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FTO (fat-mass and obesity-associated protein) deficiency aggravates age-dependent depression-like behaviors and cognitive impairment

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Abstract

Background The demethylase fat mass and obesity-related associated protein (FTO) is strongly associated with depression. Aging is a risk factor for synaptic plasticity damage in the brain and leads to neurocognitive dysfunctions. FTO-dependent m6A modification plays an important role in neurodevelopment and cognitive function. However, whether FTO is associated with susceptibility to depression in different age groups remains unknown.

Methods We subjected 3-and 12-month-old C57BL/6J male mice to chronic unpredictable mild stress (CUMS) for 6 weeks, of which 3 weeks were used for hippocampal injection of FTO knockdown adeno-associated virus 9 shRNA (FTO-KD AAV9). Finally, 36 male mice in each 3-month-old and 12-month-old groups were divided into three groups (n = 12): Sham, CUMS, and FTO-KD. After 6 weeks, we assessed behavioral deficits (depressive and anxiety-like behaviors and cognitive impairment) by behavioral tests and hippocampal neuronal damage (dendritic spine density, neuronal atrophy, and expression of proteins associated with synaptic plasticity) by molecular biochemical experiments.

Results The results showed that 12-month-old C57BL/6J mice were more likely to develop depression-like behavior and spatial learning and memory impairment induced by CUMS than 3-month-old mice. Chronic stress-induced depression-like behavior and cognitive impairment worsened after the FTO-KD intervention. In the hippocampus of 3- and 12-month-old mice, CUMS induced the downregulation of FTO, nerve growth factor (NGF), reelin, and synaptic plasticity-related proteins. It also caused abnormal brain-derived neurotrophic factor (BDNF)- the tropomyosin-related kinase B (TrkB) signaling, reduced density of dendritic spines, and an increased number of neuronal pyknotic nuclei, leading to neuronal disarray, which was more significant in 12-month-old animals. FTO deficiency accelerated neuronal damage in the hippocampus of 12-month-old CUMS mice.

Conclusions This study provides rodent evidence that FTO deficiency may increase the susceptibility to depression in older adults by impairing hippocampal neuronal function and neuronal synaptic plasticity in an age-dependent

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manner. This suggests that the development of FTO activators may be an effective treatment for depression in older adults.

Keywords FTO, Chronic unpredictable mild stress (CUMS), Cognitive impairment, Synaptic plasticity, BDNF-TrkB signaling pathway

Introduction

Major depressive disorder (MDD) is a severe psychiatric condition that adversely affects individuals' emotional and physical well-being. The WHO reports that over 280 million individuals globally experience depression, with around two-thirds exhibiting cognitive deficits [1–3]. Furthermore, by 2030, MDD is projected to be the leading cause of disease burden globally [4].

A study revealed that 94% of depressed patients display neurocognitive impairments after the alleviation of their depressive symptoms [5]. Cognitive deficits in depression are significantly correlated with age [6]. Neurocognitive deficits endure longer in older persons who have experienced depression and subsequently recovered, compared to younger individuals who recover from depression [5]. This suggests that the interaction between aging and depression may result in more pronounced neurocognitive deficits.

The hippocampus is a crucial brain region that requires examination of the factors contributing to cognitive impairment linked to depression. Moreover, the hippocampus is a significant area susceptible to age-related degeneration [7].

Neurotrophins have a crucial role in regulating neuroplasticity, neuroprotection, and brain development. Preclinical studies [8, 9] have demonstrated that NGF and BDNF are significantly concentrated in the hippocampus and prefrontal cortex, where they play crucial roles in axon growth, neuronal survival, differentiation, neuroprotection, neurogenesis, and synaptic plasticity, including postsynaptic density protein 95 (PSD-95), synaptophysin (SYN), and synaptic morphology. NFG and BDNF expression levels have been documented to be diminished in the hippocampus of individuals with mental disorders including MDD [10–12]. This indicates that the aberrant expression of NGF and BDNF is a significant pathophysiological characteristic of MDD. Reelin is intricately associated with brain development [13, 14]. Hippocampal reelin facilitates dendritic spine growth and maturation, augments synaptic plasticity and hippocampus neurogenesis, and is involved in learning and memory [15, 16].

Epigenetic changes significantly contribute to the etiology of MDD. N6-methyladenosine (m6A) is a widespread mRNA methylation alteration, influencing over 7000 mRNAs in over 25% of mammalian species [17, 18]. m6A methylation alteration plays a dynamic role in neuronal development and the aging process of the brain. Research involving humans and animals has demonstrated that m6A methylation sites markedly increase with age in the central nervous system (CNS) [19]. Fat mass and obesity-associated protein (FTO) is a crucial regulator of the dynamic equilibrium of m6A methylation modification and is significantly concentrated in the brain [20]. FTO-dependent m6A demethylation occurs in the axons and dendritic axes of neurons, indicating that the dynamic removal of m6A methylation may be crucial for brain function [21–23]. Additionally, FTO deficiency correlates with neural developmental abnormalities, growth retardation, cognitive impairment, and anxiety- and depression-related behaviors in mammals [24–26].

Nonetheless, the impact of FTO reductions and aging on stress-induced depression-like behaviors and the underlying processes remains ambiguous. Hence, this study aims to examine the impact of age-related variations in FTO on stress-induced depressive behaviors and cognitive impairments. To achieve this objective, we subjected 3- and 12-month-old C57BL/6J mice to chronic unpredictable mild stress (CUMS). FTO knockdown adeno-associated virus 9 shRNA (FTO-KD AAV9) was used to lower hippocampal FTO levels and study its effects on depression-like behaviors and cognitive deficits in CUMS animals. We hypothesized that FTO deficiency exacerbates depressive-like behaviors and cognitive deficits in 12-month-old mice by downregulating the expression of NGF and reelin proteins in the hippocampus, augmenting neuronal damage, diminishing the expression of synaptic plasticity proteins, and inhibiting the BDNF- the tropomyosin-related kinase B (TrkB) signaling axis.

Materials and methods

Animals

Male C57BL/6J mice, aged 3 and 12 months, were supplied by Henan Skobes Biotechnology Co., LTD. Mice were housed in an environment with regulated temperature (22 ± 1 ℃), humidity (50 ± 10%), and a 12-h light/dark cycle. Food and water were always accessible. Animal suffering was minimized by using the "3R" principle: (1) Reduction: reduce the number of animals used, standardized operation; (2) Replacement: using molecular biological methods instead of performing more animal

experiments; (3) Refinement: Optimize the experimental design to minimize pain. All experiments were conducted in accordance with the National Regulations on the Health and Management of Experimental Animals issued by the National Institutes of Health (NIH). All experimental protocols received approval from the Animal Ethics Committee of Chaohu Hospital, affiliated with Anhui Medical University (KYXM-202112–010).

Groups

Following a two-week adaption phase, all mice were randomly allocated into three age-based groups: (1) 3-month-old: Sham group; CUMS group; FTO knockdown (FTO-KD) group; (2) 12-month-old: Sham group; CUMS group; FTO-KD group. During weeks 1 to 6, all groups of mice, excluding the Sham group, were subjected to one or two distinct stressors daily. In the third week, all mice were treated via stereotactic brain surgery. All mice in the Sham and CUMS groups were administered green fluorescent protein (GFP), which its homologs are widely used as fluorescent markers of gene expression and for determination of protein localization and motility in living cells [27]. All animals in the FTO-KD groups were administered FTO-KD AAV9 shRNA (Shanghai Genechem Co., Ltd, China). Stressors, such as food or water deprivation, were only sparingly applied within one week of surgery. At the conclusion of week 6, behavioral assessments were conducted for a duration of 2 weeks, after which the mice were euthanized immediately following the experiment. The CUMS protocol was implemented as described previously [28]. The body weights of all mice were documented at 0, 2, 4, and 6 weeks throughout the investigation. Figure 1 illustrates the experimental methodology and CUMS stressors.

Vector construction, AAV packaging and administration

The mouse FTO shRNA targeting 5'-CGATACAAACTT TGCACCGAT-3' was cloned into the AAV expression vector. AAV-FTO-EGFP (Cat. # 68204-1) and AAV-control-EGFP (Cat. # 68205-1) were packaged by Genechem (Shanghai Genechem Co.,Ltd, China). The final titer of each AAV was 3×10^9 vector genome (v.g.)/mL.

