Translationally controlled tumor protein restores memory and synaptic function lost in animal models of dementia

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Research Article

Keywords: TCTP, transgenic, knockdown, scopolamine-induced dementia, memory, synaptic function, PSD-95, synaptophysin, synapsin-1, CREB, BDNF

Posted Date: May 24th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1675650/v1

Translationally controlled tumor protein restores memory and synaptic function lost in animal models of dementia

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Abstract

Background: The diverse roles of ubiquitously present translationally controlled tumor protein (TCTP) have been well delineated in several organs, but its possible function in the brain, especially with regard to memory function, has not received much attention. This study describes the effects of TCTP on mice with memory impaired by scopolamine (SCO) administration. Specifically, the memory and synaptic functions of 7- and 12-month-old SCO-treated wild mice (WT) were compared with those of TCTP-overexpressing (TG) and TCTP knocked down (KD) mice.

Methods: Passive-avoidance tasks were performed on WT, TG and KD mice for 4 weeks after intraperitoneal injection with SCO (1 mg/kg) or saline (CON). After completion of the behavioral studies, the hippocampi were collected and their PSD-95, synapsin-1 and synaptophysin contents analyzed by western blocking and immunohistochemical analyses, and compared with those of 5xfamilial Alzheimer's disease (5xFAD) mice and postmortem AD patients.

Results: The SCO-induced memory impairment was restored in TCTP-TG to that of WT level, but not in KD. Hippocampal expression of PSD-95, synapsin-1, and synaptophysin was increased in TG-SCO but decreased in KD-SCO mice. The decreased levels of TCTP, PSD-95, and synaptophysin were also found in the hippocampi of 5xFAD mice and AD patients. PSD-95 immunoreactivity increased particularly in dentate gyrus and CA1 in TCTP-TG, but reduced in KD. The p-CREB/CREB and brain-derived neurotrophic factor (BDNF) expressions also increased in TCTP-TG but dramatically decreased in KD.

Conclusion: TCTP restores damaged memory in mice possibly by increasing synaptic function.

Keywords

TCTP, transgenic, knockdown, scopolamine-induced dementia, memory, synaptic function, PSD-95, synaptophysin, synapsin-1, CREB, BDNF

Background

Translationally controlled tumor protein (TCTP) is a highly conserved protein present in mammalian tissues. TCTP plays important role in several biological process including cell growth, cell division, regeneration, among others [1, 2], and considered a survival and growth-promoting factor [1-3]. In addition, TCTP functions as a histamine-releasing factor that not only stimulates the release of histamine, but also modulates the release of various cytokines from immune cells [4]. We previously reported that exogenous TCTP produces an increase in neurotransmitter release from neurosecretory PC12 cells [5]. Due to these diverse and vital functions, tight regulation of TCTP expression level is essential for cells to sustain homeostasis [6], and it has been shown that unbalanced expression of TCTP can cause diseases such as cancer, diabetes [7, 8], hypertension [9], and atherosclerosis [10].

TCTP is also present in the brain as in most other organ systems. TCTP mRNA and protein are widely expressed in most regions of the adult brain, including cerebral cortex and hippocampus [3, 11-15]. TCTP transcript or protein was observed to localize in the axonal compartment of embryonic and adult neurons, implicating its synaptic functions [3,12,16]. TCTP's potential role in influencing pathophysiological conditions in the brain is suggested by TCTP's lower expression in the temporal cortices of patients with Alzheimer's disease (AD) and Down's syndrome [17], and in the schizophrenia hippocampus [18].

Chen et al reported that TCTP plays a key role in expansion of the neural precursor pool and neuronal cell survival in the newborn mouse brain, suggesting that decreases in TCTP can lead to neurodegenerative or dementia-triggering disorders [3].

The cholinergic transmission and memory processes in hippocampus in mice were shown to be impaired by the administration of scopolamine (SCO), a muscarinic receptor antagonist [19, 20]. The cAMP response element-binding protein (CREB) and of brain-derived neurotrophic factor (BDNF) have been shown to be essential for synaptic plasticity, learning and memory [21]. Therefore, loss of memory is associated with decreases in synaptic proteins such as presynaptic synaptophysin and synapsin-1, and postsynaptic density (PSD)-95 [22, 23]. Studies of SCO effects and CREB signaling and the expression of neurotrophic factor (BDNF) have become important tools in studies of memory loss.

In this study, TCTP-overexpression (TCTP-TG) and TCTP heterozygous knockdown (TCTP-KD) male mice at the age of 7-12 months were used to investigate the effects of TCTP on the memory function and synaptic and signaling in mice with SCO-induced dementia. The results demonstrated a novel memory-restoring function of TCT in the adult mouse brain. To our knowledge this study is the first to provide *in vivo* evidence linking TCTP expression and memory function.

Materials and methods

Chemicals

The following are the reagents used in this study and the sources: Anti-β-actin (sc-47778), anti-PSD-95 (sc-32290), and anti-synaptophysin (sc-17750) were purchased from Santa Cruz Biotechnology, USA; Anti-synapsin-1 (5297) was from Cell Signaling Technology, USA; and anti-pCREB (ab32096), anti-CREB (ab178322), anti-BDNF (ab108319), and anti-TCTP (ab37506) were ordered from Abcam, USA. Alexa Fluor 555-conjugated secondary antibodies (A21428) were from Invitrogen, USA; DAPI (D1306) was from Thermofisher Scientific, USA; and scopolamine (S1013) was purchased from Sigma, USA.

Animals

Adult (7 to 12-month-old) C57BL/6 male mice were used in this study. The TCTP-TG mice were generated by Macrogen (Seoul, Korea) as described in a previous study [9], and the TCTP-KD mice were kindly provided by Hsin-Fang Yang-Yen (Institute of Molecular Biology, Taipei, Taiwan). The WT/Tg-5xfamilial AD (5xFAD) mice purchased from Jackson Laboratory (Sacramento, USA) were maintained in accordance with the Animal Care and Use Guidelines of Seoul National University (permit number: SNUIBC-121018-1-1) as described in a previous study [24]. All animal studies were performed according to the Institutional Animal Care and Use Committee (IACUC) guidelines and were approved by the IACUC of Ewha Womans University (permit number: 20-010).

