

Perinatal LC- ω -3-PUFA supplementation offsets prenatal THC-induced cognitive deficit via sex-specific modulation of hippocampal neuroplasticity

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ABSTRACT

Long-chain polyunsaturated fatty acids (LC-PUFAs) are crucial for brain development, with ω -3 species supporting neuronal membrane architecture, synaptogenesis and cognitive maturation, and ω -6 species linking lipid metabolism to neuronal signalling via the endocannabinoid system (ECS), the network of receptors, lipid mediators, and enzymatic pathways that orchestrate neurodevelopmental processes. Prenatal exposure to Δ^9 -tetrahydrocannabinol (THC), the psychoactive component of cannabis, impairs memory and disrupts hippocampal plasticity markers affecting the excitatory/inhibitory (E/I) balance, in a sex-dependent manner. This dysregulation is closely associated with a lipid shift favouring ω -6-derived mediators, leading to ECS imbalance. We investigated whether perinatal LC- ω -3-PUFA supplementation could prevent prenatal THC-induced neurodevelopmental deficits. Pregnant rats received THC (2 mg/kg, gestational days 5-20) and either a standard or LC- ω -3-PUFA-enriched diet (ω -3/ ω -6 ratio = 1.4) from gestation through lactation. Adolescent offspring were evaluated for hippocampus-dependent memory (Novel object recognition) and gene expression of hippocampal plasticity markers (NR1, mGluR5, CB1R, Nlgn-1/2/3) and endocannabinoid metabolising enzymes (DAGL α , NAPE-PLD, MAGL, FAAH). LC- ω -3-PUFAs prevented prenatal THC-induced memory impairment in both sexes. Molecular analyses revealed sex-specific recovery mechanisms. In males, LC- ω -3-PUFAs counteracted prenatal THC-induced synaptic hyperexcitability by restoring NR1, mGluR5, and CB1R expression, reducing Nlgn-1 and enhancing the Nlgn-3 levels, together with increased NAPE-PLD expression. In females, LC- ω -3-PUFAs rebalanced the prenatal THC-induced predominant inhibitory shift by reducing Nlgn-2/-3- and NAPE-PLD expression levels. These findings identify perinatal LC- ω -3-PUFA supplementation as a safe, effective nutritional strategy to counteract THC-induced neurodevelopmental vulnerability by restoring ECS balance and supporting sex-specific neuronal plasticity and cognitive integrity.

1. Introduction

Long-chain (LC)- ω -3-polyunsaturated fatty acids (PUFAs) species such as docosahexaenoic acid (DHA), and ω -6-PUFA arachidonic acid (AA) are incorporated into neuronal membranes during gestation and early life (Marszalek and Lodish, 2005; Lauritzen et al., 2016). Both

LC- ω -3- and LC- ω -6-PUFAs contribute to membrane architecture, fluidity, and synaptogenesis, though their functions diverge (Prasad et al., 2010). DHA enhances membrane flexibility, supports neurite outgrowth, and promotes dendritic and synaptic development. In contrast, AA plays a prominent role in intracellular signalling and neurotransmission (Prasad et al., 2010; Calderon and Kim, 2004; Weiser

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et al., 2016; Bosetti, 2007). Metabolites derived from ω -6-PUFAs generally promote pro-inflammatory signalling, whereas ω -3-derived mediators exert anti-inflammatory, neuroprotective, and synaptotrophic effects (Calder, 2011). Clinical and epidemiological evidence indicates that higher maternal levels of LC- ω -3-PUFAs are associated with improved neurocognitive outcomes and reduced risk of neurodevelopmental disorders in the offspring (Jensen, 2006; Innis, 2007; Gould et al., 2013). On the other hand, a decreased ω -3 to ω -6 ratio, such as that provided by the Western diet, can alter membrane lipid composition, disrupt synaptic signalling, and increase the risk of developing cognitive and neuropsychiatric disorders later in life. Indeed, a high ω -3/ ω -6 ratio is crucial for maintaining normative neurodevelopment (Healy-Stoffel and Levant, 2018; Lee et al., 2025; Sarikahya et al., 2025).

A central biochemical interface between LC-PUFAs metabolism and neuronal signalling is the endocannabinoid system (ECS), composed of the cannabinoid CB1- and CB2 receptors, their endogenous ligands – anandamide (AEA) and 2-arachidonoylglycerol (2-AG) – and the associated enzymatic machinery for their synthesis [respectively N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD) and diacylglycerol lipase (DAGL α)] and degradation [respectively fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL)] (Di Marzo, 2011; Wang et al., 2003).

Both AEA and 2-AG are derived from ω -6 PUFA AA and act primarily through CB1Rs that are abundantly expressed in cortical and hippocampal neurons. In the hippocampus, CB1Rs are prominently located on both GABAergic terminals, particularly on cholecystokinin-positive (CCK+) basket cells (BCs) and onto glutamatergic excitatory terminals, where they modulate neurotransmitter release, synaptic strength and plasticity (Lu and Mackie, 2016; Tsou et al., 1999; Wang, 2003). Notably, the ω -3-derived ethanolamides, AEA-like mediators – docosahexaenoyl ethanolamide and eicosapentaenoyl ethanolamide – can activate CB1Rs and CB2Rs, interact with the endocannabinoid enzymatic machinery, and modulate the endocannabinoid tone (Di Miceli et al., 2020; Watson et al., 2019). Even phytocannabinoids such as Δ^9 -tetrahydrocannabinol (THC) bias lipid metabolism toward ω -6-derived species at the expense of ω -3 precursors, contributing to the dysregulation of ECS-related functions (Di Marzo, 2011; Watson et al., 2019).

This interaction is even more intriguing since the ECS orchestrates multiple stages of neurodevelopment, highlighting how perturbations in this lipid-endocannabinoid crosstalk are indeed contributing factors to neurodevelopmental vulnerability (Harkany et al., 2007; Vitalis et al., 2008; Galve-Roperh et al., 2009; Tringali et al., 2023; Brancato et al., 2023).

Accordingly, growing evidence indicates that prenatal THC exposure, by the abnormal activation of CB1Rs, compromises cognitive functioning and causes a profound alteration of the hippocampal excitatory/inhibitory (E/I) balance in a sex-dependent manner (de Salas-Quiroga et al., 2020; Sarikahya et al., 2023). Consistent with this evidence, our recent work demonstrated a male-specific upregulation of CB1R expression, accompanied by sex-dependent alterations in N-methyl-D-aspartate (NMDA) receptor subunit NR1 and metabotropic glutamate receptor (mGluR5) expression, which suggests the occurrence of a shift in the E/I balance upwards (Castelli et al., 2023a, 2023b). This was also confirmed by the upregulation of the synaptic adhesion molecule neuroligin-1 (Nlgn-1), which is preferentially localised to the excitatory synapse, and the downregulation of neuroligin-3 (Nlgn-3), which is selectively enriched in CB1R-expressing CCK + BCs GABAergic synapses (Castelli et al., 2024; Song et al., 1999; Uchigashima et al., 2020a,b; Chubykin et al., 2007). In the female progeny, on the other hand, an opposite condition was consequential to prenatal THC exposure, resulting in a shift of the E/I balance downwards, with an upregulation of the Nlgn-3 and a strengthening of the inhibitory synapse between CCK + BCs and hippocampal neurons (Castelli et al., 2024; Földy et al., 2013; Uchigashima et al., 2021).

Given these premises, our hypothesis is that LC- ω -3-PUFA supplementation during the perinatal period could prevent the molecular and cognitive disturbances observed in the offspring of both sexes prenatally exposed to THC. Specifically, we enriched a standard diet with DHA and EPA, increasing the ω -3/ ω -6 ratio from 0.1 to 1.4, and examined its effects on the hippocampus-dependent memory and key molecular markers of hippocampal synaptic plasticity. In detail, we measured: NR1-, mGluR5-, CB1R-, and the Nlgn-1, -2 and -3 mRNA levels in the hippocampus. Moreover, due to the close relationship between ω -6 PUFAs and the availability of endocannabinoids, we also examined the adaptive response of the endocannabinoid enzymatic machinery (DAGL α , MAGL, NAPE-PLD, FAAH) to prenatal THC exposure in the absence and in the presence of LC- ω -3-PUFA supplementation.

Our results show an overall memory-protecting activity of the enriched diet, accompanied by modifications in excitatory and inhibitory synaptic plasticity, and the endocannabinoid machinery that overall displays a sex-specific rescued neurodevelopmental profile.

2. Materials and methods

2.1. Animals

Adult Wistar rats (250–300 g, 12 weeks of age, Envigo, S. Pietro al Natisone, Italy) were housed at $22 \pm 1^\circ\text{C}$, $55 \pm 5\%$ humidity, under a 12-h light/dark cycle (lights on 7:00 a.m.–7:00 p.m.). Animal care followed Italian Ministry of Health guidelines (D.L. 26/2014, approval number 819/2021-PR) and Directive 2010/63/EU. Animals were monitored daily, with no signs of distress observed. Efforts were made to minimise animal use and suffering in compliance with ARRIVE guidelines.

