Original Article

Taurine Improved Autism-Like Behaviours and Defective Neurogenesis of the Hippocampus in BTBR Mice through the PTEN/mTOR/AKT Signalling Pathway

(taurine / hippocampal neurogenesis / Ki67 / PTEN / ASD)

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Abstract. Effective treatment of patients with autism spectrum disorder (ASD) is still absent so far. Taurine exhibits therapeutic effects towards the autism-like behaviour in ASD model animals. Here, we determined the mechanism of taurine effect on hippocampal neurogenesis in genetically inbred BTBR *T*$^{+}$ *tf/J* (BTBR) mice, a proposed model of ASD. In this ASD mouse model, we explored the effect of oral taurine supplementation on ASD-like behaviours in an open field test, elevated plus maze, marble burying test, self-grooming test, and three-chamber test. The mice were divided into four groups of normal controls (WT) and models (BTBR), who did or did not receive 6-week taurine supplementation in water (WT, WT+ Taurine, BTBR, and BTBR+Taurine). Neurogenesis-related effects were determined by Ki67 immunofluorescence staining. Western blot analysis was performed to detect the expression of phosphatase and tensin homologue deleted from chromosome 10 (PTEN)/mTOR/AKT pathway-associated proteins. Our results showed that taurine improved the autism-like behaviour, increased the proliferation of hippocampal cells, promoted PTEN expression, and reduced phosphorylation of mTOR and AKT in hippocampal tissue of the BTBR mice. In conclusion, taurine reduced the autism-like behaviour in partially inherited autism model mice, which may be associated with improving the defective neural precursor cell proliferation and enhancing the PTEN-associated pathway in hippocampal tissue.

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder that is characterized by repetitive sensory-motor behaviours, social and language deficits (Lord and Bishop, 2015). Since ASD patients cannot function independently, ASD became a social and economic burden. The therapeutic strategies for ASD patients are still limited since the aetiology of ASD is polygenic and highly heterogeneous (Abraham et al., 2019). Accumulating evidence has indicated that several brain regions are involved in the pathology of ASD, including the hippocampus, amygdala, inferior frontal gyrus, sensory cortices, and prefrontal region (Ecker et al., 2010, 2015). The hippocampus has drawn much attention in ASD research because hippocampus-dependent spatial reasoning and episodic memory are also commonly impaired in ASD (Banker et al., 2021). Increasing research reports have proved that deficits of hippocampal neurogenesis are related to the cognitive dysfunction and ASD-like psychiatric disorders (Liu et al., 2022). Preclinical research shows that the promotion of dentate gyrus (DG) neurogenesis is one of potential therapies for ASD (Liu et al., 2022).

Taurine is a β-amino acid with a natural sulphur-containing structure, which can be found in most mammalian tissues (Ji et al., 2023). Mammals cannot biosynthesize taurine to its full extent, and taurine is acquired through daily diet (Tanaka and Mataga, 1987). Taurine has various physiological functions through its antioxidant, anti-inflammatory, and blood pressure-regulating properties (ElBanna et al., 2023). Lower levels of taurine are found in ASD patients (Park et al., 2017). Although orally administered taurine is found to reduce repetitive and anxiety-like behaviour of BTBR *T*$^{+}$ *tf/J* (Black and Tan Brachyury, BTBR) mice (Sharon et al., 2019), the

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Abbreviations: ASD – autism spectrum disorder, BTBR – Black and Tan Brachyury mouse strain, DG – dentate gyrus, EPM – elevated plus maze test, mTOR – mammalian target of rapamycin, OFT – open field test, PTEN – phosphatase and tensin homologue deleted from chromosome 10, S1 – stranger mouse 1, S2 – stranger mouse 2.

exact mechanism underlying taurine improving the autism behaviour is still unknown.

Phosphatase and tensin homologue deleted from chromosome 10 (PTEN) is a phosphoinositide 3-phosphatase, and the frequent loss of PTEN expression is linked to increased cancer risks to macrocephaly and neurodevelopmental impairment (Yehia and Eng, 2018). Germline mutations in *PTEN* have been identified in up to 20 % of children with ASD (Tilot et al., 2015). PTEN variants contribute to dysregulation of cortical neurogenesis and accelerate the neuronal maturation of upper layer neurons in the ASD genetic background (Fu et al., 2023). Heterozygous *Pten*-knockout mice exhibit deficits in social behaviour and acquired repetitive behaviours (Cheung et al., 2023). The loss of PTEN induces up-regulation of mammalian target of rapamycin (mTOR)-driven transcriptional and translational machinery (Lee et al., 2018). The PTEN signalling pathway can be enhanced by taurine treatments in human-derived cancer cell lines (He et al., 2018; Wang et al., 2020).

