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RESEARCH ARTICLE

Thrombospondin-1 (TSP-1) and Neuronal Plasticity: Implications in Down Syndrome

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ABSTRACT

Down syndrome is the most common genetic cause of intellectual disability. Nevertheless, under certain stimulation processes, Down syndrome people can develop certain intellectual skills, suggesting an active mechanism of neuronal plasticity. Defects in both dendritic arborization and dendritic spines could affect neuronal plasticity and contribute to the degree of intellectual disability in people with Down syndrome. However, the cellular mechanisms involved in this process are unknown. Thrombospondin-1 (TSP-1) is an astrocyte-secreted protein involved in the development and maintenance of dendritic spines and synapses, which is altered in Down syndrome. Nonetheless, the role of TSP-1 in neuronal plasticity is not well characterized. In this study, we analyze whether TSP-1 is involved in neuronal microstructure changes induced by environmental enrichment, a model of experience-dependent neuronal plasticity. We found that the increase in dendritic spine density induced by environmental enrichment is associated with an increase in TSP-1 levels in the hippocampus of wild-type mice. The lack of TSP-1 in TSP-1$^{-/-}$ mice produce changes in the number and length of dendritic branches and a decrease in both, the number of intersections and density of dendritic spines. Exposure of TSP-1$^{-/-}$ mice to environmental enrichment did not affect the dendritic length and branching and number of intersections, but increased the density of dendritic spines significantly. These results suggest a role of TSP-1 as an important factor regulating brain microstructural plasticity, whose activity is reduced in Down syndrome.

Keywords: thrombospondin-1 (TSP-1), dendritic spines, enriched environments, neuronal plasticity, Down syndrome

Introduction

Down syndrome or trisomy 21, is the most common genetic cause of intellectual disability[1]. Cognitive alterations such as deficits in language comprehension and production[2], impairments in learning, memory[3,4], attention[5], and delayed acquisition of motor[6] and visual-spatial abilities[4] are commonly described in individuals with Down syndrome and may contribute to the degree of intellectual disability. Several studies have described neuropathological changes in the Down syndrome brain including a reduced number of neurons in the cortex and the cerebellum, a reduction in the size of the dendritic tree, alterations in the synapses and dendritic spines, abnormal neuronal differentiation and lamination, delayed myelination, defective cell proliferation and neurogenesis, abnormal neuronal migration and differentiation, and reduction in brain size, weight, and volume[7] These alterations could contribute to the degree of intellectual disability and abnormal synaptic plasticity. In addition to this, glial cells dysfunction might be involved in the development of Down syndrome neuropathology[8,9,10]. Nevertheless, under certain stimulation processes, Down syndrome people can develop certain intellectual skills, suggesting an active mechanism of neuronal plasticity. Environmental enrichment is a clinical and experimental model that involves increasing novelty and complexity in housing conditions, facilitating and reinforcing the acquisition of motor and cognitive skills in humans and rodents[11,12] due to the constant stimulation of motor, sensory, and exploration activities[11,13]. Environmental enrichment promotes changes in neuronal morphology that include complex dendritic trees[13-15], increased density of dendritic spines[14,15], and in the number of synapses and multisynaptic buttons in cortical and hippocampal areas[16,17]. Furthermore, environmental enrichment induce hippocampal neurogenesis and increases the expression of synaptic proteins and neurotrophins, such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF)[11]. The effects produced by environmental enrichment in the brain can enhance learning, cognitive abilities, and promote functional improvements in developmental disorders or recovery after lesions in adults[18]. Environmental enrichment therapies have been shown to have beneficial effects on Down syndrome[19-21], Rett syndrome[21-23], Fragile X syndrome[24-25], Autism Spectrum Disorders[26-28], Fetal Alcohol Spectrum Disorders[21], and neurodegenerative diseases such as Huntington's disease[29,30], Alzheimer's disease[31-33], and Parkinson's disease[34,35]. However, the cellular mechanisms involved in this process are not completely understood[11-13]. Thrombospondin-1 (TSP-1) is an astrocyte-secreted protein involved in cell-cell and cell-matrix communications and that participates in processes such as the growth of neurites[36-39], neuronal migration[36,40], neuronal proliferation and differentiation[41], synaptogenesis[42-45], formation of dendritic spines[46-48], and axon regeneration[49]. The absence of TSP-1 affects the recovery of motor function in cerebral ischemia[50] and spinal cord injury models[39], alters the formation of dendritic spines and synapses in Down syndrome[46,48] and fragile X syndrome[47], and favors hyperexcitability in epilepsy models[51]. The upregulation of TSP-1 causes hyperactivity[52], suggesting an important role of TSP-1 in the formation of neuronal circuits and cerebral plasticity. Nonetheless, the role of TSP-1 in neuronal plasticity is not well characterized. The objective of this work was to study whether TSP-1 is involved in changes in the neuronal microstructure induced by environmental enrichment and discuss its possible relationship with the neuropathology and plasticity in Down Syndrome

Methods

Animals

Male C57BL/6 mice (Wild type mice) and TSP-1$^{-/-}$ mice (The Jackson Laboratory), 4 weeks old were used in this study. Animals were housed under temperature (22 ± 2°C) and humidity (50 ± 10) controlled conditions, with 12 h/12 h light/dark cycles (light on at 7:00 am) with free access to food and water. All animal experiments followed the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Ethical Committee of the Facultad de Psicologia, UNAM (FPSI/DIP/CE/07/2015). All efforts were made to minimize animal suffering and to reduce the number of mice that were used.

