Journal of Functional Foods 113 (2024) 106025

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

6-Shogaol attenuates natural aging-induced locomotive and cognitive declines through suppression of p75 neurotrophin receptor *in vivo*

Hyeyoon Eo [a], Jin Hee Kim [a], Hyeri Im [b], In Gyoung Ju [c], Eugene Huh [d], Rabin Pun [e], Dongyun Shin [e], Yunsook Lim [f], Myung Sook Oh [a,b,c,*]

[a] Department of Biomedical and Pharmaceutical Sciences, Graduate School, Kyung Hee University, Seoul 02447, Republic of Korea
[b] Department of Integrated Drug Development and Natural Products, Graduate School, Kyung Hee University, Seoul 02447, Republic of Korea
[c] Department of Oriental Pharmaceutical Science, College of Pharmacy and Kyung Hee East-West Pharmaceutical Research Institute, Kyung Hee University, Seoul 02447, Republic of Korea
[d] Department of Formulae Pharmacology, College of Korean Medicine, Gachon University, Seongnam 13120, Republic of Korea
[e] College of Pharmacy, Gachon University, Incheon 21936, Republic of Korea
[f] Department of Food and Nutrition, Kyung Hee University, Seoul 02447, Republic of Korea

ARTICLE INFO

Keywords:
6-shogaol
Aging
Rejuvenation
Neurodegeneration
Senolytics
P75 neurotrophin receptor

ABSTRACT

The present study aimed to investigate the effect of 6-shogaol (6S) treatment on brain senescence. Mice were naturally aged until 25-month-old and treated with 10 mg/kg of 6S for a month. Behavioral tests were performed to measure locomotion and cognitive function. Neuronal damage, synaptic plasticity, neuroinflammation, neurogenesis and p75 neurotrophin receptor (p75NTR) expression were examined by immunohistochemistry or immunofluorescence. 6S treatment improved locomotion during open field test in the aged mice and spontaneous alternation in Y-maze. These data are in line with that 6S administration improved dopaminergic neuronal loss and dopamine signaling and attenuated hippocampal synaptic plasticity in the aged brain. Additionally, 6S treatment reduced striatal and hippocampal microgliosis and astrocytosis but promoted neurogenesis in subventricular zone. Furthermore, 6S treatment reversed the p75NTR expression in the senescent brain. The current findings suggest that 6S can be a functional food for successful aging through brain rejuvenation at the molecular level.

1. Introduction

Age-quake is a neologism meaning that there is a significant demographic change due to highly increased aged population worldwide (Sabri et al., 2022). The National Institute on Ageing and the National Institute of Health in the United states of America documented that age over 65 covers approximately 10 % of world population (Nations, Economic, & Affairs, 2013; Sabri et al., 2022). With the Age-quake, one of the leading causes of mortality and morbidity in the people aged more than 70 in 2019 worldwide was dementias including Alzheimer's disease (Collaborators, 2022). In addition to dementia, approximately 1 out of 5 elderly people was reported to have movement disorders such as Parkinson's disease which is one of the major drivers of morbidity among the aged population (Collaborators, 2022; Tse et al., 2008). Thus, it is important to properly manage neuronal dysfunction or loss in the aged population.

During the normal aging, there are some mild but progressive and significant changes in the brain at the structural, metabolic and molecular levels (Lee & Kim, 2022). Age-related alteration in the brain includes neuronal atrophy/loss, dysregulation of neurotransmitters and accumulation of damages in the neuronal environment, which result in behavioral changes such as movement deficit and cognitive decline (Lee & Kim, 2022). First, neuronal loss and impaired dopamine signaling are the major causes of movement deficit (Lubec et al., 2021). It was reported that dopaminergic signaling decreases 5–10 % every decade and the level of striatal dopamine is reduced up to 50 % with age in the human brain (Lubec et al., 2021; Morgan, 1987). In addition, secure dopamine signaling is one of the major contributors in synaptic plasticity which is closely related to memory and cognitive function as well (Madadi Asl, Vahabie, & Valizadeh, 2019; Speranza, di Porzio, Viggiano, de Donato, & Volpicelli, 2021). However, aged brain shows impairments in the synaptic plasticity and memory decline (Bergado & Almaguer,

* Corresponding author at: Department of Biomedical and Pharmaceutical Sciences, Graduate School, Kyung Hee University, Seoul 02447, Republic of Korea.
E-mail address: msohok@khu.ac.kr (M. Sook Oh).

https://doi.org/10.1016/j.jff.2024.106025
Received 17 December 2023; Accepted 16 January 2024
Available online 21 January 2024

2002; Burke & Barnes, 2006; Wong et al., 2021). This senescence-related neurodegeneration results from various biochemical changes in the neurons and one of the potential changes is p75 neurotrophin receptor (p75NTR) expression (Wong et al., 2021). The p75NTR is a superfamily of tumor necrosis factor (TNF) receptor and known to negatively regulate neuronal plasticity in a structural and functional way (Dechant & Barde, 2002; Wong et al., 2021). In a previous study, mice with p75NTR depletion had resistance to hippocampal homeostatic long-term plasticity as well as cognitive function deficits (Wong et al., 2021). Furthermore, it was reported that p75NTR signaling can regulate neuroinflammation and neurogenesis in the experimental meningitis rodent model (D. Zhang et al., 2021). Thus, it is important to regulate p75NTR expression to prevent or improve structural and functional damages found in neuronal senescence.

The current study examined the effect of 6-shogaol (6S) treatment on aging-related behavioral changes, neuronal dysfunction and p75NTR expression in naturally aged mice. The 6S is a bioactive compound found in ginger (*Zingiber officinale* Roscoe) and especially highly included when the ginger is dried (Ha et al., 2012; Moon et al., 2014; Park et al., 2013). In our previous studies, 6S showed anti-neuroinflammatory effects in the several *in vitro* and *in vivo* models (Ha et al., 2012; Park et al., 2013). Likewise, 6S treatment not only enhanced memory/cognitive function in healthy young mice but ameliorated dementia induced by scopolamine in mice (Moon et al., 2014). In this context, the current study hypothesized that 6S treatment can promote neuronal rejuvenation through improvement of neurodegeneration, synaptic plasticity, neuroinflammation and neurogenesis which are closely related to p75NTR expression in the elderly mice.

2. Materials and methods

2.1. Animals and experimental design

Eighteen male C57BL/6 mice (2-month-old, 20–24 g) were obtained from DBL Inc. (Eumseong, Republic of Korea) and housed under 12-h light/dark cycle, at a constant temperature (23 ± 1 °C) and humidity (60 ± 10 %) in standard cages. All the mice had free access to food (standard chow diet purchased from DBL Inc.) and tap water. Among them, twelve mice were naturally aged until they reached at 25-month-old.

Mice were divided into 3 groups (N = 6 per group) randomly as follows: (1) young group (2-month-old, young adult mice administered with vehicle); (2) aged group (25-month-old, naturally aged mice administered with vehicle); aged + 6S group (25-month-old, naturally aged mice administered with 10 mg/kg 6S synthesized and kindly provided from professor Dong-yun Shin at the Gachon University of Medicine and Science, Incheon, Republic of Korea as previously described (Huh et al., 2023)). Vehicle (saline with 1 % dimethyl sulfoxide) or 6S was administered by gavage once a day for twenty-eight consecutive days. All the behavioral tests were performed between twenty-first and twenty-seventh day of administration as explained in following statements.

2.2. Behavioral tests

2.2.1. Rotarod test

To evaluate motor deficit, the current study performed rotarod test. On the twenty-first day of administration, every mouse was trained on a rotating spindle (30 mm diameter, JD-A-07-TSM, Jeung Do Bio & Plant Co., Ltd., Seoul, Republic of Korea) with five individual compartments for testing five mice at the same time. The test comprised with two sessions as follows: (1) training session (accelerating rotation speed at from 5 rpm to 15 rpm for 3 min) and (2) test session (rotation speed at 15 rpm for 3 min, performed one day after training session). Latency to fall was recorded when each mouse was first dropped from rotating bar and the mouse was immediately re-located on the rotating bar. By relocating mice, total number of falls were measured during 3 min test. Test session was performed three times and data were shown as the average values over the three test trials.

2.2.2. Open field test

To test locomotor activity, mice were allowed to acclimate to the behavioral room environment for 1 h before test commencement on the twenty-third day of administration. All the mice were located in the open field arena with dimensions [W × L × H = 45 × 45 × 45 cm] and a camera placed 2.5 m above the arena was used to record locomotion for 30 min. The test was performed once to eliminate habituation issue and estimated between 9p.m. and 2 a.m. to avoid any circadian related fluctuation during performance. Total track length that each mouse travelled in the whole arena was computerized with automatic analysis system (Viewer; Biobserve, Bonn, Germany).