Human AD brains

The frozen tissues from 69- to 98-year-old AD and age-matched control subjects were obtained from the Netherlands Brain Bank (http://www.brainbank.nl/about-us/the-nbb/). Tissues from AD patients were diagnosed by neuropathological evidence using the criteria for Braak & Braak stage V or VI. The neuropathological diagnosis for non-demented controls consisted of the neuropathological criteria for classification as Braak & Braak stage 0 or I. For Western blot analysis, frozen brain tissues were used. All experimental procedures were performed in accordance with the Guidelines of the Ethics Committee at Seoul National University.

Behavioral studies

Passive avoidance learning and memory behavior tests were performed to assess memory and learning ability of the test, using step-through apparatus (Ugo Basile, USA). The experimental scheme is shown in Fig 1. During habituation (day -1), each animal was weighed and placed in the light chamber, facing away from the dark chamber, and the door to the dark chamber was opened 10 seconds later. The latency time at baseline acquisition test was measured to select outlier mice with a latency longer than the cut-off (60 seconds). WT, TCTP-TG and KD mice were injected intraperitoneally with either saline (CON) or SCO (1 mg/kg) twice on day 0 and day 27. Thirty minutes after SCO injection, mice were subjected to training session on day 0 only. During the training session, the latency time was measured at acquisition test, and when the mice entered the dark chamber, an electrical shock (0.4 mA, 3 seconds) was delivered through stainless steel rods. In retention tests after the training session, the cut-off time set to 540 seconds, and latency times were measured on days 1, 7, and 28 without foot shock.

Preparation of brain tissues and Western blot analysis

After performing behavior tests, WT and TCTP TG/KD mice were sacrificed by decapitation. The WT/Tg-5xFAD mice were euthanized with zoletil (20 mg/kg) and xylazine (9.5 mg/kg) and then perfused intracardially with 1xphosphate buffered saline (PBS). The brains were dissected, and the hippocampus taken out rapidly snap-frozen in liquid nitrogen, and stored at –80 °C. Frozen tissues were ground by tissue lyser (TissueLyser II, Qiagen, USA) and lysed in T-PER (Thermofisher Scientific, USA) containing protease inhibitor cocktails and phosphatase inhibitor cocktails (Roche, Germany) on ice for 30 minutes. The protein samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis

(SDS-PAGE) (8-12% gels) and were transferred to the nitrocellulose membrane (Cytiva, USA). The membrane was treated with Tris-buffered saline in 0.1% tween-20 (TBST) containing 5% bovine serum albumin (BSA) or 5% skim milk for 1 hour to block reactions with non-specific antibodies and incubated with the following primary antibodies at 4°C overnight. After wash with TBST, the membrane was incubated with HRP-conjugated secondary antibody for 2 hours at room temperature. The blots were visualized by enhanced chemiluminescence (Amersham Bioscience, Germany). Data collection and processing were performed using a luminescent image analyzer imaging system (ChemiDoc, Bio-Rad, USA) and immunoblots were quantified by densitometry using Image J software (http://imagej.nih.gov) and normalized to β-actin expression.

Histological analysis and immunohistochemical (IHC) studies

The mouse brains were perfused and post-fixed with 4% paraformaldehyde in PBS overnight. The tissues were dehydrated by increasing alcohol concentrations overnight. The brain tissues were paraffinized, and blocks were sectioned into 4-μm-thick slides. Hematoxylin and eosin (H&E) staining and IHC were commissioned by KP&T (Korea Pathology Technical Center, Korea). H&E and IHC sections were viewed under bright-field optical microscopy using a light microscope (Axio Scope. A1, Germany), and representative sections were photographed. The IHC sections were quantified by color deconvolution vector using Image J software.

Human AD or age-matched control brains were incubated in 10% neutral buffered formalin for 48 hours and then dehydrated and embedded in paraffin. Prior to immunostaining, slides were deparaffinized by oven heating and immersion in xylene. After dehydration through graded alcohols and water, tissue slices were immunostained overnight with a primary antibody against TCTP at 1:20, followed by Alexa Fluor 555-conjugated secondary antibodies at 1:100. Sections were stained with DAPI in PBS. After three washes in PBS, slices were mounted on microscope slides in mounting medium (P1044, Molecular Probes, USA). Confocal imaging was performed using an LSM 800 confocal microscope (Carl Zeiss, Germany). For quantification, the TCTP-positive area of 3-4 randomly selected regions was analyzed.

Statistical analysis

Experimental results are presented as the mean ± standard error of the mean (SEM) from 3 to 8 mice per group. The statistical significance of the differences between two groups was determined by the two-tailed unpaired t-test and one-way analysis of variance (ANOVA), followed by Tukey's honest significant difference test with ranks for multiple-group comparison or two-way ANOVA with Bonferroni post-hoc analysis. Statistical analyses were performed using GraphPad Prism 7 (GraphPad Software, San Diego, CA, USA). Statistical significance was considered at $P < 0.05$.

Results

Changes in memory function in SCO-induced memory impairment in TCTP-TG and TCTP-KD mice

To evaluate the effects of TCTP on memory and learning ability, the latency times in acquisition and retention were measured according to the passive avoidance test scheme presented in Fig. 1A. At the

start of the experiments, body weights were 32-42 g in C57BL/6J mice (WT & TG) and 32-38 g in C57BL/6N mice (WT & KD). On the last day of behavior test, no body weight differences were observed among groups (WT vs. TCTP-TG, WT vs. TCTP-KD, WT-CON vs. WT-SCO, TG-CON vs. TG-SCO, and KD-CON vs. KD-SCO) (Fig. 1B).

Passive avoidance tests were performed for 4 weeks after SCO administration. Latency times in acquisition test before the animals we subjected to foot shock (training session) were low (18-28 seconds) in both WT and TCTP-TG groups. In the three retention tests on 1, 7, and 28 days after the acquisition test during the training session, the TCTP-TG group did not show changes in latency (18.8±3 seconds, n=22), compared to that in the WT-CON group (22.9±4 seconds, n=17). On day 1, WT-SCO mice exhibited significantly reduced latency time (107.7±39 seconds, n=18) contrasted with that of WT-CON group (420.9±48 seconds, n=17) after foot shock, indicating SCO-induced memory impairment. Surprisingly, the SCO-induced memory impairment was sustained for weeks (day 7, 185.0±43 seconds, n=18) (day 28, 209.4±43 seconds, n=17) after a single foot shock on day 0. In TCTP-TG-SCO group, latency times were dramatically increased on day 7 (413.0±46 seconds, n=19), and on day 28 (394.2±45 seconds, n=19), compared to those in WT-SCO group on the relative days, and recovered to the levels of WT-CON group (Fig. 1C). In contrast to the TCTP-TG, the TCTP-KD group did not recover memory from the SCO-induced dementia on day 7 and even on day 28 (Fig. 1D). These behavior data establish the functional role of TCTP in memory enhancement.