Environmental Enrichment

The environmental enrichment protocol was previously described by Sztainberg and Chen[53]. Briefly, C57BL/6 and TSP-1$^{-/-}$ mice were placed in standard control cages (16 cm X 28 cm X 13 cm, 1 mouse per cage, standard condition) or environmental enrichment cages (55 cm X 25 cm X 15 cm, 4 mice per cage). Each environmental enrichment cage contained objects of various colors, shapes, and textures (wooden or plastic toys, tunnels, wood blocks, running wheels), and bedding material (shredded paper, cotton, textiles of various textures) to provide motor, olfactory, and somatosensory stimulations[11-13]. All objects were

repositioned every three days and completely substituted weekly. The animals were housed in standard condition and environmental enrichment for 5 weeks. Four independent experiments were performed by duplicate. After this period, standard condition and environmental enrichment mice were deeply anesthetized with 0.7% sodium pentobarbital; the animals were decapitated and the brains were removed and processed for immunohistochemistry, Golgi impregnation, and determination of TSP-1 levels.

Immunohistochemistry

Sections (40 µm) were cut using a semi-automatic microtome (American Optical Company), fixed with 4% paraformaldehyde/0.12 M sucrose/PBS (Sigma-Aldrich, MO, USA) for 30 min at 37°C, permeabilized 1 h with a 0.2% Triton X-100 (Sigma-Aldrich, MO, USA) in PBS solution at room temperature, followed by a 1 h incubation in a 5 % BSA (Bovine Serum Albumin, Sigma-Aldrich, MO, USA) in PBS solution at room temperature. Sections were incubated free-floating in a solution containing antibodies directed against GFAP (1:250, G4546, Sigma-Aldrich, MO, USA) and mouse anti-thrombospondin-1 (1:250, sc-59887, Santa Cruz Biotechnology, CA, USA) in 1% BSA/PBS, overnight at -4°C. Thereafter, sections were rinsed in PBS followed by a 2 h incubation in fluorescent-conjugated secondary antibody (1:500, Alexa 488 and Alexa 594, Invitrogen, Eugene, OR) and rinsed with PBS. For cell nuclei visualization, sections were incubated 2 min in PBS-solution containing Hoescht (1:10,000, Sigma-Aldrich, MO). An Axiovert 200 inverted microscope (Zeiss, Jena, Germany) was used for specimen examination and imaging. Fluorescent images were captured with a digital camera (Zeiss) and processed using the AxioVision software (Zeiss)[54].

Golgi-Impregnation

Whole brains were impregnated according to the Golgi-Cox procedure using the FD Rapid GolgiStain™ Kit (FD NeuroTechnologies, USA). Briefly, each brain was exposed to the impregnation solution (A + B). The impregnation solution was prepared with an equal volume mixture of solutions A and B. After 24 h, the solution was replaced with a new mixture of impregnation solution, and the brains were stored for 2 weeks in the dark, at room temperature. They were then transferred to solution C for 48 h at 4°C. The brains were cut into 75 µm slices (coronal sections) and mounted on gelatinized glass slides. Sections were treated with a mixture of solutions D and E and water (1:1:2 respectively); later the sections were rinsed with distilled water and dehydrated in a series of ethanol and xylene solutions. The slides were dried to 37°C and finally embedded in Permount (Fisher Scientific)[55].

Image acquisition and morphologic analysis

Analyses of dendrites and spines were conducted through double-blind to the experimental conditions. For each brain, at least 40 different hippocampal neurons per animal were captured with a digital camera (Eumex, Holland) attached to a microscope (Mediline Scientific, UK), with a 40X objective, and processed using the ImageFocus 4 software (v2.9). Only those neurons showing a completely impregnated dendritic tree and relatively isolated from neighboring cells were selected for the analysis.

The Semi-automated Sholl analysis was used as previously described[55,56]. Briefly, 8-bit images of hippocampal neurons were traced using the NeuronJ plugin for Image J (NIH, Bethesda, MD), and tracing files (*ndf files) were generated. Data were organized and converted to SWC files using MATLAB (Mathworks, MA, USA), and the connectivity of tracings was checked in NeuronStudio, where dendritic traces were confirmed. This determined the structure of each dendritic arbor in a two-dimensional form and converted it to a digital format. Once the tracing was finalized in NeuronStudio, data were exported to Excel using MATLAB. This allowed the analysis of the digitized traces into numerical data to evaluate the number, length, branching pattern (complexity) of dendrites, and the number of primary, secondary, and tertiary dendrites[55,56].