Stereotaxic surgery

The stereotactic surgical apparatus was supplied by Reward Life Technology Co., LTD (Shenzhen, China). The injection site was located in the bilateral hippocampus: anteroposterior = − 2.1 mm, mediolateral = ± 2.1

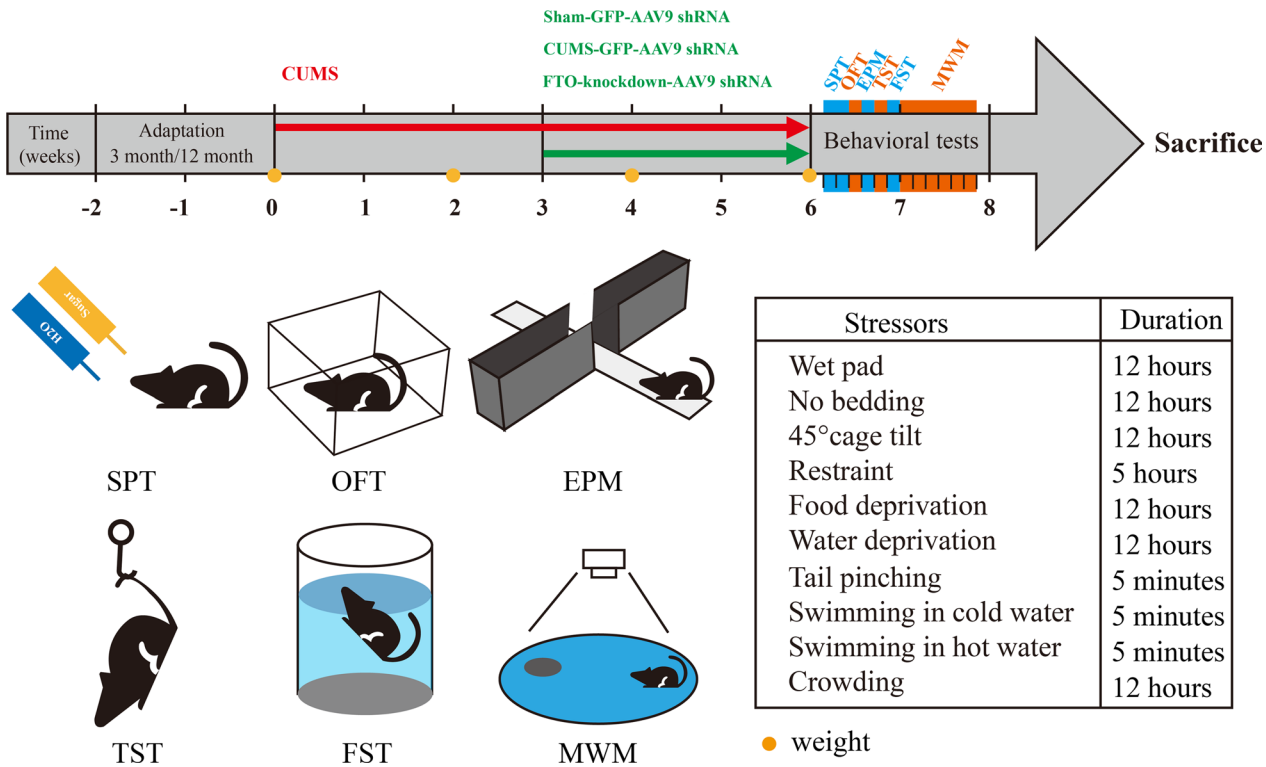


Fig. 1 Experimental design and protocol. The CUMS protocol includes 10 stressors. *CUMS* Chronic Unpredictable Mild Stress, *AAV9* adeno-associated virus 9, *SPT* Sucrose preference test, *OFT* Open field test, *EPM* the elevated plus maze, *TST* tail suspension test, *FST* forced swimming test, *MWM* Morris Water Maze

mm, dorsoventral from the bregma = − 2.0 mm. A single injection (1 μg per side) was administered at a rate of 250 ng/min over 4 min per side, followed by a 1-min post-injection needle retention period, resulting in a total duration of 5 min per side. The vital signs of the mice were carefully monitored after surgery to ensure their health.

Behavior tests

Sucrose preference test (SPT)

SPT was employed to identify anhedonia in animals, and we made minor enhancements to a prior experimental technique [29]. Prior to behavioral testing, all mice were individually housed in cages of 15 ×15 ×15 cm^3, with one cage equipped with two bottles. On the initial day, all mice were provided with two water bottles and maintained for 24 h. The following day, one of the bottles was substituted with a 1% sucrose solution, and the mice had unrestricted access for 12 h. The bottles containing water and sucrose were subsequently switched and administered for an additional 12 h. On the third day, the mice were denied access to water and food for a duration of 24 h. On the fourth day, each mouse received two premeasured solutions: 120 mL of water and 120 mL of 1% (w/v) sucrose solution. After four hours, the bottles were weighed and repositioned, and after a further four hours, they were weighed once again. Sucrose preference ratio (%) = [sucrose consumption (mL)/(sucrose consumption (mL) + water consumption (mL))] ×100%. To avoid the effect of light on the experimental mice, all mice were tested at a light intensity of 150 lx.

Open field test (OFT)

The OFT is employed to observe the spontaneous locomotion of animals in an open field setting [16]. Prior to the behavioral assessment, all mice were acclimated to the test environment for 24 h under 150 lx light. Initially, mice were permitted to explore freely in the open field for 10 min, with their activity quantified via the RWD automatic tracking system (RWD, Shenzhen, China), encompassing both the central (25 ×25 cm^2) and peripheral regions (50 ×50 cm^2).

Elevated-plus maze (EPM) test

This study employed the EPM test to evaluate anxiety-like behavior in animals [30]. The apparatus comprises two enclosed and two open arms, positioned 50 cm above the ground. Initially, the mice were positioned at the center of the maze, oriented towards the open arm. A video tracking system (RWD, Shenzhen, China) was employed to assess and document murine behavior. The distance and retention time of the mice in the open arm were evaluated throughout a 5-min period. The light intensity of the test room was 150 lx.

Tail suspension test (TST)

The TST was employed to assess depression-like behavior in animals, as described by prior research [31]. The mouse's tail was secured to a hook with tape, orienting it upside down, while its head was positioned 15 cm above the ground. The experimental area consisted of a black wooden frame. A high-definition camera filmed the mice's behavior for 6 min, comprising a 2-min latency phase followed by a 4-min test period, during which the immobility time of the mice was documented (RWD, Shenzhen, China). The experiment was conducted at a light intensity of 150 lx.

Forced swimming test (FST)

The FST was conducted in accordance with experimental methods described previously [32]. A transparent cylindrical bucket was filled with water at a temperature of 23 ± 1 ℃ to a depth of 15 cm. A camera tracking system (RWD, Shenzhen, China) was employed to document the swimming behavior of the mice in water for 10 min, and the duration of immobility (s) of the mice was recorded, accompanied by a light intensity of 150 lx. Upon completion of the experiment, the mice were dried using a heater and subsequently returned to their habitat.

Morris Water Maze (MWM)

The MWM was employed to evaluate spatial learning and memory [33]. MWM was performed in a 60 cm deep white circular pool at a temperature of 22 °C ± 1 and a light of 150 lx. Mice underwent four training trials daily for five consecutive days to locate platforms utilizing information from surrounding visual cues. During the initial five days, the mice were allotted 60 s to locate the platform. If the mice failed to locate the platform, they were aided in finding it and remaining on it for 30 s. On day six, the platform was removed, and the mice were allotted 60 s to locate the hidden platform. The camera (RWD, Shenzhen, China) documents the frequency of entries into the platform, duration spent in the target quadrant, swimming velocity, and the count of entries into the target quadrant, while the Smart program was employed to assess these metrics.

Assays

Immunohistochemistry (IHC)

Immunohistochemistry was performed on free-floating brain sections (30 μm) obtained from mice transcardially perfused with 4% paraformaldehyde (PFA). Following cryoprotection in 30% sucrose, tissues were sectioned using a cryostat and stored in PBS at 4 °C. Antigen

retrieval was conducted by incubating sections in citrate buffer (10 mM, pH 6.0) at 95 °C for 20 min. After blocking with 5% normal goat serum and 0.3% Triton X-100 in PBS for 1 h at room temperature, sections were incubated with primary antibodies (e.g., anti-Iba1 1:500, anti-GFAP 1:1000) in blocking solution for 48 h at 4 °C. Following three washes with PBST, sections were treated with fluorophore-conjugated secondary antibodies (1:1000) for 2 h at room temperature, counterstained with DAPI (1 μg/mL, 5 min), and mounted using antifade medium. Images were acquired using a confocal microscope (20 ×/40 ×objectives) and analyzed using ImageJ software. The antibodies employed were rabbit anti-FTO (Proteintech, 1:500), rabbit anti-NGF (Affinity, 1:100), and rabbit anti-Reelin (Abcam, 1:300). The secondary antibodies utilized were HRP goat anti-rabbit (Servicebio, 1:200). Three mice were included in each group, and three sections were analyzed for each mouse.

Hematoxylin–eosin (H&E) staining

H&E staining was conducted with a previously published method [34]. Following 4% paraformaldehyde perfusion, the entire brain was dissected. The hippocampus was fixed in paraffin and sectioned to a thickness of 5 μm. Dewaxing was performed with xylene, and rehydration was conducted with 100% ethanol. Hematoxylin was applied for 5 min, followed by eosin counterstaining for 3 min. Subsequently, it was processed with ethanol, xylene, and resin. H&E staining was employed for morphological examination utilizing a high-resolution optical microscope (Olympus, Japan). Three sections were analyzed for each mouse, and each section presented a whole brain image (N = 3).

Golgi-cox staining

Golgi-Cox staining was conducted in accordance with prior research [20]. Following the deep anesthesia of the mice, the entire brain was swiftly excised. The entire brain was sectioned into tissue blocks around 10 mm in thickness. Brain tissue specimens were subjected to staining using Golgi staining solution (Servicebio, Wuhan, China). The specimens were immersed in the staining solution, which was replaced after 48 h, and subsequently treated in darkness for 14 days, with the staining solution being changed every 3 days. Sections of 60 μm in thickness were excised utilizing a vibrating microtome (Leica, Germany). Three sections were analyzed for each mouse, and each section presented a whole brain image (N = 3).

