

Article

Stigmasterol Is Associated with Alterations in nNOS-PSD95/CAPON Signaling and Synaptic Plasticity in a PTSD Model

Hee Ra Park, Mudan Cai and Eun Jin Yang *

Department of KM Science Research, Korea Institute of Oriental Medicine (KIOM), 1672 Yuseong-daero, Yuseong-gu, Daejeon 34054, Republic of Korea; hrpark0109@kiom.re.kr (H.R.P.); mudan126@kiom.re.kr (M.C.)

* Correspondence: yej4823@gmail.com; Tel.: +82-42-868-9497; Fax: +82-42-868-9668

Abstract

The efficacy of stigmasterol (STG) has not been previously evaluated in post-traumatic stress disorder (PTSD) models. Mice exposed to single prolonged stress with foot shock (SPS + FS) received oral STG (25 or 50 mg/kg) for 14 days. Serum corticosterone and serotonin levels were measured, anxiety and cognition were assessed, synaptic plasticity-related proteins and genes were quantified, and neuronal nitric oxide synthase (nNOS), nitric oxide (NO) accumulation, nNOS-postsynaptic density protein 95 (PSD95), and nNOS-carboxy-terminal PDZ ligand of nNOS (CAPON) interactions were evaluated. STG significantly reduced serum corticosterone levels and increased serotonin levels altered by SPS+FS exposure. Behavioral analyses revealed attenuation of anxiety-like behavior and cognitive deficits. STG increased hippocampal synaptic plasticity-related proteins and genes and increased the number and maturation of doublecortin⁺ cells. Additionally, STG suppressed the PTSD-induced nNOS overactivation and NO accumulation in the hippocampus and serum, and altered nNOS-PSD95 and nNOS-CAPON associations in the hippocampus. Together, these findings provide integrated in vivo evidence suggesting that STG may influence stress-related neurobiological pathways relevant to PTSD.

Keywords: post-traumatic disorder; stigmasterol; synaptic plasticity; neuronal nitric oxide synthase; postsynaptic density protein 95

1. Introduction

Post-traumatic stress disorder (PTSD) is a severe mental health disorder that develops after exposure to traumatic experiences and is marked by enduring anxiety, impairments in cognitive function, and recurrent intrusive memories. Although dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis and monoaminergic neurotransmission has long been implicated in PTSD, accumulating evidence identifies oxidative stress as a central mechanism underlying its pathophysiology [1,2]. In particular, excessive production of reactive oxygen species (ROS) and nitric oxide (NO) in the hippocampus disrupts synaptic integrity and neuroplasticity [3–7]. Among brain regions affected by oxidative stress, the hippocampus is particularly vulnerable due to its high metabolic demand and dense glutamatergic synapses. Chronic stress impairs dendritic arborization, reduces neurogenesis, and compromises synaptic integrity, resulting in cognitive and affective disturbances [8,9]. Neuronal nitric oxide synthase (nNOS) plays a key role in this process, as its stress-induced overactivation leads to excessive NO production and synaptic dysfunction. Under physiological conditions, nNOS-derived NO modulates synaptic signaling and plasticity. However, excessive activation of nNOS leads to excessive production of NO, which reacts with



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ROS to form peroxynitrite, triggering oxidative damage, synaptic dysfunction, and deficits in hippocampus-dependent memory [7,10,11]. Preclinical studies have demonstrated that PTSD increases hippocampal nNOS expression and activity, resulting in excessive NO production, synaptic impairments, and memory deficits [7,12]. Pharmacological or genetic modulation of nNOS attenuates oxidative stress, restore dendritic complexity, and improve behavioral outcomes in stress-exposed animals, highlighting its critical role in the pathophysiology of PTSD [12,13]. Importantly, nNOS activity is regulated through interactions with synaptic adaptor proteins, including postsynaptic density protein 95 (PSD95) and carboxy-terminal PDZ ligand of nNOS (CAPON), which attenuate nNOS signaling. Dysregulation of this nNOS signaling axis has been implicated in stress-related cognitive and affective disturbances [14]. Accordingly, changes in nNOS-PSD95 and nNOS-CAPON associations may represent molecular pathways relevant to synaptic alterations observed under stress conditions.

Given the pivotal role of oxidative stress and nNOS dysregulation in PTSD, compounds with potent antioxidant and neuroprotective activities may offer therapeutic potential. Stigmasterol (STG) is a plant-derived phytosterol commonly found in medicinal herbs and various dietary sources and has been reported to exert anti-inflammatory, antioxidant, and neuroprotective activities [15–17]. Previous studies have demonstrated that STG alleviates oxidative stress and neuroinflammation in both in vitro oxidative stress models and in vivo ischemia/reperfusion injury models, while promoting neuronal survival by regulating apoptosis and autophagy [16,18]. However, despite its well-known neuroprotective and antidepressant-like properties, the potential efficacy and molecular mechanisms of STG in PTSD remain unstudied. Therefore, this study investigated whether STG could ameliorate anxiety and cognitive impairments and neurobiological abnormalities and elucidated the underlying molecular pathways, particularly focusing on hippocampal synaptic plasticity and nNOS signaling.

We therefore hypothesized that STG would ameliorate anxiety and cognitive impairments and neurobiological abnormalities by regulating the nNOS-PSD95 and nNOS-CAPON pathways, thereby regulating hippocampal synaptic plasticity. To test this hypothesis, mice exposed to the single prolonged stress with foot shock (SPS+FS) received STG for 14 days, followed by evaluation of behavioral performance, serum stress hormone levels, expression of synaptic plasticity-related proteins and genes, and molecular interactions. Our findings demonstrate that STG shows significant anxiolytic effects and attenuates stress-induced synaptic and neurobiological impairments by suppressing nNOS pathway activation and enhancing synaptic plasticity-associated factors. These results provide exploratory suggestions into molecular pathways that may be associated with the effects of STG in this stress model and suggest that its potential relevance to stress-related neurobiological processes warrants further investigation.

2. Materials and Methods

2.1. PTSD Mouse Model

Male C57BL/6J mice (6 weeks of old; body weight 22–23 g) were supplied by Daehan BioLink (Eumseong, Chungcheongbuk-do, Republic of Korea). Mice were allowed to acclimate for one week before the initiation of experiments and were housed under controlled environmental conditions (20–23 °C, 12 h light/dark cycle), with unrestricted access to food and water. Five mice were housed per cage. All procedures were approved by the Institutional Animal Care and Use Committee of the Korea Institute of Oriental Medicine (KIOM) (IACUC no. 24-025; 28 March 2024) and conducted in compliance with National Institutes of Health (NIH) and KIOM animal welfare guidelines. To induce PTSD phenotypes, a modified single prolonged stress (SPS) protocol was employed [19]. Mice were

sequentially subjected to 4 h of restraint stress, followed immediately by forced swimming for 20 min in a cylindrical tank (24 cm diameter, 50 cm height) filled with water maintained at 24 °C. After a 15 min recovery interval, animals were exposed to diethyl ether until loss of consciousness. After an additional 1 h resting period, mice were subjected to electric foot shocks (1 mA for 5 s, administered twice at 2-day intervals) by shock chamber (Startle and Fear Combined System; Harvard Apparatus). In vivo experimental scheme is outlined in Figure 1.

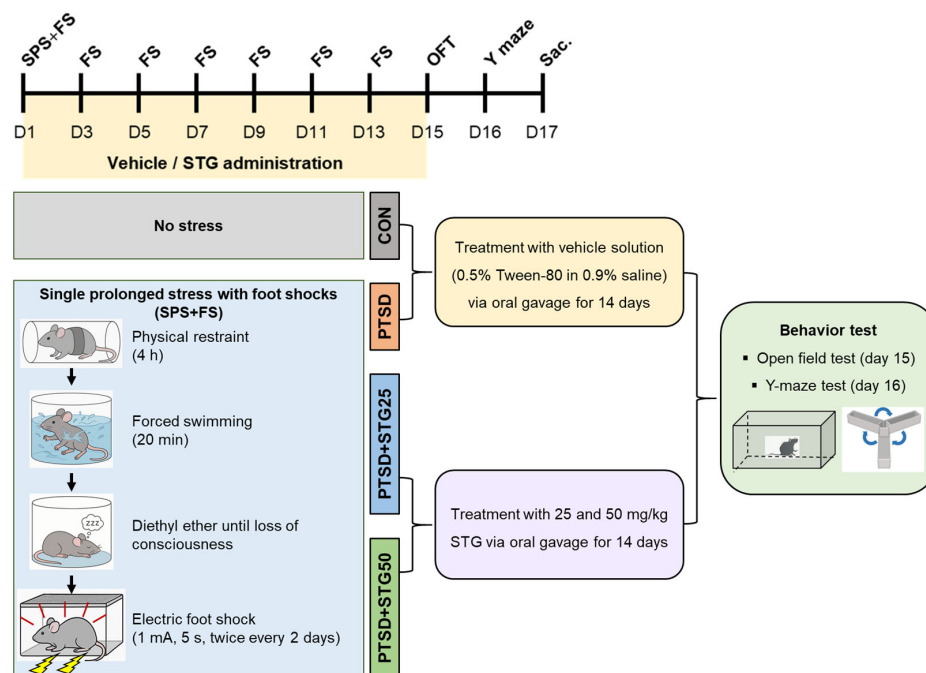


Figure 1. In vivo experimental scheme. Mice were established on the SPS+FS-induced PTSD model. Mice were orally administered vehicle solution (0.5% Tween-80 in 0.9% saline) and STG (25 and 50 mg/kg) for 14 days. On day 15 and 16, mice were subjected to behavior tests (open field test and Y-maze test). CON, control mice; PTSD, post-traumatic stress disorder; STG, stigmasterol.