Dendritic spines analysis

The estimation of the dendritic spines was performed through the semi-automated method developed by Orlowski and Bjarkam[57]. The method is based on the binarization and skeletonization functions provided by ImageJ. Only dendrites that displayed no breaks in their staining and which were not obscured by other neurons or artifacts were evaluated. Measurements were obtained from CA1, CA2/CA3, and dentate gyrus areas of the dorsal hippocampus. Primary, secondary, and tertiary dendrites branches from the apical part (stratum radiatum) and from the basal part (stratum oriens) of the pyramidal cells were analyzed. At least 20 dendrites per experimental condition were counted. Spine density was estimated as the number of spines on each terminal dendrite per 50 µm. The spines classification was based on the spine length, width, and length of head and length: width ratio obtained in RECONSTRUCT[58]. Spines were

categorized based on the following criteria: (1) Mushroom = width > 0.6 µm; (2) Long thin = length > 1 µm; (3) Thin = length:width ratio > 1 µm; (4) stubby = length:width ratio ≤ 1[58].

TSP-1 measurements

TSP-1 levels were determined from the CA1, CA2/CA3, and dentate gyrus regions of the hippocampus[59]. Dissected regions were homogenized in RIPA buffer (50mM Tris-HCl, 150mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS) plus protease inhibitors (Complete, Roche, Mannheim, Germany) with a Turrax homogenizer (Ika Works, Wilmington, NC), centrifuged at 14,000 rpm for 30 min at 4°C. TSP-1 levels in supernatant fraction were determined using a commercial ELISA kit following the manufacturer's instructions (R&D systems, Minneapolis, MN). TSP-1 levels were calculated with a standard curve of known concentrations and the results were expressed as ng/ml[46].

Statistical analysis

For all statistical evaluations, the SPSS Statistic version 22.0 software (SPSS Inc., Chicago, IL, USA) was used. Data are expressed as the mean ± SEM. Statistical analysis was performed using one-way ANOVA, Comparisons were performed using the Student's *t*-test. A value of $p < 0.05$ was considered statistically significant.

Results

TSP-1 is expressed in the mouse hippocampus

Previously, we demonstrated that TSP-1 is expressed in the human cerebral cortex[42]. To determine whether TSP-1 was expressed in the mouse brain, we analyzed the expression patterns of TSP-1. Immunohistofluorescence analyses showed the TSP-1 expression in the parenchyma, cortex, and hippocampus (Figure 1). Double labeling with anti-GFAP confirmed the expression of TSP-1 in astrocytes. These results confirm that TSP-1 is expressed in cortex and hippocampus astroglial cells.

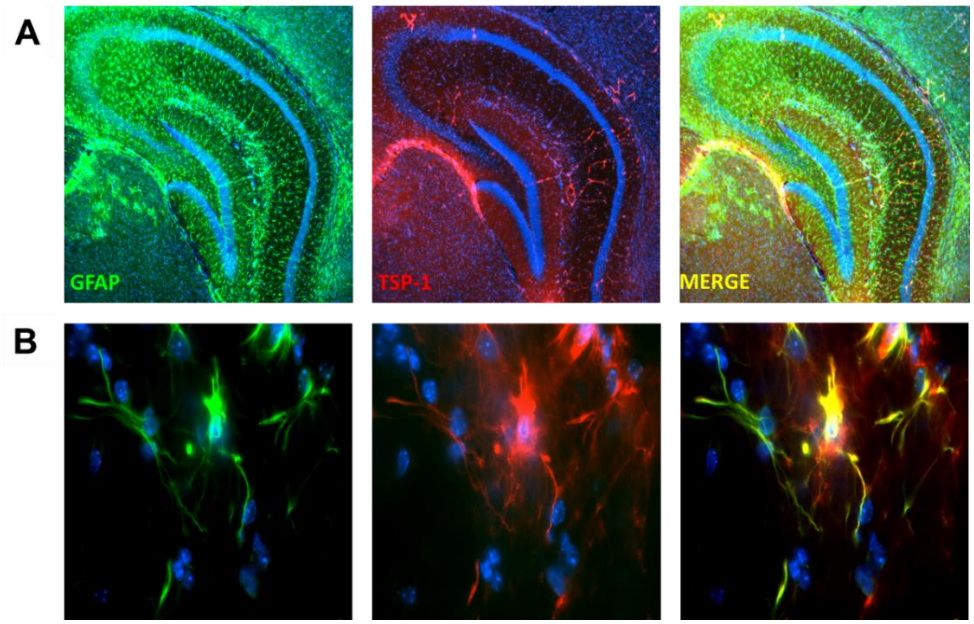

Figure 1. Expression of TSP-1 in mouse hippocampus. Double immunofluorescence with anti-GFAP (green) and anti-TSP-1 (red). Cell nuclei were counterstained with Hoechst (blue). **A)** The cortex and hippocampus showed a greater expression of TSP-1 in the brain (4X). **B)** Magnification of captured image (63X). TSP-1 was expressed in astrocytes.

