

# Traumatic Brain Injury Accelerates the Onset of Cognitive Dysfunction and Aggravates Alzheimer's-like Pathology in the Hippocampus by Altering the Phenotype of Microglia in APP/PS1 Mouse Model

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## Research

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# Abstract

**Background:** There are increasing studies suggest that Traumatic Brain Injury(TBI) might be the cause of some neurodegenerative diseases, including Alzheimer's Disease(AD). The mechanism of AD induced by TBI has also been elucidated from sorts of aspects. However, there are also researches which opponent to the viewpoint that TBI is the reason of AD. In this study, we investigate whether and how could TBI accelerated the Alzheimer's-like pathology and cognitive dysfunction in APP/PS1 mice.

**Method:** The traumatic brain injury model was established in adult male APP/PS1 and C57BL/6 mice. At the 29<sup>th</sup> and 30<sup>th</sup> day post-TBI, Novel object and novel position recognition test were performed to test the learning and memory function. After cognitive function test, all the mice were sacrificed with PBS perfusion to anatomize the brain for histopathological study. To determine whether the Alzheimer's-like pathology and the synaptic function decline can be accelerated by TBI, HE staining, IF staining and IHC staining were performed to detect the cell density in the brain, the degree of A $\beta$ -plaques deposition in the brain, and SYP expression in the brain. We also examined the activity and the phenotype of microglia by IF staining and western-blotting the after the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> week of TBI.

**Result:** In current study, we showed that, on the one hand, TBI impaired the hippocampal-dependent learning and memory, decreased the cell density in the brain, disturbed the synaptic function in the brain, aggravated A $\beta$ -plaques deposition in the hippocampus. On the other hand, TBI also quickly activated microglia in the CNS and altered the phenotype of microglia polarizing to a pro-inflammatory direction. The duration of activation of microglia post-TBI can be at least 3 weeks. We also found that microglia activity was related to the deposition of A $\beta$ -plaques in the specific region of hippocampus.

**Conclusion:** Our experiment suggested that TBI accelerate the onset of cognitive dysfunction and Alzheimer's-like pathology in APP/PS1 mouse model by altering microglia polarizing direction to mostly exhibiting pro-inflammatory phenotype.

## Introduction

Traumatic brain injury(TBI) is one of the leading causes of disability and death all over the world, with approximately 69 million people suffering from it each year<sup>1</sup>. With difficulty and costly treatment and care, there is also a huge financial and medical burden caused by TBI to the patient's family, even to the society, who suffer from it. Meanwhile, there are also a consequence of complications after recovering from TBI for many years. However, there are series of neurodegenerative diseases secondary to TBI in its chronic stage, including Alzheimer disease(AD)<sup>2</sup>, which is the most common cause of dementia.

Generally speaking, the amount of dementia patients will have increased to more than 100 million by the year 2050 all over the world with an astronomical figure global societal cost on it estimated by the World Health Organization(WHO). According to the statistics from America Alzheimer's Association, by the year 2050, the speed of patient who developed in AD will have increased by 3 folds compared with the year of

2016<sup>3</sup>. China is also a big country with millions of demented patients diagnosed with AD and the number continues to increase with years<sup>4</sup>. The pathology of AD is characterized by senile plaques(SP) and neurofibrillary tangles(NFT) deposition in the brain, which are made up of amyloid- $\beta$ (A $\beta$ ) and tau protein respectively. It has been more than 100 years since AD was first described, and researchers have never been giving up trying to find therapeutics for management of AD. With aging of population, AD is such a serious medical curiosity demanding prompt solution currently.

By observing the patients after brain injury, researchers find that there might be associations between TBI and AD. Martland reported some cases about 'punch drunk' syndrome and Raul reported a young man who experienced TBI developing in AD<sup>5,6</sup>. Although we cannot simply ascertain the cause of AD from the TBI experience, we may be able to reveal the associations between TBI and AD via detecting the changes of brain pathology. Some clinical and basic researches indicate that AD can be induced or aggregated by TBI<sup>7,8</sup>. There are also some probable mechanisms including A $\beta$  and tau protein genesis, apolipoprotein E(ApoE) $\epsilon$ 4 gene carrying, central nervous system(CNS) inflammation<sup>9</sup>, cerebral vascular factors<sup>10</sup> and so on which were proved associated with AD induced by TBI. However, there are still some researches disagree with the viewpoint that AD is associated with TBI<sup>11,12</sup>, leaving the conclusion still contradictory.

Neuroinflammation is a well elucidated pathogenesis progression during both TBI and AD, however, which needs further study. Microglia, serving as a kind of innate immunity cell in the CNS, participate in both of the above diseases and play an important role in the neuroinflammation. It is widely acknowledged that microglia have two different polarization state after activated, which is so-called classic(M1-like) and alternative(M2-like) activated state<sup>13</sup>. In the 'resting' state, microglia monitor and prevent harmful stimuli to protect the CNS environment. Whereas, in the cases of harmful stimuli, possibly neurotrauma, bacteria or abnormal folded protein, the CNS will activate microglia and switch to a specifically polarized state, M1-like or M2-like state, to fight against harmful stimuli, release proinflammatory cytokines or clean up the debris of dead neurons, repairing the CNS damage<sup>13</sup>.

In TBI progression, microglia are fast activated shift into M1-like state to respond to the damage in a few minutes to hours and last for a few weeks even months. At the same time, during AD progression, microglia can be activated and bind to the abnormally deposition of A $\beta$ , showing a proinflammatory property and producing inflammatory factors<sup>14-16</sup>. Nevertheless, microglia may also exhibit another protective function in AD by phagocytizing A $\beta$ , which is up to its phenotype<sup>17</sup>. There has been evidence suggesting that microglia in a M1-like phenotype exhibit an inhibition of A $\beta$  phagocytosis. Moreover, there are also theories inferring that M2-like phenotype of microglia might be benefit for many diseases including AD<sup>18,19</sup>.

In the current study, whether TBI could accelerate the cognitive dysfunction, aggravate the Alzheimer's-like pathology in APP/PS1 transgenic mice, as well as the potential mechanism related to the activation and phenotype of microglia post TBI were investigated.

# Materials And Methods

## Animals

Double-TG mice model (APP<sup>swe</sup>, PSEN1<sup>dE9</sup>) of Alzheimer's disease and C57BL/6 mice were used for this experiment. All the mice for the experiment were purchased from Model Animal Research Center of Nanjing University, cultured in the environment with 12 hours bright and 12 hours dark circle. All of the mice can access to the food and water freely. For all repertoires of experiments, 6-month-old male C57BL/6 mice weighted from 25 to 30 gram were selected, and were separated randomly into two groups as C57BL/6 mice and C57BL/6 TBI mice. 6-month-old male APP/PS1 mice weighted from 25 to 30 gram were selected, and were separated randomly into two groups as APP/PS1 group and APP/PS1 TBI mice.