2.2. Experimental protocol

The female rats were housed with a male for mating and subsequently housed in standard single cages (40 cm $\times$ 60 cm $\times$ 20 cm) with bedding and free access to water and food. Dams were exposed to THC modeling early life mild cannabis exposure, as previously described (Frau et al., 2019). In particular, from gestational day (GD) 5 to 20, dams were administered with vehicle (2% Tween 80 in sterile saline) (CTRL dams) or THC (2 mg/kg) (THC dams). In addition, to assess the effects of perinatal LC- ω -3-PUFA supplementation, from GD 0 to postnatal day (PND) 20, THC dams were randomly assigned to the group fed with either a standard chow diet (A5340, Mucedola) or an isocaloric LC- ω -3-PUFAs enriched diet (A5342, Mucedola), as previously described (Labrousse et al., 2012). This period of time was chosen on the basis of previous studies showing that a 2-month supplementation with a diet enriched in LC- ω -3-PUFAs increases DHA levels in neural tissues and retina (Labrousse et al., 2012; Schnebelen et al., 2009).

Standard diet contained a mixture of vegetable oils (rapeseed oil, high-oleic sunflower oil and palm oil) presenting PUFAs ($n \geq 3$) as alpha-linolenic acid only (ALA, C18:3 ω -3, 1.52 %) (Innolipid AS, Norway). LC- ω -3-PUFA supplemented diet contained a mixture of rapeseed oil, sunflower oil, palm oil and anchovy oil (Innolipid AS, Norway), resulting in a PUFA ($n \geq 3$) profile, including mainly ALA (1.13 %), EPA (C20:5 ω -3, 9.62 %) and DHA (C22:6 ω -3, 7.24 %), of 24.32 %. CTRL dams and their offspring received a standard diet. For complete macro- and micronutrient diet composition, refer to (Labrousse et al., 2012).

Pregnant dams' weight and their food consumption (g/kg) were daily monitored, and the weight gain of the dams during gestation was calculated. Upon delivery, litter size, the average weight of the pups from each litter at PND 1, and weight gain by PND 20, and the number of males and females of each litter were recorded. Weaning occurred on PND 21, when the offspring were separated by sex and housed under standard conditions (Brancato et al., 2016, 2018; Castelli et al., 2020). The male and female offspring were housed in pairs, and assigned to different groups according to the following three perinatal conditions: I)

prenatal exposure to vehicle and perinatal standard diet – CTRL; II) prenatal THC exposure and perinatal standard diet – pTHC; III) prenatal THC exposure and perinatal LC- ω -3-PUFA diet supplementation – pTHC-LC- ω -3-PUFA. Each experimental group consisted of one or two rats per sex per litter, ensuring litter effects were minimised. Starting from PND21, all offspring were maintained on the standard diet.

2.3. Prenatal THC treatment

The THC resin used for the experiment was extracted from the Forensic Laboratory of Biologically Active Substances at the University of Chemistry and Technology in Prague, Czech Republic (purity (HPLC) > 97%) (Drazanova et al., 2019). THC was dissolved in ethanol at a concentration of 20% and then sonicated for 30 min. This solution was emulsified with 2% Tween 80 and sterile saline. THC, at a dose of 2 mg/kg, or vehicle, was administered subcutaneously to the dams in a volume of 1 ml/kg from GD 5 to 20. The dose was selected to reflect real-world patterns of maternal human cannabis consumption: it corresponds to the approximate THC concentration found in mild cannabis joints. In humans, this translates to a conservative estimate of occasional to moderate cannabis use during pregnancy, particularly among users who smoke 1-2 cigarettes per day (Avalos et al., 2024; Fantauzzi et al., 2021). The dose falls within the range of experimental designs previously employed to investigate neurodevelopmental consequences in preclinical models (Frau et al., 2019; Mehmedic et al., 2010; Murru et al., 2024; Castelli et al., 2023a, 2023b, 2024, Castelli et al.; Black et al., 2025).

2.4. Behavioural procedure

On PND 31, adolescent offspring underwent the Novel Object Recognition Test (NORT) in an L-maze made of dark grey PVC, consisting of two perpendicular arms (63 cm and 50 cm in length, 10 cm wide, with 43 cm high walls). Objects were placed at the ends of the arms. The test was divided into three 5-min phases: during the habituation phase (5 min), animals freely explored the empty maze to reduce anxiety; on PND 32, in the training phase (5 min), two identical objects were introduced; and on PND 33, during the testing phase (5 min), one of the familiar objects was replaced with a novel object. Behaviour was video-recorded and analysed using AnyMaze software. The primary measure was the Discrimination Index (DI), calculated as (time spent exploring the novel object – time spent exploring the familiar object)/total exploration time (Lavanco et al., 2025).

2.5. Hippocampal tissue collection and quantitative real-time PCR

Following behavioural testing, the whole hippocampus was dissected from adolescent offspring to assess global molecular rearrangements. This approach was chosen to capture the integrated synaptic response required for cognitive tasks and to maintain methodological consistency with our previous molecular characterisations of the pTHC damage profile, specifically regarding the expression of NMDA subunits, mGluR5, and the endocannabinoid system (Castelli et al., 2023a, 2023b, 2024). The tissue was snap-frozen, and stored at -80°C . RNA was extracted with Trizol (Invitrogen), followed by chloroform layer separation and isopropanol precipitation. To remove residual salts from the precipitation step, the RNA pellets were washed with 70% ethanol (Chomczynski and Sacchi, 2006). The purified RNA was then resuspended in nuclease-free water and quantified using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Subsequently, complementary DNA (cDNA) was synthesised from the isolated RNA using SuperScript IV Reverse Transcriptase (Invitrogen). The resulting cDNA was diluted and combined with PowerUp SYBR Green Master Mix (Applied Biosystems) and specific primers. Quantitative real-time PCR (qRT-PCR) was performed with an initial denaturation at 95°C for 10 min, followed by 40 amplification cycles

consisting of 95°C for 15 s and 60°C for 1 min, and a final melting curve analysis with steps at 95°C for 15 s, 60°C for 30 s, and 95°C for 15 s. Gene expression was normalised using the delta-delta Ct method. Primers are listed in Table 1.

2.6. Statistical analysis

Data were tested for normality and variance homogeneity. If data were normal and homogeneous, comparisons were made via two-way or one-way ANOVA followed by Bonferroni post hoc test. Otherwise, Kruskal-Wallis or Brown-Forsythe and Welch tests were used. Results are expressed as mean $\pm$ SEM. Analyses were performed using GraphPad Prism v. 10.6.1; significance level was set at $\alpha = 0.05$.

3. Results

3.1. Prenatal THC exposure and perinatal supplementation with LC- ω -3-PUFAs do not alter maternal food consumption, gestational, or neonatal outcomes

Dams were monitored for body weight and food consumption. No significant difference was observed in dams' average food consumption during gestation (KW = 2.827, $p = 0.2611$). Average food intake during the postnatal epoch did not differ significantly among dams (F (2, 9) = 1.833, $p = 0.2149$).

Maternal weight gain, litter size, male-to-female ratio, litter's weight at PND1, and weight gain by PND 20 were measured to assess maternal and neonatal outcomes. Analysis of dams' weight gain during gestation showed no significant differences between groups (F (2, 9) = 2.005, $p = 0.1905$). No significant differences were found in the number of pups per litter (F (2, 9) = 3.003, $p = 0.1002$), male-to-female ratio (F (2, 9) = 2.146, $p = 0.1730$), mean pup weight at PND 1 (F (2, 9) = 0.07180,

Table 1
Primers employed in qRT-PCR experiments.

Gene Name	Primer Sequence	Product
Gapdh	GTTTGTGATGGGTGTGAACC (Forward) CTTCTGAGTGGCAGTGATG (Reverse)	–
NMDA receptor subunit NR1 - Grin1	–	Rn_Grin1_1_SG QuantiTect Primer Assay (QT00182287)
mGluR5 - Grm5	–	Rn_Grm5_1_SG QuantiTect Primer Assay (QT01081549)
CB1R - Cnr1	–	Rn_Cnr1_1_SG QuantiTect Primer Assay (QT00191737)
DAGL α	–	Rn_Dagla_2_SG QuantiTect Primer Assay (QT01597197)
MAGL	–	Rn_Magll_1_SG QuantiTect Primer Assay (QT00189742)
NAPE-PLD	–	Rn_Napepld_2_SG QuantiTect Primer Assay (QT01621704)
FAAH	–	Rn_Faah_1_SG QuantiTect Primer Assay (QT00192444)
NLGN1	–	Rn_Nlgn1_2_SG QuantiTect Primer Assay (QT01567097)
NLGN2	–	Rn_Nlgn2_1_SG QuantiTect Primer Assay (QT00191100)
NLGN3	–	Rn_Nlgn3_1_SG QuantiTect Primer Assay (QT00178304)

$p = 0.9312$), or weight gain by PND20 ($F(2, 9) = 0.2593$, $p = 0.7772$) (Table 2).