The formation of dendritic spines induced by enriched environments is dependent of TSP-1

A qualitative analysis of Golgi staining in mouse brains showed that the hippocampal neurons maintained an excellent state of impregnation, with well-defined soma and basal and apical dendrites covered by dendritic spines (Figure 2A). An analysis of the number of dendritic spines was performed in C57BL/6 mice subjected to standard condition and environmental enrichment. The results showed that environmental enrichment increased the number of dendritic spines in hippocampal neurons (Figure 2B). This increase was observed in both basal dendrites and apical dendrites (standard condition 11.05 ±

0.60 spines/50 µm; environmental enrichment 15.21 ± 0.83 spines/50 µm; n = 60) (Figure 2B). A comparative analysis of the hippocampal regions showed that environmental enrichment differentially increased the number of dendritic spines (CA1: standard condition, 11.0 ± 2.9 spines/50µm, environmental enrichment, 13.8 ± 6.1 spines/50µm; CA2/CA3: standard condition, 11.0 ± 4.0 spines/50µm, environmental enrichment 16.8 ± 7.3 spines/50µm, n = 50). The increase in dendritic spines was only statistically significant in the CA2/CA3 region of the hippocampus (Figure 2C). Interestingly, we observed that environmental enrichment did not modify the number of dendritic spines in dentate gyrus (standard condition, 13.6 ± 3.9 spines/50µm; environmental enrichment, 10.3 ± 4.3 spines/50µm, n = 30). To establish whether the formation of dendritic spines induced by environmental enrichment was dependent of TSP-1, the concentration of TSP-1 was quantified in the brains of mice exposed to standard condition and environmental enrichment (n = 30). The results showed that mice exposed to enriched conditions had a slight increase in TSP-1 levels in the hippocampus (standard condition, 11.4 ± 0.5 ng/ml, environmental enrichment, 12.7 ± 0.5 ng/ml) (Figure 2D). To determine whether this increase was selective for each hippocampal region, TSP-1 levels were measured in CA1, CA2/CA3, and dentate gyrus. The results showed a significant increase in TSP-1 levels in the CA2/CA3, as well as in the dentate gyrus, when mice were subjected to environmental enrichment (CA1: standard condition, 11.4 ng/ml; EE, 11.9 ng/ml; CA2/CA3: standard condition, 10.7 ng/ml; environmental enrichment, 12.6 ng/ml; dentate gyrus: standard condition 12.2 ng/ml; environmental enrichment, 13.5 ng/ml) (Figure 2E).

