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Perinatal omega-3 sex-selectively mitigates neuropsychiatric impacts of prenatal THC in the cortico-striatal-hippocampal circuit

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Prenatal Δ9-tetrahydrocannabinol exposure (PTE) poses long-lasting neuropsychiatric risks, as evidenced by clinical and preclinical studies, yet the neurobiological mechanisms remain poorly defined. Emerging evidence implicates the neurolipidome, a critical mediator of neurodevelopment and endocannabinoid signaling, as a potential contributor. Here, we demonstrate that dietary omega-3 fatty acid supplementation sex-selectively ameliorates neurodevelopmental deficits induced by PTE in a Wistar rat model. Omega-3 supplementation reduced cognitive and emotional disturbances in male offspring and normalized many neuronal and neurochemical abnormalities in the prefrontal cortex, nucleus accumbens, and ventral hippocampus. However, lipidomic analyses, regardless of omega-3 supplementation, uncovered pronounced, sex-specific PTE-induced disruptions in pathways critical for synaptic integrity and neurodevelopment, including those related to the endocannabinoid system. These findings provide new insights into the interplay between lipid metabolism and the endocannabinoid system in the context of PTE.

INTRODUCTION

Cannabis is one of the most commonly used substances during pregnancy, driven by expanding legalization and a widespread perception of its relative safety for managing pregnancy-related symptoms, though this trend varies geographically [1–3]. Contrary to this public perception, a growing body of evidence indicates that prenatal exposure to Δ9-tetrahydrocannabinol (THC), the primary psychoactive constituent of cannabis, in preclinical rodent models can adversely impact pregnancy outcomes and fetal neurodevelopment [2–4] and lead to subsequent behavioral abnormalities [5–10]. Prenatal THC exposure (PTE) is thus a significant developmental risk factor, and while complete abstinence from cannabis during pregnancy remains the primary clinical recommendation, the reality of continued use amongst some pregnant women necessitates the exploration of harm reduction strategies.

THC readily crosses the placental and fetal blood-brain barriers, exerting direct effects on the developing brain [3, 11]. The endocannabinoid system (ECS), comprised of cannabinoid receptors (CB1R and CB2R), endogenous ligands such as anandamide (AEA) and 2-arachidonoylglycerol (2-AG), and their metabolic enzymes, play a fundamental role in orchestrating neurodevelopmental events,

including progenitor proliferation, migration, synaptogenesis, and circuit maturation [12, 13]. THC, through partial agonism at CB1R, disrupts this finely tuned signaling system. It induces receptor desensitization and downregulation, ultimately destabilizing ECS homeostasis and interfering with brain development [4, 14, 15]. Rodent studies consistently report that PTE leads to cognitive impairments, elevated anxiety- and depressive-like phenotypes, and deficits in social interaction [5–10, 16], implicating disruptions in the PFC-striatal-hippocampal network—regions enriched in CB1Rs and integral to emotional and cognitive function [17, 18]. These outcomes have clinical correlates: longitudinal human studies report elevated incidence of childhood psychopathologies, including attentional deficits, impulsivity, emotional dysregulation, social dysfunction, and reduced cognitive performance [18–21], with neuroimaging revealing structural and functional abnormalities in the PFC, hippocampus, and amygdala of exposed children [22, 23].

Beyond its direct neural impact, THC also interferes with placental development and function. The ECS is active in placental and uterine tissues, where it modulates trophoblast invasion, angiogenesis, and maternal-fetal nutrient exchange [11, 24, 25]. Exogenous cannabinoids such as THC disrupt these processes, leading to

placental insufficiency. Rodent models reveal reduced placental weight, impaired vascularization, oxidative stress, and compromised oxygen and nutrient delivery to the fetus following PTE [5, 6, 11, 26–28]. Clinical findings parallel these observations, with PTE associated with increased rates of fetal growth restriction, preterm birth, and neonatal intensive care admissions [2, 8, 29–32]. While inconsistencies in birth weight outcomes exist, largely due to methodological and population-based variability, evidence from several large-scale studies support a link between cannabis use and impaired fetal growth [19, 29, 30, 32, 33]. Nevertheless, rodent models demonstrate postnatal compensatory catch-up growth following this fetal growth restriction, potentially exacerbating neurocognitive deficits [34, 35] and cardiometabolic dysfunction [11, 26, 27, 36] due to maladaptive resource allocation during development [37].

PTE also perturbs lipid metabolism; a critical mechanism contributing to neurodevelopmental disruption. The ECS is a lipid-based signaling system, and its endogenous ligands are derived from polyunsaturated fatty acids (PUFAs) such as arachidonic acid (AA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA). These fatty acids are essential for fetal brain development and depend upon maternal dietary intake for adequate fetal provision [18, 21, 26, 27]. Rodent models of PTE demonstrate symmetrical fetal growth restriction accompanied by altered placental structure, including reductions in placental weight and vascular development in the labyrinth zone, the key site of gas and nutrient (including lipid) exchange [5, 11, 26, 38–41]. THC-induced placental dysfunction compromises the delivery of PUFAs, oxygen, and other critical substrates, impairing both somatic and brain development. Recent work has highlighted persistent lipidomic abnormalities in PTE-exposed offspring, including reduced cerebral PUFA levels, altered lipid metabolism, and corresponding behavioral and electrophysiological disruptions [5, 6, 27, 42–44]. These findings suggest that disrupted lipid signaling may amplify ECS dysregulation, compounding the neurodevelopmental consequences of PTE that may otherwise be driven by THC's activity on cannabinoid receptors.

Maternal dietray uptake of omega-3 fatty acids (particularly DHA and EPA) during the perinatal period critically supports fetal brain development given their fundamental roles in membrane structure and fluidity (effecting neurotransmitter release), neural circuit maturation, neurite outgrowth, dendritic spine morphology, synaptogenesis, and anti-inflammatory action [45–49]. Critically, deficiencies in maternal omega-3 intake is associated with suboptimal neural outcomes [50–52]. Given that the maternal diet is the primary source of PUFAs during fetal and neonatal development [48], dietary interventions targeting omega-3 PUFAs may serve as a promising preventive strategy [53, 54] against the adverse impacts of PTE.

In the present study, we investigated whether perinatal omega-3 supplementation can ameliorate the neurodevelopmental consequences of PTE in a Wistar rat model. Our findings reveal striking sex-specific outcomes: males exhibited rescue of behavioral deficits, synaptic protein dysregulation, and electrophysiological abnormalities, despite persistent lipidomic disruptions (fatty acids and lipids). Conversely, females showed partial improvements in social motivation and ventral hippocampal activity, but retained deficits in oscillatory dynamics, lipidomic disruptions (lipids only), and protein expression. These results highlight the therapeutic potential of omega-3 fatty acid dietary interventions in reducing neuropsychiatric risks associated with PTE. Furthermore, they highlight the need to further investigate the sex-specific mechanisms underlying these alterations within the PFC-striatal-hippocampal circuitry.

MATERIALS AND METHODS
Ethics approval
All experimental procedures adhered to Canadian Council of Animal Care guidelines and were approved by the Animal Care Committee and Veterinary Services Committee at the University of Western Ontario, protocol #2022-119 (for SRL) and # 2023-129 (for DBH).

Animals
Time-pregnant Wistar rat dams (Charles River, Quebec, Canada; $n = 14$–15 dams/treatment; $n = 12$–15 offspring/treatment/sex) arrived on gestational day 3 (GD3) and were randomly assigned to receive daily intraperitoneal (i.p.) injections of either vehicle (saline with cremophor) or THC (3 mg/kg; 2.6 mg/mL in saline with cremophor) from GD7 to GD22. Concurrently, beginning on GD7, dams were randomized to one of two dietary conditions: an omega-3 enriched diet containing DHA and EPA (ω3), or a matched control diet (ct). The sole compositional difference between the two diets was the inclusion of DHA and EPA in triglyceride (TAG) form in the ω3 group; detailed composition is provided in Supplemental Table 1.

Following weaning at postnatal day 21 (PD21), all offspring were transitioned to standard laboratory chow. This standard chow is nutritionally comparable to the control purified diet administered during gestation and lactation, though it is a closed formula subject to minor batch-to-batch variability. In contrast, the purified control diet ensured consistent composition throughout the treatment period.

Maternal and fetal parameters, including maternal weight gain, food intake, offspring birth weight, and offspring weight at PD21, are summarized in Supplemental Fig. 1. All experimental details are presented in detail in the Supplemental Materials. Supplemental Table 2 lists the tentatively assigned lipids with their mass-to-charge (m/z) values and ion that were analyzed with MALDI IMS.

Statistical analyses
Analyses were performed with three-way ANOVAs (sex × treatment × omega-3 diet) for PD1 and PD21 weights, behavioral assays, electrophysiological spiking activity, bursting activity, and for local field potential analyses. Follow-up analyses of significant main effects and interactions ($p < 0.05$) were accomplished using Tukey's Honest Significant Difference (Tukey's HSD) post hoc test (α = 0.05). For the matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI IMS) data, group differences were assessed using one-sample t-tests against a hypothetical mean of 1.0, representing the VEHct group and indicating no change from baseline. This relative quantification approach is standard for MALDI IMS data interpretation [5, 6, 55, 56]. Due to inherent experimental variability requiring all compared samples to be mounted on the same slide, direct statistical comparisons across treatment groups or between sexes were not feasible for MALDI IMS. Western blot data were analyzed using three-way ANOVAs (sex × treatment × omega-3 diet) with follow-up Fisher's Least Significant Difference (LSD) post hoc test. We assessed the normality of data distributions using the Shapiro-Wilk test and applied suitable statistical adjustments for non-normal datasets; specifically, the logarithmic transformation of electrophysiological data. To identify and exclude statistically significant outliers, we utilized the Robust Regression and Outlier Removal (ROUT) method. Effect sizes for all three-way ANOVAs are reported as partial eta squared (η^2_p). Behavioral and electrophysiological datasets were analyzed offline and, where feasible, in a blinded manner to reduce experimenter bias. Western blot analyses were conducted blind to group assignment, although M.H.S. was not blinded to target proteins. For MALDI IMS, brain region selection was performed blinded to experimental conditions and analyzed using an automated MATLAB script developed by M.H.S., M.B., and S.L.C.; S.L.C. was not blinded to group assignment. Statistical analyses were performed using GraphPad Prism (version 9.0.0 for Windows 10), while R studio was used to calculate the effect sizes. Exact values ± SEM are reported.