2.2.3. Y-maze

Y-maze was conducted on the twenty-fifth day of 6S treatment by using test apparatus with three arms (3 × 40 × 12 cm) at the 120° angle from each other. Mice were located in the middle of the Y-maze and let the mice freely explore the arms for 8 min. With a camera placed 2.5 m above the apparatus, the number of arm entries and the number of triads were recorded to calculate percentage alternation, a measure of spatial working memory. The calculation formula is shown as follows: (the total number of alternations/total number of arm entries − 2) × 100. When the mice consecutively enter all three arms (i.e., ABC, BCA, or CAB but not BAB), it was considered as an alternation behavior.

2.2.4. Novel object recognition test (NORT)

NORT was performed on the twenty-sixth (training session) and twenty-seventh (test session) day of 6S administration. In the training session, mice were acclimated in the middle of a dark open field box [W × L × H = 45 × 45 × 45 cm] for 4 min and then let them observe two identical objects for another 4 min. During the training session, it was measured how long the animals spent time for examining each object. When the mice were facing, sniffing, or touching any object, the mice were considered as examining or exploring the object. On the next day, mice were allowed to explore a novel object as well as one of the familiar objects (previously observed during the training session) for 4 min. Like the training session, it was measured how long the animals spent exploring either novel or familiar object during the test session. Recognition index was calculated as follows: (time spent for novel object)/[(time spent for novel object) + (time spent for familiar object)] × 100).

2.3. Necropsy and preparation of brain tissues

On the last day of the animal experiment, mice were anesthetized with tribromoethanol (312.5 mg/kg, i.p.), perfused transcardially with 0.05 M phosphate buffered saline (PBS), and then fixed with cold 4 % paraformaldehyde (PFA) in 0.1 M phosphate buffer. Brains were removed, post-fixed 4 % PFA overnight at 4 °C and then immersed in 0.05 M PBS containing 30 % sucrose for cryoprotection. Serial 25 μm thick coronal sections were cut on a freezing microtome (Leica, Nussloch, Germany) and then stored in cryoprotectant (25 % ethylene glycol, 25 % glycerol, and 0.05 M phosphate buffer) at 4 °C before use.

2.4. Immunohistochemistry

Free floating brain sections were treated with 1 % hydrogen peroxide and then incubated overnight with primary antibodies; tyrosine hydroxylase (TH, 1:2000), dopamine transporter (DAT, 1:2000) purchased from Merck Millipore (Burlington, MA, USA); dopamine- and cyclic-AMP-regulated phosphoprotein of molecular weight 32,000 (DARPP-32, 1:1000) purchased from Cell Signaling Technology (Danvers, MA, USA); synaptophysin (SYN, 1:1000) purchased from Sigma-Aldrich (St.

Louis, MO, USA); metabotropic glutamate receptor 5 (mGluR5, 1:1000) purchased from Abcam (Cambridge, UK), ionized calcium-binding adapter molecule 1 (Iba-1, 1:500) purchased from FUJIFILM Wako Chemicals USA Corp. (Richmond, VA, USA); and doublecortin (DCX, 1:1000) purchased from Santa Cruz Biotechnology (Dallas, TX, USA). After overnight incubation, sections were incubated with biotinylated secondary antibodies (matched to host of each primary antibody) followed by incubation in avidin–biotin complex [ABC; Vector Labs (Burlingame, CA, USA)] solution. The color of every section was developed with 3,3-diaminobenzidine [DAB; Sigma-Aldrich Inc. (St. Louis, MO, USA)]. Images were captured using confocal microscopy [K1-Fluo; Nanoscope Systems (Daejeon, Republic of Korea)]. TH-immunoreactive cells in substantia nigra pars compacta (SNpc), DARPP-32-immunoreactive cells in cortex and Iba-1-immunoreactive cells with more than 3 branches in striatum and hippocampus were quantified by stereological counting, and analyzed using the Image J software [National Institutes of Health (Bethesda, MD, USA)]. Organizational area was determined by comparing images with the stereotaxic atlas of the mouse brain (Franklin & Paxinos, 2013). The optical density of DAT in striatum, SYN in hippocampal CA3 stratum lucidum (CA3:SL) and mGluR5 in the hippocampal CA1 stratum oriens (CA1:SO) region was quantified and analyzed using the Image J software. Cell counting was conducted by an experimenter who did not know the treatment condition, and the result for each animal was the average of the number from its three sections.

2.5. Immunofluorescence

For immunofluorescence, free floating brain sections were incubated overnight with primary antibodies; glial fibrillary acidic protein (GFAP) purchased from Invitrogen (Waltham, MA, USA); and neuronal-nuclei (NeuN) purchased from Merck Millipore. After then, sections were incubated with secondary antibodies; goat anti-rabbit Alexa Fluor™ 488 (dilution at 1:500, Invitrogen); and horse anti-mouse DyLight® 488 [dilution at 1:500, Vector Laboratories, Inc. (Newark, CA, USA)] at room temperature. Especially, for double-staining of p75NTR with NeuN, sections were rinsed with PBS and then incubated with anti-p75NTR purchased from ABclonal Science, Inc. (Woburn, MA, USA). Again, sections were incubated with a goat anti-rabbit DyLight® 594 (dilution at 1:500; Vector Laboratories, Inc.). All the sections were incubated with anti-4′,6-diamidino-2-phenylindole (DAPI) (dilution at 1:1000; Vector Laboratories, Inc.) at the last step of staining. Images were visualized using K1-Fluo confocal microscopy. GFAP positive cells in striatum and p75NTR positive cells in cortex were quantified by stereological counting, and analyzed using the Image J software. Like the immunohistochemistry, organizational area was determined by comparing images with the stereotaxic atlas of the mouse brain (Franklin & Paxinos, 2013). Percentage of GFAP or p75NTR positive area was quantified and analyzed using the Image J software as well. All the quantification was conducted by an experimenter who did not know the treatment condition, and the result for each animal was the average of the number from its three sections.

2.6. Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM). All statistical analyses were performed using the GraphPad Prism ver. 8.0.1 (GraphPad Software Inc., San Diego, CA, USA). A one-way analysis of variance (ANOVA) with Dunnett's post-hoc test was performed for multiple comparison, and difference between aged group and another group was considered as statistically significant at $p < 0.05$ and shown in each figure.

2.7. In silico docking analysis

The structure of 6S was downloaded in SDF format from PubChem (https://pubchem.ncbi.nlm.nih.gov/). Then it was imported into the software of Pymol and saved in mol2 format. The crystal structures of p75NTR and 6S were downloaded from RCSB Protein Data Bank (https://www.rcsb.org/). The docking simulation was conducted via SwissDock with p75NTR. The scores of full fitness and estimated ΔG were used to predict the best ranked docking pose. Generally, the lower the score, the higher affinity (Zhong, Guo, & Zheng, 2022). The lowest docking score was selected and the corresponding conformation were visualized via SwissDock.

3. Results

3.1. Effects of 6S administration on weakened motor function in the aged mice

The current study examined motor function by performing rotarod test and open field test as shown in Fig. 1. First, as shown in Fig. 1A, latency to fall during rotarod test session in the aged group was lower than that in the young group. At the same time, the number of falls during rotarod test was bigger in the aged group compared to the young group as shown in Fig. 1B. However, aged mice treated with 6S showed significantly higher latency to fall and smaller than the aged control group. Likewise, the aged group travelled less in open field test apparatus compared to the young group as shown in Fig. 1C. On the other hand, the aged + 6S group showed significantly longer track length compared to the aged group.

3.2. Effects of 6S administration on impaired cognitive function in the aged mice

To evaluate cognitive function, the current study performed Y-maze (Fig. 1D and 1E) and NORT (Fig. 1F and 1G). As shown in Fig. 1D, the aged group had lower spontaneous alternation compared to the young group but the aged + 6S group showed significant enhancement in the spontaneous alternation. Interestingly, the number of total entries which imply locomotive activity was smaller in the aged group compared to the young group as shown in Fig. 1E but there was no effect from 6S administration. Additionally, the aged group showed significantly smaller recognition index compared to the young group as shown in Fig. 1F. The aged + 6S group tended to show higher recognition index than the aged group but not significant. Furthermore, total exploring time during NORT was shorter in the aged group compared to the young group as shown in Fig. 1G. Even though the aged + 6S group showed quantitatively longer exploring time than the aged group, there was no significance between the two groups.