2.2. STG Administration

A total of eighty mice were randomly allocated into four groups ($n = 20$ per group): unstressed control (CON), PTSD (SPS+FS-exposed mice receiving vehicle solution), PTSD treated with STG at 25 mg/kg, and PTSD treated with STG at 50 mg/kg. STG was prepared in 0.5% Tween-80 dissolved in 0.9% saline and administered by oral once daily for 14 days. Mice in the CON and PTSD groups received equivalent volumes of vehicle solution.

2.3. Exclusion Criteria and Sample Retention

No animals met the predefined exclusion criteria (health abnormalities, technical malfunction, failure to complete behavioral tasks, or compromised tissue integrity). Accordingly, no behavioral or molecular samples were excluded, and the final n values reported for each assay correspond to the full number of animals assigned to each experimental group.

2.4. Behavior Test

All behavioral testing was performed during the light phase (09:00–17:00) to minimize circadian variability, as corticosterone exhibits strong diurnal fluctuations.

2.4.1. Open Field Test (OFT)

Spontaneous locomotion and anxiety-related behaviors were evaluated using the OFT. The test was conducted in a square acrylic chamber (100 × 100 × 40 cm) featuring a white

floor and black side walls. Each mouse was placed individually at the center of the arena and permitted to explore the environment freely for 20 min, consistent with commonly used for assessing locomotor and anxiety-like behavior in mouse [20,21]. Movement-related parameters, including total distance, duration in the center zone, and the number of center zone entries, were quantified using EthoVision XT software (Version XT 8.5, Noldus, Wageningen, The Netherlands). To eliminate olfactory cues, the arena was thoroughly wiped with 70% ethanol between successive trials.

2.4.2. Y-Maze Test

Cognitive function was evaluated using a Y-shaped maze composed of three identical arms (40 cm length, 3 cm width, 15 cm height) positioned at 120° angles. Each mouse was placed at to the center of the maze and allowed to explore for 8 min, consistent with commonly used to provide sufficient opportunity for arm entries and reliable alternation measurement while minimizing fatigue or stress [22,23]. Spontaneous alternation behavior was defined as consecutive entries into three different arms without repetition. The alternation percentage was calculated as:

$$\text{Spontaneous alternation (\%)} = [\text{Number of alternations} / (\text{Total arm entries} - 2)] \times 100$$

The maze was thoroughly cleaned with 70% ethanol between sessions.

2.5. Tissue Preparation

On day 17 of the experimental protocol, mice were anesthetized with 2,2,2-tribromoethanol (Avertin; 250 mg/kg). Blood was collected during the light phase between 09:00 and 17:00, matching the time window used for behavior tests to ensure consistency with respect to circadian influences. Blood was collected for hormonal measurements and centrifuged at 3000 rpm for 15 min at 4 °C to obtain serum samples. For Western blotting, hippocampal tissues were lysed in RIPA buffer supplemented with protease and phosphatase inhibitor cocktails, followed by centrifugation at 12,000 rpm for 15 min at 4 °C. The resulting supernatants, along with serum samples, were preserved at −80 °C until further analysis. For histological procedures, animals under deep anesthesia were transcardially perfused with 4% paraformaldehyde prepared in phosphate-buffered saline (PBS). Extracted brains were post-fixed overnight, cryoprotected in 30% sucrose solution, and coronally sectioned at a thickness of 40 µm using a freezing microtome. Sections spanning −2.80 to −5.80 mm from the bregma were collected according to the Franklin and Paxinos mouse brain atlas. Every sixth serial section was selected for immunostaining.

2.6. Serum Corticosterone and Serotonin Assay

Serum corticosterone and serotonin concentrations were determined using commercially available ELISA kits (Thermo Scientific, Waltham, MA, USA and MyBioSource, San Diego, CA, USA, respectively). Assays were conducted in duplicate according to the manufacturer's protocols. Absorbance values were measured at 450 nm by microplate reader (SpectraMax i3, Molecular Devices, San Jose, CA, USA), and corticosterone and serotonin concentrations were calculated from standard curves.

2.7. Western Blot Analysis

Protein concentrations were determined using the bicinchoninic acid (BCA) method. Equivalent quantities of protein (20 µg per sample) were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and subsequently transferred onto polyvinylidene difluoride membranes. The membranes were then blocked with a skim milk-based blocking solution and incubated with primary antibodies at 4 °C overnight. HRP-

conjugated secondary antibodies were applied, and signals were detected using enhanced chemiluminescence (SuperSignal™ West Femto, Thermo Scientific). The intensities were quantified using software (Image Lab software version 6.1.0, Bio-Rad, Hercules, CA, USA). Membranes were stripped and re-probed as necessary (Thermo Scientific), and molecular weight markers were used for band verification. All proteins analyzed in this study were pre-specified a priori based on their established roles in hippocampal synaptic plasticity and nNOS-associated signaling. Primary antibodies employed in this study included post-synaptic density protein 95 (PSD95; mouse, 1:1000; #sc-32290, Santa Cruz Biotechnology, Dallas, TX, USA), brain-derived neurotrophic factor (BDNF; rabbit, 1:1000; #PA5-85730, Invitrogen, Waltham, MA, USA), phosphorylated extracellular signal-regulated kinase (p-ERK, Thr202/Tyr204; rabbit, 1:1000; #4370, Cell Signaling Technology, Danvers, MA, USA), total ERK (t-ERK; rabbit, 1:1000; #9102, Cell Signaling Technology), phosphorylated cAMP response element-binding protein (p-CREB, Ser133; rabbit, 1:1000; #ab32096, Abcam, Cambridge, UK), total CREB (t-CREB; rabbit, 1:1000; #9197, Cell Signaling Technology), neuronal nitric oxide synthase (nNOS; mouse, 1:1000; #sc-5302, Santa Cruz Biotechnology), phosphorylated Ca^{2+} /calmodulin-dependent protein kinase II (p-CaMKII; rabbit, 1:1000; #12716, Cell Signaling Technology), total CaMKII (t-CaMKII; rabbit, 1:1000; #3357, Cell Signaling Technology), carboxy-terminal PDZ ligand of nNOS (CAPON; mouse, 1:1000; #sc-374504, Santa Cruz Biotechnology), and α -tubulin (mouse, 1:5000; #MA5-31466, Invitrogen), which served as a loading control.

2.8. Reverse Transcription-Quantitative Polymerase Chain Reaction (RT-qPCR)

RNA was isolated from hippocampus and reverse-transcribed into complementary deoxyribonucleic acid (cDNA). Quantitative PCR was performed using SYBR Green chemistry on a QuantStudio™ 6 Flex system (Life Technologies, Carlsbad, CA, USA). Amplification conditions consisted of an initial denaturation step followed by 40 cycles of denaturation at 95 °C for 30 s and combined annealing/extension at 60 °C for 60 s. Gene expression data were normalized against glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an internal reference using the comparative Ct ($2^{-\Delta\Delta\text{Ct}}$) approach. All genes analyzed in this study were pre-specified a priori based on their established roles in hippocampal synaptic plasticity and nNOS-associated signaling. Primer sequences are provided in Table 1.

Table 1. Primer sequences used for RT-qPCR.

Name	Primer and Sequence (5'-3')	Amplicon Size (bp)
PSD95	Forward-GCTCCCTGGAGAATGTGCTA	20
	Reverse-TGAGAAGCACTCCGTGAAC	20
BDNF	Forward-TTACTCTCTGGGTTCTCTGA	20
	Reverse-ACGTCCACTTCTGTTCTCT	20
SYP	Forward-GCCTACCTTCTCCACCCTTT	20
	Reverse-GCACTACCAACGTACAGAC	20
NeuroD2	Forward-AAGCCAGTGTCTCTTCGTGG	20
	Reverse-TTGGACAGCTTCTGCGTCTT	20
NeuroD6	Forward-ATGCGACACTCAGCCTGAAA	20
	Reverse-CTGGGATTCTGGGCAATACGA	20
Calb1	Forward-TCTGGCTTCATTTTCGACGCTG	21
	Reverse-ACAAAGGATTTCATTTCCGGTGA	23
CCK	Forward-ATACATCCAGCAGGTCCGCAA	21
	Reverse-CAGACATTAGAGGCGAGGGGT	21
TAC1	Forward-CTAAATTATTGGTCCGACTG	20
	Reverse-TTCTGCATTGCGCTTCTTTC	20
Stx1a	Forward-CCGAACCCCGATGAGAAGAC	20
	Reverse-TGCTCTTTAGCTTGGAGCGA	20

Table 1. *Cont.*

Name	Primer and Sequence (5'-3')	Amplicon Size (bp)
nNOS	Forward-CTGGTGAAGGAACGGGTCAG	20
	Reverse-CCGATCATTGACGGCGAGAAT	21
GAPDH	Forward-CCTCGTCCCGTAGACAAA	18
	Reverse-AATGAAGGGGTCGTTGATG	19

BDNF, brain-derived neurotrophic factor; Calb1, calbindin 1; CCK, cholecystokinin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; nNOS, neuronal nitric oxide synthase; NeuroD2, neuronal differentiation factor 2; NeuroD6, neuronal differentiation factor 6; PSD95, postsynaptic density protein 95; SYP, synaptophysin; Stx1a, syntaxin 1a; TAC1, tachykinin precursor 1.

