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Alterations of cognitive function and 5-HT system in rats after long term microwave exposure

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HIGHLIGHTS

- Long term microwave exposure induced dose-dependent cognitive function deficit.
- Among neurotransmitters, the increase of 5-HT was the main remote effect.
- 5-HT system may be involved in microwave-induced cognitive deficit.

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ABSTRACT

The increased use of microwaves raises concerns about its impact on health including cognitive function in which neurotransmitter system plays an important role. In this study, we focused on the serotonin system and evaluated the long term effects of chronic microwave radiation on cognition and correlated items. Wistar rats were exposed or sham exposed to 2.856 GHz microwaves with the average power density of 5, 10, 20 or 30 mW/cm^2 respectively for 6 min three times a week up to 6 weeks. At different time points after the last exposure, spatial learning and memory function, morphology structure of the hippocampus, electroencephalogram (EEG) and neurotransmitter content (amino acid and monoamine) of rats were tested. Above results raised our interest in serotonin system. Tryptophan hydroxylase 1 (TPH1) and monoamine oxidase (MAO), two important rate-limiting enzymes in serotonin synthesis and metabolic process respectively, were detected. Expressions of serotonin receptors including 5-HT$_{1A,\ 2A,\ 2C}$ receptors were measured. We demonstrated that chronic exposure to microwave (2.856 GHz, with the average power density of 5, 10, 20 and 30 mW/cm^2) could induce dose-dependent deficit of spatial learning and memory in rats accompanied with inhibition of brain electrical activity, the degeneration of hippocampus neurons, and the disturbance of neurotransmitters, among which the increase of 5-HT occurred as the main long-term change that the decrease of its metabolism partly contributed to. Besides, the variations of 5-HT$_{1A}$R and 5-HT$_{2C}$R expressions were also indicated. The results suggested that in the long-term way, chronic microwave exposure could induce cognitive deficit and 5-HT system may be involved in it.

1. Introduction

The increased use of microwaves in communications, industries and medical treatments raises the concern about its effects on health. Microwave with the frequency of 2.856 GHz and high power is widely used in radar and other communication devices. Long term impacts of high power microwave with the frequency of 2.856 GHz radiation on the population who occupationally exposed to radar and other communication devices should be given adequate attention. Accumulative studies show that the nervous system is a sensitive target of electromagnetic exposure, since many kinds of neurotransmitters exist in the nervous system and there is tremendous electrical activity in neural transmission [1]. Yet whether the alterations of neurochemistry and electrophysiology have functional consequences after microwave exposure is still under debate in terms of subject species, frequency, intensity, duration of irradiation and so on.

To date, a large number of studies have focused on the short term effect of acute microwave exposure. Evidences showed that microwave exposure (2450 MHz) induced deficits in spatial learning ability [2,3]. Nevertheless, few researches about the long term effects of chronic microwave exposure on cognitive functions have been conducted and the results remained controversial [4–6].

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The neurotransmitters play important roles in cognitive function. Studies indicated that most classical neurotransmitter systems are involved in learning and memory of rats in some way [7]. Whether neurotransmitters were involved in the effect of microwave radiation on cognitive function still needs to be further explored.

The serotonin system includes multiple receptors, including at least 15 serotonin (5-hydroxytryptamine, 5-HT) receptors which were divided into 7 classes (divided from 5-HT_1 to 5-HT_7 receptors) [8]. Growing number of studies reported that the serotonin system regulated learning and memory function of animals [9–13] and humans [14,15]. A capital essence of the modulation is 5-HT receptors' density during memory formation and amnesic states [16].

In this study, we examined whether microwave exposure produces a long term impairment of cognitive function, morphology structure of the hippocampus, related neurochemistry and electrophysiology. According to the results of above experiments, we then assessed the level of 5-HT synthesis and metabolism and measured the expressions of 5-$HT_{1A, 2A, 2C}$ receptors considering their important roles on cognitive function.

2. Material and methods

2.1. Animals and groups

Male Wistar rats weighting 140–160 g (4 weeks old) were obtained from the Laboratory Animal Center of Beijing Institute of Radiation Medicine (Beijing, China) and maintained at 22 ± 2 °C with a 12 hour light–dark cycle. All experiments were performed with the approval of the Institutional Animal Care and Use Committee. 75 rats were randomly divided into control group and microwave exposure groups which were classified into subgroups of different average power densities (5, 10, 20, 30 mW/cm^2) with 15 rats in each group.

2.2. Microwave exposure system and dosimetry

Pulsed microwaves at the frequency of 2.856 GHz were generated by the microwave exposure apparatus, which has been described at length in Wang's report [17]. Microwave energy was transmitted by rectangular waveguide and A16-dB standard-gain horn antenna to an electromagnetic shield chamber. The average power densities were measured with a waveguide antenna, the GX12M1CHP power meter (Guanghua Microelectronics Instruments, Hefei, China) and GX12M30A power heads.

