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Spatial Training Ameliorates Long-Term Alzheimer's Disease-Like Pathological Deficits by Reducing NLRP3 Inflammasomes in PR5 Mice

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Abstract

Recent studies have suggested that cognitive training could delay memory loss in Alzheimer's disease (AD). However, whether and how cognitive training produces long-term benefits remains unclear. Here, 10-month-old PR5 mice were spatially trained in a water maze for 4 consecutive weeks. The novel object recognition test (NORT), Western blots, Golgi staining, and ELISA were used to examine behavioral, biochemical, and pathological measures immediately after training and 3 months later. Immediately after training, we found that spatial training significantly improved cognitive performance; reduced tau neuropathology; increased the expression level of synaptophysin, PSD93, and PSD95 in the hippocampus; and increased the number of dendritic spines in PR5 mice. The expression levels of NLRP3, caspase-1, and interleukin (IL)-1β, which were significantly elevated in PR5 mice, were reversed by spatial training. Interestingly, these effects persisted 3 months later. To further detect the role of NLRP3 in spatial training, PR5/NLRP3−/− mice and PR5/NLRP3+/− mice were also used in our study. PR5/NLRP3−/− mice showed better cognitive performance than PR5 mice. After 1 week of spatial training, these changes (including those in expression levels of synaptophysin, PSD93, and PSD95; the number of dendritic spines; and caspase-1 and IL-1β content in PR5 mice) could be totally reversed in PR5/NLRP3−/− and PR5/NLRP3+/− mice. In addition, there was a positive correlation between NLRP3 content and the expression levels of caspase-1 and IL-1β. These results show an important role for the NLRP3/caspase-1/IL-1β axis in ameliorating the effect of spatial training on cognitive impairment in PR5 mice.

Key Words Spatial training · Alzheimer's disease · synaptic plasticity · NLRP3 inflammasome

Significance Statement

Recent studies have suggested that cognitive training could delay memory loss in Alzheimer's disease (AD). However, whether and how cognitive training produces long-term benefits remains unclear. Here, we found that spatial training significantly improves cognitive performance; reduced tau neuropathology; increased expression levels of synaptophysin, PSD93, and PSD95 in the hippocampus; and increased the number of dendritic spines in PR5 mice. The expression levels of NLRP3, caspase-1, and interleukin (IL)-1β, which were significantly elevated in PR5 mice, were reversed by spatial training. Interestingly, these effects still remained 3 months later. Furthermore, we found that PR5/NLRP3−/− mice showed better cognitive performances than PR5 mice. After 1 week of spatial training, these changes (including those in the synaptophysin, PSD93, and PSD95 expression levels; the number of dendritic spines; and the caspase-1 and IL-1β content in PR5 mice) could be totally reversed in PR5/NLRP3−/− and PR5/NLRP3+/− mice. These results show an important role for the NLRP3/caspase-1/IL-1β axis in ameliorating the effect of spatial training on cognitive impairment in PR5 mice. This study shed new light on the neuroprotective mechanisms of spatial training in AD. We think that it will be of considerable importance and interest to the neurobiological communities.

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Introduction

Alzheimer's disease (AD) is the most common type of dementia in the elderly population [1]. AD is characterized by memory loss, as well as by impaired locomotor ability, reasoning, and judgment [25]. The mechanisms underlying neurodegeneration have not been fully elucidated, and there is currently no proven, effective disease-modifying therapy for this devastating disorder.

Low levels of education increase the risk of AD. Cognitively stimulating therapy improves memory function with the same efficacy as galantamine or tacrine [40]. Studies have also shown that participation in cognitively stimulating activities are associated with reduced risks of dementia [45] and amnestic mild cognitive impairment (aMCI) onsets [42]. These studies strongly suggested that cognitive stimulation could ameliorate the memory deficits in AD and aMCI. One preclinical study also reported that spatial training with the Morris water maze (MWM) could reduce amyloid load and tau hyperphosphorylation and improve memory performance in 3xTg-AD mice [4].

Innate immunity and inflammatory responses play important roles in the pathogenesis of AD [26]. As a member of the Nod-like receptor (NLR) family, Nod-like receptor pyrin–containing 3 (NLRP3) interacts with caspase-1 to form multiprotein complexes known as inflammasomes. The inflammasomes could promote the maturation of proinflammatory cytokines, such as interleukin (IL)-1β, to initiate innate immune inflammatory responses [39]. The secretion of IL-1β could be prevented by environmental enrichment [2]. Numerous studies have provided evidence that decreased levels of IL-1β could improve synaptic structure and function in AD mice [18, 34]. The synaptic deficits occur very early in AD and correlate with the memory capacities associated with dementia in AD patients [30]. The loss of synapses and spines in various brain areas strongly correlates with clinical scores of dementia in AD [7, 38]. A recent study suggested that spatial training could ameliorate synaptic deficits in an AD model [20]. However, the neurobiological mechanisms underlying spatial training-induced cognitive improvement and the question of whether spatial training has long-term benefits are both largely unknown.

In the present study, we trained PR5 (overexpression of the longest human tau isoform together with the P301L mutation) mice, a widely used AD-like model that shows memory deficits at 6 months of age. The mice received 4 consecutive weeks of training in the MWM at 10 months of age. Then, pathophysiological changes were assessed by performing behavioral, biochemical, and pathological analyses immediately after training and 3 months later. We found that spatial training significantly ameliorated cognitive dysfunction and had long-term benefits on the cognitive performances. This cognitive benefit was associated with NLRP3/caspase-1/IL-1β axis activation and with remodeling of dendritic plasticity.

Materials and Methods

Animals

A total of 240 mice were included at the beginning of the study. Male and female mice were individually housed and kept on a 12-h light–dark schedule. All mice were given *ad libitum* access to food and water.

PR5 mice, which overexpress the longest human tau isoform together with the P301L mutation on the C57BL/6 background, were gifts from Dr. Yan-Jiang Wang (Department of Neurology, Daping Hospital, Third Military Medical University, Chongqing, China). NLRP3 KO mice on the C57BL/6 background were purchased from Jackson Laboratory. The PR5 mice and NLRP3 KO mice were bred in the Experimental Animal Central of School of Medicine, Southeast University. After weaning, the mice were housed (4 to 6 mice per cage) with free access to food and water under a 12:12-h reversed light–dark cycle, with lights on at 8:00 p.m. The PR5+NLRP3+/− and PR5+NLRP3−/− mice were PR5 mice hybridized with NLRP3 KO mice. The numbers of mouse and the sex details in this study are illustrated in Supplementary Data Tables 1–3.

Spatial Training Paradigm

The standard MWM procedure was used, with minor modifications, for spatial training [43]. Mice were trained to swim to a clear circular Plexiglas platform with a diameter of 10 cm that was submerged 1.5 cm beneath the surface of the water. The platform location was selected randomly for each mouse but was kept constant for each individual mouse throughout the training at each age. On each trial, the mouse was placed into the tank at 1 of 4 designated start locations and allowed to find and escape onto the platform. If the mouse failed to find the platform within 60 s, it was manually guided to the platform and allowed to remain there for 5 s. After this, each mouse was placed into a holding cage under a warming lamp for 25 s until the next trial. To avoid any memory difference that was attributed to insufficient task learning, the mice were subjected to 4 trials per day. Retention of spatial learning was assessed 24 h after the last training trial every week. The training paradigm was employed every day for 4 weeks, and the platform location was changed each week. The PR5+NLRP3+/− and PR5+NLRP3−/− mice were only trained for 1 week.

Behavioral Tasks

Novel Object Recognition Test

The novel object recognition test (NORT) was performed according to a previously established protocol with minor

changes. Briefly, the test procedure consisted of 3 parts: habituation, training, and testing. Each mouse was individually habituated to an open-field arena for 10 min. One day later, the mice underwent training in which 2 identical objects (object A) were placed into the 2 opposing corners of the center area, 30 cm apart from each other. Mice were then allowed to explore both the area and the objects for 5 min. The total time spent exploring the identical objects was recorded to identify any preferences for a place or an object. Exactly 1 day later, the mice underwent additional training in which they were placed back into the same arena. One familiar (object A) and 1 novel (object B) object replacing the second object were placed into the 2 opposing corners of the center area. Mice were then allowed to explore freely for 5 min, and the time spent exploring each object was recorded. Exploration of an object was identified when the animal's head faced the object at a minimum distance of 1 cm. The recording was stopped as soon as the mice turned their heads away from the object. The time spent exploring the objects during the trials was calculated and is presented as the discrimination ratio (novel object interaction/total object interaction). The arena and all objects were thoroughly cleaned with 70% ethanol solution after each trial.

