves of rats after the microwave exposure. A: the experimental design; B: the learning curves; C: the typical path of rats in each group at 3 d after exposure. Compared with Sham group, *, XS10 group $p = 0.015$ at 1 d, *, X10 group $p = 0.030$ at 2 d, *, XS10 group $p = 0.023$ at 2 d, *, XS10 group $p = 0.013$ at 3 d, *, $p < 0.05$; Compared with S10 group, ●, XS10 group $p = 0.022$ at 3 d, ●, $p < 0.05$; Compared with X10 group, ▲, XS10 group $p = 0.030$ at 3 d, ▲, $p < 0.05$ (one-way ANOVA).

difference is considered significant if $p < 0.05$.

3. Results

3.1. Effect of compound wave on spatial learning and memory

Wistar rats were exposed to compound microwave with frequency of 2.8 GHz and 9.3 GHz (Fig. 2A). Before and immediately after exposure, the anal and body surface temperature were monitored and no significant changes were observed, suggesting that no thermal effects were induced by microwave (Supplementary-Figure S1A-B). Then, the learning and memory ability was detected using MWM navigation tests at indicated time points after exposure. We found that the microwave significantly decreased the learning speed at 1 d and 3 d after exposure. Moreover, compound microwave of 2.8 GHz and 9.3 GHz could reduce learning speed much stronger than single frequency microwave at 3d after exposure. However, the learning speed was restored to the equal level with that in Sham group at 7 d after exposure, indicating self-recovery mechanisms were initiated (Fig. 2B: $F_{3,15} = 0.855$, $p > 0.05$ for 6h; $F_{3,14} = 2.734$, $p < 0.05$ for 1d; $F_{3,14} = 2.649$, $p < 0.05$ for 2d; $F_{3,13} = 3.487$, $p < 0.05$ for 3d; $F_{3,12} = 1.186$, $p > 0.05$ for 7d; $F_{3,14} = 0.226$, $p > 0.05$ for 14d; $F_{3,13} = 0.061$, $p > 0.05$ for 28d). These data suggested that compound microwave of 2.8 GHz and 9.3 GHz could impair spatial learning and memory ability in rats much more obviously than single frequency microwave.

3.2. Effect of compound wave on electroencephalogram (EEG)

Brain electrical activity play pivotal roles in maintaining learning and memory ability. Therefore, we analyzed EEG, which could clearly show the brain electrical activity, in this study. Compared to 2.8 GHz microwave (S10 group), the power of α wave was decreased at 7 d after exposure to 9.3 GHz microwave (X10 group) (Fig. 3A: $F_{3,12} = 0.773$, $p > 0.05$ for 6h; $F_{3,12} = 2.449$, $p < 0.05$ for 7d; $F_{3,12} = 0.150$, $p > 0.05$ for 14d; $F_{3,12} = 0.102$, $p > 0.05$ for 28d). Compared to Sham group, the power of β wave and θ wave were increased at 6 h after exposure to S10 group(Fig. 3B: $F_{3,12} = 5.614$, $p < 0.05$ and $p < 0.01$ for 6h; $F_{3,12} = 0.567$, $p > 0.05$ for 7d; $F_{3,12} = 0.106$, $p > 0.05$ for 14d; $F_{3,12} = 0.412$, $p > 0.05$ for 28d. Fig. 3C: $F_{3,12} = 2.588$, $p < 0.05$ for 6h; $F_{3,12} = 2.392$, $p < 0.05$ for 7d; $F_{3,12} = 0.026$, $p > 0.05$ for 14d; $F_{3,12} = 0.207$, $p > 0.05$ for 28d), while the power of δ wave was elevated at 7 d after exposure to X10 group (Fig. 3D: $F_{3,12} = 1.564$, $p > 0.05$ for 6h; $F_{3,12} = 4.081$, $p < 0.05$ for 7d; $F_{3,12} = 0.121$, $p > 0.05$ for 14d; $F_{3,12} = 0.102$, $p > 0.05$ for 28d). Importantly, both the power of θ wave and δ wave were obviously up-regulated at 7 d after radiation in compound microwave group (XS10 group) (Fig. 3C–D). Interestingly, the power of β wave in XS10 group was much lower than that in X10 group (Fig. 3B), suggesting the interaction between 2.8 GHz and 9.3 GHz microwave. Moreover, all of waves were recovered at 14 d after exposure.

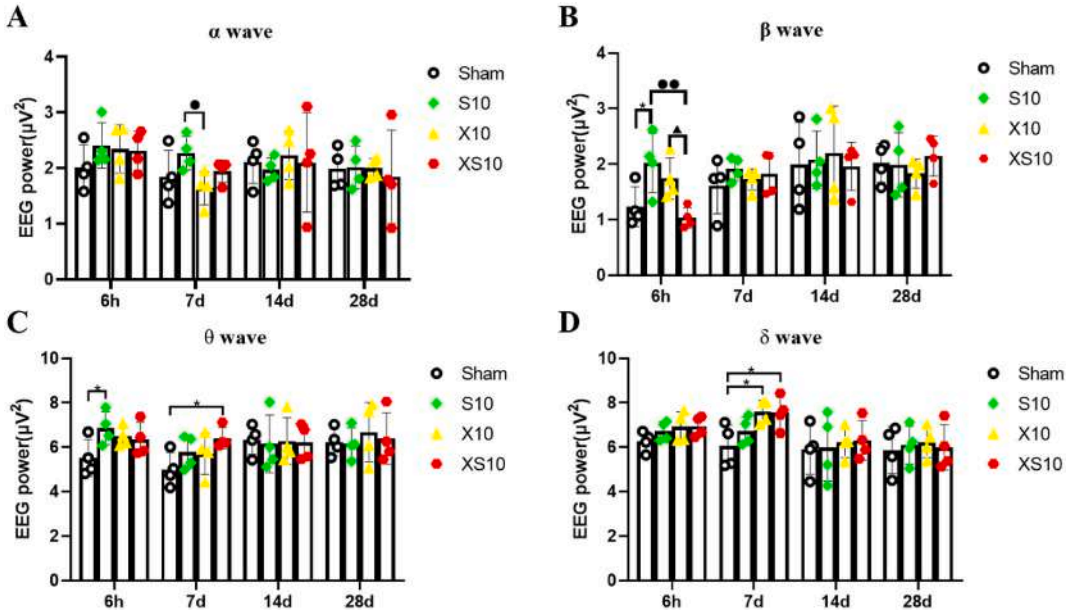


Fig. 3. The alteration of electroencephalogram (EEG) power in rats after microwave exposure. A: α wave power. Compared with S10 group, ●, $p = 0.021$ at 7 d; B: β wave power. Compared with Sham group, *, $p = 0.013$ at 6 h, Compared with S10 group, ●●, $p = 0.003$ at 6 h, Compared with X10 group, ▲, $p = 0.023$ at 6 h; C: θ wave power. Compared with Sham group, *, $p = 0.020$ at 6 h, *, $p = 0.020$ at 7 d; D: δ wave power. Compared with Sham group, *, $p = 0.011$ for X10 group, *, $p = 0.013$ for XS10 group. *, $p < 0.05$; ●●, $p < 0.01$; ▲, $p < 0.05$ (one-way ANOVA).

