Food & Function

ROYAL SOCIETY OF CHEMISTRY

PAPER

Published on 19 August 2025
Licence and permissions

Check for updates

Cite this: *Food Funct.*, 2025, **16**, 7199

Theaflavin-3,3′-digallate prevents radiofrequency radiation-induced learning and memory impairment in mice *via* regulating GSK-3α/GRα/RORα/BMAL1 expression

Jing Fu,*†[a] Yuanchen Sun,†[b] Huining Jia,[b] Hongxia Yuan,[b] Xing Zhang,[b] Wenchao Zhang,[b] Honglong Cao[c] and Fenju Qin *[b]

Radiofrequency (RF) electromagnetic radiation is an environmental hazard that causes neurobehavioral disorders and leads to learning and memory decline. In addition, theaflavin-3,3′-digallate (TFDG) is the most effective antioxidant and bioactive compound among the theaflavins in black tea, and theaflavins have been proven to provide protection against neuronal cell injury. Herein, we aimed to explore the salutary effects and relevant molecular mechanisms of TFDG on RF radiation-induced learning and memory damage in mice. Our results showed that oral administration of TFDG effectively relieved learning and memory impairment in mice caused by RF radiation (1.8 GHz, 240 μW cm^{-2}), including decreasing the number of learning attempts and increasing the memory rate in a Y-maze test, and raising the novel object recognition rate in a novel object recognition test. TFDG also reduced the pathological damage of the hippocampal pyramidal and granular neurons in RF-radiated mice, for neurotransmitters in the hippocampus, decreased the levels of glutamate (GLU), nitric oxide (NO) and acetylcholinesterase (AchE) activity and increased the acetylcholine (Ach), dopamine (DA) and dopamine transporter (DAT) levels. The decrease in the antioxidant ability in the hippocampus of RF-radiated mice was reversed by TFDG treatment by enhancing the levels of total antioxidant capacity (T-AOC), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activities and lowering the malondialdehyde (MDA) content. Furthermore, RF radiation induced the accumulation of glycogen synthase kinase-3α/glucocorticoid receptor alpha (GSK-3α/GRα) proteins and mRNA expression in the hippocampus of mice and the downregulation of retinoid-related orphan nuclear receptor alpha/brain and muscle ARNT-like 1/dopamine receptor D5 (RORα/BMAL1/DRD5) genes/protein expression. Conclusively, TFDG can improve RF radiation-induced learning and memory impairment, which may be related to the reduction of RF radiation-induced hippocampal neuron injury, the regulation of neurotransmitter disorders and antioxidant capacity reduction *via* the regulation of GSK-3α/GRα/RORα/BMAL1 pathway abnormalities.

Received 10th October 2024,
Accepted 26th July 2025

DOI: 10.1039/d4fo04752d

rsc.li/food-function

Introduction

Recently, with the rapid development of wireless communication technology and the widespread popularization of mobile phone usage, the global number of Internet users has added up to approximately 5.4 billion people (67 percent of the world's population) in 2023, which has induced a rapid increase in background radiofrequency (RF) radiation in the human environment.[1] It is well known that the central nervous system involved in many intricate electrical activities is one of the most sensitive targets of RF radiation.[2] When talking over the phone, mobile phones are often held close to the brain, which makes it vulnerable to RF radiation.[3] From the results of human epidemic statistics and animal experiments, a considerable number of researchers have found that RF exposure induces various adverse impacts such as headaches, sleep disturbances and impairment of cognitive function, including learning, memory and attention.[4,5] It is necessary to develop preventive and therapeutic strategies against learning and memory impairment caused by RF radiation.

[a]*State Key Laboratory of Biological Resources and Ecological Environment Jointly Built by Province and Ministry, School of Biological Science and Engineering, Shaanxi University of Technology, Hanzhong, 723001, China. E-mail: fujing@snut.edu.cn*

[b]*School of Chemistry and Life Sciences, Suzhou University of Science and Technology, Suzhou 215009, China. E-mail: qinfenju@usts.edu.cn*

[c]*School of Electronic and Information Engineering, Soochow University, Suzhou 215123, China*

† These authors equally contributed to this work.

This journal is © The Royal Society of Chemistry 2025

The mechanism of learning and memory impairment is complex and not completely clear at present. The hippocampal tissue of the mammalian brain plays an important role in the learning and memory function of animals, which can continuously generate new functional neurons in adult animals, integrate them into the existing neural circuits and participate in the regulation of learning and memory.[6] The significant daily circadian expression of clock genes such as brain and muscle ARNT-like 1 (BMAL1), retinoid-related orphan nuclear receptor alpha (RORα), period1 (PER1), reverse viral erythroblastosis oncogene product (REVERB) and clock-controlled cognition-related genes in the hippocampus is essential to achieve normal daily learning and memory performance.[7] Furthermore, rats exposed to glucocorticoids for a long time exhibited a disrupted rhythm of hippocampal clock gene transcription, accompanied by alterations in synaptic plasticity and memory impairment.[8] Periodic oscillations of clock genes are influenced by glucocorticoids, mainly through glucocorticoid receptor alpha (GRα).[9] The CA1 region of the hippocampus, which is known for integrating information as memory, expresses an ≈24 h oscillation in reduction–oxidation that modulates membrane excitability in hippocampal CA1 neurons.[10]

In our previous studies, RF radiation induced a decreased expression of clock genes (*clock*, *bmal1* and *Rorα*) and decreased antioxidant capacity.[11] Some studies revealed that an oxidative stress-mediated pathway was involved in the decreased learning and memory functions following electromagnetic radiation.[12,13] Glycogen synthase kinase 3β (GSK-3β), as an upstream regulator of GRα and RORα signaling, is involved in hippocampal oxidative stress damage, whereas grape seed proanthocyanidins, as potent antioxidants, could promote GSK-3 phosphorylation (inactivation) and reduce learning and memory impairments and mitochondrial oxidative stress in the hippocampus of sporadic AD mice.[14–16] Therefore, it is necessary to find safe and effective natural antioxidant products for the prevention of RF radiation damage.

Theaflavin-3,3′-digallate (TFDG or TF-3), one of the theaflavins present in black tea, is well known to possess several excellent bioactive properties, including antioxidant, anticancer, anti-inflammation, antivirus, anti-obesity and prevention of neurodegenerative diseases.[17] The theaflavins of black tea include theaflavin (TF-1), theaflavin-3-gallate (TF-2a), theaflavin-3′-gallate (TF-2b) and theaflavin-3,3′-digallate (TFDG or TF-3).[18] Among these four monomers, the proportion of TF-3 components is the highest and has the greatest antioxidant effect.[19] Previous studies on tea polyphenols have focused on the green tea extract epigallocatechin gallate (EGCG), which is a powerful antioxidant. In the hypothalamus of diet-induced obese (DIO) mouse models, EGCG treatment increased the expression levels of key circadian clock genes *Clock* and *Bmal1*.[20] EGCG can serve as a natural circadian clock modulator that protects cells against apoptosis and ECM degradation under oxidative stress in a BMAL1-dependent manner.[21] However, there are researchers who reported that the antioxidant order of theaflavins and catechins was TFDG > ECG > EGCG = TF-2b = TF-2a > TF-1 = EC > EGC using a constructed human low-density lipoprotein (LDL) oxidation model.[22,23] Therefore, TFDG, as a powerful antioxidant from black tea, might be a natural circadian clock modulator by scavenging free radicals or reactive oxygen species (ROS) and improving health through entraining circadian rhythms. Nevertheless, the functional role of TFDG in the regulation of learning and memory impairment induced by RF radiation remains unclear.

Therefore, in this study, we propose to find the preventive effects of TFDGs on RF radiation-induced learning and memory damage in mice using a Y-maze test. The regulatory effects of TFDG on hippocampal histopathology, oxidative stress and neurotransmitters were also evaluated. Furthermore, their effects on the GSK-3α-mediated GRα and clock gene pathways were analyzed to explore the possible related mechanisms.

Materials and methods

Theaflavin-3,3′-digallate (TFDG) treatments

TFDG (catalog number: 30462-35-2; purity, HPLC ≥ 98%) was provided by Shaanxi Lebo Biochemical Technology Co., Ltd (China), and was dissolved in 0.5% dimethyl sulfoxide (DMSO; Sigma-Aldrich, USA).

Animal experimental procedure

C57BL/6J male mice (3 weeks old) were obtained from the Laboratory Animal Center of Soochow University. All animal experimental procedures in this study were carried out according to the Guidelines for Experimental Animal Administration published by the State Committee of Science and Technology of the People's Republic of China, and were approved by the Academic Ethics Review Committee of Suzhou University of Science and Technology (ethical code: 2022-086). The mice were raised with a commercial diet and water *ad libitum* in an environment with a 12 h/12 h light/dark cycle, 50%–60% humidity, and a room temperature of 24 ± 2 °C. After two weeks of quarantine, first, according to the method of reference, the mice with too slow or very sensitive reactions towards Y-maze electrical stimulation were eliminated.[24] The selected animals were randomly divided into seven groups: the normal control group (NC), the vehicle control group (Vehicle), the RF exposure group (RF), the TFDG 12.5 mg kg^{-1} + RF exposure group (TFDG 12.5 + RF), the TFDG 25 mg kg^{-1} + RF exposure group (TFDG 25 + RF), the TFDG 50 mg kg^{-1} + RF exposure group (TFDG 50 + RF), and the TFDG 100 mg kg^{-1} + RF exposure group (TFDG 100 + RF), each with nine mice. The mice in these seven groups were treated with TFDG or RF radiation as shown in Fig. 1. Mice were continuously given TFDG by gastric gavage at doses mentioned in Fig. 1,[24,25] along with the same volume of distilled water or 0.5% DMSO solution for 28 days. RF radiation exposure for mice was initiated on the 15th day of the intragastric experiment. The animals in the RF and TFDG + RF groups were exposed to RF radiation for 14

 This journal is © The Royal Society of Chemistry 2025

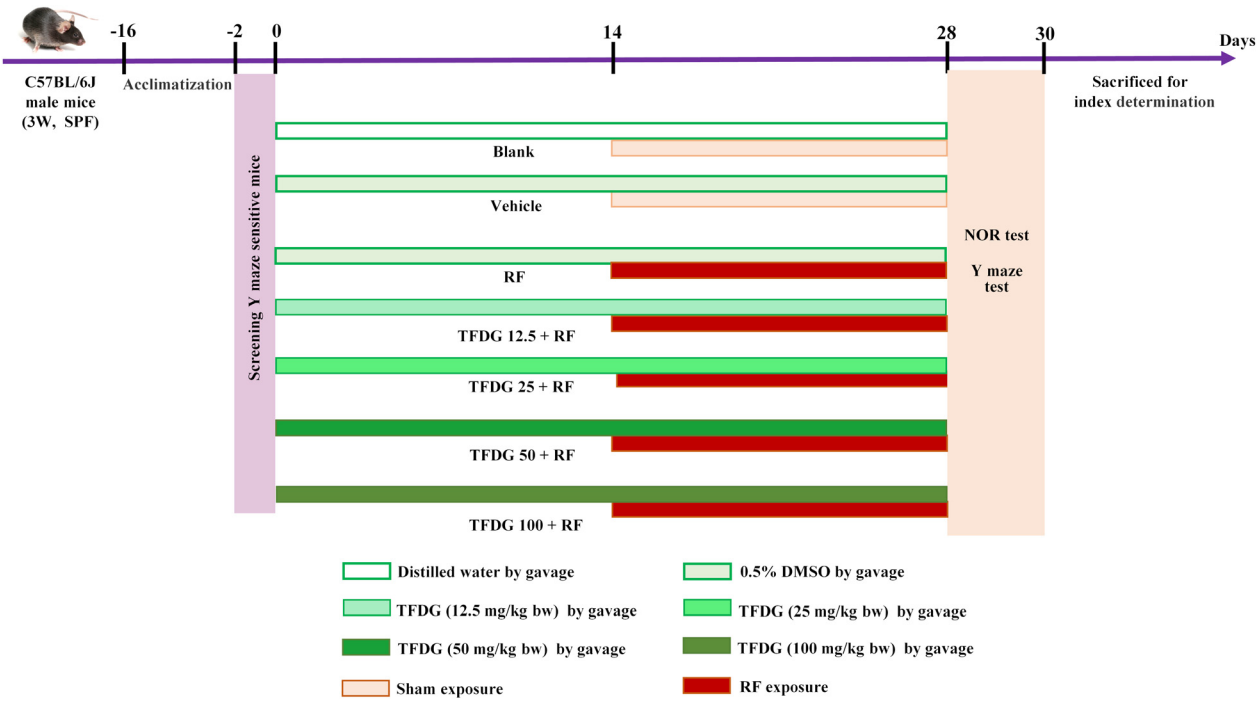


Fig. 1 Schematic of the study procedure for assessing the effects of TFDG on learning and memory in the RF exposure model mice.

consecutive days, 2 h every day. After four weeks of intervention of TFDG treatment or RF exposure, the learning and memory ability of mice was measured using the Y-type maze method and the novel object recognition (NOR) test. After the behavioral experiment, the mice were killed by cervical dislocation. The brains of three mice in each group were separated and embedded in paraffin for pathological examination, immunohistochemistry and immunofluorescence detection. Half of the brain tissue collected from the other six mice was homogenized and used for the determination of biochemical indexes, including antioxidant ability parameters (total antioxidant capacity (T-AOC), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), malondialdehyde (MDA)) and neurotransmitter parameters (glutamic acid (GLU), nitric oxide (NO), acetylcholine (Ach), acetylcholinesterase (AchE), dopamine (DA) and dopamine transporter (DAT)). The remaining brain tissue was kept frozen for real-time PCR or western blot test.