2.9. Immunostaining

Free-floating brain sections were blocked and incubated with primary antibodies overnight at 4 °C, followed by incubation with fluorescent secondary antibodies. Confocal images were acquired using an Olympus FV10i microscope (Olympus, Tokyo, Japan) under identical acquisition settings. Immunopositive cells were quantified within the granule cell layer in the hippocampus. The primary antibodies used were anti-doublecortin (DCX; 1:500, rabbit, #40619, Cell Signaling Technology), anti-nNOS (1:500, mouse, #sc-5302, Santa Cruz Biotechnology), and anti-PSD95 (1:500, rabbit, #ab18258, Abcam). DCX-positive (DCX⁺) cells in the dentate gyrus were quantified from 10 coronal sections per mouse, with each section spaced 240 µm apart, resulting in 30 sections per group (5 mice/group). Analyses were performed by investigators blinded to experimental conditions. A DCX⁺ cell was classified as one with a clearly defined soma within the granule cell layer in the dentate gyrus of the hippocampus, exhibiting fluorescence intensity at least twice the background standard deviation, and showing detectable cytoplasmic DCX labeling in a minimum two consecutive z-stacks. To ensure consistency across samples, all confocal images were acquired using identical laser power, gain, offset, and pinhole configurations. Quantitative analyses were performed independently by blinded researchers.

2.10. NO Analysis

NO levels in serum and hippocampal samples were quantified using a Griess-based assay kit (EZ-NO assay kit, DoGenBio, Seoul, Republic of Korea). Following protein removal, samples and standards were incubated sequentially with Griess reagents, and absorbance was measured at 540 nm using a microplate reader. NO concentrations were calculated using standard curves.

2.11. Co-Immunoprecipitation (Co-IP)

Co-IP analysis was performed using a Pierce Co-IP Kit (Thermo Scientific) according to the manufacturer's instructions. Briefly, pooled hippocampal tissues from five mice per group were homogenized in ice-cold IP lysis buffer supplemented with Halt protease and phosphatase inhibitor cocktail (Thermo Scientific) to obtain sufficient protein yield and relative interaction shifts. The lysates were centrifuged at 13,000 × g for 15 min at 4 °C. Supernatants were precleared by incubation with 25 µL of control agarose resin for 1 h at 4 °C, and centrifuged to remove nonspecifically bound proteins. Subsequently, mouse anti-nNOS antibody (#sc-5302, Santa Cruz Biotechnology) was added at a concentration of 2 µg per 100 µg of total protein and incubated overnight at 4 °C. The antibody–protein complexes were then incubated with protein A/G agarose for 1 h at 4 °C. Immune complexes were isolated by centrifugation, washed five times with lysis/wash buffer, and eluted by heating at 100 °C for 5 min in loading buffer. Eluted proteins were analyzed by Western blot.

2.12. Statistical Analysis

All statistical analyses were performed using GraphPad Prism (version 9.5.1). Group differences were evaluated using one-way ANOVA followed by an appropriate Tukey's test. Data are expressed as mean $\pm$ SEM. Differences were considered statistically significant at $p < 0.05$, with significance thresholds denoted as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

3. Results

3.1. Effect of STG Treatment on Serum Corticosterone and Serotonin Levels in PTSD Mice

The PTSD model was established by subjecting mice to a combination of SPS and FS. To examine the effect of STG in the PTSD group, STG (25 and 50 mg/kg) was orally administered for 14 days. We investigated the effect of STG on the concentrations of the corticosterone and serotonin in the SPS+FS-induced PTSD group. Serum analyses revealed significantly higher serum corticosterone concentration in the PTSD group than in the CON group, whereas STG treatment markedly suppressed serum corticosterone (Figure 2A). Serum serotonin levels were significantly reduced in the PTSD group compared with control animals, whereas administration of STG increased serotonin concentrations in the serum (Figure 2B).

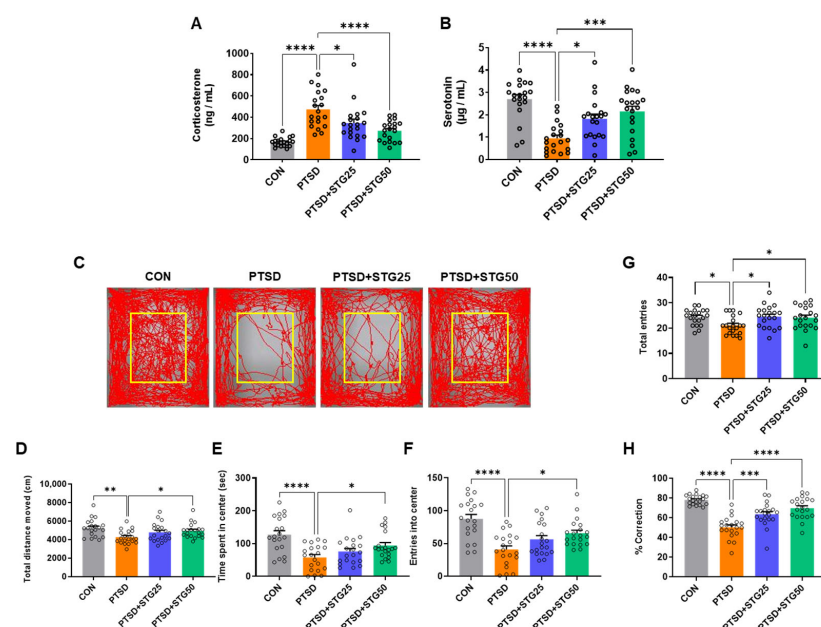


Figure 2. STG treatment reduces serum corticosterone and serotonin levels, alleviates anxiety, and improves cognitive function in PTSD mice. (A) Serum corticosterone levels across experimental groups. (B) Serum serotonin concentrations across experimental groups. (C) Representative locomotor trajectories of mice in the open field test (OFT). (D) Total distance traveled during the OFT. (E) Time spent in the central zone of the OFT. (F) Number of entries into the central zone of the OFT. (G) Total arm entry count in the Y-maze test. (H) Percentage of spontaneous alternation among the three arms in the Y-maze test. Data are expressed as the mean $\pm$ SEM ($n = 20$ mice/group); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. CON, control mice; PTSD, post-traumatic stress disorder; STG, stigmasterol.

3.2. STG Treatment Ameliorates Anxiety and Cognitive Impairments

To investigate whether STG alleviates SPS+FS-induced anxiety and cognitive deficits, mice were evaluated using the OFT and Y-maze test after 14 days of STG treatment. Relative to control (CON) animals, mice subjected to PTSD exhibited diminished locomotor activity, as evidenced by shorter durations spent in the central area and a reduced frequency of center zone entries, reflecting an anxiety-like behavior (Figure 2C). Treatment with 50 mg/kg

STG resulted in a total distance that was significantly higher than that of the PTSD group. In the open field test, mice receiving STG at 50 mg/kg exhibited increased time spent in the center zone and a higher frequency of center entries compared with the PTSD group, whereas administration of STG at 25 mg/kg failed to ameliorate PTSD-associated anxiety-like behavior (Figure 2C). In line with the hypoactivity detected in the open field test, PTSD-exposed mice showed a marked reduction in the total number of entries across all three arms in the Y-maze task (Figure 2D). In contrast, STG treatment markedly increased total arm entries in the Y-maze. Moreover, PTSD mice showed a significantly lower rate of spontaneous alternation, reflecting repeated re-entry into previously visited arms, relative to control animals; this impairment was reversed following STG administration (Figure 2D).

3.3. STG Alleviates Synaptic Plasticity Impairment in PTSD Mice

To examine the effect of STG on PTSD-induced synaptic plasticity impairment, hippocampal proteins and gene expression were analyzed using Western blot and RT-qPCR. PTSD mice exhibited reduced expressions of PSD95, BDNF, ERK, and CREB in the PTSD group compared to the CON group, whereas STG treatment significantly increased these protein expressions (Figure 3A,B). Compared with the CON group, the PTSD group also had significantly reduced synaptic plasticity-related gene expressions, including PSD95, BDNF, SYP, NeuroD2, NeuroD6, Calb1, CCK, TAC1, and Stx1a, which were significantly elevated by STG treatment (Figure 3C).