3.3. Effect of compound wave on the structure of hippocampus

To investigate the potential mechanisms, we analyzed the histological structure of the rat hippocampus, an important brain region that plays a cognitive role. At 7 d after radiation, the Sham group showed normal morphological characteristics. Deep staining and shrinkage of nuclei were observed in the CA3 and DG regions of the hippocampus in S10, X10, and XS10 groups. As expected, compound microwave produced most serious injuries to hippocampal neurons. In XS10 group, pyknotic and hyperchromatic nuclei could be detected at 6 h after exposure (Supplementary-Figure S2), and significantly increased at 7 d after exposure (Fig. 4A–D). And then, pyknotic and hyperchromatic nuclei gradually decreased and return to normal level at 28 d after exposure (Supplementary-Figure S2). Our data indicated that both 2.8 GHz and 9.3 GHz microwave, as well as compound microwave caused reversible structural injuries to rat's hippocampus, while compound microwave produced much more impressive injuries.

Nissl body participates in synthesis of structural proteins and enzyme that regulating metabolisms of organelles and neurotransmitters. Here, we analyzed the changes of Nissl body in hippocampal neurons after microwave exposure. The number of Nissl body decreased obviously at 7 d after exposure in all of microwave exposed groups. And then, the Nissl body gradually increased, and restored to normal level at 28 d after exposure. Interestingly, 2.8 GHz microwave reduced Nissl body much stronger than 9.3 GHz microwave, suggesting that Nissl body was sensitive to S band microwave (Fig. 4E–I. Fig. 4I: $F_{3,16} = 7.778$, $p < 0.05$, $p < 0.01$ and $p < 0.001$).

3.4. Effect of compound wave on ultrastructural of hippocampal tissue

Moreover, the ultrastructure of the hippocampal tissue was also analyzed at 7 d after exposure. Generally, microwave exposure caused obvious structural injuries in hippocampal neurons, and most serious injuries were observed in XS10 group. Firstly, we found that microwave exposure blurred synaptic spaces and increased postsynaptic compacts. Compared to that in S10 group, synaptic cleft in XS10 group was more blurred and the postsynaptic density was significantly increased (Fig. .5A–F. Fig. 5E: $F_{3,36} = 26.483$, $p < 0.05$ and $p < 0.001$; Fig. 5F: $F_{3,30} = 26.268$, $p < 0.05$, $p < 0.01$ and $p < 0.001$). Secondly, the mitochondrial structure was also damaged by microwave. Compared with Sham group, microwave exposure resulted in swelling, cavitation, disordered cristae and ruptured membrane of mitochondria. Compared with single frequency microwave, compound microwave damaged mitochondria much stronger (Fig. 5G–L Fig. 5K: $F_{3,48} = 178.162$, $p < 0.001$; Fig. 5L: $F_{3,48} = 55.520$, $p < 0.001$). Synapses and mitochondria are the most sensitive organelles to electromagnetic radiation, and may respond to microwaves, especially compound microwave of 2.8 GHz and 9.3 GHz.

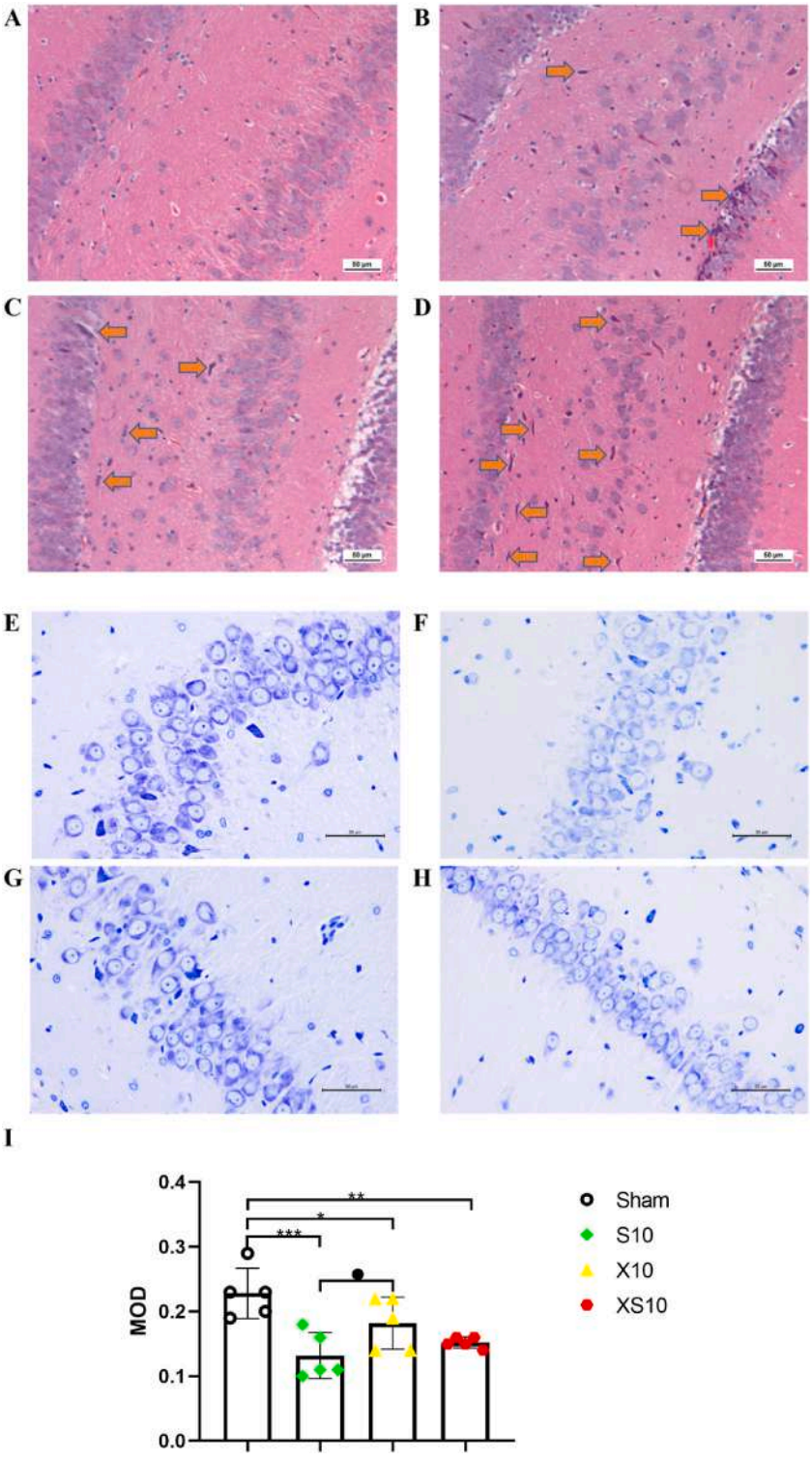
3.5. Effect of compound wave on genes expression in peripheral blood