RESULTS
Prenatal THC exposure reduces birth weights in both sexes; omega-3 supplementation mitigates this effect without altering maternal or fetal outcomes
Maternal and fetal outcomes were evaluated for all cohorts. From gestational day 7 (GD7) to GD22, dams received vehicle (VEH; saline) or 3 mg/kg i.p., THC daily [5, 6, 11, 26, 28], in conjunction with either control (ct) or an omega-3 (ω3) diet (see Supplemental Table 1 for composition) from GD7 to postnatal day (PD) 21 (Fig. 1a; Supplemental Fig. 1).
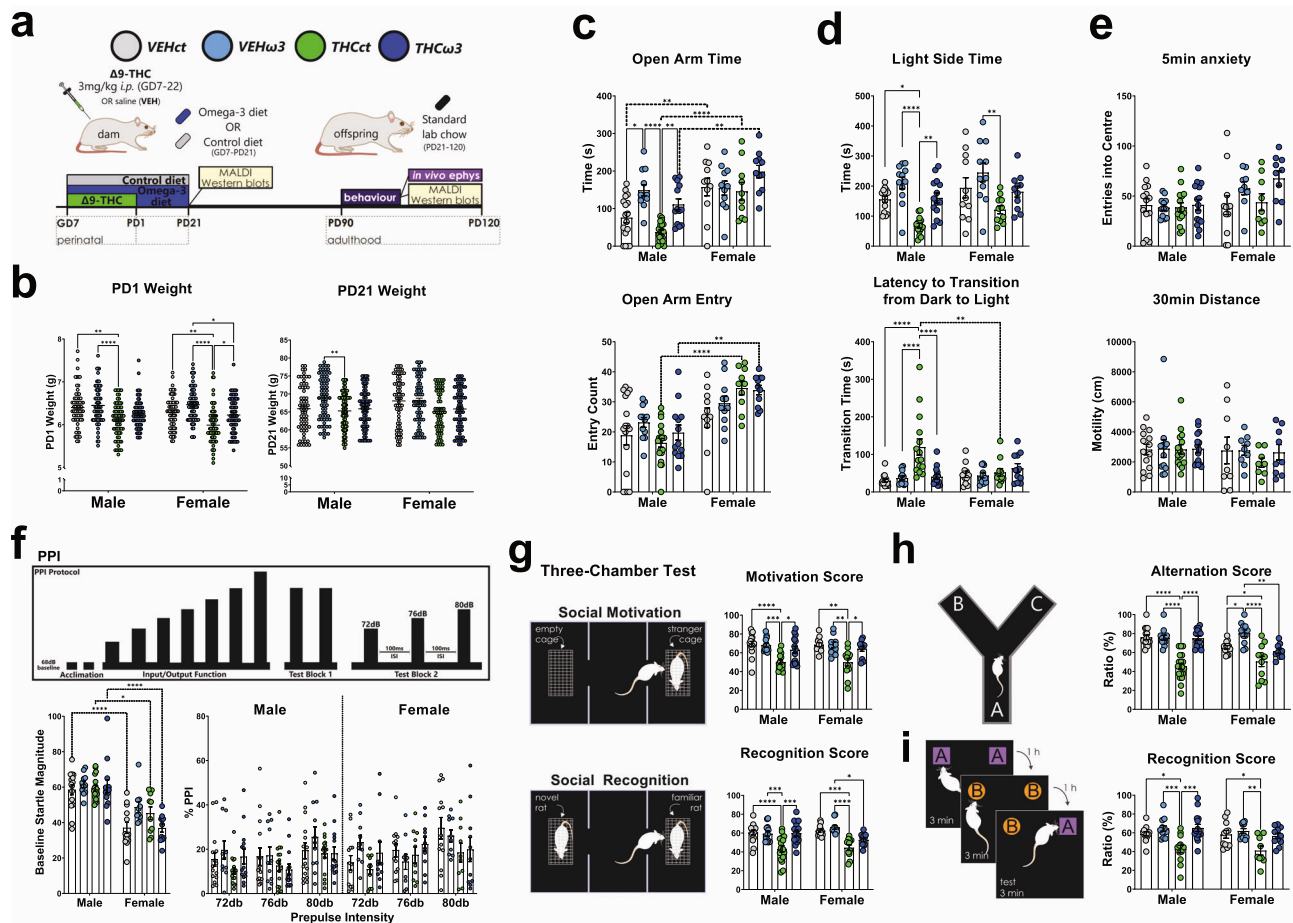
Fig. 1 Long-lasting and sex-selective effects of omega-3 supplementation on anxiety and cognition. a Schematic representation of experimental timeline. **b** Impact of Prenatal THC on Birth Weights (8 pups/dam, approximately even sex distribution): Prenatal THC exposure (*THCct*) significantly reduced birth weights at postnatal day 1 (PD1) in both male and female pups (p < 0.05). Perinatal omega-3 supplementation (*THCω3*) prevented this reduction (p < 0.05), with a significant effect in females, but males, compared to *VEHct*. By PD21, only male *VEHω3* offspring showed significant weight differences compared to *THCct* males. **c–e**, Anxiety-like behaviors assessed in adulthood (n = 12–20/treatment/sex) using EPM and LDB tests, while anxiety and motility was assessed using OFT: **c** Elevated Plus Maze (EPM): No significant anxiety-like behavior was observed in *THCct* males (p > 0.05). However, omega-3 supplementation resulted in reduced anxiety in both *VEHω3* and *THCω3* males (p < 0.05). **d** Light-Dark Box (LDB): *THCct* males exhibited increased anxiety-like behavior compared to *VEHct* males (p < 0.05). Omega-3 supplementation in *THCω3* males normalized this effect (p < 0.05). **e** Open Field Test (OFT): No significant differences were found in anxiety (first 5 minutes of center entries) or locomotor activity over 30 minutes among the groups (p > 0.05). **f** Sensorimotor Processing Assessed by Prepulse Inhibition (PPI): Schematic illustrates the PPI protocol with a 100 ms interstimulus interval at prepulse intensities of 72 dB, 76 dB, and 80 dB. Baseline startle magnitudes: *VEHω3* females > *VEHct* and *THCω3*; males > females (p < 0.05). No significant group differences were observed in PPI. **g–i**, Cognitive and Memory Assessments in Adulthood: **g** Three-Chamber Social Interaction Test: Schematic depicts the assessment of social motivation and recognition. *THCct* offspring showed impaired social motivation and memory compared to *VEHct*, *VEHω3*, and *THCω3* groups (p < 0.05). Omega-3 supplementation rescued social motivation deficits in both sexes (p < 0.05) but improved social memory only in males (p < 0.05). **h** Y-Maze Spontaneous Alternation (SA) Test: Prenatal THC exposure led to deficits in spatial working memory in both sexes (p < 0.05). Omega-3 supplementation prevented these deficits in males but not females (p > 0.05). **i** Temporal Order Novel Object Recognition (TONOR) Test: *THCct* offspring exhibited impairments in object recognition memory in both sexes. Omega-3 supplementation ameliorated these deficits in males but not in females (p > 0.05). Estrous cycles were assessed for all female offspring, but no effect on estrous cycle and behavior were observed. Outliers were excluded using the ROUT method. Statistical analyses were conducted using three-way ANOVAs with factors of sex, THC treatment, and omega-3 diet, followed by Tukey's HSD post hoc tests. Treatment effects are indicated with solid lines; sex effects with dashed lines. Significance levels are denoted as ****p < 0.0001, ***p < 0.001, **p < 0.01, *p < 0.05. Data are presented as individual points with mean±SEM.

Maternal outcomes. We assessed the impact of prenatal THC exposure and omega-3 fatty acid supplementation on several maternal variables, including gestational length, dietary intake, weight gain during pregnancy, litter size, and live birth index (Supplemental Fig. 1). Consistent with previous reports [5, 6, 11, 26, 28], no significant differences were observed in any of these parameters across the treatment groups. For live birth index, stillbirths were noted in only the *THCct* (two pups) and *THCω3* (one pup) groups. Importantly, omega-3 supplementation did not affect any maternal outcomes, regardless of THC exposure.

Pup outcomes. To evaluate fetal growth restriction (FGR), we recorded body weights at birth/postnatal day 1 (PD1) and at PD21 and monitored survival up to PD4. At PD1, a three-way ANOVA revealed significant main effects of omega-3 diet ($F_{(1528)} = 49.33$; $p < 0.0001$; $\eta^2 p = 0.05$) and THC treatment ($F_{(1528)} = 20.68$; $p < 0.0001$; $\eta^2 p = 0.03$). *Post-hoc* comparisons revealed that male pups from *THCct* dams weighed significantly less than *VEHct* pups ($p = 0.0018$) and *VEHω3* pups ($p < 0.0001$), but not *THCω3* pups ($p = 0.1353$; Fig. 1b, Supplemental Fig. 1). *Post-hoc* comparisons also revealed female pups from *THCct* dams weighed significantly

less than *VEHct* pups (p = 0.0018), *VEHω3* pups (p < 0.0001), and *THCω3* pups (p = 0.0130). Additionally, female *VEHω3* weighed significantly more then *THCω3* pups (p = 0.0198). Importantly, no differences were observed between male or female *VEHct*, and *THCω3* groups (p > 0.05).

At PD21, three-way ANOVA revealed an effect of only omega-3 diet ($F_{(1522)}$ = 18.69; p < 0.0001; η^2p = 0.04). *Post-hoc* comparisons revealed that *VEHω3* male offspring weighed significantly more than male *THCct* (p = 0.0084) offspring (Fig. 1b, Supplemental Fig. 1). The body weight normalization of *THCct* at PD21, relative to *VEHct*, indicates postnatal catch-up growth, consistent with gestational THC exposure in rodents and humans [5, 8, 26]. Three pups were dead at birth; survival until PD4 was unaffected, except in *n* = 1 pup in one *VEHω3* dam (Supplemental Fig. 1).

Omega-3 supplementation prevents PTE-induced anxiety-like phenotypes in early adulthood

Prior work has demonstrated male-specific anxiety-like phenotypes following PTE, with females largely unaffected [6, 7]. Thus, we assessed anxiety in both sexes using the elevated plus maze (EPM) and light dark box (LDB) and assessed general motility with the open field test (OFT) (Fig. 1a) to rule out motility-related effects of THC or the omega-3 diet that might confound the interpretation of anxiety (and cognitive) tasks.

Elevated plus maze. EPM data are presented in Fig. 1c. At adulthood, three-way ANOVA on times spent in open arms revealed a significant effect of sex ($F_{(1,96)}$ = 39.54, p < 0.0001; η^2p = 0.34), THC treatment ($F_{(1,96)}$ = 19.04, p < 0.0001; η^2p = 0.19), interaction Sex×omega-3 diet ($F_{(1,96)}$ = 5.831, p = 0.0176; η^2p = 0.06) and Sex×THC ($F_{(1,96)}$ = 4.655, p = 0.0334; η^2p = 0.05). Post-hoc analyses revealed a significant decrease in times spent in open arms in male *THCct* compared to *VEHct* (p < 0.0001) and *THCω3* (p = 0.0074), but not *VEHct* (p = 0.4471), and a significant increase in male *VEHω3* compared to male *VEHct* (p = 0.0178). No effect was observed in any treatment in females. Furthermore, males spent significantly less time in open arm *vs.* female counterparts in *VEHct* (p = 0.0068), *THCct* (p < 0.0001), and *THCω3* (p = 0.0059).

Three-way ANOVA on open arm entries revealed significant effects of sex ($F_{(1,95)}$ = 48.43, p < 0.0001; η^2p = 0.31) and interaction Sex×omega-3 Diet ($F_{(1,95)}$ = 6.838, p = 0.0104; η^2p = 0.08). *Post-hoc* analysis revealed no within sex differences. However, we observed significantly lower entries of males *vs.* their female counterpart in *THCct* (p < 0.0001), and *THCω3* (p = 0.0020).

Light/Dark box. LDB data are presented in Fig. 1d. At adulthood, three-way ANOVA on times spent on the light side revealed significant effects of sex ($F_{(1,91)}$ = 7.145, p = 0.00089; η^2p = 0.09), omega-3 diet ($F_{(1,91)}$ = 24.71, p < 0.0001; η^2p = 0.20), and THC treatment ($F_{(1,91)}$ = 21.93, p < 0.0001; η^2p = 0.21). Post hoc analyses revealed a significant decrease in the time spent on the light side in the male *THCct* group compared to male *VEHct* (p = 0.0177), *VEHω3* (p < 0.0001), and *THCω3* (p = 0.0096). Female *THCct* also spent significantly less time on the light side compared to female *VEHω3* (p = 0.0012). Three-way ANOVA on the latency to re-emerge from dark to light environment revealed significant effects of omega-3 diet ($F_{(1,91)}$ = 12.64, p = 0.0006; η^2p = 0.14), interaction Sex× omega-3 diet ($F_{(1,91)}$ = 4.203, p = 0.0432; η^2p = 0.05), Sex×THC ($F_{(1,91)}$ = 5.885, p = 0.0172; η^2p = 0.07), THC×omega-3 diet ($F_{(1,91)}$ = 4.659, p = 0.0335; η^2p = 0.06), and Sex×THC×omega-3 diet ($F_{(1,91)}$ = 9.270, p = 0.0030; η^2p = 0.09). *Post-hoc* analyses revealed that male *THCct* rats transitioned to the light side later male *VEHct* (p < 0.0001), *VEHω3* (p < 0.0001), and *THCω3* (p < 0.0001). No effect was observed in the female progeny (p > 0.05). Furthermore, males *THCct* exhibited significantly greater latency *vs.* their female counterpart (p = 0.0018).