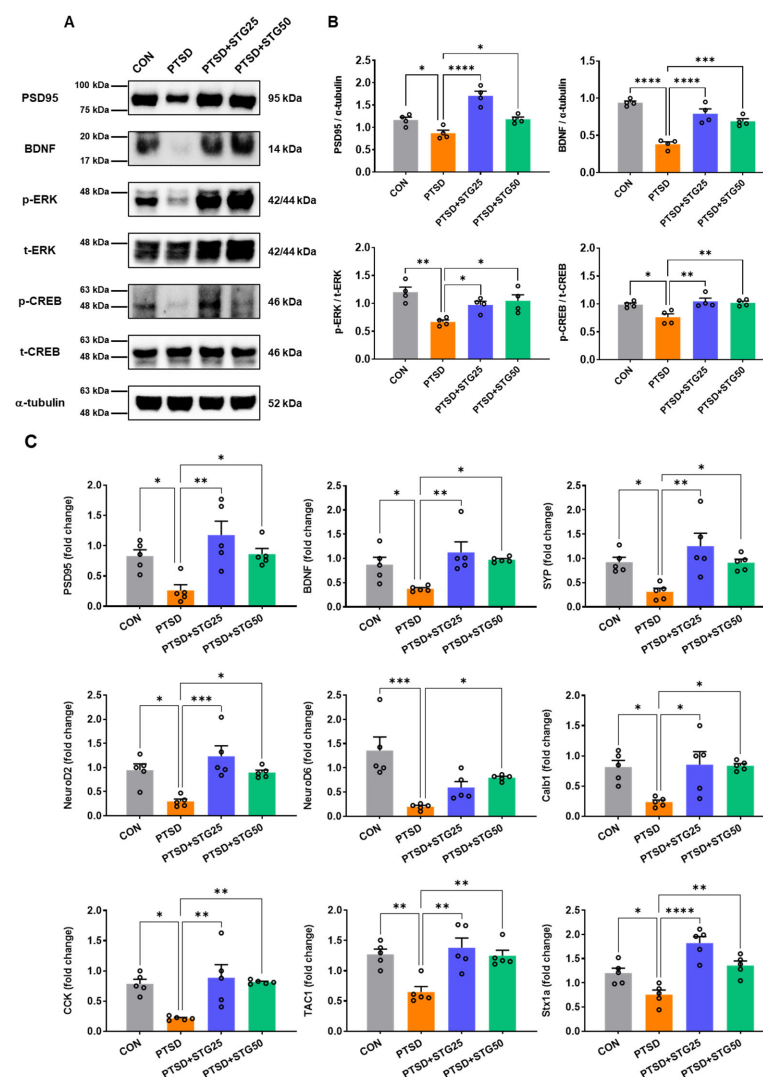


Figure 3. Cont.

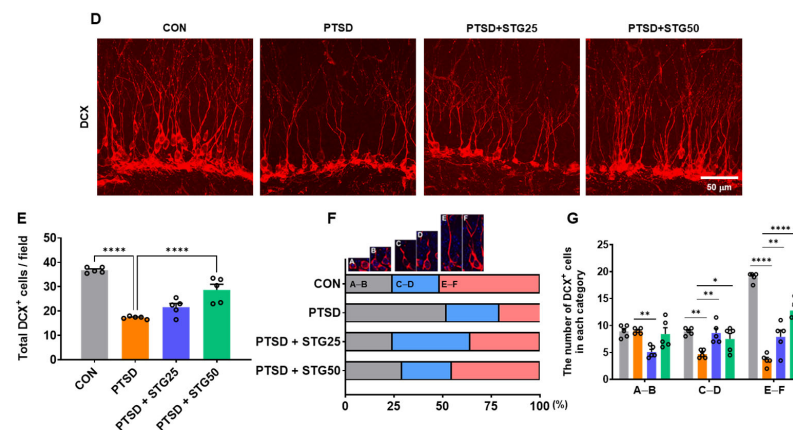


Figure 3. STG treatment protects the hippocampal synaptic plasticity impairment in PTSD mice. (A) Representative bands of synaptic plasticity-related factors (PSD95, BDNF, p-ERK, t-ERK, p-CREB, t-CREB), and α -tubulin proteins in the hippocampus. (B) Quantification of the ratio of PSD95/ α -tubulin, BDNF/ α -tubulin, p-ERK/t-ERK, and p-CREB/t-CREB by densitometric analysis, data are expressed as the mean $\pm$ SEM ($n = 4$ mice/group). (C) The quantification of mRNA expression of synaptic plasticity-related genes (PSD95, BDNF, SYP, NeuroD2, NeuroD6, Calb1, CCK, TAC1, and Stx1a) in the hippocampus. (D) Representative confocal images of the colocalization of DCX (red) in the dentate gyrus of the hippocampus. Categories AB are indicated by an asterisk, CD by an arrowhead, and EF by an arrow. Scale bar: 50 μ m. (E) Quantification of total DCX⁺ cells in the dentate gyrus of each group ($n = 10$ sections/5 mice/group). (F) Categorization of dendritic morphology from DCX⁺ cells in the dentate gyrus of the hippocampus. Category A, No processes; Category B, Short process; Category C, Medium process; Category D, Process reaching molecular layer; Category E, One dendrite branching in the molecular layer; Category F, Delicate dendritic tree branching in the granule cell layer (GCL) with quantification of the morphological distribution and number corresponding to each category. (G) Quantification of the number of DCX⁺ cells in each category. Data are expressed as the mean $\pm$ SEM ($n = 10$ sections/5 mice/group); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. BDNF, brain-derived neurotrophic factor; Calb1, calbindin 1; CCK, cholecystokinin; CON, control mice; CREB, cAMP response element-binding protein; DCX, doublecortin; ERK, extracellular signal-regulated kinase; NeuroD2, neurogenic differentiation factor 2; NeuroD6, neurogenic differentiation factor 6; p-, phosphorylated-; PSD95, postsynaptic density protein 95; PTSD, post-traumatic stress disorder; STG, stigmasterol; Stx1a, syntaxin 1a; SYP, synaptophysin; t-, total-; TAC1, tachykinin precursor 1.

DCX contributes to synaptic plasticity by regulating dendritic length and complexity, both essential for long-term potentiation (LTP) and hippocampus-dependent memory [24]. Chronic stress and PTSD have been shown to alter DCX expression and distribution in the hippocampus [25,26]. Compared with the CON group, the PTSD group exhibited a significant reduction in ex-pression and number of DCX⁺ cells in the hippocampus (Figure 3D,E). Treatment with 50 mg/kg STG significantly elevated the DCX expression and cell number (Figure 3D,E). Dendritic morphologies of DCX⁺, reflecting neuronal differentiation, were categorized and classified according to Plümpe's report [27] into proliferative (AB; no or minimal dendritic processes), intermediate (CD; moderate branching), and postmitotic (EF; full maturation of dendritic branching), comprising 24.2%, 24.2%, and 51.6% of the total population, respectively (Figure 3F,G). However, in the PTSD group, 52.1% of the DCX⁺ cells are distributed within category AB compared to the CON group (Figure 3F,G). Treatment with 50 mg/kg STG resulted in markedly increased DCX⁺ cells within category EF, reaching 45.3%, compared to the PTSD group (Figure 3F,G). These results indicate that STG ameliorates PTSD-induced impairment in hippocampal synaptic plasticity.

3.4. STG Reduces PTSD-Induced Elevation of the nNOS Pathway in the Hippocampus

To explore the mechanisms underlying the anxiolytic and synaptic plasticity-enhancing effects of STG, the expression of nNOS pathway-related factors in the hippocampus was assessed. Western blot analysis revealed that PTSD mice exhibited significantly elevated levels of hippocampal nNOS and phosphorylated CaMKII (p-CaMKII) compared to the CON group (Figure 4A,B). Treatment with 50 mg/kg STG decreased both nNOS and p-CaMKII levels, which are increased in the PTSD group (Figure 4A,B). Hippocampal nNOS mRNA expression was elevated in the PTSD group relative to control animals, whereas administration of STG at 50 mg/kg significantly reduced nNOS transcript levels (Figure 4C). Next, we analyzed the relative NO levels in the hippocampus and serum of each group. In both the hippocampus and serum, the PTSD group exhibited significantly higher NO levels than the CON group (Figure 4D,E). However, STG treatment significantly reduced the NO levels in the hippocampus and serum (Figure 4D,E).

