The Journal of Nutrition
Ingestive Behavior and Neurosciences

Prenatal Administration of Oleic Acid or Linolenic Acid Reduces Neuromorphological and Cognitive Alterations in Ts65dn Down Syndrome Mice

Susana García-Cerro,[1] Noemí Rueda,[1] Verónica Vidal,[1] Alba Puente,[1] Víctor Campa,[2] Sara Lantigua,[1] Oriol Narcís,[1] Ana Velasco,[3] Renata Bartesaghi,[4] and Carmen Martínez-Cué[1]

[1]Department of Physiology and Pharmacology, Faculty of Medicine, University of Cantabria, Santander, Cantabria, Spain; [2]Institute of Molecular Biology and Biomedicine (IBTECC), Santander, Cantabria, Spain; [3]Department of Biochemistry and Molecular Biology, Institute of Neurosciences of Castilla and Leon (INCYL), University of Salamanca, Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain; and [4]Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy

ABSTRACT

Background: The cognitive impairments that characterize Down syndrome (DS) have been attributed to brain hypocellularity due to neurogenesis impairment during fetal stages. Thus, enhancing prenatal neurogenesis in DS could prevent or reduce some of the neuromorphological and cognitive defects found in postnatal stages.

Objectives: As fatty acids play a fundamental role in morphogenesis and brain development during fetal stages, in this study, we aimed to enhance neurogenesis and the cognitive abilities of the Ts65Dn (TS) mouse model of DS by administering oleic or linolenic acid.

Methods: In total, 85 pregnant TS females were subcutaneously treated from Embryonic Day (ED) 10 until Postnatal Day (PD) 2 with oleic acid (400 mg/kg), linolenic acid (500 mg/kg), or vehicle. All analyses were performed on their TS and Control (CO) male and female progeny. At PD2, we evaluated the short-term effects of the treatments on neurogenesis, cellularity, and brain weight, in 40 TS and CO pups. A total of 69 TS and CO mice were used to test the long-term effects of the prenatal treatments on cognition from PD30 to PD45, and on neurogenesis, cellularity, and synaptic markers, at PD45. Data were compared by ANOVAs.

Results: Prenatal administration of oleic or linolenic acid increased the brain weight (+36.7% and +45%, $P < 0.01$), the density of BrdU (bromodeoxyuridine)- (+80% and +115%; $P < 0.01$), and DAPI (4′,6-diamidino-2-phenylindole)-positive cells (+64% and +22%, $P < 0.05$) of PD2 TS mice with respect to the vehicle-treated TS mice. Between PD30 and PD45, TS mice prenatally treated with oleic or linolenic acid showed better cognitive abilities (+28% and +25%, $P < 0.01$) and a higher density of the postsynaptic marker PSD95 (postsynaptic density protein 95) (+65% and +44%, $P < 0.05$) than the vehicle-treated TS animals.

Conclusion: The beneficial cognitive and neuromorphological effects induced by oleic or linolenic acid in TS mice suggest that they could be promising pharmacotherapies for DS-associated cognitive deficits. *J Nutr* 2020;150:1631–1643.

Keywords: Down syndrome, Ts65Dn mice, oleic acid, linolenic acid, prenatal treatment, neurogenesis, cognition

Introduction

Down syndrome (DS), the most common genetic cause of intellectual disability, is characterized by numerous neurobiological alterations. One of the factors partially responsible for the cognitive impairments in DS is brain hypocellularity due to alterations in neurogenesis during the early developmental stages (1–4).

The Ts65Dn (TS) mouse, the most commonly used DS model, resembles many of its phenotypes exhibiting altered cognitive abilities, hypocellularity, and reduced neurogenesis (4, 5–7). Thus, therapies aimed at rescuing neurogenesis might be a good approach to treating intellectual disability in DS individuals. Several pharmacotherapies have been reported to rescue the neurogenesis and cognitive deficits in DS murine models when administered in the pre- or postnatal stages (8–20). However, some of these drugs have failed to produce any benefit in humans or cannot be safely administered to individuals with DS. In addition, DS individuals and TS mice present altered brain development, beginning at the fetal stages (2, 3, 21). Therefore, these alterations should be corrected from the early developmental stages, using safe drugs or natural substances

Manuscript received September 19, 2019. Initial review completed December 17, 2019. Revision accepted March 2, 2020.
First published online April 3, 2020; doi: https://doi.org/10.1093/jn/nxaa074.

that can be administered during the prenatal or early postnatal stages (2, 10, 11, 22). In this context, the administration of fatty acids could be a promising strategy.

There is a direct association between the percentage of fatty acids in maternal plasma and the development of cognitive function in neonates (23), and exogenous administration of fatty acids during the pre- and postnatal periods increases brain development in humans and other animals (24). Oleic acid is a monounsaturated acid of the ω-9 series. It is present endogenously in the organism and it can also be obtained from the diet. Oleic acid acts as a neurotrophic factor in initial life stages, inducing neuronal differentiation, the growth of new neurites, neuronal migration, and synapse formation (25–30).

α-Linolenic acid is an essential PUFA of the ω-3 series, which has to be obtained from the diet. Linolenic acid is essential for proper brain development and its deficit can affect neurogenesis and neuronal function (31–33). In rodents, supplementation of linolenic acid, or its derivative DHA, in the diet of pregnant females enhances cognition and neurogenesis in the brains of their offspring (32–51).

In DS, the lower concentrations of fatty acids in brain phospholipids (52) could be partially responsible for some of the neuromorphological and cognitive alterations encountered in this syndrome. Thus, treatment with fatty acids during the critical windows of neurogenesis (the prenatal and early postnatal periods) may induce beneficial effects. In addition, oleic acid and linolenic acid do not produce important side effects, and their efficacy in treating different pathologies is currently being evaluated (53–55). Thus, the aim of this study was to analyze whether prenatal administration of oleic or linolenic acid restores the neuromorphological alterations found in TS mice, and whether these effects are maintained after discontinuation of the treatment, leading to enhanced cognitive abilities.

Methods

Animals, diets, and treatments

This study was approved by the Cantabria University Institutional Laboratory Animal Care and Use Committee and performed in accordance with the Declaration of Helsinki and the European Communities Council Directive (86/609/EEC).

TS mice were generated by repeated backcrossing of B6EiC3Sn a/A-Ts(17 < 16>)65Dn females with C57BL/6Ei × C3H/HeSNJ (B6EiCSn) F1 hybrid males. TS mice were compared with euploid littermates (Control, CO). Trisomy was determined by real-time qPCR as previously described (56).

Diet.

All pregnant and lactating TS mice were fed with Tekcal 18% protein (#2018, containing 18.6% raw protein, 6.2% fat, and 44.2% carbohydrates) Global Mouse Chow, specially formulated for gestation and lactation (INVIGO), from ED (Embryonic Day) 0 until the weaning of the pups. In order to study the long-term effects, all TS and CO mice received Tekcal Mouse Chow 14% protein (#2014, INVIGO, containing 14.3% raw protein, 4.0% fat, and 48.0% carbohydrates), designed to promote normal body weight and longevity in the rodents from weaning [Postnatal Day (PD) 21] to the end of the study.

Treatments.

A total of 85 pregnant TS females were subcutaneously treated with oleic acid (400 mg/kg), linolenic acid (500 mg/kg), or vehicle (BSA 10%) from ED10 until PD2. The doses selected are neuroprotective and/or induce neurogenesis (35, 47, 57, 58). Plasma concentrations of the 3 compounds in the PD2 pups were quantified by HPLC. Male and female TS and CO pups gestated by 45 TS females under the different treatments were used to study the short-term effects, and TS and CO mice of both sexes gestated by 40 dams under the 3 treatment conditions were used for the long-term effects study. All experimental analyses were performed on the progeny of the pregnant-treated TS mice.

The offspring of these females were assigned to 1 of the 6 experimental groups depending on their karyotype and the prenatal treatment that they received: CO pups that were treated prenatally with vehicle (CO-V), oleic acid (CO-OA), or linolenic acid (CO-LNA), and TS pups that prenatally received vehicle (TS-V), oleic acid (TS-OA), or linolenic acid (TS-LNA). For the long-term effects study, 69 male and female TS and CO pups gestated by dams under the 3 treatments were assigned to the same experimental groups. Six to 7 pups from each group were used to evaluate the short-term effects (i.e. neurogenesis, cellularity, and brain volume), and 10–13 juvenile mice prenatally treated with oleic acid, linolenic acid, or vehicle ($n = 10$–13 per group; CO-V: $n = 10$, TS-V: $n = 13$, CO-OA: $n = 12$, TS-OA: $n = 10$, CO-LNA: $n = 12$, TS-LNA: $n = 12$), were used to assess the long-term effects of the treatments [i.e. cognition in the Morris water maze (MWM), neurogenesis, cellularity, and pre- and postsynaptic markers].

Short-term effects of prenatal treatments.

To evaluate the short-term effects of oleic and linolenic acids on cell proliferation, on PD2, all pups received an intraperitoneal injection of bromodeoxyuridine (BrdU) (150 μm/g). After 2 h, they were weighed, euthanized by decapitation, and their brains were removed, weighed and fixed in paraformaldehyde (PFA) solution, transferred to 30% sucrose, and frozen at −80°C. Coronal sections of 30 μm covering the whole hippocampus were cryosectioned, and stored at −20°C. From each animal 7 series containing 6–8 hippocampal sections were obtained to perform histological analyses: granular cell layer (GCL) volume, cell proliferation (BrdU), and granule cell density [4′,6-diamidino-2-phenylindole (DAPI) staining].

Long-term effects of prenatal treatments.