RF exposure system and dosimetry

The RF exposure system consisted of a GTEM chamber, signal generator and power amplifier as shown in the previous literature published by the author.[26] A continuous wave 1800 MHz RF signal was generated, amplified and transmitted into the GTEM chamber through an antenna (Southeast University, Nanjing, China). A PMM field strength meter (Cisano sul Neva, Savona, Italy) was used to measure the 240 μW cm^{-2} power intensity in the GTEM chamber where the animals were kept for RF exposure. A computer logging system ensured the uniformity of power density by controlling and monitoring the field strength data. The specific absorption rate (SAR) was calculated using a 3-D male mouse model and SPEAG Sim4Life V3.0 electromagnetic simulation software (IT'IS Foundation, Zürich, Switzerland), whereas SAR is the mass-averaged SAR calculated by simulations in Sim4Life. The approximate SAR was 0.3575 W kg^{-1}. The mice in the sham exposure group were also placed in the RF exposure equipment, but no RF signal occurred.

Behavioral tests of learning and memory ability

After 28 days of continuous oral administration and 14 days of RF radiation, the learning and memory ability of mice was investigated by the Y-maze test and the NOR test.

Y-type maze test

The Y-maze consists of three interconnected arms (18 cm high × 55 cm long × 15 cm wide) intersecting at 120°, as shown in Fig. 2A. The determination method was as in the reference.[24] The bottom surface is a copper grid that can be electrified, and the surrounding wall is an insulating plastic board. When the experiment is performed, the three arms take turns to be the starting zone, the safety zone and the electrified zone. A light is used as the conditional stimulus, and the unconditional stimulus is the plantar electric shock. At the beginning of the test, the mice were put into the starting arm (A) and adapted to the environment for 180 s. When the mouse received the electric shock in the starting arm (A), it was regarded as a correct response if it directly ran into the safety arm (B) with a light shining at its top. Otherwise, it is the wrong response if the mouse ran into the electrical arm (C).

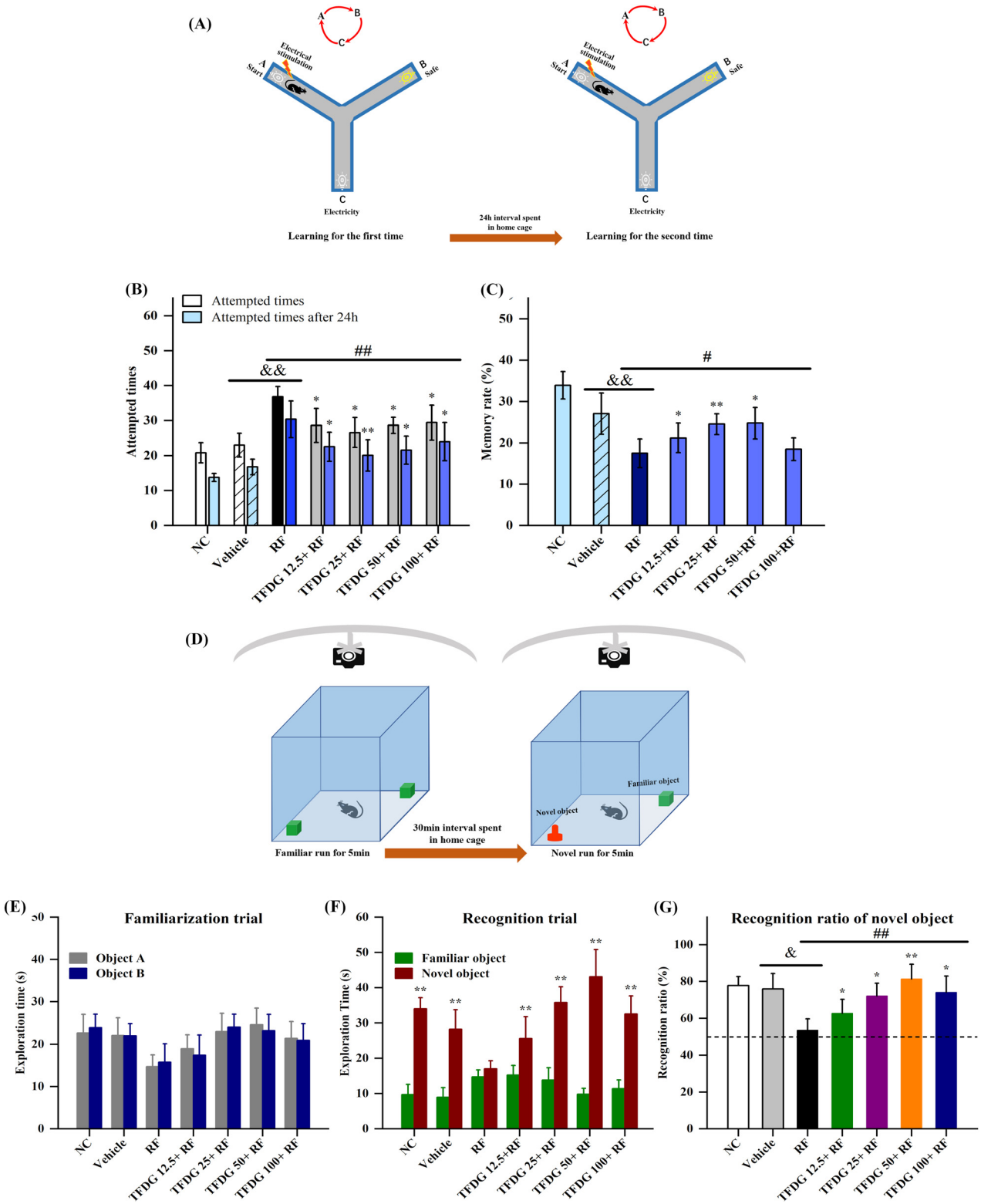


Fig. 2 TFDG alleviated RF-radiation-induced learning and memory impairment in mice. (A) Diagrammatic representation of the Y-maze test. (B and C) The attempted learning times and the memory rate. (D) Diagrammatic representation of the novel object recognition test. (E) Exploration time for two identical objects A and B during the familiarization phase. (F) Exploration time for the familiar and novel objects during the recognition/choice phase. (G) Novel object recognition rate. Data are expressed as mean ± SEM, $n = 9$. Significant difference, &/#$p < 0.05$, &&/##$p < 0.01$ by ANOVA; *$p < 0.05$ and **$p < 0.01$ compared with the RF group by Dunnett's *post hoc* test.

This journal is © The Royal Society of Chemistry 2025

After three minutes, the safety arm for the next training became the starting arm, and so on. When the mouse reached 9 correct times in 10 consecutive training attempts, the total number of electric shocks received was considered the learning time the mouse needed to navigate the maze. After 24 h, the learning ability was measured once again, and the memory rate was determined. Memory rate was calculated as the percentage of the difference between the second attempted learning times after 24 h and the first attempted learning times as a percentage of the first attempted learning times.

Novel object recognition (NOR) test

One day before the test, the mice were placed in the experiment box (50 cm × 50 cm × 50 cm) for habituation for 30 min, as showed in Fig. 2D. During the familiarization trial, the mouse was placed in the experiment box with its back facing to two identical small green objects that were fixed at both ends of the bottom diagonal, maintaining the same distance between the tip of its nose and the two objects. The mouse was allowed to explore freely for 5 min in this phase. Then, the test chamber and objects were cleaned with 20% ethanol. Thirty minutes later, one of the old objects (Familiar Object) was changed into a new object with a different shape (Novel Object) in the recognition trial, and the mice were put into the test box again for free exploration for 5 min. A video camera was used to monitor and record the movement path of the mice, and the number and time of exploring new and old objects during the recognition exploration was analyzed using the Animal Behavior Analysis System (Stoelting). Exploration time was calculated as the time spent within 2 cm of the object by the mouse nose. The exploration time of the mice in the two tests was measured, and the recognition ratio = new object exploration time/(new object exploration time + old object exploration time) × 100%.

Histopathology evaluation

Hematoxylin and eosin (Wuhan Servicebio Technology Co., Ltd, Wuhan, China) staining was used to investigate the histopathologic change of hippocampal tissues.[27] Brains randomly chosen from three mice in every group were fixed with 4% paraformaldehyde and dehydrated with the ethanol gradient. Then, they were embedded in paraffin and cut into 5 μm-thick sections using a rotary microtome (Leica RM2155, Germany) for HE staining. For every mouse, three sections were randomly selected to observe the morphological architectures in the hippocampal CA1, CA3 and DG regions, and photographed using a fluorescence microscope (Zeiss, Germany). The cell density in hippocampal subfields CA1, CA3 and DG was determined in every section according to Motaghinejad's method.[28] The neurons' damage was observed using the Image-Pro Plus 6.0 software (Media Cybernetics, USA).

Antioxidant parameters and examination of neurotransmitters

The half tissues were obtained from six brains in every group for biochemical analysis. The brain tissue was weighed, homogenized and centrifuged at 10 000*g* for 10 min at 4 °C. Afterward, for each supernatant, the GSH-Px, SOD, T-AOC, MDA, GLU, NO, Ach, AchE, DA and DAT were determined using the corresponding kits (Suzhou Comin Biotechnology Co., Ltd, Suzhou, China; Shanghai Enzyme-linked Biotechnology Co., Ltd, Shanghai, China) according to the manufacturer's instructions using a multifunction microplate reader (Thermo Fisher Scientific, Waltham, MA, USA). Each analysis was performed three times.

Immunohistochemistry

The 5 μm sections from paraffin-embedded brain tissue were deparaffinised and rehydrated. Antigen retrieval was carried out in 10 mmol L^{-1} trisodium citrate by RF at 700 W for 15 min. Then, the tissues were treated with 2% hydrogen peroxide and 50 μL blocking solution containing 3% normal goat serum in order to block endogenous peroxidase activity and nonspecific immunoglobulin binding sites. Subsequently, each tissue was incubated overnight at 4 °C in a 1/100 dilution of a rabbit polyclonal antibody recognizing GRα (NB300-633, Novus Biologicals, CO, USA). After washing, the slides were incubated with an HRP-conjugated goat anti-rabbit IgG secondary antibody (1 : 200, GB23303, Servicebio, Wuhan, China) for 2 h, followed by colorimetric detection using DAB. Next, the tissues were counterstained with hematoxylin, and photographs were taken using a fluorescence microscope (Zeiss Germany, Oberkochen, Germany). For the five slides from each animal, the optical density of stained cells to GRα antibody in the hippocampus was examined using image analysis software.

Immunofluorescence

After antigen retrieval, the sections were blocked with 3% bovine serum albumin in PBS for 30 min at room temperature, followed by incubation with a rabbit polyclonal anti-GSK-3α-1 antibody (dilution 1 : 200, NB100-81943, Novus Biologicals) in PBS at 4 °C overnight. Antigen detection was performed using a Cy3-conjugated goat anti-rabbit IgG secondary antibody (1 : 300, GB21303, Servicebio) and the nucleus was counterstained with DAPI (1 : 200, CAS 28718-90-3, Sigma-Aldrich). After staining, the slides were photographed at 400× magnification by confocal laser scanning microscopy (LSM800, Zeiss, Germany). The fluorescence intensity of the positive cells with GSK-3α immunofluorescence was determined using the Tissue Studio 4.0 software (Definiens Imaging, Munich, Germany).

Quantitative real-time PCR

The differences in the expression levels of mRNA of *Gsk-3α*, *Grα*, *Drd5*, *RORα* and *Bmal1* in brains were examined using quantitative real-time PCR (qRT-PCR). Total RNA isolation of brain tissues of mice was performed using the RNA Extraction Kit (DNase I) (Beijing Baiao Leibo Technology Co., Ltd) and quantity and quality were measured using NanoDrop™ 2000 (Thermo Fisher Scientific). The RNA was reversely transcribed into cDNA and qRT-PCR was performed according to the instructions of the PrimeScript™ One Step RT-PCR kit. The primer sequences used for real-time PCR are listed in Table 1.