Effective predictive indicators for microwave-induced neuronal injuries are useful to healthy alarming. Moreover, these indictors might be potential targets for developing preventive and therapeutic approaches. Peripheral blood is the most convenient source for screening sensitive indicators. We have previously reported that 548 upregulated genes and 311 downregulated genes were detected in peripheral blood after exposure to compound microwave of 2.8 GHz and 9.3 GHz [20]. Here, we further analyzed the DEGs that closely associated with the nervous system(https://www.jianguoyun.com/p/DQNoFnAQ98CyCxjBw_UEIAA). Only 6 neuronal functions related DEGs were found in compound microwave exposed group, including 5 up-regulated and 1 down-regulated genes (Table S1), namely huntingtin (Htt), htra serine peptidase 2 (Htra2), presenilin 1 (Psen1) and tumor protein p73 (Tp73), heterogeneous nuclear ribonucleoprotein D (Hnrnpd) and tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein epsilon (Ywhae). In the Protein-Protein Interaction (PPI) analysis, we found Htt could directly interact with brain derived neurotrophic factor (Bdnf), neurotrophic receptor tyrosine kinase 2 (Ntrk2) and microtubule associated protein tau (Mapt) to regulate neuronal activities, such as cognitive function (Fig. 6). Therefore, Htt and Bdnf might be potential indicators to predict microwave.

4. Discussion

In recent years, with the rapidly growing applications of microwave technologies, people's concerns about the health hazards of microwave are increasing [1]. Many studies have reported the biological effects induced by single microwaves with a certain frequency and power intensity in various organs and tissues, of which the brain is one of the most sensitive organs [2,8–12,21]. Studies have demonstrated that microwave exposure could cause impairment of cognitive functions, especially the learning and memory ability [18,22]. However, people always surrounded by complex environments that containing microwave with multiple frequencies and different power intensities, in daily life. Nowadays, more and more studies have been focusing on exploring biological effects induced by compound microwave exposure. Our group and others have reported that compound microwave with different frequencies could induce much more serious injuries than microwave with corresponding single frequency in various organs and tissues [4–6]. In this study, we found that exposure to compound microwave of 2.8 GHz and 9.3 GHz resulted in obvious injuries in rat's hippocampus and significant impairment of learning and memory ability.

Learning and memory ability and EEG signals are frequently used indicators to evaluate cognitive functions [23,24]. Morris Water Maze (MWM) is a common tool for assessing spatial learning and memory. Researchers found that exposure to both microwave with single frequency and multi-frequencies significantly increased the AEL in MWM navigation experiment, indicating the impairment of spatial learning and memory ability [7,13,25–27]. Wang et al. found that exposure to microwave with single frequency (1.5 GHz) or multi-frequencies (1.5 and 4.3 GHz) could prolong the AEL [5]. Tan et al. reported that radiation by S-band and L-band microwave



(caption on next page)

Fig. 4. Histopathological and Nissl analysis of hippocampal tissues of rats at 7d after exposure to microwave (HE). Neurons with normal nuclei were well-distributed in Sham group (A). However, pyknotic and hyperchromatic nuclei were observed in neurons of S10 (B) and X10 (C) groups. Importantly, more severely pyknotic and hyperchromatic nuclei with spindle shape were detected in XS10 group (D). Nissl staining images of the Sham, S10, X10 and XS10 groups were shown in E-H respectively. And, the image analysis of Nissl body changes in rat hippocampus at 7d after microwave exposure were presented in I. Generally, decrease of Nissl body content could be observed in S10, X10 and XS10 groups, while impressive changes could be detected in XS10 group. Compared with Sham group, ***, $p < 0.0001$ for S10 group, *, $p = 0.045$ for X10 group, **, $p = 0.002$ for XS10 group, *, $p < 0.05$, **, $p < 0.01$ ***, $p < 0.001$; Compared with S10 group, ●, $p = 0.031$, ●, $p < 0.05$ (one-way ANOVA). Scale bar = 50 μm for HE staining and toluidine blue staining.

with an average power density of 10 mW/cm^2 obviously increase the AEL [4]. Zhu et al. also found that exposure to L band and C band microwave at average power density of 10 mW/cm^2 extended the AEL from 1 to 7 days exposure [6]. In this study, we showed that both 2.8 GHz and 9.3 GHz microwave extend the AEL from 1d to 3d after exposure for 6 min. As expected, compound microwave of 2.8 GHz and 9.3 GHz increased AEL much more than single frequency microwave. Our data suggested that compound microwave could reduce learning and memory ability much stronger than single frequency microwave in rats.

EEG is an objective electrophysiological index to present physiological and pathological of brain [24,28]. There are four types of waves in the EEG spectrum: α (12–30 Hz), β (8–12 Hz), θ (4–8 Hz) and δ (1–4 Hz). The α and β waves appear in a relaxed or tense status of the brain, while the θ and δ waves appear in a tired or sleepy state of the brain [29]. Studies have demonstrated that both single and compound microwave could affect the waves of EEG. Wang et al. found that compound exposure to L band and C band resulted in an increase in the power of δ waves, while Zhu et al. found the power of θ wave was up-regulated on day 7 after exposure, indicating that microwaves reduce electrical excitability [5,6]. In this study, we found that 2.8 GHz microwave increased the power of θ wave at 6h after exposure. Moreover, 9.3 GHz microwave elevated the power of δ wave, while compound microwave increased the power of both θ wave and δ at 7d after exposure, indicating that cortical excitability is suppressed. These results suggested that compound microwave of 2.8 and 9.3 GHz significantly inhibited the activity of brain.

The hippocampus is a region of the brain, which is largely responsible for the learning and memory [30,31]. Previous studies have showed that microwave exposure could cause obviously damages to hippocampal tissues, in which blurred synaptic space, reduced synaptic vesicles, pyknotic and hyperchromatic nucleus could be observed [4,6,18]. Moreover, several groups reported that exposure to both single and compound microwave could injure the ultrastructure of hippocampus, especially mitochondria [6]. Mitochondria are the critical organelle for energy metabolism, and can generate energy to support the physiological functions of brain. In this study, mitochondrial swelling, cavitation, cristae disorder and rupture, and membrane broken could be observed at 7 d after exposure in all of the exposed groups. However, compound microwave produced much stronger injuries. In addition to mitochondria, Nissl bodies are also emerged as important structure of neurons. Nissl bodies could synthesize structural proteins for organelles, and enzymes for generating neurotransmitters [17]. And, it has been reported that microwave radiation can reduce the content of neuronal cytoplasmic Nissl bodies, and the decrease of Nissl bodies are positively related with the decline of learning and memory ability [4,32,33]. Our data suggested that compound microwave could destroy the metabolism of hippocampal neurons, which in turn impair the learning and memory ability of rats, while compound microwave caused most serious injuries.