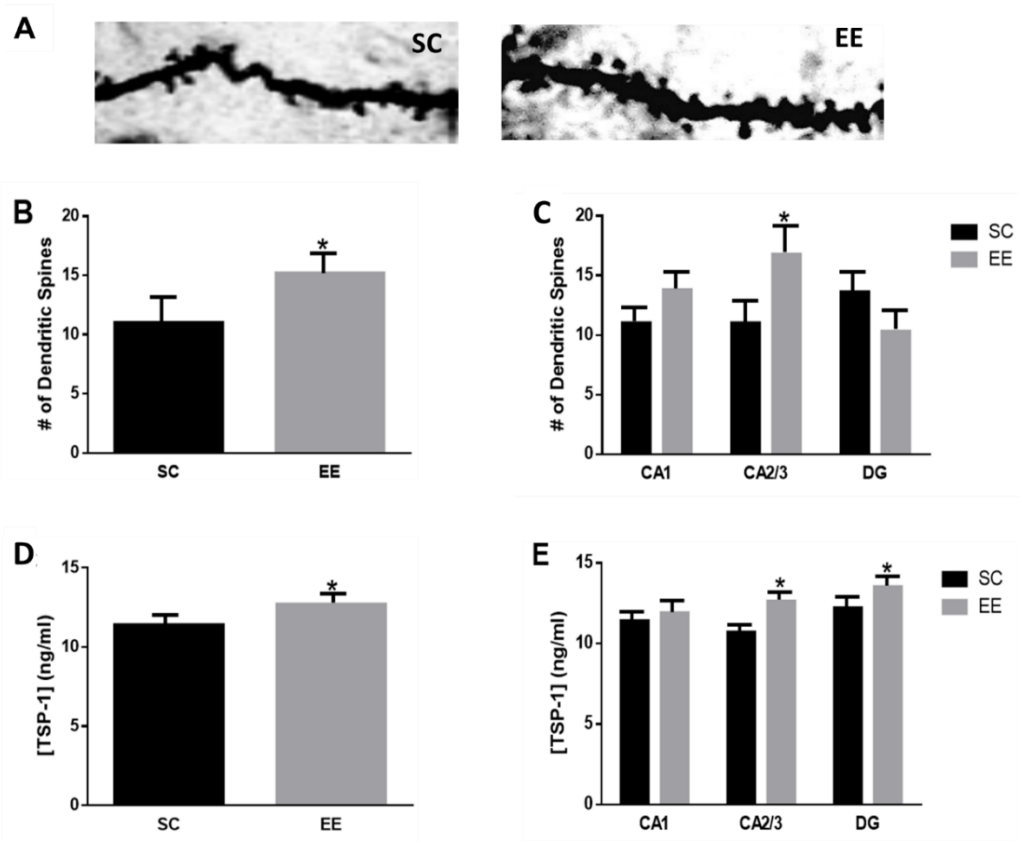

Figure 2. The formation of dendritic spines induced by environmental enrichment is dependent of TSP-1. Hippocampal dendritic segments of mice maintained under standard conditions (SC) and environmental enrichment (EE) (**A**). Quantification of dendritic spines density under the same conditions (**B**). Analysis of dendritic spines in CA1, CA2/3, and dentate gyrus (DG) hippocampal regions of mouse housed in EE (**C**). TSP-1 levels in homogenates of hippocampus (**D**) and hippocampal regions (**E**). Data were analyzed by student's *t*-test. Error bars indicate mean ± SEM, **p*<0.05.

The deficiency of TSP-1 induces alterations in neuronal morphology

The Golgi-Cox staining showed that the dendritic tree of the neurons of TSP-1$^{-/-}$ mice presented thinner arborizations (Figure 3A) and a smaller number of dendritic spines (Wt 23.2 ± 3.7 spines/50µM; TSP-1$^{-/-}$ 13.4 ± 2.7 spines/50µM n=50) (Figure 3B, C). A Sholl analysis showed that TSP-1$^{-/-}$ mice presented a significant decrease in the number of branches when compared to Wt mice (Wt: 6.7 ± 2.9; TSP-1$^{-/-}$: 3.0 ± 1.5; n=40) (Figure 3D). Additionally, it was observed that TSP-1$^{-/-}$ mice had a longer dendritic arborization than Wt mice (Wt: 55.3 ± 17.5; TSP-1$^{-/-}$: 92.4 ± 19.5; n=40) (Figure 3E). To determine whether the complexity of dendrites is affected in KO TSP-1$^{-/-}$ mice, a Sholl analysis was performed. The complexity was considered as the number of dendritic intersections along the dendrite from the center of the soma[60]. The Sholl analysis showed that the TSP-1$^{-/-}$ neurons mice had fewer intersections, suggesting less complexity with respect to the Wt neurons mice (Figure 3F). These results suggest that the loss of TSP-1 modifies neuronal morphology.

