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Taurine increases hippocampal neurogenesis in aging mice

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Abstract Aging is associated with increased inflammation and reduced hippocampal neurogenesis, which may in turn contribute to cognitive impairment. Taurine is a free amino acid found in numerous diets, with anti-inflammatory properties. Although abundant in the young brain, the decrease in taurine concentration with age may underlie reduced neurogenesis. Here, we assessed the effect of taurine on hippocampal neurogenesis in middle-aged mice. We found that taurine increased cell proliferation in the dentate gyrus through the activation of quiescent stem cells, resulting in increased number of stem cells and intermediate neural progenitors. Taurine had a direct effect on stem/progenitor cells proliferation, as observed *in vitro*, and also reduced activated microglia. Furthermore, taurine increased the survival of newborn neurons, resulting in a net increase in adult neurogenesis. Together, these results show that taurine increases several steps of adult neurogenesis and support a beneficial role of taurine on hippocampal neurogenesis in the context of brain aging.

Introduction

Neurogenesis persists during adulthood in the dentate gyrus (DG) of the hippocampus in most mammals (Altman & Das, 1965). Adult neural stem cells reside in the subgranular zone of the DG, where they give rise to intermediate progenitor cells. These progenitors proliferate rapidly to give rise to neurons, which migrate into the granule cell layer (Kronenberg et al., 2003). With their increased plasticity (Schmidt-Hieber et al., 2004), new neurons enhance synaptic plasticity in the hippocampus and participate to the mechanisms of learning and memory as well as mood control (Kheirbek et al., 2012). The age-dependent reduction in adult neurogenesis (Gebara et al., 2013; Kuhn et al., 1996; Encinas & Sierra, 2012) is associated with decreased learning performances (Gil-Mohapel et al., 2013), which can be restored by increasing adult neurogenesis with voluntary exercise (van Praag et al., 2005). Thus, manipulations aimed at increasing adult neurogenesis represent a promising approach for alleviating disease- or age-related cognitive impairment (Bolognin et al., 2014) as well as mood disorders (Drew & Hen, 2007). In this context, nutritional supplements acting on adult neurogenesis have been proposed as a beneficial approach to prevent or reduce age-related cognitive loss (van Praag et al., 2007).

Taurine is a free sulfur amino acid that is not incorporated in proteins. It is synthesized from methionine and cysteine by the rate-limiting enzyme cysteinesulfinic acid decarboxylase (CSD) that is found in the liver, the kidney and the brain,

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where it is localized in glial cells (Ripps & Shen, 2012). In the liver, CSD activity is increased by protein-rich diet (Bella et al., 1999) whereas in the brain, glutamate increases CSD activity (Wu et al., 1998). Taurine is also found in high concentrations in numerous diets such as meat and seafood (Huxtable, 1992) and crosses the blood brain barrier using a specific beta amino acid transporter TAUT (TAUrine Transporter (Benrabh et al., 1995)). Taurine is 3–4 times more abundant in the developing than in the mature brain (Miller et al., 2000) and its concentration decreases with aging (Banay-Schwartz et al., 1989), suggesting that taurine plays a role during brain development. Consistent with this, dietary taurine deficiency during gestation leads to impaired development of the cerebellum and the visual cortex of newborn cats (Sturman et al., 1985). Intriguingly, taurine also seems to play a role in the adult and aging brain: Chronic administration of taurine in aged mice (El Idrissi, 2008; Neuwirth et al., 2013) or in a mouse model of Alzheimer's disease (Kim et al., 2014) increases hippocampus-dependent learning and retention and reduces anxiety and depression (Chen et al., 2004). The mechanisms by which taurine increases learning performances are unclear, but recent work showed that taurine increases the proliferation of adult neural stem/progenitor cells from the subventricular zone *in vitro* (Ramos-Mandujano et al., 2014; Hernandez-Benitez et al., 2012), suggesting that the effect of taurine may be mediated by an increase in adult neurogenesis. However, these studies did not address whether taurine increased net hippocampal neurogenesis *in vivo*.

Here, we directly assessed the effect of taurine on the age-related decline of adult hippocampal neurogenesis. To this aim, we tested the effect of taurine injections on 10-month-old mice, an age at which adult neurogenesis has reached its minimal activity (Kuhn et al., 1996; Gil-Mohapel et al., 2013). Using the incorporation of the proliferation marker 5-bromo-2-deoxyuridine (BrdU), combined with the genetic and immunohistochemical identification of adult hippocampal stem cells, intermediate progenitors, newborn mature and immature neurons, we examined the effect of taurine on several steps of the formation of new neurons in the aging hippocampus.

Methods

Ethics statement

This study was carried out in strict accordance with the recommendations in the Guidance for the Care and Use of Laboratory Animals of the National Institutes of Health. All experimental protocols were approved by the Swiss animal experimentation authorities (Service de la consommation et des affaires vétérinaires, Chemin des Boveresses 155, 1066 Epalinges, Switzerland). Every effort was made to minimize the number of animals used and their suffering.

Animals and taurine administration

Animals used for the study were male mice of 2, 4, 6, 8 and 10 months of age at the beginning of the experiments. C57Bl/6j mice were purchased from Janvier (le Genest Saint Isle, France), nestin-GFP mice were a kind gift from the laboratory of K. Mori (PRESTO, Kyoto, Japan) (Yamaguchi et al., 2000). These mice express the green fluorescent protein (GFP) under the stem cell-specific promoter nestin. All animals were housed in a 12 h light/12 h dark cycle with free access to food and water and controlled temperature (22 °C) conditions. Taurine was prepared fresh every day and diluted in water containing 0.9% NaCl. 10-month-old mice were injected intraperitoneally every day for 40 consecutive days either with 0.2 ml of taurine (265 mg/kg, Sigma-Aldrich) or with 0.2 ml of vehicle (0.9% NaCl in water) for control animals.

BrdU administration

Mice were injected intraperitoneally with 5-bromo-2-deoxyuridine (Sigma-Aldrich, Buchs, Switzerland) at a concentration of 100 mg/kg in saline, 3 times per day at 2-h intervals, for 3 days. For proliferation studies, taurine was injected for 40 days and BrdU injection started 24 h after the last taurine injection. Mice were then sacrificed 24 h after the last BrdU injection. For survival studies, BrdU was injected for 3 days and 24 h after the last BrdU injection, taurine was injected for 40 days. 24 h after the last taurine injection, mice were sacrificed and analyzed (Chen et al., 2004).