To evaluate the long-term effects of the different treatments on new neuron survival, on PD15 all pups received an intraperitoneal injection of BrdU (150 μg/g). On PD21, all the animals were weaned and subjected to the behavioral experiments (MWM) between PD30 and PD45. On PD45, the animals were euthanized by decapitation and the brains of 6–7 animals per group were removed, fixed with PFA, and used for the following histological and immunohistochemical analyses: GCL volume, cell proliferation (Ki67 immunohistochemistry), survival (BrdU immunohistochemistry), pre- and postsynaptic density [synaptophysin (SYN) and postsynaptic density protein 95 (PSD95) immunohistochemistry]. To this end, free-floating 50 μm coronal sections covering the whole hippocampus were cryosectioned. Nine

This study was supported by "Fondazione Generali e Assicurazioni Generali", Italy, Fundación Tatiana Pérez de Guzmán el Bueno, IDIVAL (NVAL 19/23), and the Spanish Ministry of Economy and Competitiveness (PSI-2016-76194-R, AEI/FEDER, EU).
Author disclosures: The authors report no conflicts of interest.
Supplemental Figure 1 is available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn.
S-GC and NR contributed equally to this work.
Address correspondence to CM-C (e-mail: martinec@unican.es).
Abbreviations used: AFP, α-fetoprotein; ARA, arachidonic acid; BrdU, bromodeoxyuridine; CO, Control; CO-LNA, Control linolenic acid; CO-OA, Control oleic acid; CO-V, Control vehicle; DAPI, 4′,6-diamidino-2-phenylindole; DS, Down syndrome; ED, Embryonic Day; GCL, granular cell layer; ML, molecular layer; MWM, Morris water maze; NPC, neural progenitor cells; PD, Postnatal Day; PFA, paraformaldehyde; PSD95, postsynaptic density protein 95; RM, repeated measures; SGZ, subgranular zone; SYN, synaptophysin; TS, Ts65Dn; TS-LNA, TS linolenic acid; TS-OA, TS oleic acid; TS-V, TS-vehicle; TX, Triton X; VGluT, vesicular glutamate transporter.

series, containing 6–8 hippocampal sections, were obtained from each animal.

Nissl staining

Morphological analysis of GCL volume was performed on 1 of 7 series, for the short-term studies, and on 1 of 9 series, for the long-term studies. Nissl staining was performed as previously described (59). To calculate the GCL volume, each coronal section of the brain was photographed and the images were analyzed using ImageJ software (http://rsb.info.nih.gov/ij/). In all cases, the Cavaliery stereological method was used (60).

Cell proliferation (Ki67 and BrdU immunofluorescence)

To quantify the short-term effect of the treatments on cell proliferation in the GCL of PD2 animals, BrdU immunohistochemistry was performed as previously described (10). The primary antibody used was an AntiBrdU at 1:100 (Santa Cruz) in PBS-TX (Triton X) 0.1% supplemented with 1% goat serum. The total number of BrdU+ cells was counted in the selected sections with Zen 2.6 software in the GCL and calculated using the optical dissector method, as previously described (60). In each animal, the total number of positive cells per slice was divided by the volume of the GCL, to calculate the density of proliferating cells.

For the long-term analyses of cell proliferation, the protocol described by Stagni et al. (18) was followed. The primary antibodies used were a rabbit anti-Ki67 at 1:750 (Abcam) diluted in phosphate buffer (PB) with 0.5% TX-100 and 0.1% BSA and an AntiBrdU at 1:100 (Santa Cruz). The total number of Ki67-positive cells or BrdU-positive cells in the selected sections was counted using an optical fluorescence microscope (Zeiss Axioskop 2 plus, 40× objective). The total number of positive cells was divided by the area of the subgranular zone (SGZ) (defined as the length of the SGZ multiplied by the thickness of the section) to determine the Ki67+ or BrdU+ cell density in the SGZ. The total number of Ki67+ cells and BrdU+ cells in the SGZ was calculated using the optical dissector method, as previously described (59, 60).

DAPI staining

For the study of the short- and long-term effects on the number of mature cells, sections were counterstained with DAPI (Calbiochem; 1:1000). In both the short- and long-term analysis, cell counts were performed using a previously described physical dissector system coupled with confocal microscopy (61, 62).

SYN and PSD95 immunofluorescence

For the long-term synaptic effects study, SYN and PSD95 immunohistochemistry were employed following the protocol previously described in Stagni et al. (18). The antibodies used were a mouse monoclonal anti-SYN (SY38) antibody (Millipore-Biomanufacturing and Life Science Research) and a rabbit polyclonal anti-PSD95 antibody (Abcam) both diluted to 1:1000. Fluorescent images were captured by a confocal microscope (Leica SP5), using a 63 × 1.4 objective and an 8× zoom. For each marker, 4 sections per animal were used comprising the entire hippocampus, and 1 random area in the molecular layer (ML) of the dentate gyrus (DG), CA (Cornus Ammonis) 1, and CA3 per section was measured. Image analysis was performed using the NIH ImageJ software. For each marker, the number of individual puncta exhibiting SYN or PSD95 immunoreactivity was counted in a circle with an area of 325 μm^2 for each image in each hippocampal field.

Cognitive analysis, MWM

Spatial learning and memory were evaluated using a modified version of the MWM (20). Sixteen consecutive daily sessions were performed: 12 acquisition sessions (platform submerged, in 8 of these, the position of the platform changed daily, while in the other 4 it was kept constant), followed by a probe trial and 4 cued sessions (platform visible). The computerized tracking system Anymaze (Stoelting) was used to analyze the trajectories of the animals and record escape latency, distance traveled, and swimming speed of each animal in each trial.

Statistics

Shapiro–Wilk tests were used to test the normality of the data sets. As they were all normally distributed, parametric tests were used. The water maze data from the acquisition sessions (sessions 1–12) were analyzed using 2-factor ANOVA with repeated measures (RM) ('session' × 'karyotype' × 'treatment' or 'trial' × 'karyotype' × 'treatment'). The rest of the data from the short- and long-term studies was analyzed using 2-factor ('karyotype' × 'treatment') ANOVA or RM ANOVA ('quadrant'). The mean values of each experimental group were compared post hoc using Fisher's LSD (least significant difference) post hoc tests. The differences between groups were considered to be statistically significant when $P < 0.05$. All analyses were performed using IBM SPSS (Armonk) for Windows version 22.0.

Results

Short-term effects of prenatal treatment

Plasma concentrations.

After administration of the vehicle to TS females during pregnancy, their PD2 offspring showed a mean value of 9.3 μg/mL of oleic acid and 2.7 μg/mL of linolenic acid in plasma. The PD2 pups whose TS mothers received oleic acid from ED10 to PD2 presented a mean value of 20.4 μg/mL of this fatty acid, whereas PD2 pups born from pregnant TS females that received linolenic acid during gestation showed a mean plasma concentration of 8.6 μg/mL of this acid.

Body and brain weight.

At PD2, TS-V mice presented smaller body ($P < 0.05$; **Figure 1**A) and brain weights ($P < 0.01$; Figure 1B) than CO-V mice. Prenatal linolenic acid treatment increased the body weight of LNA-CO pups with respect to CO-V mice ($P < 0.001$; Figure 1A).

Prenatal administration of oleic acid increased the brain weight of TS-OA mice at PD2 with respect to TS-V pups ($P < 0.01$). Linolenic acid administration increased the brain weight of mice of both karyotypes, as demonstrated by the significant increase observed in the TS-LNA group with respect to TS-V mice ($P < 0.001$), and in CO-LNA when compared with CO-V pups ($P < 0.001$; Figure 1B).

GCL volume.

At PD2, the 3 groups of TS mice presented smaller GCL volumes than the 3 groups of controls ('karyotype': $P = 0.012$; Figure 1C and D). Oleic acid treatment increased the GCL volume in pups of both karyotypes (TS-OA compared with TS-V, $P < 0.05$, CO-OA compared with CO-V, $P < 0.05$), whereas prenatal linolenic acid administration only enhanced the GCL volume of CO mice with respect to the CO-V group ($P < 0.05$; Figure 1C and D).

BrdU immunohistochemistry.

At PD2, TS-V mice presented a lower number of BrdU+ cells per slice ($P < 0.01$, **Figure 2**A and B), and a lower total number of cells than their CO-V littermates ($P < 0.01$; Figure 2C). Both prenatal treatments increased the number of BrdU+ cells per slice in TS pups (TS-OA compared with TS-V: $P < 0.001$; TS-LNA compared with TS-V: $P < 0.01$; Figure 2B), and the total number of BrdU+ cells (TS-OA compared with TS-V: $P < 0.01$; TS-LNA compared with TS-V: $P < 0.01$; Figure 2C). However, neither the density nor the total number of this population of cells were significantly modified by any of the treatments in PD2 CO mice.

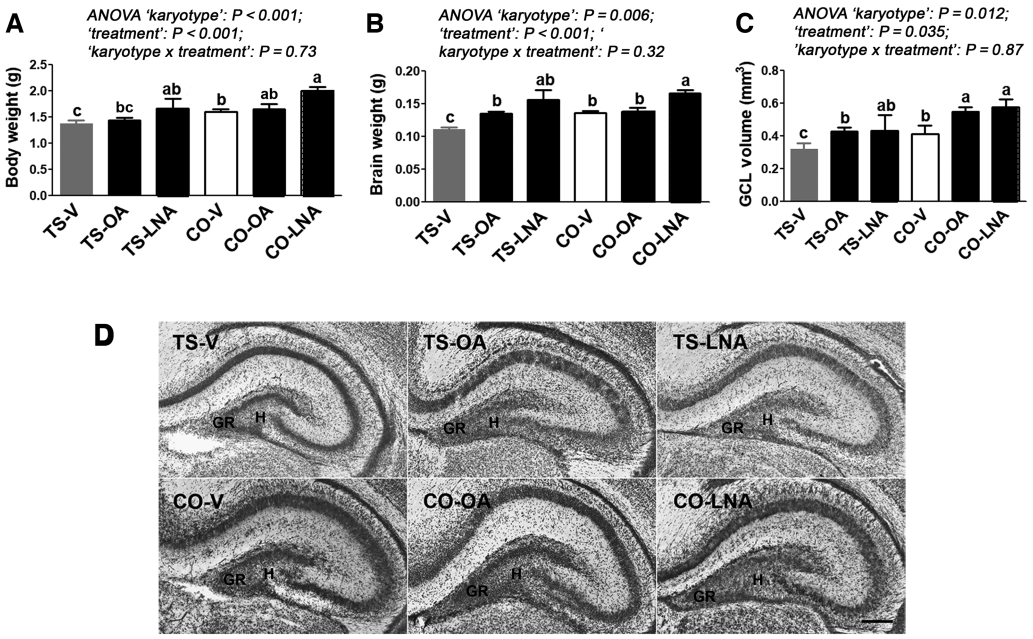

FIGURE 1 Body (A) and brain (B) weights, and GCL volume (C) of PD2 TS and CO mice prenatally treated with oleic acid, linolenic acid, or vehicle. Representative microscopy sections of Nissl staining in the hippocampus of the 6 groups of mice (D). Values in A, B, and C are means ± SEMs, $n = 6$–7 per group. Labeled bars without a common letter differ by $P < 0.05$, by Fisher's LSD post hoc tests; scale bar in D: 200 μm. CO, Control; CO-LNA, Control linolenic acid; CO-OA, Control oleic acid; CO-V, Control vehicle; GCL, granular cell layer; LSD, least significant difference; PD, Postnatal Day; TS, Ts65Dn; TS-LNA, TS linolenic acid; TS-OA, TS oleic acid; TS-V, TS-vehicle; GR, Granular Region; H, Hilus.