Western blotting

The hippocampal tissues of mice were extracted and homogenized in PIPA buffer, protease inhibitor and phosphatase inhibitor mixture at 4℃. The homogenate was transferred to a pre-cooled centrifuge, centrifuged at 12,000 g for 20 min at 4 °C, and the supernatant was collected. Protein concentration was determined with BCA Protein Quantification kit, mixed with 5× loading buffer, and denatured at 95 °C for 10 min. A total of 30 μg of each protein sample was used for electrophoresis. Proteins were separated on SDS-PAGE gels. Then, the proteins were transferred to PVDF membranes (Millipore, USA), and the membranes were incubated with blocking solution (TBST buffer containing 5% skim milk powder) for 60 min at room temperature, followed by incubation with primary antibodies at 4 ℃ overnight. The membranes were washed and incubated with the corresponding secondary antibodies for 1 h at room temperature. Protein bands were scanned and quantified using Image-Pro software. Rabbit anti-FTO (1:5000, Thermo, USA), rabbit anti-BDNF (1:1000, Cell Signaling Technology, USA), rabbit anti-TrkB (1:1000, Cell Signaling Technology, USA), rabbit anti-postsynaptic density protein 95 (PSD-95) (1:5000, Proteintech, USA), rabbit anti- synaptophysin (SYN) (1:1000, Cell Signaling Technology, USA), rabbit anti- synapse-associated protein 97 (SAP97) (1:1000, Cell Signaling Technology, USA), and mouse anti-tubulin (1:1000, Cell Signaling Technology, USA) were incubated overnight at 4 ℃. Subsequently, membranes were incubated with the respective secondary antibodies for 1 h at room temperature and analyzed using a Bio-Rad ChemiDoc MP Chemiluminescence Gel Imaging System. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and tubulin were utilized as the internal standard.

Statistical analyses

The statistical analysis was conducted using SPSS 26.0 software (SPSS, Chicago, IL, USA). The Kolmogorov–Smirnov test was employed to assess the normality of the distribution. Variations in the scale variable among groups were examined using two-way analysis of variance (ANOVA), succeeded by Tukey's test for multiple comparisons, or by the Kruskal–Wallis test, accompanied by Dunn's post hoc test. We also evaluated the treatment by age group interaction followed by analysis of simple effects. Body weights were evaluated using repeated-measures ANOVA, incorporating group and time as factors, followed by Tukey's post-hoc testing. Quantitative data were presented as mean ± standard deviation (SD). Figures were created using GraphPad Prism 10.0 software (GraphPad Software Inc., San Diego, CA, USA). Image J software was employed for image processing and analysis in immunohistochemistry, hematoxylin and eosin staining, Golgi-Cox staining, and Western blotting. The Case Viewer 2.4 program was utilized to examine and take images. P-values below 0.05, 0.01, and 0.001

were regarded as thresholds for statistically significant differences.

Results

The 12 months old FTO-KD mice are more susceptible to CUMS-induced depression-like behaviors and cognitive dysfunction

Figure 2A illustrates that the sham group experienced a consistent increase in body weight in 3- and 12-month-old mice, whereas the CUMS group experienced a minor decrease in body weight. It is important to note that the body weight difference between the sham group and CUMS group was significant ($P < 0.001$) in the sixth week. Additionally, the FTO-KD treatment substantially reduced the body weight of mice in the CUMS group in the 3- and 12-month-old groups ($P < 0.001$ and $P < 0.05$).

The behavioral experiments were conducted to assess the impact of FTO on depression-like and anxiety-like behaviors in CUMS mice of varying ages. Our findings indicated that the CUMS group exhibited a significantly lower sucrose preference (%) in SPT ($P < 0.001$), movement distance in OFT ($P < 0.001$), central area spends time in OFT ($P < 0.05$), total traveled distance in open arms ($P < 0.001$), and open arms residence time ($P < 0.001$) in EPM compared to the age-corresponding sham group in the 3- and 12-month categories (Fig. 2B–I). In addition, we also found that the movement speed of mice in OFT was not affected by FTO-KD (Supplementary file 4).

Figure 2D and H are representative images of the motion trajectories of OFT and EPM, respectively. The CUMS group exhibited a significant increase in immobility time (TST, $P < 0.001$; FST, $P < 0.001$) in comparison to the corresponding placebo group at the 3- and 12-month groups (Fig. 2J–K). These findings suggested that CUMS can induce weight loss, anhedonia, decreased activity, desperate behavior, and anxiety-like behavior in mice. The FTO-KD intervention exacerbated the aforementioned behaviors, thereby aggravating the depression-like and anxiety-like behaviors induced by CUMS in the 3- and 12-month-old groups. The behaviors induced by FTO-KD were significantly more severe in aged mice than in juvenile mice.

The MWM was implemented to evaluate the impact of FTO on neurocognitive function in CUMS mice. In the 3- and 12-month-old groups, the number of entries in the platform, time spent in the target quadrant, and number of entries in the target quadrant were substantially reduced in the CUMS group compared to the corresponding sham group, as illustrated in Fig. 3. The aforementioned indicators were further diminished by FTO-KD, and the reduction effect was more pronounced in the 12-month-old group than in the 3-month-old group. The findings indicated that FTO-KD exacerbated the spatial memory impairment induced by CUMS, and older rodents appeared to be more susceptible. The locomotor activity of rodents in water was not influenced by FTO-KD.

Additionally, we identified a significant interaction pattern between the treatment and age categories on the aforementioned behaviors (Supplementary file 1). The behavioral differences among age groups were indicated by simple effect analysis (Supplementary file 3).

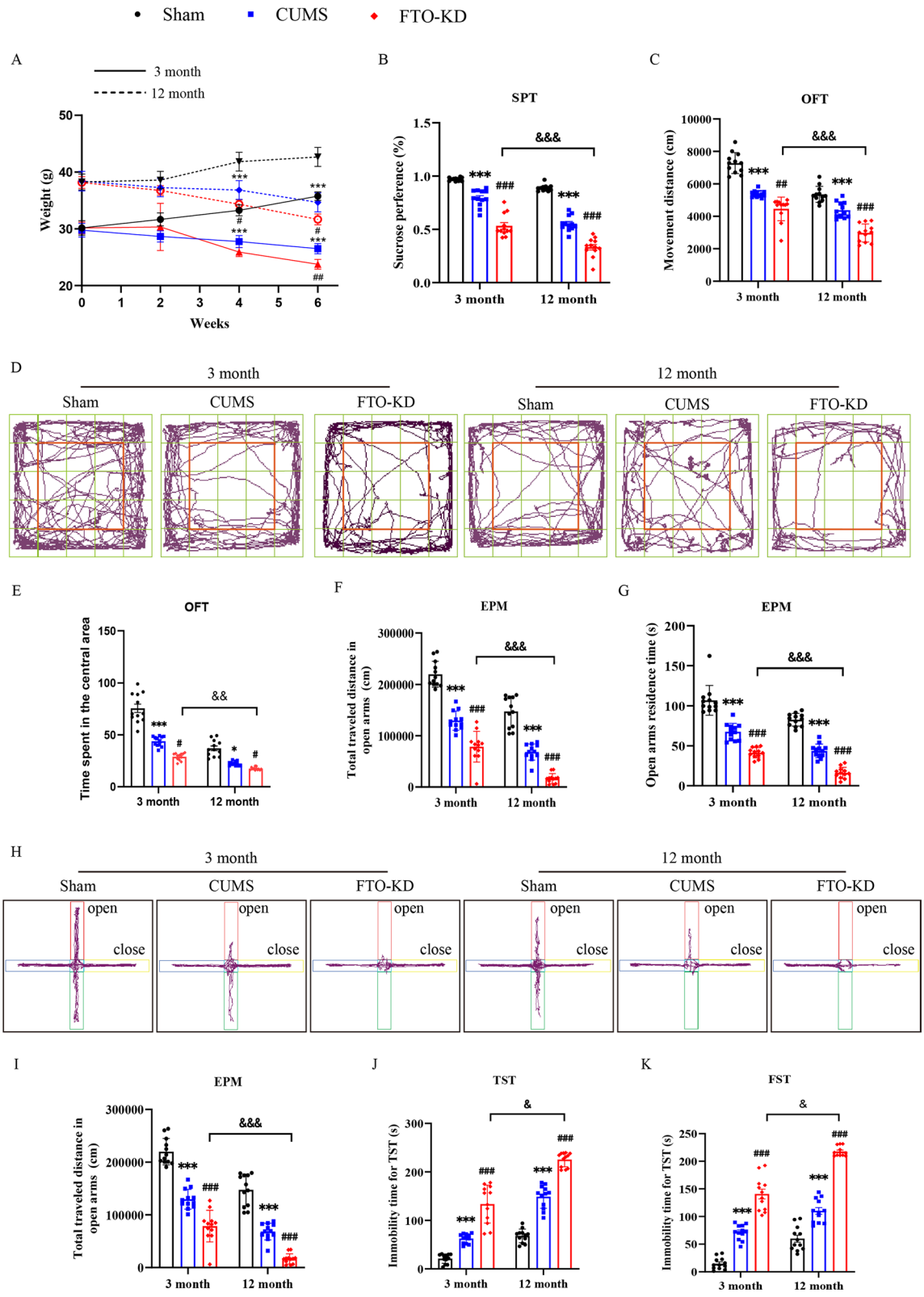
The expression of FTO protein in hippocampus of 12-month-old mice was significantly reduced after FTO-KD

Western blotting and IHC were employed to investigate the transfection of hippocampal FTO-KD AAV9 and its impact on hippocampal FTO expression. The expression of FTO in the hippocampus of mice in the CUMS group was significantly reduced in the 3-month-old ($P < 0.001$) and 12-month-old groups ($P < 0.01$) compared to the corresponding sham group, as confirmed by Western blotting results. The protein expression of FTO in the hippocampus of rodents in the CUMS group was further reduced by the intervention of FTO-KD, and the difference was statistically significant ($P < 0.05$ and $P < 0.01$, Fig. 4A, B).

In particular, the hippocampal FTO of 12-month-old rodents was more susceptible to FTO-KD. The IHC results, as illustrated in Fig. 4C, indicated that the number of anti-FTO positive neurons in CA1, CA2, and DG

(See figure on next page.)

