Associations between a Polymorphism in the rat 5-HT1A Receptor Gene Promoter Region (rs198585630) and Cognitive Alterations Induced by Microwave Exposure

Hai-Juan Li
Beijing Institute of Radiation Medicine
Yu Gao
Chinese PLA General Hospital
Yong Zou
Beijing Institute of Radiation Medicine
Si-Mo Qiao
Beijing Institute of Pharmacology and Toxicology: Academy of Military Medical Sciences Institute of Pharmacology and Toxicology
Wei-Jia Zhi
Beijing Institute of Radiation Medicine
Xin-Ping Xu
Beijing Institute of Radiation Medicine
Hong-Mei Zhou
Beijing Institute of Radiation Medicine
Li-Zhen Ma
Beijing Institute of Radiation Medicine
Lifeng Wang (fangchang_14@163.com)
Beijing Institute of Radiation Medicine https://orcid.org/0000-0003-1895-8754
Xiang-Jun Hu
Beijing Institute of Radiation Medicine

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Abstract

The nervous system is a sensitive target of electromagnetic radiation (EMR). Chronic microwave exposure can induce cognitive deficits, and the 5-HT system is involved in this effect. Genetic polymorphisms lead to individual differences. In this study, we evaluated whether the single-nucleotide polymorphism (SNP) rs198585630 of the 5-HT_{1A} receptor is associated with cognitive alterations in rats after microwave exposure. The transcriptional activity of the 5-HT_{1A} receptor promoter containing the rs198585630 C/T allele was determined *in vitro*. Electroencephalograms (EEGs), spatial learning and memory, and the mRNA and protein expression of 5-HT_{1A} receptor were evaluated *in vivo*. We demonstrated that the transcriptional activity of 5-HT_{1A} receptor promoter containing the rs198585630 C allele was higher than that of 5-HT_{1A} receptor promoter containing the T allele. The transcriptional activity of the 5-HT_{1A} receptor promoter was stimulated by 30 mW/cm^2 microwave exposure, and the rs198585630 C allele was more sensitive to microwave exposure, as it showed stronger transcriptional activation. Rats carrying the rs198585630 C allele exhibited increased mRNA and protein expression of 5-HT_{1A} receptor and were more susceptible to 30 mW/cm^2 microwave exposure, showing cognitive deficits and inhibition of brain electrical activity.

1. Introduction

In recent years, with the development of modern technology, electromagnetic radiation (EMR) has increasingly become a public health concern. Therefore, increasing attention has been paid to the biological effects and mechanisms of EMR. The nervous system is a sensitive target of EMR, although the impact of EMR on the central nervous system and the associated mechanisms remain unclear.

The 5-HT system regulates brain development, cognition and emotion. Several lines of evidence have indicated a role for 5-HT and its receptors in various aspects of cognitive functions, including learning and memory [1–4]. The main mechanism underlying the modulatory effects of 5-HT is an alteration in 5-HT receptor density during memory formation and in amnesic states [5,6]. Our previous study indicated that long-term exposure to microwaves (2.856 GHz, average power densities of 10, 20 and 30 mW/cm^2) can induce dose-dependent deficits in brain cognitive function, and an increase in 5-HT_{1A} receptor expression may mediate the disruption of spatial learning and memory caused by 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 the 5-HT_{1A} receptor gene play a role in susceptibility to the biological effects of EMR is still unclear.

2. Materials And Methods

2.1. Animals and groups

Male Wistar rats (n = 95) weighing 140–160 g were obtained from different laboratory animal centers (Beijing, China) and maintained at 22 ± 2°C under a 12-hour light-dark cycle. Food and water were freely available.

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

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

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 (100 U/ml)/streptomycin (0.1 mg/ml) (Thermo, USA). PC12 cells were cultured in DMEM supplemented with 10% FBS, 5% horse serum (Gibco, USA) and penicillin (100 U/ml)/streptomycin (0.1 mg/ml). PC12 cells were plated in culture plates that 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 a frequency of 2.856 GHz and an average power density of 30 mW/cm^2 were generated by the microwave exposure apparatus described in our previous study [7].

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

2.4. Dual-luciferase reporter assay

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

To determine the effect of the SNP rs198585630 on 5-HT_{1A} receptor promoter activity, the transcriptional activity of both versions of the promoter (the T allele and C allele) was examined in PC12 cells and 293T cells. Each of the three reporter plasmids (pGL3-Basic, pGL3-Basic-rs198585630-T and pGL3-Basic-rs198585630-C) was 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 transfection, the cells were exposed or sham-exposed to microwaves. Forty-two hours later, the cells were collected, and luciferase activity was measured 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 1 hour after microwave exposure or sham-exposure. After that, nucleoprotein was extracted from the cells using NE-PER® nuclear and cytoplasmic extraction reagents 78833 (Pierce, USA). The concentration of the extracted nucleoprotein was quantified using a bicinchoninic acid (BCA) assay, and the nucleoprotein was stored at -80°C for subsequent studies.