Tissue Preparation

Upon completion of the final behavioral tests, the animals were decapitated under deep anesthesia, and each brain was quickly removed and stored at −80 °C until further analysis.

Western Blot

The tissues were thawed and homogenized at a ratio of 9.0 ml of buffer/1.0 g tissue in ice-cold buffer containing 50 mM Tris-HCl (pH 7.0), 1.00 mM EDTA, 0.1 mM phenylmethyl sulfonyl fluoride, 1 mM benzamidine, 0.5 m isobutylmethylxanthine, and 2.0 μg/ml each of aprotinin, leupeptin, and pepstatin A. Then, the homogenized tissue was centrifuged at 12,500×g for 15 min at 4 °C, and the supernatant was collected. Protein concentrations in the supernatants were determined using the Pierce bicinchoninic acid (BCA) protein assay kit (Thermo, USA). Equal amounts of protein were isolated on 10% SDS polyacrylamide gel (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes (Immobilon Transfer Membrane, Millipore, USA). The membranes were blocked in 5% (w/v) nonfat milk in TBS-T (10 mM Tris-HCl, 150 mM NaCl, 0.02% (v/v) Tween 20, pH 7.5) and probed overnight at 4 °C with synaptophysin (1:1000; Abcam), PSD93 (1:2000; Abcam), PSD95 (1:1000; Abcam), pT231 (1:1000; Abcam), Tau-1 (1:1000; Millipore), Tau-5 (1:1000; Abcam), NLRP3 (1:2000; AdipoGen), caspase-1 (1:1000; Abcam), IL-1β (1:1000; Abcam), or GAPDH (1:4000; Sigma). The blots were developed with horseradish

peroxidase–conjugated secondary antibodies (1:5000; Pierce), visualized by an enhanced chemiluminescent substrate kit, and exposed to CL-XPosure film. The immunoreactivity of the protein bands was quantitatively analyzed by a LAS400 mini system (GE Healthcare, USA). The levels of GAPDH protein were expressed as the relative level of the mean optical density against the control.

Golgi Staining and Dendritic Spine Analysis

Golgi staining was performed in the mice using the FD Rapid GolgiStain kit (FD NeuroTechnologies), as described previously [12]. Briefly, freshly dissected brains were immersed in solutions A and B for 2 weeks at room temperature and were then transferred to solution C for 24 h at 4 °C. Neurons from the prefrontal cortex, dorsal hippocampus, and ventral hippocampus were analyzed. Five neurons were randomly selected from each region in each mouse. At least 2 segments were randomly chosen per neuron from both the apical oblique (AO) and basal shaft (BS) dendrites. The dendritic spine density was measured in a blinded manner.

ELISA

One hundred milligrams of the hippocampus tissue was rinsed with 1× PBS, homogenized in 1 ml of 1× PBS, and stored overnight at −20 °C. After 2 freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 min at 5000×g at 2–8 °C. The supernatant was removed and assayed immediately (Elabscience Life Science, Inc., China). This assay employs the competitive inhibition enzyme immunoassay technique.

Statistical Analysis

Data were analyzed using the SPSS software, version 18.0 (SPSS, Inc., Chicago). Student's t test was used to analyze the MWM test memory results, repeated measures, and multivariate analysis of variance (ANOVA) process of the general linear model and to give comparison among the different groups and different measure times pairwise in MWM spatial training, and multivariate ANOVA was used to analyze the other results. Then, to identify the origin of the difference among the groups, post hoc tests with the Bonferroni correction were conducted if the above ANOVAs observed any difference among the groups. Values are presented as the mean ± standard error of the mean (SEM). The results were considered statistically significant if the p value is < 0.05. Each experiment consisted of at least 3 replicates per condition.

Results

Effects of Spatial Training on Subsequent Performance During the Cognitive Task

We first tested the cognition of the mice using the NORT. There were no differences between the male and female mice in either the wild-type (Wt) or PR5 mice. However, the PR5 mice displayed significantly cognitive deficits compared with the Wt mice (Fig. 1a, b). After 72 h, no differences in the NORT were found between the Wt and PR5 mice (Fig. 1c). Then, the mice were trained for 4 consecutive weeks. During the training period, compared with the Wt mice, the PR5 mice exhibited significantly prolonged times in finding the hidden platform on days 4 and 5 of the first week (Fig. 1e), days 1 and 3 of the second week (Fig. 1f), and day 2 of the third week (Fig. 1g). There was no difference in the fourth week (Fig. 1h). In addition, the percentage of the time spent in a quadrant and the number of target crossings of the PR5 mice were reduced after 1 week of training compared with those of the Wt mice (Fig. 1i, j). However, during the fourth week, no differences in the performance of the MWM were found between the PR5 and Wt mice (Fig. 1o, p). The results suggested that the learning and memory performances of the PR5 mice in the MWM were gradually increased by spatial training. The discrimination ratio of the PR5 mice was significantly decreased compared with that of the Wt mice (Fig. 1q). However, after spatial training, no significant differences were found in the NORT performances between the PR5+MWM and Wt mice (Fig. 1q). Three months later, we found that the discrimination ratio of the PR5 mice that underwent spatial training was still increased compared with that of the PR5 control mice (Fig. 1r), suggesting long-term benefits of the spatial training.

Altered Expression of Tau Proteins Immediately After Training and 3 Months Later

We measured the level of tau phosphorylation in the PR5 mice. The expression levels of pS396 and pT231 were enhanced, and that of Tau-1 was decreased in the PR5 mice compared with the levels in the Wt mice (Fig. 2a–d). Spatial training significantly ameliorated tau pathology in the PR5 mice (Fig. 2a–d). The results showed that the total tau levels (Tau-5) did not change among the 4 groups (Fig. 2a, e). Importantly, 3 months later, the ameliorated effects of spatial training on the tau pathology remained as it showed immediately after training (Fig. 2f–j). These data suggested that spatial training could decrease tau pathology in PR5 mice with a long-term effect.

Fig. 1 Effects of spatial training on subsequent performance during the cognitive task. (**a–c**) The discrimination ratio of NORT in 24 h, 48 h, and 72 h. (**d**) Global timeline of the experiment in weeks. (**e–h**) The mean escape latency in the hidden platform test in the 4 weeks of training. (**i–p**) The percentage of target and target crossing in the 4 weeks of training. (**q**, **r**) The discrimination ratio of NORT immediately after training and 3 months later. The values are represented as mean ± SEM ($n = 15$). $^{\wedge}p < 0.05$, female Wt *versus* female PR5; $^{\wedge\wedge}p < 0.01$, female Wt *versus* female PR5; $^{\$}p < 0.05$, male Wt *versus* male PR5; $^{\$\$}p < 0.01$, male Wt *versus* male PR5; $^{*}p < 0.05$, PR5 *versus* Wt; $^{\#}p < 0.05$, PR5 *versus* Wt+ MWM; $^{\&}p < 0.05$, PR5 *versus* PR5+MWM

Altered Expression of Synapse-Associated Proteins and Synapse Morphology Immediately After Training and 3 Months Later

We examined synapse-associated proteins (synaptophysin, PSD93, and PSD95) in the hippocampus. The results revealed significant decreases in synaptophysin, PSD95, and PSD93 in the PR5 mice. After spatial training, the levels of synaptophysin, PSD95, and PSD93 increased significantly in the PR5 mice (Fig. 3a–d).

We also examined the dendritic spine structures of neurons in the hippocampus using Golgi staining. The dendritic spines of each neuron were counted for the AO and BS dendrites. The PR5 group exhibited significantly lower AO and BS spine densities were relative to the Wt group in the hippocampus. After spatial training, the number of dendritic spines was significantly restored (Fig. 4a–d).

Three months later, we also found that BS spine densities and synaptophysin expression were still increased in the PR5+ MWM group (Figs. 3e–h and 4e–h). These data suggested that spatial training could ameliorate synaptic plasticity deficits in AD model mice with long-term effects.

Alteration of the NLRP3/Caspase-1/IL-1β Axis Immediately After Training and 3 Months Later

We found that the expression levels of NLRP3, caspase-1, and IL-1β were significantly increased in the PR5 group compared with those in the Wt group, whereas spatial training significantly reversed these changes (Fig. 5a–d).

