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The Role of NMDAR and BDNF in Cognitive Dysfunction Induced by Different Microwave Radiation Conditions in Rats

Shiyao Liao [1,2,†], Zonghuan Liu [1,3,†], Weijia Zhi [1], Lizhen Ma [1], Hongmei Zhou [1], Ruiyun Peng [1], Xiangjun Hu [1], Yong Zou [1,*] and Lifeng Wang [1,4,*]

[1] Department of Pathology, Beijing Institute of Radiation Medicine, 27 Taiping Road, Beijing 100850, China; 724461737@qq.com (S.L.); 1052650892@qq.com (Z.L.); zhi.weijia@163.com (W.Z.); malizhen0906487@sina.com (L.M.); dexlic@126.com (H.Z.); ruiyunpeng18@126.com (R.P.); xjhu2003@vip.sina.com (X.H.)
[2] Department of Pathology, Hunan Provincial People's Hospital, The First Affiliated Hospital of Hunan Normal University, Changsha 410002, China
[3] Department of Pathology, Changzhou Maternal and Child Health Care Hospital, Changzhou 213000, China
[4] Department of Basic Medicine, University of South China, Hengyang 421001, China
* Correspondence: tjuzouyong@163.com (Y.Z.); fangchang_14@163.com (L.W.)
† These authors have contributed equally to this work.

Simple Summary: Microwaves are electromagnetic waves of which frequencies range from 300 MHz to 300 GHz, pervading diverse fields of our lives. Electromagnetic radiation can be absorbed by organisms causing a series of physiological and functional changes. The brain has been recognized as one of the most vulnerable organs to microwave radiation. However, there is no consistent conclusion about the effect of microwave radiation on human health due to the different microwave parameters, such as the frequency, modulation and radiation time. This study was focused on the role of NMDARs and BDNFs in the effects of microwave radiation on cognition.

Abstract: Background: To investigate the effects of different levels of microwave radiation on learning and memory in Wistar rats and explore the underlying mechanisms of N-methyl-D-aspartate receptor (NMDAR/NR) and Brain-derived neurotropic factor (BDNF); Methods: A total of 140 Wistar rats were exposed to microwave radiation levels of 0, 10, 30 or 50 mW/cm^2 for 6 min. Morris Water Maze Test, high-performance liquid chromatography, Transmission Electron Microscope and Western blotting were used; Results: The 30 and 50 mW/cm^2 groups exhibited longer average escape latencies and fewer platform crossings than the 0 mW/cm^2 group from 6 h to 3 d after microwave radiation. Alterations in the amino acid neurotransmitters of the hippocampi were shown at 6 h, 3 d and 7 d after exposure to 10, 30 or 50 mW/cm^2 microwave radiation. The length and width of the Postsynaptic density were increased. The expression of NR1, NR2A and NR2B increased from day 1 to day 7; Postsynaptic density protein-95 and cortactin expression increased from day 3 to day 7; BDNF and Tyrosine kinase receptor B (TrkB) expression increased between 6 h and 1 d after 30 mW/cm^2 microwave radiation exposure, but they decreased after 50mW/cm^2 exposure. Conclusions: Microwave exposure (30 or 50 mW/cm^2, for 6 min) may cause abnormalities in neurotransmitter release and synaptic structures, resulting in impaired learning and memory; BDNF and NMDAR-related signaling molecules might contribute differently to these alterations.

Keywords: microwave; NMDAR; BDNF; hippocampus; learning and memory

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1. Introduction

With the rapid development of microwave techniques, people are paying increasing attention to the potential dangerous effects caused by microwave radiation. Studies have shown that exposure to excessive electromagnetic radiation can cause dysfunction of

the central nervous system [1]. The hippocampus is the structural basis of learning and memory and a highly sensitive target of microwave radiation [2,3]. To date, studies on the mechanism of learning and memory impairment caused by microwave radiation have mainly focused on synaptic plasticity, signaling pathways, energy metabolism and neurotransmitter transmission, etc. [4–8]. However, the underlying mechanisms have not yet been illuminated.

Amino acid neurotransmitters, including excitatory transmitters such as glutamic acid (Glu) and aspartic acid (Asp) and inhibitory transmitters such as gamma-aminobutyric acid (GABA) and glycine (Gly), are the most widely distributed neurotransmitters in the central nervous system. These neurotransmitters jointly regulate learning and memory by transmitting various information, and changes in their levels are closely related to neurodegenerative diseases [9,10]. N-methyl-D-aspartate receptor (NMDAR) is a special excitatory amino acid receptor and a voltage-dependent ligand-gated ion channel distributed in the central nervous system, mainly in the cerebral cortex and hippocampus [11,12]. NMDARs consist of three subunits: the functional subunit NR1 and the regulatory subunits NR2 (A, B, C and D) and NR3 (A and B). The physiological functions of different subunits and their contributions to synaptic plasticity are closely related to the mechanisms of learning and memory [6,13]. Postsynaptic density protein-95 (PSD-95) is a scaffold protein located in the postsynaptic density (PSD) of glutamatergic synapses that play an important role in clustering and stabilizing NMDARs on the postsynaptic membrane [14–16]. Cortactin, a microfilament actin-binding protein, is a key molecule associated with the cytoskeletal reorganization signaling pathway. It regulates actin dynamics to ensure the integration of transduced signals. When PSD95 anchors NMDAR to the PSD, it connects cytoskeletal proteins and signaling molecules through multiple related regions and contributes to dynamic changes in the cortactin cycle and synapse [17,18].