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

The EMSA was performed according to the manufacturer's instructions (LightShift® Chemiluminescent EMSA Kit, 20148, Pierce, USA). Binding reactions were performed for 20 min at room temperature. Each binding system (50 μl) included 40 fmol biotin-labeled probes, 8 μg nucleoprotein from PC12 cells, 1× binding buffer, 1 μg poly(dI•dC) and 0.1 pmol $MgCl_2$. In the competition assays, 200× non-biotin-labeled probes were preincubated with nucleoprotein for 20 min at room temperature before the biotin-labeled probes were added. After the binding assay, the reaction solutions were loaded onto a 4% nondenaturing polyacrylamide gel that had been prerun 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 min in 0.5× TBE buffer. The membrane was placed facing 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 a UV lamp equipped with 254 nm bulbs for 30 minutes. Then, the biotin-labeled DNA was detected by chemiluminescence as follows. 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 light blue curtains and filled with clean water (23 ± 0.5°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 the MWM test was digitally recorded with a SLY-MW system (Beijing Sunny Instrument Co. Ltd, Beijing, China). After completion of the long-term exposure protocol, rats were trained to find the platform in five consecutive daily sessions. The average escape latency (AEL) was recorded and analyzed to assess the spatial learning ability of the rats. On the 14th day after exposure, the platform was removed, and the probe test was performed. The percentage of time that the rats stayed in the target quadrant was recorded and analyzed to evaluate the long-term memory of the rats. The time schedule of the MWM test is shown in Fig. 3A.

2.6.2. EEG recording

The rats were lightly anesthetized, and EEGs were recorded from each rat before microwave exposure and then on the 14th day after exposure. EEG signals were obtained with a BIOPAC MP-150 system (USA), and power spectral analyses were performed on spontaneous EEG segments.

2.7. Measurement of 5-HT and 5-HIAA levels in the hippocampus

Rats were anaesthetized on the 14th day after exposure. Then, the brains of the rats were removed, and the hippocampi were dissected immediately on 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 analysis. Subsequently, the 5-HT and 5-HIAA contents in the supernatants were measured by high-performance liquid chromatography with an electrochemical detector (HPLC–ECD). The HPLC system and the mobile phase were described in a previous study [7].

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

2.8.1. Quantitative real-time PCR (qRT-PCR) analysis of 5-HT_{1A} receptor mRNA levels

Total RNA was extracted from dissected hippocampal samples on the 14th day after exposure using TRIzol reagent (Invitrogen, USA). RNA levels were quantified using a Nanodrop 1000 spectrometer (Thermo Scientific, UK). Reverse transcription of mRNA was performed using a First-Strand cDNA Kit (AT311, TransGen, China) and a One-Step RT-PCR Kit (AQ131, TransGen, China) with SYBR Green (TransGen, China) according to the 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 the 5-HT_{1A} receptor gene was calculated using the $2^{-\triangle CT}$ method ($\triangle CT = CT_{(5-HT1AR)}-CT_{(GAPDH)}$). The primers were designed using Primer Premier 5.0 software. The primer sequences were as follows: 5-HT_{1A} receptor forward, 5'-AGTGAGGCAGGGTGACGACG-3', and reverse, 5'-CAGGACCAGAGCCACAATGAAA-3' (265 bp); GAPDH forward, 5'-GATTTGGCCGTATCGGAC-3', and reverse 5'-GAAGACGCCAGTAGACTC-3' (278 bp).

2.8.2. Western blot analysis of 5-HT_{1A} receptor protein levels

On the 14th day after exposure, 5-HT_{1A} receptor protein expression in the hippocampi of rats in the microwave exposure and sham exposure groups was measured using western blotting. After completion of sampling, a BCA assay (Thermo Scientific, USA) was used for quantification of the protein concentration. Western blotting was performed as previously reported [12], and an antibody against the 5-HT_{1A} receptor (Millipore, USA) was used at 1:400 dilution. Quantification of the protein band intensity was carried out using Quantity One imaging software (Bio-Rad Laboratories, USA). The results were normalized to GAPDH levels (antibody diluted 1:5000, 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 its association with microwave-induced cell alterations

3.1.1. rs198585630 modulates transcriptional activity

A dual-luciferase reporter assay was used to investigate the transcriptional activity of 5-HT_{1A} receptor fragments (967 bp) containing the − 215 T/C allele under exposure and sham-exposure conditions. The trends observed in PC12 cells and 293T cells were basically consistent (Fig. 2A). The luciferase activity of cells transfected with the pGL3-rs198585630-C plasmid was higher than that of cells transfected with the pGL3-rs198585630-T plasmid and pGL3-basic plasmid ($P < 0.05$). The results indicated that the

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

After 30 mW/cm^2 microwave exposure, the luciferase activity of cells transfected with the pGL3-rs198585630-C and pGL3-rs198585630-T plasmids was increased compared to that of cells transfected with the pGL3-basic plasmid ($P < 0.05$), and the activity of cells transfected with pGL3-rs198585630-C was increased compared to that of cells transfected with the pGL3-rs198585630-T plasmid ($P < 0.05$). Furthermore, following microwave exposure, the activity of cells transfected with the pGL3-rs198585630-C plasmid was increased compared with that of the sham exposure groups ($P < 0.05$). There were no significant changes between cells transfected with the pGL3-rs198585630-T plasmid or pGL3-basic plasmid after exposure and cells in the sham exposure groups. The results showed that the transcriptional activity of the fragments containing the − 215 C allele was increased after 30 mW/cm^2 microwave exposure, while this alteration was not observed for fragments containing the T allele.