Table 1 Primer sequences

Genes	Forward primer	Reverse primer
Gsk-3α	5′-AGGTTCTTCAGGACAAAAGGTTCAA-3′	5′-CACATACTCCAGCACCAGATTCAA-3′
Grα	5′-TACCCTGGTGTCACTGCTGGA-3′	5′-TCTGAATCCTGGTATCGCCTTT-3′
Rorα	5′-GCTGTCAAGTTTGGTCGGATGT-3′	5′-GCCCATTGGCTGAGATGTTGT-3′
Bmal1	5′-GAAGGATCAAGAATGCAAGGGA-3′	5′-CCATCCTTAGCACGGTGAGTTT-3′
Drd5	5′-CATTCTGCGACATCTGGGTG-3′	5′-CGTAGCGGAAGGGTCTGGAA-3′
Gapdh	5′-CCTCGTCCCGTAGACAAAATG-3′	5′-TGAGGTCAATGAAGGGGTCGT-3′

The mRNA expression levels of these five genes were calculated using the $2^{-(\Delta\Delta Ct)}$ method with *Gapdh* as the internal standard.

Western blot

Total protein from the brain of mice was extracted using an ExKine™ Pro Total Protein Extraction Kit (Abbkine Scientific Co., Ltd, Wuhan, China), and the quantification of protein was performed using a BCA Protein Assay Kit (Servicebio). Equal amounts of 30 μg of protein were used for western blot analysis according to the previous operation steps.[29] The bands obtained were quantified by densitometry *via* ImageJ analysis software (National Institutes of Health, Bethesda, MD, USA) and normalized to the actin housekeeping gene. The primary antibodies used and their dilutions were as follows: mouse anti-dopamine DRD5 (1 : 500, sc-33661, Santa Cruz Biotechnology, Dallas, TX, USA), rabbit anti-ROR alpha (1 : 2000, NBP3-25112, Novus Biologicals), mouse anti-BMAL1 (1 : 1000, NB100-2288, Novus Biologicals), mouse anti-β-actin (1 : 1000, NB600-501, Novus Biologicals) and the corresponding HRP-conjugated antibodies, including goat anti-mouse IgG and goat anti-rabbit IgG 1 : 5000 (Thermo Scientific, Rockford, USA).

Statistical analysis

Statistical analysis was performed using SPSS-20 (IBM SPSS Statistics Inc., Armonk, New York, USA) by ANOVA and Dunnett's *post hoc* test, and experimental data are expressed as mean ± SEM. Graphics were made using Origin 8.5 (OriginLab Corporation, Northampton, MA, USA). A $P < 0.05$ was considered statistically significant.

Results

TFDG relieves the decrease in learning and memory ability caused by radiofrequency radiation

In the Y-maze test, there is no statistical difference between the learning and memory ability of the vehicle control group and the blank group ($P > 0.05$). In the results of Fig. 2B, compared with the vehicle control group, the attempted learning times of the RF group significantly increased ($P < 0.01$). Compared with the RF radiation model group, the animals treated with TFDG, except for the 100 mg kg^{-1} dose group, significantly reduced initial attempted learning times and second-session attempted times ($P < 0.05$ or $P < 0.01$, respectively). Similarly, RF exposure lowered the memory rate of mice, and the treatment of TFDG reversed the memory damage induced by RF exposure in 25 mg kg^{-1} and 50 mg kg^{-1} doses (Fig. 2C). These results demonstrated that the right dose of TFDG addition reversed the learning and memory damage in the Y-maze test in RF-exposed mice.

In accordance with these Y-maze test results, RF-exposed mice also demonstrated memory damage in the NOR test that was reversed by TFDG treatment (Fig. 2E–G). During the familiarization phase, there was no significant difference in exploring time between two identical objects A and B in every group of mice ($P > 0.05$) (Fig. 2E). During the recognition phase, all group animals spent significantly more time exploring the novel objects than the familiar objects ($P < 0.01$) except for RF groups ($P > 0.05$), which manifested damage in recognition memory induced by RF (Fig. 2F). As is shown in Fig. 2G, there was no significant difference in the recognition rates of the novel objects between the NC group and the vehicle control group ($P > 0.05$). RF exposure dramatically lowered the NOR ratio compared with the vehicle control group mice ($P < 0.05$), whereas TFDG addition notably enhanced the NOR ratio of RF-exposed mice ($P < 0.05$ or $P < 0.01$). These results demonstrated that TFDG addition can reverse the deleterious effects of RF radiation on learning and memory.

TFDG relieves the damage to hippocampal neurons caused by radiofrequency radiation

As shown in Fig. 3A, the hippocampal neurons in the NC and Vehicle groups exhibited a regular arrangement, with distinct edges, clear nucleus and nucleolus, and no significant necrosis of pyramidal neurons. The hippocampal neurons in the RF group showed reduced density of pyramidal cells and granular cells with edema and nucleus pyknosis; neurons were arranged in a disorderly way and vacuolated, especially in the CA1 region. TFDG treatment reduced the hippocampal cell damage caused by RF, and the repair effect was most significant in the 50 mg kg^{-1} dose group. Concretely, there was no significant difference in the granular cells and pyramidal cells in hippocampal tissue between the NC group and the Vehicle group, whereas the RF radiation group had a significant loss of the granular cells in DG and pyramidal cells in the CA1 and CA3 regions, as shown in Fig. 3B–D ($P < 0.05$ or $P < 0.01$). By contrast, TFDG treatment dramatically improved the neuronal cell density in the hippocampus ($P < 0.05$ or $P < 0.01$).

 This journal is © The Royal Society of Chemistry 2025

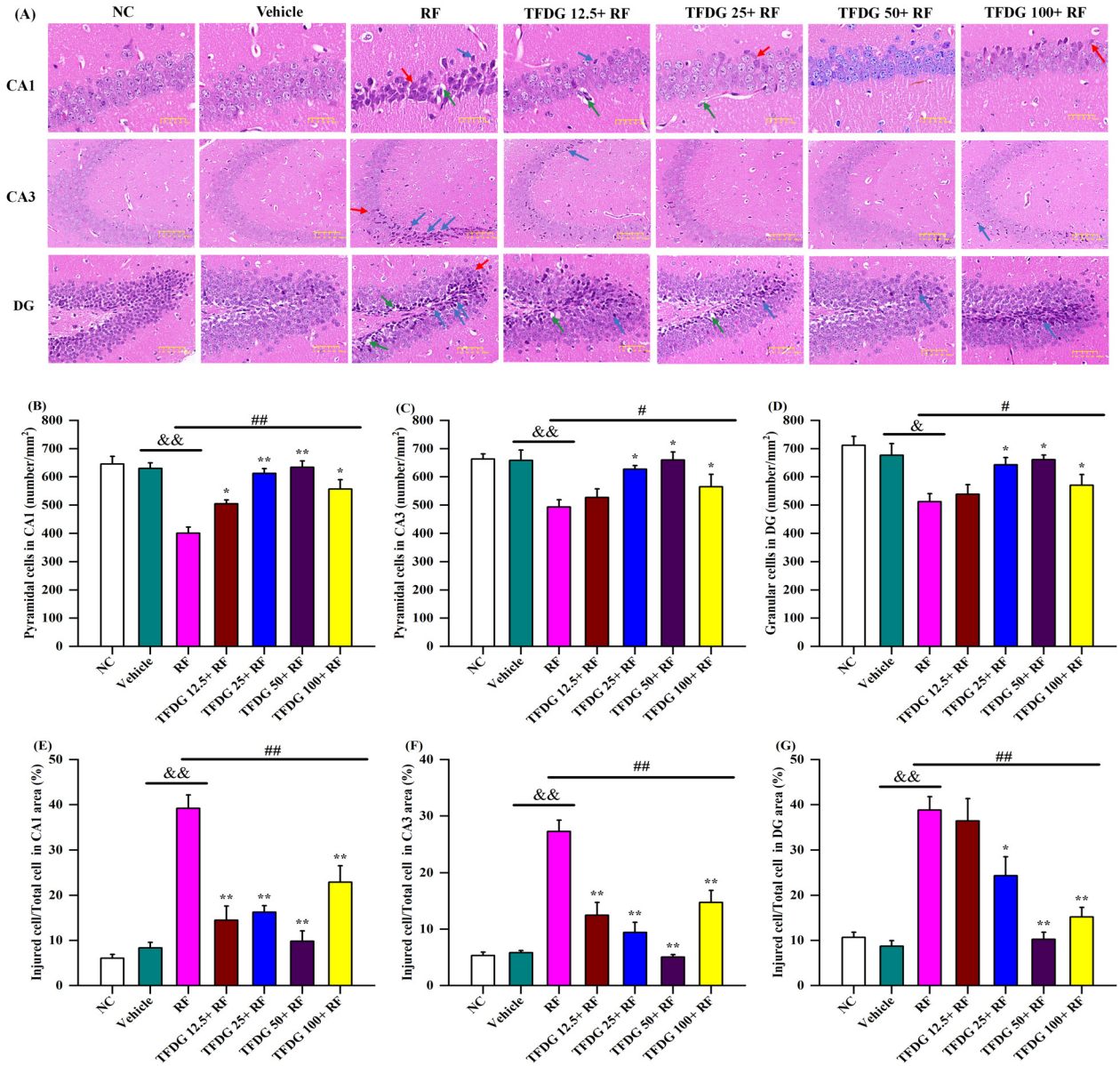


Fig. 3 TFDG alleviated RF-radiation-induced hippocampal neuron impairment in mice. (A) The morphology of neurons in subregions of the hippocampus (CA1, ×400; CA3 and DG, ×200). The blue arrow shows nuclear pyknosis. The green arrow shows cell vacuoles. The red arrow shows an unclear cell membrane boundary. The scale bar is 50 μm in CA1, 100 μm in DG and CA3. (B and C) Number of pyramidal cells in the CA1/CA3 region. (D) Number of granular cells in the DG region. (E–G) Cell damage percentage in the CA1/CA3/DG region. Data are expressed as mean ± SEM, $n = 3$. Significant difference, &/#$p < 0.05$, &&/##$p < 0.01$ by ANOVA; *$p < 0.05$ and **$p < 0.01$ compared with the RF group by Dunnett's *post hoc* test.

Furthermore, there was a sharp increase in the percentage of injured cells in the CA1, CA3 and DG regions of the RF radiation group ($P < 0.01$) (Fig. 3E–G). Nevertheless, it is TFDG addition through which reversion of cell injury can obviously be produced ($P < 0.05$ or $P < 0.01$).

TFDG relieves the disorders of the neurotransmitters in the hippocampus caused by radiofrequency radiation

As shown in Fig. 4, there is no statistical difference in neurotransmitters between the NC group and the Vehicle group ($P > 0.05$). Compared with the Vehicle group, the levels of GLU, NO and AchE activities in the RF groups increased, and the content of Ach, DA and DAT decreased ($P < 0.05$ or $P < 0.01$). Compared with the RF radiation model groups, 25 and 50 mg kg^{-1} TFDG significantly decreased the levels of GLU and NO ($P < 0.05$ or $P < 0.01$), while 12.5 and 100 mg kg^{-1} TFDG exhibited effects on any one index, GLU or NO. The addition of TFDG raised the level of Ach significantly ($P < 0.01$) but exhibited an opposite effect and reduced the activity of AchE ($P < 0.01$). Moreover, TFDG treatment distinctly raised the DA and DAT levels except in the 12.5 mg kg^{-1} dose group ($P < 0.01$).