Mature granule cell count (DAPI staining).

TS-V mice presented a lower total number of DAPI+ cells than their CO-V littermates ($P < 0.05$; Figure 2D and F), although the density of this population of cells did not significantly differ between PD2 pups of both karyotypes (Figure 2E). In TS mice, oleic acid treatment increased the density (TS-OA compared with TS-V: $P < 0.05$; Figure 2E) and the total number of DAPI+ cells (TS-OA compared with TS-V $P < 0.01$; Figure 2F), and both treatments significantly increased the total number of this population of cells in CO animals (CO-OA compared with CO-V: $P < 0.05$; CO-LNA compared with CO-V: $P < 0.05$; Figure 2F).

Long-term effects of prenatal treatment

Histology.

At PD45, TS-V mice presented a lower density ($P < 0.01$; **Supplemental Figure 1**B), and a lower total number of Ki67+ ($P < 0.01$; Supplemental Figure 1C) and BrdU+ ($P < 0.05$; Supplemental Figure 1E) cells than their CO-V littermates. Prenatal treatment with oleic or linolenic acid did not exert any long-term effect on the GCL volume, the density, or the total number of Ki67+ or BrdU+ cells in TS mice, since neither TS-OA nor TS-LNA mice differed in any of these measurements from TS-V mice (Supplemental Figure 1A–E). However, at PD45, TS-LNA mice showed a higher density of mature DAPI + cells than TS-V mice ($P < 0.05$; Supplemental Figure 1F).

At PD45, TS-V mice presented a smaller number of PSD95+ puncta than their CO-V littermates in all hippocampal areas analyzed (CA1: $P < 0.05$; CA3: $P < 0.05$; ML: $P < 0.01$; **Figure 3**A and B). Prenatal treatment with oleic acid produced a long-term enhancement of the number of PSD95+ puncta in TS mice (TS-OA compared with TS-V: CA1: $P < 0.05$; CA3: $P < 0.01$; ML: $P < 0.05$); whereas treatment with linolenic acid increased the number of PSD95+ puncta in the ML of TS-LNA mice with respect to TS-V animals, although no changes were observed in the other hippocampal areas analyzed (CA1: $P = 0.65$; CA3: $P = 0.14$; ML: $P < 0.05$).

Although TS-V mice presented a smaller number of SYN+ puncta than CO-V mice in CA1 ($P < 0.05$), CA3 ($P < 0.01$), and in the ML ($P < 0.01$; Figure 3C and D), none of the treatments modified the number of SYN + puncta in TS or CO mice.

Cognition: MMW

Reference learning and memory.

The 6 groups of mice reduced their latency to reach the platform when all sessions were taken into account (session 1–12: RM ANOVA 'session': $P < 0.001$; **Figure 4**A), both in the sessions in which the platform position was changed daily (sessions 1–8: $P < 0.001$) and in those in which the platform position was kept constant (sessions 9–12: $P < 0.001$).

The reduction in latency between sessions significantly differed between animals of both karyotypes ('session × karyotype': $P < 0.001$) and of the 3 treatment conditions ('session × treatment': $P = 0.001$).

When each pair of learning curves was analyzed separately, TS-V mice presented a deteriorated performance when compared with their CO-V littermates ($P < 0.001$; Figure 4B).

TS-OA (sessions 1–12: $P = 0.006$; sessions 1–8: $P = 0.018$; sessions: 9–12: $P = 0.026$; Figure 4C), and TS-LNA mice showed lower latencies to reach the platform than TS-V animals (sessions 1–12: $P = 0.007$; sessions 1–8: $P = 0.020$; sessions 9–12: $P = 0.059$; Figure 4E).

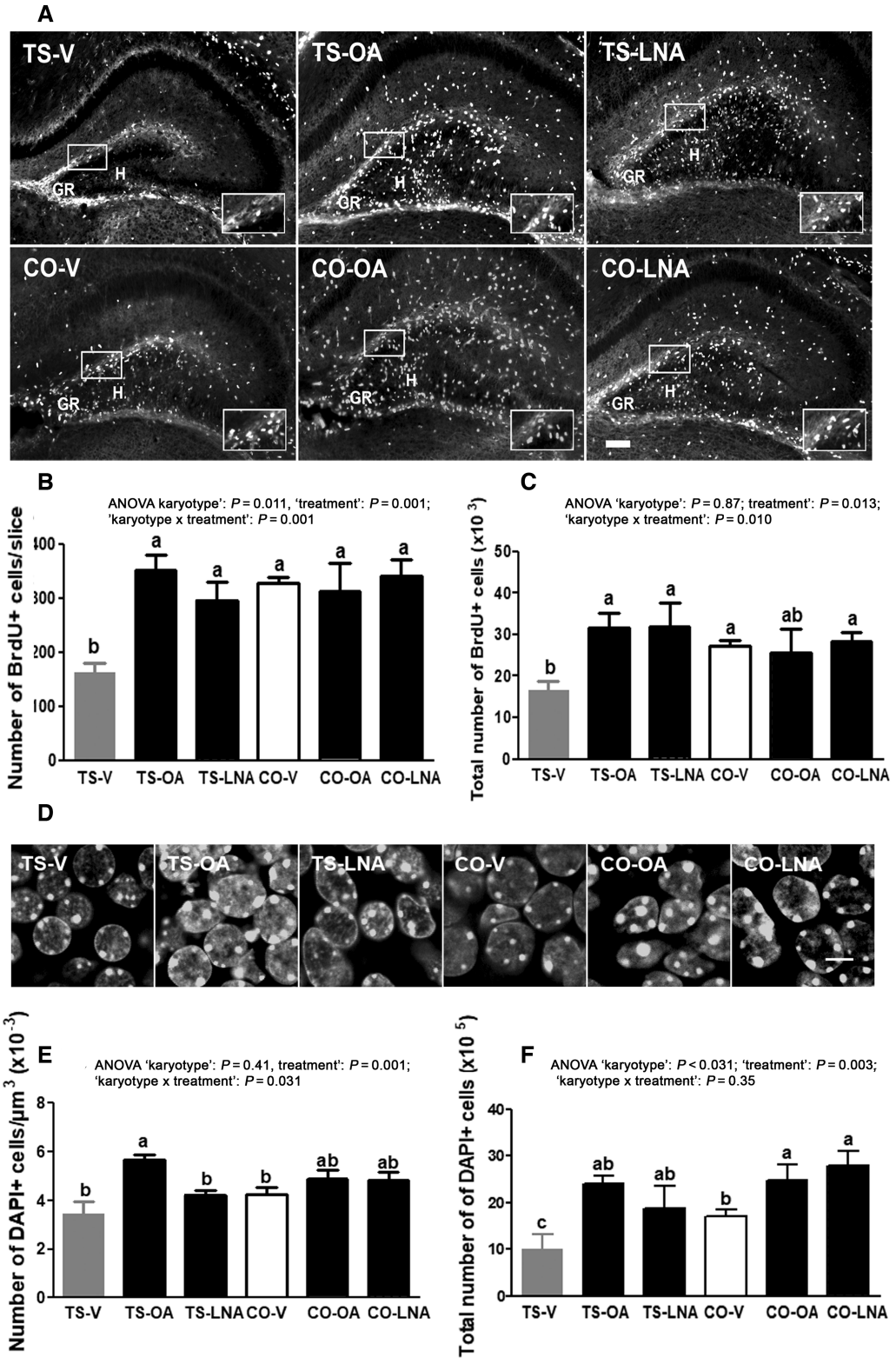

FIGURE 2 Representative confocal images of BrdU+ immunohistochemistry (A) and by DAPI staining (D), number of BrdU+ cells per slice (B), the total number (C) of BrdU+ cells, and of the density (E) and total number (F) of DAPI+ cells in the hippocampus of TS and CO pups prenatally treated with oleic acid, linolenic acid, or vehicle. Scale bar in A: 100 μm, scale bar in D: 5 μm. Values in B, C, E, and F are means ± SEMs, $n = 6$–7 per group. Labeled bars without a common letter differ by $P < 0.05$, by Fisher's LSD post hoc tests. BrdU, bromodeoxyuridine; CO, Control; CO-LNA, Control linolenic acid; CO-OA, Control oleic acid; CO-V, Control vehicle; DAPI, 4′,6-diamidino-2-phenylindole; LSD, least significant difference; PD, Postnatal Day; TS, Ts65Dn; TS-LNA, TS linolenic acid; TS-OA, TS oleic acid; TS-V, TS-vehicle; GR, Granular Region; H, Hilus.