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

To determine whether rs198585630 regulates the binding of the transcription factor to the promoter and the change after microwave exposure, the EMSA was carried out. The rs198585630 C/T variant site of the 5-HT_{1A} receptor was labeled with biotin to detect PC12 cell nuclear proteins in the EMSA. The results showed that the C and T alleles both bound to nuclear proteins, with higher levels of binding for the C allele than for the T allele. Furthermore, binding to the labeled C probes was competitively inhibited by the appropriate unlabeled C probes but not the unlabeled T probes. After 30 mW/cm^2 microwave exposure, the amount of proteins bound to the C and T alleles was bothincreased, 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 the C allele was stronger than that to the promoter containing the T allele. Additionally, the alteration in binding after microwave exposure was more sensitive for promoters containing the C allele than those containing the T allele.

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

3.2.1. Spatial learning and memory

According to repeated measures ANOVA, during the training session (the 1st day to the 5th day after the exposure), the sham-exposed rats in the TT genotype group exhibited a longer AEL than those in the TC and CC genotype groups ($P < 0.05$), as shown in Fig. 3C. At the end of the training sessions (5 days after exposure), the AEL of each group (including the control and exposure groups) gradually decreased to a stable level, indicating that rats had acquired the ability to find the platform. The rats in the TC and CC genotype groups exposed to microwaves exhibited longer AELs than control rats of the same genotype ($P < 0.05$), while rats in the TT genotype group exhibited a decreased AEL ($P < 0.05$). Analysis of AELs

suggested that in the absence of microwave exposure, rats of the TT genotype exhibited poorer spatial learning ability than those of the TC or CC genotype. Spatial learning ability was enhanced in rats of the TT genotype but decreased in rats of the TC and CC genotypes after 30 mW/cm^2 microwave exposure. In the probe test (Fig. 3D), the percentage of time spent in the target quadrant by rats in the TC and CC genotype groups was less than that by rats of the same genotype after exposure ($P < 0.05$). A lower percentage of time spent in the platform quadrant indicates poorer memory retention; thus, the results suggested that the spatial memory of rats of the TC and CC genotypes was decreased after exposure. Among the exposure groups, rats of the TT genotype spent a greater percentage of time in the target quadrant than rats of the TC and CC genotypes. Swimming speed did not differ between any of the groups ($P > 0.05$) (Fig. 3B).

3.2.2. EEG

EEGs were recorded from rats (Fig. 4A), and the delta band relative power, amplitude and frequency were assessed (Fig. 4B, 4C, 4D). On the 14th day after exposure, the amplitude and the delta band relative power were increased and the mean frequency was decreased in the rats of all three genotypes that were exposed to microwaves compared to control rats of the same genotype ($P < 0.05$). In advance, the delta band relative power was significantly increased, and the increase was greater in rats of the TC and CC genotypes than those of the TT genotype. Among the exposure groups, the delta band relative power of rats in the TC and CC genotype groups was higher than that of rats in the TT genotype group, and there was no difference between the TC and CC genotype groups. The EEG results suggested that long-term microwave exposure inhibited brain electrical activity and that rats in TC and CC genotype groups were more sensitive to these changes than those in the TT genotype group.

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

3.3.1 5-HT and 5-HIAA levels in the hippocampus

As shown in Table 1, there was a decreasing trend in 5-HIAA content and an increasing trend in 5-HT content in TT genotype group after exposure, although the differences were not significant ($P > 0.05$). The 5-HIAA/5-HT ratio was significantly lower in the TT genotype group exposed to microwaves than control rats in the TT genotype group ($P < 0.05$). There was no significant difference in 5-HIAA content, 5-HT content or the 5-HIAA/5-HT ratio between the exposure and sham exposure groups in rats of the TC and CC genotypes.

Table 1
5-HT and 5-HIAA contents in the rat hippocampus (means ± SEMs).

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

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

As shown in Fig. 5A and Fig. 5B, mRNA and protein expression of 5-HT_{1A} receptor was higher in rats of the TC and CC genotypes exposed to microwaves than sham-exposed rats of the same genotype ($P < 0.05$). Rats in the TC and CC genotype groups exhibited higher mRNA and protein expression levels of 5-HT_{1A} receptor than the TT genotype group ($P < 0.05$).

4. Discussion

The 5-HT system is involved in the regulation of cognitive functions such as learning and short- and long-term memory [2,13–15] and thus has become a pharmacological and genetic target for the treatment of memory disorders [5,16]. Serotonin signaling is mediated by 14 receptor subtypes with different functional and transduction 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. speculated that the mRNA expression of 5-HT_{1A} receptor in important brain regions may be used as a specific neuromarker of explicit memory and implicit memory [20]. Elucidating the transcription and regulation of 5-HT_{1A} receptor will help to determine its mode of action in the nervous system.

