Associations Between a rat 5-HT_{1A} Receptor Gene Promoter Region Polymorphism (rs198585630) and Cognitive Alterations Induced by Microwave Exposure

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Abstract

The nervous system is a sensitive target of electromagnetic radiation. Chronic microwave exposure could induce cognitive deficit and 5-HT system was involved in it. Genetic polymorphisms lead to individual differences. In this study, we evaluated whether the single nucleotide polymorphism rs198585630 of 5-HT_{1A} receptor were associated with cognitive alterations of rats after microwave exposure. The transcriptional activity of 5-HT_{1A} receptor promoter including rs198585630 -C/T allele was detected *in vitro* study. Electroencephalogram, spatial learning and memory functions, the expressions of 5-HT_{1A} receptor mRNA and protein were evaluated *in vivo* study. We demonstrated that the transcriptional activity of 5-HT_{1A} receptor promoter including rs198585630-C allele was higher than that of T allele. The transcriptional activity of 5-HT_{1A} receptor promoter was stimulated by 30 mW/cm² microwave exposure, and the rs198585630-C allele was more sensitive to microwave exposure with a stronger transcriptional activation. Rats carrying rs198585630-C allele presented more expressions of 5-HT_{1A} receptor mRNA and protein, and more susceptible to 30 mW/cm² microwave exposure which showed cognitive deficits and brain electrical activity inhibition.

1. Introduction

In recent years, with the developing applications of modern technology, electromagnetic radiation (EMR) has increasingly become a public health concern. Therefore, more and more attention has been attracted to focus on the biological effects and mechanisms of EMR. The nervous system is a sensitive target of EMR, though the impact of EMR on central nervous system and the mechanisms remain unconcluded.

5-HT system regulates the brain development, cognition and emotion. Several lines of evidences have indicated a role of 5-HT and its receptors in various aspects of cognitive functions, including learning and memory [1–4]. A capital essence of the modulation is 5-HT receptors' density during memory formation and amnesic states [5, 6]. Our previous study indicated that long-term exposure to microwave (2.856 GHz, with the average power density of 10, 20 and 30mW/cm²) could induce dose-dependent deficit of brain cognitive function and the increase of 5-HT_{1A} receptor expression may mediate the disruption of spatial learning and memory caused by the microwave exposure [7].

Genetic polymorphisms lead to individual differences. Single nucleotide polymorphisms (SNPs) in the 5-HT_{1A} receptor gene promoter region regulate the expression of the 5-HT_{1A} receptor in human and animal models [8–10]. Whether SNPs of 5-HT_{1A} receptor gene played a role in the susceptibility to the biological effects of EMR is still unclear.

2. Materials And Methods

2.1. Animals and groups

Male wistar rats (n = 95) weighting 140-160g were obtained from different laboratory animal centers (Beijing, China) and maintained at $22 \pm 2^{\circ}\text{C}$ under a 12 hour light-dark cycle. Food and water were freely available.

Genomic DNA of rats was extracted from the whole blood samples with Quick Gene DNA Isolation kit (Fujifilm, Japan). The extracted genomic DNA was then amplified by polymerase chain reaction (PCR) using following primers: 5'-AGTGCCCCAGGATAGGTTAA-3' (forward); 5'-CACGTCGGAGATGCTAGTAA-3' (reverse). PCR was performed as follows: an initial 5 min at 94°C was followed by 35 cycles consisting of 50 sec at 94°C , 50 sec at 56°C and 1 min at 72°C , and then a final 10 min at 72°C terminating the PCR reaction. The amplified products (1192bp) were identified by electrophoresis on 1% agarose gel. Then the genotypes of rs198585630 were determined by direct sequencing.

Rats were grouped into 3 genotype groups according to the sequencing results: rs198585630-TT, rs198585630-TC and rs198585630-CC group. The rats of the same genotype group were then randomly divided into the control group (C) and microwave exposure group (E).

This study was approved by the Institutional Animal Care and Use Committee of Beijing Institute of Radiation Medicine.

2.2. Cell culture

PC12 cells and 293T cells were used to perform the *in vitro* functional studies. 293T cells were cultured in DMEM (Gibco, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, USA) and penicillin (100U/ml) – streptomycin (0.1mg/ml) (Thermo, USA). PC12 cell were cultured in DMEM supplemented with 10% FBS, 5% horse serum (Gibco, USA) and penicillin (100U/ml) – streptomycin (0.1mg/ml). PC12 Cells were plated in culture plates which were precoated with 0.1 mg/ml poly-D-lysine (Invitrogen, USA). All cells were kept at 37°C in a humidified atmosphere of 95% air and 5% CO_2 .