3.3. Effects of 6S treatment on the dopaminergic neuronal loss and reduced dopamine signaling in the aged mice

To examine the dopaminergic neuronal loss, brain section of SNpc was stained with anti-TH which is generally recognized as a dopaminergic neuronal marker (Weihe, Depboylu, Schütz, Schäfer, & Eiden, 2006). As shown in Fig. 2A and 2B, the aged mice showed significantly lower number of TH-positive cells in SNpc than the young group. However, number of TH-positive cells in SNpc was significantly bigger than that in the aged group. In addition to the dopaminergic neuronal loss, neuronal expression of DAT and DARPP-32 was measured to evaluate dopamine signaling in the aged mice as shown in Fig. 2C – 2E. The aged mice showed significantly lower DAT expression compared to the young group but the aged + 6S group had significantly higher expression DAT in the striatum compared to the aged group (Fig. 2C and 2D). Likewise, DARPP-32 positive cells in cortex of the aged group were significantly lowered than that of the young group. However, the aged + 6S group showed significantly more DARPP-32 positive cells in the cortex compared to the aged group (Fig. 2C and 2E).

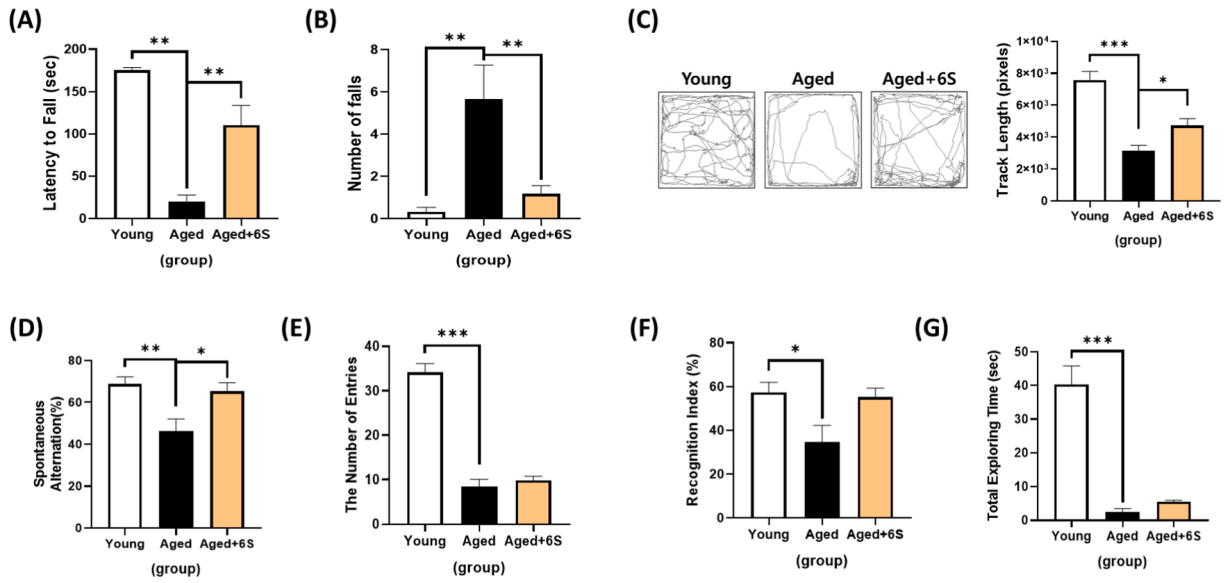

Fig. 1. 6S treatment ameliorated motor and cognitive functions in the 25-month-old C57BL/6 mice. Aged mice were treated vehicle or 10 mg/kg of 6S for 28 days (N = 6 per group). Rotarod test was performed and (A) latency to fall during and (B) the number of falls were measured during the test session. Open field test was performed to evaluate locomotive activity by measuring track length travelled by each mouse (C). Y-maze was used to measure cognitive function by calculating spontaneous alternation (D) and the number of entries to arms (E). During novel object recognition test, recognition index (F) was calculated and total exploring time (G) was measured. The data were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test: *p < 0.05, **p < 0.01 and ***p < 0.001 vs. the aged group.

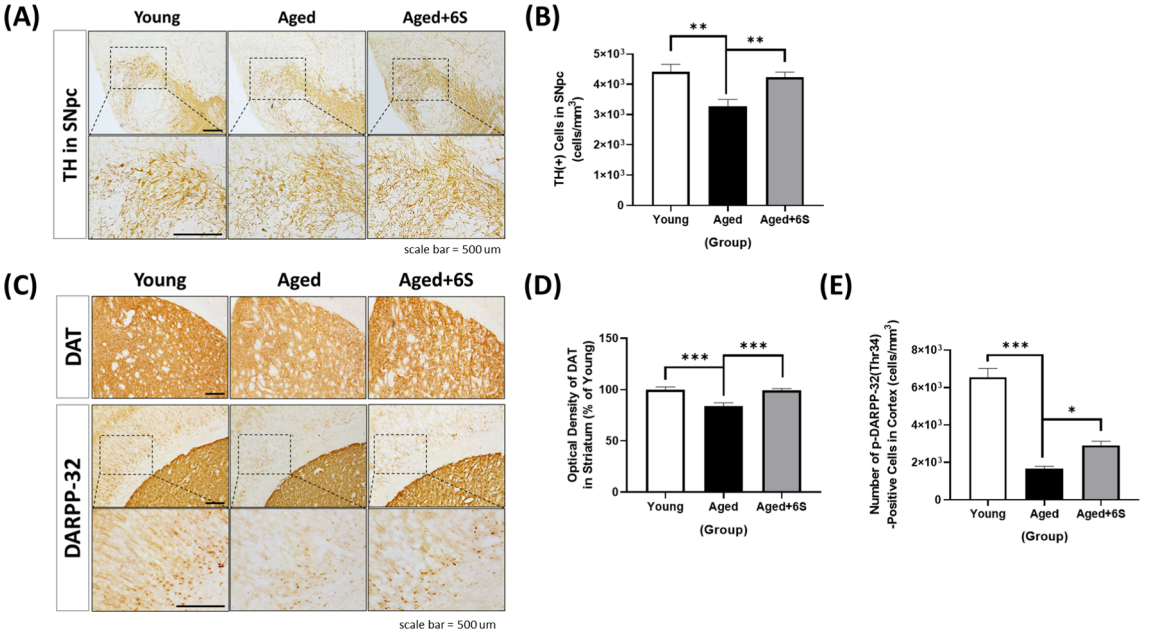

Fig. 2. 6S treatment improved dopaminergic neuronal loss and dopamine signaling in the 25-month-old C57BL/6 mice. Aged mice were treated vehicle or 10 mg/kg of 6S for 28 days (N = 6 per group). Immunohistochemistry was performed to measure TH-positive cells in the SNpc region (A), striatal expression of DAT (C, upper panel) and cortical expression of DARPP-32 (C, lower panel). Optical density of each biomarker was quantified as shown in (B), (D) and (E), respectively. The data were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test: *p < 0.05, **p < 0.01 and ***p < 0.001 vs. the aged group.

3.4. Effects of 6S treatment on hippocampal synaptic plasticity in the aged mice

The current study examined synaptic plasticity in the mouse hippocampal region by staining SYN (a marker of presynaptic transmembrane vesicles) and mGluR5 (a marker of synaptic function and plasticity) as shown in Fig. 3 (She et al., 2009; Wiedenmann & Franke, 1985). The aged mice showed lower expression of SYN in CA3:SL as well as mGluR5 in CA1:SO compared to the young group. On the other hand, the aged + 6S group showed significantly higher expression of SYN and mGluR5 than the aged group.