## Protocols Of Tbi

Traumatic brain injury mice model were made by weight drop as previous described<sup>20</sup>. Mice were anaesthetized by 10% chloral hydrate. After deeply anaesthetized, the scalp was cut apart along the sagittal suture and hypodermis was separated by vessel forceps. The periosteum was scraped with bistoury to expose the right parietal bone. A 5 mm diameter hole was made by bone drill on the right parietal bone to exposure endocranium. Release the hammer from a height of 3 cm away from the head to hit the priming wire which is connected to the endocranium to establish model of TBI mouse.

## Hemotoxin-eosin(he) Staining

HE Staining was performed on the paraffin-embedded sections of 3- $\mu$ m-thick. Sections were paraffined and hydrated in ascending concentrations of ethanol. After hydration sections were counterstained in hemotoxin and eosin solution. Then sections were washed by PBS, and dehydration in ascending concentrations of ethanol. Cover the sections with coverslip and take photos under microscope. The cell density was calculated using Photoshop CC 2018.

## Novel Object and Novel Position Recognition Tests

The test was performed in a blue plastic box (35 × 35 × 20 cm<sup>3</sup>). Test was performed without habituated to the empty field as described previously<sup>21,22</sup>. The next day (i.e., the acquisition phase), mice were exposed to two identical plastic objects that were placed at two selected contiguous corners for 10 minutes. In the test phase held 1 h later, one of the objects was replaced by a novel object in the same position, and each mouse was allowed to explore the field and objects for 5 minutes. After the novel object test, the field was cleaned thoroughly with 75% ethanol, and the novel object was replaced by the old object, which was placed at a novel place in the field. The same mouse was induced into the filed again and allowed to explore the field and objects for 5 minutes to finish the novel place recognition test. The time each mouse spent on exploring these objects and places through both acquisition and test

phases was recorded. Exploration was defined when the head of the animal was facing the object from a distance less than 1 cm away from the object or physically touch the object by upper limbs. The field and the objects were cleaned thoroughly with 75% ethanol between trials to eliminate olfactory cues. The preference index was expressed as the percentage of time exploring the displaced object to total exploration time.

## Immunohistochemistry Staining

Immunohistochemistry staining was performed on paraffin-embedded sections of 3- $\mu$ m-thick mice brain tissue. Sections were deparaffined and boiled in citric acid buffer solution for 5 minutes. After cooling down to the room temperature, sections were incubated with blocker of endogenous peroxidase (Catalog number: PV-6002, ZSGB-BIO, Beijing, China) for 15 minutes. Then sections were blocked with serum. To detect A $\beta$ -plaques deposition, sections were incubated with mouse anti-A $\beta$  primary antibody (1:100; catalog number: #15126S; Cell Signaling Technology, MA USA) overnight. Next day, sections were washed by PBS and incubated with goat anti-mouse IgG(H + L) conjugated to horseradish peroxidase secondary antibody (Catalog number: PV-6002, ZSGB-BIO, Beijing, China). Incubated the sections with dimethylaminobenzaldehyde(DAB, Beyotime Biotechnology, Zhejiang, China) reagent to chromogenic reaction. Counterstain the sections with hematoxylin solution. Cover the sections with coverslip.

## Immunofluorescence Staining

Immunofluorescence staining was performed on paraffin-embedded sections of 3- $\mu$ m-thick mice brain tissue. Sections were deparaffined and boiled in citric acid buffer solution for 5 minutes. After cooling down to the room temperature, sections were incubated with blocker of endogenous peroxidase for 15 minutes. Then sections were blocked with serum. To detect in situ antigens, sections were incubated with primary antibodies [rabbit anti-A $\beta$ (1:100; catalog number: #15126S; Cell Signaling Technology, MA USA), rabbit anti-synaptophysin(SYP)(1:100; catalog number:17785-1-AP, Proteintech., IL, USA), rabbit anti-ionized calcium-binding adapter molecule 1(IBA-1)(1:100; catalog number:10904-1-AP, Proteintech., IL, USA)] overnight. Next day, sections were washed by PBS and incubated with secondary antibodies [goat anti-rabbit IgG(H + L) cross-adsorbed ReadyProbes secondary antibody-Alexa Fluor 594 (catalog number: #R37117) or goat anti-mouse IgG(H + L) highly cross-adsorbed secondary antibody-Alexa Fluor 488 (catalog number: #A-11034) (1:2,000; Invitrogen, Thermo Fisher Scientific)].Counterstain the nuclei with 4',6-diamidino-2-phenylindole (DAPI) (1  $\mu$ g/mL; Sigma-Aldrich). Cover the sections with coverslip and take photos under the BX51 fluorescence microscope (Olympus, Tokyo, Japan).

## Western-blotting

The hippocampus was dissected from the APP/PS1 and APP/PS1 TBI mice. The protocol of Western-blot is as described previously<sup>23</sup>. Target protein expression was detected using the following primary antibodies: rabbit anti-arginase-1(Arg1) (catalog number: #93668; Cell signaling), rabbit anti-inducible nitric oxide synthase(iNOS) (catalog number: #13120; Cell signaling), and rabbit anti- $\beta$ -actin (catalog number: #4790; Cell signaling) (IgGs; 1:2000).  $\beta$ -actin expression was utilized as an internal reference. Protein blots were visualized using the goat anti-rabbit IgG (H + L) secondary antibody-HRP (1:5000;

Invitrogen, Thermo Fisher Scientific) and a Chemi-Doc™ Imaging System (Bio-Rad Laboratories, Inc., CA,USA) and measured using Image-Pro Plus 6.0.

## Statistical Analysis

Statistics were analyzed by a one-way analysis of variance (ANOVA) followed by a post hoc Bonferroni's multiple comparisons test. Data were expressed as mean  $\pm$  SEM. All the statistical analysis was performed by the software of GraphPad Prism 7.0. A value of  $P < 0.05$  was considered a statistically significant difference.

## Results

### TBI Impaired Cognitive Function of APP/PS1 Mouse Model.

To detect the cognitive function of AD mice after 4-week of TBI, we generated the TBI model with double transgenic AD mice, and novel object and position recognition test was performed to test the cognitive function in each group. The result showed that, in the acquisition phase, each group of mice spent nearly the same duration of time on exploring the two identical objects (C57BL/6, C57BL/6 TBI, APP/PS1, APP/PS1 TBI,  $P > 0.05$ ) (Fig. 1A). In the testing phase after 1 h, mice in the C57BL/6, C57BL/6 TBI and APP/PS1 group preferred to spend more time on detecting the novel object and the novel position (C57BL/6  $P > 0.05$  vs. C57BL/6 TBI,  $P > 0.05$  vs. APP/PS1; C57BL/6, C57BL/6 TBI and APP/PS1  $P < 0.05$  vs. chance level 50%) (Fig. 1B,1D), however, APP/PS1 TBI mice showed no preference to the novel object and novel position relative to other groups (APP/PS1 TBI,  $P < 0.01$  vs. C57BL/6;  $P < 0.01$  vs. C57BL/6 TBI;  $P < 0.05$  vs. APP/PS1). Of note, there is an interesting phenomenon that it is more often for the APP/PS1 TBI mice to climb on the object than any other group of mice during the test, which is never happened to the C57BL/6 mice. This result indicates that TBI impairs the hippocampal-dependent learning and memory function in APP/PS1 mice.