The transcription of the 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 site of rs198585630, which was identified in our previous study, is located in the promoter region of the 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 in the promoter region is that regulation of 5-HT_{1A} receptor expression has physiological and pathological significance. Our previous study indicated that variations in 5-HT_{1A} receptor expression are

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

An *in vitro* study indicated that the transcriptional activity of 967-bp 5-HT_{1A} receptor fragments containing the − 215 C allele was higher than that of 967-bp 5-HT_{1A} receptor fragments containing the T allele. The SNP rs198585630 may be located at the TFBS, and the − 215 T > C polymorphism affected transcription activity. Moreover, promoters containing the C allele were more sensitive to alterations in transcriptional activity after microwave exposure than those containing the T allele.

Based on the *in vitro* study of rs198585630 function, *in vivo* studies were carried out to explore the associations between rs198585630 and alterations in 5-HT_{1A} receptor induced by microwave exposure. The mRNA and protein expression levels of 5-HT_{1A} receptor in the rats' hippocampi of the TC and CC genotypes increased significantly after exposure, which was basically consistent with the results of *in vitro* experiments. rs198585630 significantly affected the expression of 5-HT_{1A} receptor. The rs198585630 C allele is related to higher expression of 5-HT_{1A} receptor and more sensitive to microwave exposure. Studies in rat and mouse models have provided evidence that performance in the MWM test is highly sensitive to changes in 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 of rats of different genotypes and found that among rats in the sham exposure groups, the spatial learning ability of rats of the TT genotype was lower than that of rats of the TC and CC genotypes. The spatial learning ability of the TT genotype exposed to microwaves was enhanced compared to that of control of the same genotypes, whereas spatial memory was not significantly altered after microwave exposure. The spatial learning and memory of the TC and CC genotypes were both significantly reduced after exposure.

An increase in 5-HT_{1A} receptor density is related to a decline in cognitive function [23–25], and 5-HT_{1A} receptor expressed on hippocampal postsynaptic neurons has a negative effect on explicit memory function [18,26]. Stimulation of 5-HT_{1A} receptors generally results in 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]. Similarly, we observed that compared with the rs198585630 T allele, the rs198585630 C allele was related to higher transcriptional activity of the 5-HT_{1A} receptor promoter and increased mRNA and protein expression of 5-HT_{1A} receptor. Rats carrying the rs198585630 C allele were more susceptible to the spatial learning and memory deficits induced by 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 in rs198585630 TT rats was significantly decreased, reflecting decreased catabolism and increased excitability of 5-HT neurons. Considering the performance of the rats in the MWM test (increased spatial learningand no

significantly change in memory), the decrease in the 5-HIAA/5-HT ratio may represent a mechanism compensating for decreased expression of 5-HT_{1A} receptor and leading to a return of the 5-HT system to the physiological range to maintain effective neurotransmission.

Alterations in EEGs can be seen in pathology and cognitive disorders, as these conditions involve cognitive deficits that are closely related to inhibition of EEG activity [32–34]. The monoamine-acetylcholine balance hypothesis is a theory related to neurophysiological markers on EEG, and an increased delta frequency band reflects an increase in the effects of inhibitory monoamine receptor subtypes such as 5-HT_{1A} receptor [35]. At a low dosage, a 5-HT_{1A} receptor agonist enhances EEG power in delta rage [36]. The EEG results showed that after 30 mW/cm^2 microwave exposure, the delta band relative powers of rats in the TC and CC genotype groups were higher than that of rats in the TT genotype group, which was consistent with the alterations in 5-HT_{1A} receptor expression after microwave exposure described previously.

In summary, the rs198585630 site 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 the 5-HT_{1A} receptor promoter containing the − 215 C allele is higher than that of the 5-HT_{1A} receptor promoter containing the T allele. The transcriptional activity of the 5-HT_{1A} receptor promoter was stimulated by 30 mW/cm^2 microwave exposure, and the 5-HT_{1A} receptor rs198585630 C allele was more sensitive to microwave exposure, showing stronger transcriptional activation. Rats with the rs198585630 C allele presented higher mRNA and protein expression of 5-HT_{1A} receptor and were more susceptible to 30 mW/cm^2 microwave exposure, as indicated by cognitive deficits and brain electrical activity inhibition, than those with the rs198585630 T allele. These findings suggest that the SNP rs198585630 of the 5-HT_{1A} receptor is an important target for further research exploring 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 analyzed during this study are included in this published article. The raw data used in 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 and 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. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Figures