Open field test. OFT data are presented in Fig. 1e. For 5 min center entries (anxiety), three-way ANOVA revealed significant effects of sex ($F_{(1,88)}$ = 7.414, p = 0.0078; η^2p = 0.07), THC treatment ($F_{(1,88)}$ = 4.827, p = 0.0306; η^2p = 0.04), and interaction Sex×THC treatment ($F_{(1,88)}$ = 4.817, p = 0.0308; η^2p = 0.04). Post-hoc analyses revealed no significant comparisons. For 30 min movement distance (motility), three-way ANOVA did not find any significant effects.

Omega-3 supplementation mitigates PTE-induced cognitive deficits in adulthood

Previous findings demonstrate that PTE induces cognitive and memory impairments in male/female adult offspring, while only males exhibited sensorimotor processing deficits [5, 6, 9, 10]. In this study, acoustic prepulse inhibition (PPI) of the startle reflex was performed to investigate sensorimotor gating. We also employed social interaction (SI) to examine social motivation and memory, spontaneous alternation (SA) to assess spatial working memory and temporal order novel object recognition (TONOR) to gauge object recognition memory.

Prepulse inhibition of the acoustic startle response. PPI data are presented in Fig. 1f. At adulthood, three-way ANOVA of startle baselines revealed a main effect of sex ($F_{(1,93)}$ = 78.75; p < 0.0001; η^2p = 0.47), and significant interactions of THC×omega-3 diet ($F_{(1,93)}$ = 7.491; p = 0.0074; η^2p = 0.06), and Sex×THC×omega-3 diet ($F_{(1,93)}$ = 4.930; p = 0.0288; η^2p = 0.05). Post-hoc analysis revealed a significant significantly greater startle baselines in males *vs.* female counterparts in *VEHct* (p < 0.0001), *THCct* (p = 0.0140), and *THCω3* (p < 0.0001), but not *VEHω3* (p = 0.0766).

Three-way ANOVA of PPI revealed significant effects of sex ($F_{(5279)}$ = 3.785; p = 0.0025; η^2p = 0.00336), and omega-3 diet ($F_{(1279)}$ = 7.641; p = 0.0061; η^2p = 0.08). *Post-hoc* analysis revealed no significant group differences.

Social interaction. SI test data are presented in Fig. 1g. At adulthood three-way ANOVA on social motivation (phase 1) revealed main effects of THC treatment ($F_{(1,96)}$ = 9.246, p = 0.0030; η^2p = 0.10), omega-3 diet ($F_{(1,96)}$ = 29.63, p < 0.0001; η^2p = 0.25), and a significant interaction THC×omega-3 diet ($F_{(1,96)}$ = 11.25, p = 0.0011; η^2p = 0.11). Post-hoc analyses revealed that the *THCct* male rats spent less time with social target over empty cage in male *THCct* compared to male *VEHct* (p < 0.0001), *VEHω3* (p = 0.0008), and *THCω3* (p = 0.0257). Similar effects were observed in the female group (*THCct vs. VEHct* p = 0.0013; *THCct vs. VEHω3* p = 0.0036; and *THCct vs. THCω3* p = 0.0386).

Moreover, at adulthood, three-way ANOVA on social memory (phase 2) revealed main effects of THC treatment ($F_{(1,96)}$ = 12.04, p = 0.0008; η^2p = 0.13), omega-3 diet ($F_{(1,96)}$ = 37.60, p < 0.0001; η^2p = 0.28), and significant interactions of THC×omega-3 diet ($F_{(1,96)}$ = 8.571, p = 0.0043; η^2p = 0.09). *Post-hoc* analyses revealed a significant decrease in time spent interacting with novel target over the familiar target in the male *THCct* compared to male *VEHct* (p < 0.0001), *VEHω3* (p = 0.0009), and *THCω3* (p = 0.0002). Furthermore, female *THCct* exhibited a reduction in social memory compared to *VEHct* (p = 0.0007) and *VEHω3* (p < 0.0001), but not *THCω3* (p = 0.5079). Additionally, female *VEHω3* exhibited significantly better performance then *THCω3* (p = 0.0445).

Spontaneous alternation. Y-maze SA test data are presented in Fig. 1h. At adulthood, three-way ANOVA revealed main effects of omega-3 diet ($F_{(1,98)}$ = 52.52; p < 0.0001; η^2p = 0.38), THC treatment ($F_{(1,98)}$ = 37.57, p < 0.0001; η^2p = 0.31), and significant interactions of THC treatment×omega-3 diet ($F_{(1,98)}$ = 8.436; p = 0.0045; η^2p = 0.11) and Sex×THC×omega-3 diet ($F_{(1,98)}$ = 14.66; p = 0.0002; η^2p = 0.13). Post-hoc analyses revealed a significant decrease in spontaneous alternations score in the male *THCct* compared to male *VEHct* (p < 0.0001), *VEHω3*

(p < 0.0001), and *THCω3* (p < 0.0001). Similarly, the female *THCct* had significantly lower scores compared to *VEHct* (p = 0.0447) and *VEHω3* (p < 0.0001), while *VEHω3* had better performance than *VEHct* (p = 0.0405) and *THCω3* (p = 0.0052).

Temporal order novel object recognition. TONOR data are presented in Fig. 1i. At adulthood, three-way ANOVA revealed significant effects of omega-3 diet ($F_{(1,87)} = 14.62$; p = 0.0002; $\eta^2 p = 0.12$), THC treatment ($F_{(1,87)} = 22.25$; p < 0.0001; $\eta^2 p = 0.22$), and an interaction THC×omega-3 diet ($F_{(1,87)} = 8.205$; p = 0.0052; $\eta^2 p = 0.09$). Post-hoc analyses revealed that male *THCct* had lower temporal recognition scores compared to male *VEHct* (p = 0.0198), *VEHω3* (p = 0.0005), and *THCω3* (p = 0.0001). For the females, the *THCct* group exhibited lower temporal recognition scores compared to *VEHct* (p = 0.0415), and *VEHω3* (p = 0.0054), but not *THCω3* (p = 0.0925).

Omega-3 supplementation prevents neuronal dysfunction in the male PFC and vHIPP, but only in the female vHIPP, of PTE offspring

PTE differentially dysregulates neuronal activity in the PFC and ventral hippocampus (vHIPP) of male and female offspring [9, 10]. To assess the impact of omega-3 supplementation on this circuit, we conducted in vivo extracellular electrophysiological recordings in the PFC and vHIPP of adult rats (Fig. 2).

Pyramidal cell activity. In PFC, three-way ANOVA comparing spontaneous firing frequency of putative pyramidal cells revealed significant effects of THC treatment ($F_{(1544)} = 7.747$, p = 0.0056; $\eta^2 p = 0.04$) and omega-3 diet ($F_{(1, 544)} = 26.78$, p < 0.0001; $\eta^2 p = 0.01$). Post-hoc analyses revealed significantly higher firing frequency in male *THCct* compared to *VEHct* (p = 0.0062), *VEHω3* (p = 0.0051), and *THCω3* (p = 0.0015). In females, the *THCct* group had significantly higher activity than the *VEHct* (p = 0.0347) and *VEHω3* (p < 0.0001), but not *THCω3* (p = 0.2475).

For bursting frequency in the PFC, three-way ANOVA revealed an effect of sex ($F_{(1468)} = 5.662$, p = 0.0177; $\eta^2 p = 0.01$) and sex×omega-3 diet ($F_{(1468)} = 6.074$, p = 0.0141; $\eta^2 p = 0.01$). Post-hoc analyses that female *VEHct* exhibited significantly higher bursting frequency then male *VEHct* (p = 0.0380).

In vHIPP, three-way ANOVA comparing spontaneous firing frequencies of putative pyramidal cells revealed significant interactions of Sex×omega-3 diet ($F_{(1474)} = 26.89$, p < 0.0001; $\eta^2 p = 0.06$), Sex×THC treatment ($F_{(1474)} = 6.069$, p = 0.0141; $\eta^2 p = 0.01$), and a Sex×THC×omega-3 diet ($F_{(1474)} = 19.60$, p < 0.0001; $\eta^2 p = 0.04$). Post-hoc analyses revealed that male *THCct* rats had significantly lower activity compared to *VEHct* (p < 0.0001), *VEHω3* (p = 0.0005) and *THCω3* (p = 0.0213). On the other hand, female *THCct* group had significantly higher activity than the *VEHct* (p < 0.0001), *VEHω3* (p = 0.0050), and *THCω3* (p = 0.0073). Additionally, male *VEHct* vs. female *VEHct* exhibited a significantly lower firing rate (p = 0.0029), while female *THCct* vs. male *THCct* exhibited significantly elevated firing rates (p < 0.0001).

For bursting frequency in the vHIPP, three way ANOVA revealed an effect of sex ($F_{(1469)} = 4.702$, p = 0.0306; $\eta^2 p = 0.00729$), omega-3 diet ($F_{(1469)} = 12.16$, p = 0.0005; $\eta^2 p = 0.03$), THC treatment ($F_{(1469)} = 21.17$, p < 0.0001; $\eta^2 p = 0.05$), Sex×THC treatment ($F_{(1469)} = 14.56$, p = 0.0002; $\eta^2 p = 0.03$), THC×omega-3 diet ($F_{(1469)} = 9.130$, p = 0.0027; $\eta^2 p = 0.02$) and sex×THC×omega-3 diet ($F_{(1469)} = 5.513$, p = 0.0193; $\eta^2 p = 0.01$). Post-hoc analyses that male *THCω3* exhibits higher burst frequencies than *VEHct* (p < 0.0001), *VEHω3* (p < 0.0001) and *THCct* (p < 0.0001). Additionally, female *THCct* exhibited higher burst frequencies than the male *THCct* (p = 0.0175).

Local field potentials. Prenatal THC exposure induced alterations in oscillatory patterns within the PFC-vHIPP circuitry of both male and female offspring (Fig. 3; statistical analyses are presented in Supplemental Table 3). Male *THCω3* normalized the THC-induced dysregulations in PFC LFP activity across the delta (0-4 Hz), theta (4-7 Hz), beta (14-30 Hz), and gamma (30-100 Hz), but not alpha bands (7-14 Hz) (Fig. 3b, c; Supplemental Table 3).

Notably, the female *THCω3* group exhibited a significant divergence in the both the PFC and vHIPP compared to *VEHct* in delta, theta, beta, and gamma oscillatory bands; as well as alpha in the vHIPP (Fig. 3c). In contrast, for males, in the vHIPP, male *THCω3* exhibit disturbances compared to *VEHct* in only theta and gamma oscillatory bands, while vHIPP delta, alpha, and beta and all bands in the PFC exhibit control level LFP (Fig. 3c). This sex-specific difference may explain the behavioral phenotype that relies on normative PFC-vHIPP communication. The restoration of normal oscillatory patterns in male *THCω3* rats may account for their improved cognitive performance relative to *THCct* males, while this is absent in females.

Omega-3 supplementation modifies lipidomic profile in PTE offspring's PFC, NAc, and hippocampal formation

To assess whether perinatal omega-3 fatty acid supplementation can counteract THC-induced lipidomic alterations, we examined the impact of PTE on PUFAs and the broader phospholipid profiles in specific brain regions (Fig. 4). Our focus included the prelimbic (PRL) and infralimbic (IL) areas of the medial PFC, the core and shell of the NAc, and the ventral and dorsal subiculum, and the CA1 area, of the hippocampus, following established methodologies [5, 6]. Using MALDI IMS, we generated heatmaps representing the ratio of each treatment group's area under the curve (AUC) to that of the *VEHct* groups AUC. Using one sample *t* test with a hypothetical mean set at 1.0, where 1.0 designates values at *VEHct* levels (statistical analyses are presented in Supplemental Table 4), MALDI-IMS analysis revealed significant changes in fatty acids due to prenatal THC exposure (*THCct*) (Fig. 4c–e); consistent with prior reports [5, 6, 26]. Perinatal omega-3 supplementation, both in the presence (*THCω3*) and absence (*VEHω3*) of THC exposure, also resulted in significant alterations in fatty acid profiles (Fig. 4c-e; Supplemental Table 4).