Comparative changes in the expression of TCTP, PSD-95, synaptophysin, or synapsin-1 in the hippocampus of TCTP-TG/KD mice

To assess the synaptic changes associated with the effect of TCTP on memory function, we monitored the expression of signaling and synaptic marker proteins involved in memory function in the hippocampal tissues obtained from mice sacrificed immediately after the last passive avoidance tasks. The expression of TCTP was significantly higher in the TCTP-TG group (1.4-fold, n=14) and significantly lower in the TCTP-KD group (4.6-fold, n=14) compared to that in the WT group. SCO produced significant decreases in TCTP expression in WT mice, while there was no change in TCTP-TG/KD mice (Fig. 2A, B). Synaptic dysfunction is associated with decreased levels of synaptic proteins, such as presynaptic synaptophysin, synapsin-1, and postsynaptic PSD-95, in AD patients and animal models [22, 24, 25].The expression levels of those three synaptic proteins increased in the TCTP-TG-SCO group compared to the WT-SCO group (Fig. 2C-F). Even under normal conditions in the absence of SCO, overexpression of TCTP (TCTP-TG) increased the levels of synaptophysin and synapsin-1. However, TCTP-KD mice have significantly reduced levels of PSD-95 and synaptophysin both in the absence and presence of SCO compared to WT-CON (Fig. 2G-J). These results suggest that TCTP enhances synaptic strength in the hippocampus, along with exerting a memory-enhancing effect.

Comparative changes in TCTP, PSD-95, and synaptophysin in the hippocampus of 5xFAD mice and postmortem AD patients

Previous genomic and proteomic analyses showed that TCTP expression decreased in postmortem brain regions including the temporal cortex, thalamus and caudate nucleus from AD patients [17]. The 5xFAD mouse model has been widely used for studying memory decline caused by an Aβ-related gene

[26]. We have demonstrated that 6-month-old 5xFAD mice show a significant decrease in freezing behavior throughout contextual fear conditioning tests compared to WT mice. In addition, the 5xFAD group showed impaired learning and long-term memory formation and exhibited a significantly decreased mean score (37.5%) compared to WT in the t-Maze test [25]. In the present study, we examined whether changes in TCTP, PSD-95, and synaptophysin levels are seen in the hippocampus of 5xFAD mice as well as in the human AD hippocampus. Interestingly, we found that TCTP and PSD-95 expression was significantly decreased in the hippocampus of 6-month-old 5xFAD mice (Fig. 3A-C). Synaptophysin also showed a tendency to decrease (Fig. 3D). Similarly, significantly decreased levels of TCTP, PSD-95, and synaptophysin were observed in the hippocampus of humans with AD compared to those without AD (CON) (Fig. 3E-H). The immunoreactivity against TCTP was significantly decreased (CON; area 1486.5±269 μm^2, AD; 391.8±155 μm^2, p=0.012) in the hippocampi of AD patients compared with age-matched control subjects (Fig. 3I-J).

Comparative changes in p-CREB/CREB and BDNF in the hippocampus of TCTP-TG/KD mice

The CREB signaling and BDNF expression have been suggested to associate with cognitive functions [21].Previous studies have demonstrated that the learning-related protein is induced activating the CREB signaling cascade in long-term potentiation [27]. It has been also shown that SCO-induced amnesia is reversed by activation of ERK/CREB signaling [28, 29].Therefore, we investigated whether the CREB signaling and BDNF can be linked to TCTP expression in memory function in SCO-induced dementia. As shown in Figure 4, the p-CREB/CREB and BDNF levels in hippocampal tissues of TCTP-TG-SCO mice were similar to those in WT mice. However, in TCTP-KD-SCO mice, p-CREB/CREB level was significantly lowered along with a decreased in BDNF level. This finding suggests that through CREB signaling and BDNF can be linked to the role of TCTP in restoring memory function.

Immunohistochemical change in BDNF expression in the hippocampus of TCTP-TG/KD mice

Hippocampus consists of subregions including CA3, CA1 and dentate gyrus (DG), which play critical roles in learning, memory consolidation, and information retrieval [30]. Quantification of BDNF-stained cells in the DG and CA1 regions revealed a significant increase in the number of stained cells in TCTP-TG compared to WT and TCP-KD groups of mice. In all groups of mice (WT, TCTP-TG and KD), SCO reduced BDNF staining in the two hippocampal regions, as was shown in other studies also [31, 32]. The BDNF level was significantly lower in TCTP-KD-SCO group than in TCTP-TG-SCO group (Fig. 5A-D). These immunohistochemical observations further indicated that TCTP may regulate BDNF reactivity particularly in the DG and CA1 regions in hippocampus, and lead to long-term memory formation.

Discussion

Although a variety of studies have been conducted to clarify roles of TCTP protein in pathological states as well as in many different living processes in the mammalian organ systems, studies on its functions in adult brain are lacking. Moreover, the *in vivo* nervous system functions of TCTP in middle-aged adult or senescent animals, to our knowledge, have not been studied yet. Here, we demonstrated for the first time the novel functional role of TCTP in learning and memory of the middle-aged adult male mice, by showing that the SCO-induced memory impairment was significantly recovered in TCTP overexpressing

TG mice, even 4 weeks after giving a single foot shock training. Such an effect was not observed in TCTP-KD mice. The memory improving effect of TCTP was found in 34- to 45-week-old C57BL/6 mice whose average life expectancy is known to be about 100 weeks [33]. Although we recently reported that the same strain of TCTP-TG mice fed normal or high fat diet displayed more weight loss than that in WT mice when measured up to 10 weeks after birth [34], TCTP-TG and KD mice aged average 35.6 weeks did not show body weight changes compared to WT mice regardless of SCO treatment (Fig. 1B). TCTP could affect metabolism in young mice, but it does not seem to be the case in older mice.