3.5. Effects of 6S administration on neuroinflammation in the aged mice

To evaluate neuroinflammation, the current study examined microglial and astrocytic cell activation through measuring expression of Iba-1 and GFAP in striatum and hippocampus, respectively (Figs. 4 and 5). First, the aged group showed significantly higher expression of Iba-1 in

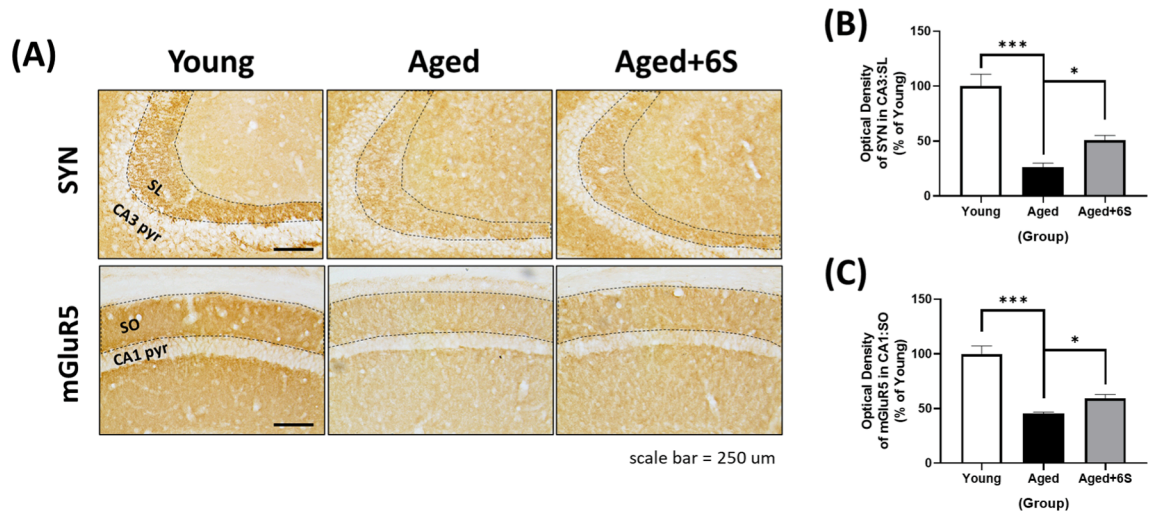

Fig. 3. 6S treatment improved synaptic plasticity in hippocampus of the 25-month-old C57BL/6 mice. Aged mice were treated vehicle or 10 mg/kg of 6S for 28 days (N = 6 per group). Immunohistochemistry was performed to measure synaptophysin expression (A, upper panel) and mGluR5 expression (A, lower panel) in the hippocampus. Optical density of each biomarker was quantified as shown in (B) and (C), respectively. The data were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test: *p < 0.05 and ***p < 0.001 vs. the aged group.

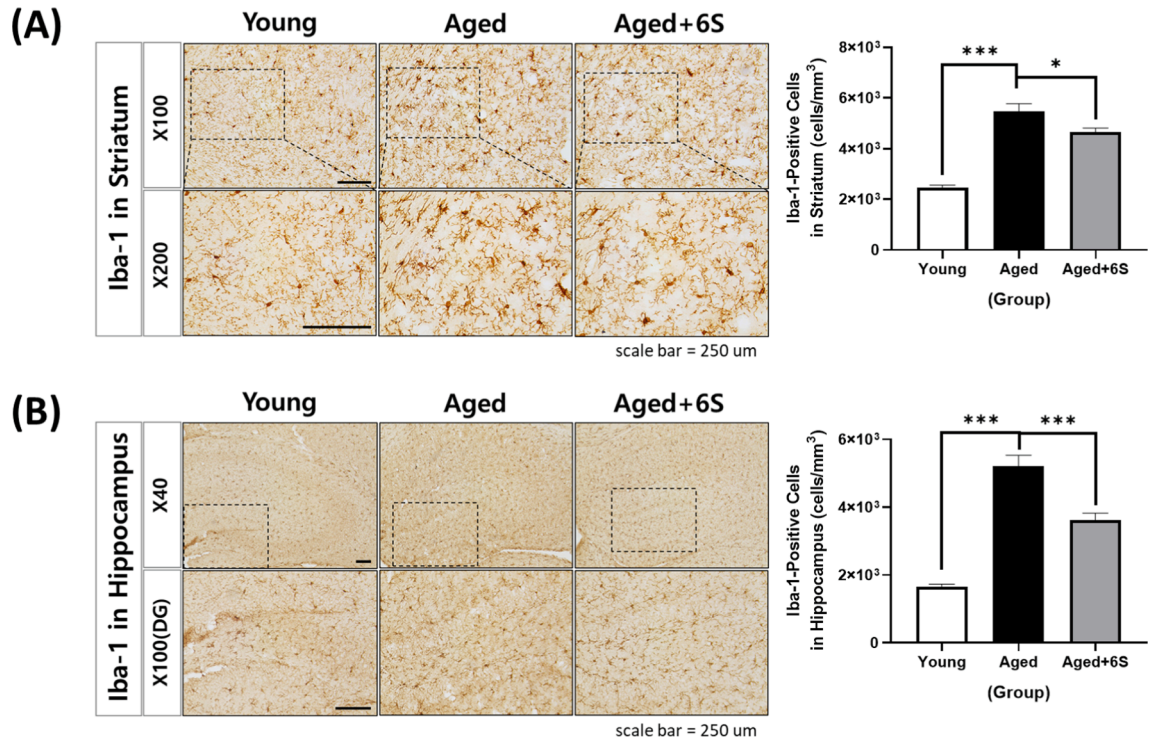

Fig. 4. 6S treatment suppressed microgliosis in the striatum and hippocampus of the 25-month-old C57BL/6 mice. Aged mice were treated vehicle or 10 mg/kg of 6S for 28 days (N = 6 per group). Immunohistochemistry was performed to investigate expression of Iba-1, a marker of microglial cell activation, in striatum (A) and hippocampus (B). Optical density was quantified as shown on the right panel of each section. The data were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test: *p < 0.05 and ***p < 0.001 vs. the aged group.

both striatum and hippocampus than the young group. However, the 6S-treated aged group had lower expression of Iba-1 in striatum and hippocampus compared to the aged group (Fig. 4). At the same time, GFAP expression in both striatum and hippocampus was higher in the aged group compared to the young group. On the other hand, the aged + 6S group had lower striatal and hippocampal GFAP expression than the aged control group (Fig. 5).

3.6. *Effects of 6S treatment on neurogenesis in the aged mice*

In the present study, the number of DCX-positive cells, a marker of neurogenesis, was measured in the subventricular zone (SVZ) to evaluate the impact of 6S on the neurogenesis (Couillard-Despres et al., 2005). As shown in Fig. 6, the aged group had smaller number of DCX-positive cells in SVZ compared to the young group. However, DCX-positive cells in the SVZ of the aged + 6S group was increased than those of the aged group.

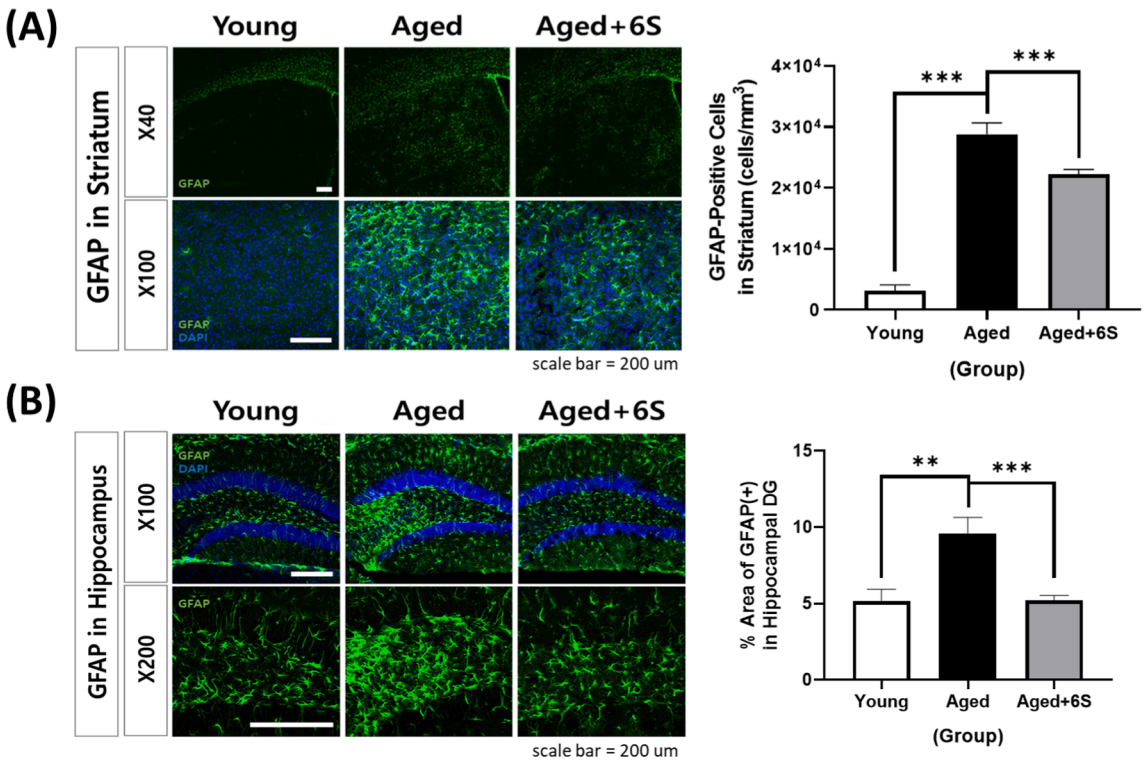

Fig. 5. 6S treatment inhibited astrocytosis in the striatum and hippocampus of the 25-month-old C57BL/6 mice. Aged mice were treated vehicle or 10 mg/kg of 6S for 28 days (N = 6 per group). Immunofluorescence was performed to investigate expression of GFAP, a marker of hyper-activated astrocytes, in striatum (A) and hippocampus (B). Optical density was quantified as shown on the right panel of each section. The data were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test: **p < 0.01 and ***p < 0.001 vs. the aged group.