### TBI Decreased Cell Density at TBI Lesion and CA3 Region of Hippocampus.

We performed TBI at the cortex of the brain, and the cell density decrease in the cortex is taken into granted (C57BL/6 TBI,  $P < 0.001$  vs. C57BL/6; APP/PS1 TBI,  $P < 0.01$  vs. APP/PS1) (Fig. 2). However, the hippocampus is not injured directly. It is well approved that hippocampus is a vital important grey matter area which is related to cognition, memory and emotion<sup>24</sup>. As cognitive function decline has been described above, we further detected the hippocampus pathological changes after TBI. HE stain was performed to determine the cell density in each region of hippocampus. By observing under the microscope, the neuron cells in the TBI brain exhibited loose and disordered in arrangement and the nucleus showed paramorphia and shrinkage. Our results revealed that neuron cell density in the region of CA3 was similar between C57BL/6 mice and APP/PS1 mice (APP/PS1,  $P > 0.05$  vs. C57BL/6) (Fig. 2). After TBI, there is a trend of decrease, although not significantly, of cell density in the C57BL/6 TBI mice compared with C57BL/6 mice (C57BL/6 TBI,  $P = 0.18$  vs. C57BL/6) (Fig. 2). Of note, the cell density in APP/PS1 TBI mice are significantly decrease compared with APP/PS1 mice (APP/PS1 TBI,  $P < 0.001$  vs.

APP/PS1) (Fig. 2). As for the cell density in the other region of hippocampus, it showed not significant difference between each group(Fig. 2). These results indicated that the cell density of APP/PS1 mice is no less than C57BL/6 mice, but TBI decrease the neuron cell in the cortex around the lesion and in CA3 region, which may be the reason of impairment of cognitive function in APP/PS1 mice post-TBI.

### **TBI Diminished Synaptic Function in the Cortex and the Specific Region of Hippocampus.**

Synaptophysin(SYP) is the marker of synapse plasticity, which reflects synaptic function<sup>25</sup>. To further evaluate synaptic function in the brain of each group, immunofluorescence staining of SYP was performed to evaluate the expression in the cortex and in the hippocampus. In the cortex, C57BL/6 mice exhibited abundant of SYP, reflecting a normal stage of synaptic function, which is paralleled with its cognitive function(Fig. 3). C57BL/6 TBI mice exhibited down regulated of SYP compared with C57BL/6 mice (C57BL/6 TBI,  $P < 0.001$  vs. C57BL/6), suggesting that TBI may impair synaptic function(Fig. 3). APP/PS1 mice expressed fewer SYP than C57BL/6 mice (APP/PS1 TBI,  $P < 0.001$  vs. APP/PS1), indicating the impairment of synaptic function in its basic level(Fig. 3). As expected, compared with APP/PS1 mice, APP/PS1 TBI mice showed a significant decrease expression of SYP in the cortex (APP/PS1 TBI,  $P < 0.05$  vs. APP/PS1), in paralleled with the cognitive function impairment of APP/PS1 TBI mice(Fig. 3). In the region of CA1 and CA3 of hippocampus, the expression of SYP in APP/PS1 TBI mice is also significantly decreased compared with APP/PS1 mice (APP/PS1 TBI,  $P < 0.05$  vs. APP/PS1), suggesting that TBI aggravated the AD dementia via devastating synaptic function in AD mouse model(Fig. 3).

### **TBI Activated Microglia in the Brain and Sustained at Least for 3 Weeks.**

Microglia that resident in the brain and differentiate from peripheral phagocyte infiltrating into CNS through injured Blood-Brain-Barrier(BBB) can be activated soon after TBI. Also, the period that microglia activated by TBI will last for days or even months<sup>26</sup>. To further determine the situation of activated microglia, we performed immunofluorescence staining to detect the IBA-1 expressed on the surface of microglia at the 1st, 2nd, 3rd and 4th week of TBI. Also, to evade the disturbance to the result by the A $\beta$ -plaques deposition which may also up-regulated the degree of activation of microglia, we just detect this marker from C57BL/6 TBI mice. The results showed that, compared with resting phenotype, in the each region of brain(cortex and hippocampus), microglia were significantly activated after the 1st week of TBI(Fig. 4), and the activation state is last at least for 3 weeks(C57BL/6  $P < 0.05$  vs. C57BL/6 TBI at the 1st week; C57BL/6  $P < 0.01$  vs. C57BL/6 TBI at the 2nd week; C57BL/6  $P < 0.01$  vs. C57BL/6 TBI at the 3rd week; C57BL/6  $P > 0.05$  vs. C57BL/6 TBI at the 4th week) (Fig. 4).

### **TBI Aggravated A $\beta$ -plaques Deposition in the Specific Region of Hippocampus in APP/PS1 Mouse Model.**

A $\beta$ -plaques deposition is concerned to be the most distinctive and classic pathological change in AD brain. Generated from the cleavage of APP by  $\beta$ -secretase, A $\beta$  molecules form into monomer, oligomer and fiber, which are both toxic to the CNS. As is reported previously, the up-regulated expression of APP mRNA and up-regulated activity of  $\beta$ -secretase has been observed in situ in the TBI mouse model, which

provides abundant materials for the generation of A $\beta$ -plaques via  $\beta$ -secretase<sup>27, 28</sup>. To further investigate whether TBI could aggravate A $\beta$ -plaques deposition in the hippocampus in APP/PS1 mice brain, immunohistochemistry staining was performed. As expected, there were not any A $\beta$ -plaques deposition in C57BL/6 and C57BL/6 TBI mice. By IHC staining, we found that the A $\beta$ -plaques deposited mostly in the specific region in the hippocampus(Fig. 5 in the region of black curly line). It showed that only a few mounts of A $\beta$ -plagues deposited in the brain of APP/PS1 mice. Whereas, after 4 weeks of TBI, APP/PS1 TBI mice exhibited significantly more A $\beta$ -plaques deposition in these specific region of hippocampus than the APP/PS1 mice did at the same month of age (APP/PS1 TBI,  $P < 0.05$ , vs. APP/PS1) (Fig. 5), which infers that TBI might be the trigger of acceleration of A $\beta$ -plaques deposition in the brain.

### **A $\beta$ -plaques Aggravated Neuroinflammation in the Specific Region of Hippocampus post-TBI.**

Microglia plays an important role in the progression of AD. It can be activated by the deposition of A $\beta$ -plaques and the potent activation of microglia results in the persistent release of proinflammatory cytokines, which causes secondary injury to the CNS<sup>29</sup>. Neuroinflammation exists both in the progression of TBI and AD. In the acute stage of TBI, there is an activation of microglia in the CNS instantly, which can persist for a long time<sup>26</sup>. To further determine the degree of neuroinflammation in the hippocampus post TBI, immunofluorescence staining was performed to detect the expression of IBA-1 which is the marker of activated microglia. In our experiment, we found that the activation of microglia was significantly elevated in the region of CA2 and DG in APP/PS1 TBI mice(APP/PS1 TBI,  $P < 0.01$  vs. APP/PS1(CA2 region); APP/PS1 TBI,  $P < 0.05$  vs. APP/PS1(DG region)) (Fig. 6), but not the other regions of hippocampus(Fig. 6). Notably, the region of up-regulated activation of microglia is paralleled with the region of accumulation of A $\beta$ -plaques deposition in the hippocampus, indicating that the possibility of correlation between potent activation of microglia and deteriorated AD pathology after TBI.