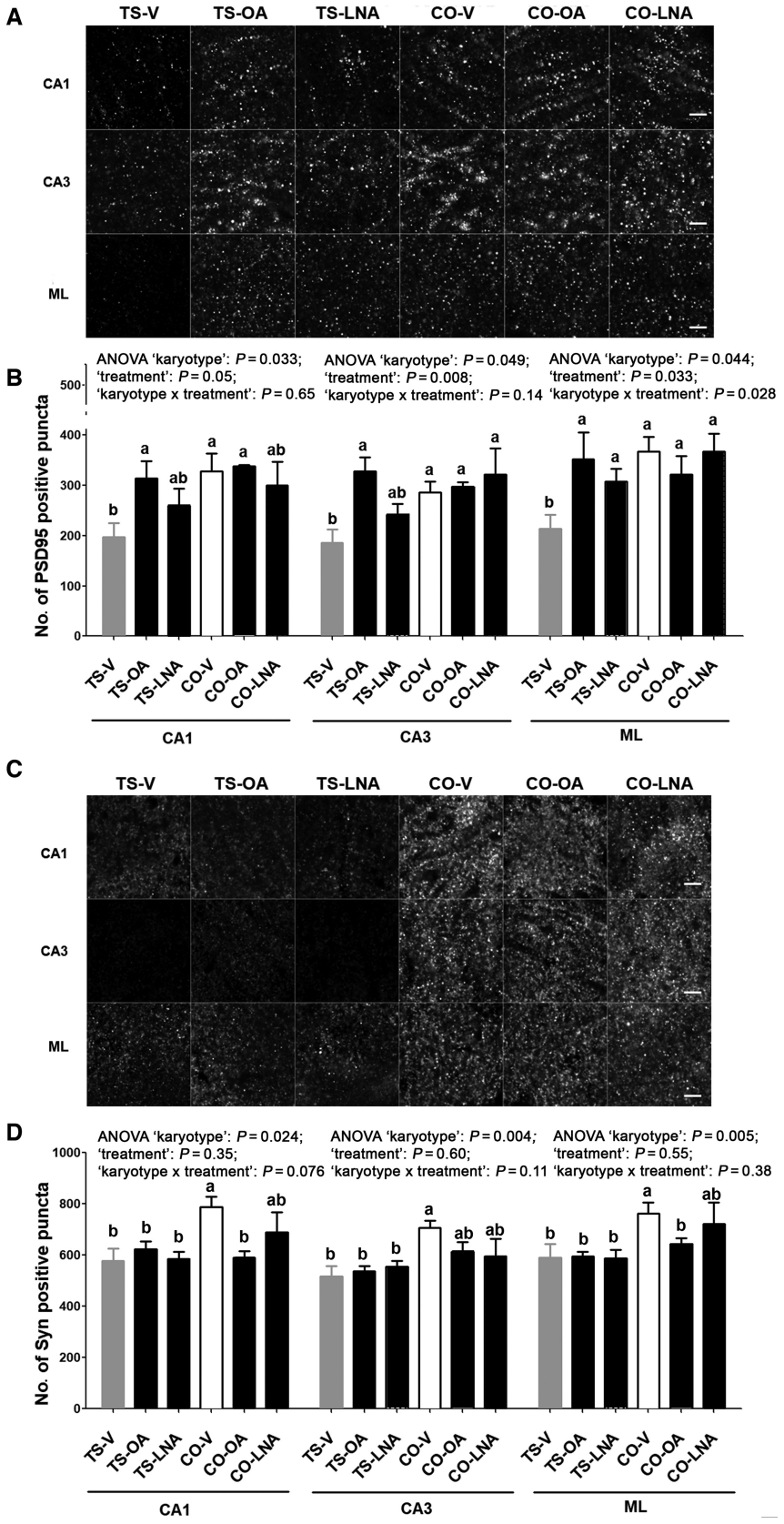

FIGURE 3 Representative confocal images PSD95 (A), and SYN (C) immunochemistry in the hippocampus, number of PSD95+ (B), and SYN+ (D) puncta in the CA1, CA3 areas and ML of the hippocampus aged 45-d TS and CO mice that received oleic acid, linolenic acid, or vehicle prenatally. Scale bars in A and C: 5 μm. Values in B and D are means ± SEMs, $n = 6$–7 per group. Labeled bars without a common letter differ by $P < 0.05$, by Fisher's LSD post hoc tests. CA1, Cornus Ammonis 1; CA2, Cornus Ammonis 2; CO, Control; CO-LNA, Control linolenic acid; CO-OA, Control oleic acid; CO-V, Control vehicle; LSD, least significant difference; ML, molecular layer; PD, Postnatal Day; PSD95, postsynaptic density protein 95; SYN, synaptophysin; TS, Ts65Dn; TS-LNA, TS linolenic acid; TS-OA, TS oleic acid; TS-V, TS-vehicle.

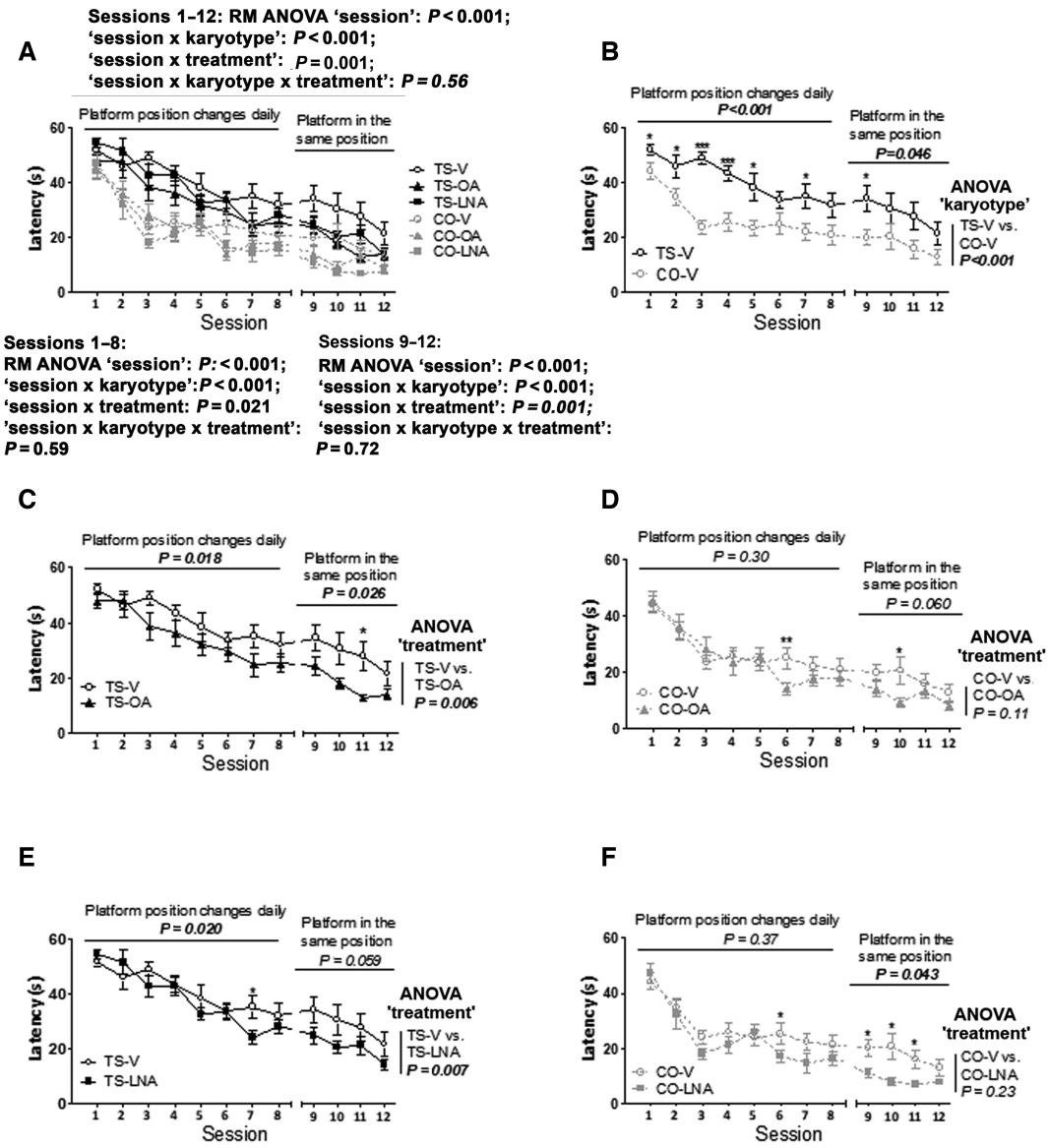

FIGURE 4 Latency to reach the platform during the 12 acquisition sessions in the MWM exhibited between PD30 and PD45 by all groups of mice (A), by TS-V and CO-V mice (B), by TS-OA and TS-V mice (C), by CO-OA and CO-V mice (D), by TS-LNA and TS-V mice (E), and by CO-LNA and CO-V mice (F). Values are means ± SEMs, n = 10–13 per group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with CO-V (in B, D, and F) or compared with TS-V (in C and E), Fisher's LSD post hoc tests. On the right side of each figure, the P value of the difference between both learning curves across the 12 sessions (RM ANOVAs) is shown. On top of each figure, the P values of the differences between the learning curves of the different groups of mice during the first 8 and the last 4 sessions are shown. CO, Control; CO-LNA, Control linolenic acid; CO-OA, Control oleic acid; CO-V, Control vehicle; LSD, least significant difference; MWM, Morris water maze; PD, Postnatal Day; RM, repeated measures; TS, Ts65Dn; TS-LNA, TS linolenic acid; TS-OA, TS oleic acid; TS-V, TS-vehicle.

In the case of CO animals, prenatal administration of oleic acid did not modify their performance in the MWM, since CO-OA mice did not differ in their latency to reach the platform when compared with CO-V mice (sessions 1–12: $P = 0.11$; sessions 1–8: $P = 0.30$; sessions 9–12: $P = 0.060$; Figure 4D). CO-LNA animals presented lower latencies to reach the platform than the CO-V group only in the sessions in which the platform position was kept constant (sessions 1–12: $P = 0.23$; sessions 1–8: $P = 0.37$; sessions 9–12: $P = 0.043$; Figure 4F).

Working memory.

When all the groups of mice were analyzed together, statistical analyses demonstrated a marked reduction in their latency to reach the platform across trials (RM ANOVA 'trial': $P < 0.001$; Figure 5A). However, the reduction in these latencies differed between the 3 groups of TS and the 3 groups of CO mice ('trial × karyotype': $P < 0.001$), and between TS and CO mice under the 3 treatment conditions ('trial × treatment': $P = 0.037$).