Notably, males and female receiving omega-3 supplementation exhibited a normalized lipidome at PD21. However, the perinatal dietary supplement in males led to a downregulation of all assessed fatty acids by PD120 particularly in the PFC and NAc regions, with minimal disturbances in hippocampus. The female phenotype across all groups at PD120 was also largely in line with *VEHct* levels, normalized, and consistent with prior reports [5, 6].

While fatty acid levels provided some insights, a comprehensive analysis of the lipidome revealed significant anomalies not apparent from fatty acid data alone (Fig. 5). We examined several classes of phospholipids: lysophospholipids (signaling molecules with a cleaved fatty acid tail; Fig. 5a), sulfatide-bound phospholipids (sulfatides; associated with the myelin sheath or in transit to it; Fig. 5b), and membrane phospholipids (containing two fatty acid tails and are either part of the membrane or in transit to it; Fig. 5e). These lipid species are products of the hepatic fatty acid pathway (Fig. 4a) and glucose metabolism mediated by CB1Rs, produced via the Kennedy pathway and then remodeled via the Land's Cycle either within the liver or the brain (Fig. 5a).

Using one sample *t* test with a hypothetical mean set at 1.0, where 1.0 designates values at *VEHct* levels (see Supplemental Table 5 for all statistics) the omega-3 diet led to numerous region- and sex- specific alterations in membrane phospholipids in both *THCω3* and *VEHω3* groups, with lysophospholipids and sulfatides largely unaffected. Heatmaps exhibit ratios where each treatment groups AUC is divided by *VEHct* AUC. Interestingly, the lipid profiles in both *THCω3* male and female offspring resembled those of *THCct*, at both postnatal days 21 and 120 in the hippocampus (Fig. 5, Supplemental Table 5). Severe deficits were observed in females within the PFC and NAc, particularly at PD120, effecting the lysophospholipids, membrane phospholipids, and sulfatides.
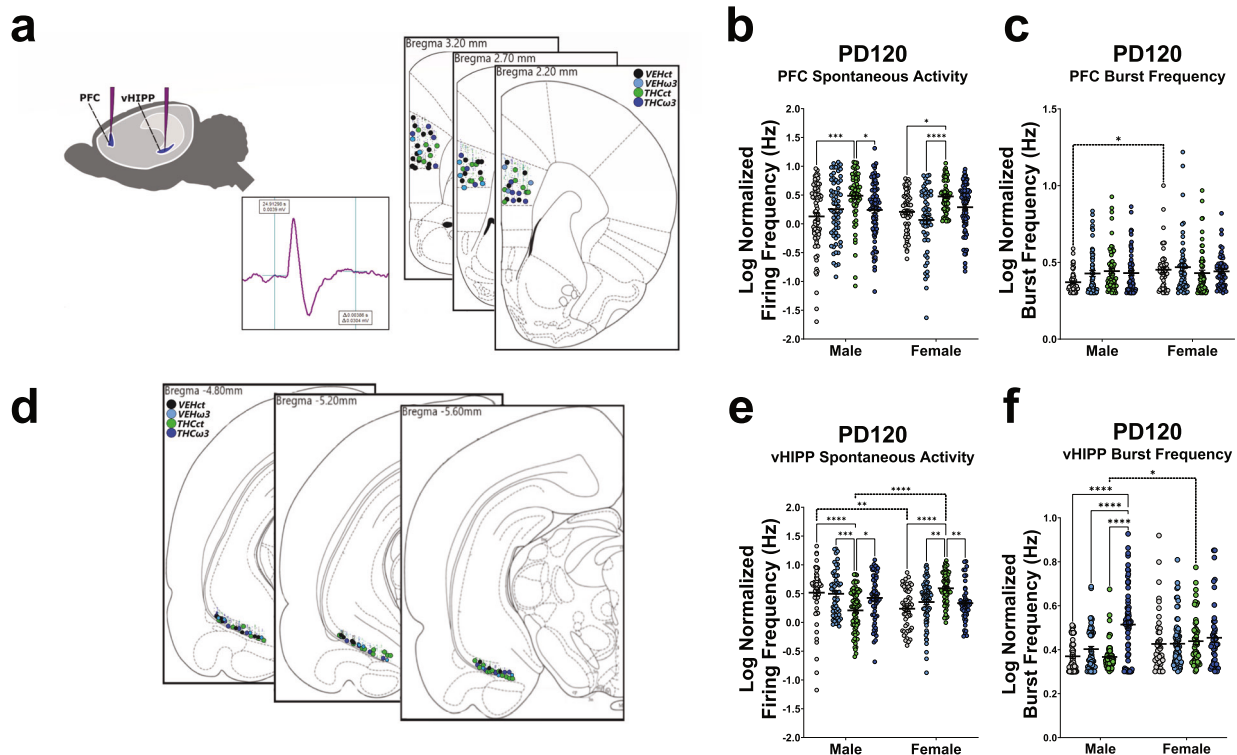
Fig. 2 Perinatal Omega-3 supplementation prevents prenatal THC induced neuronal dysfunction in the PFC and vHIPP in males, but only in female vHIPP, and elevates bursting frequency in the male, but not female, vHIPP. a End track coordinates for recorded presumptive pyramidal cells in the PFC of both hemispheres for males and females. **b** Spontaneous Firing Rates in the PFC: Prenatal THC (*THCct*) significantly increases PFC spontaneous pyramidal cell firing frequency in male and female offspring at adulthood (cells/rat: males—*VEHct* n = 78/8, *THCct* n = 67/7, *VEHω3* n = 62/8, *THCω3* n = 82/8; females—*VEHct* n = 60/8, *THCct* n = 64/7, *VEHω3* n = 57/8, *THCω3* n = 81/8). Omega-3 supplementation prevents the hyper-glutamatergic phenotype in males (p < 0.05) and mitigates the effect in females *THCω3* vs. *VEHct*. **c** Bursting Activity in the PFC: Neither prenatal THC exposure nor omega-3 supplementation significantly affected the bursting frequency of PFC pyramidal neurons. However, female PFC *VEHct* exhibit higher bursting rates then male counterparts. **d** End track coordinates for recorded presumptive pyramidal cells in the vHIPP of both hemispheres for males and females. **e** Spontaneous Firing Rates in the vHIPP: Prenatal THC induces significantly lower spontaneous pyramidal cell firing frequency in the male vHIPP and significantly higher firing frequency in the female vHIPP (cells/rat: males—*VEHct* n = 60/8, *THCct* n = 73/7, *VEHω3* n = 54/8, *THCω3* n = 60/8; females—*VEHct* n = 50/8, *THCct* n = 68/7, *VEHω3* n = 70/8, *THCω3* n = 47/8). Omega-3 intervention prevents these phenotypes in both males and females (p < 0.05). **f** Bursting Activity in the vHIPP: Omega-3 supplementation elevated bursting frequency in male *THCω3* offspring compared to *VEHct*, *THCct*, and *VEHω3* males (p < 0.05). Additionally, female *THCct* exhibited elevated bursting frequency compared to male counterparts (p < 0.05). This pattern suggests that while omega-3 fatty acids broadly confer resilience against prenatal THC-induced neuronal dysfunctions, they may also introduce sex-dependent neurophysiological alterations within hippocampal circuitry. Recordings outside the targeted brain regions were excluded based on histological confirmation. Outliers were removed using the ROUT method. Statistical analyses were conducted using three-way ANOVAs with factors of sex, THC treatment, and omega-3 diet, followed by Tukey's HSD post hoc tests. Significance levels are indicated as ****p < 0.0001, ***p < 0.001, **p < 0.01, *p < 0.05. Log-normalized data are presented as individual points with mean±SEM.

Both *VEHω3* and *THCω3* also exhibited disturbances in phospholipids associated with particularly AA or its related metabolites (Fig. 5, Supplemental Table 5). Alterations were also seen in the lysophospholipids, including lysophosphatidylethanolamines (lyso-PE), a critical precursor to the primary endocannabinoids AEA and 2-AG [57].
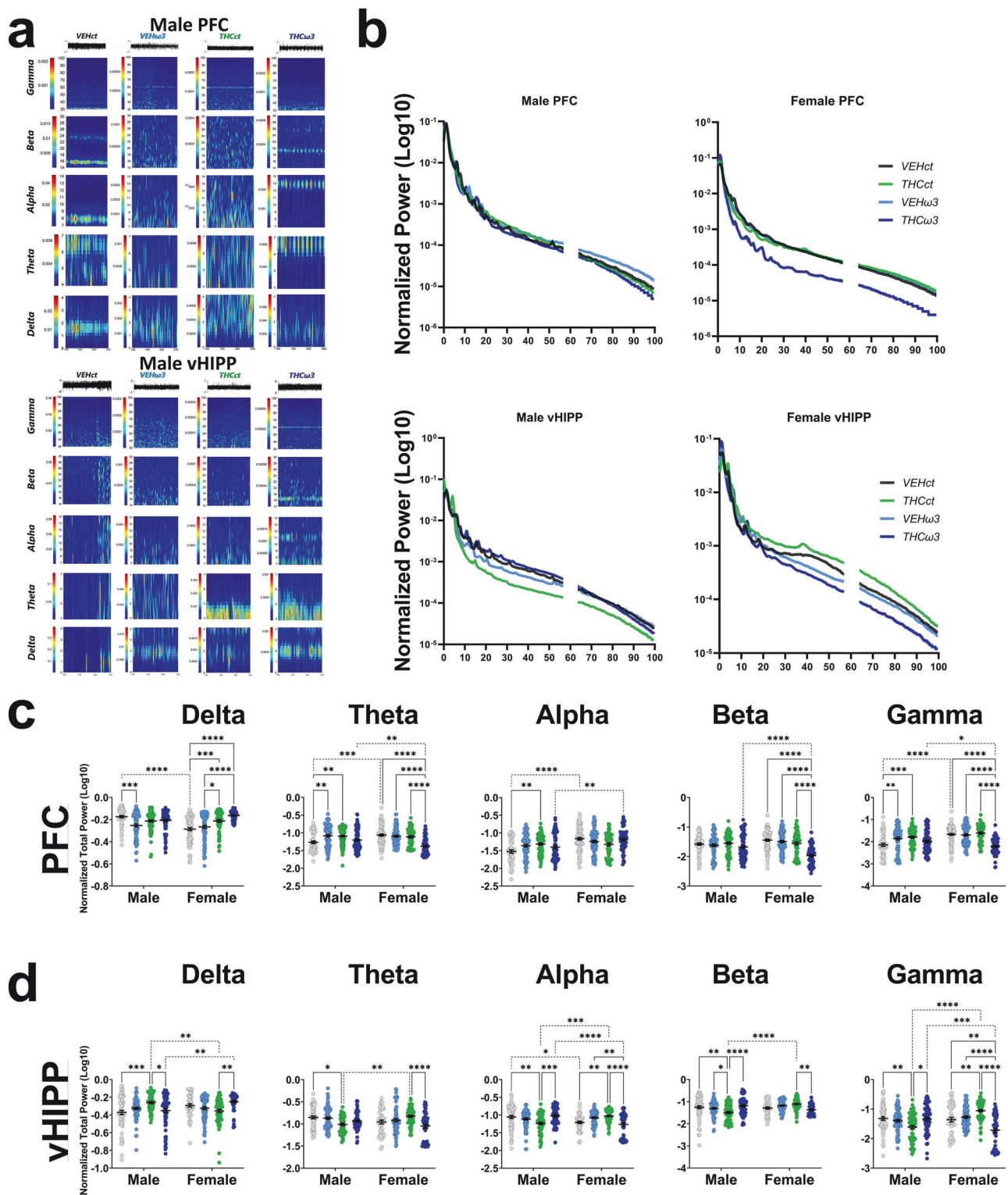
Omega-3 supplementation alters proteins involved in neural development, synaptic function, and fatty acid metabolism in the PFC, NAc, and vHIPP in a sex-dependent manner

We next examined biomarkers associated with neurodevelopment, neurotransmission, fatty acid metabolism and neuroinflammation. Targets examined in PFC, NAc, and vHIPP at PD21 and PD120 included dopamine D1R and D2R, NMDA receptor (GluN) 2 A and GluN2B, metabotropic glutamate receptor 2/3 (mGluR2/3), vesicular glutamate transporter (vGlut) 1 and vGlut2, synaptophysin (SYP), postsynaptic density protein 95 (PSD95), brain derived neurotropic factor (BDNF), GAD67, Gephyrin, peroxisome proliferator-activated receptor α (PPARα), and PPARγ (isoforms

were not observed). At PD120, three-way ANOVA revealed that PTE induced alterations to several dopaminergic, GABAergic, and glutamatergic biomarkers, as well as proteins involved in synaptic organization and development (Fig. 6; see Supplemental Table 6 for statistical analyses). Most of these effects were absent in the *THCω3* treated groups, however, some exceptions were observed in all categories of proteins examined (Fig. 6, Supplemental Table 6).