In this study, we characterized the ameliorating effects of taurine on autism-like behavioural deficits and the impact of promoting hippocampal neurogenesis, which might be related to activating the PTEN/mTOR/AKT signalling pathway.

Material and Methods

Animals and taurine supplement

C57BL/6J (referred to as WT for simplicity) and BTBR *T*$^{+}$ *Itpr3tf/J* (or *T*$^{+}$ *tf/J*) (Jackson Laboratory; referred to as BTBR) mouse strains were kept in a 12-hour light/dark cycle. BTBR is an inbred strain of mice that display similar behavioural and physiological deficits observed in patients with ASD (Leung et al., 2023). Food and water were provided *ad libitum*. All animal procedures were approved by the Ethics Committee of the Southern University of Science and Technology (Ethics number: SUSTech-JY202202040). All animals were randomly assigned to the control (WT), taurine supplement control (WT+Taurine), BTBR, or BTBR+Taurine groups. The WT and BTBR group mice were provided with ddH_2O, whereas the WT+Taurine and BTBR+Taurine group mice were administered ddH_2O containing 10 mmol/l taurine from the beginning on postnatal day 28. Behavioural tests and other experiments were performed after six weeks of taurine-water administration. Body weight was measured every seven days from postnatal day 28 to day 70.

Open field test (OFT)

Mice were habituated to the environmental room for at least one hour before the tests. The animal was placed in a custom box (40 × 50 × 50 cm) and gently put into the centre of the box, and its behaviour was video-recorded for 10 minutes. After each test, the mouse was returned to its cage, and the experimental box was cleaned with 75 % alcohol (v/v). All the collected video data were analysed by Ethovision XT2.0.

Elevated plus maze test (EPM)

The plus maze included two opposing open arms (50 × 10 cm) and two enclosed arms (50 × 10 × 40 cm). The cross shape was elevated 70 cm above the floor. Mice were placed at the junction of the four arms of the maze, facing an open arm. The total duration of the mouse stay in the elevated plus maze was 5 minutes and the duration in each arm was recorded by a video-tracking system. The maze was wiped with 75 % alcohol solution between trials to eliminate the animal odour cues before the placement of each animal.

Marble burying test

The marble burying test was performed as previously described (Tseitlin et al., 2023). Animals were placed individually into clear polycarbonate cages (22 × 30 × 28 cm), with clear Plexiglas covers, containing 5 cm of odourless bedding material. Twenty marbles were evenly spaced across the bedding. The mice were left undisturbed in the cages for 30 minutes. At the end of the experiment, animals were removed from the test cages and the number of unburied marbles was counted (less than 2/3rd of the marble's height).

Self-grooming test

Mice were scored for spontaneous self-grooming behaviours individually in a clean cage (46 × 20 cm). After a 10-minute habituation period, mice were scored for cumulative time spent grooming any region of the body over 10-minute test trials.

Three-chamber sociability test

The three-chamber test measures general sociability and interest in social novelty in rodent models and was performed as previously described (Chen et al., 2019). Before the experiments, the animal was placed in the central chamber and was given 10 minutes to freely explore the three-chamber device. In the stage I test, the test animal was placed in the central chamber and then allowed to access an empty cage and a novel mouse (Stranger 1, S1) in two opposite chambers for 10 minutes. The S1 mouse was kept under a transparent plastic box in the right chamber. In the stage II social novelty preference test, the empty cage in stage I was replaced by another novel mouse (Stranger 2, S2). The test mouse was allowed to freely navigate in the three-chamber device for 10 minutes. The amount of time spent near each cage was measured using the SocialScan software (Clever System Inc., Reston, VA).

Western blot

Western blot was carried out as described previously (Yang et al., 2022). Briefly, the brain tissue was collected and resuspended in lysis buffer (10 mM Tris-HCl

[pH7.5], 150 mM NaCl, 0.1 % Nonidet p-40, 5 mM EDTA) with 1 × protease/phosphatase inhibitor cocktail (#78447, Thermo Fisher Scientific, Waltham, MA). The protein concentration was quantitated by Coomassie brilliant blue G250 (Beyotime, Haimen, China), and the protein sample was boiled for 5 min. Protein samples (30 μg) were subjected to electrophoresis in 8–10 % SDS-polyacrylamide gel and transferred to a polyvinylidene difluoride membrane (Millipore, Burlington, MA). After being blocked with 5 % bovine serum albumin (BSA) for 1 h, the membranes were incubated with primary antibodies overnight at 4 °C, followed by secondary antibodies for 1 h. The blot membranes were developed by chemiluminescence solution (Fdbio, Hangzhou, China). The protein bands were analysed by ImageJ (National Institutes of Health, Bethesda, MD). The primary antibodies were as follows: rabbit anti-PTEN (Abcam, Cambridge, UK, #ab267787, 1 : 1000), rabbit anti-AKT (Abcam, ab18785, 1 : 1000), rabbit anti-phospho-Akt (Ser473) (Abcam, #ab285140, 1 : 1000), rabbit anti-mTOR (Abcam, # ab134903, 1 : 1000), rabbit anti-phospho-mTOR (Ser2448) (Abcam, #5536, 1 : 1000), rabbit anti-GAPDH (Proteintech, Rosemont, IL, #10494-1-AP, 1 : 5000). GAPDH served as an internal control.