Three months later, we also found that the expression levels of NLRP3, caspase-1, and IL-1β were significantly increased in the PR5 group compared with those in the Wt group, whereas spatial training significantly reversed these changes (Fig. 5e–h).

Cognitive Behavior of the PR5+NLRP3+/− and PR5+NLRP3−/− Mice

To confirm whether the NLRP3/caspase-1/IL-1β axis was involved in the regulation of cognitive improvement, we assessed the cognitive behavior of 10-month-old PR5+
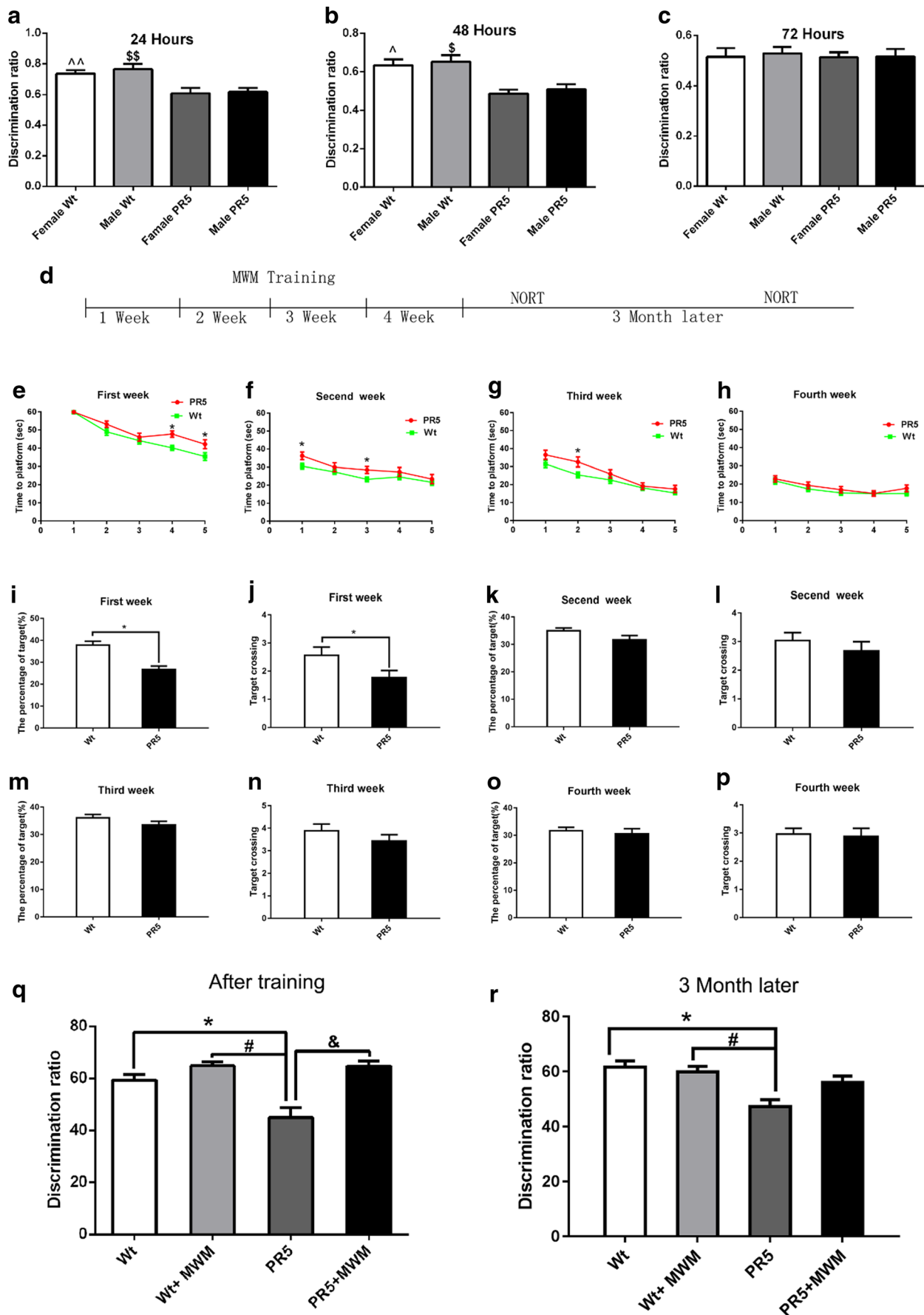
Fig. 2 Altered expression of tau proteins immediately after training and 3 months later. (**a–e**) The phosphorylation-dependent antibodies pT231, pS396, Tau-1, and Tau-5 as indicated measure the alteration of tau in different phosphorylation status immediately after training. (**f–j**) The phosphorylation-dependent antibodies pT231, pS396, Tau-1, and Tau-5 as indicated measure the alteration of tau in different phosphorylation status 3 months later. Blot images were cropped for comparison. GAPDH was used as a loading control in each sample. The values are represented as mean ± SEM ($n = 3$). *$p < 0.05$, PR5 versus Wt; **$p < 0.01$, PR5 versus Wt; ***$p < 0.001$, PR5 versus Wt; #$p < 0.05$, PR5 versus PR5+MWM; ##$p < 0.01$, PR5 versus PR5+MWM
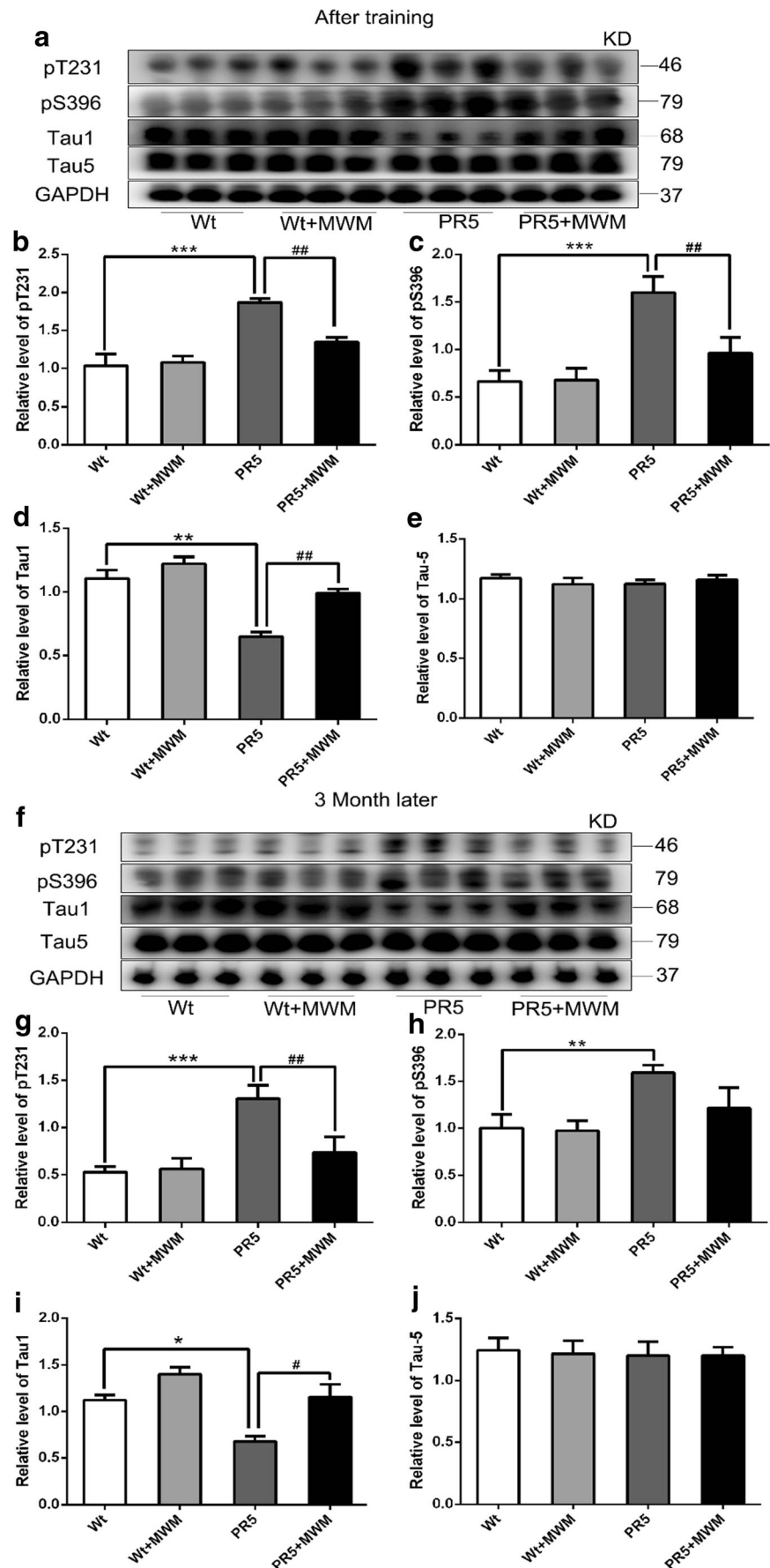
Fig. 3 Altered expression of synapse-associated proteins and synapse morphology immediately after training and 3 months later. (**a–d**) Expression of presynaptic and postsynaptic proteins synaptophysin, PSD93, and PSD95 in the hippocampus immediately after training. (**e–h**) Expression of presynaptic and postsynaptic proteins synaptophysin, PSD93, and PSD95 in the hippocampus 3 months later. Blot images were cropped for comparison. GAPDH was used as a loading control in each sample. The values are represented as mean ± SEM ($n = 3$). **$p < 0.01$, PR5 *versus* Wt; #$p < 0.05$, PR5 *versus* PR5+MWM; ##$p < 0.01$, PR5 *versus* PR5+MWM
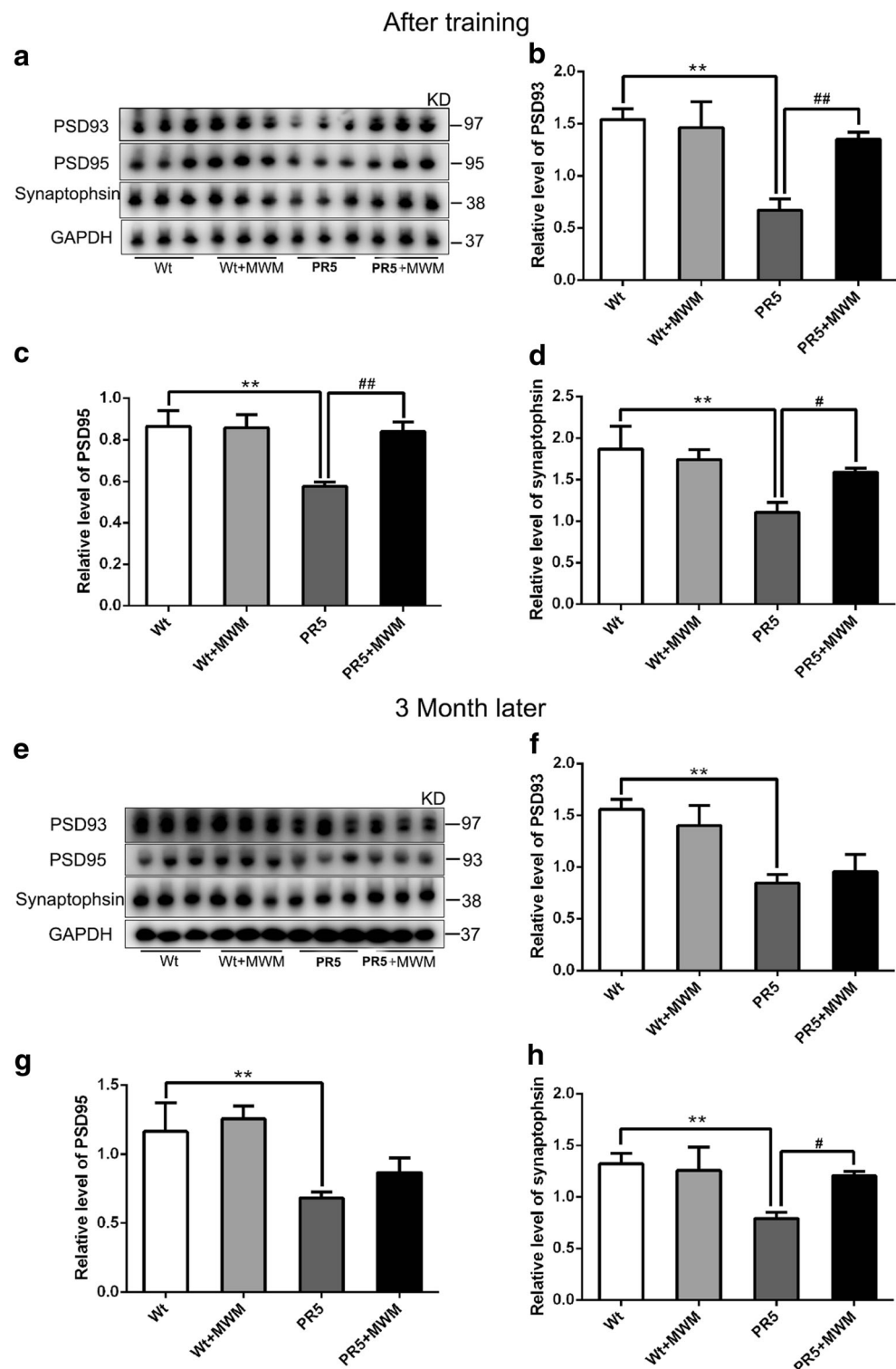
NLRP3+/− and PR5+NLRP3−/− mice using the MWM. The PR5 mice exhibited significantly prolonged times to find the hidden platform on days 4 and 5 of week 1 compared with the Wt and PR5+NLRP3−/− mice (Fig. 6a). Also, the percentage of time spent in a quadrant and the number of target crossings of the PR5 mice were decreased compared with those of the Wt, PR5+NLRP3+/−, and PR5+NLRP3−/− mice (Fig. 6b, c). In the NORT, the discrimination ratio of the PR5 group was significantly decreased compared with that of the other groups (Fig. 6d). No differences in the performance of the MWM or of the NORT were found among the Wt, PR5+NLRP3+/−, and PR5+NLRP3−/− groups (Fig. 6a–d).