2.3. Microwave exposure

Microwaves with the frequency of 2.856 GHz and the average power density of $30\text{mW}/\text{cm}^2$ were generated by the microwave exposure apparatus which has been described in our previous study [7].

The whole bodies of rats were exposed or sham exposed to microwaves for 6 minutes three times a week up to 6 weeks. PC12 cells and 293T cells were exposed or sham exposed to microwaves for 5 minutes up to 3 times with 2 intervals of 5 minutes.

2.4. Dual-Luciferase Reporter Assay

The transcription of the rat 5-HT_{1A} receptor gene is initiated from a major site located between – 967bp nucleotide and the initial ATG codon[11]. We cloned the 967bp fragments, including SNP rs198585630, into the luciferase reporter plasmid pGL-3 Basic (Promega, USA) to generate the recombinant reporter plasmids, carrying SNP rs198585630 allele T and allele C separately (Fig. 1A), which were verified by double-digestion (Fig. 1B and Fig. 1C) and sequencing (Fig. 1D).

To determine the effect of SNP rs198585630 on 5-HT_{1A} receptor promoter activity, both versions of the promoter (allele-T or allele-C) were examined for transcriptional activity in PC12 cells and 293T cells. Each of the three reporter plasmids (pGL3-Basic, pGL3-Basic-rs198585630-T or pGL3-Basic-rs198585630-C) were transiently transfected into cells using lipofectamine 2000 (Invitrogen, USA). The pRL-TK vector was cotransfected (1: 100) to normalize the transfection efficiency. At 6 hours after the transfection, cells were exposed or sham exposed to microwaves. And 42 hours later, cells were collected and assayed for luciferase activity detection using the dual-luciferase reporter® assay system (Promega, USA), following the manufacturer's instructions.

2.5. Electrophoretic mobility shift assay (EMSA)

PC12 cells were harvested at 1 hour after microwave exposure or sham exposure. After that, the nucleoprotein of the cells was extracted using NE-PER® nuclear and cytoplasmic extraction reagents 78833 (Pierce, USA). The extracted nucleoprotein was quantified using bicinchoninic acid (BCA) protein assay and stored at -80°C for next procedures.

In silico analysis by Gene-regulation (<http://gene-regulation.com/>) and MatInspector predicted that the most likely transcription regulatory factor binded to the promoter containing rs198585630 was Nkx-2.5/Csx. The transcription factor binding site (TFBS) was gcctcTGAGtgct[t/c]aggac(SNP rs198585630 was shown in square brackets). The probes (biotin labeled or non-biotin labeled) containing SNP rs198585630 -allele T/C were used in EMSA. The double-stranded probes were generated by annealing the primer 5'-GAGCCTCTGAGTGCTTAGGACCCGCGGGAG-3' (for rs198585630-allele T) with its reversed complement primer, and the primer 5'-GAGCCTCTGAGTGCTCAGGACCCGCGGGAG-3' (for rs198585630-allele C) with its reversed complement primer (the nucleotide corresponding to SNP rs198585630 is shown in bold). The CREB probes (primer 5'-AGAGATTGCCTGACGTCAGAGAGCTAG-3' with its reversed complement primer) were used as positive control. The final concentrations of biotin labeled and non-biotin labeled deoxynucleotides were 20 fmol/μl and 4 pmol/μl respectively.

The EMSA was processed according to instructions (LightShift® chemiluminescent EMSA kit 20148, Pierce, USA). Binding reactions were performed for 20 minutes at room temperature. Each binding system (50μl) included 40 fmol biotin labeled probes, 8μg nucleoprotein of PC12 cells, 1×binding buffer, 1μg Poly (dI·dC) and 0.1pmol MgCl₂. In competition assays, 200× non-biotin labeled probes were pre-incubated with the nucleoprotein for 20 minutes at room temperature before the biotin labeled probes being added. After the binding assay, the reaction solutions were loaded onto an 4% nondenaturing polyacrylamide gel which had been pre-run at 100 V for 1 h in 0.5× TBE buffer [44.5 mM Tris base, 44.5 mM boric acid, 1 mM EDTA (pH 8.0)], resolved on the gel at 100 V, and then blotted onto Ny⁺ nylon membranes at 380 mA for 40 minutes in 0.5× TBE buffer. The membrane was placed upward on a dry paper towel when the transfer was completed. The transferred DNA was crosslinked to the membrane at a distance of approximately 5 cm from UV lamp equipped with 254nm bulbs for 30 minutes. Then the biotin-labeled DNA was detected by chemiluminescence as followed. The membrane was blocked for 15 minutes and then incubated in Streptavidin-Horseradish Peroxidase conjugate/blocking buffer solution for 15 minutes