Immunofluorescence staining

The brain slices were blocked by 0.2 % (v/v) Triton X-100 and 3 % (m/v) BSA in PBS buffer for 30 minutes. Next, the brain sections were incubated with rabbit anti-PTEN (Abcam, #ab267787, 1 : 500) or rabbit-anti-Ki67 (Abcam, #ab15580, 1 : 200) in PBS buffer overnight at 4 ℃. The sections were washed with PBS three times followed by staining with goat anti-rabbit Alexa Fluor 594 secondary antibody (1 : 2000, Thermo Fisher Scientific) at room temperature for 2 hours. 4',6-Diamidino-2-phenylindole (DAPI) was used to stain the nuclei. Images of seven sections per animal were captured and used for analysis using ImageJ (National Institutes of Health).

Measurement of taurine concentration in the serum, hippocampus and cortex

The taurine concentration in the serum, hippocampus and cortex was measured according to the assay kit instructions (#CEV538Ge, Cloud-Clone Corp., Wuhan, China). The hippocampus and cortex tissue lysates were prepared as described previously (Ying et al., 2022). Briefly, the isolated tissue was homogenized in cold PBS and centrifuged at 10,000 × *g* for 15 min. The protein content of cell extracts was quantitated by Coomassie brilliant blue G250 (Bio-Rad, #5000205, Hercules, CA). Next, a 50 μl sample was added to each well. Fifty μl of Detection Reagent A (one of the reagents in the taurine assay kit) was further added and the mixed samples were incubated for 1 hour at 37 °C. Then, the liquid in each well was aspirated and the wells were washed three times. Next, 100 μl of Detection Reagent B (another reagent in the taurine assay kit) was added to each well and kept for 30 minutes at 37 °C. After aspiration and 5-time wash, each well was supplemented with 90 μl Substrate Solution and kept for 10 minutes at 37 °C. Finally, 50 μl Stop Solution was added to each well and the absorbance was measured at 450 nm immediately. Each sample was tested in three parallel repeats.

Statistical analyses

All the data are displayed as mean ± standard deviation (SD). GraphPad Prism 7 (La Jolla, CA) was used for statistical analyses and quantification graphs. One-way or two-way analyses of variance (ANOVA) were used to analyse multiple comparisons. Sidak's multiple comparison test was applied for the analyses of the three-chamber approach test. $P < 0.05$ was considered statistically significant.

Results

Taurine supplementation enhances the taurine content in the cortex and hippocampus tissue

Compared with WT groups (12 ± 1.26 mM), a lower serum taurine concentration in BTBR mice was observed (8.40 ± 3.29 mM, $P < 0.01$, Fig. 1A). The taurine concentration in the serum after taurine-water supplementation was significantly increased in both the WT (16.30 ± 1.90 mM) and BTBR (16.14 ± 2.89 mM) mice (Fig. 1A). The body weight showed no statistical difference at weeks 1–6 post taurine supplementation among WT, WT+Taurine, BTBR, and BTBR+Taurine groups (Fig. 1B). Mice in the BTBR and BTBR+Taurine groups presented significantly heavier body weight than WT mice at weeks 5–6 post taurine supplementation, which was similar with a previous study (Leung et al., 2023). The body weight showed no significant difference between the BTBR group and BTBR+Taurine group mice at weeks 5–6 post taurine supplementation (Fig. 1B). The above data suggest that the taurine supplementation had no effect on the body weight gain of BTBR mice.

Given the association between the taurine level in the hippocampus and ASD-like symptoms in humans (Bruce et al., 2023; Song et al., 2023), the taurine levels in the hippocampus and cortex of mice were further analysed. The taurine level in the cortex between WT (10.20 ± 1.43 μM/g) and BTBR (10.440 ± 1.00 μM/g) groups showed no significant difference ($P > 0.05$, Fig. 1C). In the hippocampal tissue, the taurine content in the BTBR group (5.60 ± 0.90 μM/g) was significantly reduced compared to that in the WT group (9.90 ± 1.28 μM/g) ($P < 0.001$, Fig. 1C). The taurine content in the cortex and hippocampus was significantly increased post taurine supplementation in WT or BTBR groups (Fig. 1D). This suggests that the reduced taurine level in the hippocampus of BTBR mice was improved after orally administered taurine.