It has been reported that post-training administration of SCO at 1 mg/kg impairs or does not affect memory consolidation in passive avoidance tasks performed in mice [35-37]. In the present study, the SCO-induced memory impairment in WT mice was sustained for one week and even for one month after a single foot shock (Fig. 2C-D). We observed that WT mice after a single dose of SCO (1 mg/kg) developed memory impairment that lasted until the end point of the preliminary behavior experiment. Our data indicate that SCO impairs memory consolidation, and that SCO-induced dementia mice can be used to study long-term memory as well as short-term memory.

We showed that the SCO-induced short- and long-term memory impairments in WT-SCO mice were all recovered significantly in TCTP-TG-SCO up to the control level in WT, and the initial level of passive avoidance response was conserved until week 4 after acquisition. The in vivo memory-restoring effect of TCTP was further supported by the finding from the behavior experiment in TCTP-KD mice, which showed that the memory impaired by SCO was not recovered in the TCTP-KD, unlike in the TCTP-TG group. Under normal conditions in the absence of SCO, TCTP-TG tend to increase latency times, and TCTP-KD decreases, although not significantly (Fig. 2C-D). As expected, the expression level of TCTP in the hippocampus was significantly higher in TCTP-TG but significantly lower in TCTP-KD.

In addition, SCO significantly reduced TCTP expression in CON mice but showed no change in TCTP-TG/KD mice. These data suggest that a decrease in the expression level of TCTP is involved in the SCO-induced impairment of memory consolidation and long-term memory.

Our finding of the memory-restoring effect of TCTP could be linked to its effects on neuronal survival and axonal synaptic function. The possible roles of endogenously expressed TCTP in neurons have been implicated in this phenomenon. TCTP has been located in axon terminals of the hippocampus of healthy 8-week-old male mice and has been implicated in neurotransmitter release [12], potentially via increase in intracellular Ca^{2+} concentration by regulating Na^{+} and K^{+}-ATPase [38]. Moreover, we previously demonstrated that TCTP increased the release of the neurotransmitter dopamine from PC12 cells via Ca^{2+}-independent phospholipase A_2 pathways [5]. TCTP has been also shown to be involved in axonal development through regulation of survival signaling and axonal mitochondrial function in the embryonic visual system [16]. In addition, a recent report demonstrated a key role of TCTP in expanding the neural precursor pool and neuronal cell survival in the newborn mouse brain and showed that the decreased TCTP can result in neurodegenerative or dementia-triggering disorders [3]. These age- and AD-related cognitive impairments correlate with modifications in synaptic plasticity, including deficits in the maintenance of hippocampal long-term potentiation [39]. Synaptic dysfunction related to a decreased level of synaptic marker proteins has been studied in AD patients and in animal models [22, 23].

Our results could also imply that the memory-restoring function of TCTP might be closely correlated

with increased synaptic activity by showing the accompanying changes in synaptic marker proteins, including PSD-95, synapsin-1, and synaptophysin. The Western blotting results showed that PSD-95 and synapsin-1 proteins increased in TCTP-TG-SCO compared to WT-SCO (Fig. 2C-F) but were reduced significantly in TCTP-KD and TCTP-KD-SCO compared to WT-SCO (Fig. 2G-J).

The decreased level of TCTP in the AD brain was first reported in an earlier study by Kim et al [17]. They had uses two-dimensional gel electrophoresis and matrix-assisted laser desorption/ionization mass spectrometry to show that TCTP was significantly reduced in the temporal cortex but not in the thalamus, caudate nucleus, or cerebellum of postmortem AD patients. In the present study, we found significantly reduced TCTP expression in the hippocampus of postmortem AD patients and also in the animal model (Fig. 3). The 5xFAD transgenic model has been widely used for studying memory decline caused by particular Aβ-related gene manipulation. The 6-month-old 5xFAD mice used in our study have demonstrated impaired learning and long-term memory formation, exhibiting a significantly decreased mean score (by 37.5%) compared to WT in the t-Maze test [25]. Synaptic proteins such as synaptophysin, syntaxin, and PSD-95 have been shown to decrease in the 6-month-old 5xFAD brain [24, 40]. Our result revealed that expression levels of the synaptic proteins PSD-95 and synaptophysin were also significantly reduced in the hippocampus of the 6-month-old 5xFAD mice as well as in the hippocampus of the postmortem AD patients, along with decreased TCTP expression. These results implicate the decrease in TCTP to be linked to other neurodegenerative or dementia-triggering disorders in addition to AD.

Numerous studies have reported that crosstalk between memory functions and CREB/BDNF signaling plays a critical role in promoting cell survival and protects against SCO-induced synaptic dysfunction, providing a therapeutic strategy for memory disorders [21, 41, 42]. We also investigated whether the effect of TCTP on memory function is related to the CREB/BDNF signaling pathway. Our results showed that p-CREB/CREB and BDNF expression levels in TCTP-TG-SCO were similar to the levels in WT mice, but TCTP-KD-SCO showed significantly lower p-CREB/CREB level along with a decreased BDNF level in the hippocampus (Fig. 4). Finally, BDNF-positive cells in the DG and CA1 regions were significantly increased in TCTP-TG but were not changed in TCTP-KD compared to WT (Fig. 5A-D). These results suggest that TCTP's memory restoring function is regulated by the presence or absence of TCTP protein and is linked partially through CREB signaling and BDNF.

Conclusion

Taken together, the present findings suggest that TCTP plays novel pivotal roles in restoring memory dysfunctions via regulation of memory-related synaptic and signaling proteins. We venture to speculate that this study may suggest a potential role for TCTP in designing therapies of memory loss.

Abbreviations

TCTP: Translationally-controlled tumor protein; BDNF: Brain-derived neurotrophic factor; SCO: Scopolamine; TCTP-TG: TCTP-overexpressed transgenic; TCTP-KD: TCTP knockdown; WT: Wild-type; AD: Alzheimer's disease; CREB: cAMP response element-binding protein; SYP: Synaptophysin; PSD-95: Postsynaptic density protein-95; 5xFAD: 5xfamilial AD; DG: Dentate gyrus; SDS: Sodium dodecyl sulfate; BSA: Bovine serum albumin

Author contributions

EJN and JYJ performed experiments, analyzed the data, and wrote the manuscript. HSK supervised experiments and data analyses and contributed to discussion. KL and H-JK conceived, designed, and supervised the research and wrote the manuscript. All authors reviewed the manuscript.