Predictive indicators for microwave induced injuries to nervous system, will be valuable to alarm the risks and protect people from potential health hazards. In this study, we analyzed DEGs in peripheral blood, and found that only 7 DEGs were closely related with functions of nervous system. Htt can positively regulate the transcription of Bdnf, as well as promote the transport of Bdnf in the axon of cortex and striatum. Once Bdnf is released into the synapse, it can activate the TrkB receptor to promote endocytosis of TrkB. Then, TrkB forms complex with dynein, dynactin and kinesin-1 to activate Erk1/2 and promote the survival of neurons [34]. Htra2, a serine protease located in the mitochondrial membrane gap, owns enzyme activity to degrade misfolded proteins. Htra2 has neuroprotective functions when mitochondria undergo stress response [35,36]. Psen1 play important roles in regulating lysosomal related functions during autophagy, as well as synaptic plasticity. Also, the mutations of Psen1 are closely associated with the Alzheimer's disease [37–40]. Tp73 gene, a homologue of the Tp53 family, not only can regulate autophagy and angiogenesis [41,42], but also can modulate cell senescence by intervening in mitochondrial metabolism [43]. Combined with GO and PPI analysis, we found that Htt, Htra, Psen1, Tp73 and Bdnf might be potential predictive indicators for microwave induced injuries of nervous system. Interestingly, we found that most 6 out of 7 DEGs were up-regulated at 7 d after microwave exposure, which suggested that the self-recovery mechanisms have been initiated.

In summary, the composite microwave of 2.8 GHz and 9.3 GHz caused obvious decline in the learning and memory abilities of rats, companying hippocampal structural injuries and cognition functional impairments. Mitochondrial disorder and decrease of Nissl bodies are the potential mechanisms for microwave induced injuries of structural and functional injuries. Moreover, the results of transcriptomics explored several potential indicators for diagnosis, such as Htt, Htra, Psen1, Tp73, and Bdnf, which also can be potential targets for and therapy. However, experiments are still needed to be further verify and it will be interesting and valuable to explore the interactions between microwaves with different frequencies.

5. Conclusion

The composite microwave of 2.8 GHz and 9.3 GHz can cause damage to rat hippocampal tissue, inhibit brain activity, and reduce the metabolism of hippocampal neurons through inducing mitochondrial disorder and decreasing Nissl bodies, and ultimately impaired the learning and memory abilities of rats. Notably, compound microwave can reduce the learning and memory ability of rats more than single frequency microwave. Through a combination of GO and PPI analyses, we have also identified Htt, Htra, Psen1, Tp73,