Fig. 2 FTO deficiency aggravates CUMS-induced depression-like and anxiety-like behavior in an age-dependent manner. **A** Weight changes in 3-month-old and 12-month-old mice. **B** Sucrose preference percentage in SPT. **C** Movement distance (cm) in OFT. **D** Representative picture of the movement trajectory in OFT. The red box represents the central area. **E** Time spent in the central area is in OFT (s). **F** The traveled distance in open arms (cm). **G** The time of residence in open arms (s). **H** Representative picture of the movement trajectory in EPM. The cross patterns represent open and closed arms, respectively. **I** Total traveled distance in open arms (s). **J** Immobility time (s) in TST. **K** Immobility time (s) in FST. N = 12 per group. *CUMS* Chronic Unpredictable Mild Stress, *FTO-KD* FTO knockdown, *SPT* Sucrose preference test, *OFT* open field test, *EPM* the elevated plus maze, *TST* tail suspension test, *FST* forced swimming test. Date was expressed as mean ± SD and were analyzed by two-way ANOVA followed by Tukey's multiple comparisons post hoc test. $^{*}P < 0.05$, Sham vs. CUMS; $^{\#}P < 0.05$, CUMS vs FTO-KD; $^{\&}P < 0.05$, FTO-KD (3-month-old) vs. FTO-KD (12-month-old)



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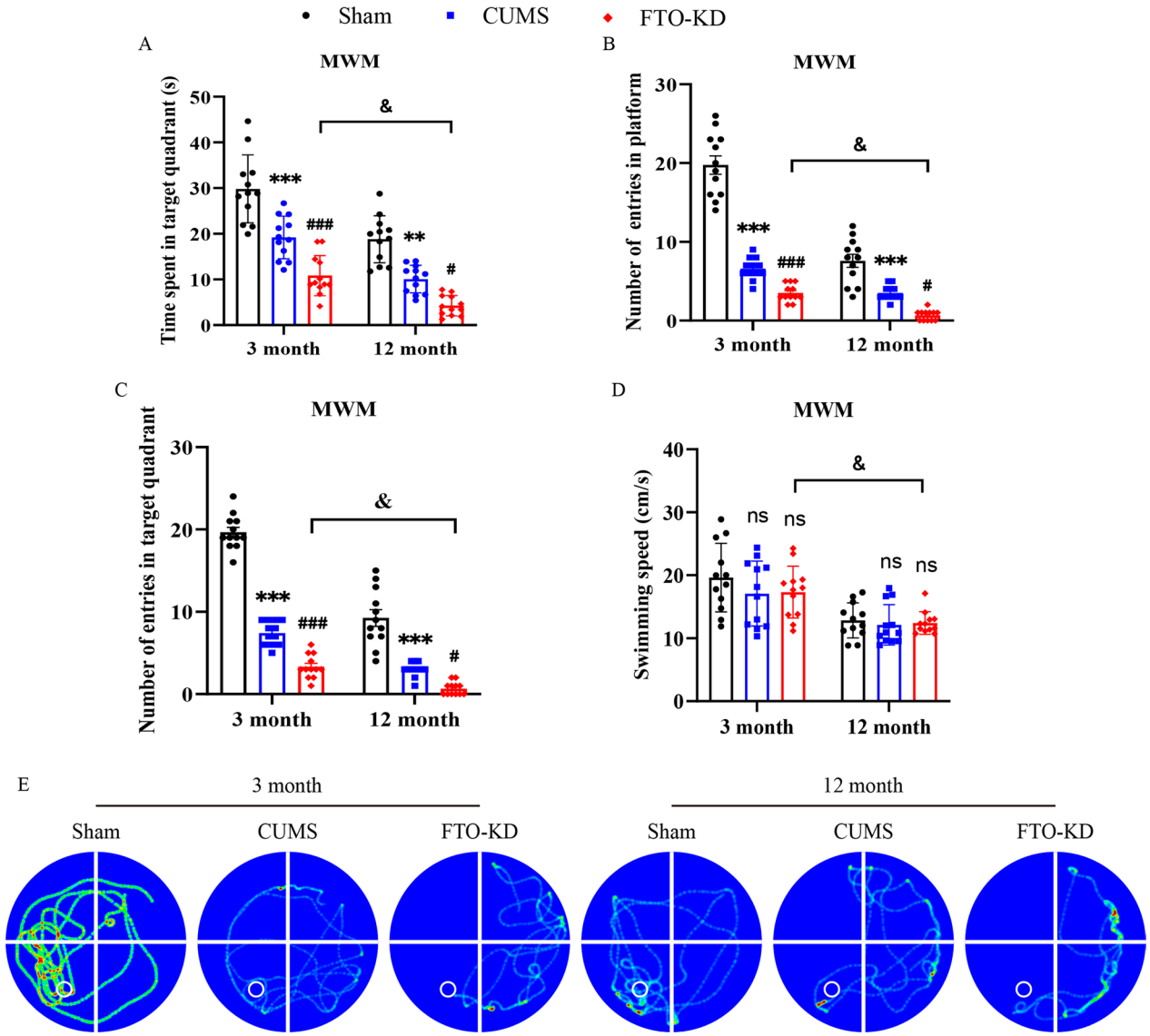

Fig. 3 FTO deficiency aggravates CUMS-induced cognitive impairment in Morris Water Maze. **A** Time spent in target quadrant (s); **B** number of entries in platform. **C** number of entries in target quadrant. **D** Swimming speed (cm/s). **E** Representative picture of the movement trajectory in MWM. N = 12 per group. *CUMS* Chronic Unpredictable Mild Stress, *FTO-KD* FTO knockdown, *MWM* Morris Water Maze. Date was expressed as mean ± SD and were analyzed by two-way ANOVA followed by Tukey's multiple comparisons post hoc test. $^{*}P < 0.05$, Sham vs. CUMS; $^{\#}P < 0.05$, CUMS vs FTO-KD; $^{\&}P < 0.05$, FTO-KD (3-month-old) vs. FTO-KD (12-month-old)

of the CUMS group was substantially lower than that of the sham group at 3 and 12 months of age. The number of anti-FTO-positive neurons in the CA1, CA2, and DG regions of CUMS mice in all age groups was significantly diminished after FTO-KD intervention. The reduction was more pronounced in the 12-month-old group. These findings suggested that CUMS induces a decrease in FTO expression in the hippocampus of mice, FTO-KD is effectively integrated in the hippocampus, and older mice are more susceptible to FTO-KD AAV9.

Western blotting and IHC revealed an interaction pattern between the treatment and age groups on FTO protein expression (Supplementary file 2). The analysis of simple effects revealed significant differences in FTO among both age groups (Supplementary file 3).

FTO-KD reduces NGF and reelin expression in hippocampus of 12-month-old CUMS mice

Early studies [40, 41] demonstrated a close relationship between hippocampal synaptic plasticity and NGF and