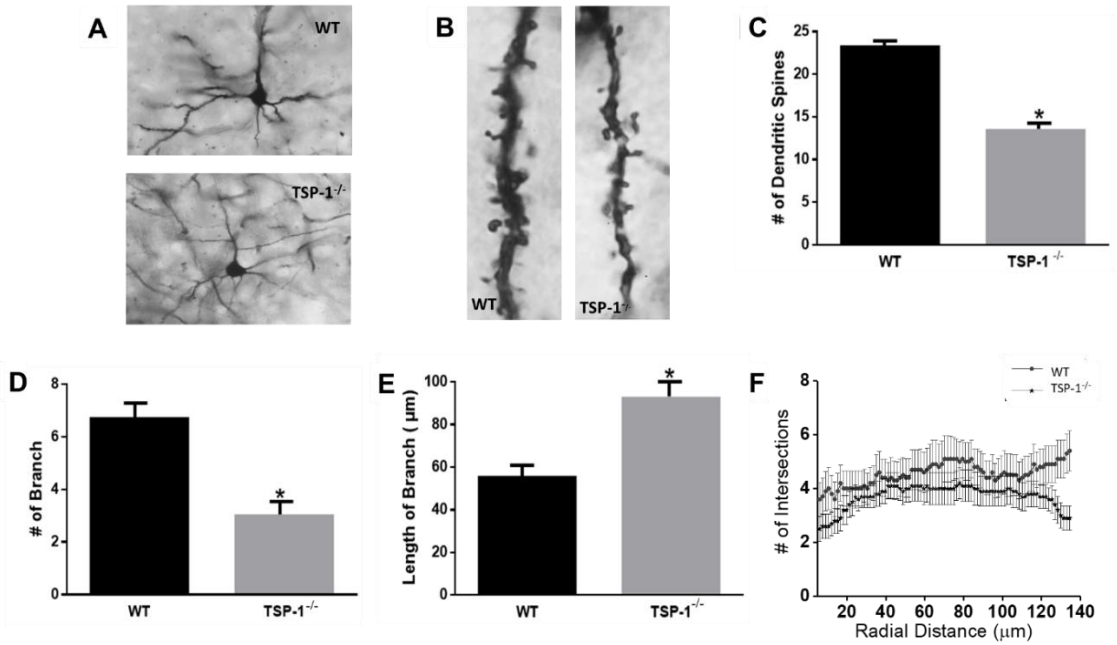

Figure 3. Deficiency TSP-1 induces alterations in neuronal morphology. Hippocampal neurons of wild type (WT) and TSP-1$^{-/-}$mice (**A**). Representative images of dendritic spines (**B**). Analysis of neuronal morphology (**C**) density of dendritic spines, (**D**) number of branches, (**E**) long branches, and (**F**) neuronal complexity. Data were analyzed by student's *t*-test. Data are presented as mean ± SEM, *$p<0.05$.

Environmental enrichment *does not modify the alterations in ramifications of TSP-1$^{-/-}$ mice*

Since the general morphology of neurons was affected in TSP-1$^{-/-}$ mice, we studied whether environmental enrichment reversed these alterations. Exposure of TSP-1$^{-/-}$ mice to environmental enrichment did not change the number (standard condition, Wt: 6.7 ± 2.9; TSP-1$^{-/-}$: 3.0 ± 2.7; environmental enrichment, Wt: 6.7 ± 2.7; TSP-1$^{-/-}$: 2.9 ± 0.4) and length (standard condition, Wt: 55.3 ± 17.6; TSP-1$^{-/-}$: 92.4 ± 19.5; environmental enrichment Wt: 92.4 ± 19.5; TSP-1$^{-/-}$: 77.3 ± 21.9) of branches (Figure 4A,B). However, environmental enrichment increased significantly the number of intersections in dendrites, between 20 and 80 µm in length, only in wild-type mice (Figure 4C). This result was also observed in the secondary and tertiary segments of dendrites, suggesting that TSP-1 may play an important role in neuronal plasticity.

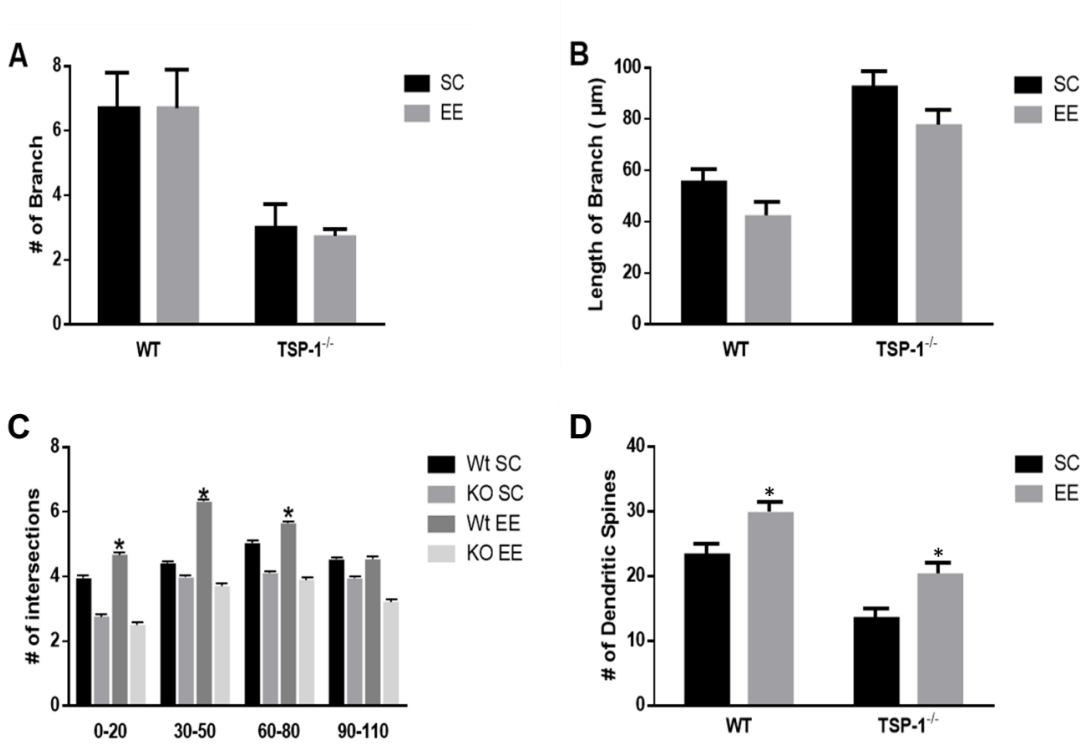

Figure 4. Environmental enrichment have a differential effect on neuronal morphology of TSP1$^{-/-}$ mice. Environmental enrichment (EE) did not produce differences in the number (**A**) and length (**B**) of neuronal branches and neuronal intersections (**C**) compared to mice exposed to standard conditions (SC). The density of dendritic spines increased in mice housed in environmental enrichment (**D**). Data were analyzed by student's *t*-test. Error bars indicate the mean ± SEM, *$p<0.05$.