Brain-derived neurotropic factor (BDNF) is an important member of the nerve growth factor family. It regulates the development and homeostasis of the central nervous system, neuronal development, differentiation, functional maintenance and synaptic plasticity by binding to the neuronal cell surface receptor, Tyrosine kinase receptor B (TrkB) [19,20]. The BDNF–TrkB signaling complex is involved in the release of glutamate through the regulation of multiple signaling pathways [21,22].

Previous studies have found that microwave radiation can cause abnormal NMDAR expression and activity [8,23], but few studies on BDNF, NMDAR and related molecules have been conducted. Therefore, changes in the expression of NMDAR (NR1, NR2A and NR2B), PSD-95, cortactin, BDNF and TrkB were assessed to elucidate the mechanism of cognitive dysfunction induced by the different microwave radiation conditions in the present study.

2. Materials and Methods

2.1. Experimental Animals and Groups

A total of 140 male Wistar rats (200 ± 20 g) were purchased from the Experimental Animal Center of Beijing Institute of Radiation Medicine (Beijing, China) and kept in a specific pathogen-free (SPF) animal room. Food and water were freely available. All rats were maintained under conditions of 23 ± 2 °C and 60% humidity with a 12 h light–dark cycle (lights on at 7 a.m.). All the experimental animals were treated complying with rules of the Institutional Animal Care and Use Committee and the ethics review number was IACUC-DWZX- 2020 - 648.

The rats were divided into 0 mW/cm^2 group, 10 mW/cm^2 group, 30 mW/cm^2 group and 50 mW/cm^2 group by the stratified random method to eliminate the effects of differences in body weight. There were 35 rats in each group.

2.2. Microwave Exposure

The details of the microwave exposure system were elaborated in a previous report [23,24]. In short, S-band pulsed microwaves with a frequency of 2.856 GHz were generated by

the microwave source of a JD 2000 klystron amplifier (Vacuum Electronics Research Institute, Beijing, China). The average power densities were 10 mW/cm^2, 30 mW/cm^2 or 50 mW/cm^2, which were measured by a waveguide antenna, GX12M30A power heads and a GX12M1CHP power meter (Guanghua Microelectronics Instruments, Hefei, China). The pattern of modulation was the pulse width of 1 μs and the repetition frequency of 250 Hz with different peak power densities. The radiation time was 6 min. Before exposure, all animals were placed in the irradiation box for 5 days for adaptive training until the animals did not show resistance and automatically entered the irradiation box. During exposure, all animals were placed in the irradiation box on a radiation table. Rats in 0 mW/cm^2 group were processed in the same way as those in the exposure groups except with no microwave radiation, to eliminate other psychophysiological effects.

2.3. Morris Water Maze Test (MWM)

The MWM test was used to assess the spatial learning and memory of rodents. The test apparatus consisted of a circular stainless-steel pool (160 cm in diameter, 45 cm in height), a submerged platform (12 cm in diameter, 15 cm in height, submerged 1.5~2 cm below the water surface), a video camera and a computer. The circular pool was divided into four quadrants (defined as the first, second, third and fourth quadrants), the depth of the water was approximately 17 cm and the water temperature was 21 ± 2 °C. The platform was placed in the appropriate position in the first quadrant. A curtain surrounding the water maze was used to block light, and the video camera was placed directly above the pool. The testing system software on the computer simultaneously recorded the rats' swimming trajectories in the pool and the relevant data. The rats rely on the distal cues on the walls, such as triangle and cross, to navigate from the start location of the four quadrants.

The navigation abilities of 48 rats (12 rats in each group) were tested at 0 h, 1 d, 2 d and 3 d after microwave irradiation. Each rat was placed in the water in each of the four quadrants in turn, and when the rats entered water, they faced the maze wall. The trajectories and data of the rats were recorded. If the rat failed to find the platform within 1 min, it was placed on the platform for 5 s. Probe trials were carried out 14 d after microwave radiation. The platform was removed, the settings remained unchanged, the rats were placed in the water in the fourth quadrant, and the swimming data of crossing the platform zone were recorded for 1 min.

2.4. Transmission Electron Microscope (Tem)

At 6 h, 1 d, 3 d, 7 d and 14 d after irradiation, seven rats were randomly selected from each group, injected intraperitoneally with 1% pentobarbital sodium (30 mg/kg) for anesthesia, and decapitated, and placed on ice. The brains were removed, and the hippocampus was dissected from each cerebral hemisphere. The left hippocampus was frozen at −80 °C for quantitative measurement of related proteins. The right hippocampus was used for electron microscopy and the measurement of amino acid neurotransmitter levels.