Tissue collection and preparation

At the end of the experiment, mice received a lethal dose of pentobarbital (10 ml/kg, Sigma-Aldrich, Buchs, Switzerland) and were perfusion-fixed with 50 ml of 0.9% saline followed by 100 ml of 4% paraformaldehyde (Sigma-Aldrich, Switzerland) dissolved in phosphate buffer saline (PBS 0.1 M, pH 7.4). Brains were then collected, postfixed overnight at 4 °C, cryoprotected 24 h in 30% sucrose and rapidly frozen. Coronal frozen sections of a thickness of 40 μm were cut with a microtome-cryostat (Leica MC 3050S) and slices were kept in cryoprotectant (30% ethylene glycol and 25% glycerin in 1× PBS) at −20 °C until processed for immunostaining.

Immunohistochemistry

Immunochemistry was performed as previously described (Gebara et al., 2013). Briefly, sections were washed 3 times in PBS 0.1 M. BrdU detection required formic acid pretreatment (formamide 50% in 2× SSC buffer; 2× SSC is 0.3 M NaCl and 0.03 M sodium citrate, pH 7.0) at 65 °C for 2 h followed by DNA denaturation for 30 min in 2 M HCl for 30 min at 37 °C and rinsed in 0.1 M borate buffer pH 8.5 for 10 min. Then, slices were incubated in blocking solution containing 0.3% Triton-X100 and 15% normal serum normal goat serum (Gibco, 16210-064) or normal donkey serum (Sigma Aldrich, D-9663), depending on the secondary antibody in PBS 0.1 M. Slices were then incubated 40 h at 4 °C with the following primary antibodies: mouse monoclonal anti-BrdU (48 h, 1:250, Chemicon International, Dietikon, Switzerland), goat anti-DCX (1:500, Santa Cruz Biotechnology, sc-8066), rabbit anti-Ki-67 (48 h, 1:200, Abcam, ab15580), rabbit anti-Tbr2 (1:200, Abcam, ab23345), goat anti-Iba1 (1:200, Abcam, ab5076), mouse anti-MHC-II (1:200, Abcam, ab23990) rabbit anti-GFAP (1:500, Invitrogen, 180063) mouse anti-Neu-N (Chemicon International 1:1000). The sections were then incubated for 2 h in either of the secondary antibodies: goat anti-mouse Alexa-594 (1:250, Invitrogen), goat anti-mouse Alexa-660

(1:250, Invitrogen), goat anti-rabbit 594 (1:250, Invitrogen), donkey anti-goat Alexa-555 (1:250, Invitrogen). After immunostaining, slices were incubated for 1 min into 4,6 diamidino-2-phenylindole (1:1000, DAPI) to reveal nuclei.

Cell culture

Adult neural progenitor cells (NPCs) were a kind gift from the laboratory of Fred Gage (Salk Institute, San Diego, USA). They were isolated from the DG of adult Fisher 344 rats and cultured as previously described (Palmer et al., 1997) at a density of 20,000 cells per well. Three wells per condition were used and experiments were replicated 3 times. Twenty-four hours after plating, the medium was supplemented with 10 mM taurine (Sigma-Aldrich) every day, for 7 days (Hernandez-Benitez et al., 2012). On the eighth day, medium was supplemented with 5 μM BrdU for 30 min then washed and fixed with 4% paraformaldehyde for 20 min, briefly washed, immunostained for BrdU and mounted.

Image analysis

All images were acquired using a Zeiss confocal microscope (Zeiss LSM 710 Quasar Carl Zeiss, Oberkochen, Germany). The total numbers of immunoreactive cells throughout the entire granule cell layer were estimated using stereological sampling, as previously described (Thuret et al., 2009), between −1.3 and −2.9 mm from the Bregma. However, no guard zones were used, which may lead to possible bias in the counting of cells at the edge of each section, spread across control and taurine groups. For each animal, a 1-in-6 series of sections was stained with the nucleus marker DAPI and used to measure the volume of the granule cell layer. The granule cell area was traced using Axiovision (Zeiss, Germany) software and the granule cell volume was determined by multiplying the traced granule cell layer area by the thickness of the corresponding section and the distance between the sections sampled (240 μm). For all the mice analyzed in this study, no difference was found between taurine-treated and control animals in the volume of the granule cell layer. All cells were counted blind with regard to the mouse status. The total number of immunolabeled cells were counted in the entire thickness of the sections in a 1-in-6 series of section (240 μm apart), for a total of 8 sections, with a 40× objective. The number of cells was then related to granule cell layer sectional volume and multiplied by the reference volume to estimate the total number of immunolabeled cells (Gebara et al., 2013; Thuret et al., 2009). Cells expressing BrdU, Ki-67, DCX or Tbr2 were counted in the granule cell layer and the subgranular zone, whereas cells expressing Iba-1 (microglia), MHC II and cells expressing GFAP with a prototypical stellar astrocyte morphology were counted in the whole dentate gyrus, as these cell types are scarce in the granule cell layer. BrdU colocalization with the neuronal marker NeuN was analyzed on confocal microscope image stacks and was confirmed on single optical sections, for 52–70 cells per animal. The proportion of double-labeled cells was then obtained for each animal and then averaged for each group (Gebara et al., 2013). 3D reconstructions were performed using Imaris 7.6.1 (Bitplane, South Windsor, CT, USA).

For *in vitro* cell quantification, images were acquired using confocal microscopy. The number of BrdU labeled NPCs was counted in 4 selected fields, systematically placed in the same position relative to the coverslips' edges. The number of BrdU labeled NPCs was reported to the total number of NPCs in each selected field to obtain a percentage of proliferation in each condition.

Statistical analysis

Hypothesis testing was two-tailed. All analyses were performed using JMP10 software. First, Shapiro–Wilk tests were performed on each group of data to test for distribution normality. For normal distribution, the equality of variances of the groups was tested and a bilateral Student t-test was performed. For non-normal distribution, we performed the Wilcoxon test. Data are presented as mean ± SEM.