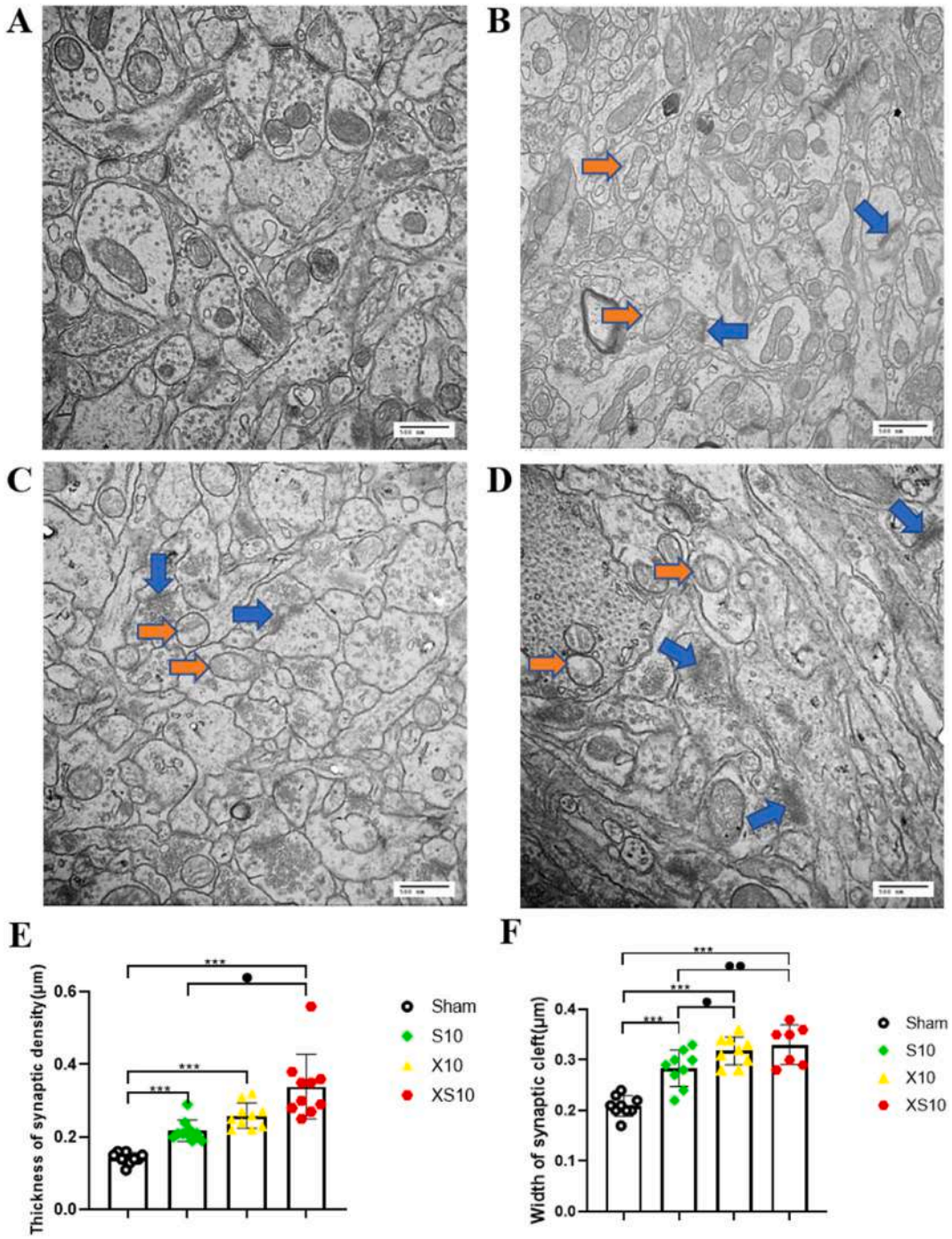


Fig. 5. Ultrastructural analysis of hippocampal neurons by transmission electron microscope (TEM). A: TEM image of synapses in the Sham group at 7 d after microwave exposure; B: TEM image of synapses in the S10 group at 7 d after microwave exposure; C: TEM image of synapses in the X10 group at 7 d after microwave exposure; D: TEM image of synapses in the XS10 group at 7 d after microwave exposure. E: Thickness of synaptic density in rat hippocampus after microwave exposure. Compared with Sham group, ***, $p < 0.001$ for S10 group, ***, $p < 0.001$ for X10 group, ***, $p < 0.001$ for XS10 group; Compared with S10 group, ●, $p = 0.010$, ●, $p < 0.05$(one-way ANOVA); F: Width of synaptic cleft in rat hippocampus after microwave exposure. Compared with Sham group, ***, $p < 0.001$ for S10 group, ***, $p < 0.001$ for X10 group, ***, $p < 0.001$ for XS10 group; Compared with S10 group, ●, $p = 0.025$, ●●, $p = 0.006$, ●, $p < 0.05$, ●●, $p < 0.01$(one-way ANOVA). G: TEM image of mitochondria in the Sham group at 7 d after microwave exposure; H: TEM image of mitochondria in the S10 group at 7 d after microwave exposure; I: TEM image of mitochondria in the X10 group at 7 d after microwave exposure; J: TEM image of mitochondria in the XS10 group at 7 d after microwave exposure. K: Length of cristae in rat hippocampus after microwave exposure. Compared with Sham group, ***, $p < 0.001$ for S10 group, ***, $p < 0.001$ for X10 group, ***, $p < 0.001$ for XS10 group; Compared with S10 group, ●●●, $p < 0.001$; Compared with X10 group, ▲▲▲, $p < 0.001$(one-way ANOVA); L:

Mitochondrial area in rat hippocampus after microwave exposure. Compared with Sham group, ***, $p < 0.001$ for S10 group, ***, $p < 0.001$ for X10 group, ***, $p < 0.001$ for XS10 group; Compared with S10 group, ●●●, $p < 0.001$; Compared with X10 group, ▲▲▲, $p < 0.001$(one-way ANOVA). The yellow arrow in the figure shows the swelling and cavitation of mitochondria; The blue arrow indicates the destruction of synaptic structure. Scale bar = 500 nm for TEM.

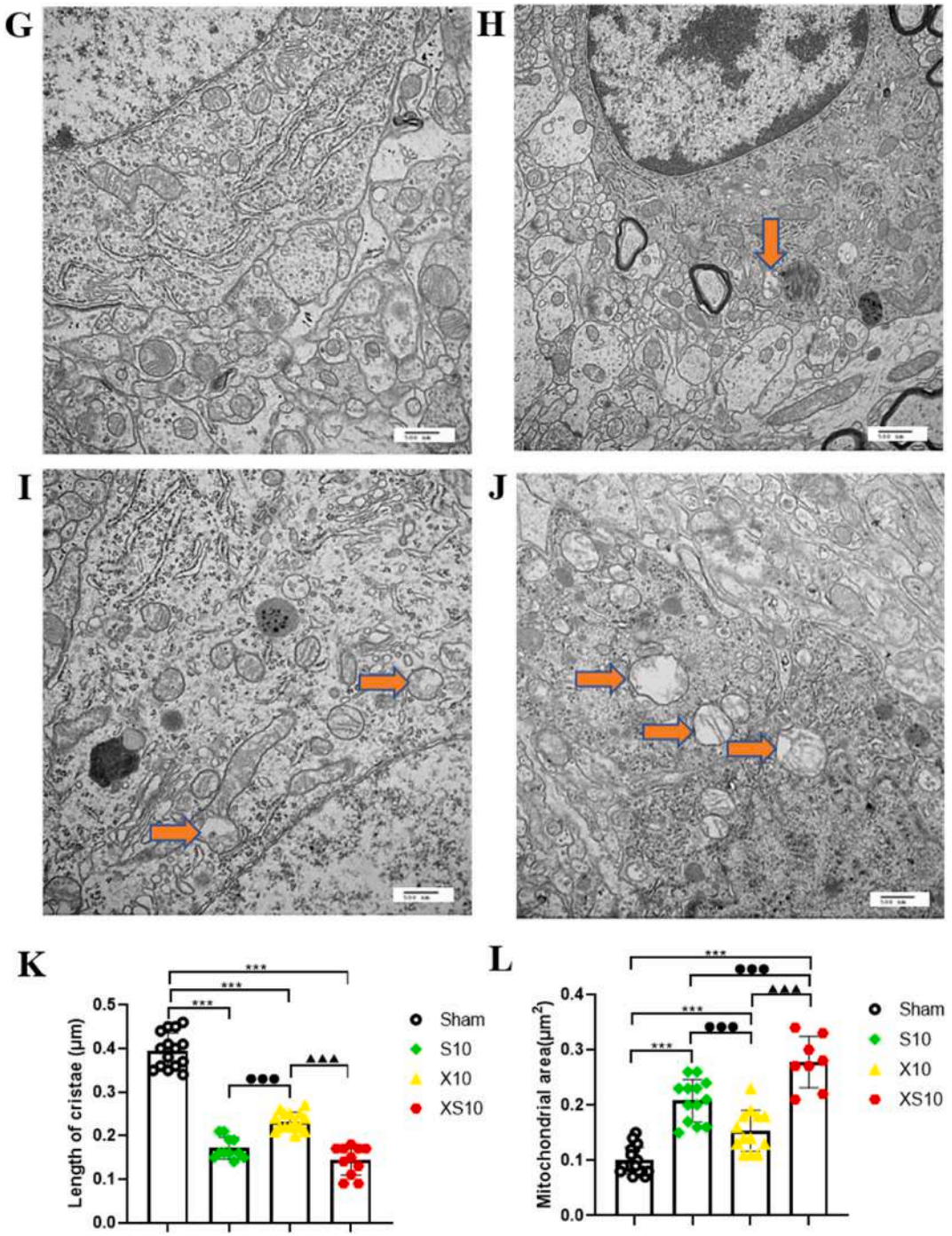


Fig. 5. (*continued*).