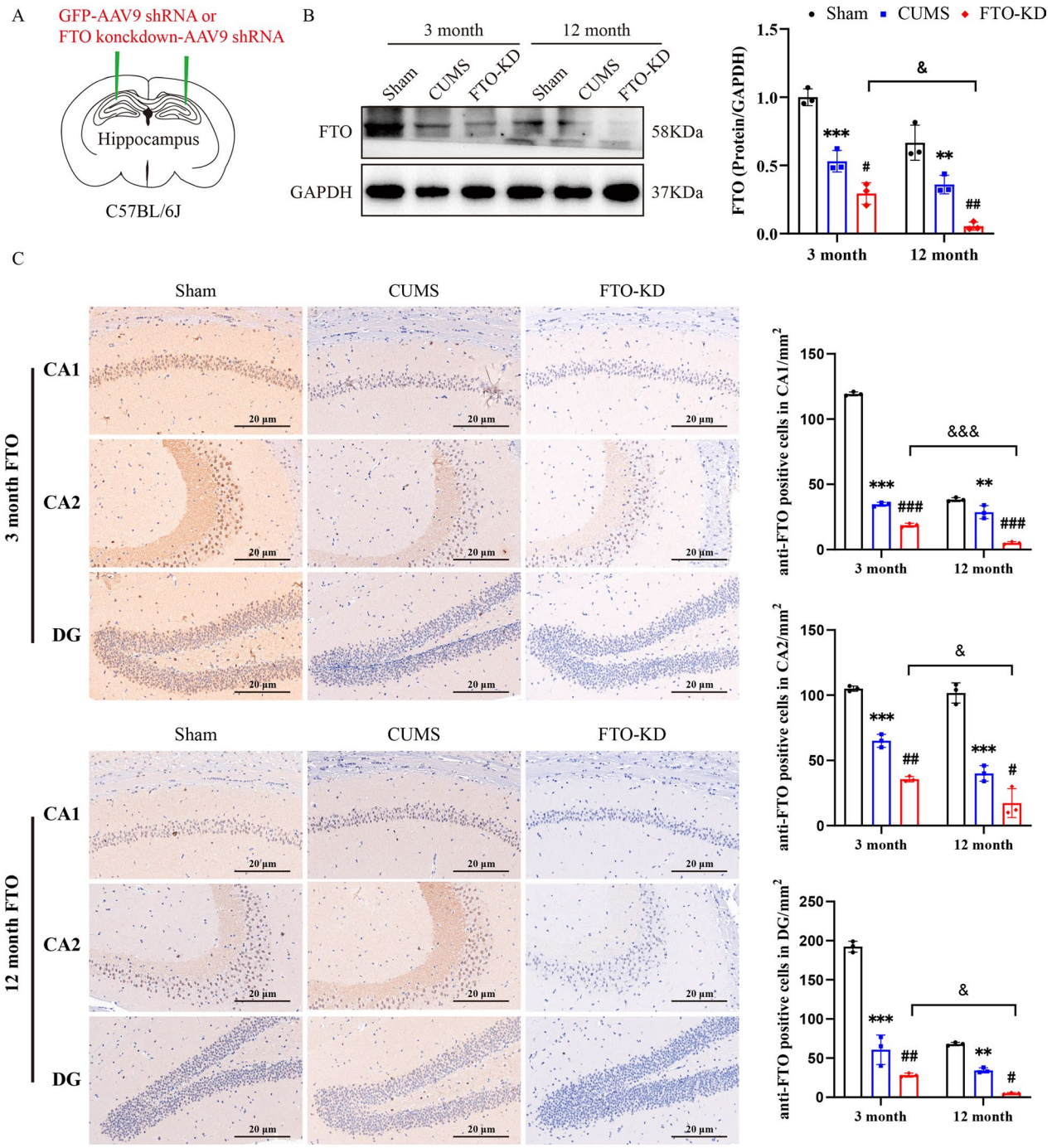

Fig. 4 Brain stereotaxic surgery and expression of FTO in the hippocampus. **A** FTO knockdown AAV9 shRNA virus injection. **B** Representative Western blotting bands of FTO in the hippocampus. Quantification of OFT in hippocampus. **C** Immunohistochemical staining of anti-FTO in the hippocampus. The anti-FTO-positive neurons were shown as dark brown dots. Scale bar = 20 μm. N = 3 per group. *CUMS* Chronic Unpredictable Mild Stress, *FTO-KD* FTO knockdown. Date was expressed as mean ± SD and were analyzed by two-way ANOVA followed by Tukey's multiple comparisons post hoc test. $^{*}P < 0.05$, Sham vs. CUMS; $^{\#}P < 0.05$, CUMS vs FTO-KD; $^{\&}P < 0.05$, FTO-KD (3-month-old) vs. FTO-KD (12-month-old)

reelin. We used IHC to detect the expression of NGF and reelin proteins in the hippocampus. IHC results showed that the number of anti-NGF-positive neurons in the DG region ($P < 0.001$ and $P < 0.05$) and the number of anti-reelin-positive neurons in CA2 mice ($P < 0.05$) in the CUMS group was significantly lower than that in the corresponding sham group in the 3- and 12-month-old groups, respectively (Figs. 5 and 6). The number of anti-NGF positive cells in the DG region was suppressed even more with FTO-KD intervention ($P < 0.001$ and $P < 0.05$), as did the number of anti-reelin positive cells in the CA2 region ($P < 0.01$ and $P < 0.05$). In particular, mice in the 12-month-old group were more susceptible to FTO-KD interference.

The interactions between treatment and age groups were significant in the CA1, CA2, and DG areas for NGF and reelin. A simple effects analysis showed that months of age changed the levels of NFG and reelin in different parts of the hippocampus, and these changes were statistically significant. Refer to Supplementary files 2 and 3 for further details.

Overall, CUMS may cause depressive-like behaviors and cognitive impairment in mice, possibly by lowering the expression of NGF and reelin proteins in the hippocampus. Furthermore, we highlight the connection

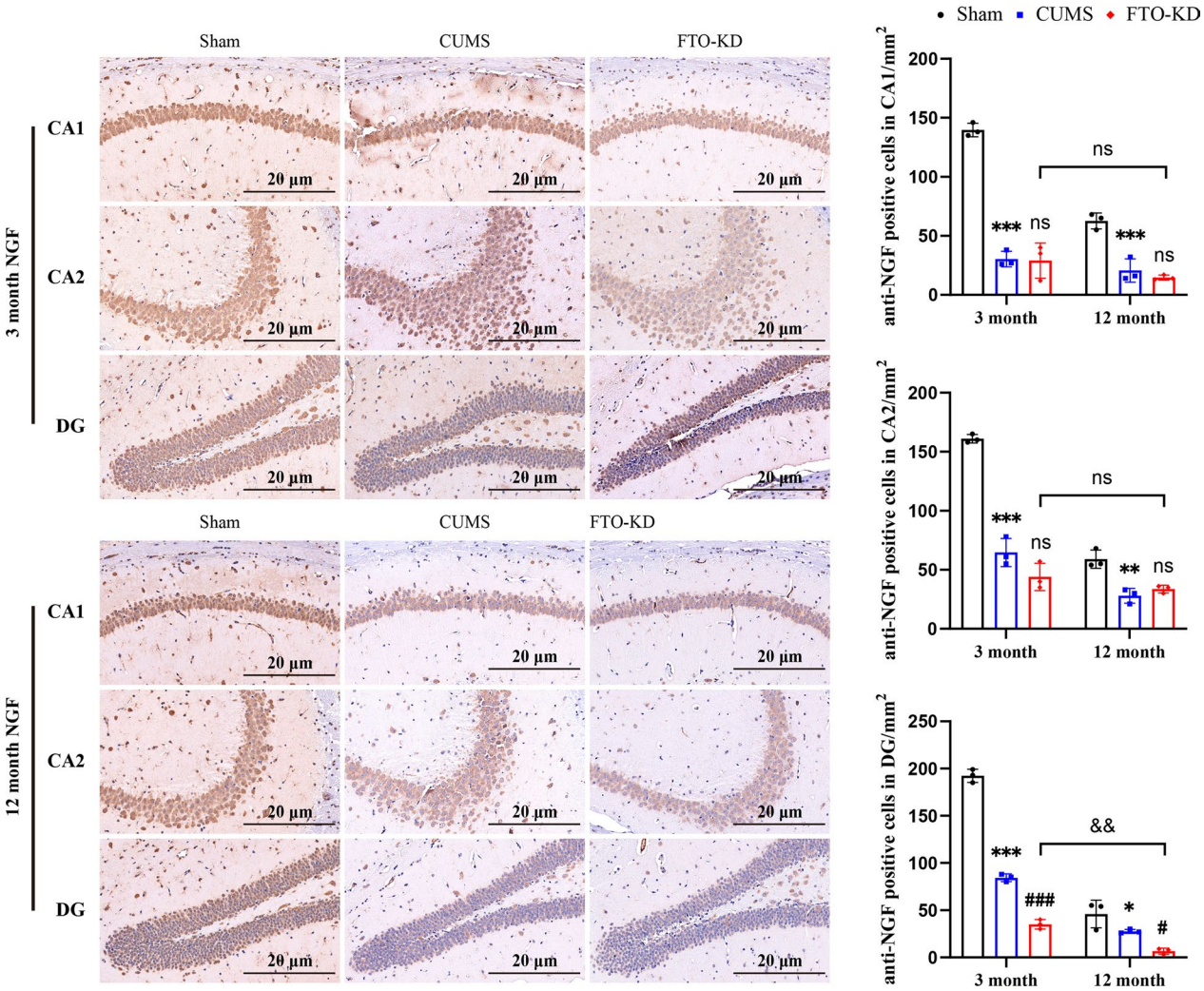

Fig. 5 NGF expression in the hippocampus. Representative picture of NGF immunohistochemical staining in hippocampus. The anti-NGF-positive neurons were shown as dark brown dots. NGF-positive cells in CA1, CA2 and DG regions of the hippocampus were quantified. Scale bar = 20 μm. N = 3 per group. *CUMS* Chronic Unpredictable Mild Stress, *FTO-KD* FTO knockdown. Date was expressed as mean ± SD and were analyzed by two-way ANOVA followed by Tukey's multiple comparisons post hoc test. $^{*}P < 0.05$, Sham vs. CUMS; $^{\#}P < 0.05$, CUMS vs FTO-KD; $^{\&}P < 0.05$, FTO-KD (3-month-old) vs. FTO-KD (12-month-old); ns, no significance