Environmental enrichment increases dendritic spines in TSP-1$^{-/-}$mice

To know whether TSP-1 has a determining role in the formation of dendritic spines in hippocampal neurons, TSP-1$^{-/-}$ mice were subjected to environmental enrichment and standard condition. Our results showed that environmental enrichment produced a significant increase in the number of dendritic spines in primary dendrites in both, wild-type mice and TSP-1$^{-/-}$ mice, but this increase was not statistically significant (standard condition, Wt: 10.2 ±1.0 spines/50 µm; TSP-1$^{-/-}$: 11.6 ± 3.3 spines/50 µm; environmental enrichment, Wt: 12.8 ±4.3 spines/50 µm; TSP-1$^{-/-}$: 13.1 ± 3.3 spines/50 µm). Similar results were found in secondary dendrites. An analysis of tertiary dendrites showed that environmental enrichment induced a significant increase in the number of dendritic spines in both, wild-type mice and TSP-1$^{-/-}$ mice (standard condition: Wt: 23.2 ± 3.7 spines/50µm; TSP-1$^{-/-}$: 13.4 ± 2.7 spines/50µm; environmental enrichment; Wt: 29.6 ± 4.6 spines/50µm, TSP-1$^{-/-}$: 20.1 ± 2.9 spines /50µm, n = 30) (Figure 4C). These results confirm the importance of TSP-1 in the formation of the dendritic spines.

The morphology of dendritic spines could be modified by the neuronal activity itself and environmental stimulation[11-13]. To determine whether environmental enrichment could have an effect on the type of spines, we analyzed the morphology of the spines with the semi-automated method proposed by Risher et al.[58]. Our results showed that the dendrites of wild-type and TSP-1$^{-/-}$ mice had spines in the form of filipodium, mushrooms, thin and long thin. We could not identify stubby spines. Wild-type mice had a predominance of mushroom spines while TSP-1$^{-/-}$ mice had fewer mushroom spines and a greater number of thin spines after the mice remained in enrichment conditions; TSP-1$^{-/-}$ mice show an increase in mushroom spines and a decrease in thin spines (data not show).

Discussion

TSP-1 is expressed and secreted by various types of cells, including fibroblasts, endothelial cells, muscle cells, immune cells, monocytes, granular pneumocytes, and platelets among others[61]. In the brain, the expression of TSP-1 occurs in astrocytes[62] and has been detected during embryonic development[63] and in large cortical projecting

neurons according to a study in post-mortem Alzheimer's disease brains[64]. TSP-1 has been located mainly in the cerebral cortex. This work showed that TSP-1 is expressed in the hippocampus of the mouse, and its expression is specific to astrocytes (Figure 1), suggesting that TSP-1 could be expressed in various brain areas. We previously demonstrated that Down syndrome brains show a significant decrease in TSP-1 levels, which could be associated with the loss of dendritic spines and synapses number[46,48]. The loss of dendritic spines would generate poor synaptic communication contributing to the cognitive deficits observed in Down synrome[65]. TSP-1 deficits could affect critical stages of neuronal development such as neurite outgrowth neuronal migration, or synaptogenesis processes that are altered in Down syndrome[7]. On the other hand, the loss of thrombospondins can leads to craniofacial dysmorphism[66], which is a common feature of Down syndrome subjects and Down syndrome mouse models[67], raising the possibility that reduced TSP-1 levels may be associated with a number of developmental anomalies present in Down Syndrome. However, under certain stimulation processes, individuals with Down syndrome improve their intellectual abilities, suggesting that brain plasticity mechanisms remain despite their genetic condition. Environmental enrichment is considered a model of experience-dependent brain plasticity. Rodents housed in environmental enrichment showed an increase in the density of dendritic spines and better execution in learning and memory models[13,15,68-70]. This could explain the beneficial effects of environmental enrichment on various neurodevelopmental disorders and neurodegenerative diseases[19-35].