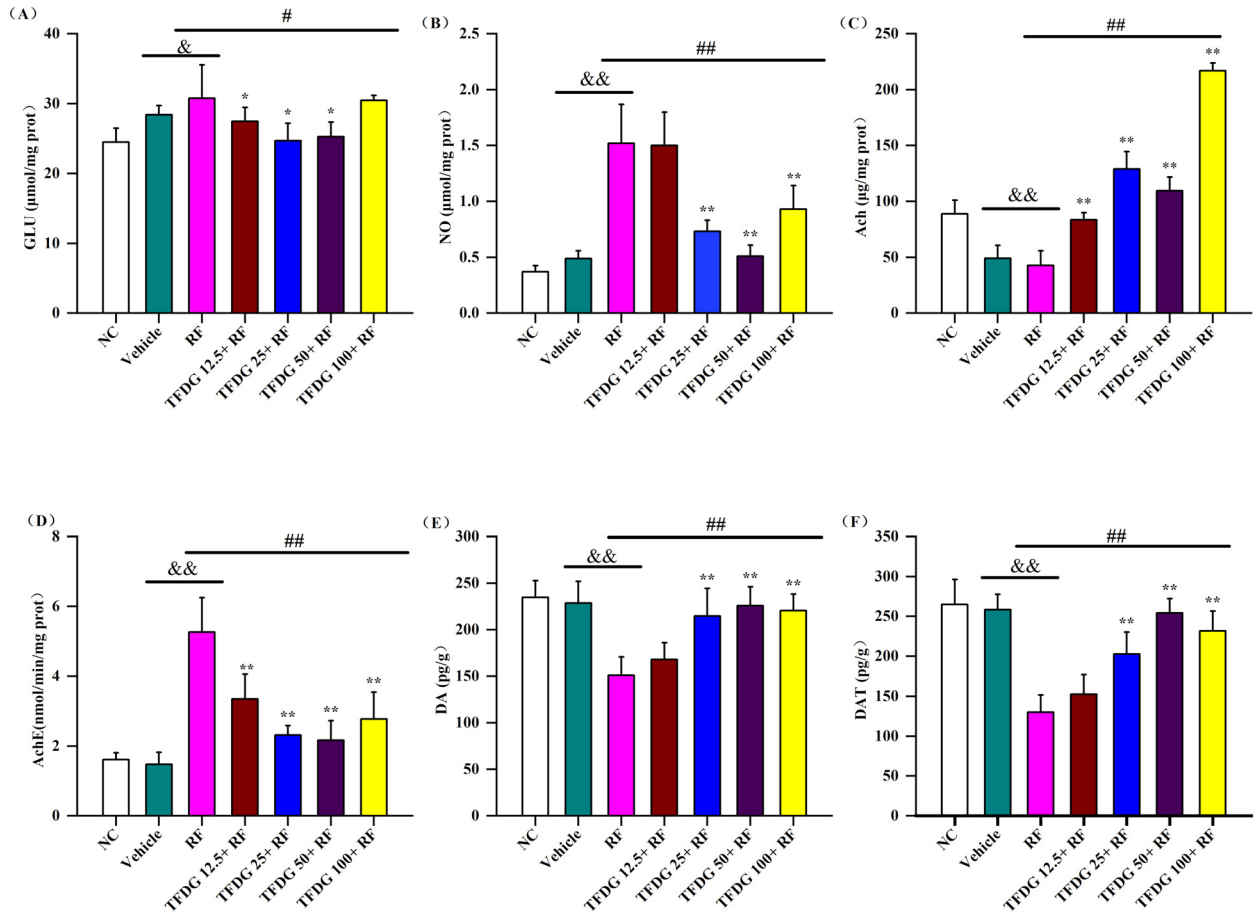


Fig. 4 The effects of TFDG on the neurotransmitters of mice exposed to RF radiation. (A) Glutamic acid (GLU), (B) nitric oxide (NO), (C) acetylcholine (Ach), (D) acetylcholinesterase (AchE), (E) dopamine (DA), and (F) dopamine transporter (DAT). Data are expressed as mean ± SEM, n = 6. Significant difference, &/#p < 0.05, &&/##p < 0.01 by ANOVA; *p < 0.05 and **p < 0.01 compared with the RF group by Dunnett's *post hoc* test.

TFDG ameliorates the decrease in antioxidant capacity of the hippocampus tissue caused by radiofrequency radiation

As shown in Fig. 5, the antioxidant index between the NC group and the Vehicle group did not show a statistical difference (P > 0.05). Compared with the Vehicle group, the T-AOC level, GSH-Px and SOD activities of the RF group significantly decreased, and the MDA content significantly increased (P < 0.05 or P < 0.01). Compared with the RF group, the four doses of TFDG treatment all increased the GSH-Px and SOD activities in RF-exposed mice, whereas the T-AOC level and the MDA content went up except for 100 mg kg^{-1} TFDG (P < 0.05 or P < 0.01).

TFDG ameliorates radiofrequency radiation-induced GSK-3α and GRα accumulation in the hippocampus

The hippocampus is an important region that maintains the continuous plasticity of neurons and performs learning and memory functions; the CA1 region is more sensitive to RF as seen in the above pathological HE staining results. GSK-3α and glucocorticoid receptor alpha (GRα) protein staining appeared red and brown, respectively, in the CA1 cell areas of the mouse hippocampus tissue (Fig. 6A and 7A). As seen in Fig. 6B and 7B, for the mean intensity values of the positive staining, there is no statistical difference between the NC group and the Vehicle group (P > 0.05). Compared with the Vehicle group, the positive staining intensity value of GSK-3α and GRα in the RF group increased (P < 0.01). The four additional doses of TFDG all lowered the mean intensity values of GRα positive staining significantly in the CA1 region of the hippocampus of mice exposed to RF (P < 0.05 or P < 0.01), and this phenomenon occurs in the GSK-3α indicator except for the 100 mg kg^{-1} TFDG treatment group. RF radiation dramatically upregulated the mRNA expression of *Gsk-3α* and *Grα* genes, whereas TFDG treatment showed markedly downregulated effects on the expression of the two mRNAs (P < 0.05 or P < 0.01) (Fig. 6C and 7C).

TFDG ameliorates the downregulation of RORα, BMAL1 and DRD5 expression caused by radiofrequency radiation in the hippocampus

As shown in Fig. 8, there is no statistical difference in protein expression of RORα, BMAL1 and DRD5 in the hippocampus of the brain between the NC group and the Vehicle group (P >

 This journal is © The Royal Society of Chemistry 2025

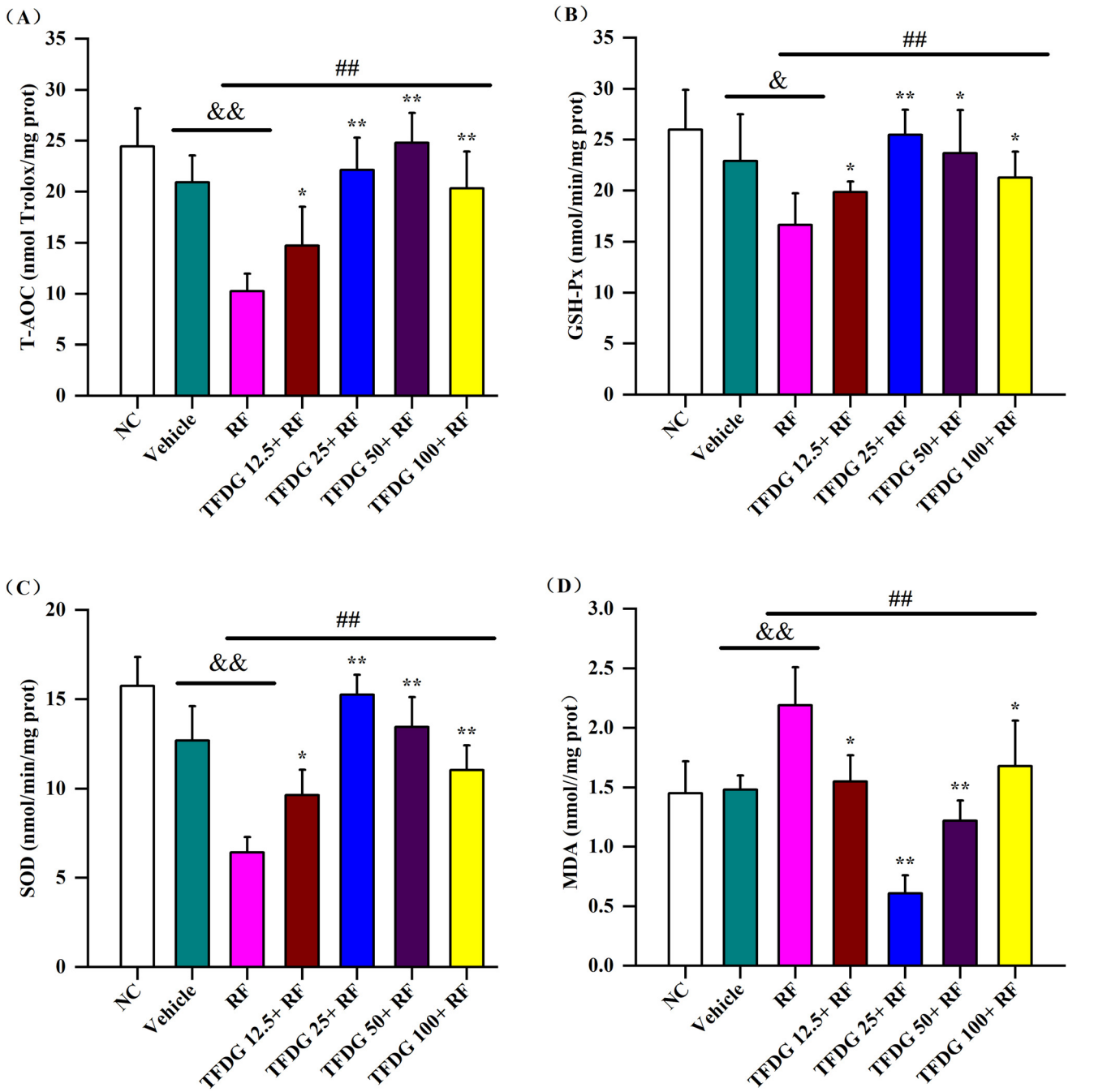


Fig. 5 The effects of TFDG on the antioxidant capacity of the hippocampus tissue of mice exposed to RF radiation. (A) Total antioxidant capacity (T-AOC), (B) glutathione peroxidase (GSH-Px), (C) superoxide dismutase (SOD), and (D) malondialdehyde (MDA). Data are expressed as mean ± SEM, $n = 6$. Significant difference, &/#$p < 0.05$, &&/##$p < 0.01$ by ANOVA; *$p < 0.05$ and **$p < 0.01$ compared with the RF group by Dunnett's *post hoc* test.

0.05). Compared with the Vehicle group, the levels of RORα, BMAL1 and DRD5 proteins in the RF group decreased ($P < 0.05$ or $P < 0.01$). Compared with the RF-exposed model group, TFDG at 25 and 50 mg kg^{-1} doses significantly increased the levels of RORα ($P < 0.05$); all four doses of TFDG addition exhibited upregulation effects for BMAL1 and DRD5 protein expression ($P < 0.01$). For RF-exposed mice, the mRNA expression of *Rorα*, *Bmal1* and *Drd5* genes showed similar upregulation effects in the four TFDG dosage groups ($P < 0.01$).

Discussion

In this study, we investigated the preventive effects and mechanisms of theaflavin monomers on learning and memory