Results

Taurine increased stem cell proliferation in the DG

We first examined the effect of taurine on cell proliferation in the DG. Ten-month-old nestin-GFP mice were injected daily for 40 days with either taurine (i.p. 265 mg/kg), or with the same volume (0.2 ml) of vehicle (0.9% NaCl) as control. This regimen has previously been shown to reduce inflammation (Yamaguchi et al., 2000) and the inflammation-induced inhibition of adult hippocampal neurogenesis (Palmer et al., 1997). Nestin-GFP mice express the GFP reporter under the control of the stem cell-specific nestin promoter thereby enabling the identification of adult neural stem cells (Kim et al., 2014). Twenty-four hours after the last taurine injection, all mice received 3 injections per day at 2-h intervals for 3 days of the synthetic thymidine analog 5-bromo-2-deoxyuridine (BrdU, i.p., 100 mg/kg) and were sacrificed 24 h after the last BrdU injection (Fig. 1A). Mice were perfused, the brains collected, sectioned and immunostained for BrdU and the endogenous proliferation marker Ki-67. The total number of immunoreactive cells was counted in the granule cell layer (GCL) and the subgranular zone of the DG. Taurine significantly increased the number of BrdU-expressing cells (Fig. 1B–C, bilateral Student's t-test $p < 0.001$) and the number of Ki-67-expressing cells (Fig. 1D–E, bilateral Student's t-test $p < 0.001$). To test whether the increased number of BrdU- or Ki-67-expressing cells could be caused by a change in hippocampal volume upon taurine treatment, we measured the volume of the GCL of all mice. There was no difference between groups (controls: 0.17 ± 0.08 mm^3; Taurine: 0.17 ± 0.07 mm^3, n = 5, bilateral Student's t-test $p = 0.26$). These results indicate that taurine increased cell proliferation in the subgranular zone of the DG.

We next identified the cell types contributing to the taurine-induced increase in cell proliferation, using immunohistochemistry and morphology. Two main types of proliferative cells co-exist in the subgranular zone: The radial glia-like (RGL), nestin-expressing stem cells, readily identifiable by their morphology consisting of a nucleus located in the subgranular zone, a large process extending through the GCL and branching into the proximal part of the molecular layer; and the intermediate neural progenitor cells, with short

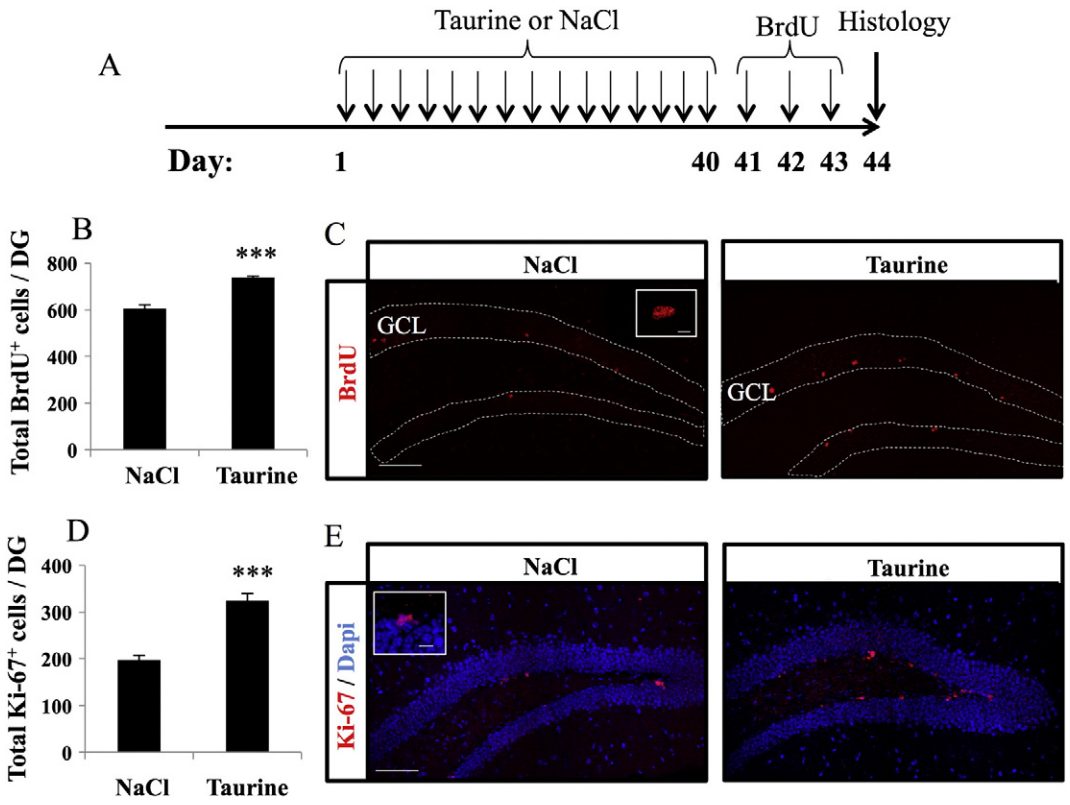

Figure 1 Taurine increased cell proliferation in the DG. (A) Experimental timeline: Mice were injected with taurine or vehicle (NaCl) for 40 days, followed immediately by 3 BrdU (5-bromo-2-deoxyuridine) injections per day for 3 days. Twenty-four hours after the last injection, brain slices were processed for histology. (B) Histogram of the total number of BrdU-immunopositive cells in the granule cell layer of the DG. (C) Confocal maximal projection micrographs of hippocampal sections immunostained for BrdU. Inset: Higher magnification confocal micrograph of a BrdU-positive cell. (D) Histogram of the total number of Ki-67-expressing cells in the GCL of the DG. (E) Confocal maximal projection micrographs of hippocampal sections immunostained for Ki-67. Inset: Higher magnification confocal micrograph of a Ki-67 expressing cell. Blue: Dapi staining. Animals, n = 5 per group. Scale bars: 100 μm, insets 10 μm, bilateral Student's t-test ***$p < 0.001$. Each value represents the mean ± SEM.

processes extending into subgranular zone/GCL area. Both cell types express the T-box brain gene-2 transcription factor (Tbr2 (Hodge et al., 2008)), but they can be differentiated by their distinct morphology. Taurine increased the number of RGL stem cells in the subgranular zone of the DG as compared to NaCl (Fig. 2A–B, bilateral Student's t-test $p < 0.001$). To test whether taurine increased the division of RGL stem cells, mitigated the age-related reduction in RGL stem cells number or both, we measured RGL stem cell number at 2, 4, 6, 8 and 10 months of age. Consistent with previous reports (Encinas et al., 2011; Walter et al., 2011; Bonaguidi et al., 2011), RGL stem cells' number decreased over time and reached its minima at 8 months (Fig. 2C). There was no significant difference between 8 months and NaCl-treated animals (*i.e.* 11.5 months, $p = 0.42$). In stark contrast, taurine treatment on 10 month-old mice restored the number of RGL stem cells to values found in 2-months-old mice (bilateral Student's t-test $p = 0.22$). This effect may be mediated by the proliferation of RGL stem cells, since taurine increased the percentage of BrdU-expressing RGL stem cells (Fig. 2D-E, bilateral Student's t-test $p < 0.05$). Taurine also significantly increased the total number of Tbr2-expressing cells (Fig. 2F–G, bilateral Student's t-test $p < 0.01$), but did not increase the proportion of Tbr2-expressing cells that incorporated BrdU (Fig. 2b H–I, bilateral Student's t-test $p = 0.6$). These results suggest that taurine increased the proliferation of RGL stem cells, resulting in an increase of both RGL stem cells and Tbr2$^+$ progenitor cells populations.