In this study, we observed that environmental enrichment increased the number of dendritic spines in both basal and apical dendrites. An analysis by regions showed that environmental enrichment produced a partial increase in dendritic spines in the CA1 region and a significant increase in the CA2/CA3 region. The increase in dendritic spines in CA1 induced by environmental enrichment has been extensively studied[13,14,71,72]; the CA1 region can be activated during spatial localization processes produced by changes in objects during the experimental process[13,14,73,74]. However, other studies suggest that the CA2 hippocampal region does not respond to plasticity as CA1 and CA3[69-72,75-78], although it is involved in the processes of social memory[79,80]. The changes observed in CA2 could be involved in the social component produced by environmental enrichment itself[11]. Hippocampal dysfunction is a common feature in individuals with Down syndrome[81]. Recently, it has been reported that Ts65Dn mice, a Down syndrome model, have memory and social learning problems[82], suggesting a dysfunction in the CA2 region. A thorough analysis of the changes in CA2 induced by each of the components of environmental enrichment would have to be evaluated to design better stimulation strategies. We observed that environmental enrichment does not produce an increase of dendritic spines in dentate gyrus, contrary to what was previously reported[83]. Dentate gyrus activity has been observed when rodents are subjected to spatial recognition tasks[84] and under voluntary exercise[83,85,86], conditions that may be presented in environmental enrichment. The discrepancy between our study and previous reports could be due to the animal model differences and the dentate gyrus subregion[87]. An analysis between the suprapyramidal or infrapyramidal subregion of the dentate gyrus could determine whether the environmental enrichment produces an increase in the number of spines or not.

To determine whether the increase in the number of spines produced by environmental enrichment was dependent on TSP-1, the levels of TSP-1 in the hippocampus were quantified (Figure 2D, E). The results showed that environmental enrichment increased TSP-1 levels in CA2/CA3 and dentate gyrus, but did not have a significant effect on CA1. The increase in TSP-1 in CA2/CA3 correlated with an increase in the number of dendritic spines, but this was not observed in the dentate gyrus. Our work suggests that TSP-1 could have a differential effect on the hippocampus. While TSP-1 could be involved in the formation of synapses and dendritic spines in the CA2/CA3 region[38,40,42,43,42,44,46,47], in the dentate gyrus, it could be associated with stimulation of neurogenesis. In this sense, previous work has shown that TSP-1 participates in the processes of proliferation and differentiation of neuronal progenitor cells, and TSP-1$^{-/-}$ mice have neurogenesis failures[41]. Other studies have shown that environmental enrichment rescues neurogenesis in Ts65Dn mice, a mouse model of Down syndrome[88]. The relationship among neurogenesis, experience-dependent brain plasticity processes and TSP-1 would have to be further analyzed.

In this work, we demonstrated that the loss of TSP-1 modifies the morphology of neurons, affecting particularly the branches and dendritic spines (Figure 3). Alterations in dendritic branches included a decrease in the number of branches and branches with greater length and less complexity. TSP-1 stimulated the growth of neurites[36] and ramifications[37], the lack of TSP-1 in mice could explain the reduction in the number of branches. Under these conditions, neurons would increase the

length of the branches as a compensatory mechanism to cover the deficit of dendritic branches and less complexity[89,90]. In Down syndrome brains presented a significant reduction of TSP-1 levels[46]; on the other hand, alterations in the Down syndrome neuronal morphology have been described, including long basilar dendrites and smaller neurons with short basilar dendrites[91], and similar results have been described in Ts65Dn mice[92]. Additionally, it has been reported that during the development of the brain with Down syndrome, the length and number, as well as the intersections of the dendritic branches, decrease with increasing age[93], suggesting an important role of TSP-1 in the maintenance of neuronal morphology in Down syndrome.

Environmental enrichment can cause changes in the dendritic arborization and density of dendritic spines[94]. We report that the environmental enrichment did not reverse the changes observed in the dendritic ramifications of the TSP-1$^{-/-}$mice (Figure 4); however, it increased the number of dendritic spines. The presence of growth factors such as NFG, BDNF and glial derivate neurotrophic factor (GDNF), could compensate for the absence of TSP-1 and stimulate the formation of dendritic spines under enrichment conditions[11,92]. Additionally, TSP-2, TSP-3, and TSP-4 could also participate in spinogenesis and synaptogenesis[42,43]. The increase of spines in TSP-1$^{-/-}$ mice could be an adaptive mechanism to generate functional synaptic circuits under constant stimulation, such as environmental enrichment. In this sense, Down syndrome animal models undergoing environmental enrichment have shown an increase in the number of dendritic spines and an improvement in learning and memory[16,89,90,92,95,96].

Spine morphology changed after the mice were subjected to environmental enrichment[94]. In the case of the TSP-1$^{-/-}$mice, the environmental enrichment produced an apparent increase in mushroom spines and a decrease in thin spines. The importance of thin and mushroom spines is associated with their functioning. Thin spines are related to the learning processes and mushroom spines to memory processes[97]. Constant stimulation by environmental enrichment could favor memory processes in TSP-1$^{-/-}$mice; however, it is necessary to perform experiments to know the ability of TSP-1$^{-/-}$ mice to solve the memory and learning paradigms after being exposed to environmental enrichment.

Conclusion

In conclusion, the results obtained in this work suggest that TSP-1 may have an important role in neuronal plasticity mechanisms associated with environmental enrichment. Strategies based on the use of environmental enrichment and drugs that stimulate the production of TSP-1 in the brain could reduce the pathology of dendritic spines and improve intellectual performance in people with Down syndrome.

Conflict of Interest

The authors have no conflicts of interest to declare

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