When each pair of learning curves was analyzed separately, it was observed that TS-V animals showed a deteriorated working memory, since they did not reduce their latency to reach the platform throughout the trials ($P = 0.82$; Figure 5B), and their latency to reach the platform was higher than that of CO-V mice ($P = 0.001$, Figure 5B). However, TS-OA (RM ANOVA 'trial': $P = 0.012$, Figure 5C) and TS-LNA mice ($P = 0.052$; Figure 5E)

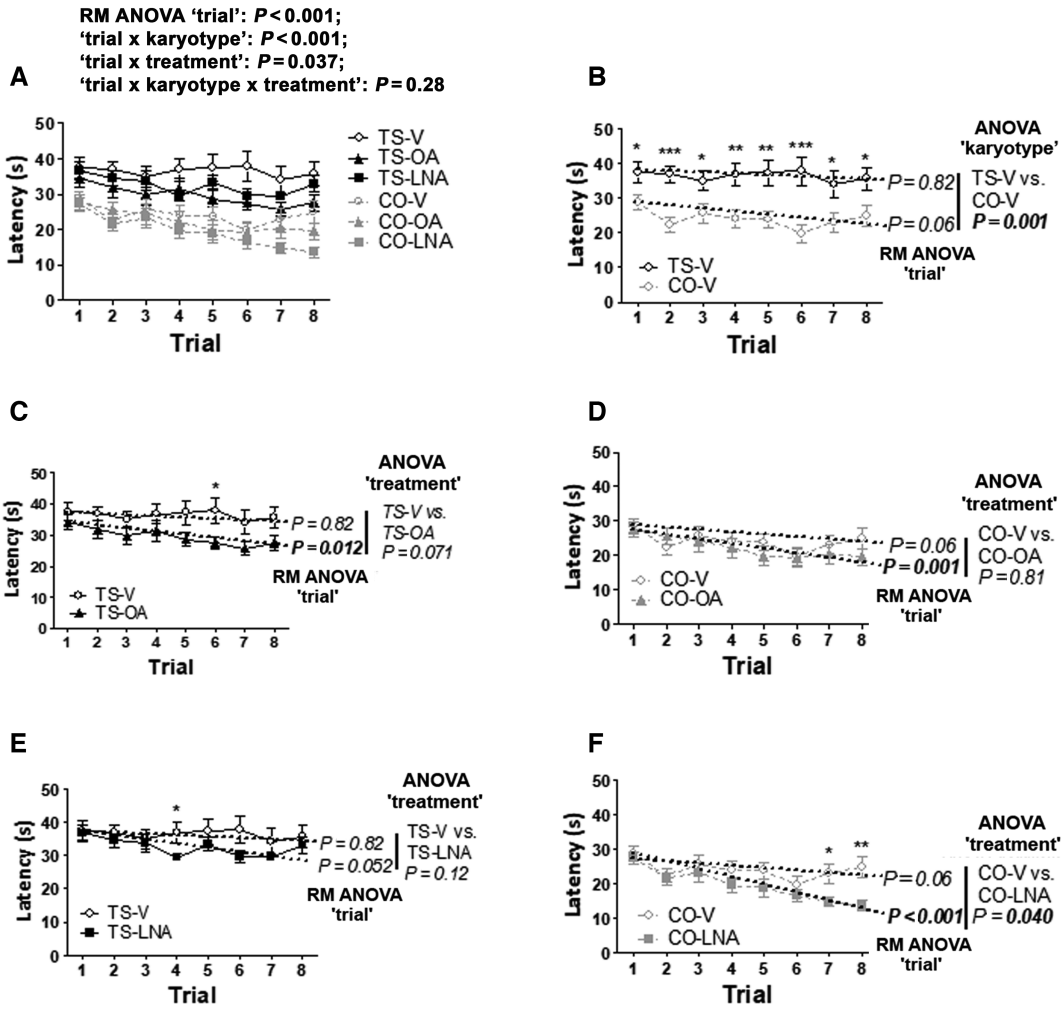

FIGURE 5 Latency to reach the platform during each trial of the first 8 acquisition sessions exhibited between PD30 and PD45 by all animals (A), by TS-V and CO-V mice (B), by TS-OA and TS-V mice (C), by CO-OA and CO-V mice (D), by TS-LNA and TS-V mice (E), and by CO-LNA and CO-V mice (F), in the MWM. Values are means ± SEMs, $n = 10$–13 per group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared with CO-V (in B, D, and F) or compared with TS-V (in C and E) Fisher's LSD post hoc tests. On the right side of each figure, the P value of the main effects for 'karyotype' (Figure 5B), or 'treatment': (Figure 5C–F) after RM ANOVAs, are shown. The dotted lines and the P values beside them represent the significance of the change in latency across the trials (RM ANOVA 'trial' of each learning curve). CO, Control; CO-LNA, Control linolenic acid; CO-OA, Control oleic acid; CO-V, Control vehicle; LSD, least significant difference; MWM, Morris water maze; PD, Postnatal Day; RM, repeated measures; TS, Ts65Dn; TS-LNA, TS linolenic acid; TS-OA, TS oleic acid; TS-V, TS-vehicle.

reduced their latency to reach the platform between trials, indicative of their ability to learn the platform position across trials.

In euploid mice, both groups of mice, CO-OA ('trial': $P < 0.001$; Figure 5D) and CO-LNA ($P < 0.001$; Figure 5F) reduced their latency to reach the platform between trials.

Cued sessions.

TS and CO mice under the different treatments did not differ in their latency to reach the platform during the cued sessions when the platform was visible ('karyotype': $P = 0.14$, 'treatment': $P = 0.30$; 'karyotype × treatment': $P = 0.038$; data not shown).

Spatial memory.

During the probe trial, TS-V mice crossed fewer times over the place where the platform was located during the training sessions ($P < 0.05$; Figure 6A) and entered the trained quadrant fewer times ($P < 0.05$; Figure 6B) than their CO-V littermates.

Prenatal treatment with oleic acid or linolenic acid increased the number of crossings that TS mice performed over the platform position with respect to vehicle-treated trisomic animals (TS-OA compared with TS-V: $P = 0.05$; TS-LNA compared with TS-V: $P < 0.05$; Figure 6A), but did not exert any significant effect on the number of entries that TS or CO mice made into the trained quadrant (Figure 6B).

TS-V mice did not show a preference for any of the quadrants ($P = 0.54$; Figure 6C and E). However, prenatal oleic acid or linolenic acid treatment improved the spatial memory of TS mice, since TS-OA and TS-LNA animals spent more time in the trained quadrant than in the rest of the quadrants (TS-OA: $P < 0.001$; TS-LNA: $P < 0.001$; Figure 6C and E). The 3 groups of CO mice spent a higher percentage of time in the trained quadrant than in the rest of the quadrants (CO-OA: $P < 0.001$; CO-LNA: $P < 0.001$; CO-V: $P < 0.001$; Figure 6D and F).

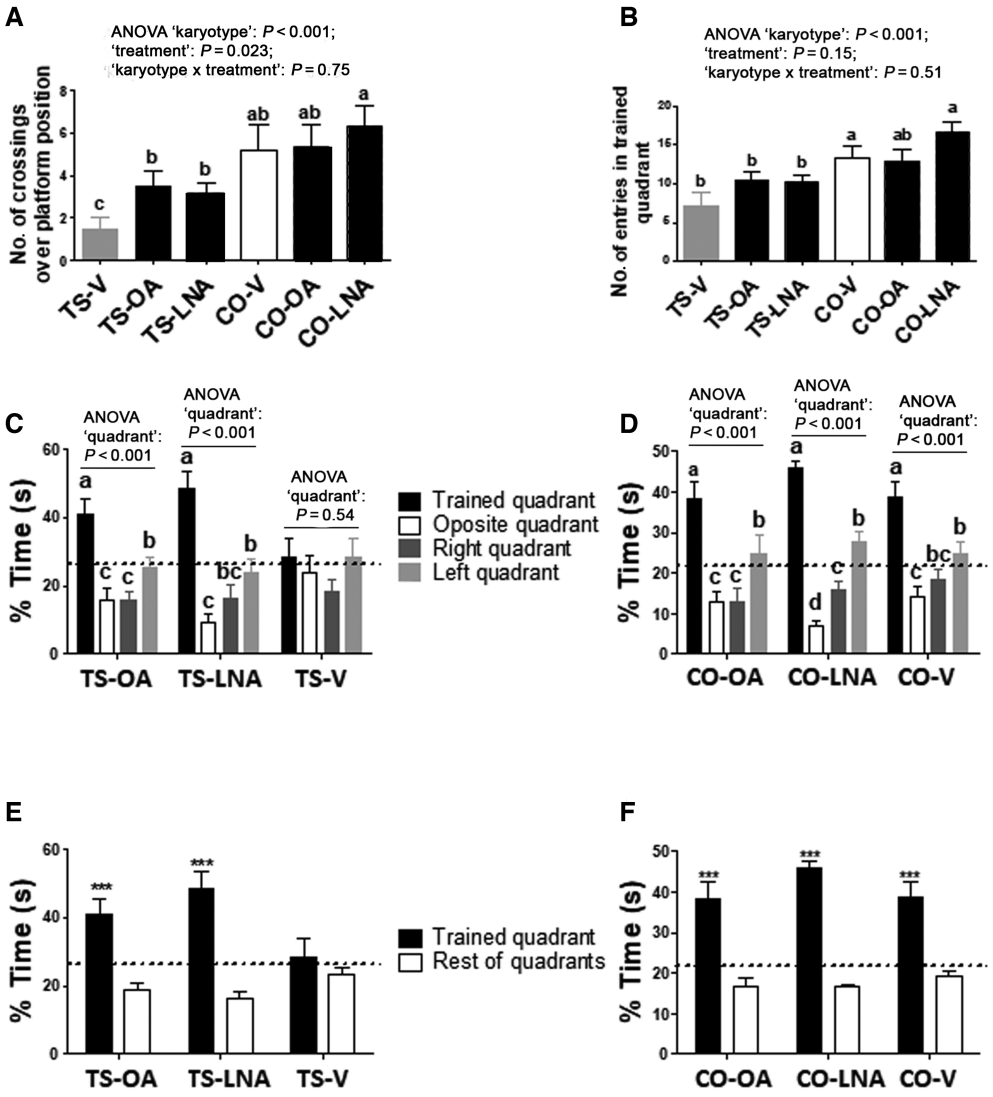

FIGURE 6 Number of crossings over the platform position (A) and number of entries in the trained quadrant (B) performed at PD45 in the probe trial by TS and CO mice prenatally treated with oleic acid, linolenic acid, or vehicle. Percentage of time spent in each quadrant during the probe trial by TS-OA, TS-LNA, and TS-V (C), and CO-OA, CO-LNA, and CO-V animals (D), and percentage of time spent in the trained quadrant compared with the rest of the quadrants by TS-OA, TS-LNA, and TS-V (E), and CO-OA, CO-LNA, and CO-V (F) mice. Values are means ± SEMs, $n = 10$–13 per group. Labeled bars without a common letter differ by $P < 0.05$, by Fisher's LSD post hoc tests. $^{***}P < 0.001$ trained quadrant compared with the rest of the quadrants, Fisher's LSD post hoc tests. The dotted lines in Figure 6C–F represent the chance level, i.e. a probability equal to 25% of the time. CO, Control; CO-LNA, Control linolenic acid; CO-OA, Control oleic acid; CO-V, Control vehicle; LSD, least significant difference; PD, Postnatal Day; TS, Ts65Dn; TS-LNA, TS linolenic acid; TS-OA, TS oleic acid; TS-V, TS-vehicle.