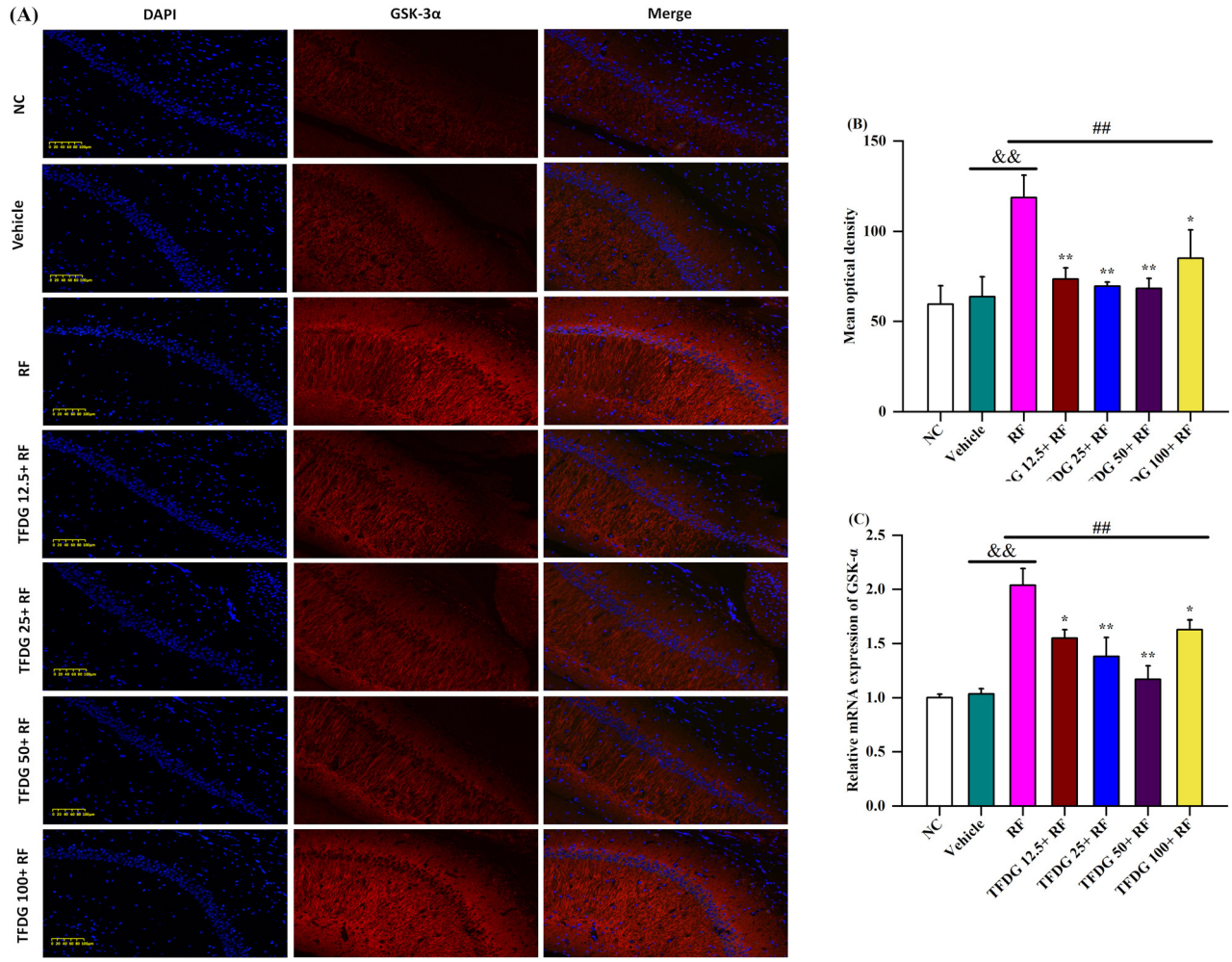


Fig. 6 The effects of TFDG on the expression of GSK-3α in the hippocampus CA1 region of mice exposed to RF radiation. (A) GSK-3α protein immunofluorescence staining (red), scale bar: 100 μm. (B) GSK-3α mean optical density, n = 3. (C) Relative mRNA expression of the GSK-3α gene, n = 6. Data are expressed as mean ± SEM. Significant difference, &&/##p < 0.01 by ANOVA; *p < 0.05 and **p < 0.01 compared with the RF group by Dunnett's *post hoc* test.

impairment induced in mice by RF electromagnetic radiation. With the widespread use of wireless communication technologies such as mobile phones, laptops, and wireless networks, there is growing concern about the impact of RF electromagnetic radiation on the neurotoxicity of the brain.[30] In learning and memory function evaluation, the Y-maze test and the NOR test are commonly used for experiments on animals. We found that 1.8 GHz RF radiation (240 μW cm^{-2}) damaged learning and memory function in mice, including an increase in learning attempt times and a decrease in the memory rate and NOR ratio. However, treatment with TFDG reversed the learning and memory damage induced by RF exposure in the Y-maze test and improved the NOR ratio in the NOR test. Theaflavins can alleviate neurological dysfunction by reducing neuronal apoptosis and affecting blood–brain barrier permeability.[31] Recent research has reported that TFDG monomers at the nanoscale may penetrate the blood–brain barrier and reach brain tissue through gavage.[32,33] As a free-radical scavenger, theaflavin monomer TFDG in DMSO solvent has shown therapeutic benefits in many clinical studies and radiation-induced tissue injury.[34] In the present study, the vehicle control group mice (0.5% DMSO) showed no statistical difference compared with the NC group mice.

The hippocampal region plays a principal role in the learning and memory systems and is particularly sensitive to RF electromagnetic radiation compared with other areas of the brain.[35,36] Some research found that 2.45 GHz RF electromagnetic radiation exposure injured neurons and cells in the mouse hippocampus and induced learning and memory impairment.[37–39] In our study, through histological examination, we found that the hippocampal neuronal cells and vesicles in the NC group and the Vehicle group exhibited a normal arrangement, with distinct edges and a clear nucleus and nucleolus, whereas neurons in the RF electromagnetic radiation group exhibited edema and irregular arrangement. In contrast, the TFDG addition groups reversed and showed

 This journal is © The Royal Society of Chemistry 2025

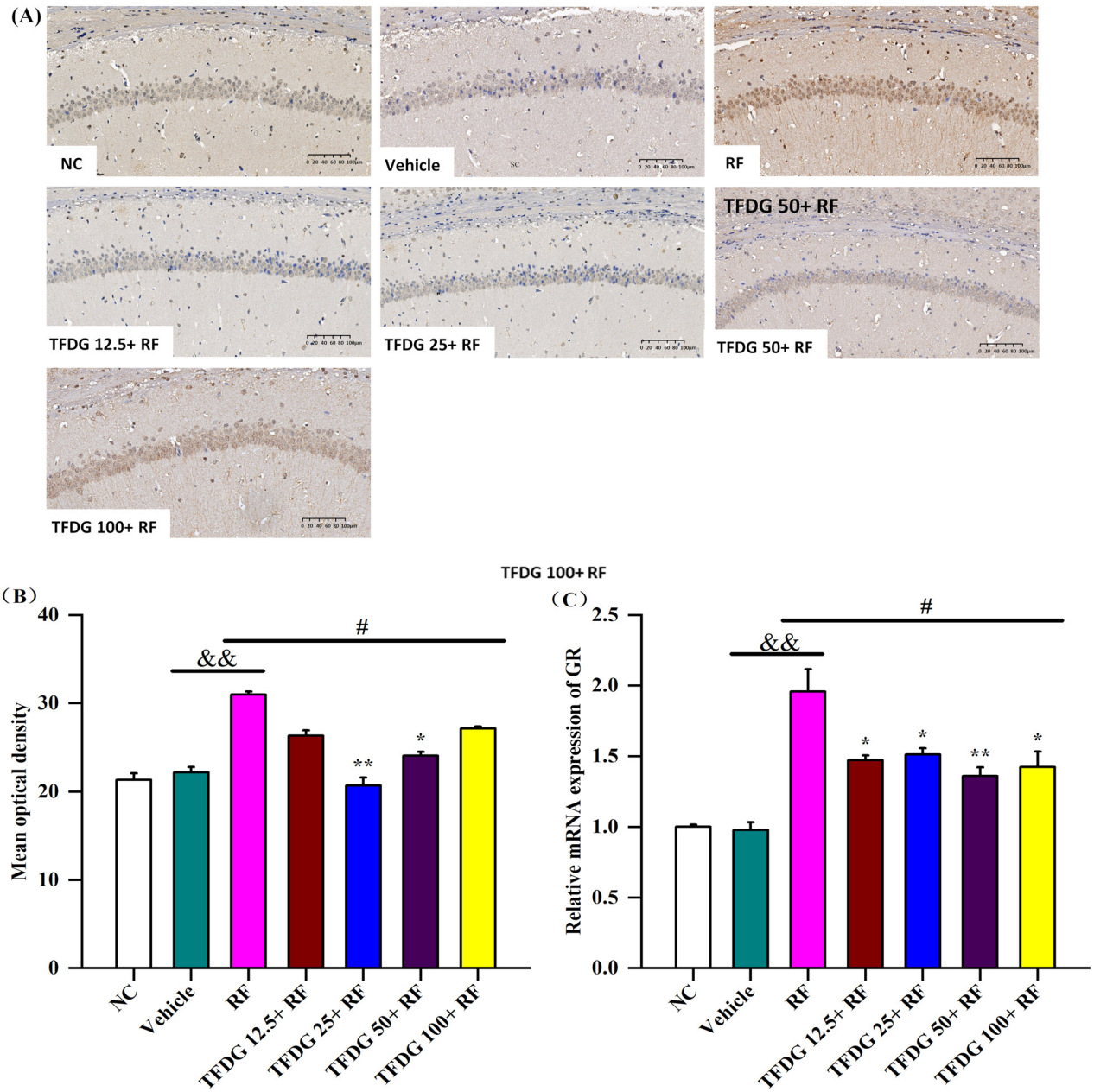


Fig. 7 The effects of TFDG on the expression of GRα in the hippocampus CA1 region of mice exposed to RF radiation. (A) GRα protein immunohistochemical staining (brown), scale bar: 100 μm. (B) GRα mean optical density, $n = 3$. (C) Relative mRNA expression of the GRα gene, $n = 6$. Data are expressed as mean ± SEM. Significant difference, #$p < 0.05$, && $p < 0.01$ by ANOVA; *$p < 0.05$ and **$p < 0.01$ compared with the RF group by Dunnett's *post hoc* test.

therapeutic effects, which indicates that TF-3 can alleviate the impairment of neurons and cells in the hippocampus caused by RF electromagnetic radiation. Meanwhile, the learning and memory function involves many different neurotransmitter systems in different brain regions.[40,41] As a major excitatory transmitter in the CNS, glutamate constitutes 50%–60% of all neurotransmitters in the brain and is intricately linked to learning and memory, whereas excess accumulation of glutamate in synapses can lead to aberrant synaptic signaling, excitotoxicity or cell death.[42,43] Nitric oxide (NO) with multifunctional signals plays an important role in learning and memory processes, and it regulates cell survival and differentiation, neuronal proliferation and synaptic activity under physiological conditions, whereas excess NO-mediated cysteine mercaptan (SNO) can cause nitrosation stress, leading to neuropathology.[44] Hippocampal DA can boost novelty-induced memory and is also required for hippocampal-dependent memory and plasticity at the CA3–CA1 synapse.[45] Ach enhances perception and sustained attention while awake.[46] AchE and DAT ensure the normal physiological function of synapses by degrading

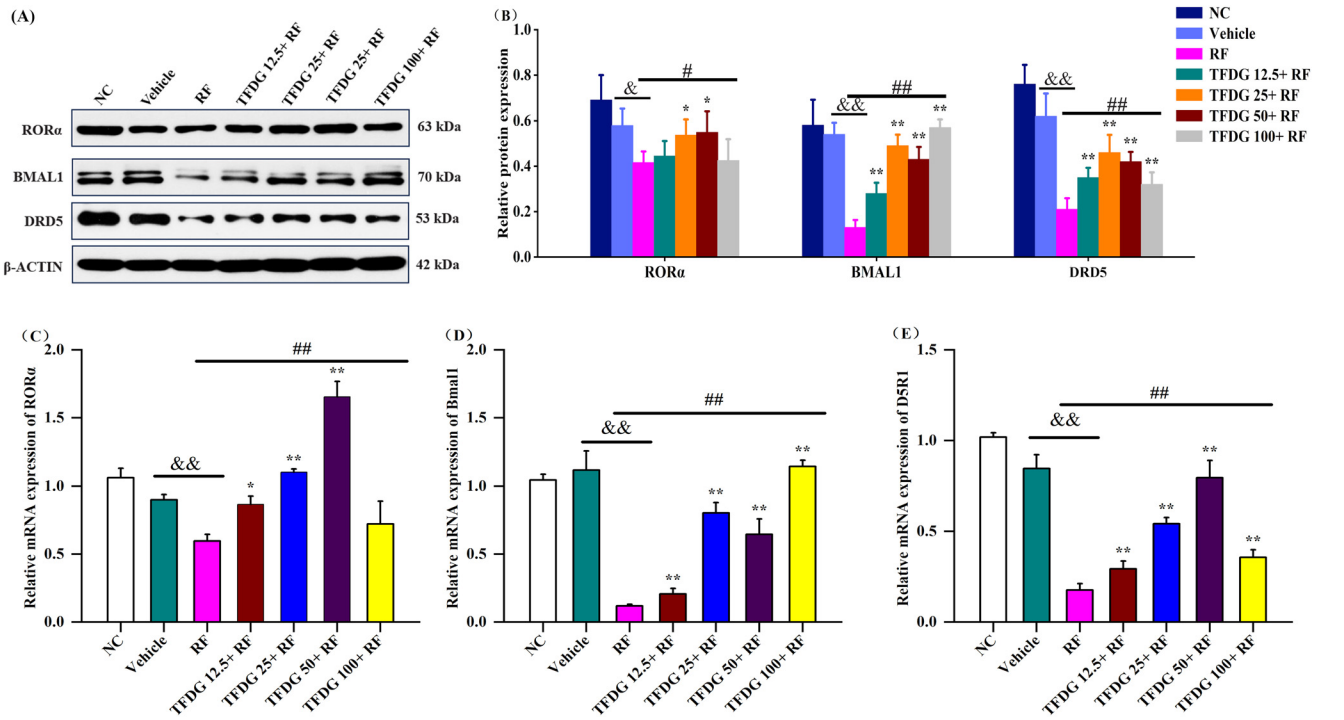


Fig. 8 The effects of TFDG on the expression of RORα, BMAL1 and DRD5 in the brain hippocampus of mice exposed to RF radiation. (A) Western blot, (B) relative protein expression, and (C–E) relative mRNA expression. Data are expressed as mean ± SEM, n = 6. Significant difference, &/#p < 0.05, &&/##p < 0.01 by ANOVA; *p < 0.05 and **p < 0.01 compared with the RF group by Dunnett's *post hoc* test.