Fig. 4 Altered synapse morphology immediately after training and 3 months later. The dendritic spine structure of neurons in the hippocampus was detected using Golgi staining. Spine density was quantified along dendrites. (**a–d**) The spine density in AO and BS dendrites immediately after training. (**e–h**) The spine density in AO and BS dendrites 3 months later. The values are represented as mean ± SEM ($n = 3$). Scale bars, 1 μm. **$p < 0.01$, PR5 *versus* Wt; *$p < 0.05$, PR5 *versus* Wt; #$p < 0.05$, PR5 *versus* PR5+MWM
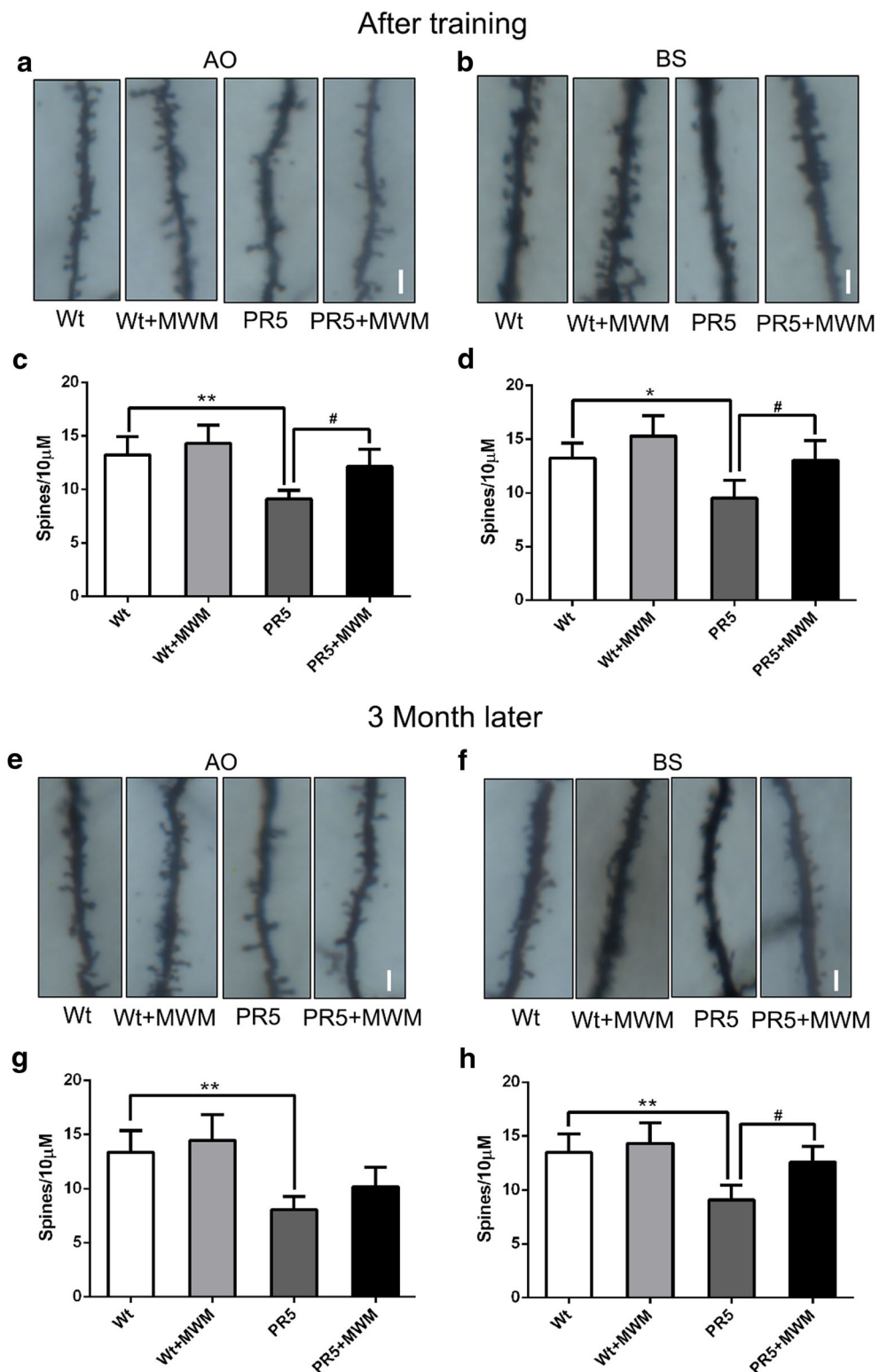