Discussion

In this study, prenatal administration of oleic acid and linolenic acid rescued several neuromorphological alterations found in newborn TS mice. In particular, these treatments increased their brain weight, the volume of their GCL, and the number of proliferating and mature granule cells in their hippocampi. When the effects of these treatments were analyzed 6 wk after the discontinuation of the treatment, no significant effects were found in the GCL volume, the number and density of Ki67+ or BrdU+ cells or the number of SYN + puncta found in the hippocampus of TS mice. However, both treatments increased the number of PSD95 + puncta. When the long-term functional effects of both prenatal treatments were analyzed, TS mice that received oleic acid or linolenic acid showed improved reference, working, and spatial memory in the MWM.

Both oleic acid and linolenic acid have been demonstrated to cross the placental and blood-brain barrier (35, 47, 63). In this study, the pups of the dams treated with these acids presented an increased concentration of both acids in plasma. Thus, oleic acid and linolenic acid were adequately transferred to the pups, incorporated into the bloodstream, and distributed throughout the organism.

Consistent with the altered prenatal neurodevelopment of TS mice and DS individuals (2, 3, 10, 11, 14, 21, 64), we found that TS mice presented reduced brain weight, GCL volume, cell proliferation, and density of mature cells. Both lipid composition and fatty acid metabolism are essential for proper neurodevelopment. DS individuals present lower concentrations of MUFAs, especially of oleic acid, in their brains (52, 65). Thus, these deficiencies could play an essential role in the neurodevelopmental alterations and/or in the functional anomalies found in DS. However, there is increasing evidence

that brain development is not only affected by the total concentrations of fatty acids, but also by the ratio and the relation between these acids. Considering DS, corn oil is a source of linoleic acid, which is a precursor of PUFAs of the omega-6 series and produces arachidonic acid (ARA), and improves brain developmentIn DS, corn oil is a source of linoleic acid, a precursor of PUFAs of the ω-6 series, which produces arachidonic acid (ARA) improves brain development (66), supporting the idea of the essential role of ω-6 and ARA in neurodevelopment. Furthermore, DS brains present an increased ratio of PUFAs of the ω-3 series with respect to those of the ω-6 series (65). These authors proposed that this ratio was modified due to the decrease of ARA in brains and that this effect plays a role in the alterations in neurodevelopment found in DS. Therefore, in the present study it is possible that adding ω-3 PUFAS to the diet induced a reduction in ARA concentrations, thereby increasing the total amount of ω-3 fatty acids and enhancing the ω-3: ω-6 ratio. This effect could be partially responsible for the benefits found after its administration.

In the present study, the prenatal administration of oleic and linolenic acids increased cell proliferation in the hippocampi of TS pups and reduced the hypocellularity in this structure. There is evidence of the proneurogenic effect of both acids under different conditions (25, 32–34, 47, 48, 66). The prenatal administration of compounds that increase neurogenesis, such as the serotonin reuptake inhibitor Fluoxetine, normalizes the neuroanatomical anomalies in TS mice (11). Thus, the increase in granule cell density induced by these compounds could be due to enhanced cell proliferation, differentiation, and/or survival.

During neurodevelopment, the primary role of oleic acid in neurogenesis is to act as a neurotrophic factor (26, 27). During this period, brain development is regulated by the α-fetoprotein (AFP)/albumin ratio that modulates the neurotrophic effects of oleic acid (67). Thus, the balance between these 2 signals seems to be essential to induce neurogenesis in embryonic development, whereas its imbalance contributes to the onset of several alterations during neurodevelopment. DS individuals present a reduction in the serum concentrations of AFP and albumin (68, 69) that correlates with a lower concentration of oleic acid in the brain (65). Thus, it is possible, that after the administration of oleic acid during the prenatal stages, adequate concentrations of this fatty acid were reached, thereby correcting the alterations in hippocampal neurogenesis in TS mice. However, after discontinuation of the treatment, these concentrations would likely have returned to their original altered states, and this might be 1 of the reasons why the beneficial effects of oleic and linolenic acids on neurogenesis in TS mice are not seen 1 mo later.

Regarding linolenic acid, it has been demonstrated that during gestation, and in the first stages of postnatal life, an adequate intake of ω-3 fatty acids, such as linolenic acid, is necessary to allow the correct development of the brain and neurogenesis (32). Most of the beneficial effects of ω-3 fatty acids are due to their conversion into DHA. Numerous studies have demonstrated that the administration of a diet rich in linolenic acid promotes hippocampal neurogenesis (33, 34, 70). In contrast, DHA deficiency during gestation, due to the reduced intake of linolenic acid, alters brain morphology, including the cortical and hippocampal areas, as well as hippocampal neurogenesis (32). These authors suggest that the inhibition of neurogenesis which occurs after linolenic acid restriction during gestation could be due to a delay in the cell cycle or the onset of neurogenesis. In TS mice, alterations in neurogenesis during prenatal stages are also due to a slowing down of the cell cycle (1, 10, 14). Thus, the administration of linolenic acid to pregnant TS females could accelerate the speed and/or reduce alterations in the cell cycle, increasing the number of neuronal precursors in TS pups.

Oleic acid and linolenic acid treatment also enhanced the number of mature granular cells, possibly due to the well-known effect of both acids on neuronal differentiation and survival (30, 70–72). This increased cellularity is likely to be responsible for the increase in the GCL volume and brain weight found in TS mice after prenatal oleic acid and linolenic acid treatment. Linolenic acid treatment increases hippocampal volume (47, 72); conversely, restriction of linolenic acid in the diet during gestation alters normal brain development (32). As low concentrations of fatty acids are found in DS brains (52, 65), their exogenous administration to pregnant TS mice could positively impact prenatal brain development, restoring the size of the hippocampal structures in their progeny.

However, most of the beneficial effects of the prenatal administration of oleic and linolenic acids on the neuromorphological alterations found in TS mice were not maintained 6 wk after the discontinuation of the treatment. These results indicate that the presence of oleic acid and linolenic acid may be necessary to induce their proliferative and prosurvival effects in TS mice.

Administration of fatty acids during adulthood also reduces the neuropathology found in TS mice. Giacomini et al. (66) showed that treatment with corn oil, which contains both oleic and linoleic acids, rescued brain weight, neurogenesis, dendritogenesis, and cognition in adult TS mice. In addition, using cultures of neural progenitor cells (NPCs) obtained from DS individuals, they demonstrated that both linolenic and oleic acids can increase the proliferation rate of NPCs (66).

One of the most relevant results of this study is the finding that prenatal administration of oleic and linolenic acid produced long-term enhancement of the cognitive abilities of TS mice in the MWM. Those animals that received this treatment during gestation demonstrated better reference, working, and spatial memory than the ones that received the vehicle, as indicated by their reduced latency to reach the platform across sessions (reference memory) and across trials in each session (working memory). Finally, both treatments also increased the memory of the platform position in TS mice, since these animals spent more time searching for the platform position in the trained quadrant and crossed over the platform position more times than the vehicle-treated TS mice. These results agree with the literature that reports the procognitive effects of oleic and linolenic acids and their derivatives. DHA supplementation improves cognition and spatial memory in normal rodents, in murine models of Alzheimer's disease (AD) (49, 50), and in the pups of rats supplemented with linolenic acid during gestation (51). Moreover, administration of corn oil to adult TS mice also induces procognitive effects (66), and treatment with a diet supplemented with fish oil that contained linolenic acid rescued cognitive deficits in mice that overexpressed the *RCAN-1* gene which is triplicated in DS and TS mice (73).

The procognitive effects found in this study cannot be attributed to changes in neurogenesis. However, changes in the characteristics of the synaptic connections might be partially responsible for these beneficial effects in the cognitive abilities of TS mice.

Although the total number of synapses was unchanged after both treatments, as demonstrated by the similar number of SYN + puncta found in TS mice under all treatments, oleic acid administration increased the number of PSD95 + puncta, a postsynaptic marker of excitatory synapses (74, 75), in all

hippocampal areas analyzed. Linolenic acid treatment only increased PSD95+ in the ML of the hippocampus. Since the number of synapses was similar in all groups, these results suggest that the altered inhibitory-excitatory balance of TS mice could have been partially corrected. The over-inhibition, due to enhanced GABA(Gamma-Aminobutyric Acid)ergic transmission and reduced glutamatergic transmission, characteristic of TS mice, has been proposed to play an essential role in the cognitive difficulties of TS mice (76). Among the mechanisms proposed as being responsible for the beneficial effects of oleic and linolenic acids is the increase in glutamatergic transmission, through the increased expression of vesicular glutamate transporters 1 and 2 (VGluT1 and VGluT2) (33, 71, 72). Oleic acid promotes the synthesis of the postsynaptic protein PSD95 (30), which is implicated in long-term potentiation (LTP) generation (77). Thus, an increase in excitatory transmission could be partially responsible for the long-term benefits exerted by oleic acid and linolenic acid in the reference memory, working memory, and spatial memory of TS mice found in this study.