Taurine increased the number of immature neurons

The increased number of Tbr2$^+$ progenitors may result in increased neuronal production. We next examined the effect of taurine on immature new neurons, identified by the expression of the immature neuronal marker doublecortin (DCX). Taurine increased the total number of DCX-immunostained neuronal soma in the subgranular zone/GCL (Fig. 3A–B, bilateral Student's t-test $p < 0.001$). Furthermore, upon taurine treatment, immature neurons displayed increased proportion of dendritic branches extending into the molecular layer, suggesting increased maturation (Fig. 3C–D, Bilateral Student's t-test for 125 μm from the GCL: $p < 0.05$). To further investigate the effect of taurine on new neurons' maturation, we quantified the three categories of DCX-expressing cells (Seri et al., 2004): cells without process, known to be the most immature subtype with the highest proliferative activity, cells with horizontal processes and cells with a radial process,

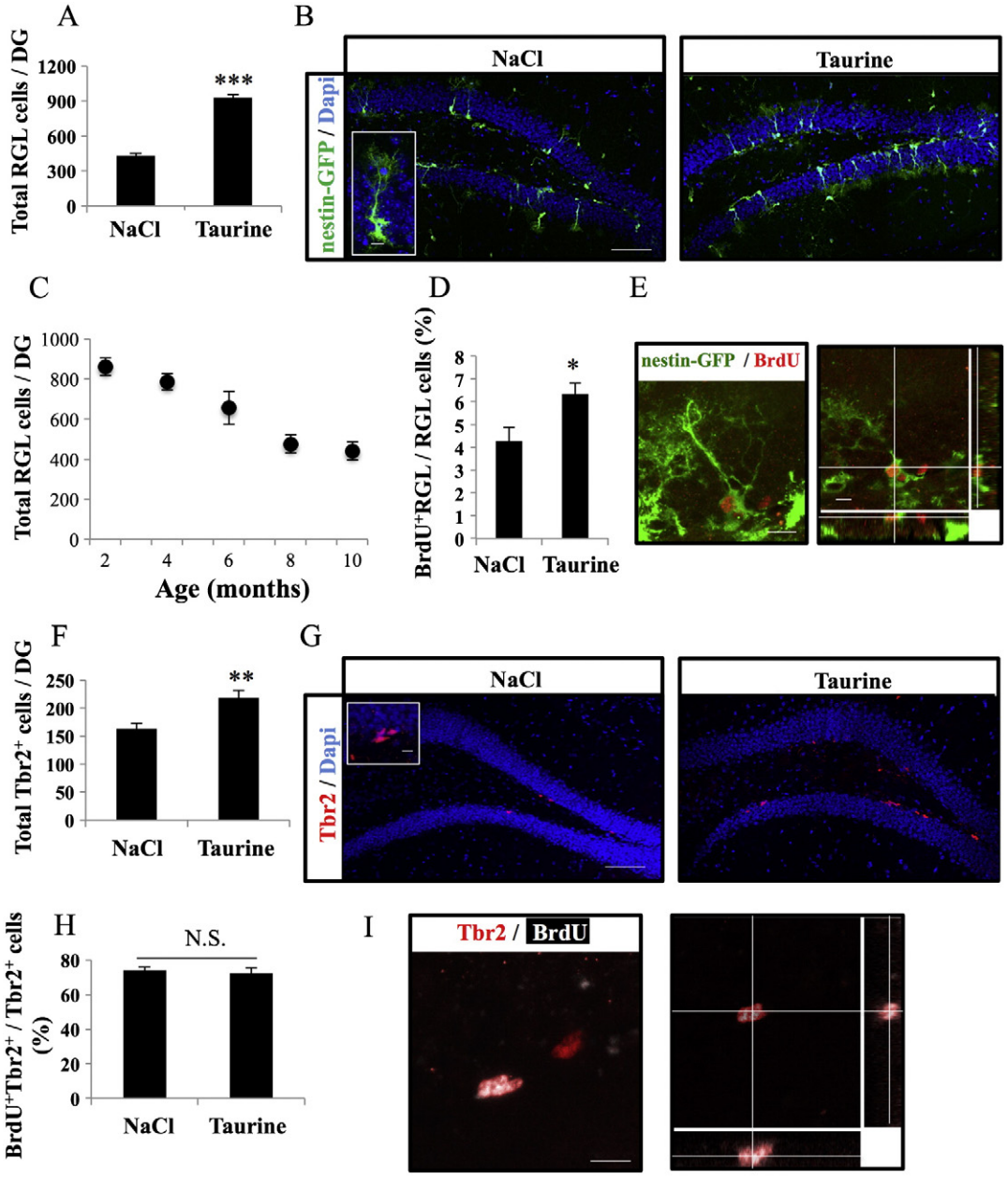

Figure 2 Taurine increased the proliferation of RGL stem cells. (A) Quantification of the total number of nestin-GFP stem cells with RGL (radial glia-like) morphology in the subgranular zone of the DG. (B) Confocal maximal projection micrographs of hippocampal sections of nestin-GFP mice. Inset: Higher magnification confocal micrograph of a GFP-expressing RGL stem cell. Scale bars: 100 μm, inset 10 μm. (C) Time course of the total number of RGL in 2, 4, 6, 8 and 10 month old mice. (D) Histogram showing the percentage of BrdU-immunostained RGL stem cells over the total number of RGL cells. (E) Left panel: Confocal micrograph of a hippocampal section (maximal projection) immunostained for BrdU (red) and GFP (green). Scale bar: 10 μm. Right panel: Orthogonal projections of the same RGL stem cell. (F) Histogram of the total number of Tbr2-expressing intermediate progenitor cells in the GCL of the DG. (G) Confocal maximal projection micrographs of hippocampal sections immunostained for Tbr2. Inset: Higher magnification confocal micrograph of a