Pieces of the hippocampus (1 mm^3) were immediately immersed in 2.5% glutaraldehyde for 2 h, fixed in 1% osmium tetroxide for 2 h, dehydrated with gradient ethanol and acetone solutions, embedded in Epon 812 resin, sliced into semithin sections, collected, sliced into ultrathin sections, and double stained with uranyl acetate and lead citrate. The ultrastructure of the tissue was observed by TEM and images were taken (H-7650, Tokyo, Japan).

2.5. Determination of Amino Acid Neurotransmitter Levels

The levels of Asp, Glu, Gly and GABA (10^{-3} mg/mL of hippocampal tissue) were measured by high-performance liquid chromatography (HPLC) 6 h, 3 d and 7 d after radiation. Before measurement, the hippocampal samples were homogenized in 10% salicylsulfonic acid and centrifuged at 15,000 rpm for 20 min at 4 °C. The supernatants were stored at −20 °C for HPLC. Every 1 μL sample was added with 5 μL O-phthalaldehyde for derivatization before injected into the HPLC detection system. The mobile phase (pH 6.8)

for HPLC consisted of 100 mM disodium hydrogen phosphate and 30% methanol. The HPLC system was used as previously described [23].

2.6. Western Blot Analysis of the Expression of NMDAR Subunits, BDNF and Related Signaling Molecules

Hippocampal tissues collected 6 h, 1 d, 3 d and 7 d after microwave radiation were removed from ice, weighed, placed at the bottom of EP tubes, quickly cut and ground with RIPA lysis buffer (20 μL/0.20 g tissue). Total protein was extracted from the tissues. The protein concentration was determined by the Pierce™ BCA Protein Assay Kit (ThermoFisher, Carlsbad, CA, USA). Then, the expression levels of NR1, NR2A, NR2B, PSD-95, cortactin, BDNF and TrkB were assessed by Western blotting according to the standard protocol. The primary antibodies were as follows: NR1 (1:1000, Abcam, Cambridge, UK), NR2A (1:1000, Abcam, Cambridge, UK), NR2B (1:1000, Abcam, Cambridge, UK), PSD-95 (1:1000, Abcam, Cambridge, UK), cortactin (1:10,000, Abcam, Cambridge, UK), BDNF (1:1000, Cambridge, UK), TrkB (1:5000, Abcam, Cambridge, UK) and GAPDH (1:10,000, Abcam, Cambridge, UK). HRP-labeled sheep anti-rabbit IgG (Beijing Zhongshan Jinqiao Biotechnology Limited Company, Beijing, China) was used as the secondary antibody. The bands were developed on autoradiographic film by using an enhanced chemiluminescence kit (Invitrogen, Carlsbad, CA, USA). The signals on the film were scanned using a high-resolution scanner, and the integral optical densities of the bands on the scanned images were quantified by AlphaVIEW SA. The densities of the target bands were normalized to the density of GAPDH for the same sample.

2.7. Statistical Analysis

All data are expressed as the mean and standard deviation. One-way ANOVA (with post hoc multiple comparisons) was used to compare the differences in the probe trials of MWM, TEM, amino acids and Western blot. Repeated-measures ANOVA were used for the analysis of average escape latency in the MWM test. Statistical analysis was performed using SPSS18.0. * indicates $p < 0.05$, and ** indicates $p < 0.01$ of the differences between 0 mW/cm^2 group and three exposure groups.

3. Results

3.1. Spatial Learning and Memory Deficits after Microwave Exposure

In the MWM experiment, the navigation trials were conducted from 3 d after microwave radiation. The average escape latency of the 50 mW/cm^2 group was significantly longer than that of the 0 mW/cm^2 group at 6 h after microwave radiation ($p < 0.05$); there was no significant difference in the average escape latency between the three radiation groups and the 0 mW/cm^2 group 1 d after microwave radiation. The average escape latency of the 30 mW/cm^2 and 50 mW/cm^2 group was significantly longer on day 2 ($p < 0.05$); and that of the 10 mW/cm^2 and 50 mW/cm^2 group was significantly longer than the 0 mW/cm^2 group 3 d after microwave radiation ($p < 0.05$) (Figure 1A).

The probe trials were conducted 14 d after microwave radiation, and it was found that the number of platform crossings was significantly reduced in the 50 mW/cm^2 group ($p < 0.05$). This result indicated that 50 mW/cm^{2}microwave radiation might impair spatial learning and memory in rats (Figure 1B).