### **TBI Aggravate the Alzheimer's-like Pathology by Altering the Phenotype of Microglia.**

As is elucidated above, microglia are activated after TBI in the specific region of hippocampus. However, that which sort of phenotype of activated microglia is predominate in the damaged brain deserves further study. In that the biological behavior of microglia, doing good or harm to the CNS, depends on its phenotype. To further investigate the direction of microglia polarization post TBI, we performed western-blotting in the APP/PS1 TBI mice to detect the production of microglia(Arg1 and iNOS) for distinguishing the predominate phenotype. The results showed that in the normal circumstance, microglia seemed to exhibit an alternative phenotype which produced high level of Arg1(Fig. 7A,7B), however, it switched the phenotype much more to a classical phenotype with the feature of producing more iNOS than the normal state post TBI(Fig. 7A,7C). These results indicated that microglia switched the phenotype from an "M2-like" to an "M1-like" post TBI, a phenotype which was disadvantaged for the clearance of Alzheimer's-like pathology.

## **Discussion**

The mechanisms that neurodegenerative disease such as AD triggered by TBI still remain elusive. In the current study, we showed that TBI impairs the hippocampal-dependent learning and memory function, decreases the cell density in hippocampus, diminishes the synaptic function in the brain, aggravates A $\beta$ -plaques accumulation in hippocampus, aggravates neuroinflammation in the CNS in APP/PS1 mice. Briefly, our experiment supports the view that the Alzheimer's-like pathology and cognitive dysfunction could be accelerated by TBI from many aspects.

AD is one of the neurodegenerative diseases which affects cognitive function primarily. Traumatic brain injury also impairs a series of cognitive fields<sup>30</sup>. It is reported that in the acute stage of mild TBI, patient who had lower education level showing poor outcomes than the normal one<sup>31</sup>. Hajime et.al reported a transient increasing production of A $\beta$  and cognitive impairments post TBI<sup>32</sup>. The degree of cognitive function decrease also depends on the degree of TBI, which exhibits like a "dose-effect relationship"<sup>33</sup>. Not only in the acute stage of TBI, the secondary effects of TBI in its chronic stage to the CNS has also been taken seriously. Kinds of neurodegenerative diseases and sorts of abnormally producing proteins induced by TBI, which also selectively impair the cognitive function<sup>34,35</sup>. In our experiment, to assess whether TBI could aggravate the cognitive impairment in the APP/PS1 mouse model, novel object and novel position recognition test, a specifically and well-accepted behavior test for APP/PS1 mouse model, was performed to evaluate the hippocampal-dependent learning and memory function. APP/PS1 TBI mice exhibited less interests to the novel object and novel position, indicating impaired in the short-period memory compared to APP/PS1 mice. The data provide an evidence that the cognitive function of APP/PS1 mice was impaired after TBI in the APP/PS1 mouse model.

It has been demonstrated that the synaptic function impairment in AD is largely associated with the abnormal accumulation of the A $\beta$ -plaques in the hippocampus<sup>36</sup>. There is also evidence showed that TBI could also affects the synaptic plasticity<sup>37</sup>. Synaptic function is rapidly disturbed after TBI<sup>38</sup>, and could also continue to the chronic stage<sup>39</sup>. Our experiment evaluates the synaptic function in the cortex and in the hippocampus of each group of mice. Our data showed that, in TBI mice, SYP in the cortex exhibited a trend of decreasing after TBI compared with each of their control group of mice. Moreover, the statistics also showed that APP/PS1 TBI mice exhibited decreased synaptic function compared with APP/PS1 mice, which paralleled with their cognitive function.

It is widely accepted that the pathology of AD is composed by abnormally accumulated of A $\beta$  and tau protein, which are both toxic to the neuron. The predominate molecular formation of A $\beta$  contains A $\beta$ <sub>1-40</sub> and A $\beta$ <sub>1-42</sub>, which can exist in a formation of monomer, oligomer and A $\beta$  fiber, and can be coagulated into deposition in the CNS finally. However, the misfold of these proteins accounts for the most cytotoxic to the CNS<sup>40</sup>. Our results showed that TBI promote the deposition of A $\beta$ -plaques in the specific region in the hippocampus in APP/PS1 TBI mice, indicating that TBI accelerates the genesis of the Alzheimer's-like pathology in the specific region.

Neuroinflammation is also a vital important component of pathology in both AD and TBI. In AD pathology, the function of microglia surrounding the SP which is still not completely cleared. In addition, the molecular, triggering receptor expressed on myeloid cells 2 (TREM2), which is only expressed on the microglia is also associated with the risk of AD, suggesting that microglia is not only a participant of AD pathology but also account for the onset of the disease<sup>9</sup>. The release of proinflammatory cytokines is also paralleled with the level of A $\beta$ -plaques deposition in the brain after TBI in AD mouse model, indicating that neuroinflammation might aggravate the pathology of AD<sup>41</sup>.

In our experiment, the activation of microglia was still potent in the CA2 and DG region of hippocampus in APP/PS1 TBI mice at the 4th week post-TBI compared to APP/PS1 mice, suggesting that TBI might trigger the neuroinflammatory response to the strike and the reaction to the abnormal accumulated protein. We also performed experiment only in the C57BL/6 mice to observe the activation of microglia *in vivo* after 4 consecutive weeks of TBI, to avoid the infectious of A $\beta$  to the microglia in the brain. Our results reflected a fluctuation activation of microglia in the brain, that microglia were directly activated after TBI, and will last for 3 weeks in our experiment. At the 4th week post-TBI, the activation of microglia seemed to decreased compared with previous 3 weeks and exhibited nearly no difference with normal state in C57BL/6 mice. Our data support the evidence that TBI could provoke the neuroinflammatory reaction in the acute stage, and there is also an activation of microglia by the abnormal accumulation of A $\beta$ -plaques.

In the previous studies, AD induced by TBI through various mechanisms, including accumulation of APP molecular cleaved by up-regulated  $\beta$ -secretase, disturbance of clearance of A $\beta$ -plaques impacted by APOE $\epsilon$ 4 gene and damaged glymphatic system<sup>42</sup> and blood vessel of brain<sup>10</sup>. Our experiment provides a new hypothesis about the mechanism, to our knowledge, that AD may induced by TBI via neuroinflammation by altering the microglia phenotype in the CNS. Then we will talk about the phenotype of microglia post-TBI in the brain.

Microglia, with two main different polarization phenotypes, serves as an important participant in the pathology of both AD and TBI. As an innate immunocyte in the CNS, microglia are activated after TBI and switches the phenotype to proinflammatory state. When resident microglia are activated and peripheral macrophage are infiltrating from the circulation in the lesion, with sorts of inflammatory factors released, including interleukin-1 $\beta$  (IL-1 $\beta$ ), interferon- $\gamma$  (IFN- $\gamma$ ), and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), CNS is attacked by the cascade of inflammation, which is disadvantaged for damage repair and neurogenesis<sup>13</sup>. After activated, microglia shift its morphology from ramified into amoeboid-like, and in this phenotype it is characterized by producing iNOS that may be neurotoxic. However, in the alternative activated phenotype, microglia show a protective function to the CNS from many aspects. The alternative phenotype could be switched by the producing of IL-4, IL-10, vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) and Arg1<sup>13</sup>.