with gentle shaking. After being washed 4 times for 5 minutes each in wash solution, the membrane was incubated in substrate equilibration buffer for 5 minutes with gentle shaking, incubated in substrate working solution for 5 minutes without shaking and then exposed to X-ray film (Kodak, Rochester, NY).

2.6. Behavioral Test and Electroencephalogram (EEG) Recording

2.6.1. Morris Water Maze (MWM) Test

The MWM apparatus consisted of a black circular pool (150 cm in diameter) surrounded by lightblue curtains and filled with clean water ($23 \pm 0.5^{\circ}\text{C}$). A movable 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 test. Rat behavior in MWM tests was digitally recorded by a SLY-MW system (Beijing Sunny Instrument Co. Ltd, Beijing, China). After the completion of the long-term exposure, rats were trained to find the platform during five consecutive daily sessions. The average escape latency (AEL) was recorded and analyzed to assess the spatial learning ability of rats. At 14th day after exposure, the platform was removed and the probe test was performed. Percentage of time when rats stayed in the target quadrant was recorded and analyzed to evaluate long memory of rats. The time schedule of MWM test is shown in Fig. 3A.

2.6.2. EEG recording

The rats were induced to be under light anesthesia conditions and the EEG of each rat was recorded before microwave exposure and then at 14th day after the exposure. The EEG signals were obtained through a BIOPAC MP-150 system (USA) and power spectral analyses were performed on spontaneous EEG segments.

2.7. Measurements of 5-HT and 5-HIAA levels of hippocampus

Rats were anaesthetized at the 14th day after the exposure. Then brains of the rats were removed and the hippocampi were dissected immediately on the ice. The hippocampi were homogenized in 5% perchloric acid on ice for 15 min and then centrifuged at 15000 rpm for 20 min at 4°C , and the supernatant was stored at -20°C for later tests. Subsequently, the 5-HT and 5-HIAA contents of the supernatants were measured by high performance liquid chromatography with electrochemical detector (HPLC–ECD) procedure. The HPLC system and the mobile phase were described in previous study [7].

2.8. Assessment of 5-HT_{1A} receptor expressions

2.8.1. qRT-PCR (quantitative real time PCR) for detection of 5-HT_{1A} receptor mRNA levels

Total RNA was extracted from dissected hippocampus samples at the 14th day after the exposure using TRIZOL reagent (Invitrogen, USA). The RNA quantification was carried out using a Nanodrop 1000 spectrometer (ThermoScientific, UK). Reverse transcription of mRNA was performed using first-strand cDNA kit AT311 (TransGen, China) and one-step RT-PCR kit AQ131 (TransGen, China) with SYBR Green (TransGen, China) according to manufacturer's instructions. Amplification was conducted using a 7500 real-time PCR system (Applied Biosystems, USA). GAPDH, a housekeeping gene, was used as an internal control to standardize 5-HT_{1A} receptor mRNA expression. Relative expression (R) of 5-HT_{1A} receptor gene were calculated using the $2^{-\Delta CT}$ method, $\Delta CT = CT_{(5-HT_{1A}R)} - CT_{(GAPDH)}$. The primers were designed using primer premier 5.0 software. Primer sequences were as follows: 5-HT_{1A} receptor forward 5'-AGTGAGGCAGGGTGACGACG-3', reverse 5'-CAGGACCAGAGCCACAATGAAA-3' (265bp). GAPDH forward 5'-GATTTGGCCGTATCGGAC-3', reverse 5'-GAAGACGCCAGTAGACTC-3' (278bp).