Ach and taking DA into the presynaptic membrane, respectively.[47] In our study, 1.8 GHz RF radiation (240 μW cm^{-2}) induced the accumulation of GLU and NO levels, an increase in AchE activities, and a decrease in Ach, DA and DAT levels, consistent with previous studies.[48–52] However, we observed that TFDG repaired the decrease in Ach and DA levels and the increase in GLU and NO levels caused by RF radiation exposure. Taken together, our findings suggest that TFDG can alleviate RF-induced learning and memory decline and neuronal damage by regulating neurotransmitter levels.

Many epidemiological researchers and animal studies have found that RF radiation of different frequencies and intensities has harmful effects on the nervous system through oxidative stress injury pathways.[53,54] Oxidative stress generally results from an imbalance between the production and scavenging of cellular oxidants (such as reactive oxygen species, ROS).[55] Excessive ROS can advance lipid peroxidation and result in the excessive production of malondialdehyde (MDA).[56] Oxidative stress damage can be countered by the antioxidant defense system, including key antioxidant enzymes such as SOD and GSH-Px. The overall resistance of the organism to ROS is reflected by the level of T-AOC.[57] Our study also proves this fact: 1.8 GHz RF radiation (240 μW cm^{-2}) induced a decrease in the T-AOC level, the downregulation of GSH-Px and SOD activity and a significant increase in MDA content in the hippocampus tissue. Theaflavins possess antioxidative activity as radical scavengers and metal chelators, whereas the phenolic hydroxyl groups and the gallic acid moiety are essential for their antioxidative function.[58] Among the four major compounds of theaflavins, the activity hierarchy of antioxidants is TFDG > TF-2A = TF-2B > TF-1.[59] Further studies found that TFDG could upregulate the expression of antioxidant genes such as *Nrf2*, *Prx2*, *Gsh-px1* and *Sod1*, enhance free-radical scavenging ability and reduce the degree of lipid peroxidation.[24,60] Under oxidative stress, nuclear factor erythroid 2-related factor 2 (NRF2) translocates to the nucleus and binds to antioxidant response elements (AREs) in the genome, promoting transcription of antioxidant related genes, including heme oxygenase-1, NAD(P)H dehydrogenase, c-glutamylcysteine synthetase, glutathione S-transferase, glutathione reductase, GSH-Px, and SOD.[61] In our study, TFDG can alleviate RF-induced oxidation damage by improving the T-AOC level and GSH-Px and SOD activities and lowering MDA accumulation.

GSK-3β is a member of the Ser/Thr kinase family, an essential regulator of the oxidative stress response, and can regulate cell division, differentiation, autophagy, apoptosis and neurological degeneration.[62,63] In the context of neurodegenerative disorders such as Alzheimer's disease (AD), the upregulated activity of GSK-3α is involved in the proteolytic processing of the Aβ peptide precursor.[64] Therefore, GSK-3α is related to learning and memory. Our study showed that the expression of GSK-3α significantly increased in the RF radiation group, which came with learning and memory impairment. A research group found that GSK-3-mediated glucocorticoid and RORα pathways are involved in the regulation of autophagy

 This journal is © The Royal Society of Chemistry 2025

activation of hippocampal neurons under white LED irradiation.[14] Hence, GSK-3 was identified as an upstream regulator of the GRα/RORα signaling pathway. Excessive glucocorticoid leads to hippocampal damage by regulating genes involved in ROS production and apoptosis, which in turn leads to impairment of learning and memory function.[65] GRα is highly expressed in the hippocampus to mediate the central response to peripheral glucocorticoids under basal and stressful conditions.[66] In our study, 1.8 GHz RF radiation expression of GRα significantly increased in hippocampal tissue, which is consistent with the 2.45 GHz exposure results in previous research.[67] A recent study found that GR antagonist FX5 significantly promotes the performance of learning and memory function in diabetic mice, and learning and memory improvement was observed following brain GR-specific knockdown by injecting AAV-si-GR.[68] Recent studies have proved that inhibitors of GSK-3 have become new targets for treating traumatic brain injury by promoting neuroprotective processes and inhibiting cell apoptosis.[69] Isoorientin (a 6-*C*-glycosylflavone), as a highly selective inhibitor of GSK-3β, has been found to rescue synaptic dysfunction and improve spatial memory deficits.[70] In our study, TFDG alleviated RF-induced accumulation of GSK-3 and GRα in hippocampal tissue. The results indicated that TFDG improves the ability of learning and memory in mice exposed to RF, which is related to the decrease of hippocampus GSK-3 and GRα protein expression.

The primary function of GRα in the brain is to regulate the expression of many components of the circadian oscillator network and its major target genes.[71] A range of physiological functions is controlled by circadian cycles, including memory processes. The molecular biological clock consists of four key genes/gene families, *Per*, *Clock*, *Cryptochrome* and *Bmal1*, which cycle rhythmically in a continuous transcription–translation negative feedback loop that maintains a cycle of approximately 24 h within the cells of the brain and body. Recent research has suggested that these clock genes may act locally within brain regions associated with memory to regulate memory in the day/night cycle.[72] BMAL1 is an essential component of the circadian pacemaker in mammals, and its dysfunction underlies a variety of neurodegenerative-like diseases including learning and memory impairments.[73] As a member of the orphan nuclear receptor family, retinoic acid receptor-associated orphan receptor-α (RORα) acts as a transcriptional activator in response to circadian changes and regulates circadian transcription of the core-clock *Bmal1*.[74] Oxidative stress turned out to be one of the common mechanisms by which Bmal1 deficiency promotes AD and associated aging-related diseases. Therefore, promising antioxidant therapeutic strategies involved in the regulation of Bmal1 have been provided, such as melatonin, natural compounds, and other interventions.[75] This present study showed that RF exposure downregulated the genes and proteins of RORα and BMAL1, whereas strong antioxidant TFDG from black tea reversed this change induced by RF radiation in mouse hippocampus tissue.

Learning and memory performance in people varies according to the time of day. The hippocampal clock is controlled by the circadian-dependent transcription factor BMAL1.[76] Germline *Bmal1* deletion causes brain oxidative stress and synapse loss in mice, and it can exacerbate dopaminergic neurodegeneration.[77] Lowered recognition memory in mice with inducible BMAL1 negative expression was accompanied by decreased expression of dopamine receptor D5 (DRD5) in the hippocampus, whereas pharmacological activation of DRD5 salvages impaired memory.[78] There is research that found that a significantly reduced density of DRD5 receptors in the hippocampus may explain the learning difficulties observed in ADHD patients.[79] A study showed that a significant reduction in the basal hippocampal Ach level was found in the DRD5 receptor knockout mouse model, which suggests that the DRD5 receptor plays a tonic role in Ach release.[80] Genetic deficiency of DRD5 significantly impairs spatial memory and

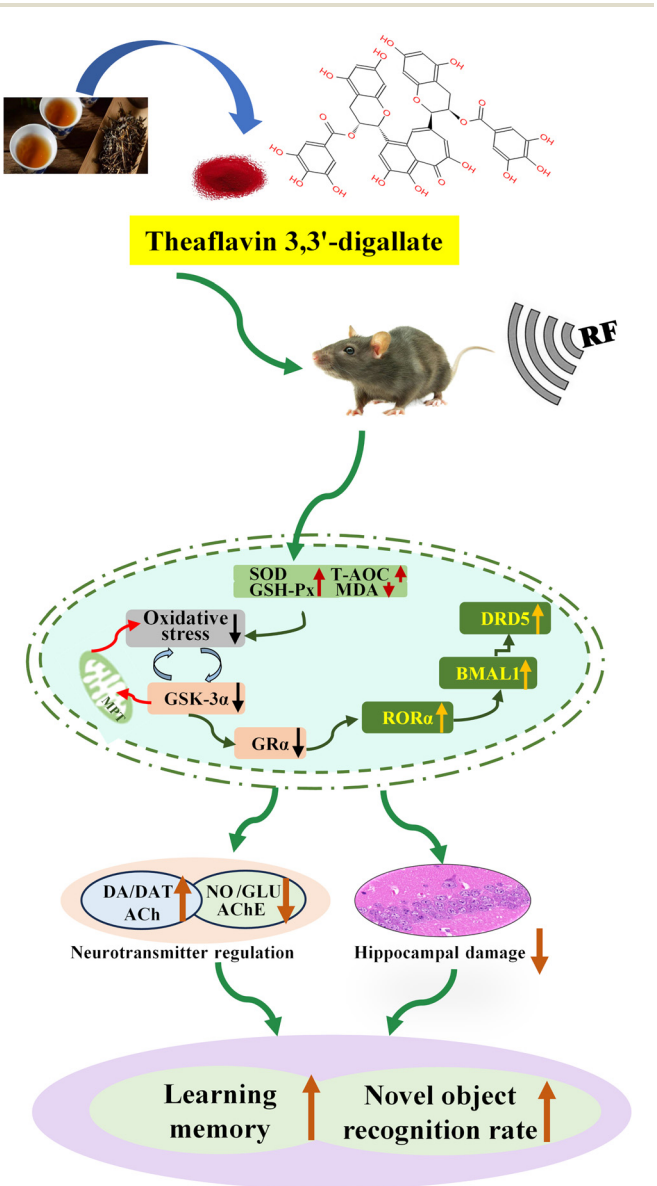


Fig. 9 Mechanism of TFDG preventing radiofrequency radiation-induced learning and memory impairment in mice.

recognition memory performance and results in a strong and selective reduction in the expression of the ionic glutamate receptor NMDA subunit NR2B in the hippocampus, which suggests a functional interaction of DRD5 with hippocampal glutamatergic pathways.[81] DRD5 knockdown in the prefrontal cortex also induced learning and memory deficits, and these changes were accompanied by an increase in the activity of the prefrontal cortex GSK-3β.[82] In our study, there was a significant decrease in DRD5 expression in the RF-exposed mice, whereas a DRD5 expression increase appeared in the TFDG addition group animals. These results suggest that TFDG can improve the learning and memory ability of mice exposed to RF by inhibiting GSK-3-mediated GRα expression and restoring ROR, BMAL1 and DRD5 expression.

Conclusion

Overall, the present study demonstrated that TFDG treatment could dose-dependently reduce the learning and memory impairment of mice exposed to 1.8 GHz RF electromagnetic radiation. Mechanistically, TFDG supplementation activated RORα/BMAL1/DRD5 pathways by inhibiting GSK-3α and GRα expression to restore neurotransmitter release and boost the learning and memory ability, which could be attributed to the peptides possessing excellent hydroxyl radical-scavenging ability (Fig. 9). Moreover, this finding might open a possibility for the exploitation of TFDG as a novel preventive and therapeutic natural antioxidant ingredient for RF radiation damage.

Abbreviations

Ach	Acetylcholine
AchE	Acetylcholinesterase
BMAL1	Brain and muscle arnt-like1
CA1	Cornu ammonis 1
CA3	Cornu ammonis 3
DA	Dopamine
DAT	Dopamine transporter
DG	Dentate gyrus
DRD5	Dopamine receptor D5
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GLU	Glutamic acid
GRα	Glucocorticoid receptor alpha
GSH-Px	Glutathione peroxidase
GSK-3α	Recombinant glycogen synthase kinase 3 alpha
MDA	Malondialdehyde
NO	Nitric oxide
RF	Radiofrequency
RORα	Retinoic acid receptor-related orphan receptor-α
SOD	Superoxide dismutase
T-AOC	Total antioxidant capacity
TFDG	Theaflavin-3,3′-digallate
REVERB	Reverse viral erythroblastosis oncogene product

Author contributions

Jing Fu: data analysis, funding acquisition, and writing – original draft; Yuanchen Sun: methodology and data analysis; Huining Jia: methodology; Hongxia Yuan: writing review and editing; Xing Zhang: investigation; Wenchao Zhang: supervision and validation; Honglong Cao: software and RF exposure system management; Fenju Qin: project administration, conceptualization, funding acquisition, and writing – review and editing.

Conflicts of interest

There are no conflicts to declare.

Data availability

Data supporting the current study are available from the corresponding author upon reasonable request.

Supplementary Material for Western blot in Fig. 8(A). See DOI: **https://doi.org/10.1039/d4fo04752d**.