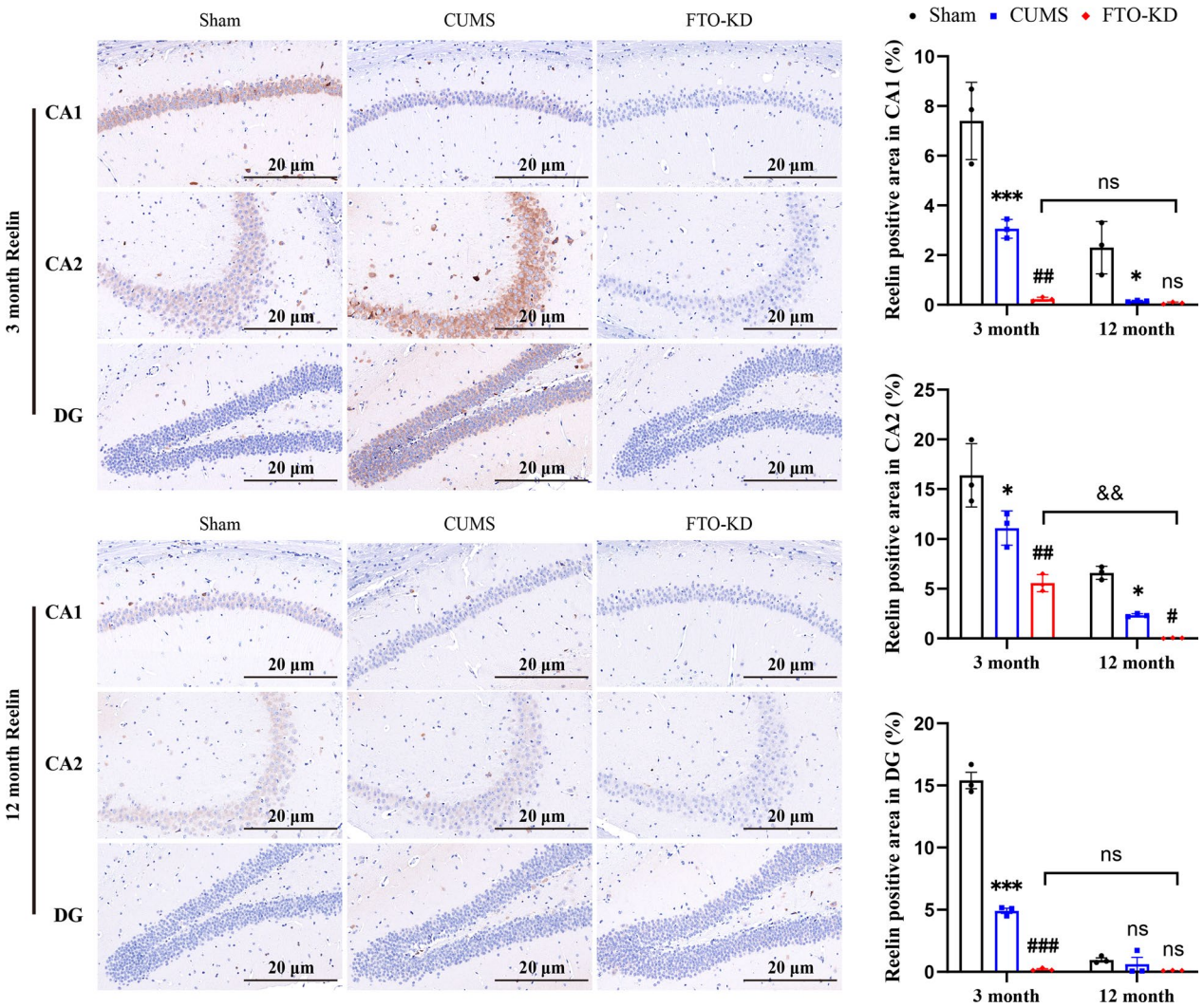

Fig. 6 Reelin expression in the hippocampus. Representative picture of Reelin immunohistochemical staining in hippocampus. The anti-Reelin-positive neurons were shown as brown area. Reelin-positive cells in CA1, CA2 and DG regions of the hippocampus were quantified. Scale bar = 20 μm. N = 3 per group. *CUMS* Chronic Unpredictable Mild Stress, *FTO-KD* FTO knockdown. Date was expressed as mean ± SD and were analyzed by two-way ANOVA followed by Tukey's multiple comparisons post hoc test. $^{*}P < 0.05$, Sham vs. CUMS; $^{\#}P < 0.05$, CUMS vs FTO-KD; $^{\&}P < 0.05$, FTO-KD (3-month-old) vs. FTO-KD (12-month-old); ns, no significance

between FTO loss and age in relation to cognitive impairment. FTO-KD exacerbated depression-like behaviors and cognitive deficits in an age-dependent manner.

FTO-KD aggravates hippocampal neuronal damage in 12-month-old CUMS mice

We used H&E staining to investigate the effect of FTO on hippocampal neurons. Figure 7 shows that in the hippocampal CA1 ($P < 0.05$), CA2 ($P < 0.05$), and DG ($P < 0.05$ and $P < 0.01$) areas, the CUMS group had more pyknotic nuclei than the sham group at both 3 and 12 months. FTO-KD intervention increased the number of pyknotic nuclei in hippocampal CA1 ($P < 0.01$) and DG ($P < 0.01$) areas of 12-month-old CUMS mice.

FTO-KD reduces hippocampal dendritic spine density in 12-month-old CUMS mice

Dendritic spines play an important role in neuronal plasticity [42]. We applied Golgi-cox staining to explore the impact of FTO on hippocampal synaptic plasticity. Using Golgi-cox staining, we found that the density of hippocampal dendritic spines was significantly reduced in the 3-and 12-month-old groups of CUMS mice compared to the corresponding sham group ($P < 0.05$). Interestingly, the density of dendritic spines in 12-month-old

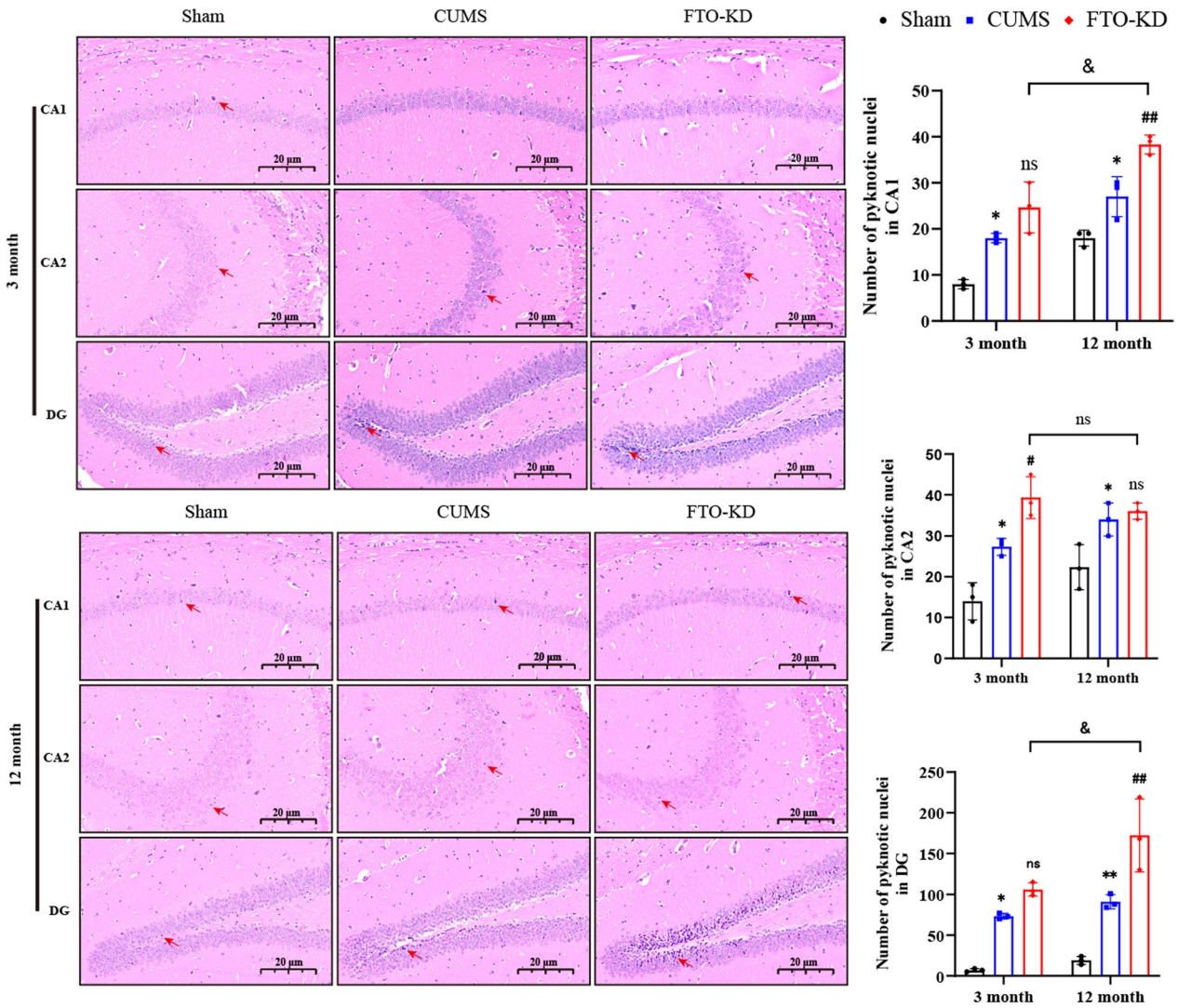

Fig. 7 FTO deficiency aggravates CUMS-induced hippocampal neuronal damage. Representative image of H&E staining of the hippocampus. Quantification of neuronal pyknotic nuclei in CA1, CA2, and DG regions. Pyknotic nuclei are shrunken, hyperchromatic nuclei with condensed chromatin, characteristic of apoptotic or degenerating cells. Scale bar = 20 μm. N = 3 per group. *CUMS* Chronic Unpredictable Mild Stress, *FTO-KD* FTO knockdown. Date was expressed as mean ± SD and were analyzed by two-way ANOVA followed by Tukey's multiple comparisons post hoc test. $^{*}P < 0.05$, Sham vs. CUMS; $^{\#}P < 0.05$, CUMS vs FTO-KD; $^{\&}P < 0.05$, FTO-KD (3-month-old) vs. FTO-KD (12-month-old); ns, no significance

rats was significantly lower than that in 3-month-old rats after FTO-KD intervention (Fig. 8). These results showed that FTO-KD aggravated synaptic plasticity in the mouse hippocampus in an age-dependent manner.

FTO-KD attenuated the hippocampal expression of synaptic plasticity proteins in 12-month-old CUMS mice

Neurotrophins and synaptic plasticity proteins are directly involved in neuronal plasticity and play an important role in learning and memory [43]. In addition, evidence [44] suggests that the BDNF-TrkB signaling pathway regulates the development of neurons and the function of mature synapses. We used Western blotting and found that the levels of BNDF ($P < 0.001$), TrkB ($P < 0.001$ and $P < 0.05$), PSD-95 ($P < 0.05$), SYN ($P < 0.01$), and SAP97 ($P < 0.01$ and $P < 0.05$) proteins were significantly lower in the CUMS group than in the sham group for both the 3- and 12-month-old groups.