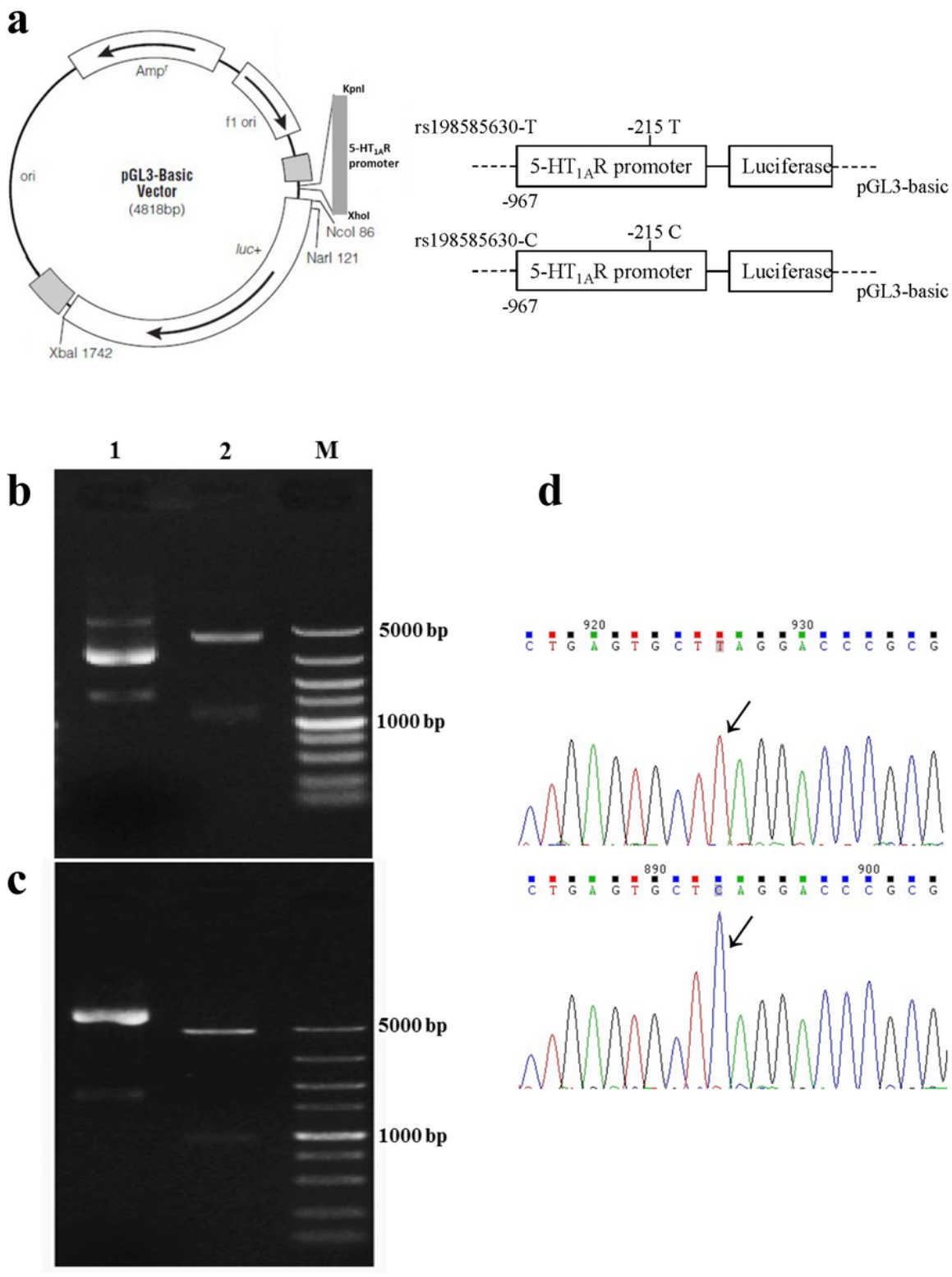

Figure 1

Recombinant reporter plasmids carrying the 5-HT1A receptor rs198585630 T and C alleles A: The recombinant reporter plasmids. B: Results of double digestion of the 5-HT1A receptor promoter containing the -215 T allele (A) and C allele (B) in the pGL3-basic vector. Lane 1: Undigested fragment;

lane 2: digested with KpnI and XhoI enzymes; M: DNA marker. C: Sequencing of the 5-HT1A receptor promoter containing the -215T allele and the C allele in the pGL3-basic vector.