DISCUSSION

Our study uncovers various novel effects of PTE on neuropsychiatric biomarkers within the cortico-striatal-hippocampal network, encompassing cognitive, affective, neuronal, protein, and lipidomic alterations. We demonstrate that perinatal omega-3 fatty acid supplementation can mitigate many adverse outcomes of THC exposure, albeit with sex-specific efficacy. Male offspring exhibited significant improvements across behavioral, electrophysiological domains, effectively counteracting THC-induced deficits in all but

protein and lipidomics. In stark contrast, female offspring exhibited improvements in only social behavior and neuronal activity but retained residual THC-related disturbances in their PFC, NAc, and hippocampus. These sex-dependent outcomes likely reflect variations in developmental responses to PTE, sensitivity to FGR, differential neuroinflammatory and metabolic pathways, and distinct reactions to omega-3 interventions. These findings also highlight the need to consider sex-specific

neurobiological responses when designing interventions for ECS-related neurodevelopmental disorders.

We focused on the PFC, vHIPP, and NAc, regions central to cognition, affect, and reward [17, 58, 59] and highly sensitive to cannabinoid-induced disruptions [4–6, 10, 19, 60, 61]. These areas form the core of the cortico-striatal-hippocampal network and are modulated by dopaminergic VTA signaling [62–65]. A hallmark of rodent models of neurodevelopmental PTE is hyperdopaminergia

Fig. 3 Perinatal omega-3 supplementation modulates prenatal THC-Induced oscillatory field dysfunctions in the PFC and vHIPP with sex-specific effects. a Representative Spectrograms: Local field potential (LFP) recordings from male PFC and vHIPP across treatment groups. Spectrograms highlight small-amplitude, fast oscillations. Power values between 59–61 Hz (reflecting power line frequency) were excluded. **b** Normalized Power Spectra: Average power spectra of LFP recordings in the adult male and female PFC and vHIPP. Spectral analyses examined delta (0–4 Hz), theta (4–7 Hz), alpha (7–14 Hz), beta (14–30 Hz), and gamma (30–100 Hz) bands. **c** PFC Oscillations: Prenatal THC exposure and omega-3 supplementation induced significant changes in PFC oscillatory activity across sexes (recording sites/rat: males—VEHct n = 54/8; THCct n = 90/7; VEHω3 n = 75/8; THCω3 n = 85/8; females—VEHct n = 82/8; THCct n = 61/7; VEHω3 n = 88/8; THCω3 n = 68/8); see Supplemental Table 3 for statistical analyses. Males: THC exposure increased theta, alpha, and gamma power compared to *VEHct*. Male THCω3 did not exhibit significant differences from either *VEHct* or *THCct*; suggesting that some underlying THC-induced effects persist. Females: THC exposure increased only alpha power compared to *VEHct*. Omega-3 supplementation elevated delta, and reduced theta, beta, and gamma power in the PFC compared to *VEHct*. **d** vHIPP Oscillations: THC and omega-3 supplementation induced significant changes in vHIPP oscillations (recording sites/rat: males—VEHct n = 66/8; THCct n = 60/7; VEHω3 n = 77/8; THCω3 n = 60/8; females—VEHct n = 50/8; THCct n = 68/7; VEHω3 n = 76/8; THCω3 n = 48/8). Males: THC exposure elevated delta, and reduced theta, alpha, beta, and gamma oscillations. Omega-3 supplementation normalized delta, alpha, beta, and gamma oscillations to *VEHct* levels, compared to *THCct*, but had no effect on theta oscillations. Females: THC exposure increased alpha and gamma power compared to *VEHct*. Omega-3 supplementation significantly reduced total power across all frequency bands compared to *THCct*, except delta oscillations. Outliers were removed if failing the ROUT Outlier Test. Comparisons were made using Three-Way ANOVA with Sex, THC treatment, and omega-3 diet as factors, followed by post hoc Tukey's HSD. ****p < 0.0001, ***p < 0.001, **p < 0.01, *p < 0.05; individual data points with mean ± SEM are shown.
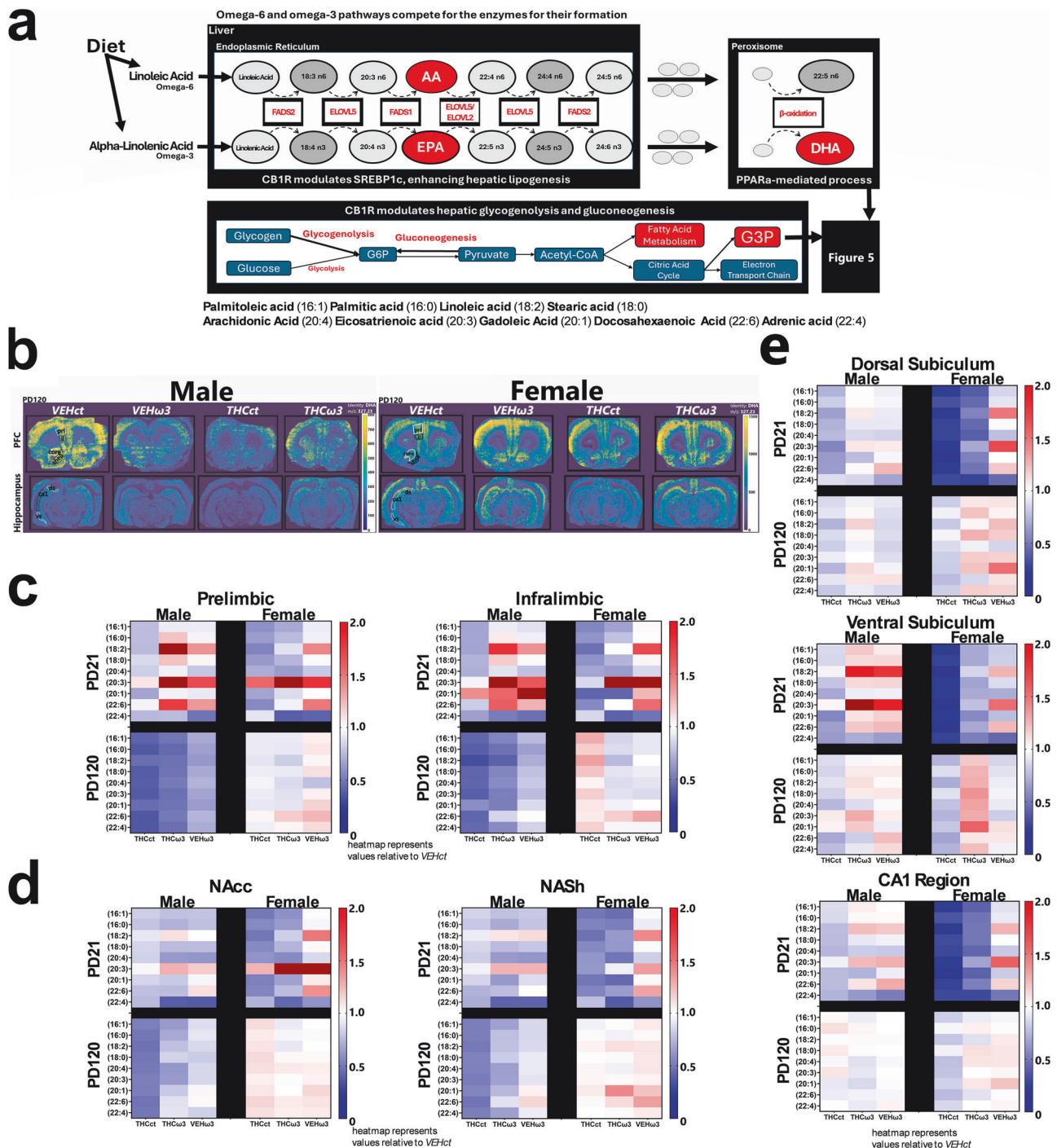
in the VTA, which disrupts the balance of excitation and inhibition across downstream targets [6, 60, 61]. The ECS plays a central role in regulating this balance by modulating GABAergic and glutamatergic tone, particularly via CB1R-dependent signaling in GABAergic interneurons [57]. These regions, in turn, regulate dopamine release by providing inhibitory and/or excitatory tone to the VTA [66, 67]. Dysregulation of this system during development leads to enduring circuit-level perturbations [4–6, 9, 10, 60] and may impact crucial developmental processes like cell migration, axonal guidance, and synaptogenesis [4, 18, 57].

In the PFC, CB1Rs regulate GABAergic inhibitory control via parvalbumin (PV)- and cholecystokinin (CCK)-positive interneurons [68]. PFC cannabinoid signaling regulates inhibitory tone and influences anxiety and affective behaviors [62, 63]. Disruption of PFC PV+ interneuron function alters gamma oscillations and impairs cognitive processes such as attention and working memory [68, 69]. Interestingly, disrupting PFC PV+ neuronal activity disrupts normal social behaviors [70], consistent with the disturbed sociability phenotypes observed in our THC-treated cohorts, and other prenatal THC studies [71–74]. Our analyses also revealed dysregulation in PFC gamma power in THC-treated males, which was prevented by the omega-3 intervention, demonstrating a protective effect of omega-3 against THC-induced cortical disruptions. These findings align with reports of reduced PFC PV+ interneurons in rodent models of FGR-induced schizophrenia, specifically sociability dysfunction, and impaired sensorimotor gating [75], consistent with our sociability, but not PPI, findings. Importantly, during perinatal brain development, DHA directly modulates glutamatergic and GABAergic activity in the PFC and vHIPP by promoting normal synaptic maturation and fatty acid signaling [45, 76]. Thus, omega-3 supplementation restored gamma oscillations in males, potentially by normalizing PV+ interneuron activity. However, in females, this intervention failed to rescue PFC function, suggesting fundamental sex differences in the response to omega-3 supplementation. The impact of the supplementation on interneuron function was not examined and requires further exploration.

Sex-dependent differences extended to the vHIPP, a region critical for regulating anxiety and memory. vHIPP→PFC inputs activate CCK+ and PV+ interneurons, providing feed-forward inhibition of pyramidal cells [77, 78]. Optogenetic inhibition of PFC CCK+ interneurons has been shown to disrupt working memory [79]. Previous studies have reported male-specific reductions in CCK+ cells following PTE, a phenotype linked to spatial memory deficits [10, 80]. Consistent with these findings, male THC-treated (*THCct*) cohorts in our study displayed PFC hyperactivity and vHIPP hypoactivity, reflecting a disrupted balance within the PFC-vHIPP

network. In contrast, females exhibited hyperactivity in both regions, suggesting divergent alterations in circuit excitability. Reduced vHIPP spiking activity and increased vGlut1 expression in males suggest impaired glutamatergic tone [5, 9, 10, 71, 80], whereas females showed no changes in vGlut1 or vGlut2 expression despite increased pyramidal cell activity. These findings indicate that omega-3 supplementation effectively normalized excitatory/inhibitory balance in males but failed to address similar disruptions in females. Male *THCct* offspring also exhibited increased mGluR2/3 expression in the PFC, a compensatory response to pyramidal cell hyperactivity, as mGluR2/3 suppresses local glutamate release [81]. Notably, this upregulation was absent in *THCω3* males, consistent with normalized pyramidal cell activity in the omega-3-supplemented cohort. Both male and female *THCω3* offspring displayed reduced PFC expression of NMDA receptor subunits GluN2A and GluN2B. A similar reduction was observed in *VEHω3* groups, indicating a direct effect of omega-3 supplementation on NMDA receptor expression, consistent with previous studies [82]. However, the sustained decrease in DHA levels in the PFC of *THCω3* males by PD120 may have contributed to this downregulation, highlighting the long-term limitations of early-life supplementation. Interestingly, while females are more efficient in DHA synthesis [5, 83], their NMDA receptor expression patterns in the PFC mirrored those of males, indicating complex sex-specific omega-3 interactions that warrant further study.