In conclusion, the prenatal administration of oleic and linolenic acid restored several neuromorphological alterations in the TS65Dn mouse model of DS. In addition, they produced long-term enhancement of the cognitive abilities of these animals, possibly by normalizing the excitatory-inhibitory synaptic balance. These results provide evidence for the potential therapeutic effect of oleic and linolenic acids in DS. Given that both are natural substances present in the human diet, they could be administered prenatally and thereby exert stronger effects on the neurodevelopment of the DS population.

Acknowledgments

The authors' contributions were as follows—SG-C and NR: conducted the research and analyzed data; VV, AP, VC, SL, ON, and AV: conducted the research; SG-C, NR, and CM-C: wrote the manuscript; RB and CM-C: designed the research and analyzed data; CM-C: had primary responsibility for the final content; and all authors read and approved the final manuscript.

References

1. Contestabile A, Fila T, Ceccarelli C, Bonasoni P, Bonapace L, Santini D, Bartesaghi R, Ciani E. Cell cycle alteration and decreased cell proliferation in the hippocampal dentate gyrus and in the neocortical germinal matrix of fetuses with Down syndrome and in Ts65Dn mice. Hippocampus 2007;17:665–78.
2. Guidi S, Bonasoni P, Ceccarelli C, Santini D, Gualtieri F, Ciani E, Bartesaghi R. Neurogenesis impairment and increased cell death reduce total neuron number in the hippocampal region of fetuses with Down syndrome. Brain Pathol 2008;18:180–97.
3. Guidi S, Ciani E, Bonasoni P, Santini D, Bartesaghi R. Widespread proliferation impairment and hypocellularity in the cerebellum of fetuses with Down syndrome. Brain Pathol 2011;21:361–73.
4. Rueda N, Flórez J, Martínez-Cué C. Mouse models of Down syndrome as a tool to unravel the causes of mental disabilities. Neural Plast 2012:584071.
5. Chakrabarti L, Galdzicki Z, Haydar TF. Defects in embryonic neurogenesis and initial synapse formation in the forebrain of the Ts65Dn mouse model of Down syndrome. J Neurosci 2007;27:11483–95.
6. Das I, Reeves RH. The use of mouse models to understand and improve cognitive deficits in Down syndrome. Dis Model Mech 2011;4:596–606.
7. Xing Z, Li Y, Pao A, Bennett AS, Tycko B, Mobley W, Yu E. Mouse-based genetic modeling and analysis of Down syndrome. Br Med Bull 2016; 120:111–22.
8. Malberg JE, Eisch AJ, Nestler EJ, Duman RS. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J Neurosci 2000 20:9104–10.
9. Clark S, Schwalbe J, Stasko MR, Yarowsky PJ, Costa ACS. Fluoxetine rescues deficient neurogenesis in hippocampus of the Ts65Dn mouse model for Down syndrome. Exp Neurol 2006;200: 256–61.
10. Bianchi P, Ciani E, Guidi S, Trazzi S, Felice D, Grossi G, Fernandez M, Giuliani A, Calzà L, Bartesaghi R. Early pharmacotherapy restores neurogenesis and cognitive performance in the Ts65Dn mouse model for Down syndrome. J Neurosci 2010;30:8769–79.
11. Guidi S, Stagni F, Bianchi P, Ciani E, Giacomini A, De Franceschi M, Moldrich R, Kurniawan N, Mardon K, Giuliani A, et al. Prenatal pharmacotherapy rescues brain development in a Down's syndrome mouse model. Brain 2014;137:380–401.
12. Stagni F, Giacomini A, Guidi S, Ciani E, Ragazzi E, Filonzi M, De Iasio R, Rimondini R, Bartesaghi R. Long-term effects of neonatal treatment with fluoxetine on cognitive performance in Ts65Dn mice. Neurobiol Dis 2015;74:204–18.
13. Contestabile A, Greco B, Ghezzi D, Tucci V, Benfenati F, Gasparini L. Lithium rescues synaptic plasticity and memory in Down syndrome mice. J Clin Invest 2013;123:348–61.
14. Bianchi P, Ciani E, Contestabile A, Guidi S, Bartesaghi R. Lithium restores neurogenesis in the subventricular zone of the Ts65Dn mouse, a model for Down syndrome. Brain Pathol 2010;20:106–18.
15. Zhou WB, Miao ZN, Zhang B, Long W, Zheng FX, Kong J, Yu B. Luteolin induces hippocampal neurogenesis in the Ts65Dn mouse model of Down syndrome. Neural Regen Res 2019;14:613–20.
16. Parrini M, Ghezzi D, Deidda G, Medrihan L, Castroflorio E, Alberti M, Baldelli P, Cancedda L, Contestabile A. Aerobic exercise and a BDNF-mimetic therapy rescue learning and memory in a mouse model of Down syndrome. Sci Rep 2017;7:16825.
17. Nakano-Kobayashi A, Awaya T, Kii I, Sumida Y, Okuno Y, Yoshida S, Sumida T, Inoue H, Hosoya T, Hagiwara M. Prenatal neurogenesis induction therapy normalizes brain structure and function in Down syndrome mice. Proc Natl Acad Sci U S A 2017;114: 10268–73.
18. Stagni F, Giacomini A, Emili M, Trazzi S, Guidi S, Sassi M, Ciani E, Rimondini R, Bartesaghi R. Short- and long-term effects of neonatal pharmacotherapy with epigallocatechin-3-gallate on hippocampal development in the Ts65Dn mouse model of Down syndrome. Neuroscience 2016;333:277–301.
19. Corrales A, Vidal R, García S, Vidal V, Martínez P, García E, Flórez J, Sanchez-Barceló EJ, Martínez-Cué C, Rueda N. Chronic melatonin treatment rescues electrophysiological and neuromorphological deficits in a mouse model of Down syndrome. J Pineal Res 2014;56: 51–61.
20. Martínez-Cué C, Martínez P, Rueda N, Vidal R, García S, Vidal V, Corrales A, Montero JA, Pazos Á, Flórez J, et al. Reducing $GABA_A$ $\alpha 5$ receptor-mediated inhibition rescues functional and neuromorphological deficits in a mouse model of Down syndrome. J Neurosci 2013;33:3953–66.
21. Guihard-Costa AM, Khung S, Delbecque K, Ménez F, Delezoide AL. Biometry of face and brain in fetuses with trisomy 21. Pediatr Res 2006; 59: 33–8.
22. Incerti M, Horowitz K, Roberson R, Abebe D, Toso L, Caballero M, Spong CY. Prenatal treatment prevents learning deficit in Down syndrome model. PLoS One 2012;7(11):e50724.
23. Gil-Sánchez A, Koletzko B, Larqué E. Current understanding of placental fatty acid transport. Curr Opin Clin Nutr Metab Care 2012;15:265–72.
24. Gaete MG, Atalah SE, Araya J. Efecto de la suplementación de la dieta de la madre durante la lactancia con ácidos grasos omega 3 en la composición de los lípidos de la leche. Revista Chilena Pediátrica 2002;73:239–47.
25. Tabernero A, Lavado EM, Granda B, Velasco A, Medina JM. Neuronal differentiation is triggered by oleic acid synthesized and released by astrocytes. J Neurochem 2001;79:606–16.
26. Medina JM, Tabernero A. Astrocyte-synthesized oleic acid behaves as a neurotrophic factor for neurons. J Physiol Paris 2002;96:265–71.
27. Velasco A, Tabernero A, Medina JM. Role of oleic acid as a neurotrophic factor is supported in vivo by the expression of GAP-43 subsequent to the activation of SREBP-1 and the up-regulation of stearoyl-CoA desaturase during postnatal development of the brain. Brain Res 2003;977:103–11.
28. Rodríguez-Rodríguez RA, Tabernero A, Velasco A, Lavado EM, Medina JM. The neurotrophic effect of oleic acid includes dendritic