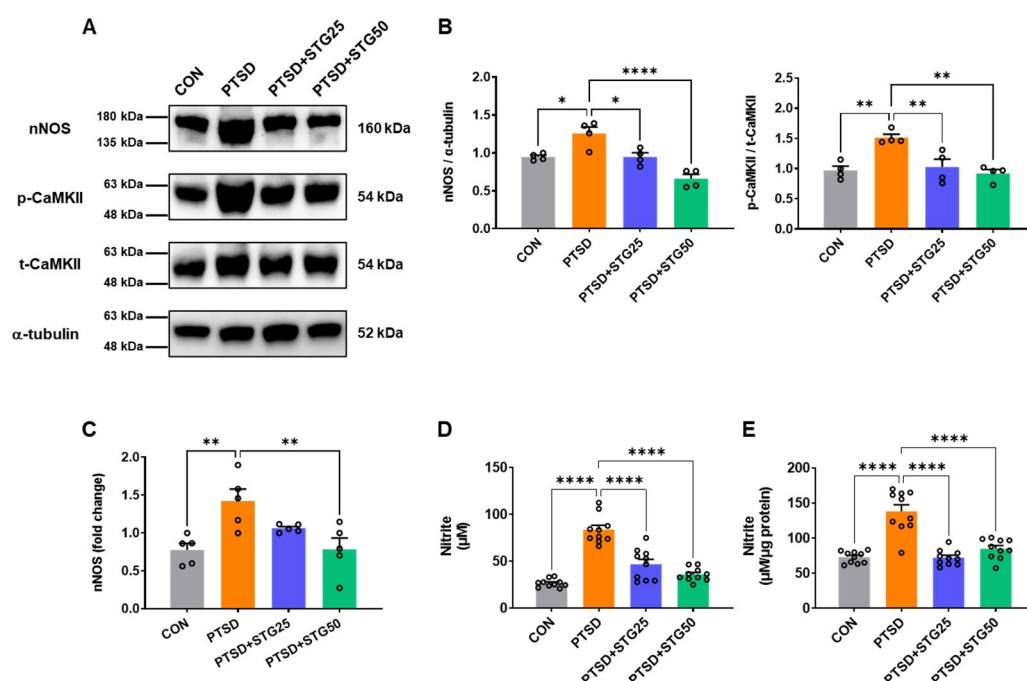


Figure 4. STG treatment regulates the nNOS pathway and NO production in the hippocampus of PTSD mice. (A) Representative immunoblot images of nNOS signaling-related proteins, including nNOS, p-CaMKII, t-CaMKII, and α-tubulin, in hippocampal tissue. (B) Densitometric quantification of nNOS normalized to α-tubulin and the ratio of p-CaMKII to t-CaMKII. Data are expressed as the mean ± SEM ($n = 4$ mice/group). (C) Relative mRNA expression levels of nNOS in the hippocampus. Data are expressed as the mean ± SEM ($n = 5$ mice/group). (D) Relative nitrite concentrations in serum samples. (E) Relative nitrite concentrations in hippocampal tissue. Data are expressed as the mean ± SEM ($n = 10$ mice/group); * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$. CaMKII, calcium/calmodulin-dependent protein kinase II; CON, control mice; nNOS, neuronal nitric oxide synthase; p-, phosphorylated-; PTSD, post-traumatic stress disorder; STG, stigmasterol; t-, total-.

3.5. STG Reduces PTSD-Induced nNOS-PSD95 Interaction

Co-IP analyses were performed using pooled hippocampal lysates to obtain sufficient protein yield; therefore, these results do not represent independent biological replicates and should be interpreted as supportive rather than standalone mechanistic evidence. We explored whether STG-treated mice showed reduced nNOS-PSD95 association in the hippocampus. In the PTSD group, the interaction between nNOS and PSD95 was higher than in the CON group, and representative images showed greater colocalization of these proteins in the hippocampus (Figure 5A,B). STG-treated mice showed lower nNOS-PSD95

association compared with the PTSD group (Figure 5A,B). In contrast, PTSD exposure was associated with reduced nNOS-CAPON interaction relative to CON, and STG treatment was accompanied by an increase in this association (Figure 5A). Taken together, these patterns suggest that PTSD is associated with opposite shifts in nNOS-PSD95 and nNOS-CAPON interactions, and that STG treatment coincides with a mitigation of these alterations of these protein-protein associations.

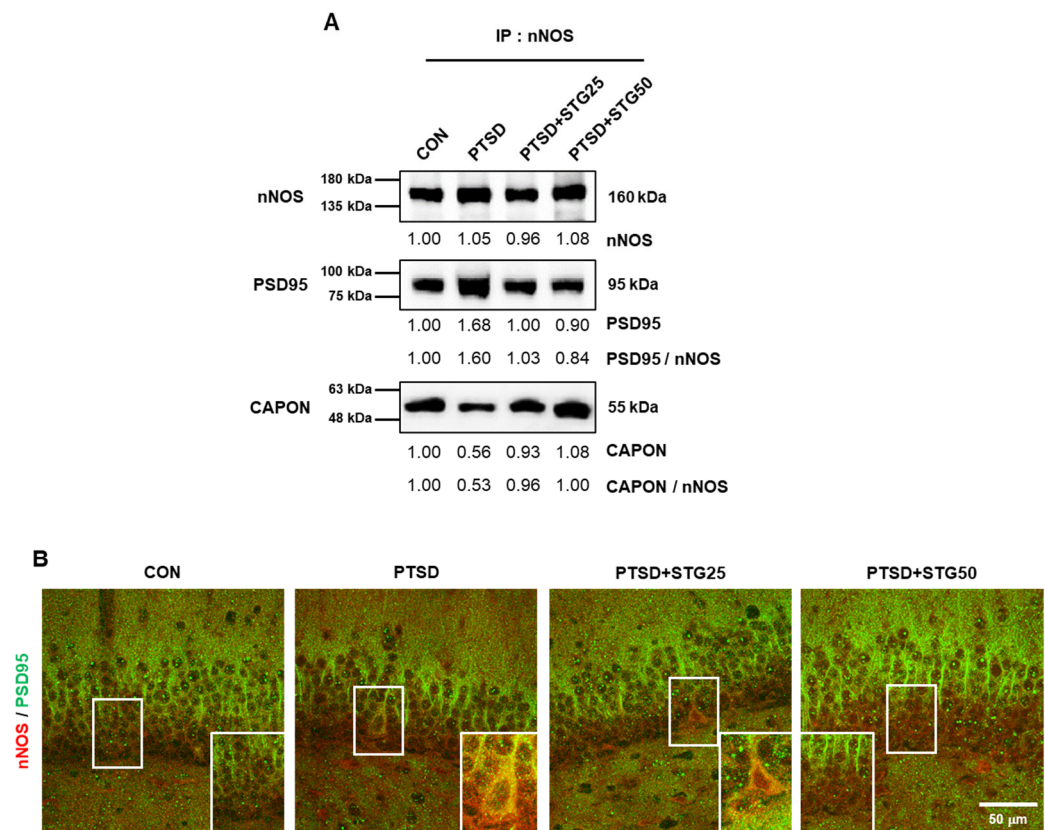


Figure 5. STG treatment is associated with altered nNOS-PSD95 and nNOS-CAPON associations in the hippocampus of PTSD mice. **(A)** Co-IP was conducted on pooled hippocampal tissue; thus, findings reflect pooled samples rather than independent biological replicates. Representative bands indicate that pooled hippocampi of each group were immunoprecipitated with nNOS antibody and immunoblotted with nNOS, PSD95, and CAPON antibodies. The intensity of each band is indicated. **(B)** Representative confocal images of the colocalization of nNOS (red) and PSD95 (green) in the dentate gyrus of the hippocampus. Scale bar: 50 μ m. CAPON, carboxy-terminal PDZ ligand of nNOS; CON, control mice; nNOS, neuronal nitric oxide synthase; PSD95, postsynaptic density protein 95; PTSD, post-traumatic stress disorder; STG, stigmasterol.

4. Discussion

In the present study, we demonstrated that STG treatment improves anxiety and cognitive impairments, synaptic plasticity-related markers, and the nNOS pathway in the hippocampus. These observations indicate a close relationship between STG treatment and reversal of stress-induced neurobiological alterations.

Emerging evidence indicates that STG confers broad neuroprotective benefits, including the suppression of inflammatory responses, mitigation of neurotoxicity, preservation of neuronal integrity, and enhancement of synaptic function, collectively supporting cognitive and brain function [15,17,28]. Phytosterols, including STG, can penetrate the blood–brain barrier and accumulate in the brain [29], further supporting their therapeutic potential for central nervous system disorders. These properties suggest that STG may have relevance for neurobiological pathways implicated in stress-related brain conditions, although further

mechanistic and translational studies are required to determine its potential significance. Although hippocampal concentrations of stigmasterol (STG) were not directly measured in this study, previous evidence supports its central availability. In particular, dietary plant sterols, including stigmasterol, have been shown to accumulate in the murine brain following peripheral administration, indicating their ability to cross the blood–brain barrier [30]. Verena et al. reported the detection of STG in the brain using GC-MS quantitative analysis of serum and brain STG after STG-enriched dietary uptake. Thus, STG may be able to penetrate the brain and exert central effects. The doses used in this study were selected based on prior *in vivo* reports demonstrating neuroprotective efficacy without overt toxicity. Nevertheless, future studies incorporating pharmacokinetic and target engagement analyses will be required to establish precise dose–exposure relationships. The doses of STG used in this study (25 and 50 mg/kg) were selected based on previous *in vivo* studies demonstrating neuroprotective and anti-inflammatory effects at comparable concentrations without overt toxicity, representing a pharmacologically relevant range for evaluating the therapeutic potential of STG in a PTSD model [17,31–33].