Tbr2-expressing cell. Blue: Dapi staining. Scale bars: 100 μm, inset 10 μm. (H) Histogram of the percentage of $BrdU^+$ $Tbr2^+$ cells over the total number of $Tbr2^+$ cells. (I) Left panel: Confocal micrograph of a hippocampal section (maximal projection) immunostained for Tbr2 (red) and BrdU (white). Scale bar: 10 μm. Right panel: Orthogonal projections of a $BrdU^+$ $Tbr2^+$-immunolabeled cell. Animals, n = 5 per group. Bilateral Student's t-test N.S.: $p > 0.05$ *$p < 0.05$ **$p < 0.01$ ***$p < 0.001$. Each value represents the mean ± SEM.

representing the most mature phenotype (Fig. 3E). Taurine significantly increased the proportion of DCX-expressing cells with a process (Student's t-test: $p < 0.001$), an effect that was almost entirely due to an increase in cells with a radial process (Fig. 3F, Student's t-test: $p < 0.001$). All together, these results suggest that taurine increased the number of new, immature

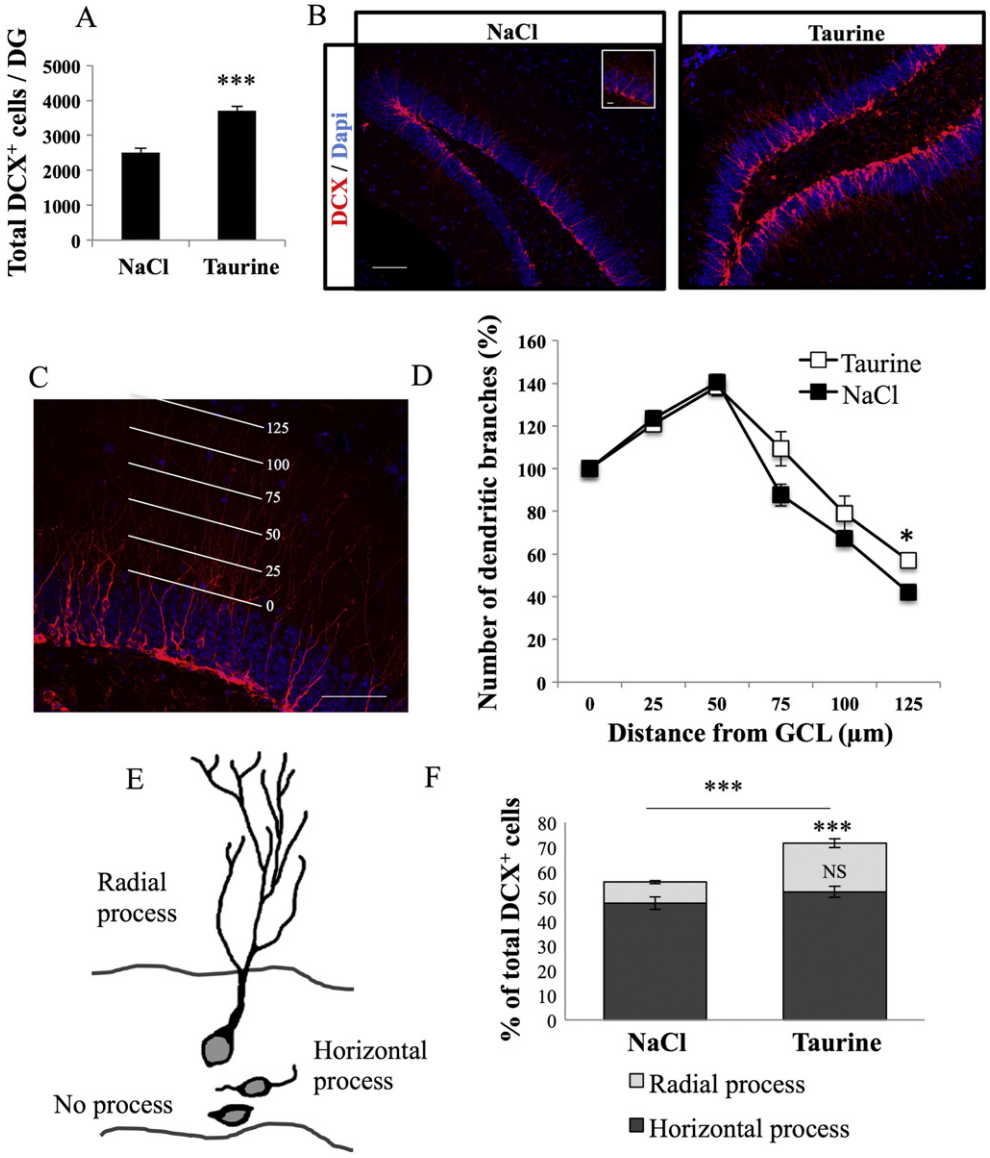

Figure 3 Taurine increased the number of immature neurons. (A) Histogram of the number of DCX^+ cells in the GCL of the DG. (B) Confocal maximal projection micrographs of hippocampal sections immunostained for DCX. Inset: Higher magnification confocal micrograph of a DCX-positive cell. Dapi staining. Scale bars: 100 μm, insets 10 μm. (C) Confocal micrograph of DCX^+ cells, extending dendrites in the molecular layer. Scale bar: 100 μm. (D) The number of dendritic branches crossing each white bar (shown in C) was counted and normalized to the value measured at 0 μm, to account for the increased number of cells. (E) Schematic illustration of the three subtypes of DCX expressing cells, based on the presence and orientation of their processes. (F) Histogram showing the proportion of DCX expressing cells without process, with a radial or a horizontal process in NaCl and taurine-treated animals. Animals, n = 5 per group. Bilateral Student's t-test N.S.: $p > 0.05$ *$p < 0.05$ ***$p < 0.001$. Each value represents the mean ± SEM.

neurons and increased the proportion of cells with mature morphological characteristics.