Functional vHIPP-PFC interactions are critical for anxiety regulation [84], sensorimotor gating and cognition [85–87]. PTE disrupts key axonal proteins required for normative hippocampal neuronal connectivity [15] and impedes DHA transfer to neural membranes [5, 6, 11, 26, 88, 89]. DHA is critical for hippocampal neurogenesis and functional connectivity, and supplementation during gestation can improve spatial learning, cognition, and anxiety behaviors [48, 53, 90, 91]. Gestational omega-3 deficiency impairs myelin integrity and hippocampus-PFC connectivity, further exacerbating network disruptions [92]. Synaptic phospholipids such as lysophosphatidic acid (Lyso-PA) are also implicated in cortical excitation/inhibition balance [93]. As the several classes of membrane phospholipids are significantly reduced at PD120, they may contribute to the observed oscillatory disruptions within the male *THCct* and female *THCω3* offspring. These early-life phospholipid reductions, evident as early as PD21, suggest a disrupted neural architecture that persists into adulthood and highlights the limited efficacy of omega-3 supplementation in females [93, 94]. Persistent lipid disturbances in *THCω3* offspring, however, may also reflect systemic effects of PTE, particularly in peripheral organs like the liver and pancreas that are also developing during gestation [42]. Both are sites of high CB1R expression and are vulnerable to cannabinoid-induced

dysregulation of fatty acid metabolism [11, 28, 42, 95–97]. Hepatic dyslipidemia, resulting in reduced fatty acid transport to the brain, may contribute to persistent DHA deficits in males. Astrocytes, the primary sites of brain lipid remodeling, may also be affected, as even adolescent THC exposure disrupts astrocyte structure and function [98–100]. These effects, though speculative, provide a potential framework for understanding the sex-specific lipidomic alterations observed in this study. Further exploration is required of the gestational aspect of this intervention to understand how these effects persist to PD120.

The NAc, a target of dopaminergic VTA projections, mediates anxiety, motivation, and reward-related behaviors and is also

regulated by CB1Rs [17, 101, 102]. Hyperactive VTA dopamine signaling, a well-documented phenotype of PTE [6, 60], may disrupt downstream circuits, including those involving the PFC and NAc [6, 60, 61]. Given the PFC's regulatory influence on the NAc through direct and indirect pathways, alterations in PFC activity may also exert downstream effects on NAc functionality [58, 71–74]. Alterations in PFC gephyrin, mGluR2/3, and GluN2A/B expression and associated neurophysiological adaptations likely influence dopaminergic and glutamatergic signaling within the NAc, contributing to anxiety-related behaviors observed in male *THCct* cohorts [71, 103]. Importantly, omega-3 supplementation prevented many of these molecular changes and abolished

Fig. 4 MALDI-IMS analyses reveal significant age- and sex- specific fatty acid abnormalities in male and female offspring following PTE and perinatal omega-3 supplementation. a Graphical representation illustrates the origin of the assessed fatty acids. The biosynthesis of PUFAs involves several enzymatic steps that largely take place within the endoplasmic reticulum of hepatocytes in the liver; a more limited capacity for fatty acid synthesis does exist within astrocytes in the brain. The ECS, through CB1R and PPAR, regulates fatty acid synthesis and glucose metabolism. These fatty acids and glycerol-3-phosphate (G3P) are then utilized in lipid formation (see Fig. 5). For omega-6 PUFAs, linoleic acid is converted to arachidonic acid (AA), while, for omega-3 PUFAs, alpha-linolenic acid is converted to EPA, and via a PPAR-mediated mechanism in peroxisomes, into DHA. These fatty acids are then incorporated into lipids within the brain. Their release from the membrane is mediated by the Land's Cycle and various phospholipases (here, PLA$_2$, which has a preference for AA cleavage), that then allow these fatty acids to be converted to inflammatory mediators, or endocannabinoids. **b** Representative male and female MALDI IMS images of DHA in each of the treatment groups. As mass spectrometry is subject to run-to-run variability, all experimental values are normalized as ratios to *VEHct*, which is treated as the value 1.0. Mass-to-charge (m/z) values are based on previously described mass target [55]. The assessed fatty acids and their m/z are detailed in Supplemental Table 2. **c-e**, Male and female offspring at PD21 (Male: n = 12/treatment, Female: n = 8/treatment) and PD120 (n = 10/treatment/sex, Female: n = 8/treatment) exposed to THC (*THCct*) exhibited significant age- and sex-specific fatty acid alterations in the (**c**) prelimbic and infralimbic regions of the PFC, (**d**) the NAc core (NAcc) and shell (NASh), and (**e**) the dorsal and ventral subiculum, and CA1 region, of the hippocampus. The omega-3 intervention (*VEHω3* and *THCω3*) also led to significant changes in numerous fatty acids, notably AA and its metabolites. Female offspring exhibited largely normalized fatty acids by adulthood, while male *THCct* offspring showed substantial PFC, NAc, and hippocampal abnormalities. Area under the curve (AUC) ratio comparisons were conducted with one-sample *t* tests, with a hypothetical mean of 1.0; where 1.0 suggests no difference between the *VEHct* compared to each respective treatment's offspring. Heatmaps present AUC ratio means, while statistical analyses, compared to *VEHct*, is presented in Supplemental Table 4.

anxiety phenotypes in male offspring, as assessed by the EPM and LDB. However, female offspring showed no significant changes in DHA levels, anxiety phenotypes, or dopaminergic markers in the PFC, NAc, or vHIPP, suggesting a sex-specific resilience of the dopaminergic system to PTE [7].

The vHIPP-NAc pathway, which preferentially targets D2 receptor (D2R)-expressing medium spiny neurons, plays a critical role in anxiety modulation [104]. Inhibition of the vHIPP produces anxiolytic effects, while local THC administration induces acute anxiogenesis in rodent models [105, 106]. Our findings suggest significant dysregulation of the vHIPP-NAc circuit following PTE, with distinct sex-specific alterations. For instance, both the vHIPP and NAc exhibited changes in GAD67 expression, a key marker of GABA synthesis. THC-treated (*THCct*) males showed reduced NAc GAD67 expression in these regions, while females displayed significant elevations in NAc GAD67 levels, potentially as a compensatory response to heightened excitatory input from the PFC and/or vHIPP. Regional variations in dopamine D2R expression further highlight the role of the vHIPP-NAc circuit, particularly in male offspring. In *THCct* males, significant increases in D2R expression were observed in the vHIPP, paired with pronounced downregulation in the NAc. These changes likely reflect disrupted signaling along the vHIPP-NAc axis, where D2R-expressing medium spiny neurons in the NAc shell integrate hippocampal inputs critical for regulating anxiety and reward-related behaviors. Interestingly, omega-3 supplementation normalized D2R expression in the vHIPP and PFC of *THCω3* males but failed to rescue the deficits in the NAc. This outcome contrasts with reports of adult omega-3 supplementation, which is associated with decreased D2R expression, whereas prenatal deficiency correlates with increased D2R expression during early development [107, 108]. These findings suggest that early-life supplementation may offset some developmental disruptions, though its benefits appear incomplete.

By PD120, DHA levels were significantly reduced in the prelimbic and infralimbic PFC, the NAc core and shell, and showed trends toward reductions in the vHIPP of *THCω3* males. This depletion of DHA, despite early-life omega-3 supplementation, may contribute to alterations in D2R expression, as DHA is critical for dopaminergic signaling and D2R function [108]. Furthermore, DHA influences ECS-mediated anxiety regulation [109]. In contrast, *THCct*-treated females did not exhibit reductions in DHA at PD120, nor did they exhibit anxiety or alterations in D2R expression in the PFC, NAc, or vHIPP. This sex-selective resilience suggests that omega-3 supplementation may target mechanisms specific to male dopaminergic vulnerability following PTE [6, 7, 60].

The pronounced sex differences observed in anxiety-related behavioral tests align with established sexual dimorphism in both baseline anxiety behaviors and endocannabinoid system function in rodents. Female rodents typically display lower baseline anxiety levels in tests such as the elevated plus maze and light/dark box, characterized by greater exploratory behavior and reduced thigmotaxis [110, 111]. This behavioral divergence is partly mediated by estrogenic modulation of the ECS, including elevated AEA levels and enhanced CB1R sensitivity in limbic regions [112, 113]. PTE appears to disrupt this endocannabinoid-mediated anxiety regulation more severely in males, potentially due to their lower baseline endocannabinoid tone and distinct developmental trajectories of ECS maturation [114, 115]. The differential response to omega-3 supplementation between sexes further supports this interpretation, as the restoration of appropriate membrane lipid composition may more effectively normalize endocannabinoid signaling in males, who exhibit more pronounced baseline disruptions following THC exposure [5, 6, 116, 117].