2.8.2. Western Blot for detection of 5-HT_{1A} receptor protein levels

At the 14th day after the exposure, 5-HT_{1A} receptor protein expressions in rats' hippocampi of microwave exposure and sham exposure groups were measured using western blot. After the completion of sampling, bicinchoninic acid (BCA) protein assay (Thermo Scientific, USA) was used for quantification of protein. The detailed procedure of western blot was performed as previously reported[12], and the antibody against 5-HT_{1A} receptor (Millipore, USA) was at 1:400 dilution. Quantification of the protein band intensity was carried out using Quantity One imaging software (Bio-Rad Laboratories, USA). Results were normalized to GAPDH (antibody at 1:5000 dilution, Kangchen Bio-tech, China).

2.9. Data analysis

Analysis of variance (ANOVA) was used to compare the differences among groups with SPSS 16.0 software. The accepted level of significance for all tests was $P < 0.05$.

3. Results

3.1. Functional analysis of rs198585630 and association with microwave induced cells alterations

3.1.1. rs198585630 modulates the transcriptional activity

Dual-luciferase reporter assay was used to investigate the transcriptional activity of the 5-HT_{1A} receptor fragments (967-bp) containing - 215 T/C allele under the circumstances of exposure and sham exposure. It turned out that the trends in PC12 cell and 293T cell were basically consistent (Fig. 2A). The luciferase activity of cells which were transfected with pGL3-rs198585630-C plasmids was higher compared to those with pGL3-rs198585630-T plasmids and pGL3-basic plasmids ($P < 0.05$). The results indicated that

the transcriptional activity of the 5-HT_{1A} receptor fragments (967-bp) containing – 215 C allele was higher than T allele.

After 30 mW/cm² exposure, the luciferase activity of cells which were transfected with the pGL3-rs198585630-C and pGL3-rs198585630-T plasmids was increased compared to those with pGL3-basic plasmids ($P < 0.05$), and the activity of cells which were transfected with pGL3-rs198585630-C was increased compared to those with pGL3-rs198585630-T plasmids ($P < 0.05$). Furthermore, following the microwave exposure, the activity of cells which were transfected with pGL3-rs198585630-C plasmids was increased than that of sham exposure groups ($P < 0.05$). And there were no significant changes in cells which were transfected with pGL3-rs198585630-T plasmids or pGL3-basic plasmids after the exposure compared to those of sham exposure groups respectively. It showed that the transcriptional activity of the fragments containing – 215 C allele was increased while the alteration didn't happen among those containing T allele after 30mW/cm² exposure.

3.1.2. rs198585630 modulates the recruitment of the transcription factor to the promoter

In order to determine if rs198585630 regulates the binding of the transcription factor to the promoter and the change after the microwave exposure, EMSA experiment was carried out. rs198585630 C/T variant site of 5-HT_{1A} receptor was biotin-labeled to probe PC12 cell nuclear proteins in EMSA. The results showed that the C and T alleles both bound at nuclear proteins, with higher levels of binding for the C allele compared to the T allele. Furthermore, binding for the labeled C probes was competitively inhibited by the appropriate unlabeled C probes but not T probes. After 30 mW/cm² microwave exposure, the binding proteins to the C and T alleles were both increased, with a more significant effect observed for the C allele (Fig. 2B). The results suggested that the binding of the transcription factor to the promoter containing C allele was stronger than that containing T allele. Also the alteration of binding after microwave exposure was more sensitive for promoter containing C allele than that containing T allele.

3.2. Association of rs198585630 with microwave-induced functional brain abnormalities

3.2.1. spatial learning and memory ability

According to repeated-measures ANOVA analysis as shown in Fig. 3C, during the training session (over the 1st day to the 5th day after the exposure), rats in TT genotype group performed prolongations in AEL compared to TC and CC genotype groups among sham exposed rats ($P < 0.05$). At the end of the training sessions (5 days after the exposure), the AEL of each group (including the control and exposure groups) was gradually decreased to a stable level, which meant rats had acquired the ability to find the platform. After exposure, rats in TC and CC genotype groups behaved longer AEL separately compared to their own genotype ($P < 0.05$), while rats in TT genotype group behaved decreased AEL ($P < 0.05$). The results of AEL suggested that in the absence of microwave exposure, rats carrying TT genotype performed lower

spatial learning abilities than those carrying TC or CC genotype. Spatial learning abilities were enhanced in TT-genotypic rats while decreased in TC and CC-genotypic rats after 30 mW/cm² microwave exposure. In probe test (Fig. 3D), the percentage of time in the target quadrant of rats in TC and CC genotype groups was separately less than those of their own genotype after exposure ($P < 0.05$). A lower percentage of time spent in the platform quadrant is interpreted as a lower level of memory retention, which meant the spatial memories of TC and CC-genotypic rats were decreased after exposure. Among exposure groups, a longer percentage of time spent in the target quadrant of TT-genotypic rats than those of TC- and CC-genotypic rats. The swimming speed did not differ among all groups ($P > 0.05$) (Fig. 3B).