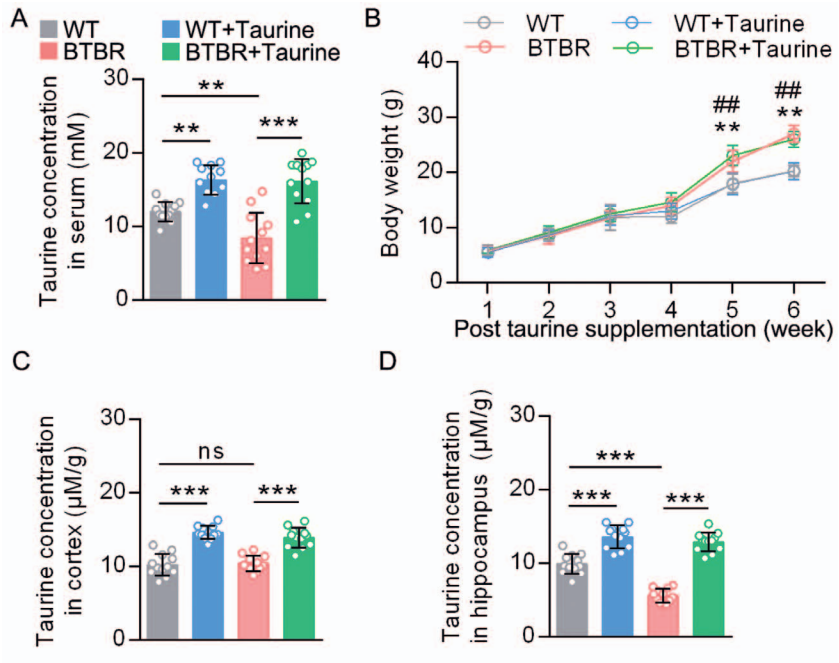

Fig. 1. Taurine concentration in the serum and hippocampus tissue increased after taurine supplementation, and the effect of taurine supplementation on body weight of mice in WT, WT+Taurine, BTBR, and BTBR+Taurine groups. **(A)** Taurine concentration test in the serum in WT, WT+Taurine, BTBR, and BTBR+Taurine groups. N = 12/group. **(B)** Body weight changes over the six weeks of taurine supplementation (N = 12/group). **(C, D)** Taurine concentration test in the cortex (C) and hippocampus (D) in WT, WT+Taurine, BTBR, and BTBR+Taurine groups. N = 12/group. Data are shown as the mean ± SD. $^{**}P < 0.01$, $^{***}P < 0.001$, ns: non-significant. ##P < 0.01, BTBR+Taurine vs WT group. BTBR: BTBR *T+tf/J.*

Taurine-water treatment reduces the anxiety-like and repetitive behaviour and increases the sociability and social memory of BTBR mice

The OFT, EPM, and marble burying tests were conducted to examine the effects of taurine supplementation on the anxiety-like behaviour in BTBR mice. The taurine supplementation in WT mice had no significant impact on the centre stay duration in the OFT test, the time spent in the closed arms in the EPM test, and the buried marbles in the marble burying test (Fig. 2A–C). BTBR mice showed significantly reduced time in the central zone in the OFT test, the time spent in the open arms in the EPM test, and the number of buried marbles in the marble burying test, while the taurine-water treatment attenuated this reduction (Fig. 2A–C).

The self-grooming test was used to examine the effect of taurine supplementation on repetitive behaviour in mice. We observed that the self-grooming time was significantly increased in BTBR mice compared with WT mice (Fig. 2D) ($P < 0.001$), whereas taurine supplementation reduced the self-grooming time in BTBR mice.

The three-chamber social test was used to determine the social behaviour abnormality (Fig. 2E). Mice in the WT and WT+Taurine groups spent more time with S1, whereas mice in the BTBR group spent time with no significant difference between the object (Ob) and S1, indicating social interaction deficits (Fig. 2E). In contrast, mice treated with taurine spent more time with S1 while spending significantly less time with Ob. In the social memory trials, the Ob in the cage was replaced with a stranger (Stranger 2, S2). We observed that mice spent significantly more time with S2 than with S1 in the WT, WT+Taurine, and BTBR+Taurine groups. There was no significant difference in the social time in the BTBR groups. The results show that taurine improves social deficits in BTBR mice.