Taurine increased neurogenesis

We next examined the effect of taurine on the fate of newly-divided cells. Ten-month-old C57Bl/6 mice were injected with BrdU for 3 days (i.p., 100 mg/kg, 3 injections per day at 2-h intervals) and 24 h after the last injection, were treated with daily injections of taurine for 40 days. Owing to the short half-life of BrdU (11 min (Taupin, 2007)), this protocol ensures that BrdU is not incorporated into cells after 24 h (Sultan et al., 2013a). One day after the last taurine injection, mice were sacrificed, the brains removed, sliced and immunostained against BrdU and the mature neuronal marker Neu-N (Fig. 4A). The number of BrdU-labeled cells was significantly increased by 66% in taurine-injected animals (Fig. 4B bilateral Student's t-test $p < 0.05$). BrdU can be retained in daughter cells after several divisions. Since taurine increased proliferation by 22% (Fig. 1B), the effect observed here is consistent with an increase in both proliferation and survival of the new cells.

We then assessed the neuronal differentiation of newborn cells into neuronal lineage by measuring the proportion of BrdU-labeled cells that also expressed NeuN (Fig. 4C–E). In taurine-treated mice, neurons accounted for 85 ± 8% of the surviving BrdU-positive cells as compared to 60.5 ± 10% in NaCl mice. This difference was however not significant (Wilcoxon test, $p = 0.07$, Fig. 4C), indicating that taurine did not significantly increase neuronal differentiation. Nonetheless, when the number of surviving cells was multiplied by the fraction of cells that differentiated into neurons, taurine-treated mice had about 128% more newly-formed neurons than vehicle-treated mice (Fig. 4D, bilateral Student's t-test $p < 0.01$). Thus taurine increased net adult neurogenesis.

Taurine decreased microglia

Taurine is known to have anti-inflammatory properties (Menzie et al., 2013; Kim & Cha, 2014) and we recently found that the number of microglia in the dentate gyrus is inversely correlated with the proliferation of stem/progenitor cells in aged mice (Gebara et al., 2013). To test the effect of taurine on microglia in the DG, we examined microglia using immunostaining against the microglia-specific marker Iba1 (Fig. 5). Taurine significantly decreased the total number of Iba1-expressing microglia in the DG (Fig. 5A–B, bilateral Student's t-test $p < 0.001$), but did not affect the number of GFAP-immunolabeled astrocytes (Supplementary Fig. 1, bilateral Student's t-test $p = 0.57$), indicating that the effect of taurine was specific for microglia. Brain inflammation results in morphological changes of microglia upon activation: Resting microglia (stage I) have rod-shaped cell bodies with fine, ramified processes whereas activated microglia (stage II) have elongated cell bodies with long thick processes (Fig. 5C) (Mathieu et al., 2010; Preissler et al., 2015; Streit et al., 1999). We therefore examined the morphology of microglia in taurine-treated and control animals. Taurine decreased the soma area (Fig. 5D, bilateral Student's t-test $p < 0.001$), increased the territory projection area (Fig. 5E, bilateral Student's t-test $p < 0.001$) and the number of branches (Fig. 5F, bilateral Student's t-test $p < 0.001$) of microglia.

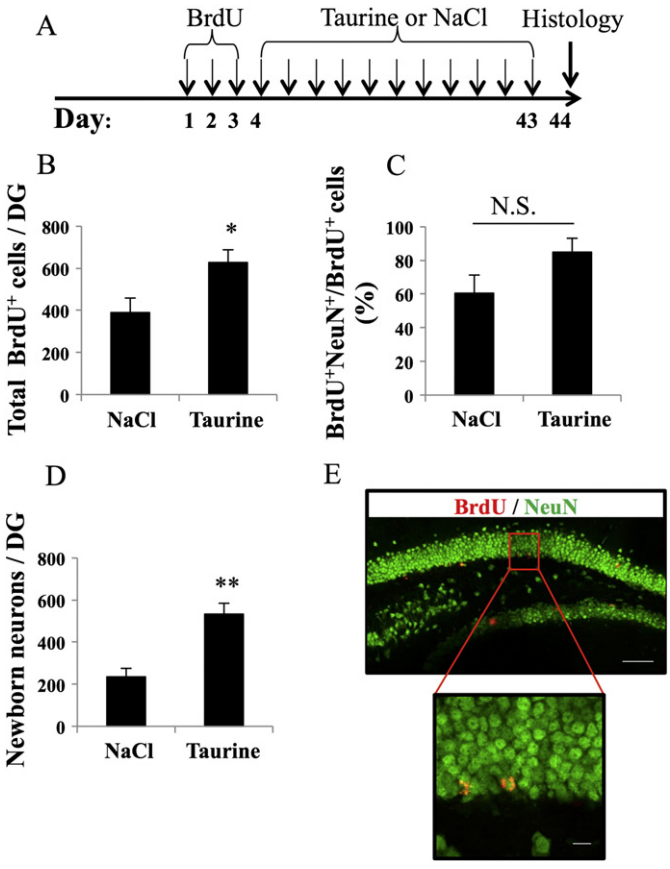

Figure 4 Taurine increased the survival of new-born neurons. (A) Experimental timeline: Mice were injected with BrdU 3 times a day for 3 days. 24 h after the last BrdU injection, mice were injected with taurine or NaCl (n = 5 mice per group) daily for 40 days. 24 h after the last taurine injection, mice were sacrificed and their brains processed for histology (B) Histogram showing the total number of BrdU-expressing cells. (C) Histogram showing the percentage of $BrdU^+$ $NeuN^+$ new neurons over the total number of $BrdU^+$ cells. Wilcoxon test N.S.: $p = 0.07$ (D) Histogram of the total number of new, $BrdU^+$ newborn neurons. (E) Upper panel: confocal micrograph of a hippocampal section immunostained for BrdU (red) and NeuN (green). Lower panel. Higher magnification micrograph of BrdU-NeuN expressing cells. Scale bar: 100 μm, inset 10 μm. For the NeuN–BrdU co-localization study, we analyzed 3 animals per group and 52–70 cells per animal. Bilateral Student's t-test: $^*p < 0.05$, $^{**}p < 0.01$. Each value represents the mean ± SEM.

These morphological modifications are consistent with a decreased activation state of microglia upon taurine treatment. Consistently, activated microglial cells accounted for 8.2 ± 0.75% of the total number of microglia in taurine-treated animals and for 37.8 ± 1.8% in NaCl-treated animals (bilateral Student's t-test $p < 0.001$). Finally, the expression of the activated microglia marker MHC-II (Frank et al., 2006) was significantly reduced in the DG of taurine-treated mice (Fig. 5G–H, bilateral Student's t-test $p < 0.001$). Thus, consistently with previous studies (Menzie et al., 2013; Kim & Cha, 2014; Kim & Kim, 2005; Chan et al., 2014) these results suggest that taurine decreased microglia number.