3.2. Prenatal THC exposure causes memory deficits in adolescent offspring of both sexes that are prevented by perinatal exposure to a LC- ω -3-PUFA-enriched diet

Two-way ANOVA was performed on data from the NORT in the L-maze, considering treatment (prenatal THC and perinatal LC- ω -3-PUFA-enriched diet exposure) and sex as statistical factors. The analysis of DI data revealed a significant main effect of treatment ($F(2, 42) = 20.8$, $p < 0.001$), while the main effect of sex ($F(1, 42) = 0.0646$, $p = 0.80$) and their interaction ($F(2, 42) = 0.330$, $p = 0.72$) were not significant.

In male rats, Bonferroni post hoc test showed a reduced DI in the pTHC group compared to CTRL counterpart ($t = 4.12$, $df = 42$, $p < 0.001$), and an increased DI in the pTHC- LC- ω -3-PUFAs rats compared to pTHC group ($t = 4.02$, $df = 42$, $p < 0.0011$) (Fig. 1A). Similarly, in female offspring we observed a decreased DI in the pTHC rats compared to the CTRL group ($t = 4.34$, $df = 42$, $p < 0.001$), and increased DI in pTHC - LC- ω -3-PUFAs rats compared to pTHC group ($t = 3.15$, $df = 42$, $p = 0.009$) (Fig. 2A).

When total object exploration time was analysed, no difference was observed (treatment: $F(2, 42) = 1.39$, $p = 0.26$; sex: $F(1, 42) = 2.92$, $p = 0.10$; interaction: $F(2, 42) = 1.16$, $p = 0.32$) (Fig. 1B; 2 B).

In addition, two-way ANOVA on exploration time of familiar vs. novel object in the male offspring revealed a significant effect of the object ($F(1, 42) = 19.75$, $p < 0.001$) and the interaction with treatment

($F(2, 42) = 9.642$, $p = 0.0004$). Bonferroni post hoc test showed novel object discrimination in CTRL rats, which displayed increased exploration of the novel object in comparison with the familiar one ($t = 4.205$, $df = 42$, $p < 0.001$), and in pTHC - LC- ω -3-PUFAs group ($t = 4.508$, $df = 42$, $p < 0.001$) (Fig. 1C). A significant effect of the object ($F(1, 42) = 18.84$, $p < 0.001$) and treatment/object interaction ($F(2, 42) = 5.061$, $p = 0.0107$) were also observed when data from the female offspring were analysed. Bonferroni post hoc test showed increased novel object exploration in CTRL- ($t = 4.613$, $df = 42$, $p < 0.001$) and pTHC - LC- ω -3-PUFAs - ($t = 2.77$, $df = 42$, $p = 0.0249$) groups (Fig. 2C).

3.3. Sex is a significant factor in hippocampal molecular outcomes induced by prenatal THC and perinatal LC- ω -3-PUFAs-enriched diet exposure

To evaluate the interaction between perinatal treatments (prenatal THC and perinatal diet exposure) and sex on molecular outcomes measured in the hippocampus, we performed a two-way ANOVA (Table 3). The analysis indicated significant interactions between treatments and sex for almost all the genes evaluated, thus, detailed analyses were conducted separately for each sex.

3.3.1. Prenatal THC exposure induced alterations in the markers of hippocampal synaptic plasticity that are prevented by the perinatal LC- ω -3-PUFA-enriched diet in the male offspring

Expression levels of key molecular markers associated with excitatory and inhibitory synaptic plasticity were measured in the hippocampus of the adolescent male offspring. NMDA receptor subunit NR1 expression showed significant group differences ($F(2, 15) = 423.9$, $p < 0.0001$), with increased NR1 in pTHC- vs. CTRL group ($t = 25.49$, $df = 15$, $p < 0.0001$), and reduced NR1 in pTHC - LC- ω -3-PUFAs vs. pTHC group ($t = 24.94$, $df = 15$, $p < 0.0001$) (Fig. 3A). Also mGluR5 expression differed significantly between groups ($F(2, 15) = 223.9$, $p < 0.0001$), with markedly increased levels in pTHC- vs. CTRL group ($t = 20.13$, $df = 15$, $p < 0.0001$), and a significant reduction in the pTHC - LC- ω -3-PUFA-compared to the pTHC group ($t = 15.72$, $df = 15$, $p < 0.0001$) (Fig. 3B). CB1R expression was also significantly altered ($F(2, 15) = 193.3$, $p < 0.0001$), with an increase in pTHC- vs. CTRL group ($t = 17.02$, $df = 15$, $p < 0.0001$), and a decrease in pTHC - LC- ω -3-PUFAs- vs. pTHC group ($t = 17.04$, $df = 15$, $p < 0.0001$) (Fig. 3C).

3.3.2. Prenatal THC-induced disruption in hippocampal Neuroligin expression is prevented by perinatal LC- ω -3-PUFA supplementation in the male offspring

In the hippocampus of male adolescent offspring, one-way ANOVA revealed a significant group difference in the relative expression of the cell adhesion molecule Nlgn-1 ($F(2, 15) = 7.352$, $p = 0.0060$). Post hoc analysis indicated that prenatal THC exposure significantly increased Nlgn-1 mRNA levels ($t = 3.606$, $DF = 15$, $p = 0.0078$), whereas perinatal supplementation with an LC- ω -3-PUFA-enriched diet resulted in a significant reduction in Nlgn-1 expression in pTHC offspring ($t = 2.932$, $DF = 15$, $p = 0.0309$) (Fig. 4A). In contrast, Nlgn-2 expression levels did not differ significantly among groups ($F(2, 15) = 1.588$, $p = 0.2368$) (Fig. 4B). Finally, one-way ANOVA revealed a significant group difference in the expression of Nlgn-3 ($F(2, 15) = 45.73$, $p < 0.0001$). While prenatal THC exposure was associated with a reduced Nlgn-3 expression ($t = 2.749$, $DF = 15$, $p = 0.0447$), LC- ω -3-PUFA supplementation markedly increased Nlgn-3 mRNA levels in pTHC offspring ($t = 9.307$, $DF = 15$, $p < 0.0001$) (Fig. 4C).

3.3.3. The hippocampal endocannabinoid enzymatic machinery is differentially sensitive to prenatal THC exposure and to the perinatal LC- ω -3-PUFA-enriched diet in the male offspring

DAGL α expression, the main enzyme responsible for 2-AG synthesis, showed significant group differences ($F(2, 15) = 5.226$, $p < 0.0001$), with higher DAGL α levels in pTHC- vs. CTRL group ($t = 2.834$, $df = 15$,

Table 2

Maternal and neonatal outcomes. n.s. = non-significant.

Group	Parameter	N	Mean	SEM	p
CTRL dams	Body weight gain (g)	4	125.5	11.06	n.s.
THC dams		4	110.3	3.20	
THC - LC- ω -3-PUFAs dams		4	128.3	3.01	
CTRL dams	Gestational average food consumption (g/kg)	4	72.72	2.21	n.s.
THC dams		4	70.45	2.63	
THC - LC- ω -3-PUFAs dams		4	82.09	8.02	
CTRL dams	Postnatal average food consumption (g/kg)	4	283.9	19.62	n.s.
THC dams		4	346.8	33.48	
THC - LC- ω -3-PUFAs dams		4	285.0	24.83	
CTRL dams	Pups per litter (n)	4	13.25	0.75	n.s.
THC dams		4	13.75	0.90	
THC - LC- ω -3-PUFAs dams		4	11.67	0.47	
CTRL dams	Male-to-Female ratio	4	0.85	0.14	n.s.
THC dams		4	1.30	0.25	
THC - LC- ω -3-PUFAs dams		4	4.12	2.08	
CTRL litters	Litter's weight at PND 1 (g)	4	7.02	0.14	n.s.
pTHC litters		4	7.01	0.20	
pTHC - LC- ω -3-PUFAs litters		4	6.94	0.17	
CTRL litters	Litter's weight gain by PND 20 (g)	4	31.76	3.27	n.s.
pTHC litters		4	34.10	2.2	
pTHC - LC- ω -3-PUFAs litters		4	31.99	1.96	