Taurine supplementation increases hippocampal cell proliferation in BTBR mice

Ki67 was used to determine the neural progenitor proliferation in the cortex and dentate gyrus (DG) of the BTBR mice. In the cortex, the number of $Ki67^+$ among the WT, WT+Taurine, BTBR, and BTBR+Taurine groups showed similar results (Fig. 3A–B). In the DG region of the hippocampus, there was no difference in $Ki67^+$ cell numbers between the WT and WT+Taurine groups (Fig. 3C–D). The amount of neurogenic marker Ki67 was reduced in the DG of the BTBR mice compared with that in the WT mice (Fig. 3C–D). Taurine supplementation normalized the $Ki67^+$ cell number in the DG region of BTBR mice (Fig. 3C–D). The above data demonstrate that taurine supplementation enhances cell proliferation in the DG region but not in the cortex of BTBR mice.

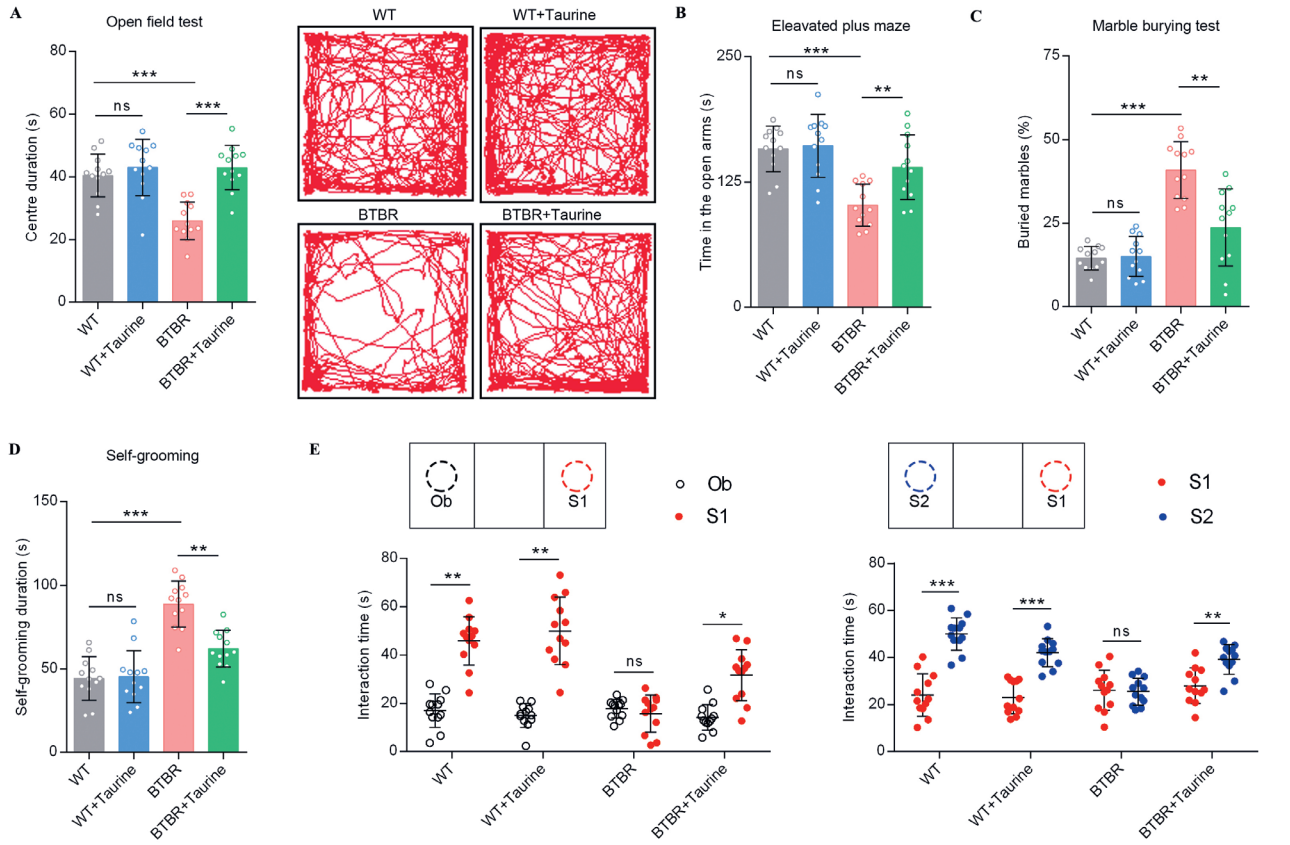

Fig. 2. Taurine supplementation ameliorated repetitive and anxiety-like behaviour and improved the sociability in the BTBR mouse model for ASD. **(A)** Open field test. Bar graph showing alterations in the time spent in the central zone and the representative track-sheets of the open field test. **(B)** Elevated plus maze test. **(C)** Marble burying test. **(D)** Grooming test. **(E)**Three-chamber test. N = 12/group. Data are shown as the mean ± SD. ns: non-significant, $^{*}P < 0.05$, $^{**}P < 0.05$, $^{***}P < 0.001$. Ob, object; S1: stranger mouse 1; S2: stranger mouse 2.