Funding

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education (2019R1F1A1057931) to H.-J. Kim and (2020R1A6A1A03043528 and 2021R1A2C2003629) to K. Lee.

Availability of data and materials

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

Declarations

Ethics approval and consent to participate

All procedures were performed according to the National Institute of Health Guide for the Care and Use of Experimental Animals and were approved by the University of Ewha Womans University Institutional Animal Care and Use Committee.

Consent for publication

Not applicable.

Competing interests

The authors have no competing interests.

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Conflicts of interest

We declare no conflict of interest in this manuscript.

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Figure legend

Fig. 1 (a) Changes in memory function in the SCO-induced dementia model of TCTP-TG/KD mice. Schematic timeline of drug treatment and passive avoidance behavior tasks in mice. (b) Saline or scopolamine (SCO, 1 mg/kg) was administered twice, on day 0 and day 27. The training session (foot shock) was conducted 30 minutes after SCO injection on day 0 only, and retention trials were performed on day 1 (1 D), day 7 (7 D), and day 28 (28 D). The latency times were measured during acquisition and retention tests. (c-d) Body weights of mice were measured during habituation and retention trials in all groups Latency times were measured in WT/TCTP-TG and WT/TCTP-KD mice.

Fig. 2 Changes in the expression of TCTP, PSD-95, synaptophysin, or synapsin-1 in the hippocampus of TCTP-TG/KD mice. a-j The hippocampal tissues from saline (CON)- or scopolamine (SCO)-administered mice were lysed and subjected to Western blotting and densitometric analyses to determine the expression levels for TCTP in WT/TG/KD mice (a, b) and those of PSD-95, synapisn-1, and synaptophysin (SYP) in WT/TG mice (c-f) and WT/KD mice (g-j). Each protein was quantified relative to β-actin. Values are presented as the mean ± SEM (n=4-8) and analyzed by two-tailed t-test (*$p < 0.05$, **$p < 0.01$ WT vs. TG, $^{\dagger}p<0.05$, WT-SCO vs. TG-SCO, $^{\#}p < 0.05$, $^{\#\#}p<0.01$ WT vs. WT-SCO, $^{++}p < 0.01$ WT vs. TCTP-KD, ‡‡ $p < 0.01$ KD vs. KD-SCO, $^{\$}p < 0.05$, $^{\$\$}p < 0.01$ WT-SCO vs. KD-SCO).

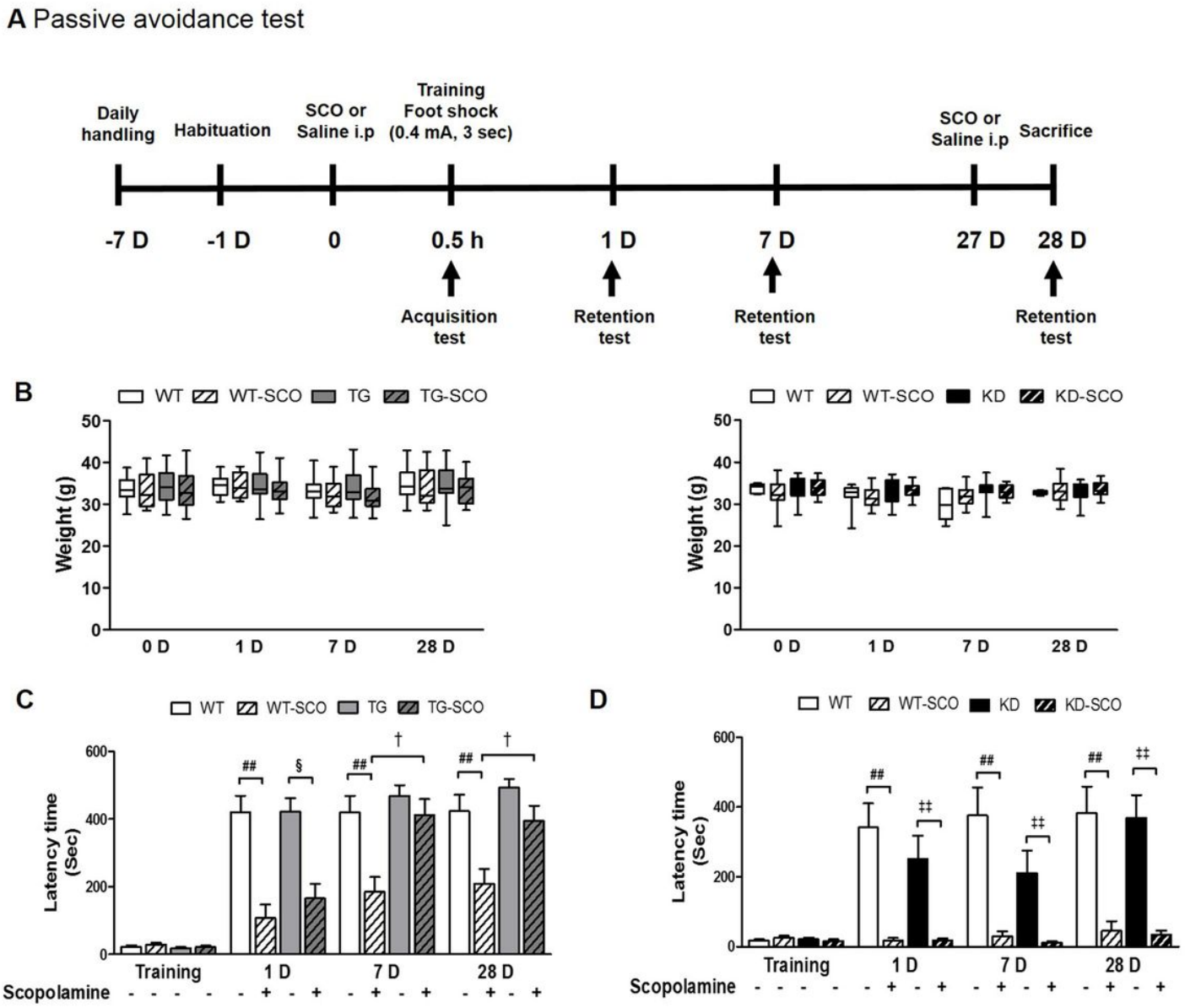
Fig. 3 Changes in TCTP, PSD-95, and synaptophysin in the hippocampus of 5xFAD mice and postpartum AD patients. a-l The hippocampal tissues from WT/5xFAD mice were lysed and subjected to Western blotting and densitometric analyses to determine the expression levels for TCTP (a, b), PSD-95 (a, c), and synaptophysin (SYP) (a, d). The hippocampal tissues from CON/AD human brains were lysed and subjected to Western blotting of TCTP (e, f), PSD-95 (e, g), and synaptophysin (SYP) (e, h). Each protein was quantified relative to β-actin. Representative images of the dentate gyrus in the hippocampus of an AD patient (70-years-old, Braak stage 5) compared with an age-matched control subject (71-years-old, Braak stage 1). TCTP immunoreactivity was measured by immunohistochemistry. Scale bars, 100 μm (inset, white square box) and 20 μm (magnified panel). (i) Quantitative graphs for TCTP immunoreactivity in age-matched control subjects and AD (n=4, Student's t-test) (j). Values are presented as the mean ± SEM (n=4-6) and analyzed by two-tailed t-test ($^{++}p < 0.01$ WT vs. Tg-5xFAD, *$p < 0.05$ CON vs. AD patient, **$p < 0.01$ CON vs. AD patient).