Behaviorally, reduced locomotor activity, decreased time spent, and fewer entries into the center zone are interpreted as indices of heightened anxiety and emotional withdrawal, reflecting avoidance of potentially threatening environments. Mice were explored for 20 min OFT because increasing the OFT duration allowed for the assessment of both novel stimulus-induced exploratory behavior in the early phase and the subsequent decline of activity due to habituation in the later phase, thereby providing a more complete profile of anxiety and hypoactivity in PTSD. Prolonged immobility or hypoactivity in the OFT has also been associated with anxiety induced by chronic stress or trauma exposure [34,35]. PTSD-exposed mice exhibited anxiety-like behavior and impaired cognitive function. Treatment with STG, particularly at 50 mg/kg, significantly ameliorated these behavioral deficits, as demonstrated by increased locomotor activity, more frequent entries, and longer time spent in the center zone in the OFT. Although reduced locomotor activity in the OFT is commonly interpreted as an index of anxiety-like behavior, it may also reflect hypoactivity, emotional blunting or low motivation induced by PTSD [36–38]. Therefore, the behavior alterations observed in this study represent a composite of anxiety-related and motivational changes rather than a single behavior dimension. The Y maze test evaluates the spatial working memory and cognitive flexibility, which depend on hippocampal integrity and synaptic plasticity [39]. The spontaneous alternation behavior, defined as consecutive entries into all three arms without repetition, reflects the ability of mice to retain and update spatial information over short time intervals [40]. Reduced alternation percentage and total arm entries indicate impairments in exploratory motivation and cognitive function including working memory commonly observed in PTSD and stress-induced cognitive dysfunction models [41,42]. Our results suggest that mice exposed to PTSD showed cognitive impairments, which were improved by STG treatment in the Y maze test. Because spontaneous alternation depends on sufficient exploratory activity, reduced arm entries in the PTSD group introduce an interpretational limitation, as lower alternation ratios may partially reflect diminished locomotor/exploratory drive rather than cognitive deficits alone. These behavioral improvements were accompanied by improvement of serum corticosterone and serotonin levels [43,44], suggesting that STG may enhance behavior improvement by reducing corticosterone and elevating serotonin. Together, these results suggest that STG may influence neurochemical and synaptic alterations associated with traumatic stress, in a manner consistent with the specific behavioral constructs assessed in this study. Because we did not evaluate fear conditioning or extinction, the behavioral conclusions are limited to anxiety-like behavior and recognition memory, and should not be generalized to broader PTSD-related domains.

nNOS is localized to the postsynaptic density through a canonical interaction between its N-terminal PDZ domain and the PDZ2 domain of PSD95, facilitating N-methyl-D-aspartate receptor (NMDAR)-dependent signaling [45]. CAPON binds to the PDZ domain of nNOS via its C-terminal PDZ-binding motif [46], competitively disrupting this canonical nNOS-PSD95 association and reducing nNOS synaptic localization and activity. Thus, PSD95 promotes canonical synaptic anchoring of nNOS, whereas CAPON functions as a negative regulator. At the molecular level, the interaction between nNOS and PSD95 plays a critical role in regulating synaptic signaling and neuronal survival under stress conditions [47,48]. PSD95 anchors nNOS to NMDAR complexes at the postsynaptic membrane, facilitating calcium-dependent activation of nNOS and subsequent NO production. Under physiological conditions, this signaling cascade supports normal synaptic transmission and plasticity. However, excessive or sustained activation of the nNOS-PSD95 complex under chronic stress or traumatic exposure leads to overproduction of NO and reactive nitrogen species, resulting in oxidative and nitrosative stress, mitochondrial dysfunction, and synaptic degeneration [49,50]. Preclinical studies show that stress-induced hyperactivation of the nNOS-PSD95 complex in the hippocampus and prefrontal cortex contributes to neuronal atrophy, reduced dendritic complexity, and behavioral manifestations resembling anxiety and depression [47,51]. In rodent models of PTSD, elevated nNOS expression and its enhanced coupling with PSD95 have been associated with impaired LTP and memory deficits [47,52]. Pharmacological disruption of the nNOS-PSD95 interaction, using selective small-molecule inhibitors such as ZL006 or IC87201, has been shown to attenuate stress-induced neuronal loss, increasing NO signaling, and reverse depressive- and anxiety-like behaviors [53–55]. In line with these findings, our study demonstrates that STG treatment was associated with attenuation of PTSD-induced overactivation of the nNOS-PSD95 interaction and a concurrent increase in nNOS-CAPON interaction, suggesting a shift toward a neuroprotective interaction profile. STG treatment markedly reduced nNOS-PSD95 interaction, while enhancing hippocampal nNOS-CAPON association. This molecular shift was accompanied by a significant reduction in NO levels, increase in synaptic plasticity-related genes and proteins (PSD95, BDNF, ERK, and CREB), and increased maturation of DCX⁺ neurons. These molecular and cellular findings were interpreted within an integrated neuroplasticity framework, reflecting coordinated patterns rather than isolated molecular effects. STG-treated mice showed changes consistent with partial preservation of hippocampal structure and function, potentially involving alterations in nNOS-associated signaling. Although the precise mechanisms underlying these protein-protein interaction changes remain undetermined, STG treatment coincided with lower NO metabolite levels and reduced nNOS-PSD95 association, suggesting a possible relationship. Overall, the results indicate that a naturally occurring phytosterol can engage components of a well-characterized synaptic signaling axis *in vivo*, yielding convergent behavioral, molecular, and cellular patterns within this PTSD-like model. By considering upstream protein associations alongside downstream signaling and neuroplasticity markers, these findings broaden existing mechanistic hypotheses derived from synthetic nNOS-PSD95 disruptors and highlight the potential relevance of endogenous compounds for stress-related neurobiological processes.

Some limitations should be acknowledged. First, future studies are needed to assess the pro-neurogenic effects of STG on behavior or neurological parameters in healthy, non-stressed mice. Second, only male mice were used; therefore, potential sex-specific differences in STG efficacy or the nNOS pathway remain unexamined. Third, although the hippocampus is central in PTSD pathophysiology, other relevant regions, such as the amygdala and prefrontal cortex, were not examined, limiting conclusions regarding regional specificity. Fourth, although STG-induced changes in nNOS-associated interactions

were observed, potential off-target effects and alternative signaling pathways cannot be excluded. Additionally, incorporating fear memory paradigms in future studies will be necessary to fully characterize associative learning components of the stress response. Finally, the lack of pharmacokinetic and target engagement analyses precludes definitive conclusions regarding direct molecular mechanisms.

5. Conclusions

Collectively, these findings provide hypothesis-generating *in vivo* evidence that STG is associated with reductions in stress-related anxiety-like behavior, cognitive deficits, and accompanying molecular alterations, including changes in nNOS-PSD95/CAPON associations and synaptic-related markers.

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Abbreviations

BDNF	brain-derived neurotrophic factor
BSA	bovine serum albumin
Calb1	calbindin 1
CaMKII	calcium/calmodulin-dependent protein kinase II
CAPON	carboxy-terminal PDZ ligand of nNOS
CCK	cholecystokinin
Co-IP	co-immunoprecipitation
CON	control
CREB	cAMP response element-binding protein
DCX	doublecortin
ERK	extracellular signal-regulated kinase
FS	foot shock
HPA	hypothalamic–pituitary–adrenal
NeuroD2	neuronal differentiation factor 2
NeuroD6	neuronal differentiation factor 6
nNOS	neuronal nitric oxide synthase
NO	nitric oxide
OD	optical density
OFT	open field test
PSD95	postsynaptic density protein 95
PTSD	post-traumatic stress disorder
qPCR	quantitative polymerase chain reaction
ROS	reactive oxygen species
SDS-PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
SPS	single prolonged stress

STG	stigmasterol
Stx1a	syntaxin 1a
SYP	synaptophysin
TAC1	tachykinin precursor 1
TBS	tris-buffered saline