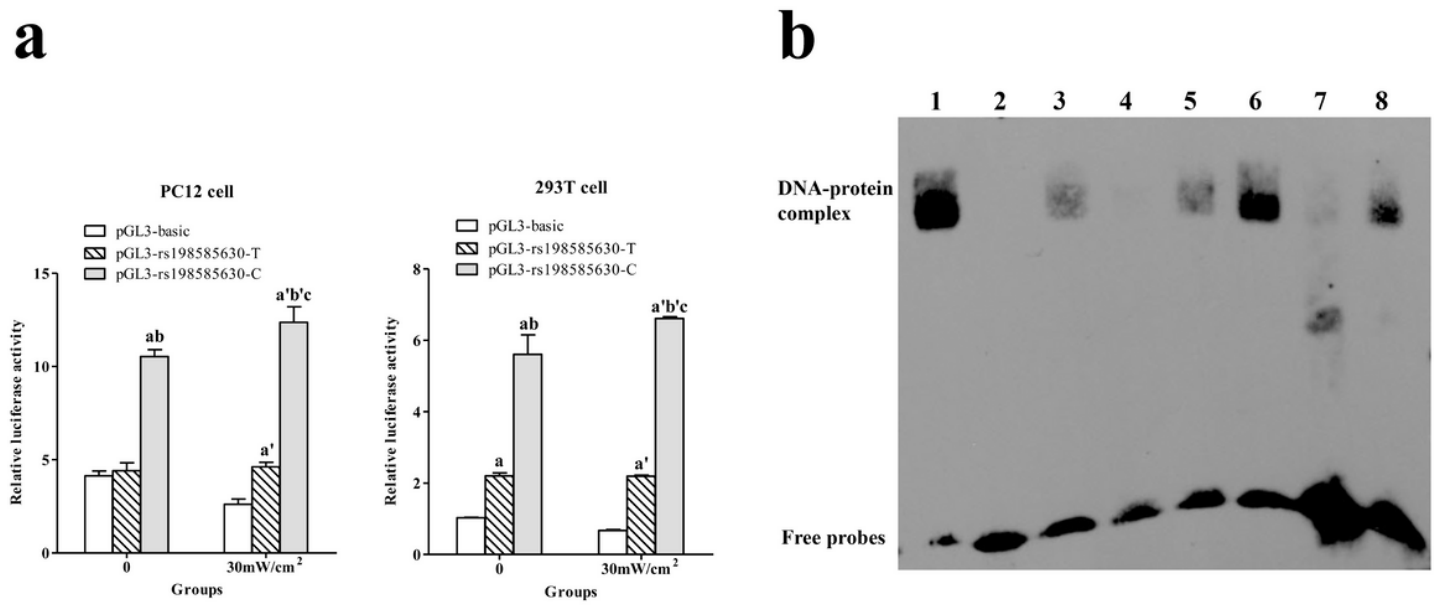

Figure 2

Functional analysis of rs198585630 and its association with the alterations induced by microwave exposure A: Relative luciferase activity driven by either the 5-HT1A receptor promoter -215T or C allele-pGL3 plasmid in PC12 and 293T cells treated with or without 30 mW/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: Analysis of 5-HT1A receptor -215 T/C oligonucleotides incubated with nuclear extracts derived from PC12 cells by the EMSA. Lane 1: labeled CREB probe with nuclear extract from sham exposed cells; lane 2: labeled C probe without nuclear extract; lane 3: labeled C probe with nuclear extract from sham exposed cells; lane 4: labeled C probe and 200-fold unlabeled C probe with nuclear extract from sham exposed cells; lane 5: labeled C probe and 200-fold unlabeled T probe with nuclear extract from sham exposed cells; lane 6: labeled C probe with nuclear extract from 30 mW/cm2-exposed cells; lane 7: labeled T probe with nuclear extract from sham exposed cells; lane 8: labeled T probe with nuclear extract from 30 mW/cm2-exposed cells.