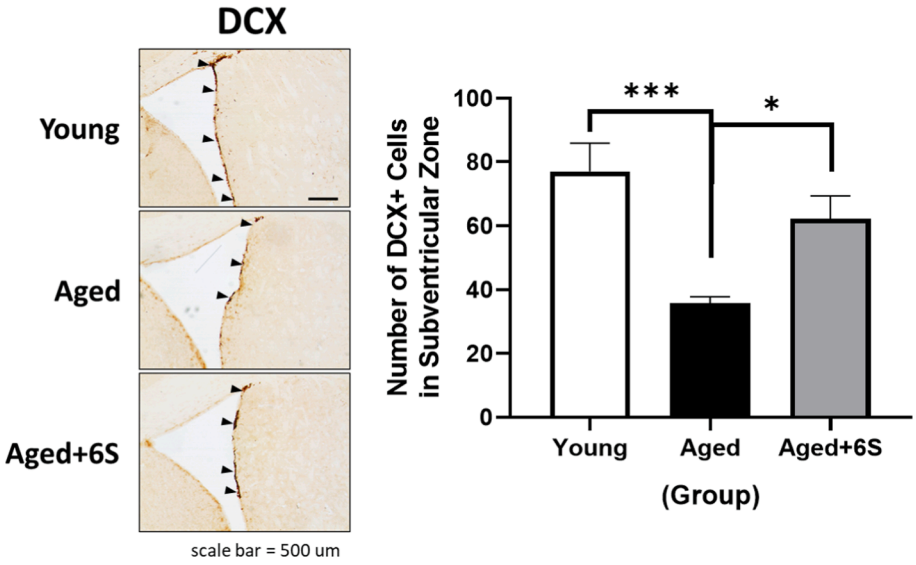

Fig. 6. 6S treatment stimulated neurogenesis in the subventricular zone of the 25-month-old C57BL/6 mice. Aged mice were treated vehicle or 10 mg/kg of 6S for 28 days (N = 6 per group). Immunohistochemistry was performed to evaluate expression of doublecortin (DCX), a marker of neurogenesis, in the subventricular zone (SVZ) of the 25-month-old C57BL/6 mice. Representative images were shown on the left panel and the optical density was quantified as shown on the right panel. The data were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test: *p < 0.05 and ***p < 0.001 vs. the aged group.

3.7. Effects of 6S administration on p75NTR expression in the brain of the aged mice

As shown in Fig. 7, p75NTR expression in the cortex and hippocampus was measured to evaluate whether effects of 6S treatment on neuronal rejuvenation is related to p75NTR signaling. The aged group showed increase in both cortical and hippocampal p75NTR expression contrary to the young group. Interestingly, the aged mice treated with 6S showed lower p75NTR expression in both cortex and hippocampus than the aged mice.

3.8. Molecular docking analysis of 6-shogaol with p75NTR

To estimate the interaction between 6-shogaol and p75NTR at the

atomic level, the current study performed *in silico* molecular docking analysis using SwissDock. The full fitness value is −3462.77 kcal/mol and the estimated ΔG value is −7.35 kcal/mol, respectively (Table 1). The three-dimensional interaction of 6S and p75NTR was visualized using SwissDock as shown in Fig. 8.

4. Discussion

The current study demonstrated that 6S treatment can attenuates weakened motor function as well as impaired cognitive function in the 25-month-old mice (Fig. 1). In the current study, 6S administration improved dopaminergic neuronal loss in SNpc and dopamine signaling which are associated with the motor deficits (Fig. 2). Likewise, 6S treatment significantly stimulated hippocampal synaptic plasticity which is closely related to the memory decline in aged mice (Fig. 3). Here, our group found that 6S administration not only reduced neuroinflammation in the striatum and the hippocampus (Figs. 4 and 5) but promoted neurogenesis found in SVZ (Fig. 6). Moreover, 6S treatment inhibited p75NTR expression which is a potential key modulator of the brain rejuvenation (Fig. 7).

As life expectancy has risen in last decades, people have more chance to encounter neurodegenerative diseases such as Parkinsonian and dementia (Kang et al., 2023). Thus, as the desire for a higher quality of life in old age so called 'successful aging' grows higher, as the people get motivated with desires for anti-aging medicines or products more (Barbaccia, Bravi, Murmura, Savelli, & Viganò, 2022; Flatt, Settersten, Ponsaran, & Fishman, 2013). Especially, brain fitness is highlighted in the successful aging because older adults and their behaviors are affected by multi-dimensional constructs including (i) biological/physical, (ii) psychological/cognitive, (iii) social and (iv) environmental/contextual perspectives which are all under control of central nervous system (CNS) (Barbaccia et al., 2022; Flatt et al., 2013). Hence, healthy brain aging can protect the older people from possible social isolation due to relatively low age of retirement and/or limited economic activity (Barbaccia et al., 2022; Murman, 2015).

Unfortunately, normal aging is inevitably accompanied with performance decline mainly in terms of motor function (Noda, Sato, Fukuda, Tada, & Hattori, 2020) and memory/cognition (Murman, 2015). In this context, the current study first examined whether 6S treatment can ameliorate motor deficits in the elderly mice (Fig. 1A – 1C). As shown in Fig. 1, aged mice showed motor deficits in terms of both involuntary (Fig. 1A and 1B) and voluntary movement (Fig. 1C) compared to the young mice. However, 6S treatment significantly improved forced locomotion and general activity in the elderly mice. The result is in line with that the aged mice had lower TH expression in SNpc, striatal DAT expression and cortical DARPP-32 expression but 6S-treated aged mice showed higher TH expression in SNpc and promotion in both DAT and DARPP-32 expression (Fig. 2). Generally, TH is a biomarker of dopaminergic neurons (Weihe et al., 2006). As shown in Fig. 2A, aged mice had dopaminergic cell loss in SNpc compared to the young mice, which implies lower production of dopamine, a neurotransmitter involving in movement, cognition, mood and reward (Vaughan & Foster, 2013). In addition to dopamine production, availability of dopamine is controlled by DAT which plays a role in reuptake of extracellular transmitter into presynaptic neurons (Vaughan & Foster, 2013). For this reason, DAT is often considered as a target for many diseases such as depression and Parkinson's disease (Vaughan & Foster, 2013). Additionally, DARPP-32 is a key target for dopamine signaling in striatal neurons and crucial integrator of dopamine and glutamate signals (Fernandez, Schiappa, Girault, & Le Novère, 2006). Thus, our data implies that 6S can attenuate locomotion through promotion of dopamine metabolism and signaling in the neurodegeneration naturally occurred in senescence.

Table 1
Full fitness and estimated ΔG values predicted for ligands docked with p75NTR (3IJ2) by SwissDock.

Protein[a]	Full fitness (kcal/mol)	Estimated ΔG (kcal/mol)
3IJ2	−3462.77	−7.35

[a] 4-character denotes PDB identifier or PDB ID.

In addition to motor deficits, the current study found that cognitive decline in the aged mice was attenuated by 6S treatment by measuring spontaneous alternation during Y-maze test (Fig. 1D). Moreover, the current study showed that 6S treatment improved hippocampal synaptic plasticity (Fig. 3). Synaptic plasticity plays a key role in learning and memory consolidation (Goto, 2022; Takeuchi, Duszkiewicz, & Morris, 2014). Among the several molecules in synaptic plasticity, SYN is one of the major synaptic vesicle proteins sustaining presynaptic plasticity by regulation of synaptic structure and neurotransmitter release (Niu et al., 2020). In addition to SYN, mGluR5 involves in synaptic plasticity as well by regulating glutamate-associated hyperactivity and postsynaptic excitatory synaptic transmission (Niu et al., 2020). In the current study, 6S treatment significantly increased SYN expression in CA3:SL and mGluR5 expression in CA1:SO regions in the aged mice (Fig. 3). Thus, our data suggest that improvement of cognitive decline from 6S treatment was mediated by restoring senescence-induced impairments of synaptic plasticity in hippocampus.