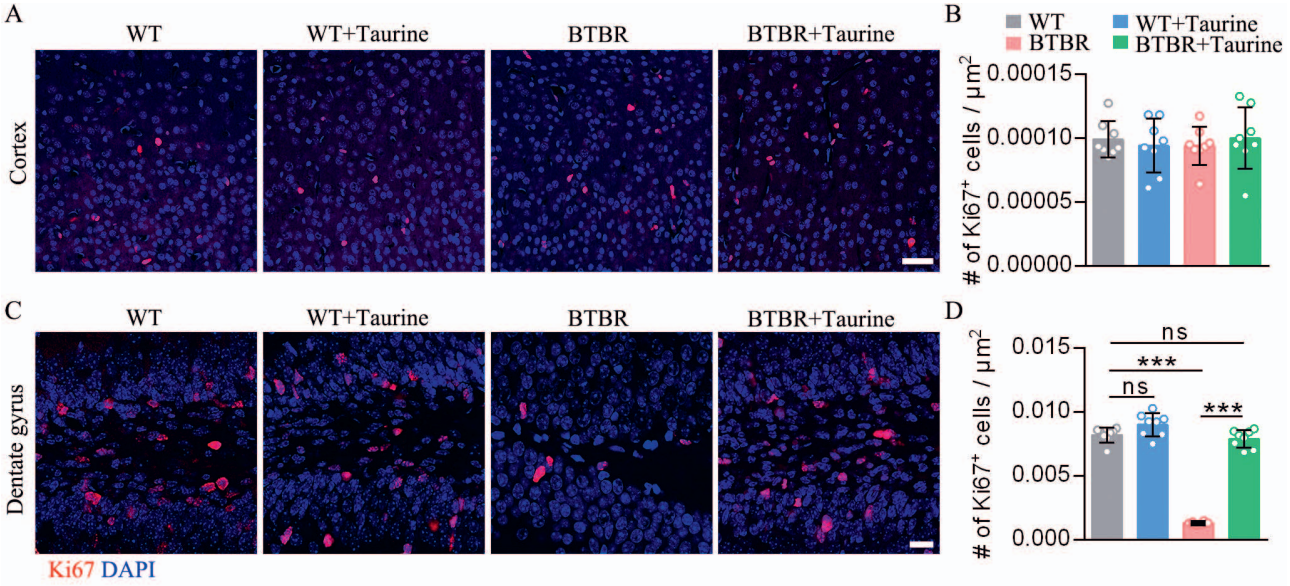

Fig. 3. Ki67 immunofluorescent expression in the cortex and dentate gyrus (DG) of the hippocampus. **(A)** Representative microphotography of immunofluorescent expression of Ki67 in WT, WT+Taurine, BTBR, and BTBR+Taurine mice with the labelled area of the cortex. Scale bars = 50 µm. **(B)** The number of $Ki67^{+}$ cells/μm^2 in the cortex in WT, WT+Taurine, BTBR, and BTBR+Taurine mice. **(C)** Representative microphotography of immunofluorescent expression of Ki67 in DG. Scale bars = 20 µm. **(D)** The number of $Ki67^{+}$ cells/μm^2 in hippocampi in WT, WT+Taurine, BTBR, and BTBR+Taurine mice. N = 8/group. Data are shown as the means ± SD. ns: non-significant, $^{***}P < 0.001$.

Taurine supplementation enhances the PTEN/mTOR/AKT pathway in the hippocampal tissue of BTBR mice

Since PTEN contributes to the proliferation of cells upon taurine treatment in *in vitro* studies, the PTEN expression was further analysed in the hippocampus of BTBR mice upon taurine supplementation. No differences in PTEN expression in the DG region were observed in the WT and WT+taurine groups (Fig. 4A–B). Significantly down-regulated PTEN expression in the DG regions of BTBR mice was observed when compared with WT groups, but taurine supplementation recovered the PTEN level in the DG regions of BTBR mice (Fig. 4A–B). Confirmative results by Western blotting supported this finding, showing that the PTEN expression level in the hippocampal tissue of BTBR+ Taurine mice was significantly increased when compared to BTBR mice (Fig. 4C–D).