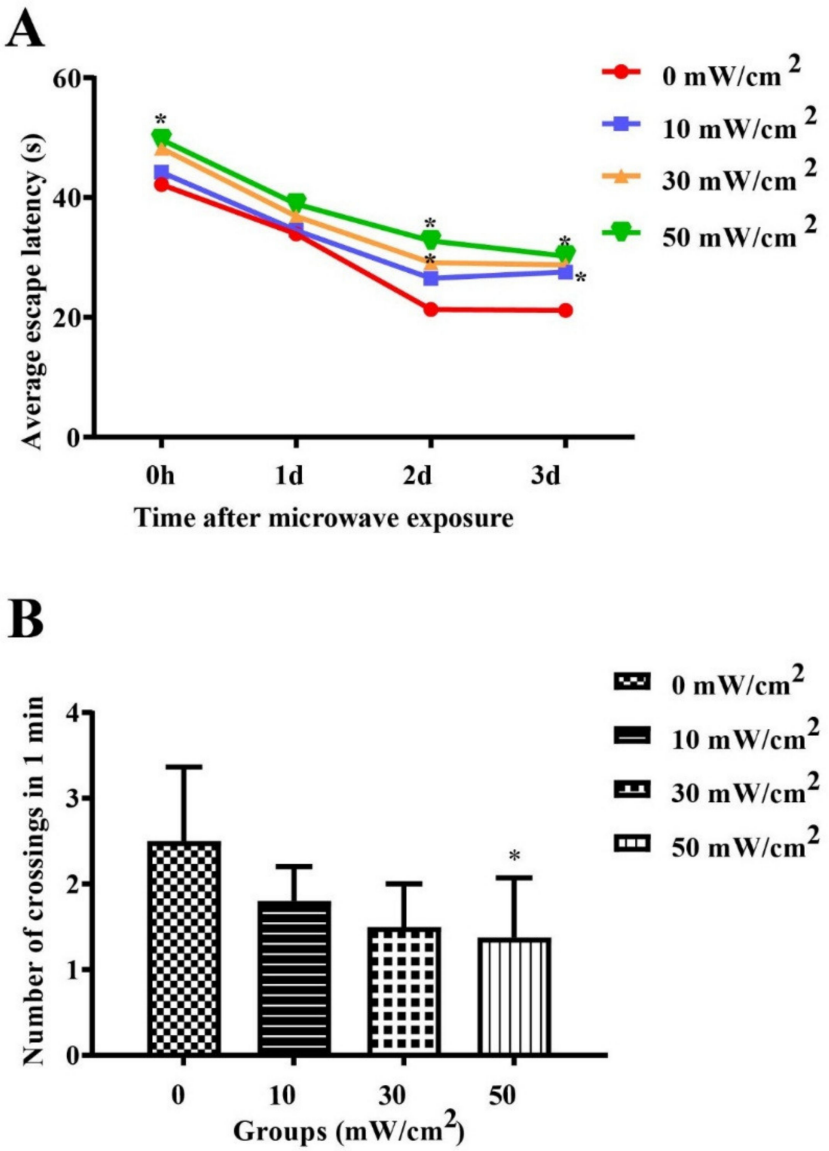

Figure 1. Effect of microwave radiation on spatial learning and memory in the MWM. (**A**) The average escape latency in the navigation test 0 h–3 d after 10, 30 and 50 mW/cm^2 microwave radiation. (**B**) The number of crossings in the probe trials 14 d after 10, 30 and 50 mW/cm^2 microwave radiation. Compared with 0 mW/cm^2 group, * $p < 0.05$.

3.2. Ultrastructural Changes in the Rat Hippocampus after Microwave Radiation

To observe the effect of microwave radiation on synaptic ultrastructure, a transmission electron microscope was used in this study. The ultrastructure of the hippocampus in 0 mW/cm^2 group was normal (Figure 2(A1)). However, the thickness and length of the PSD were increased (Figure 2(A2–4)–C) in the hippocampi of rats exposed to microwaves, especially those in the 30 and 50 mW/cm^2 groups.