3.2.2. EEG

EEG was recorded in rats (Fig. 4A), and the delta band relative power, the amplitude and frequency were assessed (Fig. 4B, 4C, 4D). At the 14th day after exposure, the amplitude and the delta band relative power of rats in all three genotype groups were increased, while the mean frequency was decreased, compared to those of their own genotype ($P < 0.05$). In advance, the delta band relative power was significantly increased presenting higher increasing in TC and CC-genotypic rats than TT-genotypic rats. Among exposure groups, the delta band relative power of rats in TC and CC genotype groups were both higher than that of TT genotype group, with no difference between TC and CC genotype groups. The EEG results suggested that long-term microwave exposure could induce the inhibition of brain electrical activity, and the alterations were more sensitive for rats of TC and CC genotype groups than TT genotype group.

3.3 Association of rs198585630 with microwave-induced alterations of 5-HT system

3.3.1 5-HT and 5-HIAA levels of hippocampus

As shown in Table 1, there was a decreasing trend in 5-HIAA content and a rising trend in 5-HT content of rats in TT genotype group after exposure without statistical difference ($P > 0.05$). 5-HIAA/5-HT ratio was significantly lower for rats in TT genotype group after exposure ($P < 0.05$). There was no significant difference of 5-HIAA content, 5-HT content and 5-HIAA/5-HT ratio between exposure and sham exposure groups for rats of TC and CC genotypes separately.

Table 1
5-HT and 5-HIAA contents in rats' hippocampi (means $\pm$ SEM).

Groups	Contents (pg/mg)		
	5-HIAA	5-HT	5-HIAA/5-HT
TT-C	75.27 $\pm$ 6.956	99.11 $\pm$ 10.27	0.79 $\pm$ 0.07
TT-E	55.75 $\pm$ 7.179	118.00 $\pm$ 14.58	0.48 $\pm$ 0.04*
TC-C	59.68 $\pm$ 8.321	101.20 $\pm$ 11.16	0.58 $\pm$ 0.05
TC-E	69.12 $\pm$ 16.50	93.00 $\pm$ 14.94	0.68 $\pm$ 0.08
CC-C	58.01 $\pm$ 8.599	107.50 $\pm$ 17.43	0.56 $\pm$ 0.05
CC-E	67.98 $\pm$ 6.538	113.60 $\pm$ 8.511	0.60 $\pm$ 0.03
Note: * $P < 0.05$ versus TT-C group.			

3.3.2 Expression of 5-HT_{1A} receptor mRNA and protein

As shown in Fig. 5A and Fig. 5B, after exposure, higher expressions of 5-HT_{1A} receptor mRNA and protein were detected in rats of TC and CC genotypes separately compared to those of sham exposed rats of their own genotypes ($P < 0.05$). Among exposure groups, higher expressions of 5-HT_{1A} receptor mRNA and protein were measured in rats of TC and CC genotype than those of TT genotype ($P < 0.05$).

4. Discussion

The 5-HT system is involved in the regulation of cognitive functions such as learning, short- and long-term memory [2, 13–15], becoming a pharmacology and genetics target in the treatment of memory disorders [5, 16]. Serotonin signaling is mediated by 14 receptor subtypes with different functional and transductional properties. The 5-HT_{1A} subtype is of particular interest, since it is one of the main mediators of the action of 5-HT and plays an important regulatory role in the 5-HT system [5, 17–19]. Perez-Garcia G et al. believed that the expression of 5-HT_{1A} receptor mRNA may be used as a specific neuromarker of explicit memory and implicit memory in important brain regions [20]. Understanding the transcription and regulation of 5-HT_{1A} receptor helps to understand its mode of action in the nervous system.

The transcription of rat 5-HT_{1A} receptor gene is mainly initiated by the sequence from – 967 bp upstream to the ATG segment of the start codon [11]. The rs198585630 site which was screened in our previous study is located in the promoter region of 5-HT_{1A} receptor gene. No functional studies on this site have been reported yet. The premise for studying the functional significance of rs198585630 as a SNP site in the promoter region is that the regulation of 5-HT_{1A} receptor expression has physiological and pathological significances. Our previous study indicated that the variations of 5-HT_{1A} receptor

expressions were involved in microwave exposure induced cognitive deficit. The rs198585630 which is located in the promoter region has the potential to regulate the expression of 5-HT_{1A} receptor. So the *in vivo* and *in vitro* experiments were conducted to study the function and the association with the individual different cognitive alterations after the microwave exposure.