As the downstream pathway of PTEN, mTOR inactivation promoted self-renewal regulation of neural progenitor cells (NPCs) (Han et al., 2013). To explore whether the PTEN/mTOR/AKT pathway was related to the effects of taurine on hippocampal neurogenesis, we analysed the expression of PTEN and its downstream signalling molecules, including mTOR and AKT, in the hippocampus and cortex. As shown in Fig. 4C–D, the PTEN level and total or phosphorylated protein levels of mTOR and AKT in the hippocampi did not show significant differences between the WT and WT+Taurine groups. The p-mTOR and p-AKT levels in the hippocampi of the BTBR group were significantly increased as compared to WT mice. Taurine supplementation recovered the PTEN level and reduced the p-mTOR and p-AKT levels in the hippocampi of BTBR mice (Fig. 4C–D). In the cortex, the PTEN level and total or phosphorylated protein levels of mTOR and AKT showed no significant difference among the WT, WT+Taurine, BTBR, and BTBR+Taurine groups. This shows that taurine supplementation enhanced the PTEN/mTOR/AKT signalling pathway in the hippocampus, but not in the cortex of BTBR mice.

Discussion

In our study, the administration of taurine from conception through adulthood reduced the anxiety-like and repetitive behaviour, and increased sociability and social memory in BTBR mice, which is similar to previous reports (Sharon et al., 2019). The BTBR mouse strain has a high tendency for obesity. Several quantitative trait loci have been identified to promote metabolic disease in the BTBR strain (Leung et al., 2023), which explains the higher body weight in the BTBR mice than in WT mice in our study. A previous report shows that taurine transporter knockout mice have lower body weights and visceral fat (Ito et al., 2015). Taurine reduced obesity and body weight in WT mice fed a diet containing 60 % fat (Murakami, 2017). The body weight and height of the newborn are significantly higher in the high taurine intake group of pregnant women (Jung and Choi, 2019). Here, we found that taurine supplementation had no effect on the body weight of WT and BTBR mice, which may suggest that taurine reduced obesity and increased energy expenditure of mice under special conditions, such as a high-fat diet.

Deficits of hippocampal neurogenesis are involved in ASD development and are one of the pathological changes in ASD (Liu et al., 2022). Also, abnormal neural proliferation has been reported in the hippocampus of the ASD mouse model. In the VPA-treated model, the neural proliferation of the hippocampus is enhanced in the early stage, followed by a significant decrease in later stages (Kinjo et al., 2019). In Ube3a transgenic adult mice, impaired neural proliferation of the hippocampus was observed (Godavarthi et al., 2015). We provided novel evidence that taurine supplementation enhanced the proliferation of hippocampal neural cells in the hippocampal DG region of BTBR mice. This supports the assumption that restoring neurogenesis may be a novel therapeutic approach for ASD. Deficits of adult hippocampal neurogenesis can cause social behavioural defects and anxiety-like and repetitive behaviour (Liu et al., 2022). The recovered effect on hippocampal neurogenesis by taurine supplementation may contribute to the remission of anxiety-like, repetitive, and defective sociability and social memory in ASD.

PTEN is among the most common ASD-predisposing genes (Tilot et al., 2015). Heterozygous *Pten*-knockout mice exhibit deficits in social behaviour and acquired repetitive behaviours (Cheung et al., 2023). Pten deficiency leads to ASD-like behaviours and long-term memory impairment in mice (Chen et al., 2019). As one of the predominantly downstream molecules, the inhibition of mTOR rescued the ASD-like behaviour associated with Pten deficiency (Chen et al., 2019). Based on the results in our study, we suggest that the activation of PTEN/mTOR signalling by taurine supplementation contributed to improving behaviour in the BTBR mice.

Conclusion

In summary, we uncovered that the remission of anxiety-like, repetitive, and defective sociability and social memory behaviour in BTBR mice by taurine supplementation may be related to promoting the proliferation of hippocampal neural precursor cells and activation of the PTEN/AKT/mTOR signalling pathway, providing new evidence for the prevention of ASD with taurine supplementation.

Authors' contributions

Huang Xiaoyan designed and supervised the study. Huang Xiaoyan, Chen Jinyuan and Qin Wen performed the experiments and collected the data. Yang Zhaoxi and Zhang Lingli summarized and analysed the data. Huang Xiaoyan wrote and finalized the manuscript. All authors have given approval to the final version of the manuscript.