Also, after FTO-KD intervention, the levels of BNDF, TrkB, PSD-95, SYN, and SAP97 proteins in the hippocampus of 12-month-old CUMS mice were lower than those in 3-month-old CUMS mice (Fig. 9). The results showed that FTO-KD aggravated synaptic plasticity damage in the hippocampus by lowering the levels of BNDF,

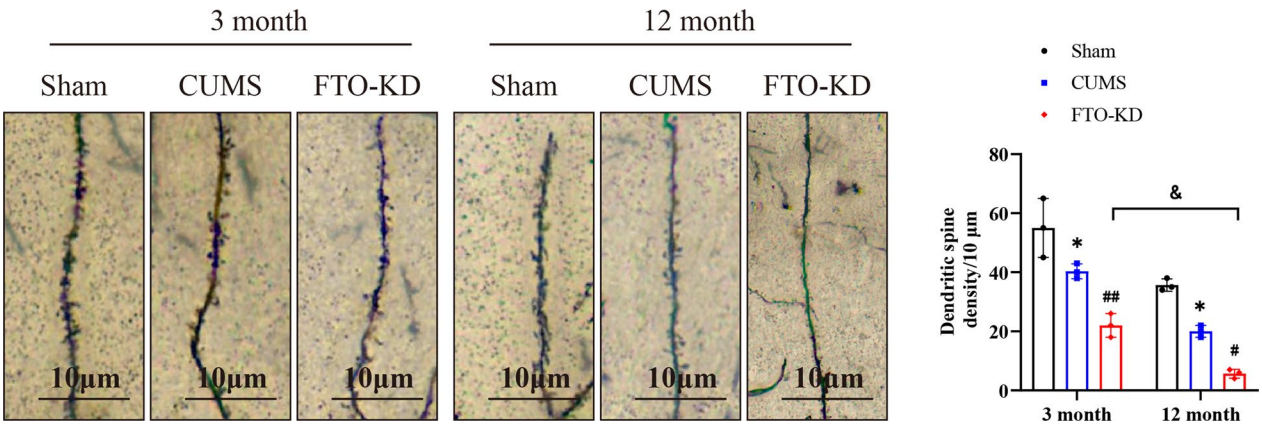

Fig. 8 FTO deficiency aggravated CUMS-induced hippocampal dendritic spine abnormalities. Representative image of hippocampal dendritic spines. Quantification of hippocampal dendritic spine density. Scale bar = 10 μm. N = 3 per group. *CUMS* Chronic Unpredictable Mild Stress, *FTO-KD* FTO knockdown. Date was expressed as mean ± SD and were analyzed by two-way ANOVA followed by Tukey's multiple comparisons post hoc test. $^{*}P < 0.05$, Sham vs. CUMS; $^{\#}P < 0.05$, CUMS vs FTO-KD; $^{\&}P < 0.05$, FTO-KD (3-month-old) vs. FTO-KD (12-month-old)

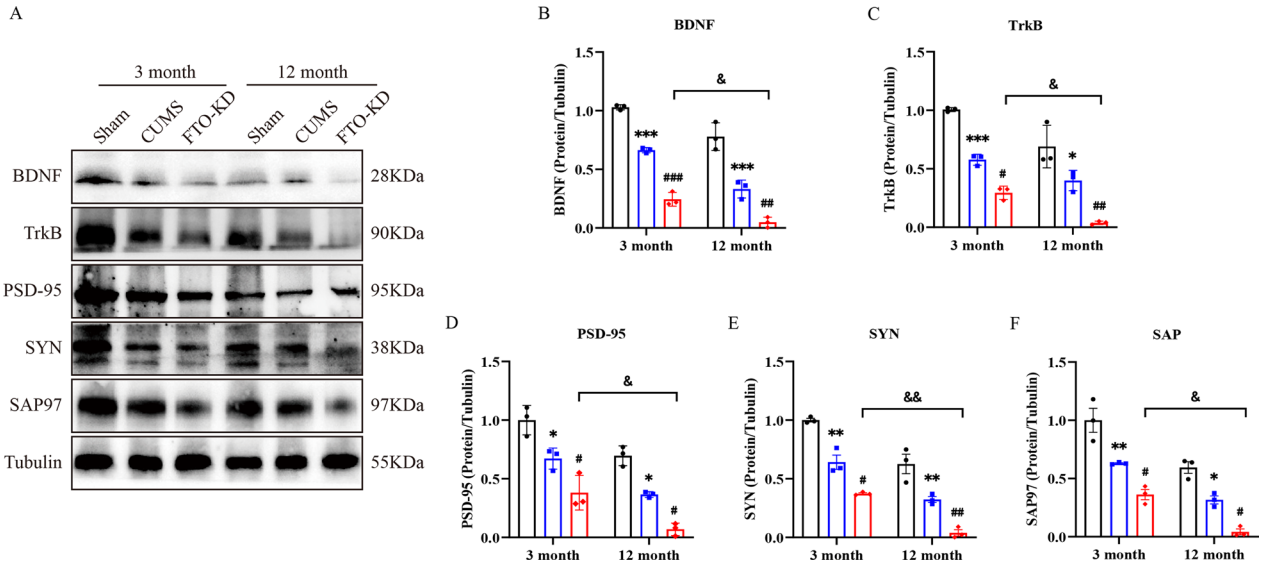

Fig. 9 FTO deficiency aggravates synaptic plasticity damage in hippocampus. **A** Representative image of Western blotting bands. **B**–**F**. Quantification of hippocampal BDNF, TrkB, PSD-95, SYN, and SAP97 expression. N = 3 per group. *CUMS* Chronic Unpredictable Mild Stress, *FTO-KD* FTO knockdown, *BDNF* brain-derived neurotrophic factor, *TrkB* tyrosine Kinase receptor B, *PSD-95* postsynaptic density protein 95, *SYN* synaptophysin, *SAP97* synapse associated protein of 97. Date was expressed as mean ± SD and were analyzed by two-way ANOVA followed by Tukey's multiple comparisons post hoc test. $^{*}P < 0.05$, Sham vs. CUMS; $^{\#}P < 0.05$, CUMS vs FTO-KD; $^{\&}P < 0.05$, FTO-KD (3-month-old) vs. FTO-KD (12-month-old)

TrkB, PSD-95, SYN, and SAP97 proteins. In addition, 12-month-old CUMS mice seem to be more susceptible to FTO-KD. Supplementary file 2 provides detailed data.

Discussion

FTO-KD, age, and depression- and anxiety-like behaviors and cognitive deficits

This study effectively induced depression-like behavior and cognitive deficits in 3- and 12-month-old C57BL/6 J mice following a 6-week CUMS paradigm. Notably, in comparison to the age-matched sham group, the 12-month-old mice exhibited a greater propensity for developing CUMS-induced depressive-like behaviors and cognitive impairments. Significantly, 12-month-old CUMS mice exhibited greater susceptibility to FTO than 3-month-old CUMS mice, and FTO deficiency exacerbated depressive-like behavior and cognitive impairment more in older CUMS mice than in their younger

counterparts. In addition, we found that FTO-KD caused depression-like behaviors, but not dyskinesia. Moreover, relative to the age-matched sham group, the expression of FTO protein in the hippocampus of CUMS group mice was markedly diminished, and FTO-KD AAV9 further attenuated the FTO expression levels in the hippocampus of CUMS mice, demonstrating that FTO-KD AAV9 effectively integrated into the hippocampus.

We found that the CUMS paradigm markedly decreased the expression levels of NGF and reelin in the hippocampus, leading to neuronal nuclear atrophy, disorganized neuronal architecture, diminished dendritic spine density, impaired BDNF-TrkB signaling, and reduced expression of synaptic plasticity-related proteins (PSD-95, SYN, and SAP97) in 3- and 12-month-old mice. FTO deficiency exacerbated hippocampal neuronal loss and pathological alterations in the hippocampus of CUMS animals, particularly in mice aged 12 months. The findings indicate that FTO deficiency exacerbates hippocampus neuronal dysfunction in CUMS animals in an age-dependent manner, resulting in depression-like behaviors and cognitive deficits. Consequently, FTO deficiency is a crucial facilitator of depressive-like behaviors in aged mice.

FTO variants correlate with food consumption and energy expenditure [35]. Our investigation revealed that FTO-KD markedly decreased the body weight of CUMS mice in both the 3-month and 12-month age groups. The reason may be that FTO inhibition activates signal transducer and activator of transcription (STAT3) via extracellular regulated protein kinases (ERK1/2), hence decreasing food consumption and body weight [36]. Results from SPT, OFT, TST, and FST demonstrated that CUMS exposure significantly diminished sucrose preference (%) and locomotor activity while augmenting immobility duration in 3- and 12-month-old mice compared to the age-matched sham group. Nonetheless, 12-month-old mice exhibited lower performance compared to 3-month-old mice in these tests. This suggests that the detrimental effects of CUMS in murine models are significantly more evident in older mice, thereby potentially heightening susceptibility to depression-like behaviors, consistent with previous studies [37, 38]. Concurrently, there is accumulating evidence that aging in rodents heightens their vulnerability to anhedonia [39].

Furthermore, we observed that FTO deficiency exacerbated CUMS-induced anhedonia, diminished exploratory activity, and increased desperate behavior in the SPT, OFT, TST, and FST behavioral assessments, particularly in aged mice. The findings indicate that FTO deletion heightens vulnerability to stress-induced depression-like behaviors in an age-dependent fashion, confirming that FTO downregulation markedly elevates the likelihood of developing depressive-like behaviors, particularly in older populations. Consequently, the cumulative effects of FTO deficiency and aging may serve as catalysts for the onset of depression-like behavior and cognitive impairment due to prolonged stress. It was previously shown that FTO knockout or knockdown can induce depression-like and anxiety-like behaviors in mice [40], as these mice exhibit increased anhedonia, reduced activity, and prolonged immobility time [41]. On the other hand, the overexpression of FTO or the introduction of exogenous FTO mitigated stress-induced depression-like behaviors [42].

Anxiety is among the most prevalent emotional symptoms in individuals with MDD. The World Health Organization reports that 4% of the global population today experiences anxiety [43]. Spychala et al. [44] discovered that FTO deficiency heightened anxiety and compromised long-term working memory in the hippocampus. Our work demonstrated that OFT and EPM revealed a substantial reduction in the crossing number, travel distance in open arms, and open arms residence time of mice following CUMS administration, indicating anxiety-like behaviors. The FTO-KD AAV9 intervention exacerbated anxiety-like behaviors triggered by CUMS exposure, aligning with prior findings. Nevertheless, several investigations have reported [45, 46] that FTO does not influence anxiety-like behaviors following acute or chronic stress in mice. These contradictory findings may be ascribed to differences in rodent species or the stress stimulation paradigm.