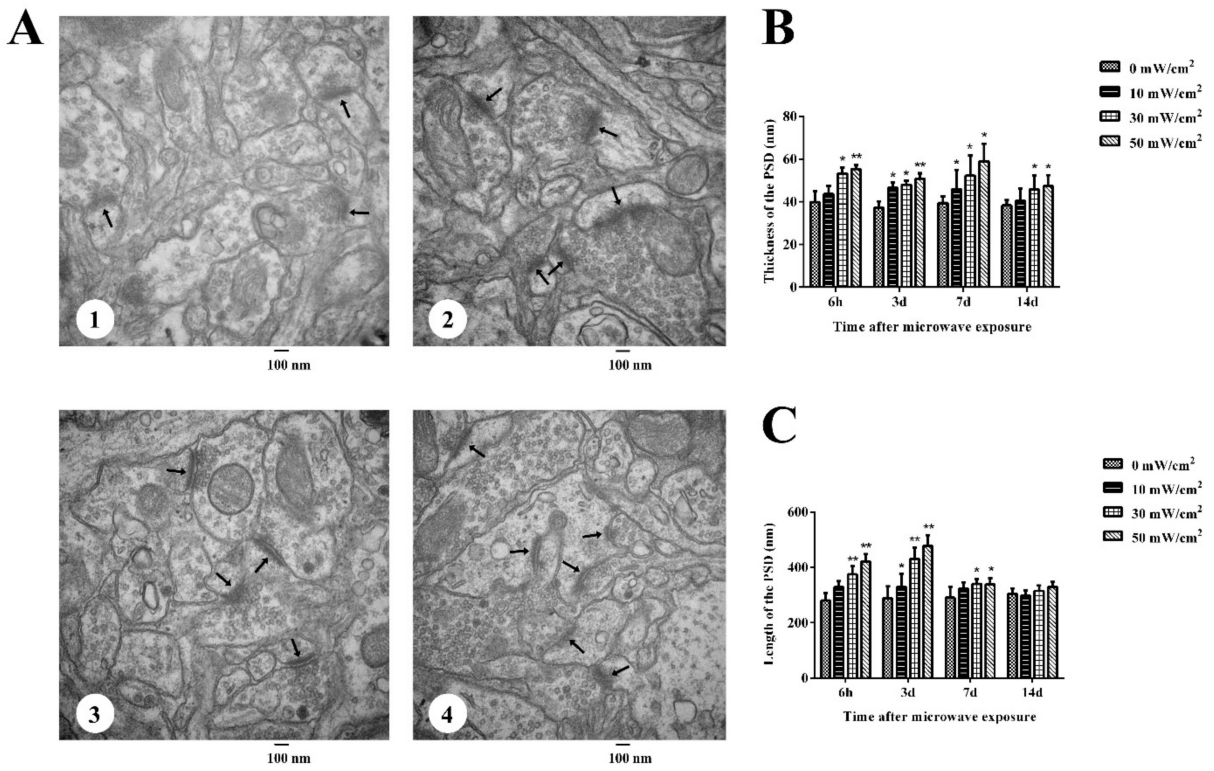

Figure 2. Hippocampal synaptic ultrastructure after microwave radiation. (**A**) 1–4: Images of 0 mW/cm^2, 10 mW/cm^2, 30 mW/cm^2 and 50 mW/cm^2 groups at 3 d after microwave radiation. Scale bars = 100 nm. (**B**,**C**) Quantitative analysis of PSD (→) thickness and length. Compared with 0 mW/cm^2 group, * $p < 0.05$, ** $p < 0.01$.

3.3. Levels of Amino Acid Neurotransmitters in the Hippocampus

Based on the results of the MWM test and hippocampal ultrastructure analysis, the levels of Asp, Glu, Gly and GABA in the hippocampus were measured at 6 h, 3 d and 7 d after microwave radiation. For Asp, the effects of 10 mW/cm^2 and 30 mW/cm^2 microwave radiation on its contents in the hippocampus were roughly the same (Figure 3A). Firstly, the levels of Asp decreased at 6 h after microwave radiation, then they were upregulated 3 d after microwave radiation, which was equivalent to that of the 0 mW/cm^2 group, and finally returned to a relatively low levels 7 d after microwave radiation. The influence of 50 mW/cm^2 microwave radiation on the levels of ASP was completely opposite to that of the 10 mW/cm^2 and 30 mW/cm^2 groups. The expression of ASP did not change at 6 h, but decreased significantly on day 3 and 7 after 50 mW/cm^2 microwave radiation compared with the 0 mW/cm^2 group ($p < 0.05$).

For Glu, the effects of 10 mW/cm^2 and 50 mW/cm^2 microwave radiation on its contents in the hippocampus were basically similar (Figure 3B). Results showed that the levels of Glu were significantly upregulated at 6 h, and tended to be stable on day 3 and 7 after 10 mW/cm^2 and 50 mW/cm^2 radiation, which were equivalent to the level of the 0 mW/cm^{2}group ($p > 0.05$). The effect of 30 mW/cm^2 microwave radiation on Glu contents was completely the opposite to that of the 10 mW/cm^2 and 50 mW/cm^2 groups. At 6 h after 30 mW/cm^2 microwave radiation, the initial level was basically the same as that of the 0 mW/cm^2 group, but then the levels of Glu were significantly and extremely significantly upregulated on day 3 ($p < 0.05$) and 7 d($p < 0.01$) after microwave radiation, respectively.

The effects of 10 mW/cm^2, 30 mW/cm^2 and 50 mW/cm^2 microwave radiation on Gly levels were almost alike (Figure 3C), showing that the contents of Glu decreased at 6 h and 3 d after radiation, and recovered to the normal level 7 d after microwave radiation ($p > 0.05$ in the three groups).

For GABA (Figure 3D), the contents decreased significantly at 6 h ($p < 0.01$), then increased on day 3 ($p < 0.01$), and finally returned to the normal level on day 7 after 10 mW/cm^2 and 30 mW/cm^2 microwave radiation. However, the effect of 50 mW/cm^2 on GABA contents was different. There was no significant difference between the 0 mW/cm^2 and 50 mW/cm^2 groups at 6 h, then it was significantly upregulated on day 3 ($p < 0.05$) and returned to normal levels on day 7 ($p < 0.01$) after 50 mW/cm^2 microwave radiation.