In AD, microglia exhibit also A $\beta$ -plaques degrading function<sup>43</sup>. However, that the ability of microglia phagocytosis A $\beta$  peptides will be diminished by the exposure of pro-inflammatory cytokines medium, but

it will be restored by applying anti-inflammatory chemicals<sup>43</sup>. There is also experiment indicating that the “M2-like” phenotype microglia induced by anti-inflammatory treatment is benefit for both cognitive function and A $\beta$ -plaques clearance in APP/PS1 mice in the early stage of AD<sup>44</sup>. In our experiment, we supposed that A $\beta$ -plaques deposition accumulated in the brain is associated with the altered proportion of “M1/M2” microglia phenotype post TBI. Consistent with our presume, microglia were quickly activated and exhibited a proinflammatory phenotype after TBI last for at least 3 weeks, with the high expression of iNOS and down-regulated expression of Arg1. These results suggest that TBI down-regulated the proportion of alternative phenotype of microglia which may exhibit protective function to the brain. Then the clearance of the abnormal protein, A $\beta$ , which is toxic to the brain, will be decreased, resulting in the accumulation of A $\beta$ -plaques in the brain.

In summary, our data give an evidence that TBI impaired the hippocampal-dependent learning and memory, decreased the cell density in hippocampus, diminished the synaptic function in the cortex and in the specific region of hippocampus, aggravated A $\beta$ -plaques deposition in hippocampus in APP/PS1 mice. TBI may deteriorate the pathology of AD by altering the phenotype of microglia, advancing the onset of AD in APP/PS1 mouse model.

## Abbreviations

A $\beta$ , amyloid- $\beta$ ; AD, Alzheimer’s disease; ANOVA, analysis of variance; APOE, apolipoprotein E; APP, amyloid- $\beta$  precursor protein; Arg1, Arginase-1; IBA-1, ionized calcium-binding adapter molecule 1; IL, interleukin; CNS, central nervous system; HRP, conjugated to horseradish peroxidase; iNOS, inducible nitric oxide synthase; PBS, phosphate buffer saline; PS1, Presenilin 1; SP, senile plaques; SEM, standard error of the mean; SYP, Synaptophysin; TBI, traumatic brain injury.

## Declarations

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### Authors’ contributions

SL, BL and YFZ conceived the study; XDL, YXL, XDT and XL collected and administered the serum; JPPK, XDL, YXL and DSM conducted the novel object and novel position recognition tests; DW and MTN performed the HE staining; DW, YXL and MTN performed the immunostaining; DSM, YXL and XL performed the Western blotting; DW, JPPK and XDL analyzed the data; DW wrote the manuscript; LS, BL and YFZ modified the manuscript; All authors read and approved the final manuscript.

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None

## Availabilities of data and material

The datasets during and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Ethics approval and consent to participate

All the animal experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23) revised 1996 and associated guidelines, and all the procedures were approved by the ethical committee of Harbin Medical University.

## Consent for publication

Not applicable

## Competing interests

The authors declare that they have no competing interests.

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## Figures



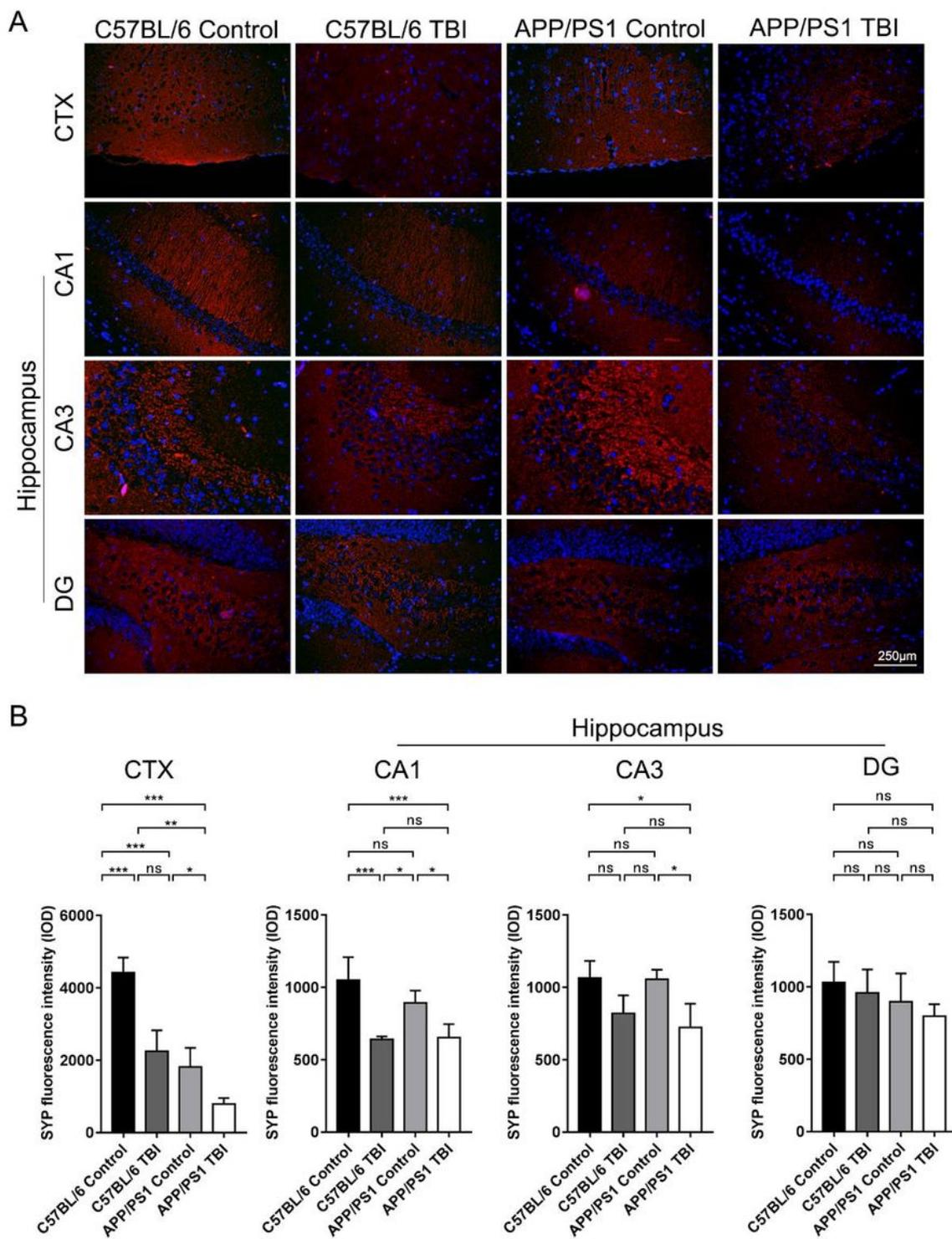
### Figure 1

TBI impaired the cognitive function of APP/PS1 mouse model. Quantitative analyses of discrimination indexes of C57BL/6, C57BL/6 TBI, APP/PS1 and APP/PS1 TBI mice at the acquisition (A) and test phases (B) in the novel-object recognition test (n = 5/group). Quantitative analyses of discrimination indexes of C57BL/6, C57BL/6 TBI, APP/PS1 and APP/PS1 TBI mice at the acquisition (C) and test phases (D) in the novel-position recognition test (n = 5/group). ns, nonsignificant ; \*P  $\leq$  0.05; \*\*P  $\leq$  0.01;