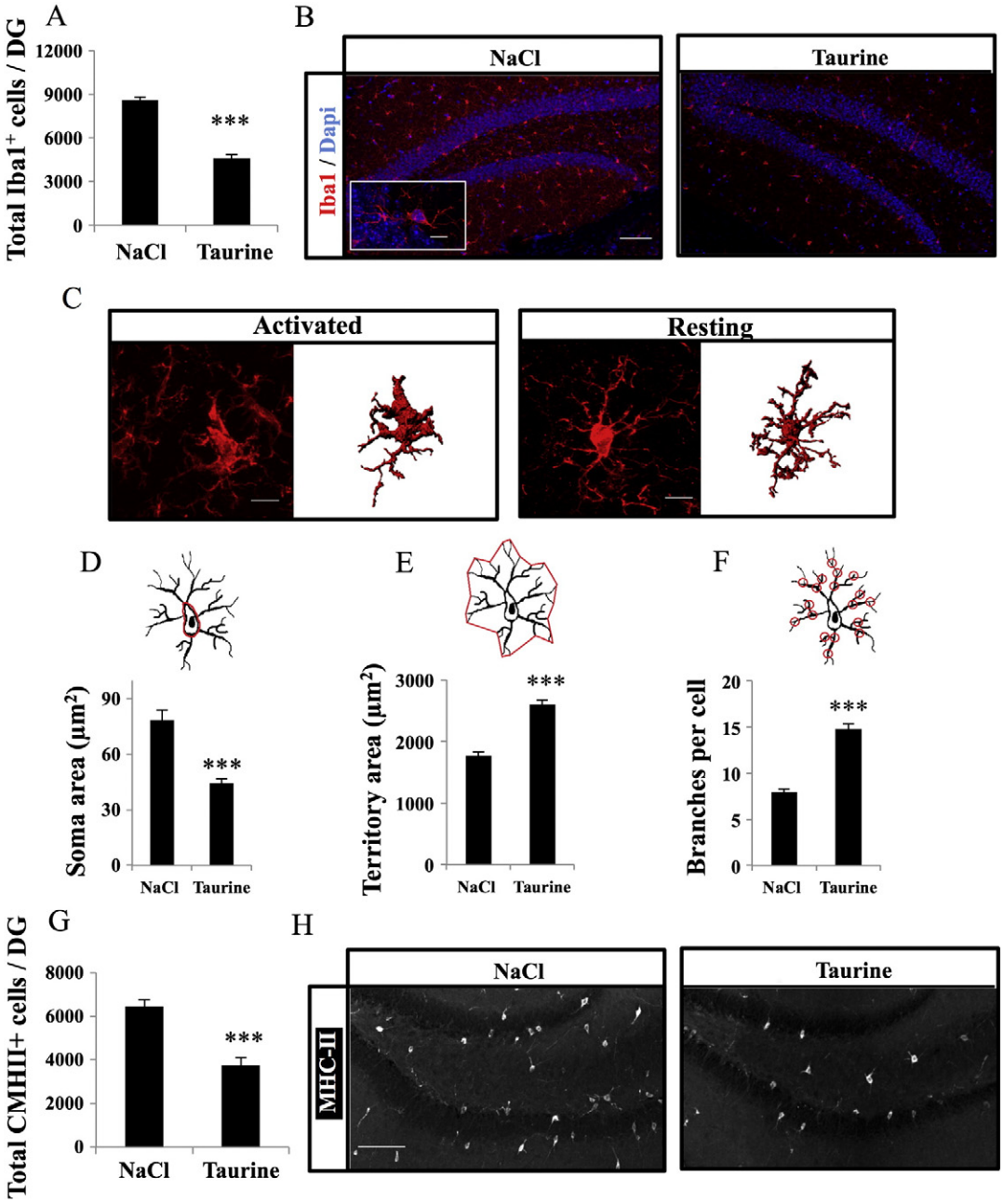

Figure 5 Taurine decreased microglia number and markers of microglia activation. (A) Histogram showing the total number of Iba1-expressing cells in the DG. (B) Confocal micrographs (maximal projections) of hippocampal sections immunostained for Iba1. Inset: Higher magnification confocal micrograph of an Iba1-immunolabeled cell. (C) Confocal micrographs and 3D reconstruction of microglia in activated (left) and resting state (right). (D) Drawing (upper panel) and histogram (lower panel) showing the soma area of microglia. (E) Drawing and histogram showing the territory area of microglia. (F) Drawing and histogram showing the number of branches per microglia. (G) Histogram showing the total number of MHC-II-expressing microglia in the DG. (H) Confocal micrographs (maximal projections) of hippocampal sections immunostained for MHC-II. Blue: Dapi staining. Animals: n = 5 per group. Scale bars: 100 µm, inset 10 µm. Bilateral Student's t-test ***$p < 0.001$. Each value represents the mean ± SEM.

Taurine increased the proliferation of neural stem/progenitor cells in vitro

Finally, we examined whether taurine may have a direct effect on stem/progenitor cells. For this, we performed *in vitro* experiments on purified adult hippocampal stem/progenitor cells (NPCs). 20,000 NPCs per well were plated and treated everyday with either 10 mM taurine or the equivalent volume of PBS 0.1 M for half an hour. After 7 days, the medium was supplemented with 5 μM BrdU for 30 min then washed and immediately fixed and immunostained for BrdU (Hernandez-Benitez et al., 2012). Taurine increased the proportion of NPCs that incorporated BrdU (Fig. 6A–B, Student's t-test, $p < 0.01$), indicating that taurine directly increased the proliferation of NPCs.

Discussion

In the present study, we tested the effect of chronic administration of taurine on hippocampal neurogenesis in aging mice. We found that taurine increased cell proliferation in the DG. More specifically, RGL stem cells showed enhanced proliferation that resulted in an increase in the number of RGL stem cells, $Tbr2^+$ intermediate progenitors and DCX^+ immature neurons. Moreover, taurine increased the survival of new neurons, resulting in a net increase in adult neurogenesis. Taurine also a reduced microglia number, morphological parameters associated with activation, MHC-II expression and increased stem/progenitor cell proliferation *in vitro*. Together, these results indicate that, in the aging brain, taurine increases the production of new neurons by stimulating several steps of adult neurogenesis and plays a role in microglia function.