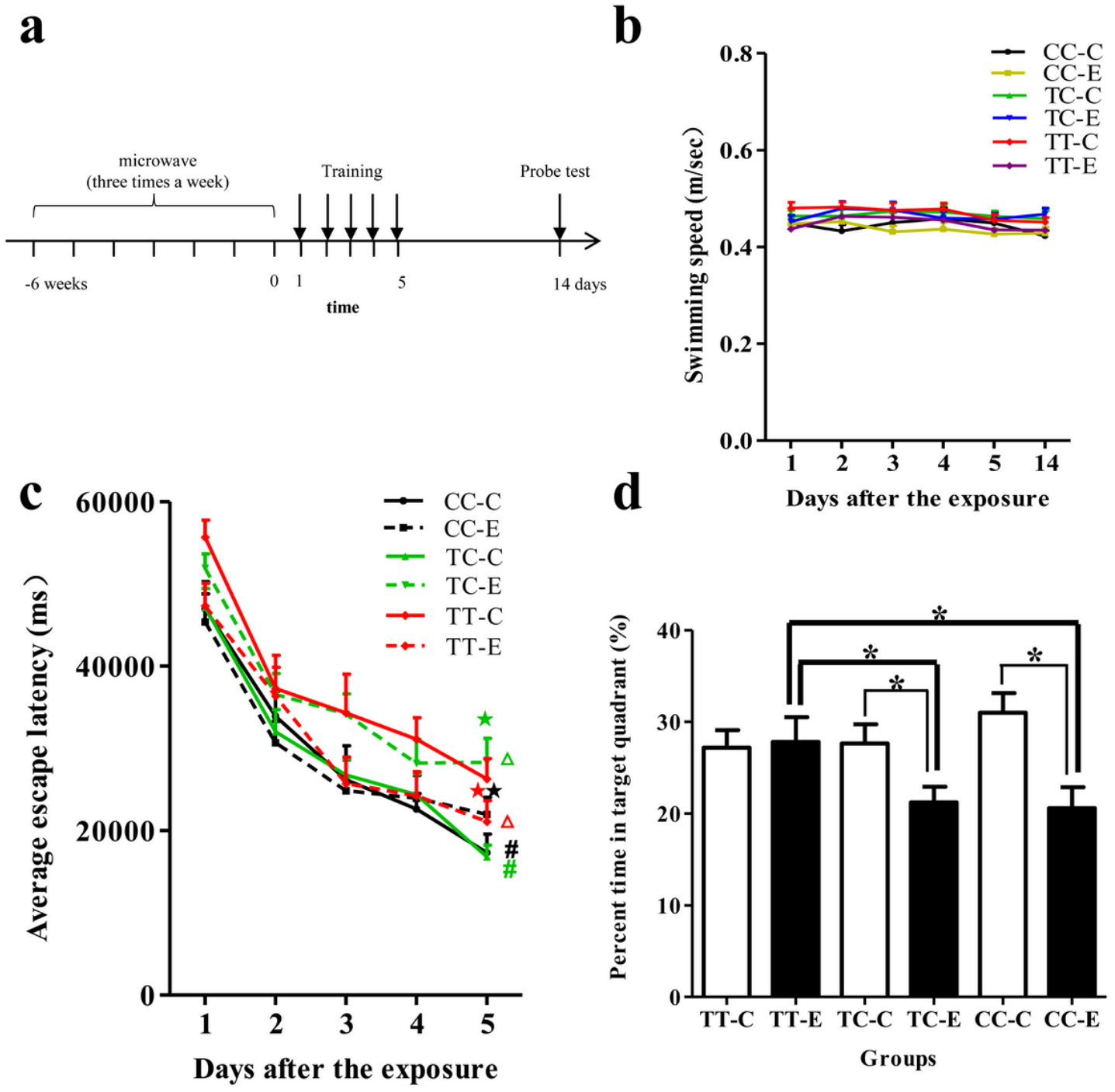

Figure 3

Performance of rats in the MWM test. A: Time schedule of the experiments. B: Swimming speed of the rats. C: AELs in the navigation test. Statistical significance (repeated measures ANOVA); changes in the exposed TC genotype (TC-E) and exposed TT genotype (TT-E) groups: △ $P<0.05$ versus the sham exposure group of the same genotype; changes in the control TC genotype (TC-C) and control CC genotype (CC-C) groups: # $P<0.05$ versus the control TT genotype (TT-C) group; ★ $P<0.05$ versus sham exposed rats of the same genotype at the corresponding time point; D: percentage of time spent in the target quadrant in the probe test. * $P<0.05$.