Altered Expression of Tau Proteins After Training in the PR5+NLRP3+/− and PR5+NLRP3−/− Mice

We measured the levels of tau phosphorylation after training in PR5+NLRP3+/− and PR5+NLRP3−/− mice.

The expression levels of pS396 and pT231 were decreased, and that of Tau-1 was increased in the PR5+NLRP3+/− and PR5+NLRP3−/− mice compared with the levels in the PR5 mice (Fig. 7a–d). We found that spatial training could significantly ameliorate tau

Fig. 5 Alteration of the NLRP3/
caspase-1/IL-1β axis
immediately after training and
3 months later. (**a–d**) Expression
of NLRP3, caspase-1, and IL-1β
in the hippocampus immediately
after training. (**e–h**) Expression of
NLRP3, caspase-1, and IL-1β in
the hippocampus 3 months later.
Blot images were cropped for
comparison. GAPDH was used as
a loading control in each sample.
The values were represented as
mean ± SEM ($n = 3$). ** $p < 0.01$,
PR5 versus Wt; *** $p < 0.001$,
PR5 versus Wt; # $p < 0.05$, PR5
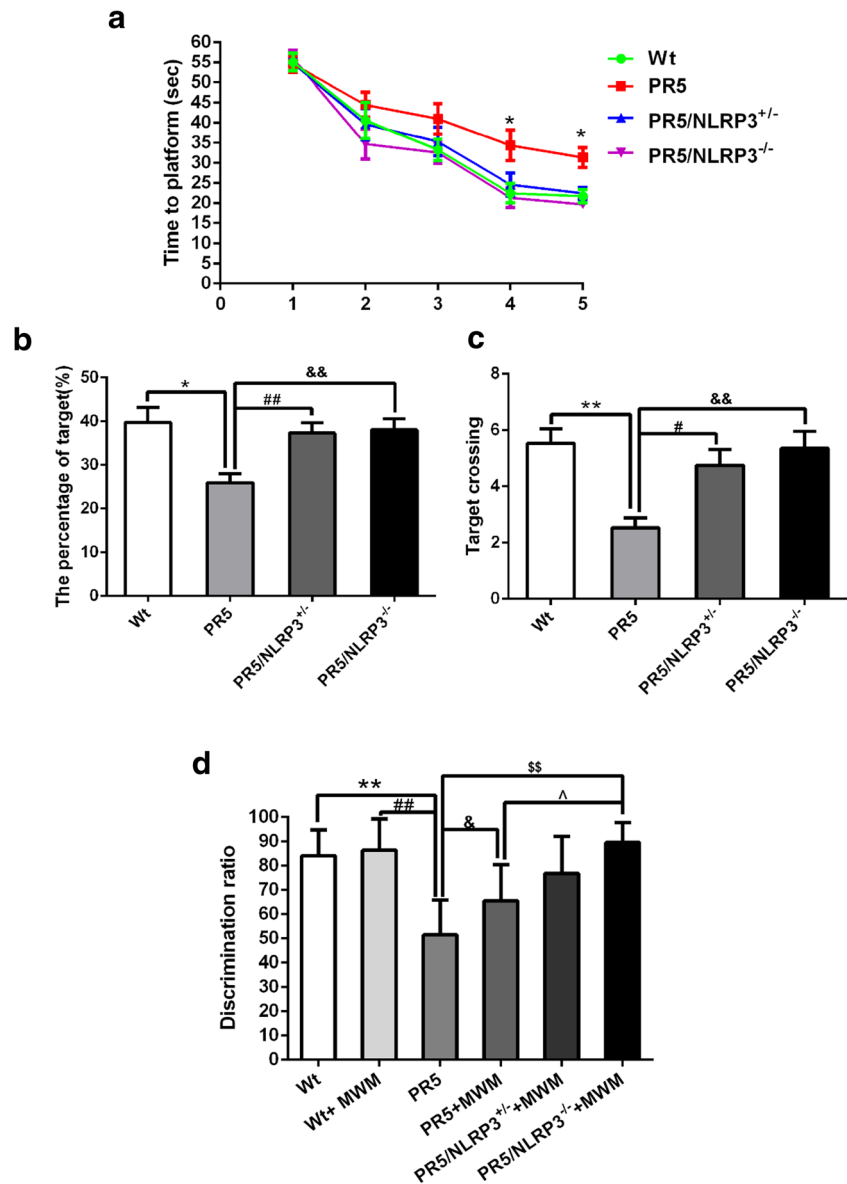versus PR5+MWM; ## $p < 0.01$,
PR5 versus PR5+MWM
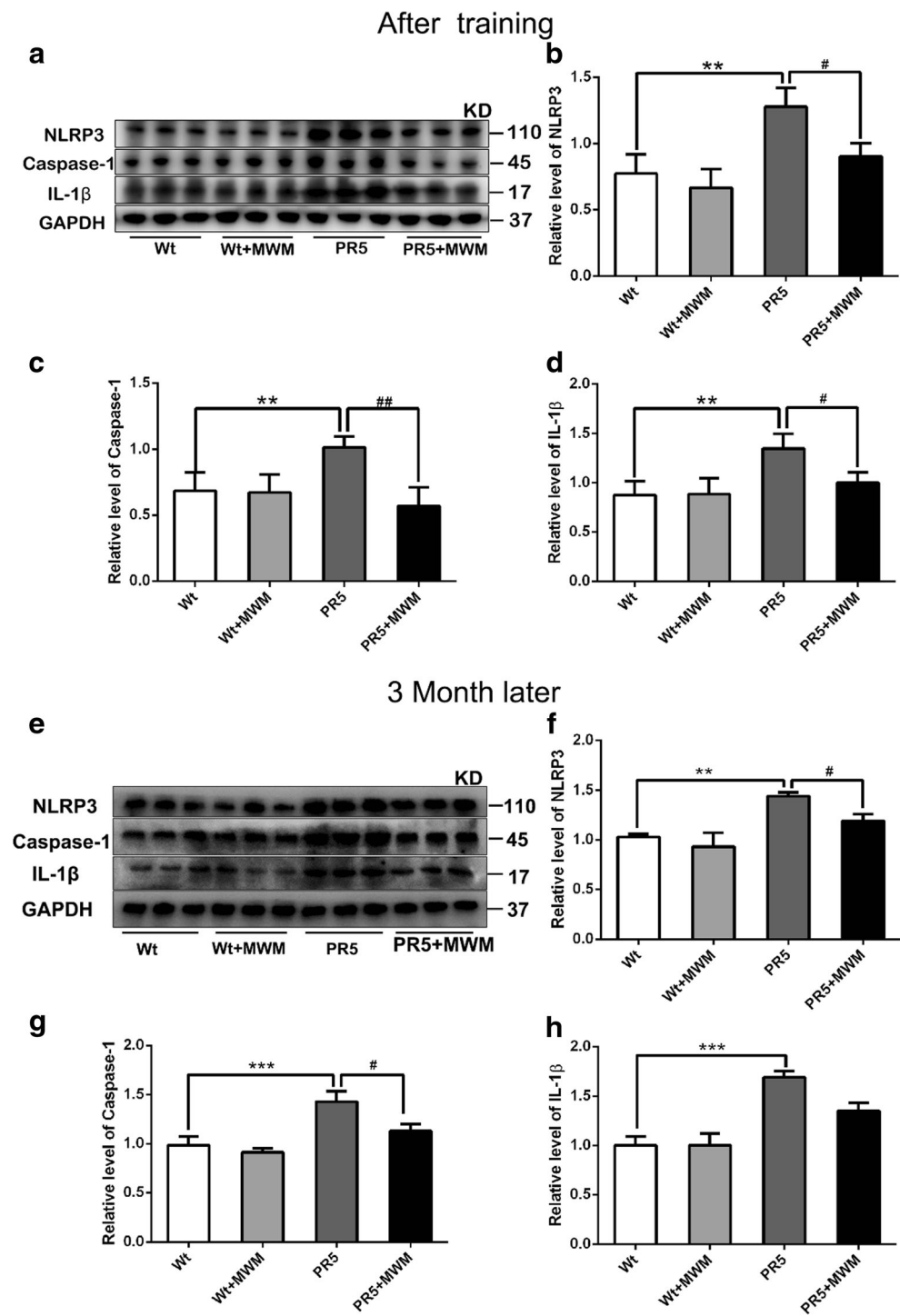
pathology in the PR5 mice (Fig. 7a–d). However, no differences were found in terms of tau phosphorylation between the PR5+NLRP3+/− and PR5+NLRP3+/−+WMW mice or between the PR5+NLRP3−/− and PR5+NLRP3−/−+WMW mice (Fig. 7a–d). The results showed that the total tau levels (Tau-5) did not change in these groups (Fig. 7a, e).