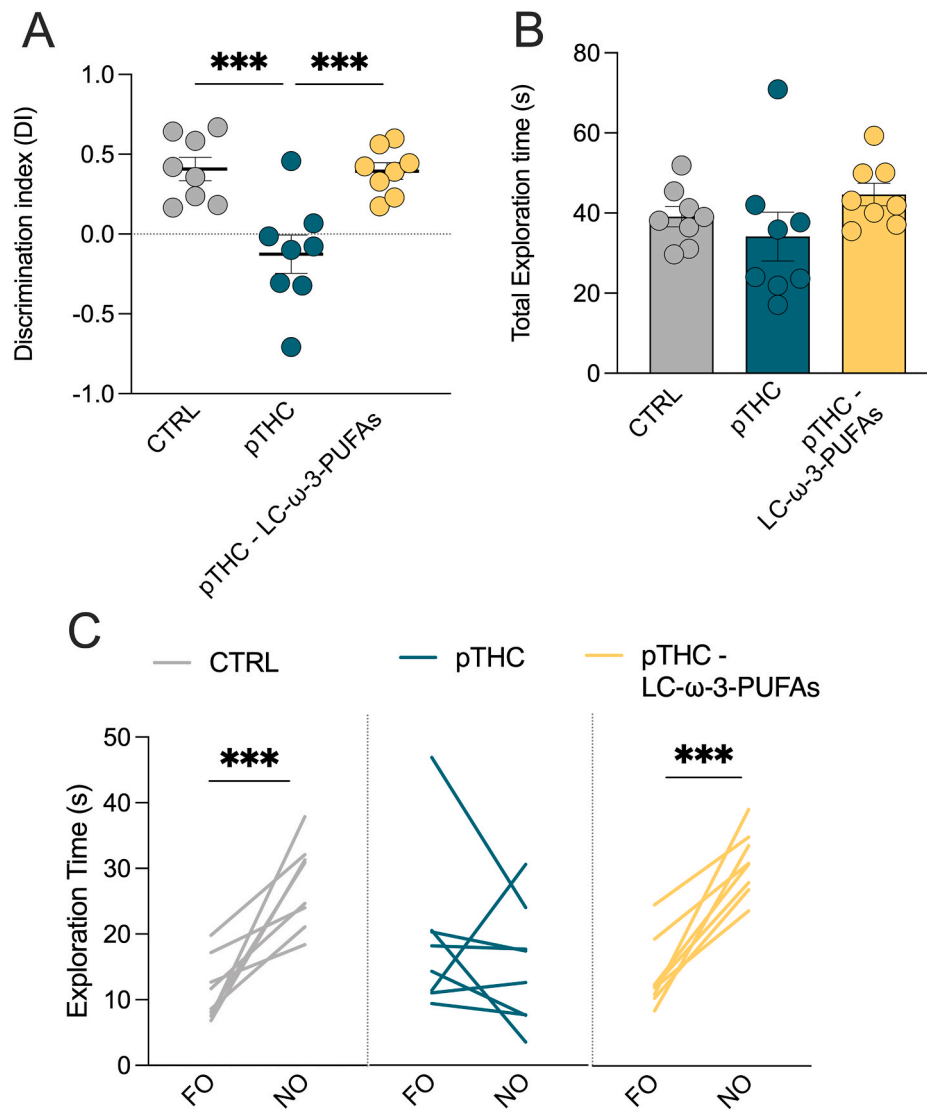


Fig. 1. Effect of prenatal THC exposure and perinatal exposure to an LC- ω -3-PUFA-enriched diet in the NORT. Male offspring were tested in the Novel Object Recognition Test (NORT) using the L-maze to assess declarative memory. (A) The discrimination index (DI) was reduced in the pTHC- compared to the CTRL group, and increased in the pTHC - LC- ω -3-PUFAs group compared to the pTHC group. (B) Total object exploration time did not differ among groups. (C) The exploration of the novel object rather than the familiar one was increased in CTRL- and pTHC - LC- ω -3-PUFAs groups. Each bar represents the group mean $\pm$ SEM. ** $p < 0.01$; *** $p < 0.001$. CTRL: male rat offspring prenatally exposed to vehicle; pTHC: male rat offspring prenatally exposed to THC; pTHC - LC- ω -3-PUFAs: male rat offspring prenatally exposed to THC and perinatally exposed to an LC- ω -3-PUFA-enriched diet.

$p = 0.0377$), and reduced gene expression levels in pTHC - LC- ω -3-PUFAs vs. pTHC group ($t = 2.765$, $df = 15$, $p = 0.0434$) (Fig. 5A). MAGL mRNA levels, the enzyme responsible for 2-AG degradation, did not differ significantly between groups (KW = 0.1527, $p = 0.9377$) (Fig. 5B). NAPE-PLD expression, the enzyme involved in AEA synthesis, was significantly different among groups (KW = 11.11, $p = 0.0007$). Prenatal THC exposure did not alter NAPE-PLD expression levels ($Z = 0.5951$, $p > 0.9999$), while perinatal supplementation with an LC- ω -3-PUFA-enriched diet increased NAPE-PLD mRNA levels in the pTHC group ($Z = 3.138$, $p = 0.0051$) (Fig. 5C). On the other hand, FAAH expression, which catalyses AEA degradation, remained unchanged across groups ($F(2, 15) = 0.3366$, $p = 0.7195$) (Fig. 5D).

3.3.4. Prenatal THC-induced alterations in hippocampal synaptic plasticity markers are not prevented by the perinatal LC- ω -3-PUFA-enriched diet in the female offspring

In the female hippocampus, NR1 expression did not differ between groups ($F(2, 15) = 3.185$, $p = 0.0703$) (Fig. 6A). The expression of mGluR5 showed a significant overall group effect ($F(2, 15) = 18.60$,

$p < 0.0001$) and the post hoc Bonferroni comparison revealed a decrease of gene expression levels in pTHC- vs. CTRL group ($t = 4.492$, $DF = 15$, $p = 0.0013$), but no difference between pTHC - LC- ω -3-PUFAs and pTHC group ($t = 1.328$, $DF = 15$, $p = 0.6125$) (Fig. 6B). No significant differences among groups were detected for CB1R expression levels ($F(2, 15) = 1.072$, $p = 0.3671$) (Fig. 6C).

3.3.5. LC- ω -3-PUFA supplementation is able to prevent prenatal THC-induced increase in Nlgn-3 and decreases Nlgn-2 expression in the hippocampus of female offspring

In the hippocampus of female adolescent offspring, Nlgn-1 expression levels did not significantly differ among groups ($F(2, 15) = 0.1470$, $p = 0.8645$) (Fig. 7A). In contrast, a Kruskal-Wallis test revealed a significant group effect on Nlgn-2 expression (KW = 11.56, $p = 0.0004$). Post hoc comparisons showed no significant difference between the CTRL- and pTHC groups ($Z = 0.2176$, $p > 0.9999$), while perinatal supplementation with an LC- ω -3-PUFA-enriched diet significantly reduced Nlgn-2 expression in pTHC offspring ($Z = 0.2176$, $p = 0.0140$) (Fig. 7B). A significant group difference for Nlgn-3 mRNA levels was