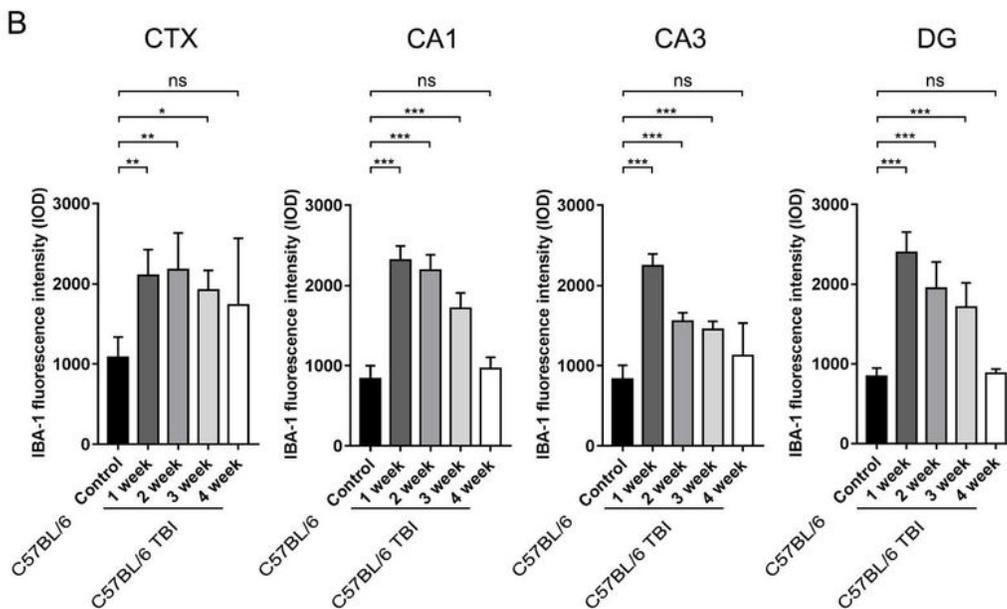
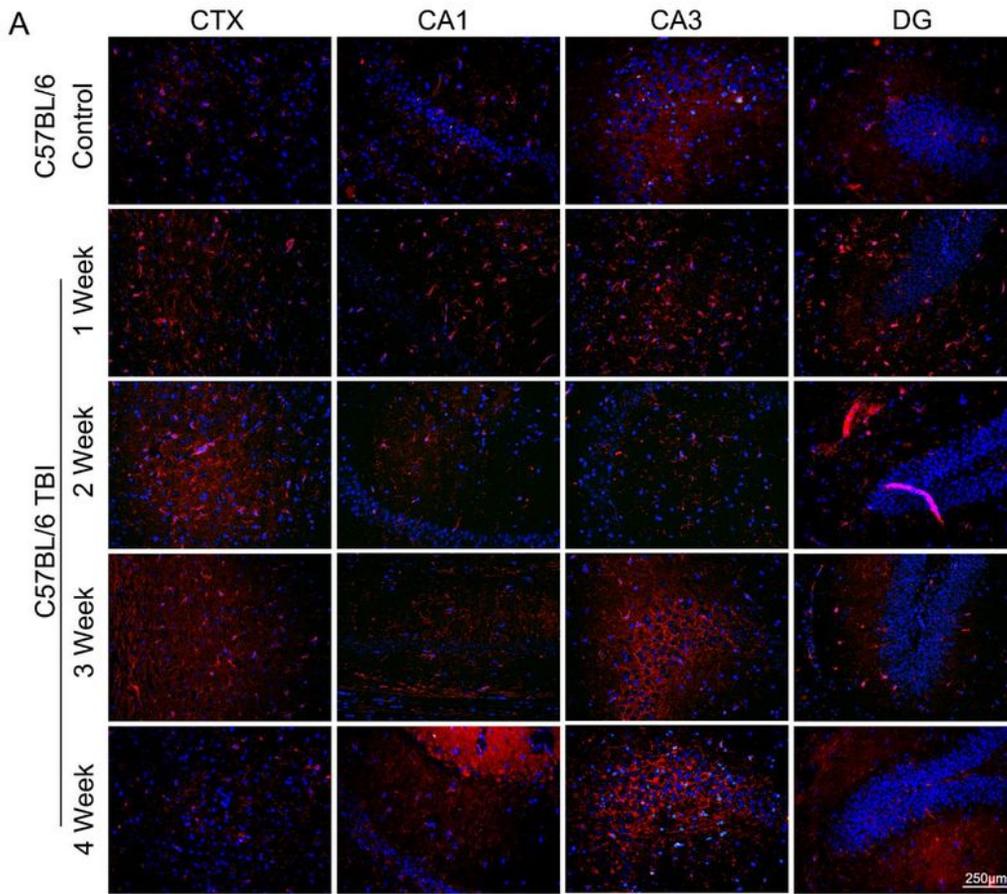
### Figure 2

TBI decreased cell density at TBI lesion and CA3 region of hippocampus.(A) Photographs of HE staining in the cortex and hippocampus(CA1,CA3 and DG) of C57BL/6, C57BL/6 TBI, APP/PS1 and APP/PS1 TBI mice. (B) Cell density in the cortex and in the hippocampus(CA1, CA3 and DG) of C57BL/6, C57BL/6 TBI, APP/PS1 and APP/PS1 TBI mice. Error bars represent the mean  $\pm$  SEM(n=5/group). ns, nonsignificant ; \*\*P  $\leq$  0.01; \*\*\*P  $\leq$  0.001.