In *in vitro* study of our research indicated that the transcriptional activity of the 967-bp 5-HT_{1A} receptor fragments containing -215 C allele was higher than that containing T allele. The SNP site rs198585630 may be located at the transcription factor binding site and the polymorphism of -215 T > C affected the transcription activity. Moreover, the alteration of transcriptional activity after microwave exposure was more sensitive for promoter containing C allele than that containing T allele.

Based on *in vitro* functional study of rs198585630, *in vivo* studies were carried out to explore the associations of rs198585630 with the alterations of 5-HT_{1A} receptor induced by microwave exposure. The expression levels of 5-HT_{1A} receptor mRNA and protein in hippocampus of TC and CC rats increased significantly after exposure, which were basically consistent with the results of *in vitro* experiments. The SNP site rs198585630 significantly affected the expression of 5-HT_{1A} receptor. The rs198585630 C allele is related to the higher expression of 5-HT_{1A} receptor and more sensitive to the microwave exposure. Studies in the rat and the mouse models have provided evidences that the MWM task is highly sensitive to the changes of 5-HT_{1A} receptor function [20–22]. Considering the important role of 5-HT_{1A} receptor in cognitive function, we tested the spatial learning and memory ability of rats with different genotypes and found that among rats in the sham exposure groups, the spatial learning ability of TT rats was lower than TC and CC rats. Comparing to that of the same genotype rats, the spatial learning ability of TT rats was enhanced, and the spatial memory ability was not significantly altered after the microwave exposure. The spatial learning and memory ability of TC and CC rats are both significantly reduced after the exposure.

The increase of 5-HT_{1A} receptor density is related to the decline of cognitive function [23–25], and 5-HT_{1A} receptor of hippocampal postsynaptic has a negative effect on explicit memory function [18, 26]. The stimulation of 5-HT_{1A} receptors generally produces learning impairments by interfering with memory-encoding mechanisms, while antagonists of 5-HT_{1A} receptors facilitate certain types of memory by enhancing hippocampal/cortical cholinergic and/or glutamatergic neurotransmission [20, 27, 28]. Correspondingly, we observed that rs198585630-C allele was related to higher transcriptional activity of 5-HT_{1A} receptor promoter and increased expressions of 5-HT_{1A} receptor mRNA and protein than rs198585630-T allele. And the rats carrying rs198585630-C allele were more susceptible to the spatial learning and memory deficit induced by the microwave exposure.

The 5-HIAA/5-HT ratio reflects the relative metabolic rate of 5-HT, which can be used to evaluate serotonergic system activity [29–31]. After microwave exposure, the 5-HIAA/5-HT ratio of rs198585630-TT rats decreased significantly, reflecting the decreased catabolism and increased excitability of 5-HT neurons. Combined with its performance in MWM (spatial learning function increased, memory function did not change significantly), the decrease of 5-HIAA/5-HT ratio could be some mechanism to

compensate for lower expression of 5-HT_{1A} receptors. The rat 5-HT system was then adjusted to the physiological range to maintain effective neurotransmission.

The alterations of EEG can be seen in pathological and cognitive disorders, in which the cognitive deficits occurred in close relation to the inhibition of EEG [32–34]. The monoamine-acetylcholine balance hypothesis is a theory of neurophysiological markers of the EEG and the delta frequency band reflects the increased effects of inhibitory monoamine receptor subtypes such as 5-HT_{1A} receptor [35]. The 5-HT_{1A} receptor agonist at a low dosage enhanced the EEG power in delta range [36]. The EEG results showed that after 30 mW/cm² microwave exposure, the delta band relative power of rats in TC and CC genotype groups were both higher than that of TT genotype group, which was consistent with the alterations of 5-HT_{1A} receptor expression after microwave exposure in our study described before.