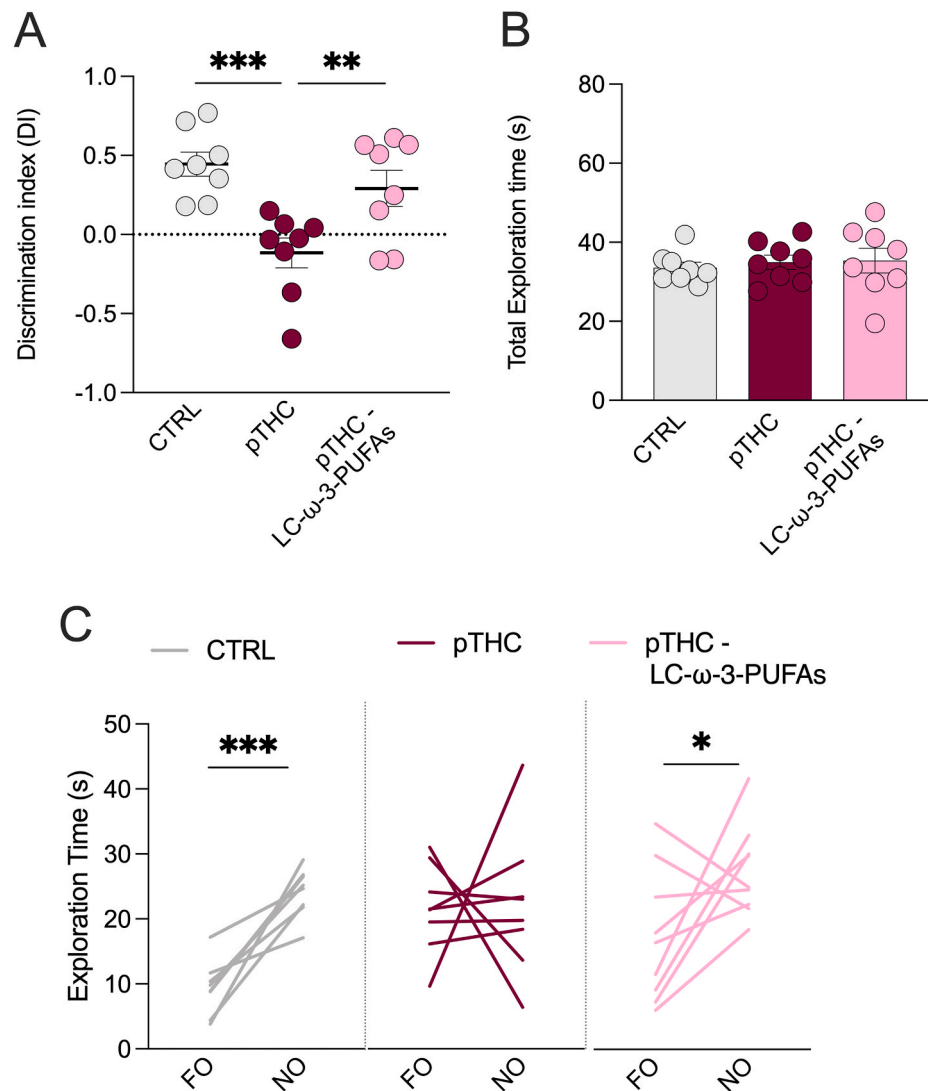


Fig. 2. Effect of prenatal THC exposure and perinatal exposure to an LC-ω-3-PUFA-enriched diet in the NORT. (A) The discrimination index (DI) was significantly lower in the pTHC-compared with the CTRL group, and higher in the pTHC - LC-ω-3-PUFAs relative to the pTHC group. (B) Total object exploration time was comparable across groups. (C) Both CTRL and pTHC - LC-ω-3-PUFAs groups showed increased exploration of the novel object over the familiar one. Each bar represents the group mean $\pm$ SEM. * $p < 0.05$; * $p < 0.01$; *** $p < 0.001$. CTRL: female rat offspring prenatally exposed to vehicle; pTHC: female rat offspring prenatally exposed to THC; pTHC - LC-ω-3-PUFAs: female rat offspring prenatally exposed to THC and perinatally exposed to an LC-ω-3-PUFA-enriched diet.

Table 3

Effect of perinatal treatments and sex on molecular outcomes measured in the hippocampus.

Gene	Statistical factor (F (DFn, DFd), p value)		Sex		Interaction	
	Treatments					
NR1	F (2, 30) = 229	$p < 0.001$	F (1, 30) = 231	$p < 0.001$	F (2, 30) = 176	$p < 0.001$
mGluR5	F (2, 30) = 199	$p < 0.001$	F (1, 30) = 447	$p < 0.001$	F (2, 30) = 227	$p < 0.001$
CB1R	F (2, 30) = 107	$p < 0.001$	F (1, 30) = 106	$p < 0.001$	F (2, 30) = 88.1	$p < 0.001$
Nlgn-1	F (2, 30) = 4.98	$p = 0.01$	F (1, 30) = 7.78	$p = 0.009$	F (2, 30) = 5.69	$p = 0.008$
Nlgn-2	F (2, 30) = 0.16	$p = 0.86$	F (1, 30) = 8.73	$p = 0.006$	F (2, 30) = 6.06	$p = 0.006$
Nlgn-3	F (2, 30) = 7.73	$p = 0.002$	F (1, 30) = 9.47	$p = 0.004$	F (2, 30) = 33.7	$p < 0.001$
DAGLα	F (2, 30) = 3.50	$p = 0.04$	F (1, 30) = 2.44	$p = 0.13$	F (2, 30) = 5.37	$p = 0.01$
MAGL	F (2, 30) = 1.17	$p = 0.32$	F (1, 30) = 0.11	$p = 0.74$	F (2, 30) = 0.66	$p = 0.52$
NAPE-PLD	F (2, 30) = 8.75	$p = 0.001$	F (1, 30) = 12.5	$p = 0.001$	F (2, 30) = 21.9	$p < 0.001$
FAAH	F (2, 30) = 0.279	$p = 0.76$	F (1, 30) = 0.424	$p = 0.52$	F (2, 30) = 0.191	$p = 0.83$

detected ($F(2, 15) = 16.49$, $p = 0.0002$). Specifically, Nlgn-3 expression was significantly elevated in the hippocampus of pTHC- compared to CTRL female offspring ($t = 5.182$, $DF = 15$, $p = 0.0003$), while LC-ω-3-PUFA supplementation significantly attenuated this increase in the pTHC group ($t = 4.736$, $DF = 15$, $p = 0.0008$) (Fig. 7C).

3.3.6. The hippocampal endocannabinoid enzymatic machinery of the female offspring prenatally exposed to THC is sensitive to the perinatal LC-ω-3 PUFA supplementation

No differences were observed for DAGLα- ($F(2, 15) = 1.332$, $p = 0.2935$) (Fig. 8A) or MAGL- ($F(2, 15) = 2.746$, $p = 0.0964$)

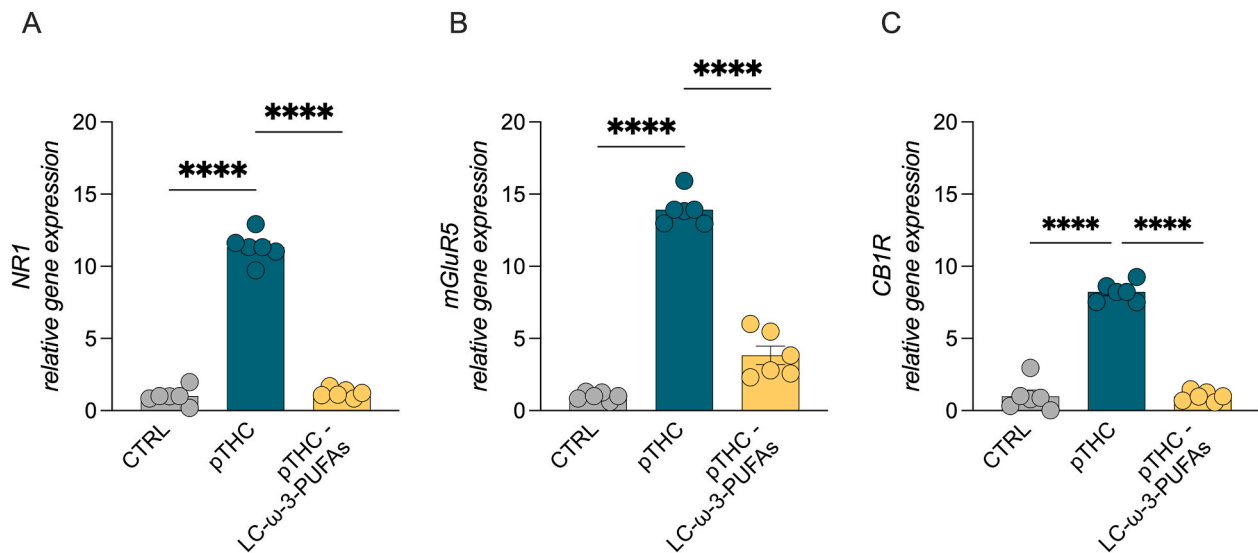


Fig. 3. Prenatal exposure to THC induced an alteration in markers of hippocampal synaptic plasticity that is prevented by LC-ω-3-PUFA supplementation. Expression levels of key molecular markers involved in excitatory and inhibitory synaptic plasticity were analysed in the hippocampus of adolescent male offspring. (A) Expression levels of NMDA receptor subunit NR1 showed a significant increase in the pTHC group compared to CTRL, and a significant reduction in the pTHC - LC-ω-3-PUFAs group compared to the pTHC group. (B) mGluR5 expression levels differed significantly among groups, with elevated levels in pTHC vs. CTRL, and a marked reduction in the pTHC - LC-ω-3-PUFAs group compared to pTHC. (C) The expression levels of CB1R were also significantly affected, with higher levels in pTHC vs. CTRL, and a significant decrease in the pTHC - LC-ω-3-PUFAs- compared to the pTHC group. Data are expressed as mean $\pm$ SEM. **** $p < 0.0001$. CTRL: male rat offspring prenatally exposed to vehicle; pTHC: male rat offspring prenatally exposed to THC; pTHC - LC-ω-3-PUFAs: male rat offspring prenatally exposed to THC and perinatally exposed to an LC-ω-3-PUFA-enriched diet; NR1: NMDA receptor subunit NR1; mGluR5: group I metabotropic glutamate receptor 5; CB1R: cannabinoid receptor type 1.