Next, the current study examined the effect of 6S administration on neuroinflammation which provides detrimental condition to the neurons toward neurodegeneration directly and indirectly (W. Zhang, Xiao, Mao, & Xia, 2023). Glial cells such as microglia and astrocytes are major immune cells during inflammatory process in CNS (Singh, 2022). When the microglia and astrocytes are highly activated, the cells produce substantial amount of pro-inflammatory cytokines including nitric oxide, interleukin (IL)-1 beta, IL-6 and TNF alpha which contribute to generate reactive astrocytes (also known as astrocytosis) and further microgliosis as well as cause synaptotoxicity and neurotoxicity (Singh, 2022). Interestingly, when the microgliosis and astrocytosis remains uncontrolled, pathogenic proteins such as amyloid beta, alpha-synuclein and tau can be aggregated and accumulated in the CNS and consequently result in promotion of neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease (Singh, 2022; W. Zhang et al., 2023). As proteinopathy in CNS cause further neuroinflammation, it is important to modulate neuroinflammation appropriately to stop vicious cycle of neurodegeneration and ensure healthy brain (Singh, 2022). In the current study, aged mice had microgliosis and astrocytosis in both striatum and hippocampus measured by Iba-1 and GFAP expression, respectively but 6S-treated aged mice showed lower Iba-1-positive and GFAP-positive cells in the CNS (Figs. 4 and 5). The data were in line with our previous researches elucidated that 6S reduced neuroinflammation induced by endotoxins or oligomeric form of amyloid beta (Ha et al., 2012; Moon et al., 2014). Therefore, our data implies that the effects of 6S treatment on aging-associated neuronal damages were also supported by its anti-neuroinflammatory attribute.

Furthermore, the current study examined impact of 6S treatment on neurogenesis in the aged brain as shown in Fig. 6. In mammals, limited neurogenic brain regions such as SVZ and subgranular zone (SGZ) in hippocampus operate neurogenesis throughout life (Culig, Chu, & Bohr, 2022; Kase, Shimazaki, & Okano, 2020). However, the number of neural stem cells (NSCs) or neural progenitor cells (NPCs) located in those neurogenic areas are decreased with advancing years (Culig et al., 2022; Seib & Martin-Villalba, 2015). Aged mice over 21 months had severe reduction of cells expressing doublecortin (DCX) which is a marker of immature neuroblasts in SVZ (Ahlenius, Visan, Kokaia, Lindvall, & Kokaia, 2009) and in SGZ (Yang et al., 2015). To evaluate effect of 6S on neurogenesis, the current study measured the number of DCX-positive cells in SVZ region where the largest pool of NSCs in rodents exists (Cutler & Kokovay, 2020). As shown in Fig. 6, aged mice had reduction of DCX + cells in SVZ region compared to the young mice. However, 6S-treated aged mice showed greater number of DCX + cells in SVZ region than the aged control group. Thus, the data suggests that 6S treatment

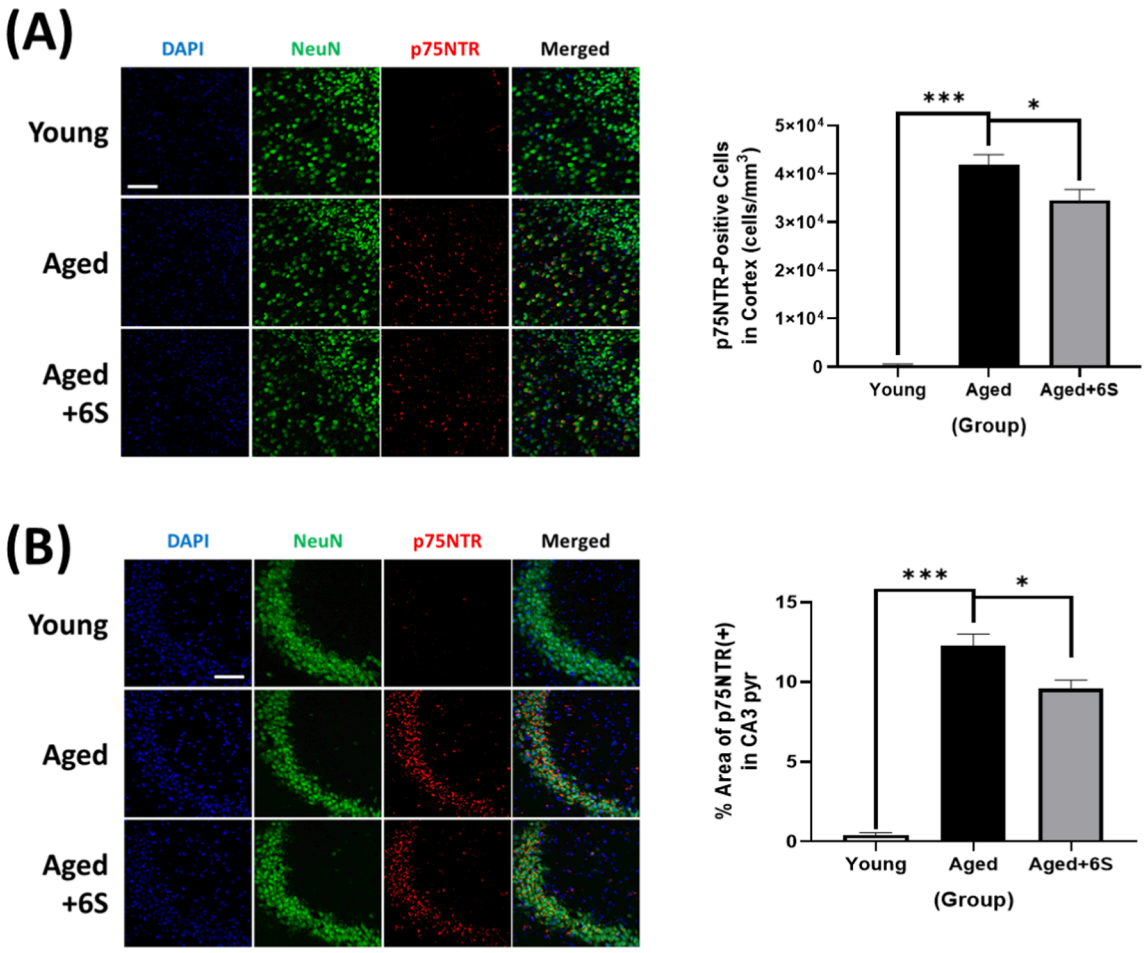

Fig. 7. 6S treatment reduced neuronal p75NTR expression in the 25-month-old C57BL/6 mice. Aged mice were treated vehicle or 10 mg/kg of 6S for 28 days (N = 6 per group). Immunofluorescence was performed to measure p75NTR expression in the cortex (A) and hippocampus (B). Area of p75NTR-positivev neurons were was quantified as shown on the right panel of each section. The data were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test: *p < 0.05 and ***p < 0.001 vs. the aged group.

can promote neurogenesis which might control the behavioral changes found in the aged mice accompanied with prevention of neuronal damages.

Lastly, our group investigated the effect of 6S treatment on p75NTR expression in the brain (Fig. 7). As mentioned above, p75NTR plays a key role in the brain fitness in terms of synaptic plasticity, neuroinflammation and neurogenesis in a negative way (Dechant & Barde, 2002; Wong et al., 2021; D. Zhang et al., 2021). Indeed, aged mice and mice with neurologic disorders had higher expression of p75NTR (Manucat-Tan et al., 2019; Wang et al., 2011). Likewise, as shown in Fig. 7, the aged mice had significantly greater p75NTR expression in cortex and hippocampal CA3 region compared to the young mice. However, 6S administration substantially reduced p75NTR expression both in cortex and CA3 in the aged mice. Both data are in line with the results that the aged mice showed typical neurodegenerative features including more neuronal damages, less synaptic plasticity, higher neuroinflammation and lower neurogenesis but 6S treatment repaired all the detrimental conditions. Similarly, a previous report demonstrated genetic and chemical normalization of p75NTR delayed the onset of motor deficits in rodent model of Huntington's disease (Suelves et al., 2019). Another group documented that p75NTR depletion inhibited tau phosphorylation and behavioral alteration in the tau-P301L transgenic mice (Manucat-Tan et al., 2019). Especially, our *in silico* data exhibited that 6S have high affinity to p75NTR (Fig. 8 and Table 1). Thus, the results imply that neuronal rejuvenating efficacy of 6S is mediated by its inhibitory effect on p75NTR. Additionally, 6S can be further considered as potent agent for any other human disease related to p75NTR expression such as schizophrenia, bronchial asthma and other autoimmune disorders (Schor, 2005).

5. Conclusions

Taken together, the present findings provide the evidence that 6S can be a novel senolytic for brain senescence through attenuation of neuronal damages and neuroinflammation but promotion of synaptic plasticity and neurogenesis. Especially, this study is the first to demonstrate that 6S can normalize p75NTR expression in the aged brain which is potential target of brain rejuvenation. Thus, 6S has highly potential role in nutraceutical effects and treatment strategies for aging-related neurologic disorders.