References

1. Aliev, G.; Beeraka, N.M.; Nikolenko, V.N.; Svistunov, A.A.; Rozhnova, T.; Kostyuk, S.; Cherkesov, I.; Gavryushova, L.V.; Chekhonatsky, A.A.; Mikhaleva, L.M.; et al. Neurophysiology and Psychopathology Underlying PTSD and Recent Insights into the PTSD Therapies-A Comprehensive Review. *J. Clin. Med.* **2020**, *9*, 2951. [\[CrossRef\]](#)
2. Kmita, H.; Pinna, G.; Lushchak, V.I. Potential oxidative stress related targets of mitochondria-focused therapy of PTSD. *Front. Physiol.* **2023**, *14*, 1266575. [\[CrossRef\]](#)
3. Perkovic, M.N.; Milkovic, L.; Uzun, S.; Mimica, N.; Pivac, N.; Waeg, G.; Zarkovic, N. Association of Lipid Peroxidation Product 4-Hydroxynonenal with Post-Traumatic Stress Disorder. *Biomolecules* **2021**, *11*, 1365. [\[CrossRef\]](#)
4. Sun, X.R.; Zhang, H.; Zhao, H.T.; Ji, M.H.; Li, H.H.; Wu, J.; Li, K.Y.; Yang, J.J. Amelioration of oxidative stress-induced phenotype loss of parvalbumin interneurons might contribute to the beneficial effects of environmental enrichment in a rat model of post-traumatic stress disorder. *Behav. Brain Res.* **2016**, *312*, 84–92. [\[CrossRef\]](#)
5. Wilson, C.B.; McLaughlin, L.D.; Nair, A.; Ebenezer, P.J.; Dange, R.; Francis, J. Inflammation and oxidative stress are elevated in the brain, blood, and adrenal glands during the progression of post-traumatic stress disorder in a predator exposure animal model. *PLoS ONE* **2013**, *8*, e76146. [\[CrossRef\]](#)
6. Xie, P.; Chen, L.; Wang, J.; Wang, X.; Yang, S.; Zhu, G. Polysaccharides from *Polygonatum cyrtoneura* Hua prevent post-traumatic stress disorder behaviors in mice: Mechanisms from the perspective of synaptic injury, oxidative stress, and neuroinflammation. *J. Ethnopharmacol.* **2024**, *319*, 117165. [\[CrossRef\]](#)
7. Jankovic, T.; Bogicevic, M.; Knezevic, N.N. The role of nitric oxide and hormone signaling in chronic stress, anxiety, depression and post-traumatic stress disorder. *Mol. Cell Endocrinol.* **2024**, *590*, 112266. [\[CrossRef\]](#)
8. Zhou, J.; Hu, Y.; Jing, P. Antidepressant-Like Effect of Saffron (*Crocus sativus* L.) in Mice Exposed to Chronic Unpredictable Mild Stress via Attenuating Neuroinflammation and Recovering Neuroplasticity. *Mol. Nutr. Food Res.* **2025**, *69*, e70201. [\[CrossRef\]](#)
9. Hei, M.; Chen, P.; Wang, S.; Li, X.; Xu, M.; Zhu, X.; Wang, Y.; Duan, J.; Huang, Y.; Zhao, S. Effects of chronic mild stress induced depression on synaptic plasticity in mouse hippocampus. *Behav. Brain Res.* **2019**, *365*, 26–35. [\[CrossRef\]](#)
10. Shi, H.J.; Wu, D.L.; Chen, R.; Li, N.; Zhu, L.J. Requirement of hippocampal DG nNOS-CAPON dissociation for the anxiolytic and antidepressant effects of fluoxetine. *Theranostics* **2022**, *12*, 3656–3675. [\[CrossRef\]](#)
11. Munzuroglu, M.; Gokcek-Sarac, C.; Sinen, A.G.; Derin, N. Nitric oxide plays a role in anxiety- and depression-like behaviors induced by sodium metabisulfite intake in rats. *Toxicol. Lett.* **2025**, *411*, 111–121. [\[CrossRef\]](#)
12. Sadeghi, M.A.; Hemmati, S.; Yousefi-Manesh, H.; Fekrvand, S.; Foroutani, L.; Nassireslami, E.; Yousefi Zoshk, M.; Hosseini, Y.; Dehpour, A.R.; Chamanara, M. Neuronal nitric oxide synthase inhibition accelerated the removal of fluoxetine's anxiogenic activity in an animal model of PTSD. *Behav. Brain Res.* **2023**, *437*, 114128. [\[CrossRef\]](#)
13. Zhou, Q.G.; Hu, Y.; Hua, Y.; Hu, M.; Luo, C.X.; Han, X.; Zhu, X.J.; Wang, B.; Xu, J.S.; Zhu, D.Y. Neuronal nitric oxide synthase contributes to chronic stress-induced depression by suppressing hippocampal neurogenesis. *J. Neurochem.* **2007**, *103*, 1843–1854. [\[CrossRef\]](#)
14. Zhu, L.J.; Li, F.; Zhu, D.Y. nNOS and Neurological, Neuropsychiatric Disorders: A 20-Year Story. *Neurosci. Bull.* **2023**, *39*, 1439–1453. [\[CrossRef\]](#)
15. Park, S.J.; Kim, D.H.; Jung, J.M.; Kim, J.M.; Cai, M.; Liu, X.; Hong, J.G.; Lee, C.H.; Lee, K.R.; Ryu, J.H. The ameliorating effects of stigmasterol on scopolamine-induced memory impairments in mice. *Eur. J. Pharmacol.* **2012**, *676*, 64–70. [\[CrossRef\]](#)
16. Pratiwi, R.; Nantasenamat, C.; Ruankham, W.; Suwanjang, W.; Prachayasittikul, V.; Prachayasittikul, S.; Phopin, K. Mechanisms and Neuroprotective Activities of Stigmasterol Against Oxidative Stress-Induced Neuronal Cell Death via Sirtuin Family. *Front. Nutr.* **2021**, *8*, 648995. [\[CrossRef\]](#)
17. Jie, F.; Yang, X.; Yang, B.; Liu, Y.; Wu, L.; Lu, B. Stigmasterol attenuates inflammatory response of microglia via NF-kappaB and NLRP3 signaling by AMPK activation. *Biomed. Pharmacother.* **2022**, *153*, 113317. [\[CrossRef\]](#)
18. Sun, J.; Li, X.; Liu, J.; Pan, X.; Zhao, Q. Stigmasterol Exerts Neuro-Protective Effect Against Ischemic/Reperfusion Injury Through Reduction of Oxidative Stress and Inactivation of Autophagy. *Neuropsychiatr. Dis. Treat.* **2019**, *15*, 2991–3001. [\[CrossRef\]](#)
19. Souza, R.R.; Noble, L.J.; McIntyre, C.K. Using the Single Prolonged Stress Model to Examine the Pathophysiology of PTSD. *Front. Pharmacol.* **2017**, *8*, 615. [\[CrossRef\]](#)
20. Ackert-Bicknell, C.L.; Anderson, L.C.; Sheehan, S.; Hill, W.G.; Chang, B.; Churchill, G.A.; Chesler, E.J.; Korstanje, R.; Peters, L.L. Aging Research Using Mouse Models. *Curr. Protoc. Mouse Biol.* **2015**, *5*, 95–133. [\[CrossRef\]](#)