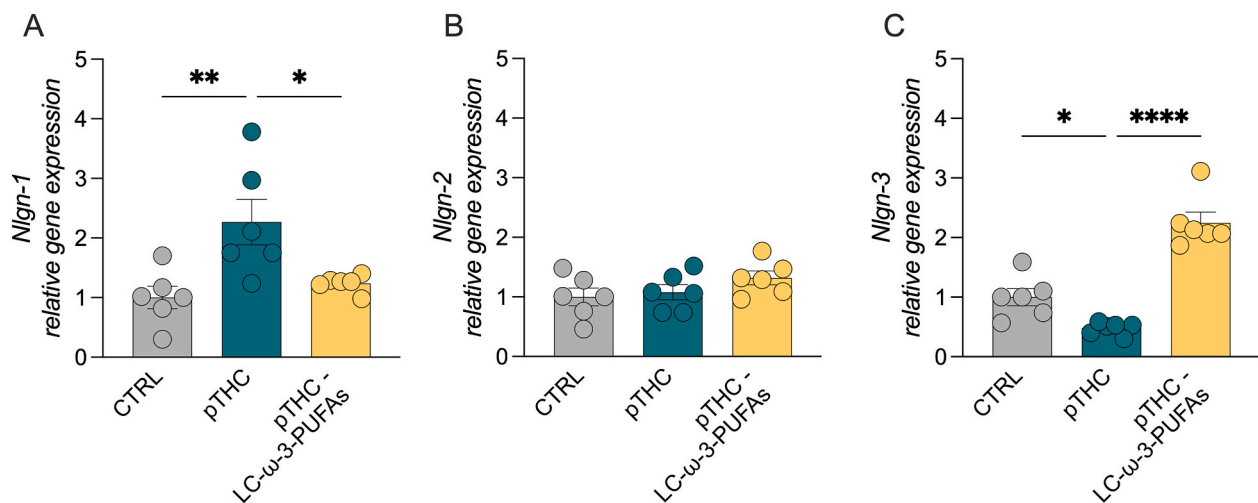


Fig. 4. Prenatal THC exposure alters Neuroligins gene expression, while perinatal LC-ω-3-PUFA supplementation prevents it. (A) Prenatal THC exposure significantly increased Nlgn-1 expression compared to controls, whereas perinatal supplementation with an LC-ω-3-PUFA-enriched diet prevented this upregulation. (B) Nlgn-2 expression did not differ significantly among groups. (C) Prenatal THC exposure reduced Nlgn-3 mRNA levels, while perinatal LC-ω-3-PUFA supplementation increased Nlgn-3 expression in prenatally THC-exposed offspring. Data are expressed as mean $\pm$ SEM. * $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$. CTRL: male rat offspring prenatally exposed to vehicle; pTHC: male rat offspring prenatally exposed to THC; pTHC - LC-ω-3-PUFAs: male rat offspring prenatally exposed to THC and perinatally exposed to an LC-ω-3-PUFA-enriched diet; Nlgn: Neuroligin.

expression levels (Fig. 8B). The expression of NAPE-PLD showed a significant overall group effect ($F(2, 15) = 4.235$, $p = 0.0348$), post hoc Bonferroni comparison revealed a decrease in pTHC - LC-ω-3-PUFA-vs. pTHC group ($t = 2.725$, $DF = 15$, $p = 0.0470$), while no significant difference in CTRL-vs. pTHC group comparison was detected ($t = 0.4770$, $DF = 15$, $p > 0.9999$) (Fig. 8C). FAAH expression showed no significant difference among groups ($KW = 0.08221$, $p = 0.9663$) (Fig. 8D).

4. Discussion

The results of this study indicate that the perinatal supplementation with LC-ω-3-PUFAs is able to prevent the cognitive impairment and the dysregulation of hippocampal markers of synaptic plasticity in the offspring that was exposed to THC during gestation. The efficacy of this dietary intervention is evident in both sexes, although it is accompanied by sex-specific molecular adaptations.

Neither prenatal THC exposure nor perinatal supplementation with LC-ω-3-PUFAs affected maternal food intake, body weight, or neonatal

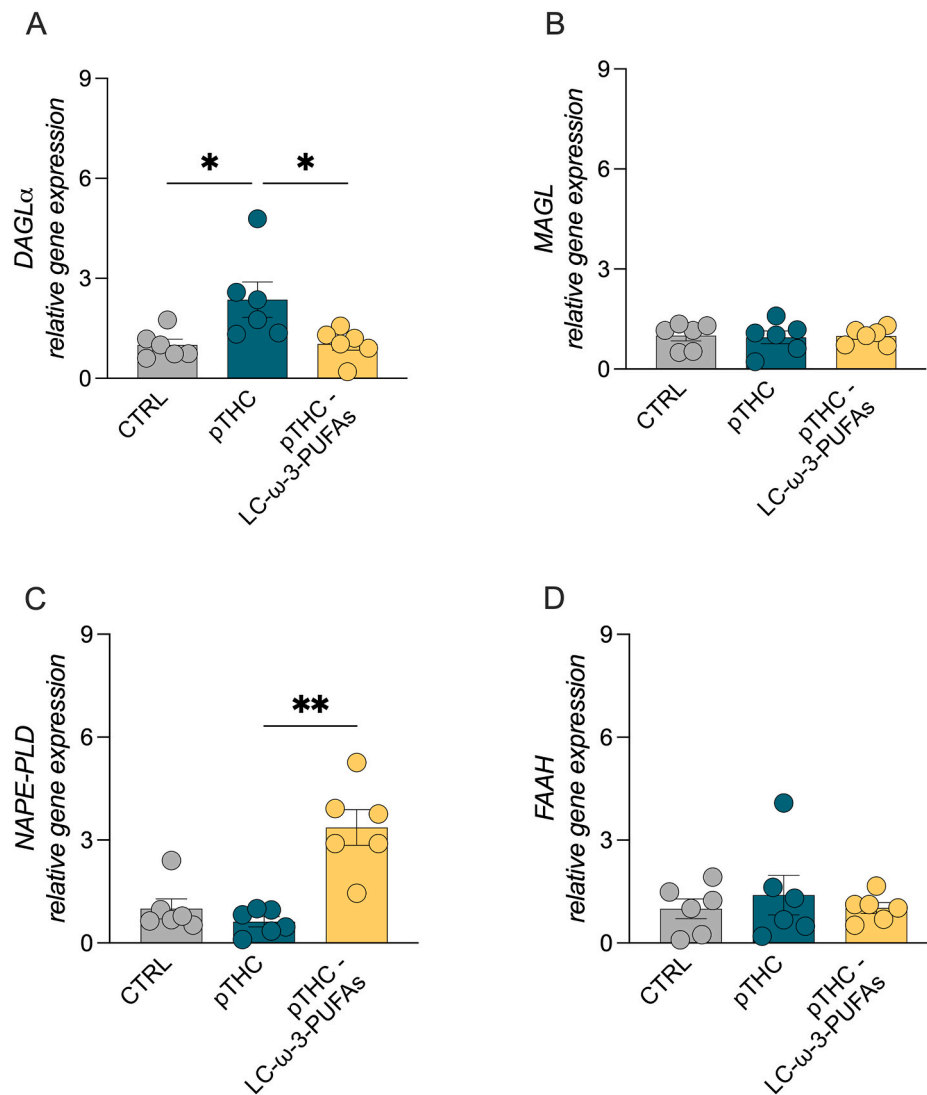


Fig. 5. Prenatal exposure to THC-induced alterations in the hippocampal endocannabinoid enzymatic machinery of male offspring are prevented by perinatal LC- ω -3-PUFA supplementation. (A) Prenatal THC exposure increased DAGL α levels relative to controls, whereas perinatal supplementation with an LC- ω -3-PUFA-enriched diet normalised its expression. MAGL mRNA levels did not differ among groups. (C) Although NAPE-PLD expression was not altered by prenatal THC exposure, perinatal LC- ω -3-PUFA supplementation increased NAPE-PLD levels in the pTHC group. (D) FAAH expression remained unchanged across experimental groups. Data are expressed as mean $\pm$ SEM. * $p < 0.05$; ** $p < 0.01$. CTRL: male rat offspring prenatally exposed to vehicle; pTHC: male rat offspring prenatally exposed to THC; pTHC - LC- ω -3-PUFAs: male rat offspring prenatally exposed to THC and perinatally exposed to an LC- ω -3-PUFA-enriched diet; DAGL α : diacylglycerol lipase α ; MAGL: monoacylglycerol lipase; NAPE-PLD: N-acyl phosphatidylethanolamine phospholipase D; FAAH: fatty acid amide hydrolase.

outcomes, indicating that these interventions did not exert unintended effects on pup development.