Taurine is known to be involved in variety of cellular processes, including calcium homeostasis (El Idrissi, 2008; Wu et al., 2005; Foos & Wu, 2002), protection from glutamate excitotoxicity and apoptosis (Foos & Wu, 2002; Leon et al., 2009), inflammation (Kim & Cha, 2014), oxidative stress (Menzie et al., 2013), and epilepsy (El Idrissi et al., 2003), all of which contribute to the regulation of adult neurogenesis. However, since there is currently no known taurine receptor, its role as an osmolyte is believed to participate to these processes. Our results suggest that taurine can regulate adult neurogenesis both through an indirect effect on microglia and a direct effect on stem/progenitor cells.

The indirect effect of taurine on adult neurogenesis may be mediated by its anti-inflammatory properties: taurine reduces the production of inflammatory cytokines such as TNFα or IL-1β (Kim & Cha, 2014) and its derivative, taurine-chloramine, reduces the activation of NFkb in several models of inflammation (Kim & Kim, 2005). In the brain, taurine administration reduces cell damage and cytokines expression after traumatic brain injury (Chan et al., 2014) and mitigates lipopolysaccharide-induced inflammation and microglia activation (Menzie et al., 2013). In physiological conditions, brain aging is accompanied by increased expression of genes involved in cellular stress and inflammation (Sturman et al., 1985) and increased microglia proliferation and activity (Kohman, 2012). In turn, this increased inflammation negatively correlates with hippocampal RGL stem cells proliferation (Gebara et al., 2013). Thus, the increased inflammation in the aging brain inhibits hippocampal neurogenesis and conversely, anti-inflammatory treatments that reduce microglia activation, increase adult neurogenesis (Gebara et al., 2013; Sultan et al., 2013b). Consistent with these observations, taurine partially restores cell proliferation in the DG after lipopolysaccharide-induced inflammation (Menzie et al., 2013). Altogether, these results suggest that the effect of taurine on adult neurogenesis that we observed in the aging brain may be, at least partially, mediated by a reduction in microglia activation. Although we did not directly measure levels of inflammatory cytokines in this study, this possibility is consistent with our observations of decreased microglia number, reduced morphological markers that are normally associated with activation and reduced MHC-II expression upon taurine treatment.

In addition to its indirect effect, taurine directly increased the proliferation of purified adult hippocampal stem/progenitor cells *in vitro*, similarly to what was previously observed on subventricular zone progenitors (Ramos-Mandujano et al., 2014). The antioxidant (Schaffer et al., 2009) and antiapoptotic properties of taurine (Taupin, 2007; Sultan et al., 2013a), can potentially contribute to the increased proliferation and survival rate of the highly proliferative and metabolically active neural stem cells and new neurons. Of particular interest, taurine has recently been shown to interact with the polyamine site of the NMDA receptor and modulate the activity of the NMDA receptor (Chan et al., 2014). NMDA receptors are expressed by RGL stem cells and regulate their activity (Muth-Kohne et al., 2010), as demonstrated by the increased proliferation induced

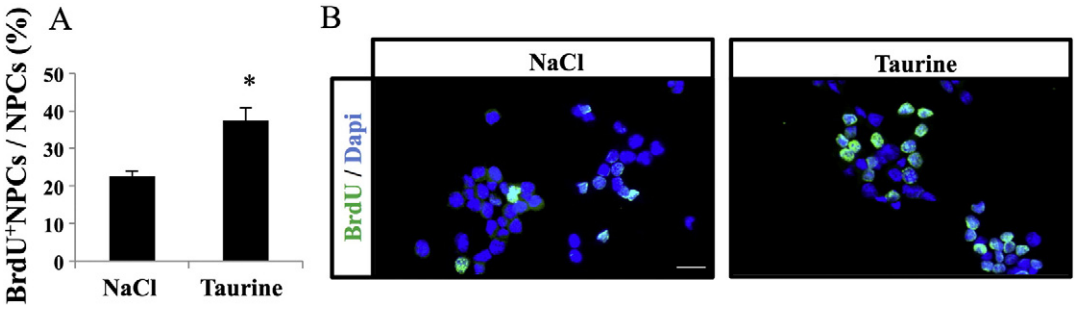

Figure 6 Taurine increased the proliferation of adult hippocampal stem/progenitor cells (NPCs) *in vitro*. (A) Histogram showing the percentage of NPCs immunostained for BrdU over the total number of NPCs. (B) Confocal micrographs of NPCs treated with NaCl (left panel) or taurine (right panel). n = 3 culture wells per group. *$p < 0.05$. Each value represents the mean ± SEM. Scale bar: 20 μm.

by the NMDA receptor antagonists memantine or MK-801 (Namba et al., 2009), and the co-agonist D-serine (Sultan et al., 2013a). NMDA receptors are also expressed on immature adult-born hippocampal neurons and are required for the survival of these cells (Tashiro et al., 2006). Thus, by directly modulating NMDA receptor activity on RGL stem cells and on immature neurons, taurine may increase the proliferation of the former and the survival of the latter and thereby contribute to the increased neurogenesis we observed. Endogenous taurine is released by astrocytes (Choe et al., 2012) and may contribute to the regulation of adult neurogenesis by the microenvironment. With age, the dysregulation of the neurogenic niche (Bernal & Peterson, 2011; Katsimpardi et al., 2014) may involve a reduction of astrocytic function, leading to reduced taurine production, thereby causing age-related impaired neurogenesis.

Reduced adult neurogenesis contributes to age-related cognitive impairment and several therapeutic approaches for stroke or neurodegenerative disorders target adult neurogenesis. The renewal of stem cells in the adult brain being limited (Calzolari et al., 2015), approaches that increase the stem cell pool may yield a more promising outcome for restoring neurogenesis in the aged brain, than targeting later stages of the process. In light of our results, the potent activating effect of taurine on RGL stem cells may underlie its beneficial cognitive effects and may represent a promising approach for the treatment of age-related reduction in adult neurogenesis and cognition.

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Author contribution

E.G., F.U., S.S. and N.T. designed the experiment. E.G., F.U. and S.S. performed the experiments and analyzed the data. E.G., F.U. and N.T. prepared the manuscript, N.T. provided financial support.

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