21. Selak, S.; Fritzler, M.J. Altered neurological function in mice immunized with early endosome antigen 1. *BMC Neurosci.* **2004**, *5*, 2. [[CrossRef](#)] [[PubMed](#)]
22. Yanagisawa, D.; Hamezah, H.S.; Durani, L.W.; Taguchi, H.; Tooyama, I. Study of tau pathology in male rTg4510 mice fed with a curcumin derivative Shiga-Y5. *PLoS ONE* **2018**, *13*, e0208440. [[CrossRef](#)] [[PubMed](#)]
23. Kim, J.; Kang, H.; Lee, Y.B.; Lee, B.; Lee, D. A quantitative analysis of spontaneous alternation behaviors on a Y-maze reveals adverse effects of acute social isolation on spatial working memory. *Sci. Rep.* **2023**, *13*, 14722. [[CrossRef](#)] [[PubMed](#)]
24. Cohen, D.; Segal, M.; Reiner, O. Doublecortin supports the development of dendritic arbors in primary hippocampal neurons. *Dev. Neurosci.* **2008**, *30*, 187–199. [[CrossRef](#)]
25. Dioli, C.; Patricio, P.; Sousa, N.; Kokras, N.; Dalla, C.; Guerreiro, S.; Santos-Silva, M.A.; Rego, A.C.; Pinto, L.; Ferreira, E.; et al. Chronic stress triggers divergent dendritic alterations in immature neurons of the adult hippocampus, depending on their ultimate terminal fields. *Transl. Psychiatry* **2019**, *9*, 143. [[CrossRef](#)]
26. Kurilova, E.; Sidorova, M.; Tuchina, O. Single Prolonged Stress Decreases the Level of Adult Hippocampal Neurogenesis in C57BL/6, but Not in House Mice. *Curr. Issues Mol. Biol.* **2023**, *45*, 524–537. [[CrossRef](#)]
27. Plumpe, T.; Ehninger, D.; Steiner, B.; Klempin, F.; Jessberger, S.; Brandt, M.; Romer, B.; Rodriguez, G.R.; Kronenberg, G.; Kempermann, G. Variability of doublecortin-associated dendrite maturation in adult hippocampal neurogenesis is independent of the regulation of precursor cell proliferation. *BMC Neurosci.* **2006**, *7*, 77. [[CrossRef](#)]
28. Brimson, J.M.; Brimson, S.J.; Brimson, C.A.; Rakkhitawattana, V.; Tencomnao, T. Rhinacanthus nasutus extracts prevent glutamate and amyloid-beta neurotoxicity in HT-22 mouse hippocampal cells: Possible active compounds include lupeol, stigmasterol and beta-sitosterol. *Int. J. Mol. Sci.* **2012**, *13*, 5074–5097. [[CrossRef](#)]
29. Vanmierlo, T.; Weingartner, O.; van der Pol, S.; Husche, C.; Kerksiek, A.; Friedrichs, S.; Sijbrands, E.; Steinbusch, H.; Grimm, M.; Hartmann, T.; et al. Dietary intake of plant sterols stably increases plant sterol levels in the murine brain. *J. Lipid Res.* **2012**, *53*, 726–735. [[CrossRef](#)]
30. Burg, V.K.; Grimm, H.S.; Rothhaar, T.L.; Grosgen, S.; Hundsdorfer, B.; Hauptenthal, V.J.; Zimmer, V.C.; Mett, J.; Weingartner, O.; Laufs, U.; et al. Plant sterols the better cholesterol in Alzheimer's disease? A mechanistical study. *J. Neurosci.* **2013**, *33*, 16072–16087. [[CrossRef](#)]
31. Antwi, A.O.; Obiri, D.D.; Osafo, N.; Forkuo, A.D.; Essel, L.B. Stigmasterol inhibits lipopolysaccharide-induced innate immune responses in murine models. *Int. Immunopharmacol.* **2017**, *53*, 105–113. [[CrossRef](#)] [[PubMed](#)]
32. Antwi, A.O.; Obiri, D.D.; Osafo, N.; Essel, L.B.; Forkuo, A.D.; Atobiga, C. Stigmasterol Alleviates Cutaneous Allergic Responses in Rodents. *Biomed. Res. Int.* **2018**, *2018*, 3984068. [[CrossRef](#)] [[PubMed](#)]
33. Huo, R.; Yang, W.J.; Liu, Y.; Liu, T.; Li, T.; Wang, C.Y.; Pan, B.S.; Wang, B.L.; Guo, W. Stigmasterol: Remodeling gut microbiota and suppressing tumor growth through Treg and CD8+ T cells in hepatocellular carcinoma. *Phytomedicine* **2024**, *129*, 155225. [[CrossRef](#)] [[PubMed](#)]
34. Prut, L.; Belzung, C. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: A review. *Eur. J. Pharmacol.* **2003**, *463*, 3–33. [[CrossRef](#)]
35. Crawley, J.N. Exploratory behavior models of anxiety in mice. *Neurosci. Biobehav. Rev.* **1985**, *9*, 37–44. [[CrossRef](#)]
36. Gencturk, S.; Unal, G. Rodent tests of depression and anxiety: Construct validity and translational relevance. *Cogn. Affect. Behav. Neurosci.* **2024**, *24*, 191–224. [[CrossRef](#)]
37. Sequeira-Cordero, A.; Salas-Bastos, A.; Fornaguera, J.; Brenes, J.C. Behavioural characterisation of chronic unpredictable stress based on ethologically relevant paradigms in rats. *Sci. Rep.* **2019**, *9*, 17403. [[CrossRef](#)]
38. Vu, P.A.; McNamara, E.H.; Liu, J.; Tucker, L.B.; Fu, A.H.; McCabe, J.T. Behavioral responses following repeated bilateral frontal region closed head impacts and fear conditioning in male and female mice. *Brain Res.* **2021**, *1750*, 147147. [[CrossRef](#)]
39. Kraeuter, A.K.; Guest, P.C.; Sarnyai, Z. The Y-Maze for Assessment of Spatial Working and Reference Memory in Mice. *Methods Mol. Biol.* **2019**, *1916*, 105–111. [[CrossRef](#)]
40. Hughes, R.N. The value of spontaneous alternation behavior (SAB) as a test of retention in pharmacological investigations of memory. *Neurosci. Biobehav. Rev.* **2004**, *28*, 497–505. [[CrossRef](#)]
41. Jacob, S.N.; Dodge, C.P.; Vasterling, J.J. Posttraumatic stress disorder and neurocognition: A bidirectional relationship? *Clin. Psychol. Rev.* **2019**, *72*, 101747. [[CrossRef](#)]
42. Cai, M.; Park, H.R.; Yang, E.J. Hominis Placenta modulates PTSD-like behaviors in SPSS-induced PTSD mice: Regulating energy metabolism and neuronal activity. *Biomed. Pharmacother.* **2024**, *178*, 117243. [[CrossRef](#)] [[PubMed](#)]
43. Spinelli, S.; Muller, T.; Friedel, M.; Sigrist, H.; Lesch, K.P.; Henkelman, M.; Rudin, M.; Seifritz, E.; Pryce, C.R. Effects of repeated adolescent stress and serotonin transporter gene partial knockout in mice on behaviors and brain structures relevant to major depression. *Front. Behav. Neurosci.* **2013**, *7*, 215. [[CrossRef](#)] [[PubMed](#)]
44. Dieterich, A.; Srivastava, P.; Sharif, A.; Stech, K.; Floeder, J.; Yohn, S.E.; Samuels, B.A. Chronic corticosterone administration induces negative valence and impairs positive valence behaviors in mice. *Transl. Psychiatry* **2019**, *9*, 337. [[CrossRef](#)] [[PubMed](#)]

45. Kamel, E.M.; Allam, A.A.; Rudayni, H.A.; Ahmed, N.A.; Aba Alkhayl, F.F.; Lamsabhi, A.M. Uncoupling toxic NO signaling: Progress, challenges, and therapeutic promise of disrupting the PSD-95/nNOS protein-protein interaction. *Eur. J. Med. Chem.* **2025**, *298*, 117994. [\[CrossRef\]](#)
46. Xie, W.; Xing, N.; Qu, J.; Liu, D.; Pang, Q. The Physiological Function of nNOS-Associated CAPON Proteins and the Roles of CAPON in Diseases. *Int. J. Mol. Sci.* **2023**, *24*, 15808. [\[CrossRef\]](#)
47. Li, L.P.; Dustrude, E.T.; Haulcomb, M.M.; Abreu, A.R.; Fitz, S.D.; Johnson, P.L.; Thakur, G.A.; Molosh, A.I.; Lai, Y.; Shekhar, A. PSD95 and nNOS interaction as a novel molecular target to modulate conditioned fear: Relevance to PTSD. *Transl. Psychiatry* **2018**, *8*, 155. [\[CrossRef\]](#)
48. Gu, Y.; Zhu, D. nNOS-mediated protein-protein interactions: Promising targets for treating neurological and neuropsychiatric disorders. *J. Biomed. Res.* **2020**, *35*, 1–10. [\[CrossRef\]](#)
49. Piao, W.H.; Campagnolo, D.; Dayao, C.; Lukas, R.J.; Wu, J.; Shi, F.D. Nicotine and inflammatory neurological disorders. *Acta Pharmacol. Sin.* **2009**, *30*, 715–722. [\[CrossRef\]](#)
50. Sattler, R.; Xiong, Z.; Lu, W.Y.; Hafner, M.; MacDonald, J.F.; Tymianski, M. Specific coupling of NMDA receptor activation to nitric oxide neurotoxicity by PSD-95 protein. *Science* **1999**, *284*, 1845–1848. [\[CrossRef\]](#)
51. Yu, H.; Ma, L.; Liu, D.; Wang, Y.; Pei, X.; Duan, Z.; Ma, M.; Zhang, Y. Involvement of NMDAR/PSD-95/nNOS-NO-cGMP pathway in embryonic exposure to BPA induced learning and memory dysfunction of rats. *Environ. Pollut.* **2020**, *266*, 115055. [\[CrossRef\]](#)
52. Wang, L.; Koutelou, E.; Hirsch, C.; McCarthy, R.; Schibler, A.; Lin, K.; Lu, Y.; Jeter, C.; Shen, J.; Barton, M.C.; et al. GCN5 Regulates FGF Signaling and Activates Selective MYC Target Genes during Early Embryoid Body Differentiation. *Stem Cell Reports* **2018**, *10*, 287–299. [\[CrossRef\]](#)
53. Smith, A.E.; Xu, Z.; Lai, Y.Y.; Kulkarni, P.M.; Thakur, G.A.; Hohmann, A.G.; Crystal, J.D. Source memory in rats is impaired by an NMDA receptor antagonist but not by PSD95-nNOS protein-protein interaction inhibitors. *Behav. Brain Res.* **2016**, *305*, 23–29. [\[CrossRef\]](#)
54. Bach, A.; Pedersen, S.W.; Dorr, L.A.; Vallon, G.; Ripoche, I.; Ducki, S.; Lian, L.Y. Biochemical investigations of the mechanism of action of small molecules ZL006 and IC87201 as potential inhibitors of the nNOS-PDZ/PSD-95-PDZ interactions. *Sci. Rep.* **2015**, *5*, 12157. [\[CrossRef\]](#)
55. Doucet, M.V.; Levine, H.; Dev, K.K.; Harkin, A. Small-molecule inhibitors at the PSD-95/nNOS interface have antidepressant-like properties in mice. *Neuropsychopharmacology* **2013**, *38*, 1575–1584. [\[CrossRef\]](#)

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