Fig. 4 Changes in p-CREB/CREB and BDNF in the hippocampus of TCTP-TG/KD mice. a-f The hippocampal tissues from saline (CON)- or scopolamine (SCO)-administered mice were lysed and subjected to Western blotting and densitometric analyses to quantify the levels for p-CREB/CREB (a, b) and BDNF (a, c) in WT/TG mice and p-CREB/CREB (d, e) and BDNF (d, f) in WT/KD mice. BDNF level was quantified relative to β-actin. Values are presented as the mean ± SEM (n=3-4 or 6-8) and analyzed by two-tailed t-test ($^{++}p < 0.01$ WT vs. KD, $^{\#\#}p < 0.01$ WT vs. WT-SCO, $^{\$\$}p < 0.01$ WT-SCO vs. KD-SCO, $^{\ddagger\ddagger}p < 0.01$ KD vs. KD-SCO.)

Fig. 5 Immunohistochemical (IHC) change in BDNF expression in the hippocampus of TCTP-TG/KD mice. The brains from WT/TG/KD mice administered saline (CON) or scopolamine (SCO) were

perfused and post-fixed overnight; the tissues were dehydrated, paraffinized, and sectioned into 4-μm-thick slides and IHC staining was performed for BDNF. a-d The BDNF-stained cells in the dentate gyrus (DG) region (a) and CA1 region (b) were quantified by color deconvolution vector using Image J software. Relative optical densities are expressed as a percentage of the BDNF immunoreactivity detected in the DG (c) and CA 1 (d) for each section (n=3 per group). The images were magnified by 10X, and the scale bar is 100 μm. Values are presented as the mean ± SEM (n=3-4 or 6-8) and analyzed by two-tailed t-test (*$p < 0.05$ WT vs. TG, $^{\S}p < 0.05$ TG vs. TG-SCO, $^{\&}p < 0.01$ TCTP-TG vs. KD).

Figures



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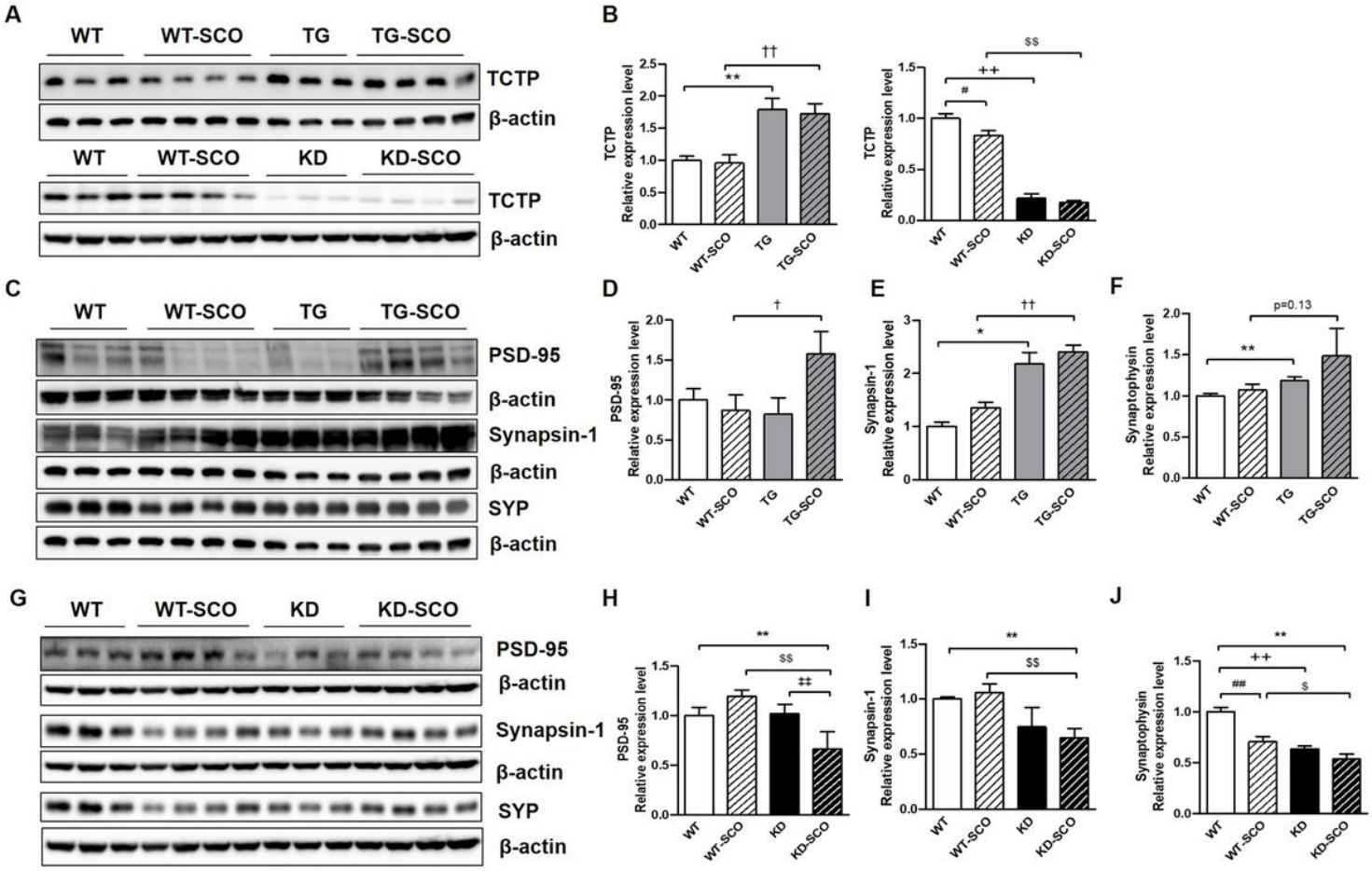