Our findings also highlight the potential of maternal omega-3 supplementation to mitigate PTE-induced FGR phenotypes, particularly cognitive deficits associated with FGR-related "catch-up" growth [34–36, 40]. Early nutritional interventions are known to improve growth and cognitive outcomes [118], suggesting that improving early childhood nutrition may counteract gestational nutritional imbalances [35]. Recent studies using similar omega-3 supplementation protocols in rodent models of autism support this hypothesis, demonstrating enhanced placental nutrient and fatty acid transfer, modulation of neuroinflammatory mediators, and improved fetal growth [53, 54, 119, 120]. This likely explains the increased birth weights observed in *VEHω3* and the elevated PD21 weights of male offspring in our study. However, sex differences in FGR resilience may influence the efficacy of omega-3 supplementation. Female fetuses demonstrate greater resilience to FGR stressors, exhibiting superior placental function and adaptive responses to inflammation compared to males [121]. Despite their resilience to FGR, female offspring may remain particularly susceptible to direct THC-induced effects [5], likely due to higher CB1R expression in the hippocampus [122]. Consistent with this, while female *THCω3* offspring performed comparably to *VEHct* in social motivation tasks, they retained deficits in hippocampus-dependent behaviors. These behavioral outcomes may also be influenced by maternal factors, as maternal socialization during early life shapes social motivation later in life. In males, preventing FGR alleviated cognitive deficits in *THCct* offspring, although protein and lipidomic deficits persisted. This suggests that omega-3 supplementation effectively targets certain developmental disruptions in males, but its benefits remain incomplete; lipidomic and protein abnormalities persisted in *THCω3* males, even in the absence of overt neuropathology.
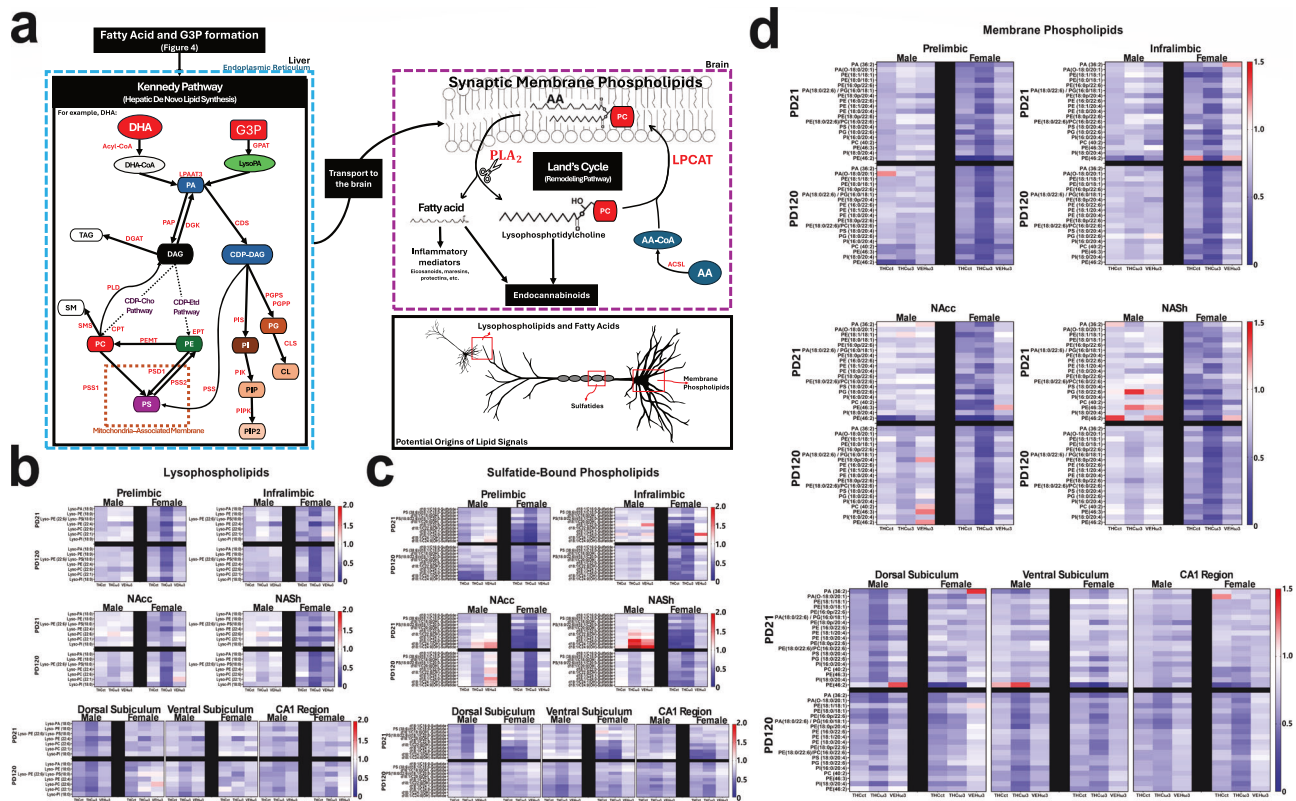
Fig. 5   MALDI-IMS analyses reveal significant age- and sex- specific lipidomic abnormalities in male and female offspring following PTE and perinatal omega-3 supplementation. a The Kennedy Pathway and Land's cycle are central in lipid formation. Once fatty acids and glyercol-3-phosphate (G3P) are formed they can be converted into a phosphatidic acid (PA). PA is the simplest phospholipid. PA is then converted into diacylglycerol; (DAG). DAG, central in the Kennedy Pathway, yields phosphatidylcholine (PC) through CDP-choline pathway, or phosphatidylethanolamine (PE) via the CDP-Eto pathway. PE and PC can be metabolized to form phosphatidylserine (PS), which are major synaptic phospholipids typically enriched with DHA. PA can also produce phosphatidylinositol (PI; a key fatty acid utilized for endocannabinoid production and other signaling pathways), phosphatidylglycerol (PG), and cardiolipin (CL; critical to mitochondrial membrane structure and function) via the CDP-DAG pathway. Land's Cycle remodels brain phospholipids utilizing phospholipases, like PLA$_2$, to modify fatty acyl chains; a process essential for membrane flexibility and endocannabinoid signaling. Lastly, a graphical representation of a neuron illustrates the potential origins of the assessed fatty acids and phospholipids: lysophospholipids are membrane phospholipids released by action of phospholipases and have a fatty acyl chain cleaved; membrane phospholipids, intact phospholipids with both fatty acid tails found within or being incorporated into membranes; and sulfatide-containing phospholipids (i.e., sulfatides), primarily located in myelin or in transit into myelin. **b–d**, Mass-to-charge (m/z) values are based on previously described mass target [55]. Following standard fatty acid and lipid nomenclature for the fatty acids, the brackets for each phospholipid indicate the carbon chain that constitutes the associated fatty acid. The assessed lipids and their m/z are detailed in Supplemental Table 2; statistical analyses are presented in Supplemental Table 5. Male and female offspring (PD21, n = 8/treatment/sex; PD120, n = 8/treatment/sex) exposed to THC (*THCct*) exhibited significant age- and sex- specific lipidomic alterations. The omega-3 intervention (*VEHω3* and *THCω3*) also led to significant changes. Female *THCω3* exhibited substantial alterations at PD21 and PD120 in the PFC, NAc, and hippocampal regions. On the other hand, male *THCω3* exhibited disturbances in select phospholipids, particularly those containing AA (heatmaps present means and statistical significance compared to *VEHct*, see Supplemental Table 5 for statistical analyses). Area under the curve (AUC) ratio comparisons were conducted with one-sample t tests, with a hypothetical mean of 1.0; where 1.0 suggests no difference between the *VEHct* compared to each respective treatment's offspring; ****p < 0.0001, ***p < 0.001, **p < 0.01, *p < 0.05, two-tailed.

The mitigation of the male *THCω3* phenotype may stem from preventing FGR, thus avoiding postnatal catch-up growth or reducing gestational neuroinflammation, as seen in other studies using this diet, particularly in the placenta [53, 54, 123]. Maternal cannabinoid use also reduces oxygen supply, alongside nutrients and fatty acids, potentially causing hypoxic damage and elevated inflammation [11, 124–126]. The upregulation of DHA and EPA, two fatty acids known to minimize hypoxic damage and enhance anti-inflammatory processes [127], might explain the long-term effects observed [53, 54, 119, 120]. DHA and EPA lower inflammation, inhibit neuronal cell death [46, 128, 129], with EPA playing a vital role in placental protective mechanisms against oxidative stress [130–133]. Neuroprotectin D1, a DHA derived mediator, is critical in preventing oxidative stress damage to the brain [130, 134]. In the NAc, PPARα was reduced in only the *THCω3* females, while males exhibited elevations in PPARα and PPARγ.

This may suggest that the female ability to counteract inflammation may be impaired, while males upregulation may be a compensatory response to address inflammation. Sex differences in PPARα expression and activation by agonists have been previously reported to be more effective in males than females [135–137]. Prenatal hypoxia and adverse neuroinflammatory responses may better explain the sex-specificity of the observed outcomes and responses to *THCω3*, although more direct examination of neuroinflammation is required.

Notably, while cognitive and memory deficits are recognized outcomes of FGR, no direct association has been established between anxiety and placental anomaly-related FGR [38, 88, 138–143]. However, prenatal hypoxia, a co-incident occurrence with THC-induced FGR (i.e., reduced maternal oxygen transfer) [11, 31], is linked with changes in the hypothalamic-pituitary-adrenal (HPA) axis and heightened anxiety [144, 145] as
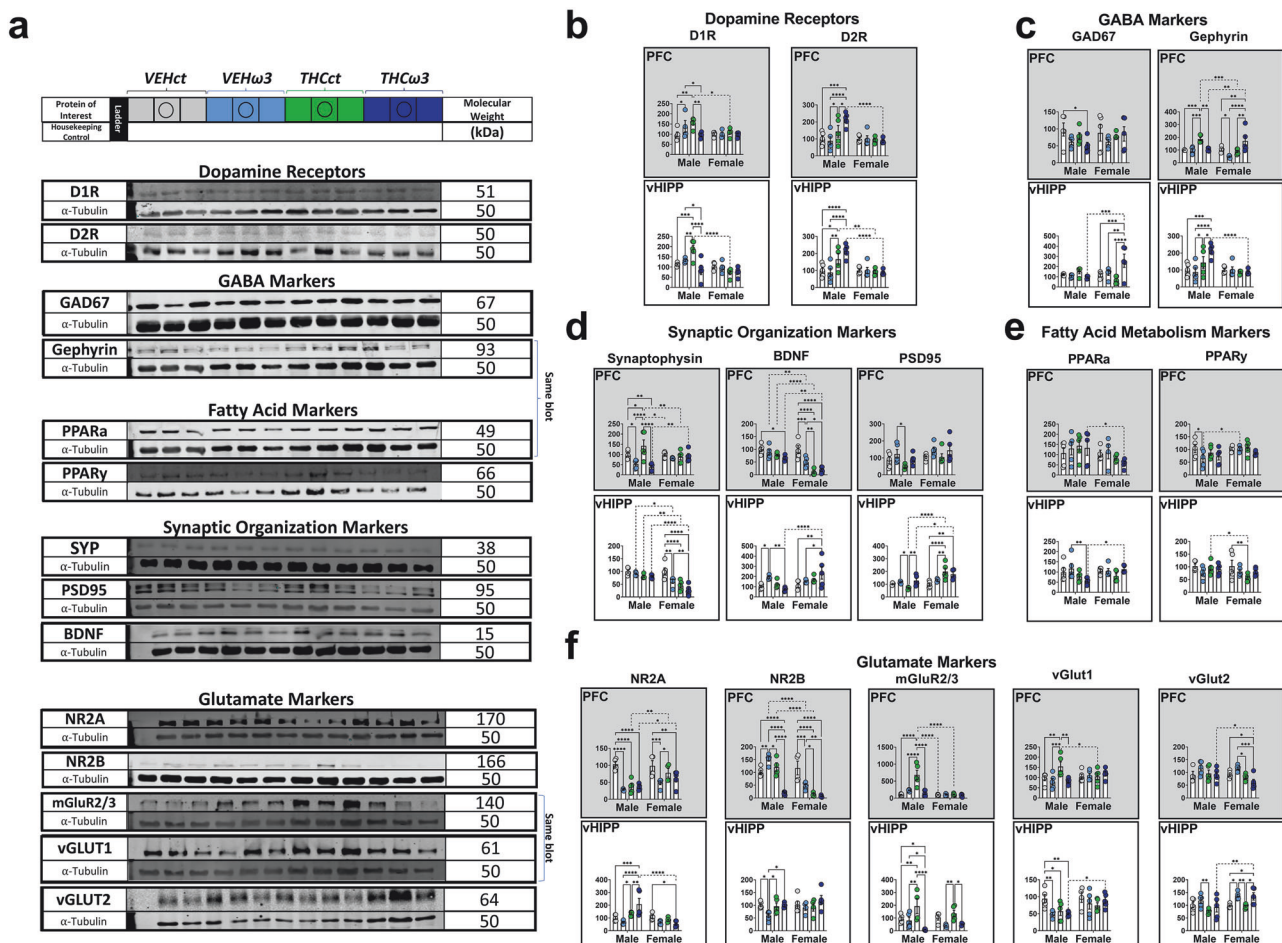
Fig. 6 Protein Analyses reveal sex- specific alterations to markers of neural development, synaptic function, and neurotransmission. Western Blot protein analyses revealed sex- and region-specific changes in critical neural markers within the prefrontal cortex (PFC) and ventral hippocampus (vHIPP) at postnatal day 120 (PD120). Supplemental Fig. 2 presents nucleus accumbens (NAc) protein analyses. Detailed statistical analyses for the PFC, NAc, and vHIPP are provided in Supplemental Table 6. The proteins examined are key components of the dopamine, glutamate, and GABA neurotransmitter systems, synaptic organization, and lipid metabolism. **a** Protein bands of interest are shown for each treatment group, normalized to their respective α-tubulin controls and then to the vehicle control diet group (*VEHct*) for each sex. All blots displayed are PFC. **b Dopamine Receptors (D1R and D2R):** Dopamine D1R and D2R modulate excitatory and inhibitory responses, respectively, and have distinct roles in the PFC, NAc, and vHIPP affecting anxiety, reward processing, and cognition. **c Glutamate Markers:** NMDA receptor subunits GluN2A and GluN2B are critical for excitatory signaling, especially in the corticostriatal network. Metabotropic glutamate receptor 2/3 (mGluR2/3) inhibits glutamate release, helping maintain excitatory balance. Vesicular glutamate transporters vGlut1 and vGlut2 facilitate glutamate release, supporting excitatory neurotransmission. **d Synaptic Organization Proteins:** Synaptophysin (SYP), postsynaptic density protein 95 (PSD95), and brain-derived neurotrophic factor (BDNF) are essential for excitatory synapse integrity. BDNF promotes synaptic plasticity and neurogenesis, while PSD95 serves as a scaffolding protein at excitatory synapses. **e GABAergic Markers:** Glutamate decarboxylase 67 (GAD67) synthesizes GABA from glutamate, vital for maintaining baseline inhibitory neurotransmission. Gephyrin supports GABA$_A$ receptor clustering at inhibitory synapses. **f Nuclear Receptors (PPARα and PPARγ):** Peroxisome proliferator-activated receptors are involved in fatty acid metabolism(particularly DHA), contextual fear extinction, cognition, and inflammation. Outliers were removed if failing ROUT Outlier Test. Comparisons were made with Three-Way ANOVA with Sex, THC treatment, and the omega-3 diet as factors, with post hoc Fisher's LSD, ****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$; individual data points with mean and $\pm$ SEM are presented.