The whole bodies of Wistar rats were sham exposed or exposed to microwaves with the average power density of 5, 10, 20 or 30 mW/cm^2 respectively for 6 min three times a week up to 6 weeks.

2.3. Behavioral test and electroencephalogram (EEG) recording

2.3.1. Morris water maze (MWM) behavioral test

The MWM test [18] was implemented to assess cognitive evaluation of rats (spatial learning and long-term memory) and the MWM apparatus used in this study was described in Qiao's study [19]. The MWM consisted of a black circular pool (150 cm in diameter) filled with clear water (23 ± 0.5 °C). The pool was surrounded by thick curtains to hide extra-maze visual cues from the rats. A movable escape platform (12 cm in diameter) was submerged 1.5 cm below the surface of the water in the center of an arbitrarily defined quadrant of the pool and remained in the same position throughout the testing.

After the completion of long-term exposure, the MWM training sessions were initiated. Rats were trained to find the submerged escape platform during four consecutive daily sessions. Four trials were given to the rats per day and began by placing the rats into one of the four starting positions in a fixed order. Rats were positioned to face the wall of the pool and released into the water. Each trial had a maximum duration of 60 s and rats that failed to locate the platform in 60 s were guided to the platform. All rats remained on the platform for 10 s before proceeding to the next trial. Rat behavior in the MWM experiments during the training and memory test procedures was digitally recorded using a computer-assisted tracking system (SLY-MWM system, Beijing Sunny Instrument Co. Ltd., Beijing, China), and the average escape latency (AEL) was analyzed. 5 days after the exposure, the platform was removed and the probe test was performed. The rats were placed in a start position which was farthest from the platform quadrant used in navigation test. The time for probe trials lasted 60 s. Percentage of time in the target quadrant was recorded and analyzed. To test the long-term effect of microwave on spatial memory, navigation tests were performed 14 and 28 days after the exposure. The time schedule of MWM test is shown in Fig. 1A.

2.3.2. EEG recording

14 days after microwave exposure, the rats of exposed and sham exposed groups were evaluated under light anesthesia conditions using a four-electrode configuration. The EEG recorded the collective activity of neurons through electrodes placed on the surface of the scalp. The EEG signals were obtained through a BIOPAC MP-150 system (USA) and power spectral analyses were performed on spontaneous EEG segments.

2.4. Hematoxylin and eosin (H&E) staining of the hippocampus

14 days, 28 days and 2 months after exposure, rats' brains were removed and fixed in 10% buffered formalin solution. Coronal brain sections (3.5 μm) including the hippocampal area were prepared for H&E staining. The brain sections were deparaffinized and rehydrated with different concentrations of xylene and alcohol, and then dipped in hematoxylin for 5 min, de-stained in 1% hydrochloric acid ethanol for several seconds, and redyed in eosin for 2 min. Following dehydration in an alcohol gradient, xylene clearance and coverslipping, stained brain sections were examined under a microscope and the hippocampi were photographed at a 200× magnification.

2.5. Measurements of neurotransmitter contents of the hippocampus and cerebrospinal fluid (CSF)

2.5.1. Sample processing and pretreatment of samples

Rats from microwave exposure and sham exposure groups were anesthetized 14 days, 28 days and 2 months after exposure. CSF samples were obtained by cisternal puncture using a 1 mL syringe. Then brains of the rats were removed and the hippocampi were dissected immediately on the ice and stored at −80 °C.

For pretreatment of samples, the hippocampi and CSF were homogenized in 10% salicylsulfonic acid (for amino acid measurements) or 5% perchloric acid (for monoamine measurements). The homogenate was centrifuged at 15,000 rpm for 20 min at 4 °C and the supernatant was transferred to a clean tube for the next test.

2.5.2. Amino acid neurotransmitter measurements

Aspartic acid (Asp), glutamic acid (Glu), glycine (Gly), taurine (Tau) and γ-aminobutyric acid (GABA) in the hippocampus and CSF were measured by high performance liquid chromatography with fluorescence detector (HPLC–FLD) procedure. The HPLC system consisted of a microbore reverse-phase column (particle size 5 μm, 150 mm × 4.6 mm; Model Venusil AA, Bonna-Agela Technologies, China), an Agilent 1100 pump (Agilent Technologies, USA) and a fluorescence detector (Agilent Technologies, USA). The mobile phase (pH 6.8) consisted of 100 mM disodium hydrogen phosphate and 30% methanol. 1 μL sample was derivated with 5 μL o-phthalaldehyde before being injected to the detection system.

2.5.3. Monoamine neurotransmitter measurements

Noradrenalin (NA), homovanillic acid (HVA), dopamine (DA), dihydroxy-phenyl acetic acid (DOPAC), and 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in the hippocampus and CSF