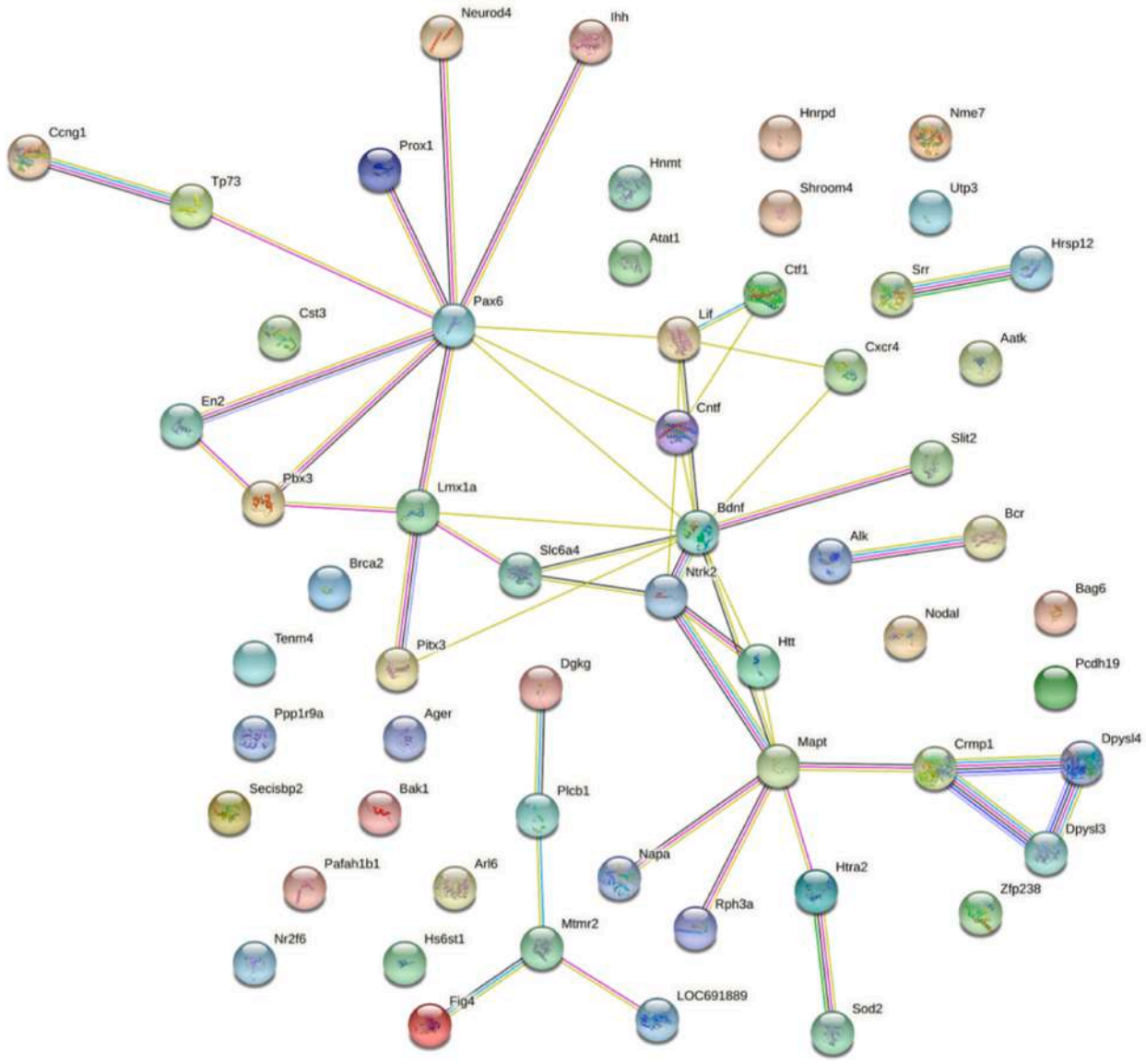


Fig. 6. Protein-protein interaction (PPI) analysis of differential expressed genes that closely related with functions of nervous system. Network nodes represent proteins. Node Color: colored nodes: query proteins and first shell of interactors, white nodes: second shell of interactors; Node Content: empty nodes: proteins of unknown 3D structure, filled nodes: some 3D structure is known or predicted. Edges represent protein-protein association. Known Interactions: from curated databases, experimentally determined; Predicted Interactions: gene neighborhood, gene fusions, gene co-occurrence; Others: textmining, co-expression, protein homology.

and Bdnf as potential predictive indicators of microwave-induced neuronal damage in the nervous system.

CRediT authorship contribution statement

Liu Sun: Writing – original draft, Methodology, Formal analysis, Data curation. **Xiaoya Wang:** Methodology, Formal analysis, Data curation. **Ke Ren:** Methodology, Formal analysis. **Chuanfu Yao:** Methodology, Formal analysis. **Haoyu Wang:** Methodology, Formal analysis. **Xinping Xu:** Methodology, Formal analysis. **Hui Wang:** Methodology, Formal analysis. **Ji Dong:** Methodology, Formal analysis. **Jing Zhang:** Methodology, Formal analysis. **Binwei Yao:** Methodology, Formal analysis. **Xiaohui Wei:** Methodology, Formal analysis. **Ruiyun Peng:** Writing – review & editing, Project administration. **Li Zhao:** Writing – review & editing, Project

administration, Methodology, Formal analysis.

Ethics approval and consent to participate declaration

All protocols were approved by Institutional Animal Care and Use Committee at Beijing Institute of Radiation Medicine (IACUC-DWZX-2020-781)

Availability of data and materials declaration

Not applicable.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2025.e41626.