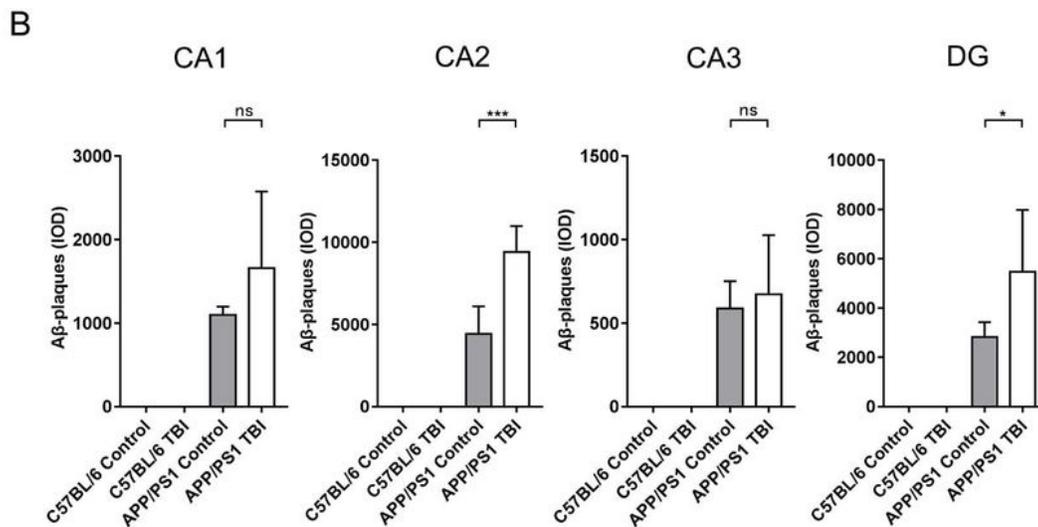
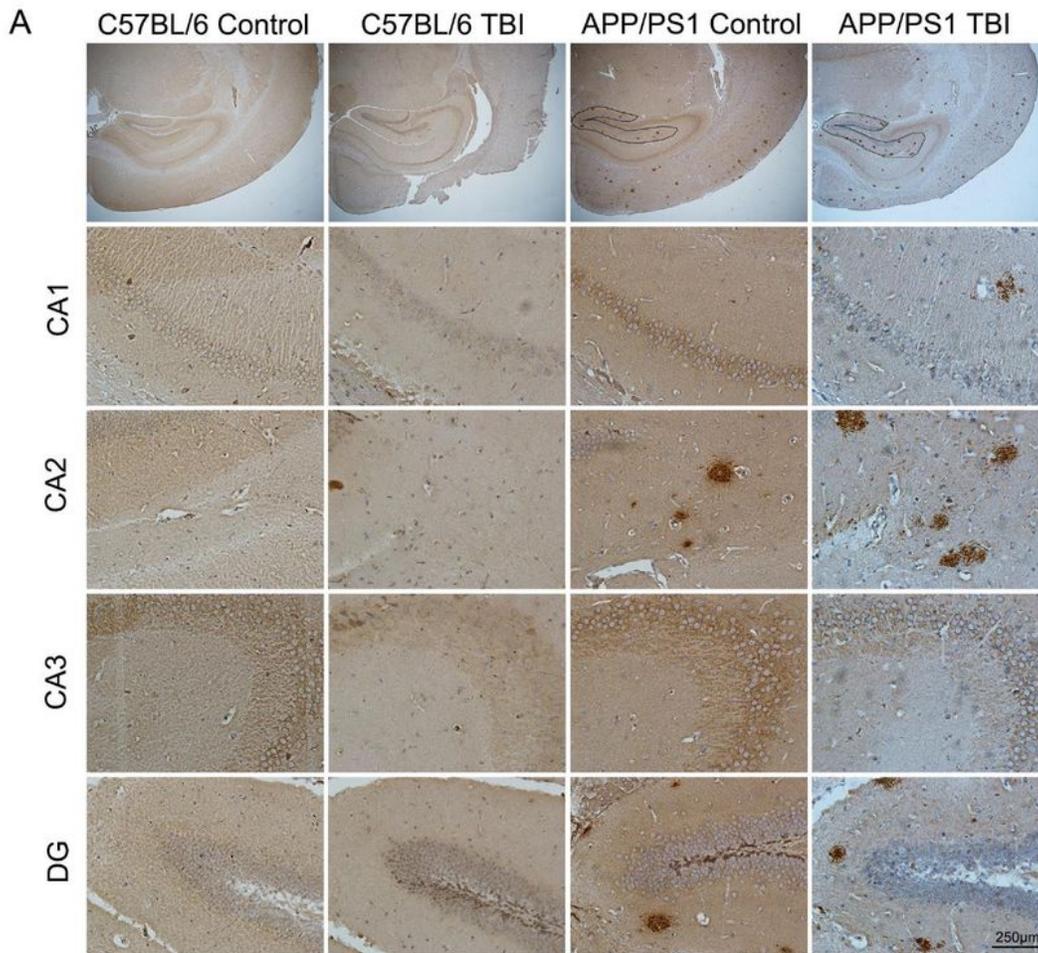
**Figure 3**

TBI diminished synaptic function in the cortex and the specific region of hippocampus.(A) Representative fluorescence images of cortex and hippocampus(CA1, CA3 and DG) immunostained for SYP(in red) and DAPI nuclear staining(in blue) of C57BL/6, C57BL/6 TBI, APP/PS1 and APP/PS1 TBI mice.(B) The fluorescence intensity of SYP in the cortex and in the hippocampus(CA1, CA3 and DG). Error bars represent the mean  $\pm$  SEM(n=5/group). ns, nonsignificant ; \*P  $\leq$  0.05; \*\*P  $\leq$  0.01; \*\*\*P  $\leq$  0.001.



**Figure 4**

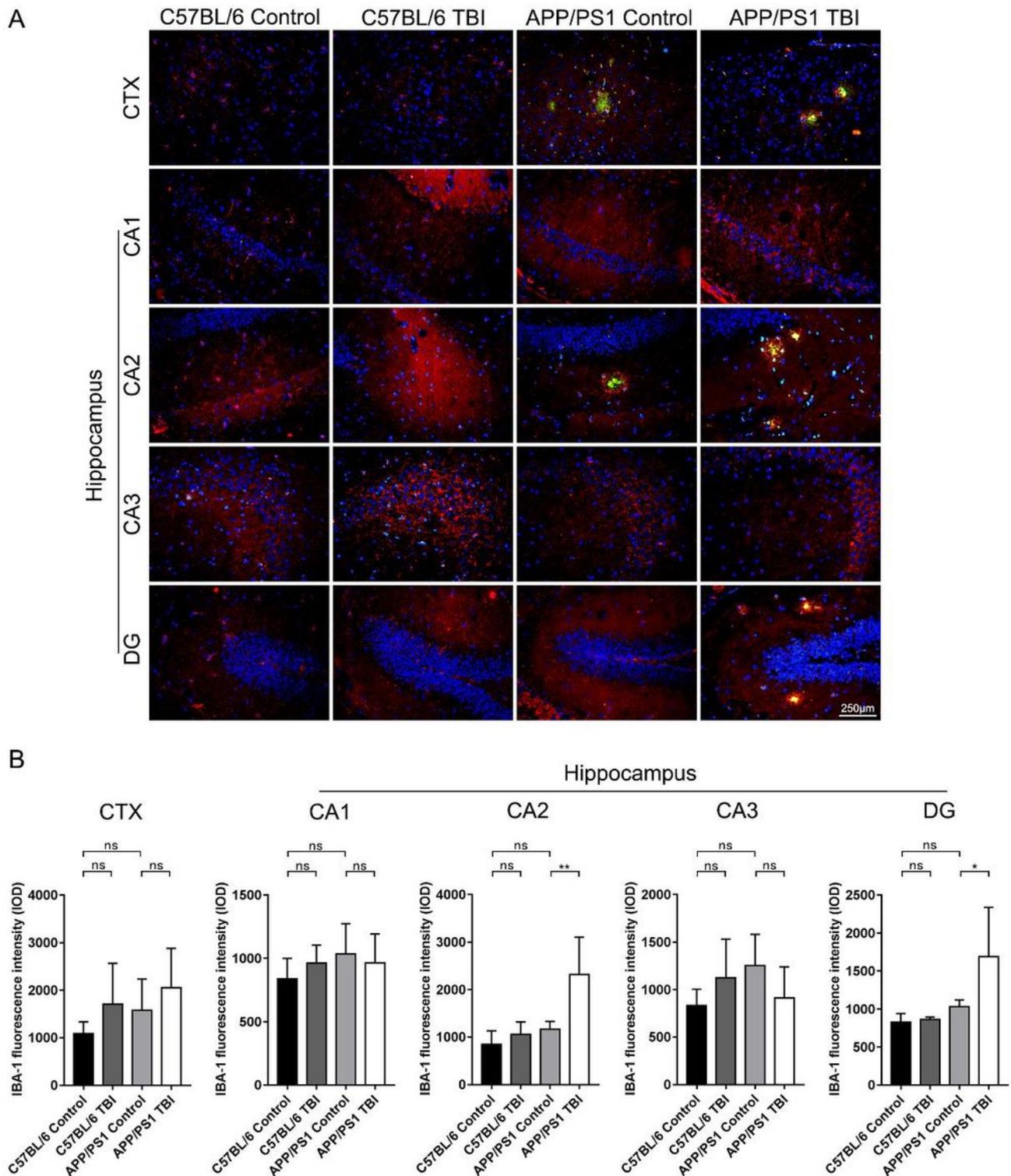
TBI activated microglia in the brain and sustained at least for 3 weeks. (A) Representative fluorescence images of cortex and hippocampus (CA1, CA3 and DG) immunostained for IBA-1 (in red) and DAPI nuclear staining (in blue) of C57BL/6 and C57BL/6 TBI mice. (B) The fluorescence intensity of IBA-1 in the cortex and in the hippocampus (CA1, CA3 and DG). Error bars represent the mean  $\pm$  SEM (n=5/group). ns, nonsignificant; \*P  $\leq$  0.05; \*\*P  $\leq$  0.01; \*\*\*P  $\leq$  0.001.