Effects of Spatial Training on the Expression of Synapse-Associated Proteins and Synaptic Morphology in PR5+NLRP3+/− and PR5+NLRP3−/− Mice

We examined synapse-associated proteins (synaptophysin, PSD93, and PSD95) in the hippocampus. The results revealed

Fig. 6 Cognitive behavior of the PR5+NLRP3+/− and PR5+ NLRP3−/− mice. (**a**) The mean escape latency in the hidden platform test among the trainings. (**b**, **c**) The percentage of target and target crossing after training. (**d**) The discrimination ratio of NORT among those groups. The values were represented as mean ± SEM ($n = 11$). *$p < 0.05$, PR5 *versus* Wt; **$p < 0.01$, PR5 *versus* Wt; ##$p < 0.01$, PR5 *versus* PR5/ NLRP3+/−; &&$p < 0.01$, PR5 *versus* PR5/NLRP3−/−; ##$p < 0.01$, PR5 *versus* Wt+ MWM; &$p < 0.05$, PR5 *versus* PR5+MWM; ^$p < 0.05$, PR5+ MWM *versus* PR5/NLRP3 −/−+MWM; $$$p < 0.01$, PR5 *versus* PR5/NLRP3−/−+MWM

that the levels of synaptophysin, PSD95, and PSD93 were decreased in the PR5 group (Fig. 8a–d). Spatial training increased synaptophysin, PSD95, and PSD93 expression in the PR5+MWM group (Fig. 8a–d). In addition, the PR5+NLRP3 −/−+MWM group exhibited significantly increased expression of synapse-associated proteins compared with the PR5+ MWM group (Fig. 8a–d). These data suggested that NLRP3 KO in PR5 mice significantly enhanced the effect of spatial training on the expression of synapse-associated proteins.
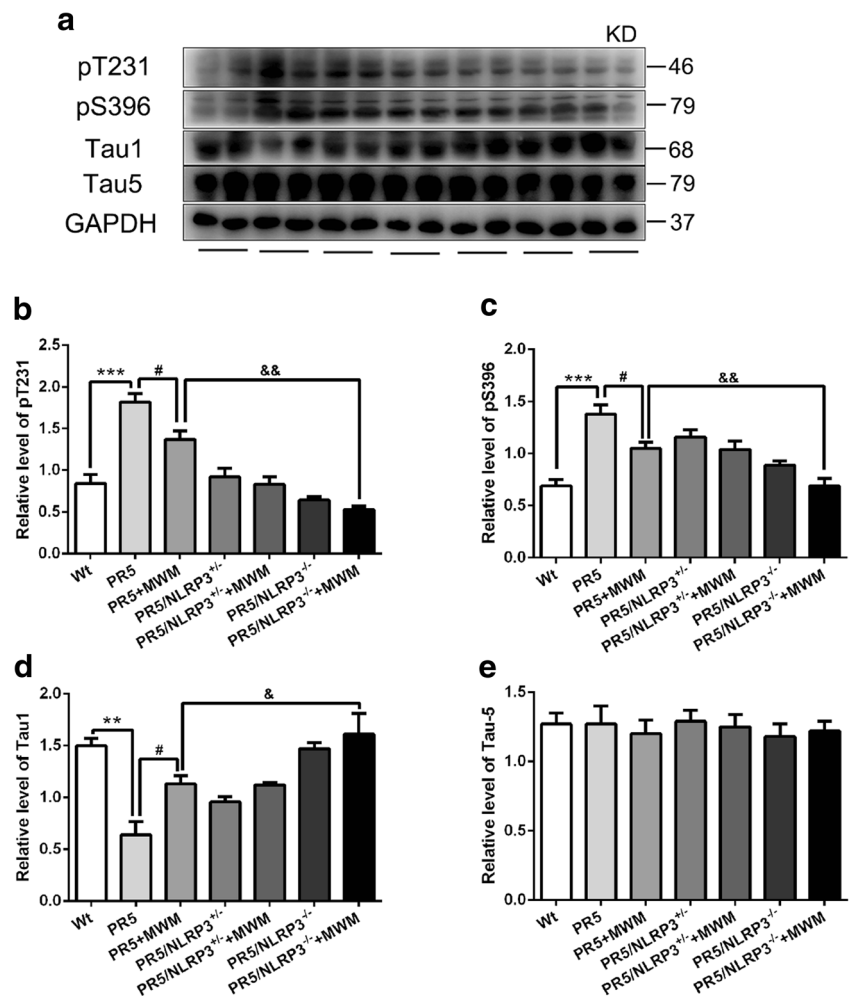
We also examined the dendritic spine structure of neurons in the hippocampus after spatial learning in the 6 groups. We found that the AO and BS spine densities were significantly lower in the PR5 group than those in the Wt group (Fig. 8e–h). The AO and BS spine densities were significantly increased in the PR5+MWM, PR5+NLRP3+/−+MWM, and PR5+NLRP3 −/−+MWM groups compared with those in the PR5 group

(Fig. 8e–h). In addition, the AO and BS spine densities in the PR5+NLRP3−/− group were significantly increased compared with those in the PR5+MWM group (Fig. 8e–h).

The NLRP3/Caspase-1/IL-1β Axis Changes in PR5+NLRP3−/− Mice After Spatial Training

To further explore the involvement of the NLRP3/caspase-1/IL-1β axis in the training-induced upregulation of synaptic plasticity, we measured changes in the NLRP3/caspase-1/IL-1β axis. The results revealed that the expression levels of NLRP3, caspase-1, and IL-1β significantly increased in the PR5 group compared with those in the Wt group (Fig. 9a–d). These levels significantly decreased in the PR5+MWM, PR5+NLRP3+/ −+MWM, and PR5+NLRP3−/−+MWM groups compared with those in the PR5 group (Fig. 9a–d). Also, the expression levels

Fig. 7 Altered expression of Tau proteins after spatial training in the PR5+NLRP3+/− and PR5+NLRP3−/− mice. (**a**–**e**) The phosphorylation-dependent antibodies pT231, pS396, Tau-1, and Tau-5 as indicated measured the alteration of tau in different phosphorylation status after training in the PR5+NLRP3+/− and PR5+NLRP3−/− mice. Blot images were cropped for comparison. GAPDH was used as a loading control in each sample. The values were represented as mean ± SEM ($n = 3$). **$p < 0.01$, PR5 versus Wt; ***$p < 0.001$, PR5 versus Wt; #$p < 0.01$, PR5 versus PR5+MWM; &$p < 0.05$, PR5+MWM versus PR5/NLRP3 −/−+MWM; &&$p < 0.01$, PR5+MWM versus PR5/NLRP3 −/−+MWM

of NLRP3, caspase-1, and IL-1β in the PR5+NLRP3 −/−+MWM group significantly decreased compared with those in the PR5+MWM group (Fig. 9a–d). We also detected the secretion of IL-1β and TNF-α. The results revealed that the secretion of IL-1β was aligned with the expression of IL-1β; however, no significant difference was found in the secretion of TNF-α among the 6 groups (Fig. 9e, f).