References

[1] A. Kocaman, G. Altun, A.A. Kaplan, Ö.G. Deniz, K.K. Yurt, S. Kaplan, Genotoxic and carcinogenic effects of non-ionizing electromagnetic fields, Environ. Res. 163 (2018) 71–79, https://doi.org/10.1016/j.envres.2018.01.034. PubMed PMID: 29427953.
[2] M. Esmekaya, C. Ozer, N. Seyhan, 900 MHz pulse-modulated radiofrequency radiation induces oxidative stress on heart, lung, testis and liver tissues, Gen. Physiol. Biophys. 30 (1) (2011) 84–89.
[3] C. Liu, W. Duan, S. Xu, C. Chen, M. He, L. Zhang, et al., Exposure to 1800 MHz radiofrequency electromagnetic radiation induces oxidative DNA base damage in a mouse spermatocyte-derived cell line, Toxicol. Lett. 218 (1) (2013) 2–9.
[4] S. Tan, H. Wang, X. Xu, L. Zhao, J. Zhang, J. Dong, et al., Study on dose-dependent, frequency-dependent, and accumulative effects of 1.5 GHz and 2.856 GHz microwave on cognitive functions in Wistar rats, Sci. Rep. 7 (1) (2017) 10781, https://doi.org/10.1038/s41598-017-11420-9. PubMed PMID: 28883530; PubMed Central PMCID: PMCPMC5589914.
[5] H. Wang, Y. Liu, Y. Sun, L. Zhao, J. Dong, X. Xu, et al., Changes in rat spatial learning and memory as well as serum exosome proteins after simultaneous exposure to 1.5 GHz and 4.3 GHz microwaves, Ecotoxicol. Environ. Saf. 243 (2022) 113983, https://doi.org/10.1016/j.ecoenv.2022.113983. PubMed PMID: 35985199.
[6] R. Zhu, H. Wang, X. Xu, L. Zhao, J. Zhang, J. Dong, et al., Effects of 1.5 and 4.3 GHz microwave radiation on cognitive function and hippocampal tissue structure in Wistar rats, Sci. Rep. 11 (1) (2021) 10061, https://doi.org/10.1038/s41598-021-89348-4. PubMed PMID: 33980913; PubMed Central PMCID: PMCPMC8115682.
[7] H.J. Li, R.Y. Peng, C.Z. Wang, S.M. Qiao, Z. Yong, Y.B. Gao, et al., Alterations of cognitive function and 5-HT system in rats after long term microwave exposure, Physiol. Behav. 140 (2015) 236–246, https://doi.org/10.1016/j.physbeh.2014.12.039. PubMed PMID: 25542888.
[8] A.F. Fragopoulou, A. Polyzos, M.D. Papadopoulou, et al., Hippocampal lipidome and transcriptome profile alterations triggered by acute exposure of mice to GSM 1800 MHz mobile phone radiation: An exploratory study [JJ, Brain and behavior 8 (6) (2018) e01001.
[9] K. Hu, Y. Li, H. Yu, Y. Hu, CTBP1 confers protection for hippocampal and cortical neurons in rat models of Alzheimer's disease, Neuroimmunomodulation 26 (3) (2019) 139–152, https://doi.org/10.1159/000500942. PubMed PMID: 31340205.
[10] N. Karimi, M. Bayat, M. Haghani, H.F. Saadi, G.R. Ghazipour, 2.45 GHz microwave radiation impairs learning, memory, and hippocampal synaptic plasticity in the rat, Toxicol. Ind. Health 34 (12) (2018) 873–883, https://doi.org/10.1177/0748233718798976. PubMed PMID: 30345889.
[11] S.N. Narayanan, N. Mohapatra, P. John, N. K, R.S. Kumar, S.B. Nayak, et al., Radiofrequency electromagnetic radiation exposure effects on amygdala morphology, place preference behavior and brain caspase-3 activity in rats, Environ. Toxicol. Pharmacol. 58 (2018) 220–229, https://doi.org/10.1016/j.etap.2018.01.009. PubMed PMID: 29413766.
[12] M.L. Pall, Microwave frequency electromagnetic fields (EMFs) produce widespread neuropsychiatric effects including depression, J. Chem. Neuroanat. 75 (Pt B) (2016) 43–51, https://doi.org/10.1016/j.jchemneu.2015.08.001. PubMed PMID: 26300312.
[13] H. Wang, R. Peng, Y. Gao, L. Zhao, S. Wang, X.U. Xinping, et al., Long term effect of different power density microwave radiation on the learning and memory ability and hippocampal structure in rats, Chinese Journal of Stereology and Image Analysis (2015).
[14] L.F. Wang, L. Wei, S.M. Qiao, X.N. Gao, Y.B. Gao, S.M. Wang, et al., Microwave-induced structural and functional injury of hippocampal and PC12 cells is accompanied by abnormal changes in the NMDAR-PSD95-CaMKII pathway, Pathobiology : journal of immunopathology, molecular and cellular biology 82 (5) (2015) 181–194, https://doi.org/10.1159/000398803. PubMed PMID: 26337368.
[15] S.M. Mortazavi, S. Taeb, N. Dehghan, Alterations of visual reaction time and short term memory in military radar personnel, Iranian journal of public health 42 (4) (2013) 428–435. Epub 2013/06/21. PubMed PMID: 23785684; PubMed Central PMCID: PMCPMC3684731.
[16] F. Ma, H.Y. Xiong, Y. Zhang, X.M. Zhang, F. Ma, C.L. Yang, et al., Epidemiological investigation of effects of high intensity eletromagnetic radiation on neurobehavioral functions of military task population, J Third Mil Med Univ. (22) (2004) 2048–2050.
[17] A. Beaudet, A. Rambourg, The tridimensional structure of Nissl bodies: a stereoscopic study in ventral horn cells of rat spinal cord, Anat. Rec. 207 (4) (1983) 539–546, https://doi.org/10.1002/ar.1092070402. PubMed PMID: 6670751.
[18] A. Sharma, K.K. Kesari, V.K. Saxena, R. Sisodia, Ten gigahertz microwave radiation impairs spatial memory, enzymes activity, and histopathology of developing mice brain, Mol. Cell. Biochem. 435 (1–2) (2017) 1–13, https://doi.org/10.1007/s11010-017-3051-8. PubMed PMID: 28470342.
[19] J. Lin, Electromagnetic Interaction with Biological Systems. Springer Science & Business Media, PLenum Press, New York, NY, USA, 2012. Berlin/Heidelberg, Germany.
[20] C. Yao, H. Wang, L. Sun, K. Ren, J. Dong, H. Wang, et al., The biological effects of compound microwave exposure with 2.8 GHz and 9.3 GHz on immune system: transcriptomic and proteomic analysis, Cells 11 (23) (2022) 3849, https://doi.org/10.3390/cells11233849. PubMed PMID: 36497106.
[21] H. Wang, R.Y. Peng, Y.B. Gao, L. Zhao, S.M. Wang, X.P. Xu, et al., Long term effect of different power density microwave radiation on the learning and memory ability and hippocampal structure in rats, Chin J Stereology and Image Analysis 20 (4) (2015) 434–442, https://doi.org/10.13505/j.1007-1482.2015.20.04.016.