**Figure 5**

TBI aggravated A $\beta$ -plaques deposition in the specific region of hippocampus in APP/PS1 mouse model. (A) Representative immunohistochemistry images of hippocampus(CA1, CA2, CA3 and DG) immunostained for A $\beta$ -plaques of C57BL/6, C57BL/6 TBI, APP/PS1 and APP/PS1 TBI mice.(B) Quantitative analyses of A $\beta$ -plaques deposition in the CA1, CA2, CA3 and DG region of hippocampus in

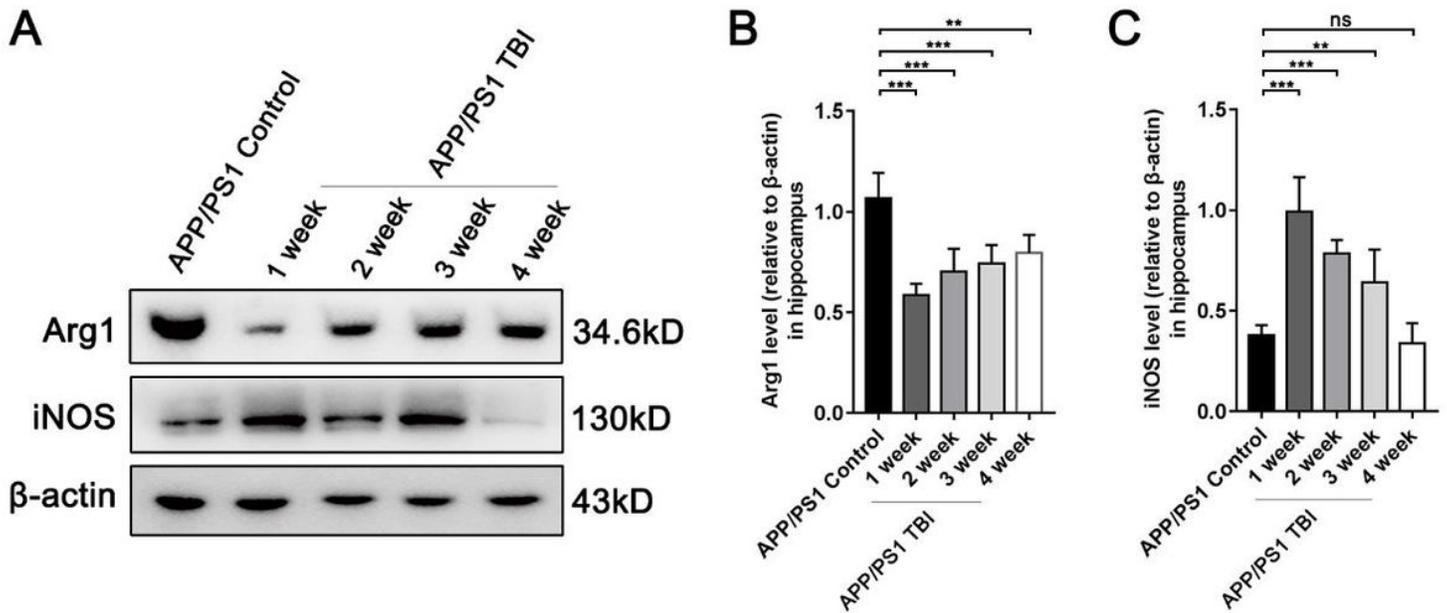
C57BL/6, C57BL/6 TBI, APP/PS1 and APP/PS1 TBI mice. Error bars represent the mean  $\pm$  SEM(n=5/group). ns, nonsignificant ; \*P  $\leq$  0.05; \*\*\*P  $\leq$  0.001.



**Figure 6**

A $\beta$ -plaques aggravated neuroinflammation in the specific region of hippocampus post-TBI. (A) Representative fluorescence images of cortex and hippocampus(CA1, CA2, CA3 and DG) immunostained for IBA-1(in red) and for A $\beta$ -plaques(in green) and DAPI nuclear staining(in blue) of C57BL/6, C57BL/6

TBI, APP/PS1 and APP/PS1 TBI mice.(B) The fluorescence intensity of IBA-1 in the cortex and in the hippocampus(CA1, CA2, CA3 and DG). Error bars represent the mean  $\pm$  SEM(n=5/group). ns, nonsignificant ; \*P  $\leq$  0.05; \*\*P  $\leq$  0.01;



**Figure 7**

TBI Aggravate the Alzheimer's-like Pathology by Altering the Phenotype of Microglia. (A) Representative Western blotting images of Arg1, iNOS and  $\beta$ -actin in the hippocampus of APP/PS1 and APP/PS1 TBI mice in each group. (B-C) Quantitative analyses of the relative Arg1 (B), iNOS(C) levels to  $\beta$ -actin in hippocampus of APP/PS1 and APP/PS1 TBI mice (n = 5/group). Error bars represent the mean  $\pm$  SEM. ns, nonsignificant; \*\*P < 0.01; \*\*\*P < 0.001.