In this study, pTHC male offspring exhibit memory deficits associated with aberrant hippocampal excitatory synapse, as shown by increased NMDA receptor subunit NR1 expression levels and the compensatory upregulation of mGluR5 and CB1R mRNA levels, in accordance with previous data from this and other research groups (de Salas-Quiroga et al., 2020; Castelli et al., 2023a, 2023b, 2024). Currently, multiple hypotheses may explain the modifications produced by the abnormal early interference of prenatal THC exposure with the ECS, basically focusing on the central and essential role played by the endocannabinoids during the ontogenesis (Wu et al., 2011; Noble et al., 2025). Growing evidence supports the key role played by the prenatal THC exposure in the dysregulation of the ω -3/ ω -6 ratio, as a result of an increase in ω -6-derived mediators in the brain (Sarikahya et al., 2022, 2023; Murru et al., 2024). During the perinatal window, LC-PUFAs from the ω -3 and ω -6 families are indispensable for brain development and neuronal functioning, but it is a ratio in favour of ω -3s that confers a

nurturing effect on brain development (Sheppard and Cheatham, 2013; Vinot et al., 2011). Indeed, in non-pathological conditions, a low ω -3/ ω -6 ratio can impair the development of hippocampal glutamatergic circuits by altering the expression of ionotropic NMDA- and AMPA receptor subunits during postnatal maturation (Moreira et al., 2010). Such alterations are associated with memory deficits and anxiety-like behaviour (Madore et al., 2014; Moreira et al., 2010; Larrieu et al., 2016). Furthermore, previous evidence shows that a low ω -3/ ω -6 ratio can compromise ECS functionality, leading to CB1R uncoupling from its G-protein effectors and, consequently, a reduced efficiency of the endocannabinoid-mediated synaptic plasticity and the hippocampal-related cognitive functions (Zamberletti et al., 2017; Lafourcade et al., 2011).

Interestingly, in our study, the perinatal dietary supplementation with LC- ω -3-PUFAs in pTHC male offspring prevented the hippocampus-dependent memory deficit observed in the NORT as well as the occurrence of the aberrant excitatory synaptic plasticity, as shown by normative NR1- and mGluR5 expression. Moreover, the perinatal LC-

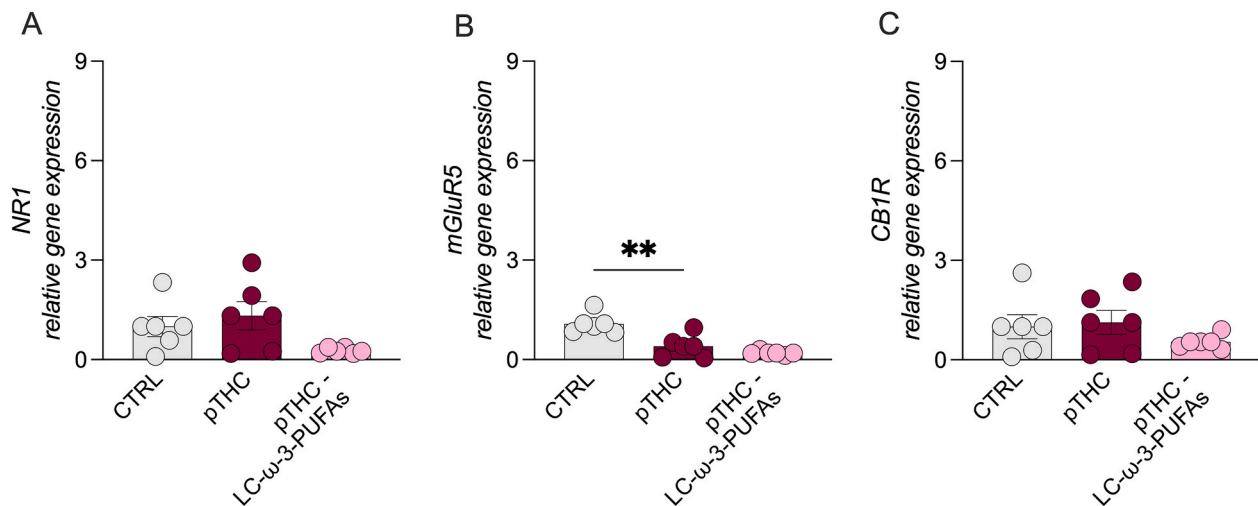


Fig. 6. Prenatal THC-induced alterations in hippocampal synaptic plasticity markers in female offspring are not prevented by the perinatal LC-ω-3 PUFA supplementation. (A) NR1 mRNA expression levels in the hippocampus of female offspring did not differ significantly among experimental groups. (B) mGluR5 expression showed a significant reduction in the pTHC group compared to controls, whereas perinatal LC-ω-3-PUFA supplementation did not significantly modify mGluR5 expression relative to the pTHC group. (C) CB1R expression levels were not significantly different among groups. Data are expressed as mean $\pm$ SEM. ** $p < 0.01$. CTRL: female rat offspring prenatally exposed to vehicle; pTHC: female rat offspring prenatally exposed to THC; pTHC - LC-ω-3-PUFAs: male rat offspring prenatally exposed to THC and perinatally exposed to an LC-ω-3-PUFA-enriched diet; NR1: NMDA receptor subunit NR1; mGluR5: group I metabotropic glutamate receptor 5; CB1R: cannabinoid receptor type 1.

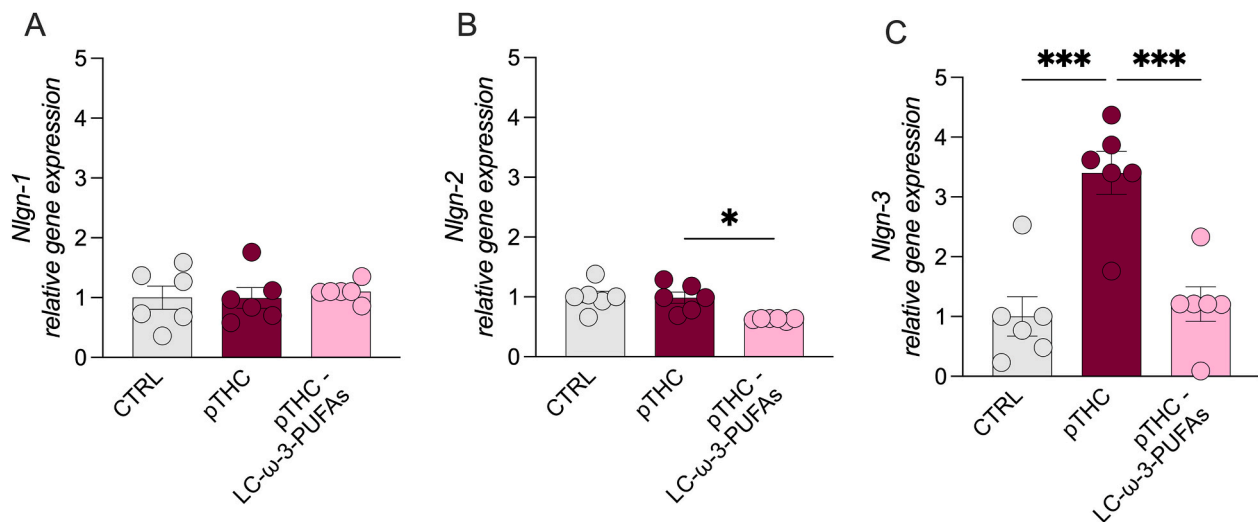


Fig. 7. Prenatal THC exposure and perinatal LC-ω-3-PUFA supplementation modify Neuroligin-associated excitatory/inhibitory synaptic balance in the hippocampus of female offspring. (A) Nlgn-1 mRNA expression levels in the hippocampus of female adolescent offspring did not differ significantly among experimental groups. (B) Nlgn-2 expression was not altered by prenatal THC exposure, whereas perinatal LC-ω-3-PUFA supplementation significantly reduced Nlgn-2 mRNA levels in pTHC-exposed offspring. (C) Prenatal THC exposure markedly increased Nlgn-3 mRNA levels compared to controls, while LC-ω-3-PUFA supplementation significantly attenuated this elevation. Data are expressed as mean $\pm$ SEM. * $p < 0.05$; *** $p < 0.001$. CTRL: female rat offspring prenatally exposed to vehicle; pTHC: female rat offspring prenatally exposed to THC; pTHC - LC-ω-3-PUFAs: male rat offspring prenatally exposed to THC and perinatally exposed to an LC-ω-3-PUFA-enriched diet; Nlgn: Neuroligin.