Discussion

AD, which is characterized by memory loss, is the most common type of dementia in the elderly population. An increasing amount of research has supported that our brains retain the capacity to change in response to experience until late adulthood. This suggests the potential of cognitive training to ameliorate age-associated cognitive decline by inducing training-specific neural plastic changes at both the neural and behavioral levels. Previous studies have shown that environmental enrichment or spatial training using a water maze could delay the development of neuropathology or memory decline in 3xTg-AD mice [4, 19]. Some groups have indicated that

spatial learning in the MWM influences the production and fate of newly born cells [46]. However, the underlying mechanisms of spatial training amelioration on cognitive decline in AD are still unclear. In our study, we observed decreased synaptic plasticity in the 10-month-old PR5 mice. Spatial training could ameliorate these deficits with long-term effects. The NLRP3/caspase-1/IL-1β axis plays an important role in regulating synaptic plasticity in spatial training.

PR5 mice, which serve as a model of the neurofibrillary tangle pathology of AD and show hippocampus-dependent behavioral impairments related to AD, have been widely used as AD models [13, 14, 37]. Neurofibrillary tangles first appear at 6 months in the amygdala, followed by the CA1 region of the hippocampus [8]. The synergistic effects of amyloid beta and tau are evident in the 8-month-old (triple) PR5 mice [37]. The MWM and Y-maze revealed intact spatial working memory but impairments in spatial reference memory at 6 months of age [14]. Therefore, in the current study, we used the 10-month-old PR5 mice with remarkable pathological and behavioral impairments to study whether spatial training could improve the cognitive abilities of the mice. First, we demonstrated that the memory duration of the normal mice was less than

Fig. 8 Effects of spatial training on the expression of synapse-associated proteins and on the synaptic morphology in PR5+NLRP3+/− and PR5+NLRP3−/− mice. (**a–d**) Expression of pre-synaptic and postsynaptic proteins synaptophysin, PSD93, and PSD95 in the hippocampus after training. (**e–h**) The spine density in AO and BS dendrites in the hippocampus after training. Blot images were cropped for comparison. GAPDH was used as a loading control in each sample. The values were represented as mean ± SEM ($n = 3$ or $n = 5$). Scale bars, 1 μm. *$p < 0.05$, PR5 versus Wt; **$p < 0.01$, PR5 versus Wt; #$p < 0.01$, PR5 versus PR5+MWM; &$p < 0.05$, PR5+MWM versus PR5/NLRP3−/−+MWM; &&$p < 0.01$, PR5+MWM versus PR5/NLRP3−/−+MWM; &&&$p < 0.001$, PR5+MWM versus PR5/NLRP3−/−+MWM; $$p < 0.01$, PR5 versus PR5/NLRP3+/−+MWM
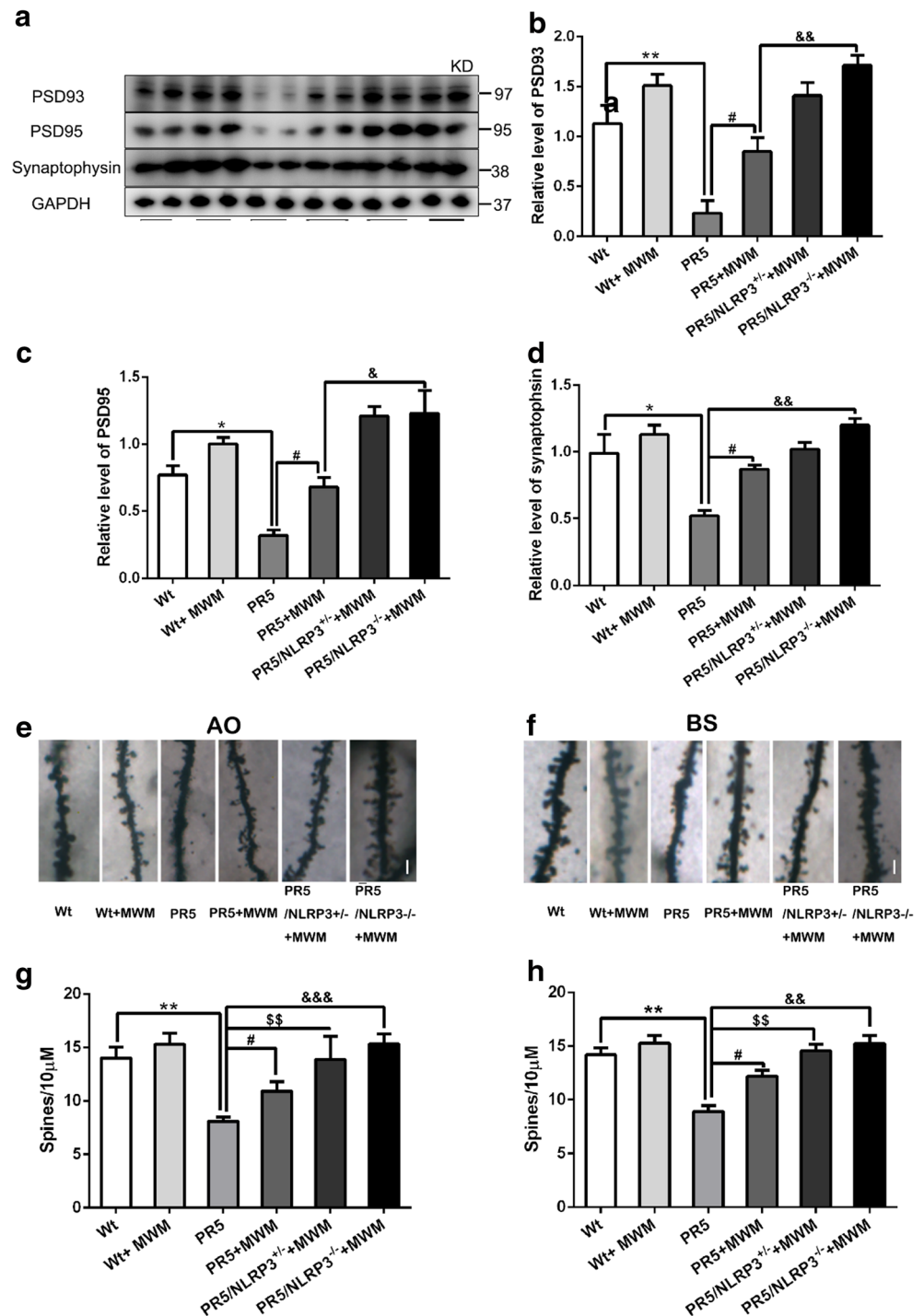

72 h by using the NORT to exclude the possibility of the long-term effects of spatial training being due to memory abilities. We found that spatial training significantly improved both learning and memory abilities. No differences in the performances of learning and memory abilities were found between the PR5 mice and the Wt mice during the fourth week or the third–fourth weeks, respectively, suggesting that with increased spatial training, the learning and memory abilities of the PR5 mice could be improved significantly. We also found similar results using the NORT. Interestingly, 3 months later, the effects still remained, suggesting that cognitive training has long-term effects, which might explain the finding that high education levels are a protective factor against AD.