Funding statement

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2022R1A6A3A01087061, and NRF-2017R1A5A2014768). This work was also supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT, 2021R1A2C2007708).

Ethics statements

All the maintenance and experimental procedure were carried out in accordance with the 'Guide for the Care and Use of Laboratory Animals, 8th edition' (National Institutes of Health, 2011) and the 'Animal Care and Use Guidelines' of Kyung Hee University, Seoul, Korea [approval

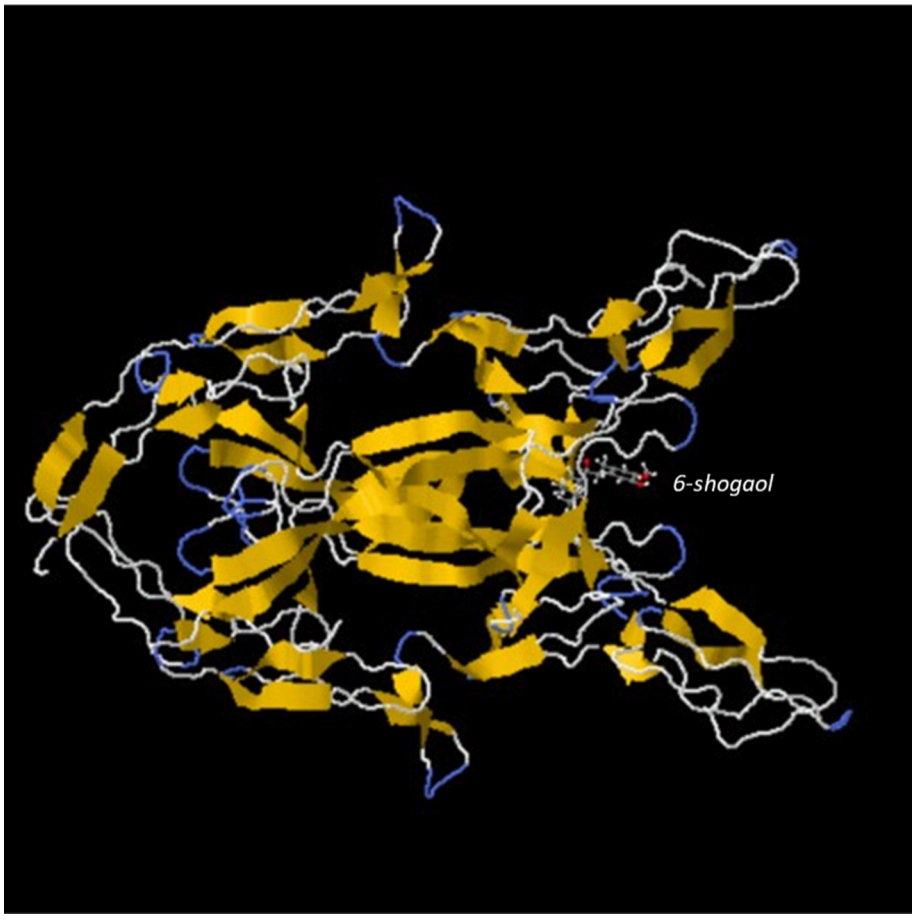

Fig. 8. Molecular docking analysis of 6-shogaol Docking result of SwissDock showing the three-dimensional interaction of 6-shogaol with p75NTR (PDB ID: 3IJ2).

number: KHUASP(SE)–22–415].

CRediT authorship contribution statement

Hyeyoon Eo: Conceptualization, Formal analysis, Funding acquisition, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Jin Hee Kim:** . **Hyeri Im:** Methodology, Resources. **In Gyoung Ju:** Validation, Writing – review & editing. **Eugene Huh:** Validation. **Rabin Pun:** Resources. **Dongyun Shin:** Resources. **Yunsook Lim:** Conceptualization, Funding acquisition, Project administration, Supervision, Validation. **Myung Sook Oh:** Conceptualization, Validation, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgement

Graphical abstract was created with BioRender.com.

References

Ahlenius, H., Visan, V., Kokaia, M., Lindvall, O., & Kokaia, Z. (2009). Neural stem and progenitor cells retain their potential for proliferation and differentiation into functional neurons despite lower number in aged brain. *The Journal of Neuroscience, 29*(14), 4408–4419. https://doi.org/10.1523/jneurosci.6003-08.2009

Barbaccia, V., Bravi, L., Murmura, F., Savelli, E., & Viganò, E. (2022). Mature and older adults' perception of active ageing and the need for supporting services: insights from a qualitative study. *International Journal of Environmental Research and Public Health, 19*(13). https://doi.org/10.3390/ijerph19137660

Bergado, J. A., & Almaguer, W. (2002). Aging and synaptic plasticity: A review. *Neural Plasticity, 9*(4), 217–232. https://doi.org/10.1155/np.2002.217

Burke, S. N., & Barnes, C. A. (2006). Neural plasticity in the ageing brain. *Nature Reviews Neuroscience, 7*(1), 30–40. https://doi.org/10.1038/nrn1809

Collaborators, G. B. D. A. (2022). Global, regional, and national burden of diseases and injuries for adults 70 years and older: Systematic analysis for the Global Burden of Disease 2019 Study. *BMJ, 376*, e068208.

Couillard-Despres, S., Winner, B., Schaubeck, S., Aigner, R., Vroemen, M., Weidner, N., & Aigner, L. (2005). Doublecortin expression levels in adult brain reflect neurogenesis. *The European Journal of Neuroscience, 21*(1), 1–14. https://doi.org/10.1111/j.1460-9568.2004.03813.x

Culig, L., Chu, X., & Bohr, V. A. (2022). Neurogenesis in aging and age-related neurodegenerative diseases. *Ageing Research Reviews, 78*, Article 101636. https://doi.org/10.1016/j.arr.2022.101636

Cutler, R. R., & Kokovay, E. (2020). Rejuvenating subventricular zone neurogenesis in the aging brain. *Current Opinion in Pharmacology, 50*, 1–8. https://doi.org/10.1016/j.coph.2019.10.005

Dechant, G., & Barde, Y.-A. (2002). The neurotrophin receptor p75NTR: Novel functions and implications for diseases of the nervous system. *Nature Neuroscience, 5*(11), 1131–1136. https://doi.org/10.1038/nn1102-1131

Fernandez, E., Schiappa, R., Girault, J. A., & Le Novère, N. (2006). DARPP-32 is a robust integrator of dopamine and glutamate signals. *PLoS Computational Biology, 2*(12), e176.

Flatt, M. A., Settersten, R. A., Jr., Ponsaran, R., & Fishman, J. R. (2013). Are "anti-aging medicine" and "successful aging" two sides of the same coin? Views of anti-aging practitioners. *The Journals of Gerontology. Series B, Psychological Sciences and Social Sciences, 68*(6), 944–955. https://doi.org/10.1093/geronb/gbt086

Franklin, K. B. J., & Paxinos, G. (2013). *Paxinos and Franklin's The mouse brain in stereotaxic coordinates (Fourth* (edition. ed.). Amsterdam: Academic Press, an imprint of Elsevier.

Goto, A. (2022). Synaptic plasticity during systems memory consolidation. *Neuroscience Research, 183*, 1–6. https://doi.org/10.1016/j.neures.2022.05.008

Ha, S. K., Moon, E., Ju, M. S., Kim, D. H., Ryu, J. H., Oh, M. S., & Kim, S. Y. (2012). 6-Shogaol, a ginger product, modulates neuroinflammation: A new approach to neuroprotection. *Neuropharmacology, 63*(2), 211–223. https://doi.org/10.1016/j.neuropharm.2012.03.016

Huh, E., Choi, J. G., Choi, Y., Ju, I. G., Noh, D., Shin, D. Y., & Oh, M. S. (2023). 6-shogaol, an active ingredient of ginger, improves intestinal and brain abnormalities in proteus mirabilis-induced parkinson's disease mouse model. *Biomol Ther (Seoul), 31*(4), 417–424. https://doi.org/10.4062/biomolther.2023.098

Kang, S. H., Moon, S. J., Kang, M., Chung, S. J., Cho, G. J., & Koh, S. B. (2023). Incidence of Parkinson's disease and modifiable risk factors in Korean population: A longitudinal follow-up study of a nationwide cohort. *Frontiers in Aging Neuroscience, 15*, 1094778. https://doi.org/10.3389/fnagi.2023.1094778

Kase, Y., Shimazaki, T., & Okano, H. (2020). Current understanding of adult neurogenesis in the mammalian brain: How does adult neurogenesis decrease with age? *Inflammation and Regeneration, 40*(1), 10. https://doi.org/10.1186/s41232-020-00122-x