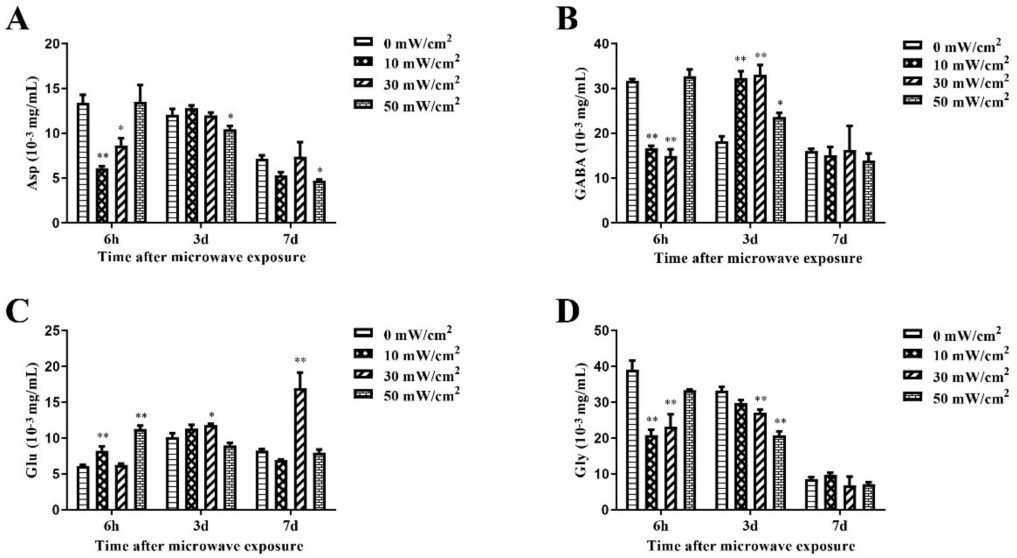

Figure 3. The levels of amino acid neurotransmitters in the rat hippocampus after microwave radiation. (**A**–**D**) Compared with 0 mW/cm^2 group, * $p < 0.05$, ** $p < 0.01$.

3.4. Effects of Microwave Exposure on the Levels of NMDAR, BDNF and Related Molecules

To investigate the mechanism underlying the effect of microwave radiation on cognition, changes in the levels of NMDAR, BDNF and related molecules at different times after exposure to different microwave radiation conditions were investigated. First, we studied the changes in NMDAR levels under different conditions, as shown in Figure 4A–C. There was no significant change in the protein expression of NR1, NR2A and NR2B between the 10 mW/cm^2 group and 0 mW/cm^2 group at 6 h, 1 d, 3 d and 7 d after microwave radiation; the expression of NR1, NR2A and NR2B in the 30 mW/cm^2 group was increased at 1 d, 3 d, 7 d after microwave radiation, and the expression of NR1, NR2A and NR2B in the 50 mW/cm^2 group was decreased at 6 h, 1 d, 3 d and 7 d after microwave radiation.

To evaluate the effect of BDNF on NMDAR, we assessed the expression of BDNF and TrkB at 6 h, 1 d and 3 d after microwave radiation. As shown in Figure 4D,E, the expression of BDNF was increased in the 30 mW/cm^2 group and decreased in the 50 mW/cm^2 group at 6 h after microwave radiation. The expression of BDNF and TrkB was increased in the 30 mW/cm^2 group and decreased in the 50 mW/cm^2 group at 1 d after microwave radiation.

The expression of the downstream molecules PSD95 and cortactin was measured 1 d, 3 d and 7 d after microwave radiation. As shown in Figure 4F,G, the expression levels of PSD95 and Cortactin were increased in the 30 mW/cm^2 group 3 d after microwave radiation and decreased in the 50 mW/cm^2 group. PSD95 showed the same trend 7 d after microwave radiation.