[22] L.J. Sun, Y. Gao, Y. Guo, C.G. Zhang, Y.Z. Zhang, Y.F. Li, Behavioral tests of mood-cognitive impairment of mice exposed to high-frequency electromagnetic radiation, Chin. J. Comp. Med. 28 (3) (2018) 15–20.
[23] Y. Deng-Bryant, L.Y. Leung, K. Caudle, F. Tortella, D. Shear, Cognitive evaluation using Morris water maze in neurotrauma, Methods Mol. Biol. 1462 (2016) 539–551, https://doi.org/10.1007/978-1-4939-3816-2_29. PubMed PMID: 27604737.
[24] M. Rashno, A. Sarkaki, S. Ghaderi, S.E. Khoshnam, Sesamin: insights into its protective effects against lead-induced learning and memory deficits in rats, J. Trace Elem. Med. Biol. 72 (2022) 126993, https://doi.org/10.1016/j.jtemb.2022.126993. PMID: 35550983.
[25] G.P. Lu, Research on the Prevention Effects and Mechanism of Hyperbaric Oxygen on Brain Injury Induced by Microwave Radiation in Rats, Southern Medical University, 2016.
[26] N. Wang, S.J. Lv, Effect of microwave radiation on learning and memory damage in mice, Jilin Yiyao Xueyuan Xuebao 40 (5) (2019) 321–323, https://doi.org/10.13845/j.cnki.issn1673-2995.2019.05.001.
[27] S.M. Qiao, X.J. Hu, L.F. Wang, R.Y. Peng, Y.B. Gao, S.M. Wang, et al., Effect of microwave radiation on hippocampal structure and function in Wistar rats and its relationship with synapsin I, Mil Med Sci. 37 (2) (2013) 126–129+40.
[28] H. Hinrikus, M. Bachmann, J. Lass, R. Tomson, V. Tuulik, Effect of 7, 14 and 21 Hz modulated 450 MHz microwave radiation on human electroencephalographic rhythms, Int. J. Radiat. Biol. 84 (1) (2008) 69–79.
[29] D. Karamacoska, R.J. Barry, G.Z. Steiner, Resting state intrinsic EEG impacts on go stimulus-response processes, Psychophysiology 54 (6) (2017) 894–903, https://doi.org/10.1111/psyp.12851. PubMed PMID: 28258583.
[30] J.L. McClelland, B.L. McNaughton, R.C. O'Reilly, Why there are complementary learning systems in the hippocampus and neocortex: insights from the successes and failures of connectionist models of learning and memory, Psychol. Rev. 102 (3) (1995) 419–457, https://doi.org/10.1037/0033-295x.102.3.419. PubMed PMID: 7624455.
[31] M.J. Sekeres, G. Winocur, M. Moscovitch, The hippocampus and related neocortical structures in memory transformation, Neurosci. Lett. 680 (2018) 39–53, https://doi.org/10.1016/j.neulet.2018.05.006. PubMed PMID: 29733974.
[32] J. Li, P.Y. Wen, W.W. Li, J. Zhou, Upregulation effects of Tanshinone IIA on the expressions of NeuN, Nissl body, and IκB and downregulation effects on the expressions of GFAP and NF-κB in the brain tissues of rat models of Alzheimer's disease, Neuroreport 26 (13) (2015) 758–766, https://doi.org/10.1097/wnr.0000000000000419. PubMed PMID: 26164608.
[33] J. Chen, D.W. Wang, H.Y. Zuo, R.Y. Peng, S.M. Wang, Y.B. Gao, et al., Effects of electromagnetic radiations on the morphology in hippocampus and the expression of injury-related proteins, J. Prev. Med. Chin. People's Liberation Army (2) (2007) 82–86.
[34] Saudou, Frederic, Humbert, Sandrine.The Biology of Huntingtin.
[35] C. Chen, X. Chen, Q. Cai, Y.E. Lichao, Q. Wan, R. Cai, et al., Association of omi/HTRA2 gene with Parkinson's disease in huazhong region han population, Chin. J. Neuroimmunol. Neurol. (2018).
[36] X.Y. Zhao, Y.S. Chen, Research progress of Omi/HtrA2 gene and neurodegenerative diseases, J. Xinxiang Med. Coll. (5) (2008) 530–533.
[37] S. Chakroborty, I. Goussakov, M.B. Miller, G.E. Stutzmann, Deviant ryanodine receptor-mediated calcium release resets synaptic homeostasis in presymptomatic 3xTg-AD mice, J. Neurosci.: the official journal of the Society for Neuroscience 29 (30) (2009) 9458–9470, https://doi.org/10.1523/jneurosci.2047-09.2009. PubMed PMID: 19641109; PubMed Central PMCID: PMCPMC6666542.
[38] G.E. Stutzmann, A. Caccamo, F.M. LaFerla, I. Parker, Dysregulated IP3 signaling in cortical neurons of knock-in mice expressing an Alzheimer's-linked mutation in presenilin1 results in exaggerated Ca2+ signals and altered membrane excitability, J. Neurosci.: the official journal of the Society for Neuroscience. 24 (2) (2004) 508–513, https://doi.org/10.1523/jneurosci.4386-03.2004. PubMed PMID: 14724250; PubMed Central PMCID: PMCPMC6729995.
[39] G.E. Stutzmann, I. Smith, A. Caccamo, S. Oddo, F.M. Laferla, I. Parker, Enhanced ryanodine receptor recruitment contributes to Ca2+ disruptions in young, adult, and aged Alzheimer's disease mice, J. Neurosci.: the official journal of the Society for Neuroscience. 26 (19) (2006) 5180–5189, https://doi.org/10.1523/jneurosci.0739-06.2006. PubMed PMID: 16687509; PubMed Central PMCID: PMCPMC6674246.
[40] S. Zhang, M. Zhang, F. Cai, W. Song, Biological function of Presenilin and its role in AD pathogenesis, Transl. Neurodegener. 2 (1) (2013) 15, https://doi.org/10.1186/2047-9158-2-15. PubMed PMID: 23866842; PubMed Central PMCID: PMCPMC3718700.
[41] F. Huang, B.R. Wang, Y.G. Wang, Role of autophagy in tumorigenesis, metastasis, targeted therapy and drug resistance of hepatocellular carcinoma, World J. Gastroenterol. 24 (41) (2018) 4643–4651, https://doi.org/10.3748/wjg.v24.i41.4643. PubMed PMID: 30416312; PubMed Central PMCID: PMCPMC6224467.
[42] J. Xu, M. Hao, Progress of TP53 family gene TP53 and tumorigenesis, Cancer Res Prev Treat 48 (2) (2021) 186–190.
[43] P. Lopriore, N. Capitanio, E. Panatta, N. Di Daniele, A. Gambacurta, G. Melino, et al., TAp73 regulates ATP7A: possible implications for ageing-related diseases, Aging 10 (12) (2018) 3745–3760, https://doi.org/10.18632/aging.101669. PubMed PMID: 30530920; PubMed Central PMCID: PMCPMC6326685.
[44] L. Zhao, C. Yao, H. Wang, J. Dong, J. Zhang, X. Xu, et al., Immune responses to multi-frequencies of 1.5 GHz and 4.3 GHz microwave exposure in rats: transcriptomic and proteomic analysis, Int. J. Mol. Sci. 23 (13) (2022), https://doi.org/10.3390/ijms23136949. PubMed PMID: 35805954; PubMed Central PMCID: PMCPMC9266614.
[45] R. Zhu, H. Wang, X. Xu, L. Zhao, J. Zhang, J. Dong, et al., Effects of 1.5 and 4.3 GHz microwave radiation on cognitive function and hippocampal tissue structure in Wistar rats, Sci. Rep. 11 (1) (2021) 10061, https://doi.org/10.1038/s41598-021-89348-4. PubMed PMID: 33980913; PubMed Central PMCID: PMCPMC8115682.