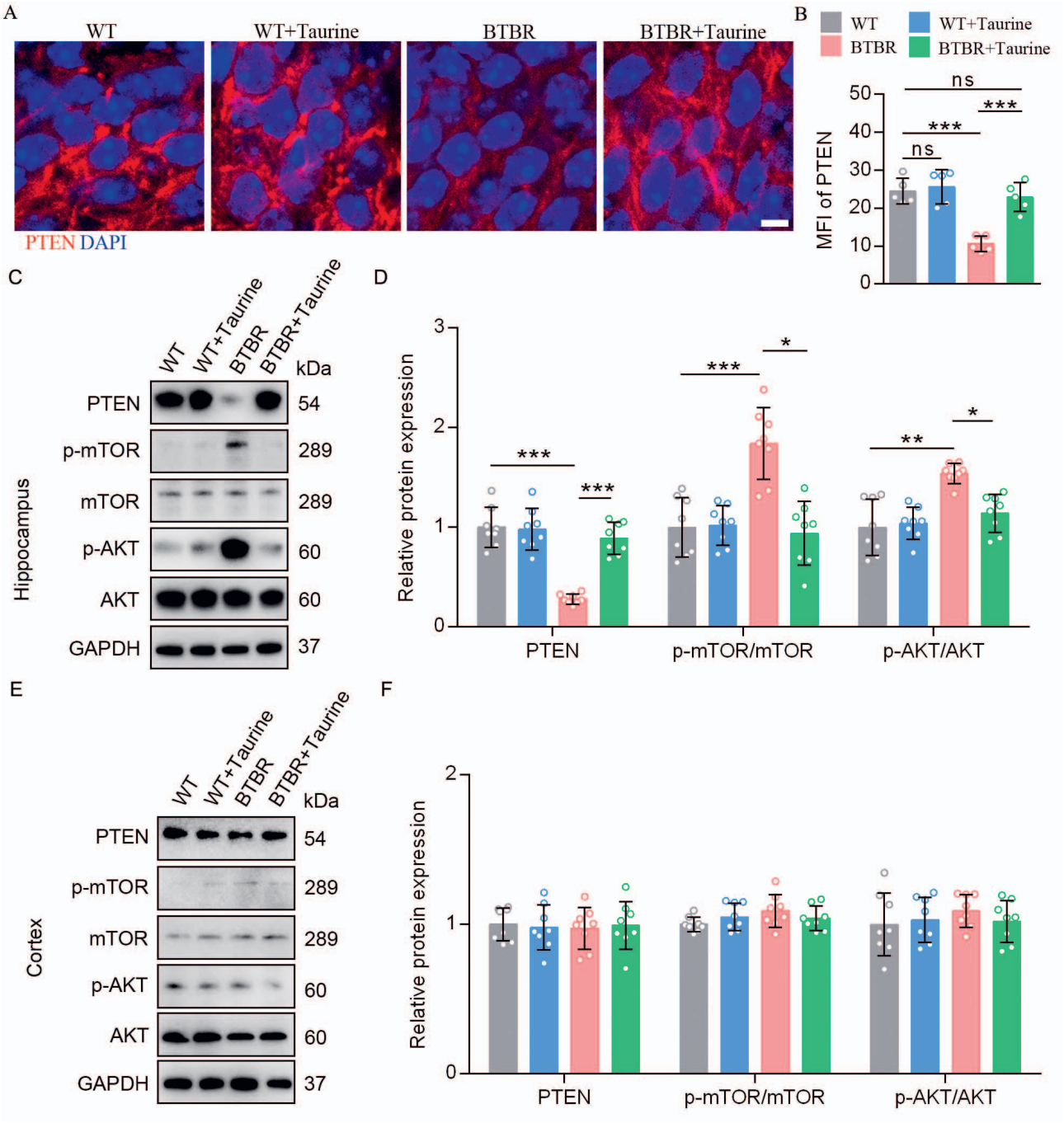

Fig. 4. Taurine activated the PTEN/AKT/mTOR pathway. **(A)** Representative microphotography of immunofluorescent expression of PTEN in the subgranular zone (SGZ) of dentate gyrus (DG) of WT, WT+Taurine, BTBR, and BTBR+Taurine mice. Scale bars = 10 μm. **(B)** Mean fluorescent intensity (MFI) of PTEN in SGZ of DG of WT, WT+Taurine, BTBR, and BTBR+Taurine mice. N = 5/group. **(C)** Western blotting was used to test the protein levels of PTEN, p-mTOR, mTOR, p-AKT, and AKT in the hippocampal tissue of WT, WT+Taurine, BTBR, and BTBR+Taurine groups. GAPDH served as the internal control. **(D)** Quantification of immunoblots as shown in (C), expressed as normalized intensity compared to the control (WT) group that was set to 1 (N = 8/group). **(E)** Western blotting was used to test the protein levels of PTEN, p-mTOR, mTOR, p-AKT, and AKT in the cortex of WT, WT+Taurine, BTBR, and BTBR+Taurine groups. GAPDH served as the internal control. **(F)** Quantification of immunoblots as shown in (E), expressed as normalized intensity compared to the control (WT) group that was set to 1 (N = 8/group). Data are shown as the means ± SD. ns: non-significant, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Data availability statement

Original data in our study are available upon request.

Conflict of interest

The authors have no conflict of interest to declare.

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