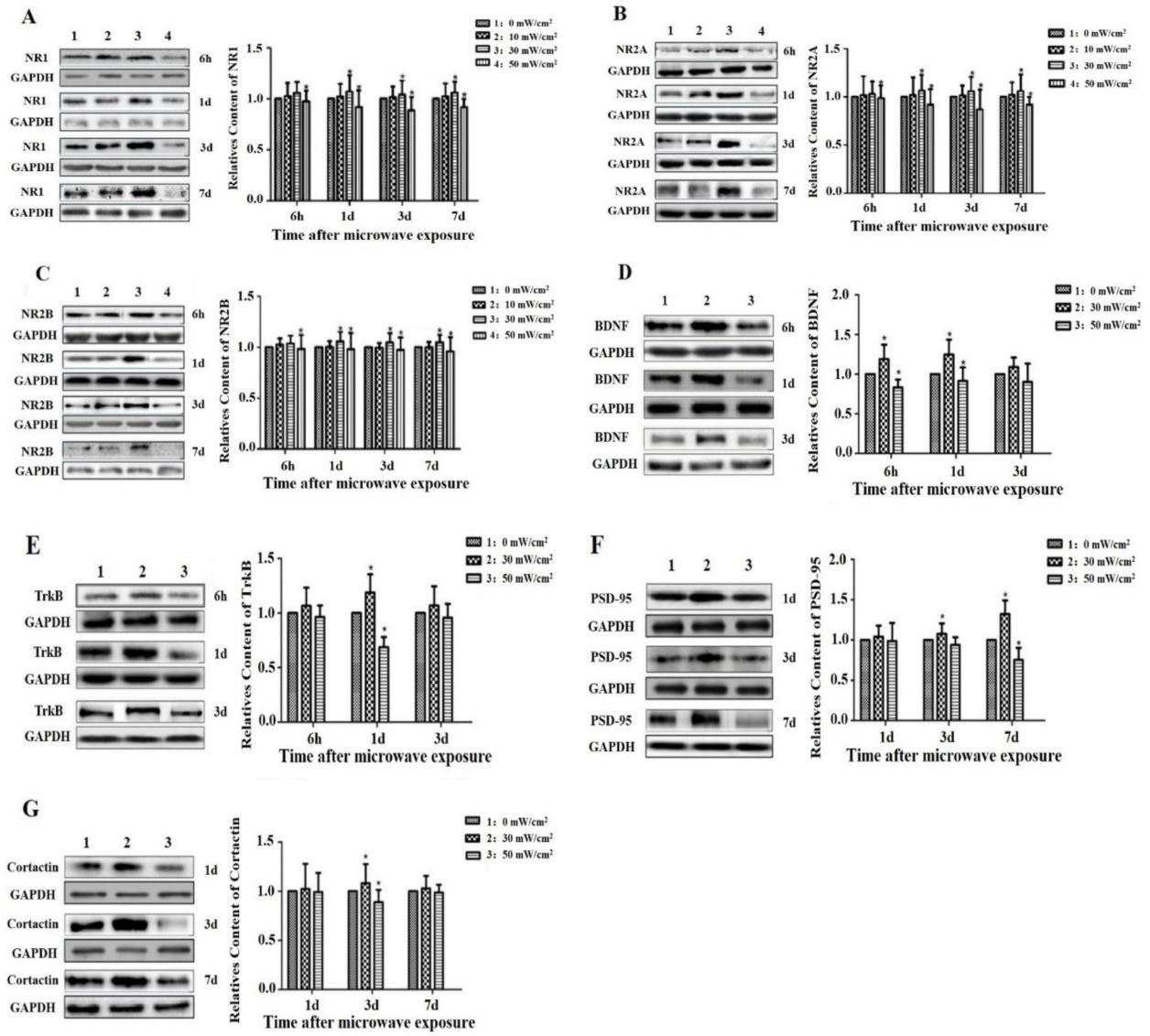

Figure 4. The protein expression of NR1, NR2A, NR2B, BDNF, TrkB, PSD95 and Cortactin after microwave radiation. (**A**) The protein expression level of NR1. (**B**) The protein expression level of NR2A. (**C**) The protein expression level of NR2B. (**D**) The protein expression level of BDNF. (**E**) The protein expression level of TrkB. (**F**) The protein expression level of PSD95. (**G**) The protein expression level of Cortactin. The data are expressed as the means and standard deviations. 1, 2, 3, 4: Compared with 0 mW/cm^2 group, * $p < 0.05$.

4. Discussion

Many studies have shown that learning and memory abilities, which are advanced functions of the brain, especially spatial memory ability, are dependent on the integrity of the hippocampus [25–27]. Cognitive impairment is one of the most important effects of microwave-induced damage [28,29]. The MWM experiment is a classic experiment used to evaluate the spatial learning and memory abilities of rodents [30–34]. Zhao L [35] found that learning and memory were impaired in Wistar rats exposed to microwaves with an average power density of 10 mW/cm^2 and an average calculated specific absorption rate (SAR) of 4.2 W/kg. Deshmukh PS [36] demonstrated that exposure to 900, 1800 or 2450 MHz microwave radiation with a SAR of 5.953×10^{-4} W/kg, 5.835×10^{-4} W/kg or 6.672×10^{-4} W/kg, respectively, for 90 d (2 h/d, 5 d/week) could lead to a decline in cognitive function. In our study, there was no significant change in the average escape latency of the 10 mW/cm^2 group, but the 30 mW/cm^2 and 50 mW/cm^2 groups exhib-

ited significantly longer average escape latencies and fewer platform crossings than the 0 mW/cm^2 group 14 d after 50 mW/cm^2 microwave radiation. The results may indicate that 30 mW/cm^2 and 50 mW/cm^2 microwave radiation can lead to cognitive dysfunction and that the greater the average power density, the more severe the cognitive dysfunction.

Amino acid neurotransmitters are widely distributed in the central nervous system [37]. According to their functions, they can be divided into excitatory and inhibitory amino acids. Glu is the most important excitatory amino acid, and GABA is the most important inhibitory amino acid, and together they regulate learning and memory functions in the brain [38,39]. Changes in amino acid neurotransmitter levels are closely related to neurodegeneration, brain injury and learning and memory [40–42]. Xiong L found that following exposure of PC12 cells to 30-mW/cm^2 microwave radiation for 5 min, the ratio of released Glu and GABA increased markedly, suggesting that Glu, as a basic excitatory neurotransmitter, might play a key role in microwave-associated synaptic plasticity impairment [24]. In another study, Wistar rats were exposed to a 2.856-GHz pulsed microwave radiation at a power density of 50 mW/cm^2 for 6 min. The results showed than Glu levels were significantly decreased at 3, 9 and 12 months after microwave exposure. The ratio of Glu and GABA was significantly decreased at 6 months. This indicated that microwave exposure can cause changes in the excitability, which may be related to the accumulation of metabolic products or the imbalance of the internal environment [43]. Our previous research revealed that following microwave exposure for 5 min at an average power density of 10 mW/cm^2, there was a significant decrease in Glu levels at 6 h, 1 d and 7 d indicating that amino acid metabolism was disturbed, which may play an important role in the neuronal damage induced by microwave exposure [44]. In this study, there was an increase in Glu levels and fluctuations in GABA levels in the 10 mW/cm^2 and 30 mW/cm^2 groups and increases in Glu and GABA levels in the 50 mW/cm^2 group. The results suggest that microwave radiation can cause the disturbance of amino acid neurotransmitters, resulting in excitability changes after exposure to 10 mW/cm^2 or 30 mW/cm^2 microwave radiation and inhibitory changes after exposure to 50 mW/cm^2 microwave radiation. These results are not consistent with those of previous studies, but the damage mechanisms may have been different due to differences in the radiation dosage and exposure time.