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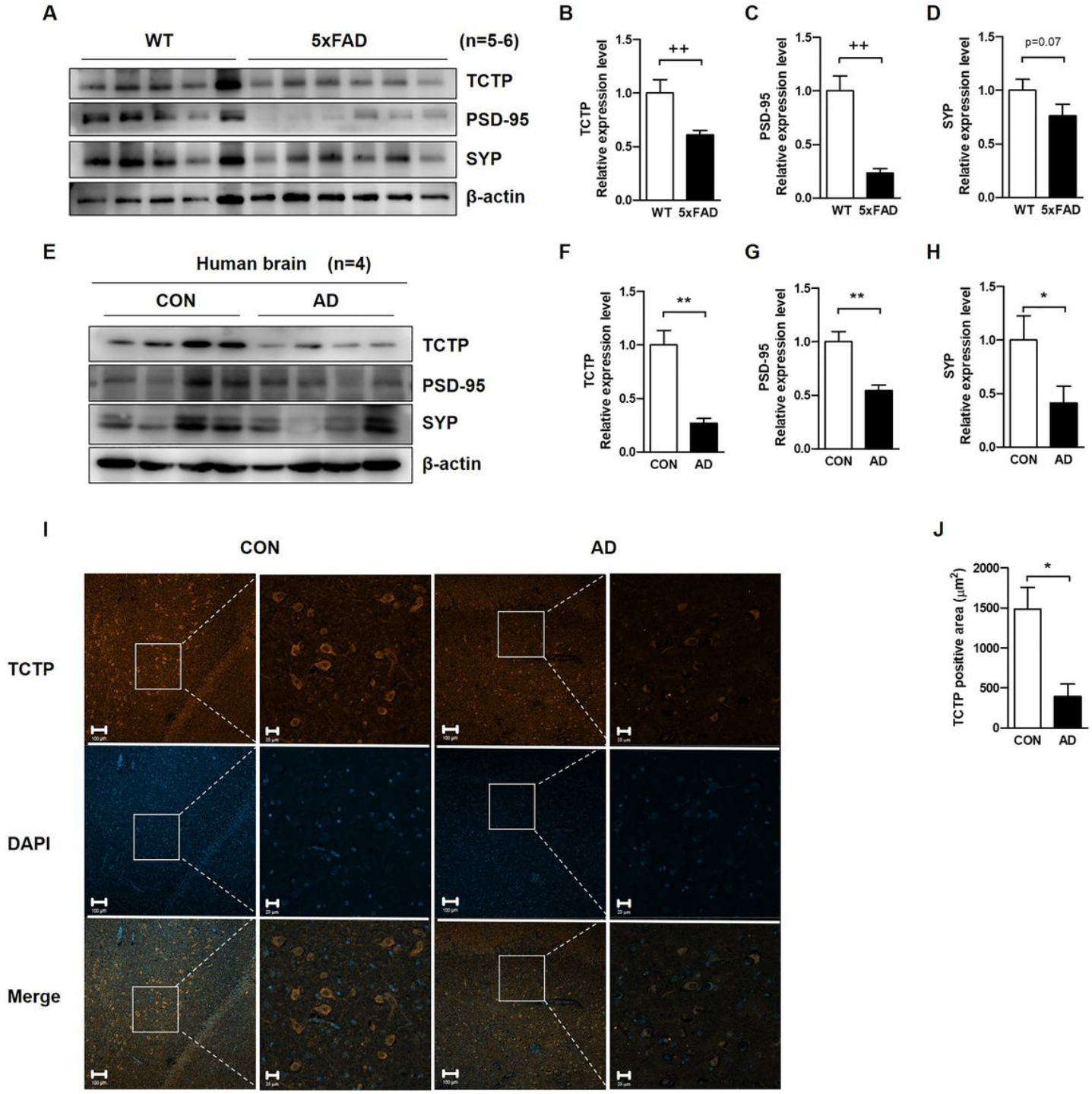