PR5 mice overexpress the longest human tau isoform, together with the P301L mutation. In accordance with the

Fig. 9 The NLRP3/caspase-1/IL-1β axis changes in PR5+NLRP3 −/− mice after spatial training. (a–d) Expression of NLRP3, caspase-1, and IL-1β in the hippocampus after training. (e, f) The IL-1β and TNF-α levels in the hippocampus after training was detected by ELISA. Blot images were cropped for comparison. GAPDH was used as a loading control in each sample. The values were represented as mean ± SEM (n = 3 or n = 6). *p < 0.05, PR5 versus Wt; **p < 0.01, PR5 versus Wt; #p < 0.01, PR5 versus PR5+MWM; &&p < 0.01, PR5+MWM versus PR5/NLRP3 −/−+MWM; &&&p < 0.001, PR5+ MWM versus PR5/NLRP3 −/−+MWM
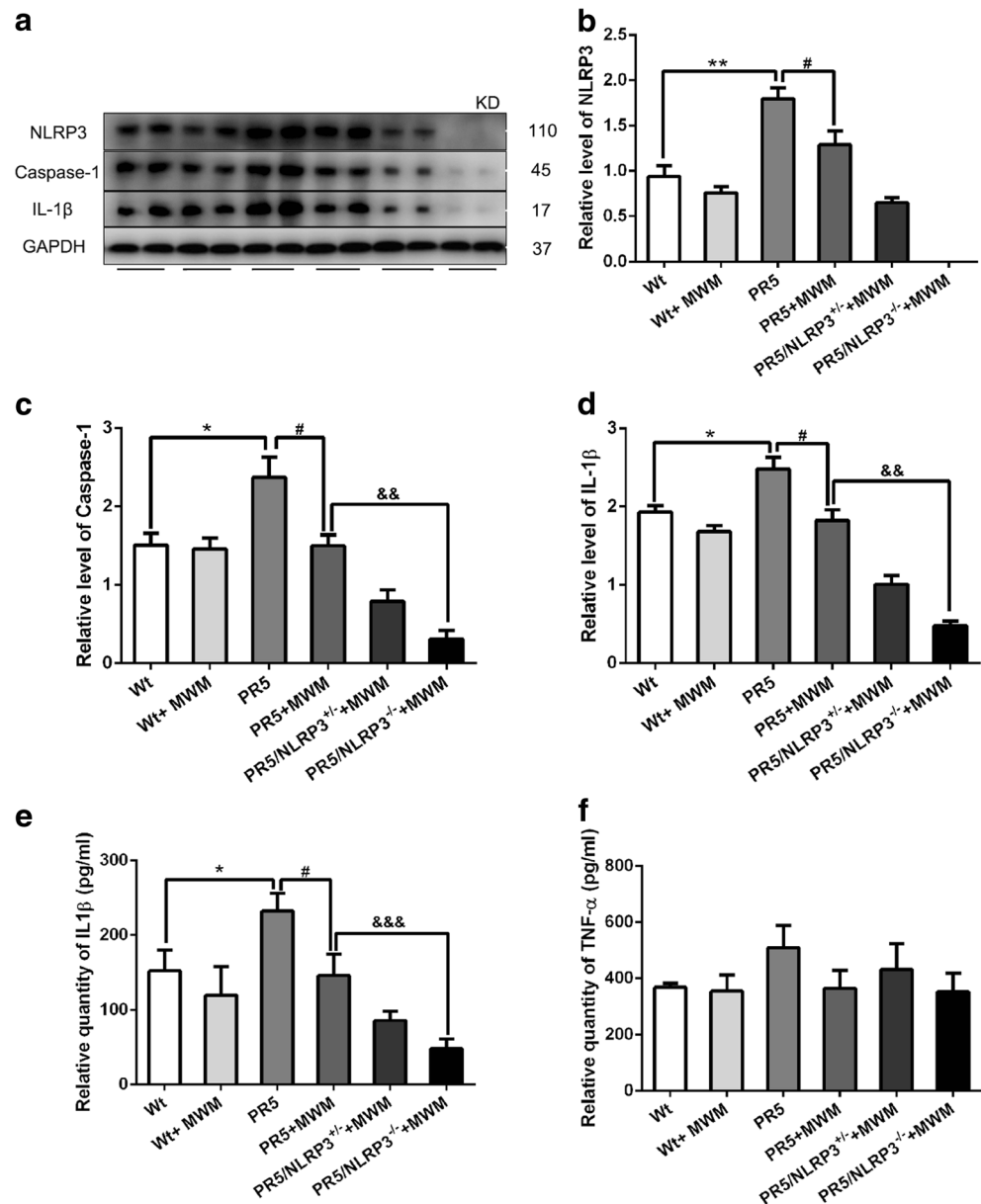
findings of previous studies [4, 20], we found accumulations of phosphorylated tau in the hippocampi of PR5 mice, which were significantly attenuated by spatial training. To further investigate the involvement of NLRP3 in tau changes, we detected the expression of phosphorylated tau in PR5/NlRP3 −/− and PR5/NlRP3+/− mice and found that the levels of phosphorylated tau were reduced in PR5/NLRP3−/− and PR5/NlRP3+/− mice. However, spatial training could not further ameliorate tau phosphorylation in PR5/NlRP3−/− mice or NlRP3+/− mice, suggesting that NIRP3 was the main molecule in the effects of spatial training.

Synapses are the functional units of neuronal communication, and synaptic dysfunction is directly linked to cognitive disturbances [3, 7, 28]. In AD, synaptic failure strongly correlates with cognitive decline [3, 5, 36]. Dendritic spines are tiny protrusions of dendrites that are essential for excitatory synaptic transmission [6, 7, 22]. AD brains show a marked reduction in synaptic density and a loss of dendritic spines in the cortex and the hippocampus [9, 31]. Synaptic impairment is likely to be the major contributor to memory loss in AD [23, 41]. In our research, we assessed synapse-associated protein expression after 4 consecutive weeks of spatial training. We observed that the expression levels of the presynaptic protein synaptophysin or of the postsynaptic proteins PSD93 and PSD95 significantly increased in PR5 mice after spatial training. Our data also showed that the dendritic spine density decreased significantly in PR5 mice. More importantly, we found that the dendritic spine density was significantly increased by spatial training. Three months later, we also found that there were increased levels of synapse-associated protein expression and dendritic

spine densities in PR5 mice who underwent spatial training. In accordance with the results of previous studies, our results suggested that these synapse-associated protein and dendritic spine density enhancements might facilitate learning and memory.

Innate immunity and inflammatory responses play important roles in the pathogenesis of AD. It has been demonstrated that the inhibition of NLRP3 inflammasomes could be largely protective against memory loss and may decrease Aβ deposition in AD transgenic mouse models [16, 44]. A recent study further showed that NLRP3 or caspase-1 deficiency completely prevented long-term potentiation (LTP) suppression in hippocampal slices and improved spatial memory in AD transgenic mice [10, 24]. NLRP3 inflammasome inhibition regulates synaptic dysfunction, cognitive impairment, and the restriction of beneficial microglial clearance functions [11, 17, 33]. NLRP3 inflammasomes are composed of NLRP3, the adaptor molecule apoptosis–associated speck-like protein containing a CARD (ASC), and the cysteine protease caspase-1. The characterized consequence of caspase-1 activation in inflammasomes is the secretion of proinflammatory cytokines. Particularly, the most important of the proinflammatory cytokines is IL-1β, which may mediate a variety of local and systemic immune responses [21, 27]. Several studies have already demonstrated that higher levels of IL-1β can exacerbate AD pathogenesis and memory deficits by inducing tau hyperphosphorylation [15, 29], affecting synaptic plasticity, and inhibiting LTP [32, 35]. Additionally, inhibition of IL-1β signaling *in vivo* has been shown to provide disease-modifying benefits in an AD mouse model [24]. In addition, the secretion of IL-1β could be prevented by environmental enrichment [2]. In our study, we first found that the expression levels of NLRP3, caspase-1, and IL-1β were significantly increased in PR5 mice and could be decreased by spatial training. Then, when NLPR3 was knocked out in PR5 mice, the effect of spatial training on cognition was significantly enhanced in PR5 mice and was accompanied by increased expression levels of synapse-associated protein and an increased number of dendritic spines. In addition, a positive correlation was observed between NIRP3 levels and the expressions of caspase-1 and IL-1β. Meanwhile, spatial training significantly decreased the secretion of IL-1β but not that of TNF-α, suggesting that IL-1β is a specific inflammatory factor involved in the ameliorating effect of spatial training on cognitive impairment. Together, these results suggested that the NLRP3/caspase-1/IL-1β axis plays an important role in the spatial training-induced synaptic plasticity repair in PR5 mice.

In conclusion, the present results demonstrated that spatial training ameliorates cognitive performance and AD-like pathology with a long-term effect in PR5 mice. This cognitive benefit was associated with NLRP3/caspase-1/IL-1β axis activation and with remodeling of dendritic plasticity.

Compliance with Ethical Standards

Conflict of Interest   The authors declare that they have no competing interests.

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