A recent study [47] indicated that naturally aging C57BL/6J mice exhibit cognitive decline from 6 months of age, with further deterioration observed at 22 months of age. In our study, 12-month-old CUMS mice exhibited significantly poorer performance than age-matched sham mice in the MWM. Furthermore, FTO-KD exacerbated the spatial learning and memory deficits caused by CUMS exposure, suggesting that FTO deletion heightened vulnerability to chronic stress-induced cognitive impairment in mice. Prior research [25, 48] has demonstrated that FTO deficiency can lead to reduced brain size in mice, alterations in brain region structures, and the inhibition of proliferation and differentiation of adult neural stem cells (aNSCs), resulting in compromised spatial learning and memory, aligning with our findings.

Polymorphisms in the FTO gene have been linked to a heightened incidence of depressive symptoms [49–51]. Nonetheless, there is conflicting information concerning the relationship between FTO and MDD. Preclinical findings [52] indicate that FTO deleted mice exhibit less resilience to stressors and a higher propensity for anxiety-like and depression-like behaviors. Conversely, another study [53] demonstrated that FTO whole-gene

knockout mice lowered inflammatory factors via specific alterations in the gut microbiota, rendering the mice less vulnerable to stress stimuli and exhibiting diminished anxiety-like and depression-like behaviors. These differences may be due to a number of factors, including the heterogeneity of depression.

FTO-KD, age and the hippocampus

Our study revealed that following CUMS exposure, FTO expression in the hippocampus of mice was diminished. Additionally, FTO-KD AAV9 treatment further decreased FTO expression in the hippocampus, exacerbating depressive-like behavior and cognitive deficits, with aged mice exhibiting increased vulnerability to chronic stress. The data unequivocally demonstrated that the loss of FTO following CUMS treatment adversely affects the hippocampus, with this damage exhibiting age-dependent characteristics. Furthermore, epigenetic modifications may have occurred with aging. In accordance with this, prior research [46] indicated that FTO expression was diminished in the hippocampus of individuals with significant depression and in animal models of depression. Exogenous FTO led to diminished m6A hypermethylation and ameliorated the depression-like phenotype [54].

There is growing evidence [55–57] that hippocampus neuronal plasticity, dendritic spine atrophy, and neurogenesis are significant factors in the pathophysiology of depression and cognitive impairment. Neurotrophins, including BDNF and NGF, play a role in synaptic plasticity [58], learning and memory [59], aging, neurogenesis [10], and neuronal maturation [60]. Furthermore, as a secreted glycoprotein, reelin is essential for neuronal development, dendritic growth and maturation, and synaptic plasticity [16]. Transcripts associated with BDNF are similarly implicated in synaptic plasticity inside the hippocampus, encompassing molecules pertinent to synaptic plasticity (PSD-95, SYN) and synaptic morphology (dendritic spine density and branching quantity). Studies [10, 61] indicates that mice subjected to prolonged unpredictable mild stress exhibit reduced expression levels of BDNF, NGF, reelin, PSD-95, and SYN in the hippocampus. Our findings indicate that NGF levels in the DG region, reelin in the CA2 region, dendritic spine density, and synaptic plasticity-related proteins such as PSD-95, SYN, and SAP97 were significantly diminished, while the activation of the BDNF-TrkB signaling pathway was aberrant in the hippocampus of mice subjected to CUMS at 3 and 12 months of age.

Furthermore, we observed pathological alterations including neuronal nucleus shrinkage and disordered neuronal cell shape in the hippocampus dentate gyrus region of the CUMS model. It is important to acknowledge that the interaction among the treatment and age groups significantly influenced the expression of both NGF and reelin proteins in the hippocampal subregions (CA1, CA2, and DG). Previous findings indicated that aberrant expression of these proteins and other pathological alterations in the hippocampus are prevalent neuropathological characteristics in patients with MDD and in animal models [62, 63].

Numerous studies indicate that FTO is integral to various biological processes, such as the regulation of neurogenesis [64], memory formation [65, 66], mood fluctuations [67], and synaptic plasticity [23]. FTO can modulate the expression of synaptic plasticity-related factors via its demethylase activity [68]. FTO may potentially regulate synaptic dysfunctions in hippocampus primary neurons by modulating synaptic activity and memory-associated mRNA [65, 69]. The present study revealed that FTO deficiency expedited the decline in hippocampal dendritic spine quantity, exacerbated neuronal disorganization and injury, and diminished the expression of proteins associated with synaptic plasticity, along with the aberrant transmission of the BDNF-TrkB signaling pathway in the CUMS model, particularly in aged mice. These findings reaffirm that FTO serves a significant neuroprotective function in depression. The absence of FTO results in depression-like behavior and cognitive deficits, as FTO deficiency induces m6A hypermethylation, causing the degradation of essential transcripts for synaptic plasticity (dendrites, BDNF, PSD-95, SYN, and SAP97) and neurogenesis (NGF, reelin), thereby downregulating their expression in the brain. Moreover, aging may serve as a trigger for neural deterioration. Wang et al. [69] similarly showed that the absence of FTO elevated m6A methylation levels of SYN mRNA and reduced SYN expression. Conversely, FTO overexpression reduced the m6A methylation level on SYN mRNA and elevated SYN expression. FTO, functioning as a demethylating agent of m6A in RNA, may be involved in synaptic plasticity by regulating the methylation modification of substrate mRNA.

Limitation

This study has certain shortcomings that warrant acknowledgment. First, we did not conduct a reverse verification of the impact of FTO on depressive-like behavior and cognitive impairment in the CUMS model using exogenous FTO (FTO overexpression or FTO activator), particularly regarding neuroprotective benefits in aged mice. Second, this study utilized mice aged 3 and 12 months. Therefore, the effects of FTO in older (> 12 months) mice has remained elusive. Third, this study only examined the impact of the demethylase FTO on depression, although other factors associated with m6A

methylation, such as human Alk B homolog 5 (ALKBH5), WT1 Associated Protein (WTAP), methyltransferase like 3 (METTL3), and methyltransferase-like 14 (METTL14), are linked to mental depression. Fourth, our investigation focused solely on the influence of FTO in the hippocampus regarding depression-like behaviors, leaving other brain sub-regions (amygdala, prefrontal cortex, hypothalamus, striatum) unexplored. Fifth, sex-biased FTO targets have been documented to participate in brain development [70], although the present study does not include female mice. Sixth, in our study, the sample size of histological staining and protein detection experiments was relatively small (N = 3). Therefore, our data deserve replication using larger sample sizes to enhance statistical power. Seventh, hippocampal neuronal cell subtypes were not classified in this study; future work will investigate the role of FTO in specific neuronal cell functions.

Conclusion

We discovered that 12-month-old mice exhibited greater susceptibility to CUMS and demonstrated inferior performance in depression-like and cognitive tasks relative to 3-month-old mice. Following FTO-KD treatment, the protein expression levels of FTO, NGF, and reelin in the hippocampus of 3- and 12-month-old CUMS mice were markedly diminished. Additionally, neuronal atrophy was pronounced, dendritic spine density was reduced, and the expression of proteins associated with synaptic plasticity was down-regulated, particularly in the 12-month-old group. This study reinforces the association between FTO and depression, elucidating the mechanism by which diminished FTO exacerbates age-related vulnerability to stress-induced depressive behaviors and cognitive deficits.

FTO deficiency and aging exacerbate vulnerability to depression-like behaviors and cognitive impairment, as well as hippocampus neuronal dysfunction. FTO is significant in the pathophysiology of MDD and the age-related vulnerability to develop MDD. The development of specific small molecule FTO activators may provide a novel therapeutic strategy for depression-like behaviors.

Abbreviations

FTO	Fat mass and obesity-related protein
CUMS	Chronic unpredictable mild stress
FTO-KD AAV9	FTO knockdown adeno-associated virus 9 shRNA
NGF	Nerve growth factor
MDD	Major depressive disorder
BDNF	Brain-derived neurotrophic factor
TrkB	The tropomyosin-related kinase B
PSD-95	Postsynaptic density protein 95
SYN	Synaptophysin
GABA	γ-Aminobutyric acid
m6A	N6-methyladenosine
CNS	Central nervous system
GFP	Green fluorescent protein
SPT	Sucrose preference test
OFT	Open field test
EPM	Elevated-plus maze
TST	Tail suspension test
FST	Forced swimming test
MWM	Morris Water Maze
IHC	Immunohistochemistry
H&E	Hematoxylin–eosin
ANOVA	Analysis of variance
SD	Standard deviation
aNSCs	Adult neural stem cells
ALKBH5	Human Alk B homolog 5
WTAP	WT1 Associated Protein
METTL3	Methyltransferase like 3
METTL14	Methyltransferase like 14

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12993-025-00280-3.

Supplementary file 1: Table 1. Behavior tests

Supplementary file2: Table 2. Assay

Supplementary file 3: Table 3. Simple effects for different age in 3 groups

Supplementary file 4

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Not applicable.

Author contributions

Original Draft Preparation, ML; Methodology, YZ; Visualization, YY, TC, and YL; Review & Editing, MM; Supervision, HL and MM; Project Administration, HL and MM; Funding Acquisition, HL All authors read and approved the final manuscript.

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Data availability

The dataset analyzed in this study is available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

The Ethics Committee of Chaohu Hospital affiliated to Anhui University of Medical Science approved all animal experiments (KYXM-202112-010).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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