The hippocampus is an important component of the limbic system in humans and other vertebrates. Two of its important functions are learning and memory, which are highly sensitive to microwave exposure [45,46]. The normal morphological structure of the hippocampus is the structural basis for the maintenance of normal behavior and cognition. It has been reported that microwave radiation can induce synaptic plasticity impairment, which is characterized by a decrease in the number of synaptic vesicles, mitochondrial swelling, postsynaptic membrane perforation, abnormal postsynaptic membrane length and density distribution and rough endoplasmic reticulum expansion [3,47–49]. In our experiment, ultrastructural analysis of the hippocampus showed increases in the PSD and the length of the active area, which became more pronounced as the average power density increased. These changes may have been due to the dysfunction of amino acid neurotransmitter metabolism after microwave radiation and may have caused compensatory changes in synaptic structure.

It is well known that synaptic plasticity is the neurobiological basis of learning and memory [50]. NMDARs present on the presynaptic and postsynaptic membrane bound with glutamate released from presynaptic terminals to regulate synaptic plasticity [6,13]. NMDARs are composed of NR1 and NR2 or NR3 subunits, among which NR2A and NR2B play key roles in activity-dependent synaptic plasticity [51]. Wang H [23] studied the effects of 2.856 GHz radiation with an average power density of 2.5, 5 or 10 mW/cm^2 for 6 min/day (5 d/week, up to 6 weeks) on NMDARs expressions and phosphorylation. It was found that NR2A, NR2B and p-NR2B expression were decreased in the 10 mW/cm^2 group compared with the 0 mW/cm^2 group. In our study, the expression of NR1, NR2A and NR2B in the 10 mW/cm^2 group was not statistically significant from that in the 0 mW/cm^2 group due to the radiation exposure time; the expression levels of the NMDAR

subunits NR1, 2A and 2B were increased in the 30 mW/cm^2 group but decreased in the 50 mW/cm^2 group 1 and 3 d after microwave radiation. Therefore, we chose 30 and 50 mW/cm^2 radiation for subsequent studies.

PSD-95 affects the redistribution of the postsynaptic structural protein cortactin, regulates synaptic plasticity and promotes the recovery of learning and memory abilities. Cortactin is an F-actin-binding protein that is related to the stabilization and differentiation of actin filaments and participates in neuronal activity-dependent synaptic plasticity [52,53]. The Shank–Cortactin interaction provides a potential connection between the postsynaptic NMDAR-PSD-95 complex and the actin cytoskeleton of dendritic spines [18]. This study revealed that microwave radiation caused an increase in the expression of PSD-95 and Cortactin in the 30 mW/cm^2 group but a decrease in the expression of these proteins in the 50 mW/cm^2 group, which was in accordance with the change in NMDAR. BDNF is synthesized in the brain and is distributed in the central nervous system, which plays an important role in the survival, differentiation and growth of neurons [19,22]. Studies have found that BDNF activates NMDAR by acting on the TrkB receptor, causing cytotoxicity [15,21,54]. This study revealed that microwave radiation caused an increase in the expression of BDNF and its receptor TrkB in the 30 mW/cm^2 group and a decrease in the expression of these proteins in the 50 mW/cm^2 group. These results show that after 30 mW/cm^2 microwave exposure, BDNF expression is upregulated, and BDNF specifically binds to the TrkB receptor on the target cell membrane, activating its signal transduction pathway and possibly upregulating the expression of the NR1, NR2A and NR2B subunits and causing PSD-95 and Cortactin to anchor NMDARs to the PSD, allowing the activation of NMDAR. However, the levels of NMDAR and related proteins were decreased after 50 mW/cm^2 microwave radiation, and BDNF may have downregulated the expression of NMDAR subunits, leading to downregulated PSD activity and cognitive dysfunction.

5. Conclusions

In summary, microwave radiation (2.856 GHz, average power density of 30 or 50 mW/cm^2 for 6 min) may cause disturbances in amino acid neurotransmitters, abnormalities in synaptic structure and cognitive dysfunction, and BDNF and NMDAR play an important role in these alterations via their signaling pathways. However, further study of this mechanism is required.

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Institutional Review Board Statement: All the experimental animals were treated complying with rules of the Institutional Animal Care and Use Committee and the ethics review number was IACUC-DWZX-2020-648.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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