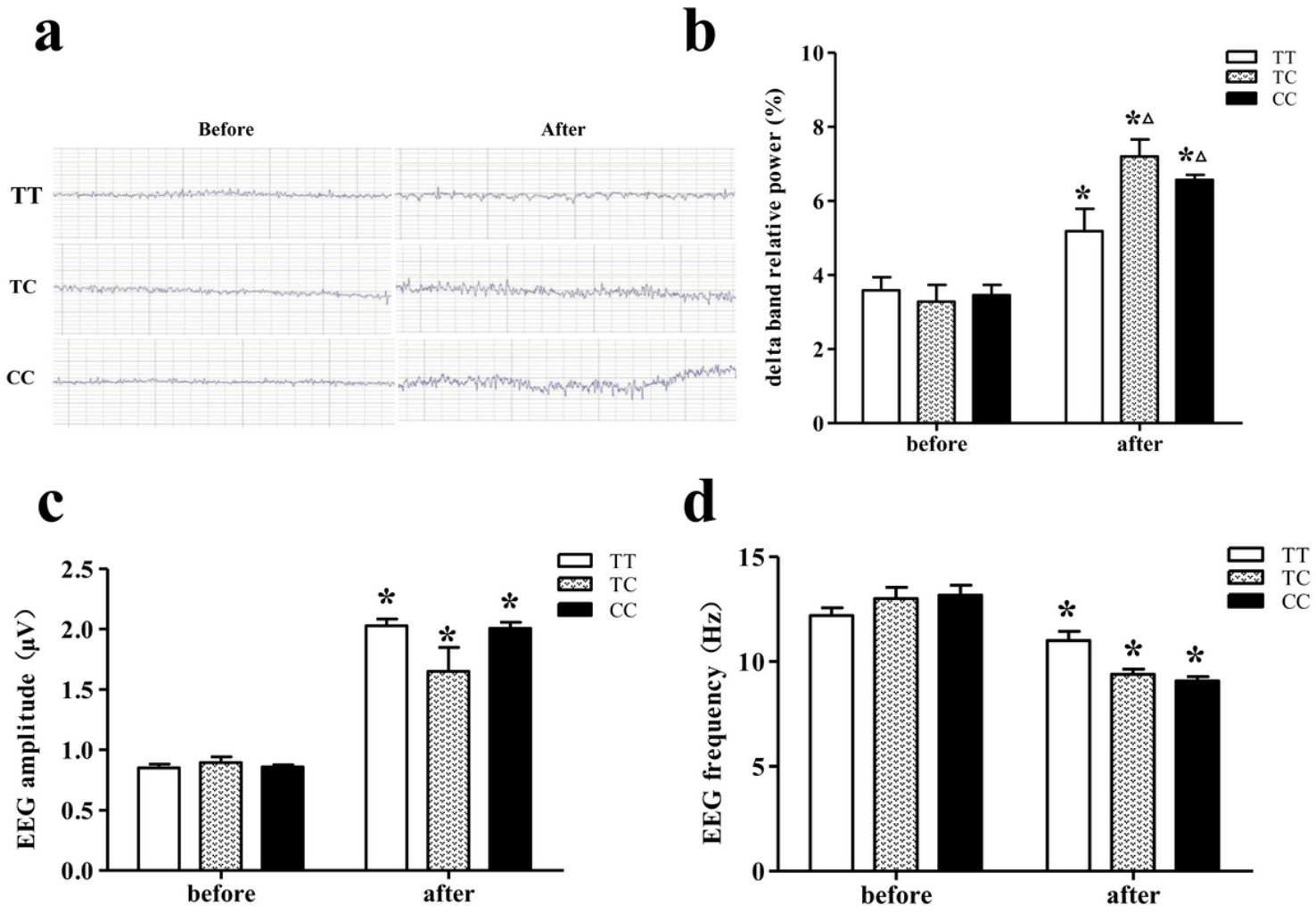

Figure 4

EEGs from rats on the 14th day after 30 mW/cm2 microwave exposure. A: EEG. B: Delta band relative power. C: Amplitude. D: Frequency. Statistical significance (repeated measures ANOVA); changes in the TC and CC genotype groups: △ $P<0.05$ versus the TT genotype group.* $P<0.05$ versus prior to exposure.

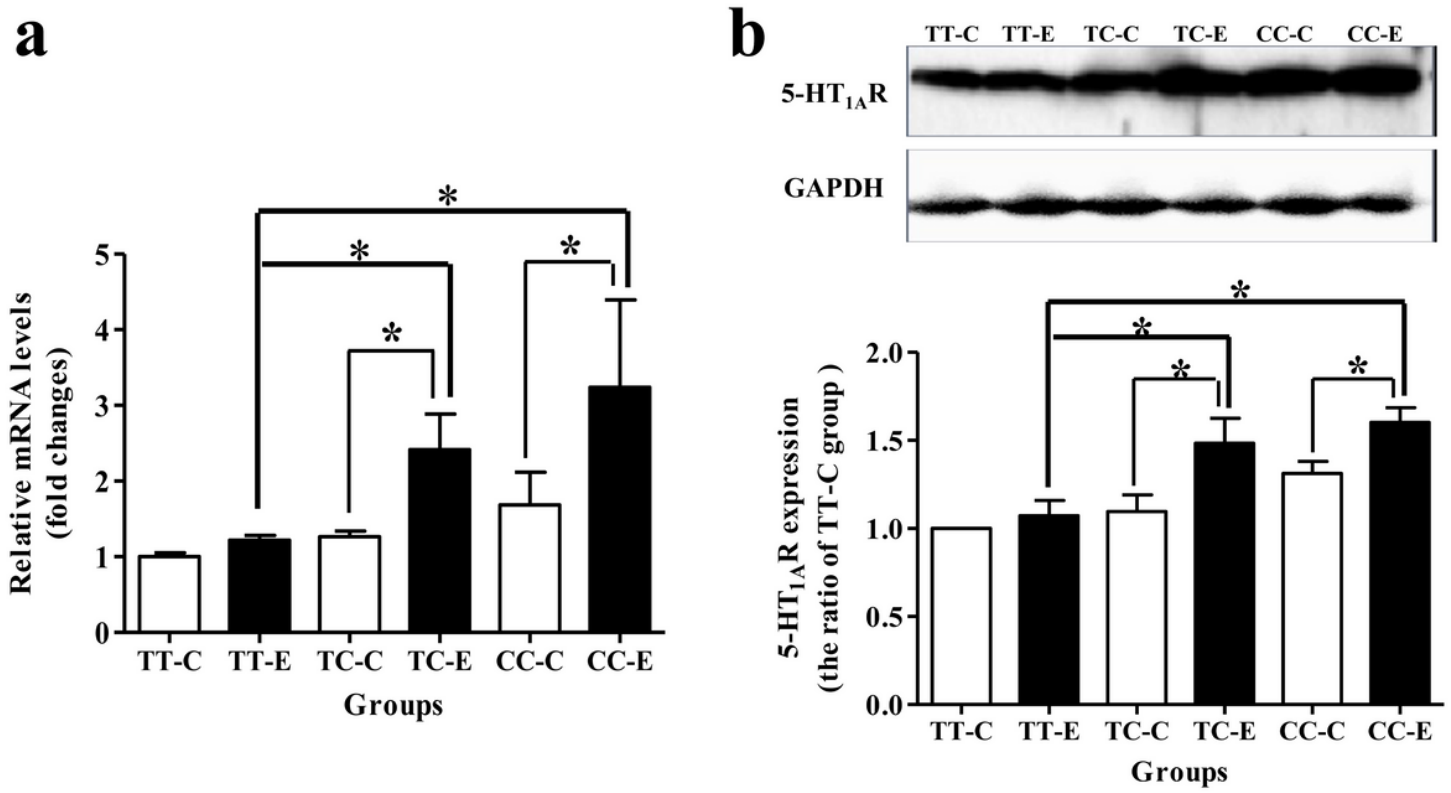

Figure 5

5-HT1A receptor mRNA and protein levels in the rat hippocampus after 30 mW/cm2 microwave exposure. * P<0.05. The histogram panels show semiquantified protein level data. Each value is expressed as the ratio of the level in the indicated group to the level in the control TT genotype (TT-C) group. * P<0.05.