Acknowledgements

This study was funded by the Special Research Foundation of Qinba State Key Laboratory of Biological Resources and Ecological Environment (SXZC-2102), the National Natural Science Foundation of China (81773463), and the Key Research and Development Program of Shaanxi Province (2021NY-121).

References

1 International Telecommunication Union, End-2023 estimates for key ICT indicators, Measuring digital development: Facts and figures 2023 (itu.int), 2024.
2 H. Deng, L. Liu, X. Tang, Y. Lu, X. Wang, Y. Zhao and Y. Shi, Impact of specific electromagnetic radiation on wakefulness in mice, *Proc. Natl. Acad. Sci. U. S. A.*, 2024, **121**, e2313903121.
3 J. Moon, The relationship between radiofrequency-electromagnetic radiation from cell phones and brain tumor: The brain tumor incidence trends in South Korea, *Environ. Res.*, 2023, **226**, 115657.
4 A. Magiera and J. Solecka, Mobile telephony and its effects on human health, *Rocz. Panstw. Zakl. Hig.*, 2019, **70**, 225–234.
5 H. Li, Y. Gao, Y. Zou, S. Qiao, W. Zhi, L. Ma, X. Xu, X. Zhao, J. Zhang, L. Wang and X. Hu, Associations between a polymorphism in the rat 5-HT1A receptor gene promoter region (rs198585630) and cognitive alterations induced by microwave exposure, *Front. Public Health*, 2022, **10**, 802386.

 This journal is © The Royal Society of Chemistry 2025

6 B. Zhang, L. Wang, A. Zhan, M. Wang, L. Tian, W. Guo and Y. Pan, Long-term exposure to a hypomagnetic field attenuates adult hippocampal neurogenesis and cognition, *Nat. Commun.*, 2021, **12**, 1174.

7 R. S. Golini, S. M. Delgado, L. S. Navigatore Fonzo, I. T. Ponce, M. G. Lacoste and A. C. Anzulovich, Daily patterns of clock and cognition-related factors are modified in the hippocampus of vitamin A-deficient rats, *Hippocampus*, 2012, **22**, 1720–1732.

8 M. T. Birnie, M. D. B. Claydon, O. Troy, B. P. Flynn, M. Yoshimura, Y. M. Kershaw, Z. Zhao, R. C. R. Demski-Allen, G. R. I. Barker, E. C. Warburton, Z. A. Bortolotto, S. L. Lightman and B. L. Conway-Campbell, Circadian regulation of hippocampal function is disrupted with corticosteroid treatment, *Proc. Natl. Acad. Sci. U. S. A.*, 2023, **120**, e2211996120.

9 Z. Nagy, A. Marta, H. Butz, I. Liko, K. Racz and A. Patocs, Modulation of the circadian clock by glucocorticoid receptor isoforms in the H295R cell line, *Steroids*, 2016, **116**, 20–27.

10 G. Naseri Kouzehgarani, M. Y. Bothwell and M. U. Gillette, Circadian rhythm of redox state regulates membrane excitability in hippocampal CA1 neurons, *Eur. J. Neurosci.*, 2020, **51**, 34–46.

11 F. Qin, T. Shen, H. Cao, J. Qian, D. Zou, M. Ye and H. Pei, CeO(2)NPs relieve radiofrequency radiation, improve testosterone synthesis, and clock gene expression in Leydig cells by enhancing antioxidation, *Int. J. Nanomed.*, 2019, **14**, 4601–4611.

12 S. Tan, H. Wang, X. Xu, L. Zhao, J. Zhang, J. Dong, B. Yao, H. Wang, H. Zhou, Y. Gao and R. Peng, Study on dose-dependent, frequency-dependent, and accumulative effects of 1.5 GHz and 2.856 GHz microwave on cognitive functions in Wistar rats, *Sci. Rep.*, 2017, **7**, 10781.

13 D. P. Jiang, J. H. Li, J. Zhang, S. L. Xu, F. Kuang, H. Y. Lang, Y. F. Wang, G. Z. A. J. Li and G. Z. Guo, Long-term electromagnetic pulse exposure induces Abeta deposition and cognitive dysfunction through oxidative stress and overexpression of APP and BACE1, *Brain Res.*, 2016, 10–19.

14 Y. Yang, Y. Jia, Q. Sun, H. Dong and R. Zhao, White light emitting diode induces autophagy in hippocampal neuron cells through GSK-3-mediated GR and RORα pathways, *Aging*, 2019, **11**, 1832–1849.

15 Y. Fan, J. Wang, N. He and H. Feng, PLK2 protects retinal ganglion cells from oxidative stress by potentiating *Nrf2* signaling via GSK-3β, *J. Biochem. Mol. Toxicol.*, 2021, **35**, e22815.

16 Q. Sun, N. Jia, X. Li, J. Yang and G. Chen, Grape seed proanthocyanidins ameliorate neuronal oxidative damage by inhibiting GSK-3β-dependent mitochondrial permeability transition pore opening in an experimental model of sporadic Alzheimer's disease, *Aging*, 2019, **11**, 4107–4124.

17 G. Luan, Z. Zhu, K. Wu and S. Yin, Theaflavin-3,3′-digallate attenuates cigarette smoke extract-induced pulmonary emphysema in mice by suppressing necroptosis, *Exp. Ther. Med.*, 2022, **23**, 11.

18 T. He, X. Lin, C. Yang, Z. Chen, L. Wang, Q. Li, J. Ma, F. Zhan, Y. Wang, J. Yan and Z. Quan, Theaflavin-3,3′-Digallate plays a ROS-mediated dual role in ferroptosis and apoptosis via the MAPK Pathway in human osteosarcoma cell lines and xenografts, *Oxid. Med. Cell. Longevity*, 2022, 8966368.

19 J. He, G. Yao, Q. He, T. Zhang, H. Fan, Y. Bai, J. Zhang, G. Yang, Z. Xu, J. Hu and Y. Sun, Theaflavin 3, 3′-Digallate delays ovarian aging by improving oocyte quality and regulating granulosa cell function, *Oxid. Med. Cell. Longevity*, 2021, 7064179.

20 H. Li, H. C. Kek, J. Lim, R. W. Gelling and W. Han, Green tea (-)-epigallocatechin-3-gallate counteracts daytime overeating induced by high-fat diet in mice, *Mol. Nutr. Food Res.*, 2016, **60**, 2565–2575.

21 L. Mei, Y. Zheng, T. Ma, B. Xia, X. Gao, Y. Hao, Z. Luo and J. Huang, (-)-Epigallocatechin-3-gallate ameliorates intervertebral disc degeneration through reprogramming of the circadian clock, *Front. Pharmacol.*, 2021, **12**, 753548.

22 L. K. Leung, Y. Su, R. Chen, Z. Zhang, Y. Huang and Z. Y. Chen, Theaflavins in black tea and catechins in green tea are equally effective antioxidants, *J. Nutr.*, 2001, **131**, 2248–2251.

23 H. F. He, Research progress on theaflavins: efficacy, formation, and preparation, *Food Nutr. Res.*, 2017, **61**, 1344521.

24 Y. Cao, Y. Zhang, Z. Jia, H. Jia, Y. Sun, H. Yuan, Y. Bian, B. Xu, J. Fu and F. Qin, Theaflavin-3,3′-digallate ameliorates learning and memory impairments in mice with premature brain aging induced by D-galactose, *Physiol. Behav.*, 2023, **261**, 114077.

25 W. Wang, Y. Sun, J. Liu, J. Wang, Y. Li, H. Li, W. Zhang and H. Liao, Protective effect of theaflavins on cadmium-induced testicular toxicity in male rats, *Food Chem. Toxicol.*, 2012, **50**(9), 3243–3250.

26 F. Qin, H. Cao, H. Yuan, W. Guo, H. Pei, Y. Cao and J. Tong, 1800 MHz radiofrequency fields inhibits testosterone production via CaMKI /RORα pathway, *Reprod. Toxicol.*, 2018, **81**, 229–236.

27 X. Ma, O. Cheng, Q. Jiang, J. Yang, H. Xiao and H. Qiu, Activation of ephrinb1/EPHB2/MAP-2/NMDAR mediates hippocampal neurogenesis Promoted by transcranial direct current stimulation in cerebral-ischemic mice, *NeuroMol. Med.*, 2021, **23**, 521–530.

28 M. Motaghinejad, N. Farokhi, M. Motevalian and S. Safari, Molecular, histological and behavioral evidences for neuroprotective effects of minocycline against nicotine-induced neurodegeneration and cognition impairment: Possible role of CREB-BDNF signaling pathway, *Behav. Brain Res.*, 2020, **386**, 112597.

29 F. Qin, J. Zhang, L. Zan, W. Guo, J. Wang, L. Chen, Y. Cao, O. Shen and J. Tong, Inhibitory effect of melatonin on testosterone synthesis is mediated via GATA-4/SF-1 transcription factors, *Reprod. BioMed. Online*, 2015, **31**, 638–646.

30 G. Q. Zhou, X. Wang, P. Gao, T. Z. Qin, L. Guo, Z. W. Zhang, Z. F. Huang, J. J. Lin, Y. T. Jing, H. N. Wang, C. P. Wang and G. R. Ding, Intestinal microbiota via NLRP3 inflammasome dependent neuronal pyroptosis mediates anxiety-like behaviour in mice exposed to 3.5 GHz radiofrequency radiation, *Sci. Total Environ.*, 2024, **927**, 172391.
31 R. R. Pan, Y. H. Zhi, Y. X. Jin and X. H. Zhou, Influences of theaflavins on neuronal apoptosis and blood-brain barrier in rats with cerebral hemorrhage by regulating CaMKK2/AMPK signaling pathway, *Chin. J. Clin. Pharmacol. Ther.*, 2022, **27**, 1240–1246.
32 X. Dong, J. Miao, L. Wu, Z. Kong, Z. Liu, D. Jia, Q. Zhai, D. Zhang and Y. Xu, Diabetic wound healing breakthrough: theaflavin-3, 3′-digallate nanoparticles@hydrogel activates the TGF-β1/SMAD3 pathway, *Phytomedicine*, 2025, **141**, 156617.
33 V. Kopatz, K. Wen, T. Kovács, A. S. Keimowitz, V. Pichler, J. Widder, A. D. Vethaak, O. Hollóczki and L. Kenner, Micro- and Nanoplastics Breach the Blood-Brain Barrier (BBB): Biomolecular Corona's Role Revealed, *Nanomaterials*, 2023, **13**, 1404.
34 R. Peng, W. Zhang, Z. Zuo, Y. Shan, X. Liu, Y. Tang, Z. Yu, L. Wang and Y. Cong, Dimethyl sulfoxide, a potent oral radioprotective agent, confers radioprotection of hematopoietic stem and progenitor cells independent of apoptosis, *Free Radicals Biol. Med.*, 2020, **153**, 1–11.
35 F. L. Hitti and S. A. Siegelbaum, The hippocampal CA2 region is essential for social memory, *Nature*, 2014, **508**, 88–92.
36 S. Mumtaz, J. N. Rana, E. H. Choi and I. Han, Microwave radiation and the brain: mechanisms, current status, and future prospects, *Int. J. Mol. Sci.*, 2022, **23**, 9288.
37 N. Karimi, M. Bayat, M. Haghani, H. F. Saadi and G. R. Ghazipour, 2.45 GHz microwave radiation impairs learning, memory, and hippocampal synaptic plasticity in the rat, *Toxicol. Ind. Health*, 2018, **34**, 873–883.
38 P. Chauhan, H. N. Verma, R. Sisodia and K. K. Kesari, Microwave radiation (2.45 GHz)-induced oxidative stress: Whole-body exposure effect on histopathology of Wistar rats, *Electromagn. Biol. Med.*, 2017, **36**, 20–30.
39 R. Varghese, A. Majumdar, G. Kumar and A. Shukla, Rats exposed to 2.45 GHz of non-ionizing radiation exhibit behavioral changes with increased brain expression of apoptotic caspase 3, *Pathophysiology*, 2018, **25**, 19–30.
40 W. Koh, H. Kwak, E. Cheong and C. J. Lee, GABA tone regulation and its cognitive functions in the brain, *Nat. Rev. Neurosci.*, 2023, **24**, 523–539.
41 S. Vaseghi, M. Nasehi and M. R. Zarrindast, How do stupendous cannabinoids modulate memory processing via affecting neurotransmitter systems?, *Neurosci. Biobehav. Rev.*, 2021, **120**, 173–221.
42 S. F. Gao and A. M. Bao, Corticotropin-releasing hormone, glutamate, and γ-aminobutyric acid in depression, *Neuroscientist*, 2011, **17**, 124–144.
43 Y. Zhang, J. M. Chu and G. T. Wong, Cerebral Glutamate Regulation and Receptor Changes in Perioperative Neuroinflammation and Cognitive Dysfunction, *Biomolecules*, 2022, **12**, 597.
44 M. K. Tripathi, M. Kartawy and H. Amal, The role of nitric oxide in brain disorders: Autism spectrum disorder and other psychiatric, neurological, and neurodegenerative disorders, *Redox Biol.*, 2020, **34**, 101567.
45 I. Espadas, O. Ortiz, P. García-Sanz, A. Sanz-Magro, S. Alberquilla, O. Solis, J. M. Delgado-García, A. Gruart and R. Moratalla, Dopamine D2R is required for hippocampal-dependent memory and plasticity at the CA3-CA1 synapse, *Cereb. Cortex*, 2021, **31**, 2187–2204.
46 M. Rima, Y. Lattouf, M. Abi Younes, E. Bullier, P. Legendre, J. M. Mangin and E. Hong, Dynamic regulation of the cholinergic system in the spinal central nervous system, *Sci. Rep.*, 2020, **10**, 1–13.
47 N. P. Kurzina, I. Y. Aristova, A. B. Volnova and R. R. Gainetdinov, Deficit in working memory and abnormal behavioral tactics in dopamine transporter knockout rats during training in the 8-arm maze, *Behav. Brain Res.*, 2020, **390**, 112642.
48 S. K. Gupta, M. K. Mesharam and S. Krishnamurthy, Electromagnetic radiation 2450 MHz exposure causes cognition deficit with mitochondrial dysfunction and activation of intrinsic pathway of apoptosis in rats, *J. Biosci.*, 2018, **43**, 263–276.
49 N. A. Ahmed, N. M. Radwan, H. S. Aboul Ezz, Y. A. Khadrawy and N. A. Salama, The chronic effect of pulsed 1800 MHz electromagnetic radiation on amino acid neurotransmitters in three different areas of juvenile and young adult rat brain, *Toxicol. Ind. Health*, 2018, **34**, 860–872.
50 B. Avci, A. Akar, B. Bilgici and Ö. K. Tunçel, Oxidative stress induced by 1.8 GHz radio frequency electromagnetic radiation and effects of garlic extract in rats, *Int. J. Radiat. Biol.*, 2012, **88**, 799–805.
51 J. H. Kim, C. H. Lee, H. G. Kim and H. R. Kim, Decreased dopamine in striatum and difficult locomotor recovery from MPTP insult after exposure to radiofrequency electromagnetic fields, *Sci. Rep.*, 2019, **9**, 1201.
52 C. Hu, H. Zuo and Y. Li, Effects of radiofrequency electromagnetic radiation on neurotransmitters in the brain, *Front. Public Health*, 2021, **9**, 691880.
53 K. V. Singh, R. Gautam, R. Meena, J. P. Nirala, S. K. Jha and P. Rajamani, Effect of mobile phone radiation on oxidative stress, inflammatory response, and contextual fear memory in Wistar rat, *Environ. Sci. Pollut. Res.*, 2020, **27**, 19340–19351.
54 H. Elyasi, M. Ghanbari and F. Nadri, Investigation of the adverse health effects of cell phone radiation and propose solutions to minimize them: A systematic review, *Indian J. Occup. Environ. Med.*, 2024, **28**(1), 18–22.
55 J. Li, H. Jiang, P. Wu, S. Li, B. Han, Q. Yang, X. Wang, B. Han, N. Deng, B. Qu and Z. Zhang, Toxicological effects of deltamethrin on quail cerebrum: Weakened antioxidant defense and enhanced apoptosis, *Environ. Pollut.*, 2021, **286**, 117319.