ω-3-PUFA supplementation maintains CB1R mRNA levels within normative ranges, thereby contributing to the proper endocannabinoid-dependent modulation of synaptic plasticity (Di Miceli et al., 2020; Lafourcade et al., 2011; Cinquina et al., 2023). In accordance with the prominent presence of CB1R mRNA in hippocampal GABAergic interneurons, previous evidence shows that prenatal THC exposure results in a reduced number of hippocampal CB1R-expressing CCK + interneurons and profound alterations in the expression of the Nlgn (de Salas-Quiroga et al., 2020; Castelli et al., 2021, 2024; Chubykin et al., 2007; Földy et al., 2013; Varoquaux et al., 2004; Song et al., 1999). Indeed, in pTHC male offspring, the expression of Nlgn-1, predominantly localised at excitatory glutamatergic synapses, was increased,

whereas mRNA levels of Nlgn-3, a key mediator of the endocannabinoid tone, particularly localised at inhibitory synapses, were reduced (Castelli et al., 2024; Song et al., 1999; Uchigashima et al., 2020a,b; Chubykin et al., 2007). The latter finding is particularly relevant since Nlgn-3 acts as a synaptic organiser that selectively targets GABAergic synapses formed by CB1R-expressing CCK + interneurons, where it contributes to the maintenance of the tonic endocannabinoid signalling (Földy et al., 2013; Uchigashima et al., 2020b; Gonzalez et al., 2025; Cao and Tabuchi, 2017).

In pTHC male offspring, the LC-ω-3-PUFA supplementation maintained the Nlgn-1 at normal levels, while enhancing Nlgn-3 expression, thus suggesting the occurrence of a balanced E/I tone. The measurement

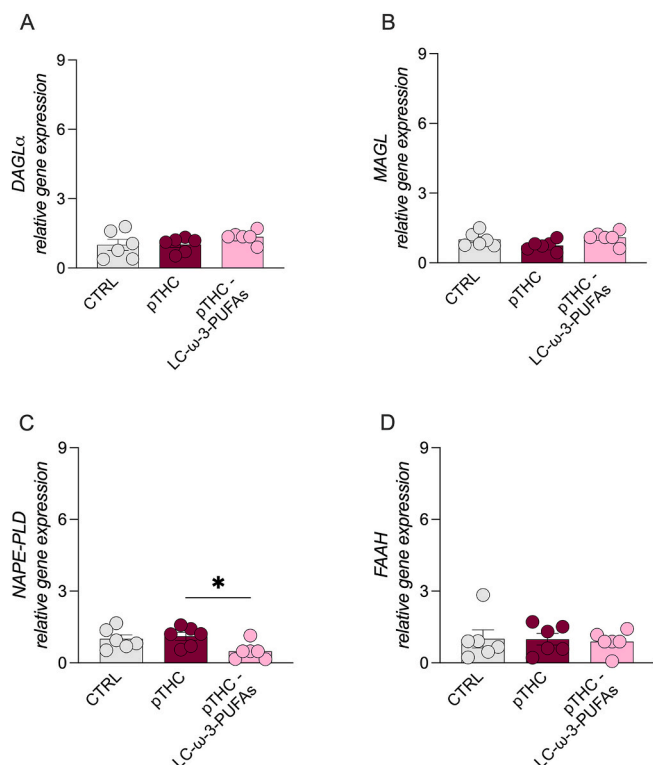


Fig. 8. Perinatal LC- ω -3-PUFA supplementation, but not prenatal THC exposure, modulates hippocampal endocannabinoid enzymatic machinery in female offspring. (A-B) DAGL α and MAGL expression did not differ among groups. (C) Decreased NAPE-PLD expression was found in the pTHC - LC- ω -3-PUFAs- compared to the pTHC group, whereas no significant difference was observed between the CTRL and pTHC groups. (D) FAAH remained unchanged among groups. Data are expressed as mean $\pm$ SEM. * $p < 0.05$. CTRL: female rat offspring prenatally exposed to vehicle; pTHC: female rat offspring prenatally exposed to THC; pTHC - LC- ω -3-PUFAs: male rat offspring prenatally exposed to THC and perinatally exposed to an LC- ω -3-PUFA-enriched diet; DAGL α : diacylglycerol lipase α ; MAGL: monoacylglycerol lipase; NAPE-PLD: N-acyl phosphatidylethanolamine phospholipase D; FAAH: fatty acid amide hydrolase.

of the CB1R-expressing CCK + interneurons will tell more about the protective role exerted by the LC- ω -3-PUFA enrichment towards the developmental effects of prenatal THC exposure.

A further novelty of this study is the analysis of the enzymatic machinery involved in the synthesis and degradation of major endocannabinoids – an area that has been scarcely explored in relation to prenatal THC exposure and LC- ω -3-PUFA supplementation.

Our results show that prenatal THC exposure induced significant alterations in the hippocampal endocannabinoid metabolic pathway. Specifically, in males, we observed an increase in DAGL α levels, with unchanged expression of MAGL, suggesting an elevated 2-AG tone. Importantly, we found an up-regulation of mGluR5 expression, which is mechanistically linked to the synthesis and release of endocannabinoids, particularly 2-AG: this directly couples glutamatergic signalling to endocannabinoid-mediated modulation of neuroplasticity (Izumi and Zorumski, 2012) as an adaptive response to the aberrant excitatory synapse (Castelli et al., 2023a, 2023b, 2024).

The LC- ω -3-PUFA supplementation effectively prevented the increase in DAGL α - and mGluR5 expression, promoting a normative 2-AG signalling. Moreover, the supplementation with LC- ω -3-PUFAs induced an increase in NAPE-PLD expression levels, in the absence of a prenatal THC-related effect. Prior evidence shows that supplementation with DHA and EPA provides an increased availability of LC- ω -3-derived ethanolamide mediators by increasing NAPE-PLD mRNAs (Kim and

Watkins, 2014). Therefore, we could expect NAPE-PLD upregulation to mechanistically contribute to enhanced availability of LC- ω -3-derived ethanolamide mediators (Wood et al., 2010; D'Angelo et al., 2020). A further investigation on the levels of ethanolamide mediators, including AEA, will let us validate this hypothesis.

The LC- ω -3-PUFA supplementation allowed a normative cognitive performance in pTHC female offspring, although the associated hippocampal molecular markers displayed a sex-specific pattern. In particular, in the absence of signs of hyperexcitability and CB1R dysregulation, prenatal THC exposure induced an increase in Nlgn-3 that is associated with a potentiation in the hippocampal inhibitory synaptic transmission via the presynaptic release of GABA (Uchigashima et al., 2020b). This is also supported by the compensatory down-regulation of mGluR5, just as opposed to that in the pTHC male progeny. Notably, no alterations in the enzymatic endocannabinoid machinery were observed. The LC- ω -3-PUFA supplementation reduced the expression of Nlgn-3, as well as of Nlgn-2, which is exclusively localised at GABAergic synapses (Ali et al., 2020). These effects, together with the concomitant reduction in NAPE-PLD expression, suggest that LC- ω -3-PUFAs effectively prevented the prenatal THC-induced imbalance in the E/I tone downwards, limiting the synthesis of inhibitory signalling molecules within the hippocampus.

5. Conclusions

Overall, our findings confirm that there is no intrinsically “spared” sex from the early perturbations induced by the prenatal THC exposure in the offspring. At the transcriptional level, the changes induced by prenatal THC exposure are not limited to a direct interaction with CB1R, but involve modifications in markers of neuroplasticity and in the availability of endocannabinoids in a sex-dependent manner.

Recent work identified the hippocampus as a primary hotspot for behavioural deficits in anxiety-like behaviour and discrete forms of memory deficits associated with prenatal THC exposure. Besides regional functional specialisations, lipidomic evidence indicates that pTHC-induced deficiencies in essential fatty acids like DHA and ARA are widespread, affecting the ventral subiculum, dorsal subiculum, and the CA1 region. By analysing the whole hippocampus, our study complements these subregional findings, highlighting that pTHC acts as a broad “lipidome disruptor” that destabilises global E/I balance (Sarikahya et al., 2023), according to previous whole-hippocampus characterizations (Castelli et al., 2023a, 2023b, 2024). Importantly, when LC- ω -3-PUFA supplementation is provided, prenatal THC-related effects are abolished, confirming that the restoration of NR1, mGluR5, and CB1R expression by LC- ω -3-PUFA supplementation provides a comprehensive “metabolic shield” across the hippocampal formation.

Interestingly, although the molecular effects elicited by LC- ω -3-PUFAs differed in both direction and magnitude between males and females, the biological responses align with the sex-specific nature of prenatal THC-induced alterations. Our results identify perinatal LC- ω -3-PUFA supplementation as a viable nutritional strategy to mitigate cannabinoid-induced developmental vulnerability and highlight the critical role of maternal lipid balance in supporting healthy neurodevelopment.

Declaration of generative AI and AI-assisted technologies in the writing process

During the writing of this manuscript, the authors used Grammarly to improve its grammar and readability. After using these tools, the authors reviewed and edited the content as needed and took full responsibility for the publication's content.

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CRediT authorship contribution statement

Gianluca Lavanco: Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Valentina Castelli:** Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Cesare D'Amico:** Formal analysis, Investigation, Writing – original draft. **Salvatore Feo:** Methodology, Resources. **Martin Kuchar:** Methodology, Resources. **Petr Palivec:** Methodology, Resources. **Anna Brancato:** Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Carla Cannizzaro:** Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The Authors declare that they have no competing interests.

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Data availability

Data will be made available on request.

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