differentiation and the expression of the neuronal basic helix-loop-helix transcription factor NeuroD2. J Neurochem 2004;88: 1041–51.
29. Polo-Hernández E, De Castro F, García-García AG, Tabernero A, Medina JM. Oleic acid synthesized in the periventricular zone promotes axonogenesis in the striatum during brain development. J Neurochem 2010;114:1756–66.
30. Polo-Hernández E, Tello V, Arroyo AA, Domínguez-Prieto M, de Castro F, Tabernero A, Medina JM. Oleic acid synthesized by stearoyl-CoA desaturase (SCD-1) in the lateral periventricular zone of the developing rat brain mediates neuronal growth, migration and the arrangement of prospective synapses. Brain Res 2014;1570:13–25.
31. Yehuda S, Rainovitz S, Mostofsky DI. Effects of essential fatty acids preparation, (SR-3) on brain biochemistry and on behavioral and cognitive functions. Eur J Pharmacol 1997;328:23–9.
32. Coti Bertrand P, O'Kusky JR, Innis SM. Maternal dietary (n-3) fatty acid deficiency alters neurogenesis in the embryonic rat brain. J Nutr 2006;136:1570–5.
33. Tang M, Zhang M, Cai H, Li H, Jiang P, Dang R, Liu Y, He X, Xue Y, Cao L, et al. Maternal diet of polyunsaturated fatty acid altered the cell proliferation in the dentate gyrus of hippocampus and influenced glutamatergic and serotoninergic systems of neonatal female rats. Lipids Health Dis 2016;15:71.
34. Cao D, Kevala K, Kim J, Moon H-S, Jun SB, Lovinger D, Kim H-Y. Docosahexaenoic acid promotes hippocampal neuronal development and synaptic function. J Neurochem 2009;111:510–21.
35. Cutuli D. Functional and structural benefits induced by omega-3 polyunsaturated fatty acids during aging. Curr Neuropharmacol 2017;15:534–42.
36. Tixier-Vidal A, Picart R, Loudes C, Bauman AF. Effects of polyunsaturated fatty acids and hormones on synaptogenesis in serum-free medium cultures of mouse fetal hypothalamic cells. Neuroscience 1986;17:115–32.
37. Kawakita K, Kawai N, Kuroda Y, Yasashita S, Nagao S. Expression of matrix metalloproteinase-9 in thrombin-induced brain edema formation in rats. J Stroke Cerebrovasc Dis 2006;15:88–95.
38. Wurtman RJ, Ulus IH, Cansev M, Watkins CJ, Wang L, Marzloff G. Synaptic proteins and phospholipids are increased in gerbil brain by administering uridine plus docosahexaenoic acid orally. Brain Res 2006;1088:83–92.
39. Beltz BS, Tlusty MF, Benton JL, Sandeman DC. Omega-3 fatty acids upregulate adult neurogenesis. Neurosci Lett 2007;415:154–8.
40. Maekawa M, Takashima N, Matsumata M, Ikegami S, Kontani M, Hara Y, Kawashima H, Owada Y, Kiso Y, Yoshikawa T, et al. Arachidonic acid drives postnatal neurogenesis and elicits a beneficial effect on prepulse inhibition, a biological trait of psychiatric illnesses. PLoS One 2009;4:e5085.
41. Robson LG, Dyall S, Sidloff D, Michael-Titus AT. Omega-3 polyunsaturated fatty acids increase the neurite outgrowth of rat sensory neurones throughout development and in aged animals. Neurobiol Aging 2010;3:678–87.
42. Sakayori N, Maekawa M, Numayama-Tsuruta K, Katura T, Moriya T, Osumi N. Distinctive effects of arachidonic acid and docosahexaenoic acid on neural stem/progenitor cells. Genes Cells 2011;16: 778–90.
43. Kim HY, Spector AA, Xiong ZM. A synaptogenic amide N-docosahexaenoylethanolamide promotes hippocampal development. Prostaglandins Other Lipid Mediat 2011;96:1–4.
44. Rashid MA, Katakura M, Kharebava G, Kevala K, Kim HY. N-Docosahexaenoylethanolamine is a potent neurogenic factor for neural stem cell differentiation. J Neurochem 2013;125:869–84.
45. Kang JX, Wan JB, He C. Concise review: regulation of stem cell proliferation and differentiation by essential fatty acids and their metabolites. Stem Cells 2014;32:1092–8.
46. Tokuda H, Kontani M, Kawashima H, Kiso Y, Shibata H, Osumi N. Differential effect of arachidonic acid and docosahexaenoic acid on age-related decreases in hippocampal neurogenesis. Neurosci Res 2014;88:58–66.
47. Cutuli D, Pagani M, Caporali P, Galbusera A, Laricchiuta D, Foti F, Neri C, Spalletta G, Caltagirone C, Petrosini L, et al. Effects of omega-3 fatty acid supplementation on cognitive functions and neural substrates: a Voxel-based morphometry study in aged mice. Front Aging Neurosci 2016;8:38.
48. Hashimoto M, Hossain S, Al Mamun A, Matsuzaki K, Arai H. Docosahexaenoic acid: one molecule diverse functions. Crit Rev Biotechnol 2016;37:579–97.
49. Tanabe Y, Hashimoto M, Sugioka K, Maruyama M, Fujii Y, Hagiwara R, Hara T, Hossain SM, Shido O. Improvement of spatial cognition with dietary docosahexaenoic acid is associated with an increase in Fos expression in rat CA1 hippocampus. Clin Exp Pharmacol Physiol 2004;10:700–3.
50. Lee AY, Choi JM, Lee J, Lee MH, Lee S, Cho EJ. Effects of vegetable oils with different fatty acid compositions on cognition and memory ability in Aβ25-35-induced Alzheimer's disease mouse model. J Med Food 2016;19:912–21.
51. Bhatia HS, Agrawal R, Sharma S, Huo Y, Ying Z, Gomez-Pinilla F. Omega-3 fatty acid deficiency during brain maturation reduces neuronal and behavioral plasticity in adulthood. PloS One 2007;6(12): e28451.
52. Shah SN. Fatty acid composition of lipids of human brain myelin and synaptosomes: changes in phenylketonuria and Down's syndrome. Int J Biochem 1979;10:477–82.
53. Avila-Martin G, Galan-Arriero I, Gómez-Soriano J, Taylor J. Treatment of rat spinal cord injury with the neurotrophic factor albumin-oleic acid: translational application for paralysis, spasticity and pain. PLoS One 2011;6(10):e26107.
54. Bloch MH, Qawasmi A. Omega-3 fatty acid supplementation for the treatment of children with attention-deficit/hyperactivity disorder symptomatology: systematic review and meta-analysis. J Am Acad Child Adolesc Psychiatry 2011;50:991–1000.
55. Vacca RA, Valenti D. Green tea EGCG plus fish oil omega-3 dietary supplements rescue mitochondrial dysfunctions and are safe in a Down's syndrome child. Clin Nutr 2015;34:783–4.
56. Liu DP, Schmidt C, Billings T, Davisson MT. Quantitative PCR genotyping assay for the Ts65Dn mouse model of Down syndrome. BioTechniques 2003;35:1170–74.
57. Trépanier MO, Taha AY, Mantha RL, Ciobanu FA, Zeng QH, Tchkhartichvili GM, Domenichiello AF, Bazinet RP, Burnham WM. Increases in seizure latencies induced by subcutaneous docosahexaenoic acid are lost at higher doses. Epilepsy Res 2012;99: 225–32.
58. Pan H, Hu XZ, Jacobowitz DM, Chen C, McDonough J, Van Shura K, Lyman M, Marini AM. Alpha-linolenic acid is a potent neuroprotective agent against soman-induced neuropathology. Neurotoxicology 2012;33:1219–29.
59. García-Cerro S, Martínez P, Vidal V, Corrales A, Flórez J, Vidal R, Rueda N, Arbonés ML, Martínez-Cué C. Overexpression of Dyrk1A is implicated in several cognitive, electrophysiological and neuromorphological alterations found in a mouse model of Down syndrome. PLoS One 2014;9:e106572.
60. Trejo JL, Carro E, Torres-Aleman I. Circulating insulin-like growth factor I mediates exercise-induced increases in the number of new neurons in the adult hippocampus. J Neurosci 2001;21: 1628–34.
61. Llorens-Martín M, Torres-Alemán I, Trejo JL. Pronounced individual variation in the response to the stimulatory action of exercise on immature hippocampal neurons. Hippocampus 2006;16:480–90.
62. Rueda N, Vidal V, García-Cerro S, Narcís JO, Llorens-Martín M, Corrales A, Lantigua S, Iglesias M, Merino J, Merino R, et al. Anti-IL17 treatment ameliorates Down syndrome phenotypes in mice. Brain Behav Immun 2018;73:235–51.
63. Liu JJ, Green P, John Mann JJ, Rapoport S, Sublette ME. Pathways of polyunsaturated fatty acid utilization: implications for brain function in neuropsychiatric health and disease. Brain Res 2015;1597: 220–46.
64. Lorenzi HA, Reeves RH. Hippocampal hypocellularity in the Ts65Dn mouse originates early in development. Brain Res 2006;1104: 153–9.
65. Brooksbank BW, Martinez M. Lipid abnormalities in the brain in adult Down's syndrome and Alzheimer's disease. Mol Chem Neuropathol 1989;11:157–85.
66. Giacomini A, Stagni F, Emili M, Guidi S, Salvalai ME, Grilli M, Vidal-Sanchez V, Martinez-Cué C, Bartesaghi R. Treatment with corn oil improves neurogenesis and cognitive performance in the Ts65Dn mouse model of Down syndrome. Brain Res Bull 2018;140: 378–91.

67. García-García AG, Polo-Hernández E, Tabernero A, Medina JM. Alpha-fetoprotein (AFP) modulates the effect of serum albumin on brain development by restraining the neurotrophic effect of oleic acid. Brain Res 2015;1624:45–58.
68. Nelson TL. Serum protein and lipoprotein fractions in mongolism. Am J Dis Child 1961;102:369–74.
69. Kronquist KE, Dreazen E, Keener SL, Nicholas TW, Crandall BF. Reduced fetal hepatic alpha-fetoprotein levels in Down's syndrome. Prenat Diagn 1990;10:739–51.
70. Niculescu MD, Lupu DS, Craciunescu CN. Maternal α-linolenic acid availability during gestation and lactation alters the postnatal hippocampal development in the mouse offspring. Int J Dev Neurosci 2011;29:795–802.
71. Blondeau N, Nguemeni C, Debruyne DN, Piens M, Wu X, Pan H, Hu X, Gandin C, Lipsky RH, Plumier JC, et al. Subchronic alpha-linolenic acid treatment enhances brain plasticity and exerts an antidepressant effect: a versatile potential therapy for stroke. Neuropsychopharmacol 2009;34:2548–59.
72. Venna VR, Deplanque D, Allet C, Belarbi K, Hamdane M, Bordet R. PUFA induce antidepressant-like effects in parallel to structural and molecular changes in the hippocampus. Psychoneuroendocrinology 2009;39:199–211.
73. Zmijewski PA, Gao LY, Saxena AR, Chavannes NK, Hushmendy SF, Bhoiwala DL, Crawford DR. Fish oil improves gene targets of Down syndrome in C57BL and BALB/c mice. Nutr Res 2015;35:440–8.
74. Fukaya M, Watanabe M. Improved immunohistochemical detection of postsynaptically located PSD-95/SAP90 protein family by protease section pretreatment: a study in the adult mouse brain. J Comp Neurol 2000;426:572–86.
75. Chen X, Nelson CD, Li X, Winters CA, Azzam R, Sousa AA, Leapman RD, Gainer H, Sheng M, Reese TS. PSD-95 is required to sustain the molecular organization of the postsynaptic density. J Neurosci 2011;31:6329–38.
76. Martínez-Cué C, Delatour B, Potier MC. Treating enhanced GABAergic inhibition in Down syndrome: use of GABA alpha5-selective inverse agonists. Neurosci Biobehav Rev 2014;46:218–27.
77. Stein V, House DR, Bredt DS. Postsynaptic density-95 mimics and occludes hippocampal long-term potentiation and enhances long-term depression. J Neurosci 2003;2:5503–6.