 This journal is © The Royal Society of Chemistry 2025

56 Y. Chen, R. Cheng, W. Lu, Y. Fan, Y. Yu, L. Huang, Z. Wan and S. Zheng, Metformin promotes the survival of random skin flaps via the activation of Nrf2/HO-1 signaling, *Chem.-Biol. Interact.*, 2024, **401**, 111188.

57 H. Mazdak, G. Z. Tolou and M. Gholampour, Bladder cancer: total antioxidant capacity and pharmacotherapy with vitamin-E, *Int. Urol. Nephrol.*, 2020, **52**, 1255–1260.

58 Z. Yang, G. Jie, F. Dong, Y. Xu, N. Watanabe and Y. Tu, Radical-scavenging abilities and antioxidant properties of theaflavins and their gallate esters in H_2O_2-mediated oxidative damage system in the HPF-1 cells, *Toxicol. in Vitro*, 2008, **22**, 1250–1256.

59 Y. Y. Wu, W. Li, Y. Xu, E. H. Jin and Y. Y. Tu, Evaluation of the antioxidant effects of four main theaflavin derivatives through chemiluminescence and DNA damage analyses, *J. Zhejiang Univ., Sci., B*, 2011, **12**, 744–751.

60 Y. Teng, Z. Jin, W. Ren, M. Lu, M. Hou, Q. Zhou, W. Wang, H. Yang and J. Zou, Theaflavin-3,3′-Digallate protects cartilage from degradation by modulating inflammation and antioxidant pathways, *Oxid. Med. Cell. Longevity*, 2022, 3047425.

61 B. Han, S. Li, Y. Lv, D. Yang, J. Li, Q. Yang, P. Wu, Z. Lv and Z. Zhang, Dietary melatonin attenuates chromium-induced lung injury via activating the Sirt1/Pgc-1α/Nrf2 pathway, *Food Funct.*, 2019, **10**, 5555–5565.

62 S. Huang, Y. Wang, S. Xie, Y. Lai, C. Mo, T. Zeng, S. Kuang, G. Deng, C. Zhou, Y. Chen, S. Huang, L. Gao and Z. Lv, Hepatic TGFβr1 deficiency attenuates lipopolysaccharide/D-Galactosamine-induced acute liver failure through inhibiting GSK3β-Nrf2-mediated hepatocyte apoptosis and ferroptosis, *Cell. Mol. Gastroenterol. Hepatol.*, 2022, **13**, 1649–1672.

63 O. Silva-García, R. Cortés-Vieyra, F. N. Mendoza-Ambrosio, G. Ramírez-Galicia and V. M. Baizabal-Aguirre, GSK3α: an important paralog in neurodegenerative disorders and cancer, *Biomolecules*, 2020, **10**, 1683.

64 C. J. Phiel, C. A. Wilson, M. Y. Lee and V. P. S. Klein, GSK-3α regulates production of Alzheimer's disease amyloid-β peptides, *Nature*, 2003, **423**, 435–439.

65 O. V. Podgorny and N. V. Gulyaeva, Glucocorticoid-mediated mechanisms of hippocampal damage: Contribution of subgranular neurogenesis, *J. Neurochem.*, 2021, **157**, 370–392.

66 J. M. You, S. J. Yun, K. N. Nam, C. Kang, R. Won and E. H. Lee, Mechanism of glucocorticoid-induced oxidative stress in rat hippocampal slice cultures, *Can. J. Physiol. Pharmacol.*, 2009, **87**, 440–447.

67 M. J. Misa-Agustiño, J. M. Leiro-Vidal, J. L. Gomez-Amoza, M. T. Jorge-Mora, F. J. Jorge-Barreiro, A. A. Salas-Sánchez, F. J. Ares-Pena and E. López-Martín, EMF radiation at 2450 MHz triggers changes in the morphology and expression of heat shock proteins and glucocorticoid receptors in rat thymus, *Life Sci.*, 2015, **127**, 1–11.

68 D. Y. Zhu, J. Lu, R. Xu, J. Z. Yang, X. R. Meng, X. N. Ou-Yang, Q. Y. Yan, R. F. Nie, T. Zhao, Y. D. Chen, Y. Lu, Y. N. Zhang, W. J. Li and X. Shen, FX5, a non-steroidal glucocorticoid receptor antagonist, ameliorates diabetic cognitive impairment in mice, *Acta Pharmacol. Sin.*, 2022, **43**, 2495–2510.

69 S. S. Shim and G. E. Stutzmann, Inhibition of Glycogen Synthase Kinase-3: An Emerging Target in the Treatment of Traumatic Brain Injury, *J. Neurotrauma*, 2016, **33**, 2065–2076.

70 X. Tan, Z. Liang, Y. Li, Y. Zhi, L. Yi, S. Bai, K. H. Forest, R. A. Nichols, Y. Dong and Q. X. Li, Isoorientin, a GSK-3β inhibitor, rescues synaptic dysfunction, spatial memory deficits and attenuates pathological progression in APP/PS1 model mice, *Behav. Brain Res.*, 2021, **398**, 112968.

71 H. Eachus, L. Oberski, J. Paveley, I. Bacila, J. P. Ashton, U. Esposito, F. Seifuddin, M. Pirooznia, E. Elhaik, M. Placzek, N. P. Krone and V. T. Cunliffe, Glucocorticoid receptor regulates protein chaperone, circadian clock and affective disorder genes in the zebrafish brain, *Dis. Models Mech.*, 2023, **16**, dmm050141.

72 C. W. Smies, K. K. Bodinayake and J. L. Kwapis, Time to learn: The role of the molecular circadian clock in learning and memory, *Neurobiol. Learn. Mem.*, 2022, **193**, 107651.

73 Y. Zheng, L. Pan, F. Wang, J. Yan, T. Wang, Y. Xia, L. Yao, K. Deng, Y. Zheng, X. Xia, Z. Su, H. Chen, J. Lin, Z. Ding, K. Zhang, M. Zhang and Y. Chen, Neural function of *Bmal1*: an overview, *Cell Biosci.*, 2023, **13**, 1.

74 J. M. Lee, H. Kim and S. H. Baek, Unraveling the physiological roles of retinoic acid receptor-related orphan receptor α, *Exp. Mol. Med.*, 2021, **53**, 1278–1286.

75 R. Fan, X. Peng, L. Xie, K. Dong, D. Ma, W. Xu, X. Shi, S. Zhang, J. Chen, X. Yu and Y. Yang, Importance of *Bmal1* in Alzheimer's disease and associated aging-related diseases: Mechanisms and interventions, *Aging Cell*, 2022, **21**, e13704.

76 I. Barone, N. M. Gilette, H. Hawks-Mayer, J. Handy, K. J. Zhang, F. F. Chifamba, E. Mostafa, E. M. Johnson-Venkatesh, Y. Sun, J. M. Gibson, A. Rotenberg, H. Umemori, P. T. Tsai and J. O. Lipton, Synaptic BMAL1 phosphorylation controls circadian hippocampal plasticity, *Sci. Adv.*, 2023, **9**, eadj1010.

77 M. F. Kanan, P. W. Sheehan, J. N. Haines, P. G. Gomez, A. Dhuler, C. J. Nadarajah, Z. M. Wargel, B. M. Freeberg, H. R. Nelvagal, M. Izumo, J. S. Takahashi, J. D. Cooper, A. A. Davis and E. S. Musiek, Neuronal deletion of the circadian clock gene *Bmal1* induces cell-autonomous dopaminergic neurodegeneration, *JCI Insight*, 2024, **9**, e162771.

78 S. Hasegawa, H. Fukushima, H. Hosoda, T. Serita, R. Ishikawa, T. Rokukawa, R. Kawahara-Miki, Y. Zhang, M. Ohta, S. Okada, T. Tanimizu, S. A. Josselyn, P. W. Frankland and S. Kida, Hippocampal clock regulates memory retrieval via Dopamine and PKA-induced GluA1 phosphorylation, *Nat. Commun.*, 2019, **10**, 5766.

79 T. Medin, J. E. Rinholm, S. G. Owe, T. Sagvolden, A. Gjedde, J. Storm-Mathisen and L. H. Bergersen, Low dopamine D5 receptor density in hippocampus in an animal model of attention-deficit/hyperactivity disorder (ADHD), *Neuroscience*, 2013, **242**, 11–20.

80 F. Laplante, D. R. Sibley and R. Quirion, Reduction in acetylcholine release in the hippocampus of dopamine D5 receptor-deficient mice, *Neuropsychopharmacology*, 2004, **29**, 1620–1627.
81 R. Moraga-Amaro, H. González, V. Ugalde, J. P. Donoso-Ramos, D. Quintana-Donoso, M. Lara, B. Morales, P. Rojas, R. Pacheco and J. Stehberg, Dopamine receptor D5 deficiency results in a selective reduction of hippocampal NMDA receptor subunit NR2B expression and impaired memory, *Neuropharmacology*, 2016, **103**, 222–235.
82 A. M. Albeely, C. J. Nolan, D. J. Rasmussen, C. D. C. Bailey and M. L. Perreault, Cortical dopamine D5 receptors regulate neuronal circuit oscillatory activity and memory in rats, *CNS Neurosci. Ther.*, 2023, **29**, 2469–2480.

This journal is © The Royal Society of Chemistry 2025