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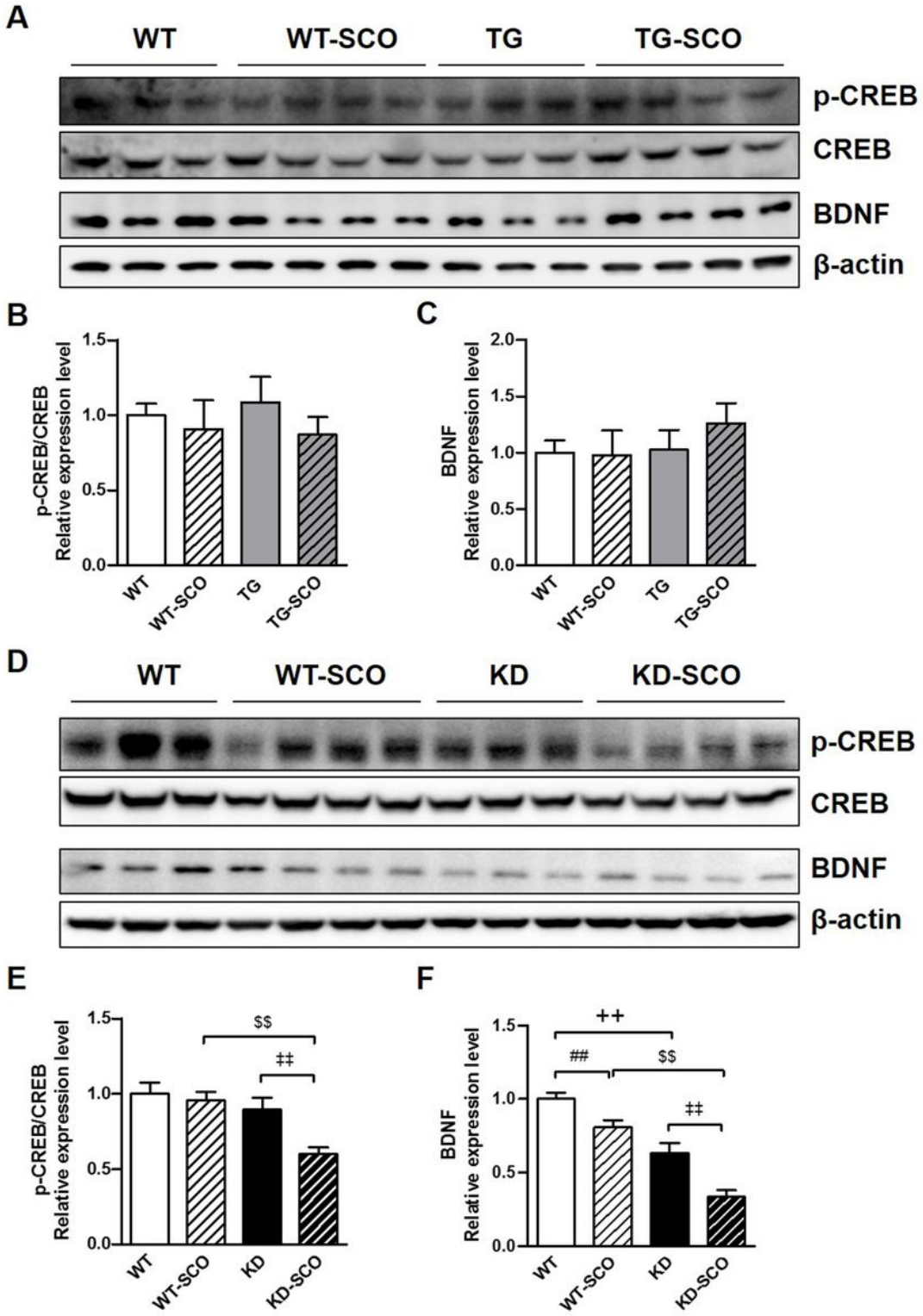

Figure 4

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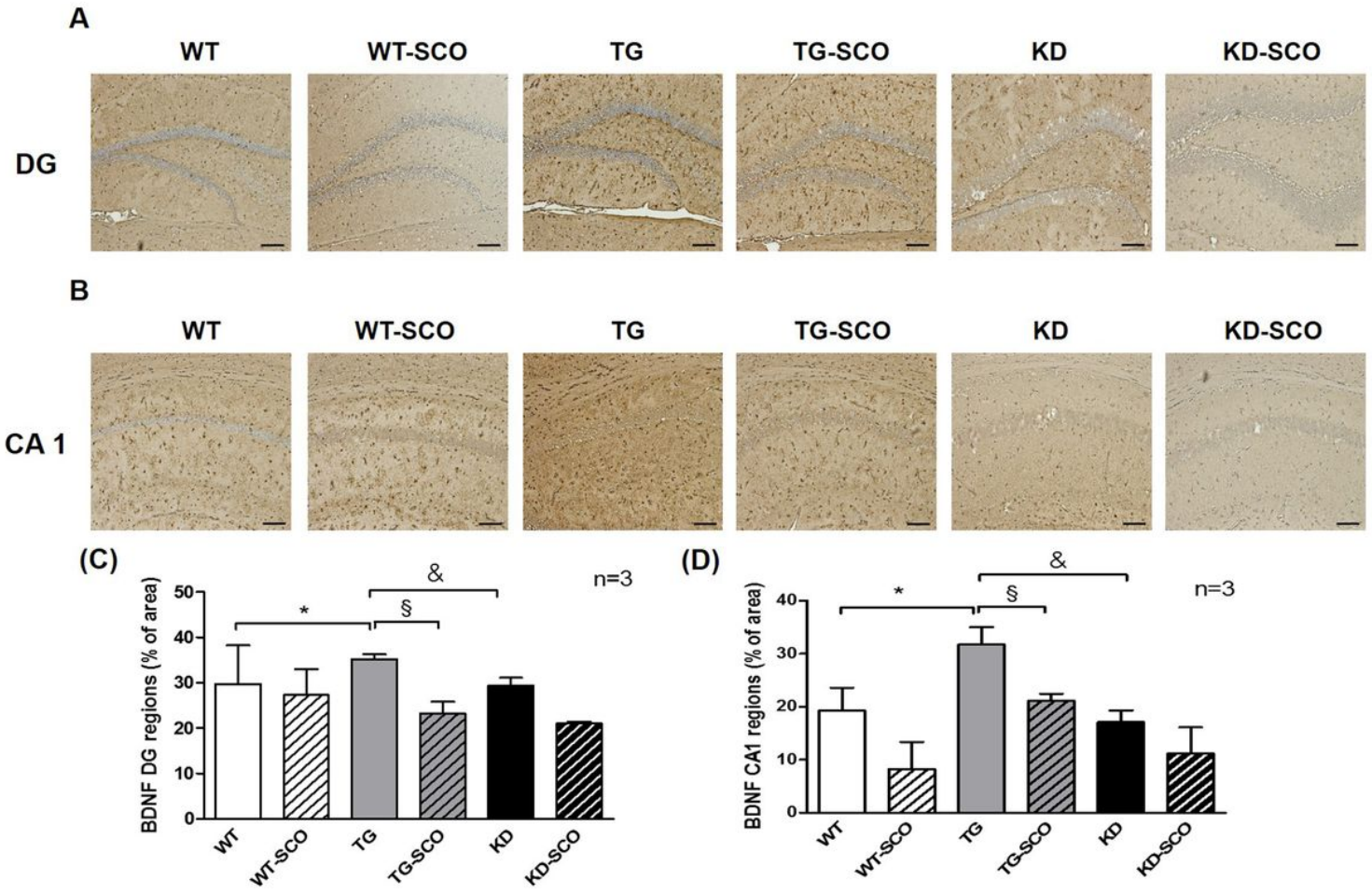

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