In summary, the SNP site rs198585630 in the rat 5-HT_{1A} receptor promoter region is a functional site that regulates the transcription of 5-HT_{1A} receptor. The transcriptional activity of 5-HT_{1A} receptor promoter including – 215 C allele is higher than that of T allele. The transcriptional activity of 5-HT_{1A} receptor promoter was stimulated by 30 mW/cm² microwave exposure, and 5-HT_{1A} receptor rs198585630-C allele was more sensitive to microwave exposure with a stronger transcriptional activation. Rats with rs198585630-C allele presented more expressions of 5-HT_{1A} receptor mRNA and protein, more susceptible to 30 mW/cm² microwave exposure shown as cognitive deficits and brain electrical activity inhibitions than those with rs198585630-T allele. These findings suggest that rs198585630 of 5-HT_{1A} receptor is an important target for further research to explore the mechanisms of hypersensitivity to microwave exposure.

Declarations

- **Ethics approval and consent to participate**

This study was approved by the Institutional Animal Care and Use Committee of Beijing Institute of Radiation Medicine.

- **Consent for publication**

Not applicable.

- **Availability of data and material**

All data generated or analysed during this study are included in this published article. The raw data during the current study are available from the corresponding author on reasonable request.

- **Competing interests**

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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- **Authors' contributions**

Xiang-Jun Hu and Li-Feng Wang contributed to the study conception and design. Material preparation, data collection and analysis were performed by Hai-Juan Li, Yong Zou, Si-Mo Qiao, Wei-Jia Zhi, Xin-Ping Xu and Hong-Mei Zhou. The first draft of the manuscript was written by Yu Gao, Hai-Juan Li and Li-Zhen Ma. And all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Figures

The recombinant reporter plasmids carrying 5-HT1A receptor rs198585630 allele T and C A: The recombinant reporter plasmids; B: Results of double-digestion experiments of 5-HT1A receptor promoter containing -215T allele A and C allele B in pGL3-basic vector: Lane 1: Undigested fragment, Lane 2: Digested with KpnI and XhoI enzymes, M: DNA marker. C: Sequencing results of 5-HT1A receptor promoter containing -215T allele and C allele in pGL3-basic vector.

Figure 2

Functional analysis of rs198585630 and association with the alterations induced by microwave exposure A: Relative luciferase activity driven by either 5-HT1A receptor promoter -215T or C allele-pGL3 plasmids in PC12 and 293T cells treated with or without 30mW/cm2 microwave exposure. a P<0.05 versus pGL3-basic-0, b P<0.05 versus pGL3-T-0, c P<0.05 versus pGL3-C-0, a' P<0.05 versus pGL3-basic-30 mW/cm2, b' P<0.05 versus pGL3-T-30 mW/cm2. B: EMSA analysis of 5-HT1A receptor -215 T/C oligonucleotides incubated with nuclear extracts derived from PC12 cell. Lane 1: labeled CREB probe with nuclear extract of sham exposed cells; Lane 2: labeled C probe without nuclear extract; Lane 3: labeled C probe with nuclear extract of sham exposed cells; Lane 4: labeled C probe and 200 fold unlabeled C probe with nuclear extract of sham exposed cells; Lane 5: labeled C probe and 200 fold unlabeled T probe with nuclear extract of sham exposed cells; Lane 6: labeled C probe with nuclear extract of 30mW/cm2 exposed cells; Lane 7: labeled T probe with nuclear extract of sham exposed cells; Lane 8: labeled T probe with nuclear extract of 30mW/cm2 exposed cells.

Figure 3

Performances of rats in the Morris water maze test. A: Time schedule of experiments; B: Swimming speed of rats; C: Average escape latencies during navigation test. Statistical significances (repeated measures ANOVA): Effect of TC-E and TT-E group: Δ $P < 0.05$ versus sham exposure group of the same genotype; Effect of TC-C and CC-C group: # $P < 0.05$ versus TT-C group; $\boxtimes$ $P < 0.05$ versus sham exposed rats of the same genotype at corresponding time point; D: Percent time in the target quadrant in probe test. * $P < 0.05$.

Figure 4

EEG of rats at 14th day after 30 mW/cm² microwave exposure. A: Electroencephalogram; B: Delta band relative power; C: Amplitude; D: Frequency. Statistical significances (repeated measures ANOVA): Effect of TC and CC group: Δ $P < 0.05$ versus TT group. * $P < 0.05$ versus data before the exposure.

Figure 5

5-HT_{1A} receptor mRNA and protein levels of rats' hippocampi after 30 mW/cm² microwave exposure. * P<0.05. The histogram panels showed the semi-quantified data of protein levels. Each value was expressed as a ratio versus the level of TT-C group. * P<0.05.