Lee, J., & Kim, H. J. (2022). Normal aging induces changes in the brain and neurodegeneration progress: review of the structural, biochemical, metabolic, cellular, and molecular changes. *Frontiers in Aging Neuroscience, 14*, Article 931536. https://doi.org/10.3389/fnagi.2022.931536

Lubec, J., Kalaba, P., Hussein, A. M., Feyissa, D. D., Kotob, M. H., Mahmmoud, R. R., & Lubec, G. (2021). Reinstatement of synaptic plasticity in the aging brain through specific dopamine transporter inhibition. *Molecular Psychiatry, 26*(12), 7076–7090. https://doi.org/10.1038/s41380-021-01214-x

Madadi Asl, M., Vahabie, A. H., & Valizadeh, A. (2019). Dopaminergic modulation of synaptic plasticity, its role in neuropsychiatric disorders, and its computational modeling. *Basic Clin Neurosci, 10*(1), 1–12. https://doi.org/10.32598/bcn.9.10.125

Manucat-Tan, N. B., Shen, L. L., Bobrovskaya, L., Al-Hawwas, M., Zhou, F. H., Wang, Y. J., & Zhou, X. F. (2019). Knockout of p75 neurotrophin receptor attenuates the hyperphosphorylation of Tau in pR5 mouse model. *Aging (Albany NY), 11*(17), 6762–6791. https://doi.org/10.18632/aging.102202

Moon, M., Kim, H. G., Choi, J. G., Oh, H., Lee, P. K., Ha, S. K., & Oh, M. S. (2014). 6-Shogaol, an active constituent of ginger, attenuates neuroinflammation and cognitive deficits in animal models of dementia. *Biochemical and Biophysical Research Communications, 449*(1), 8–13. https://doi.org/10.1016/j.bbrc.2014.04.121

Morgan, D. G. (1987). The dopamine and serotonin systems during aging in human and rodent brain. A brief review. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 11*(2–3), 153–157. https://doi.org/10.1016/0278-5846(87)90053-4

Murman, D. L. (2015). The impact of age on cognition. *Seminars in Hearing, 36*(3), 111–121. https://doi.org/10.1055/s-0035-1555115

Nations, U., Economic, D. o., & Affairs, S. (2013). World Population Ageing: United Nations New York.

Niu, Y., Zeng, X., Zhao, L., Zhou, Y., Qin, G., Zhang, D., & Chen, L. (2020). Metabotropic glutamate receptor 5 regulates synaptic plasticity in a chronic migraine rat model through the PKC/NR2B signal. *The Journal of Headache and Pain, 21*(1), 139. https://doi.org/10.1186/s10194-020-01206-2

Noda, S., Sato, S., Fukuda, T., Tada, N., & Hattori, N. (2020). Aging-related motor function and dopaminergic neuronal loss in C57BL/6 mice. *Molecular Brain, 13*(1), 46. https://doi.org/10.1186/s13041-020-00585-6

Park, G., Kim, H. G., Ju, M. S., Ha, S. K., Park, Y., Kim, S. Y., & Oh, M. S. (2013). 6-Shogaol, an active compound of ginger, protects dopaminergic neurons in Parkinson's disease models via anti-neuroinflammation. *Acta Pharmacologica Sinica, 34*(9), 1131–1139. https://doi.org/10.1038/aps.2013.57

Sabri, S. M., Annuar, N., Rahman, N. L. A., Musairah, S. K., Mutalib, H. A., & Subagja, I. K. (2022). Major trends in ageing population research: a bibliometric analysis from 2001 to 2021. *Proceedings, 82*(1), 19.

Schor, N. F. (2005). The p75 neurotrophin receptor in human development and disease. *Progress in Neurobiology, 77*(3), 201–214. https://doi.org/10.1016/j.pneurobio.2005.10.006

Seib, D. R., & Martin-Villalba, A. (2015). Neurogenesis in the normal ageing hippocampus: a mini-review. *Gerontology, 61*(4), 327–335. https://doi.org/10.1159/000368575

She, W. C., Quairiaux, C., Albright, M. J., Wang, Y. C., Sanchez, D. E., Chang, P. S., & Lu, H. C. (2009). Roles of mGluR5 in synaptic function and plasticity of the mouse thalamocortical pathway. *The European Journal of Neuroscience, 29*(7), 1379–1396. https://doi.org/10.1111/j.1460-9568.2009.06696.x

Singh, D. (2022). Astrocytic and microglial cells as the modulators of neuroinflammation in Alzheimer's disease. *Journal of Neuroinflammation, 19*(1), 206. https://doi.org/10.1186/s12974-022-02565-0

Speranza, L., di Porzio, U., Viggiano, D., de Donato, A., & Volpicelli, F. (2021). Dopamine: The neuromodulator of long-term synaptic plasticity, reward and movement control. *Cells, 10*(4). https://doi.org/10.3390/cells10040735

Suelves, N., Miguez, A., Lopez-Benito, S., Barriga, G. G., Giralt, A., Alvarez-Periel, E., & Brito, V. (2019). Early downregulation of p75(NTR) by genetic and pharmacological approaches delays the onset of motor deficits and striatal dysfunction in huntington's disease mice. *Molecular Neurobiology, 56*(2), 935–953. https://doi.org/10.1007/s12035-018-1126-5

Takeuchi, T., Duszkiewicz, A. J., & Morris, R. G. (2014). The synaptic plasticity and memory hypothesis: Encoding, storage and persistence. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 369*(1633), 20130288. https://doi.org/10.1098/rstb.2013.0288

Tse, W., Libow, L. S., Neufeld, R., Lesser, G., Frank, J., Dolan, S., & Halbig, T. D. (2008). Prevalence of movement disorders in an elderly nursing home population. *Archives of Gerontology and Geriatrics, 46*(3), 359–366. https://doi.org/10.1016/j.archger.2007.05.008

Vaughan, R. A., & Foster, J. D. (2013). Mechanisms of dopamine transporter regulation in normal and disease states. *Trends in Pharmacological Sciences, 34*(9), 489–496. https://doi.org/10.1016/j.tips.2013.07.005

Wang, Y. J., Wang, X., Lu, J. J., Li, Q. X., Gao, C. Y., Liu, X. H., & Zhou, X. F. (2011). p75NTR regulates Abeta deposition by increasing Abeta production but inhibiting Abeta aggregation with its extracellular domain. *The Journal of Neuroscience, 31*(6), 2292–2304. https://doi.org/10.1523/JNEUROSCI.2733-10.2011

Weihe, E., Depboylu, C., Schütz, B., Schäfer, M. K., & Eiden, L. E. (2006). Three types of tyrosine hydroxylase-positive CNS neurons distinguished by dopa decarboxylase and VMAT2 co-expression. *Cellular and Molecular Neurobiology, 26*(4–6), 659–678. https://doi.org/10.1007/s10571-006-9053-9

Wiedenmann, B., & Franke, W. W. (1985). Identification and localization of synaptophysin, an integral membrane glycoprotein of Mr 38,000 characteristic of presynaptic vesicles. *Cell, 41*(3), 1017–1028. https://doi.org/10.1016/s0092-8674(85)80082-9

Wong, L. W., Chong, Y. S., Lin, W., Kisiswa, L., Sim, E., Ibáñez, C. F., & Sajikumar, S. (2021). Age-related changes in hippocampal-dependent synaptic plasticity and memory mediated by p75 neurotrophin receptor. *Aging Cell, 20*(2), e13305.

Yang, T. T., Lo, C. P., Tsai, P. S., Wu, S. Y., Wang, T. F., Chen, Y. W., & Kuo, Y. M. (2015). Aging and exercise affect hippocampal neurogenesis via different mechanisms. *PLoS One1, 10*(7), e0132152.

Zhang, D., Zhao, S., Zhang, Z., Xu, D., Lian, D., Wu, J., & Li, L. (2021). Regulation of the p75 neurotrophin receptor attenuates neuroinflammation and stimulates hippocampal neurogenesis in experimental Streptococcus pneumoniae meningitis. *Journal of Neuroinflammation, 18*(1), 253. https://doi.org/10.1186/s12974-021-02294-w

Zhang, W., Xiao, D., Mao, Q., & Xia, H. (2023). Role of neuroinflammation in neurodegeneration development. *Signal Transduction and Targeted Therapy, 8*(1), 267. https://doi.org/10.1038/s41392-023-01486-5

Zhong, Z., Guo, X., & Zheng, Y. (2022). Network pharmacology-based and molecular docking analysis of resveratrol's pharmacological effects on type i endometrial cancer. *Anti-Cancer Agents in Medicinal Chemistry, 22*(10), 1933–1944. https://doi.org/10.2174/1871520621666211015140455