well as impairments in spatial memory and exploration [146]. A series of studies by Howell and Pillai found that prenatal hypoxia particularly impacts blood flow to the hippocampus in mice [147, 148]. This diminished blood flow adversely affects reelin expression, a extracellular matrix protein essential for neuronal migration and cortical lamination [147–149]. Reelin deficiency itself leads to cortical dysgenesis and heightened anxiety phenotypes [150]. In our MALDI IMS data, adrenic acid—a metabolite of arachidonic acid critical for endothelial integrity and associated with reelin—was markedly reduced in both sexes at PD21, suggesting compromised cerebrovascular function that may exacerbate hypoxia's impact on hippocampal development and later-life anxiety [147–149, 151]. PTE likely perturbs HPA axis

maturation both directly and indirectly. THC itself may alter HPA functionality of the developing fetus, but HPA levels in the placenta, that is, maternal responses to stressors (i.e., from the injection [152] or from THC itself [153]) can alter levels of corticotrophin-releasing hormones (CRH) [154, 155]. Elevated CRH can thin the developing cortex by dysregulating the release of hormones necessary for proper neural development from the hypothalamus [156]. Additionally, as maternal stressors can elevate corticosterone levels, this may in turn lead to elevated oxidative stress damage in the placenta [157], which has been associated with sex-specific neurobiological deficits [158, 159]. These include damage to hippocampal dendrite length, reductions in PV+ interneurons, and decreased GABAAR expression in

the hippocampus and amygdala of male offspring; demonstrated in 2D cell culture studies [159, 160]. Prenatal stress is also thought to particularly impact GABAergic control within the corticolimbic circuit in a sex-dependent manner [161]. The prevailing phenotype observed from PTE offspring included disrupted glutamatergic activity, altered GABAergic markers, and modulation of dopaminergic signaling, with the hippocampus emerging as the most vulnerable brain region, in both males and females [5–7, 14, 60, 71, 162]. This hippocampal vulnerability aligns with findings from prenatal hypoxia models [146, 163–165], suggesting that mitigating hypoxic damage could potentially reverse or modulate the observed phenotype [159]. In the developing brain, reactive oxygen species (ROS) can disrupt critical neurodevelopmental processes such as synaptogenesis, neuronal differentiation, and myelination [166, 167]. The brain is particularly vulnerable to oxidative stress due to its high lipid content and relatively low antioxidant capacity [168]. Excessive ROS leads to lipid peroxidation, protein oxidation, and DNA damage, contributing to neuronal death and neural network disruption, and alterations in lipid levels beyond those caused by insufficient placental transfer [42, 169, 170]. Persistent disturbances to fatty acid levels, then, may be a consequence of not just glucose/lipid metabolic disturbances to the offspring liver [11, 26, 42, 153], but potentially stem from disturbed inflammatory balance within the fetal brain that persists into adulthood.

Interestingly, despite overall phenotype improvements, omega-3-supplemented groups continued to exhibit low fatty acid and phospholipid levels, at least at the time points measured. One possible explanation for the persistence of DHA, and notably AA and adrenic acid, reductions may be due to the timing of withdrawal of the dietary intervention, which allowed the subsequent emergence of underlying deficits in fatty acid levels previously masked by the omega-3 enriched diet (assessed at PD21). Temporary supplementation with DHA does not necessarily lead to permanent changes in the lifelong accumulation of these fatty acids in the brain. Additionally, considering the broader physiological impacts of THC, the brain's vulnerability to both the indirect effects (i.e., FGR) and the direct effects of THC should be noted. For example, THC may cause lasting damage to fatty acid metabolic machinery outside the brain, such as the liver; the site where these large-chain polyunsaturated fatty acids, like DHA and AA, are produced and then trafficked to brain [11, 26–28]. In males, we found that phospholipids containing omega-3 PUFAs, especially DHA, were largely protected from PTE. Conversely, females are capable of upregulating endogenous fatty acid levels [83, 171], which may mean that levels observed at PD120 are less contingent on earlier life levels. Nevertheless, the neurophysiological relevance of fatty acid levels diminishes in favor of phospholipid levels with age, as they continue to be incorporated into cell and synaptic membranes, myelin sheath components, or become lysophospholipids and influence numerous signaling cascades.

Disruptions in lysophospholipid pools impose a potential "double hit" on the ECS by simultaneously depleting both primary endocannabinoid precursors and lipid mediators of neuroinflammation. Lyso-PE species serve as direct substrates for N-acyltransferase and subsequent N-acyl phosphatidylethanolamine-specific phospholipase D (NAPE-PLD) to generate AEA, while lyso-PC species feed into diacylglycerol lipase (DAGL) pathways for 2-AG synthesis [172]. In CB1R$^{-/-}$ mice, loss of CB1R signaling produces heightened anxiety-like behavior and altered stress responsivity—phenotypes mirrored in our PTE cohorts with depleted lyso-PE and lyso-PC levels [173]. Moreover, AA, DHA, and EPA are precursors not only for endocannabinoids but also for cyclooxygenase- and lipoxygenase-derived eicosanoids and specialized pro-resolving mediators which temper neuroinflammation [57, 174]. The marked reductions in these lysophospholipid and PUFA pools—observed in male *THCct*, male *THCω3*, and female

THCω3 offspring across the PFC, NAc, and vHIPP—suggest widespread PTE-induced ECS hypofunction and impaired resolution of inflammatory signaling. Such a state may perpetuate maladaptive neuroimmune crosstalk, locking neural circuits into heightened excitability and anxiety phenotypes.

Complementing these deficits, decreases in sulfatide-bound AA threaten myelin integrity and action potential fidelity. In cerebroside sulfotransferase (CST)$^{-/-}$ mice, absence of sulfogalactolipids leads to myelin sheath decompaction, slowed nerve conduction, and elevated anxiety-like behaviors [175, 176]; phenotypes that parallel our electrophysiological findings of disrupted PFC-vHIPP oscillations. Sulfatide-AA constitutes one of the most abundant PUFA species in central myelin [177], and when these lipids are deficient, oligodendrocytes may incorporate alternative phospholipids with differing biophysical properties, altering membrane capacitance and ion channel gating [92, 177]. Concurrent reductions in DHA further compound membrane instability: DHA-rich lipid rafts are essential for clustering of NMDA and GABA$_A$ receptors, and their depletion compromises receptor trafficking, synaptic vesicle fusion, and ion channel conductance [116, 133, 178, 179]. Likewise, diminished PE and PC levels weaken membrane curvature and fusion competence, providing a mechanistic substrate for the altered bursting and aberrant synchrony we observed [180]. Importantly, these lipidomic signatures, and the associated down-regulation of synaptic scaffolding proteins such as synaptophysin, PSD95, and gephyrin, map onto the sex-specific behavioral and electrophysiological phenotypes, with males exhibiting more severe disruptions in lipid species pivotal for synaptic plasticity within the PFC and NAc [116, 181]. The persistence of sulfatide deficits at PD120 in both sexes, despite omega-3 supplementation, indicates that early-life PTE triggers enduring alterations to lipid metabolic pathways that may require more targeted interventions to restore myelin and membrane function.

Our study has several limitations. First, we did not explore the neuroinflammatory effects, particularly during gestation when the dietary effects of omega-3 supplementation would be most evident. This limits our understanding of the maternal and placental impacts of the diet. Although omega-3 and omega-6 fatty acids are also known to play critical roles in neuroinflammation, and other studies have shown positive neuroinflammatory effects in the placenta leading to cognitive benefits [53, 54, 119, 120, 123], we did not directly assess these effects. Additionally, while the observed alterations in various fatty acid signaling pathways serve as reliable biomarkers for neuroinflammation, it is important to directly assay specific markers for neuroinflammation during key periods of gestation, within the placenta, and at the later-life timepoints, in future analyses. This will help elucidate the mechanisms by which omega-3 supplementation mitigates the neuropsychiatric risks associated with prenatal THC exposure. Of course, the supplementation protocol was prolonged and during THC exposure; we examined this extended GD6-PD21 period utilizing previously established protocols [53, 54, 119, 120, 123], and as such, is not directly translatable. Further research should focus on the gestational and maternal effects to provide a more comprehensive understanding of the intervention's benefits. Our lab is presently pursuing these interventions.

While we demonstrated that omega-3 fatty acids can counteract many pathological impacts of prenatal THC exposure on behavior, molecular markers, and neuronal activity within the PFC-NAc-vHIPP network, the exact pathways remain unclear. Omega-3 supplementation potentially exerts its protective effects on the PFC-NAc-vHIPP network through several converging mechanisms that extend beyond simple replacement of deficient DHA. First, DHA/EPA remodel synaptic PE/PS composition, alter lipid-raft architecture, and thereby regulate GPCR (including CB1R) clustering and G-protein signaling [182–186]. By competing with AA during phospholipid remodeling, omega-3s lower the immediate substrate pool used by DAGL and NAPE-PLD, thereby constraining

the excess 2-AG and AEA formation that often follows PTE-induced phospholipase activation [117, 174, 187]. Second, enzymatic conversion of DHA and EPA also yields resolvins and protectins that dampen microglial NFκB signaling, downregulate IL-1β transcription, and bias macrophage polarization toward an M2 reparative phenotype; mechanisms shown to attenuate LPS-induced neuroinflammation in CB1R knockout and wild-type mice alike [47, 153]. Third, sex differences in both lipid metabolism and steroid signaling shape the therapeutic window: estrogen upregulates FADS1/2, MFSD2A, and several pro-resolving mediators, accelerating endogenous DHA synthesis and incorporation into dendritic spines [188–191]. Consequently, females enter gestation with higher baseline membrane DHA and greater CB1R reserve capacity, coupled with sexual dimorphism in ECS receptor density, signaling efficiency, and developmental trajectories, potentially render the marginal gains from supplementation smaller than those seen in males [113, 115, 190, 192–194]. Yet estrogen also increases synaptic ERβ signaling, which interacts with omega-3 derived pro-resolving mediators to stabilize PSD95 scaffolds and GAD67 expression, despite the observed lingering PFC lipid alterations [195–198]. Taken together, these complementary pathways highlight why omega-3's partially, but not completely, normalized many PTE-related endophenotypes. They also reinforce that cannabis cessation remains the most reliable strategy for preventing long-term neurobehavioral morbidity, particularly given the dose, frequency, and potency-dependent escalation of PTE risk [29, 32]. Future work should test brief but strategically timed supplementation windows, for example, during lactation or early adolescence, to determine whether these targeted interventions can match the efficacy of prolonged perinatal dosing while improving translational feasibility.

Perinatal omega-3 supplementation may exert its protective effects in part by attenuating placental inflammation, a known driver of both FGR and downstream neurodevelopmental deficits [199]. These conditions are characterized by elevated levels of pro-inflammatory cytokines such as IL-1β, IL-8, IL-6, and TNF-α [200–203]. THC disrupts trophoblast integrity via TLR4–MyD88 signaling (TLR4/MyD88/PI3K interactions) [204], triggering NFκB-mediated transcription of IL-1β, IL-6, and TNF-α in placental macrophages and endothelial cells. DHA and EPA counteract this cascade through several complementary pathways. First, DHA is converted by ALOX15 into resolvin D1 and protectin D1, which bind GPR32 and ALX/FPR2 on trophoblasts to inhibit IKKβ phosphorylation (NFκB pathway) and reduce COX-2 expression, thereby lowering PGE$_2$ synthesis in LPS-challenged murine placental eells [205, 206]. In parallel, EPA competes with AA for COX-2 and 5-LOX enzymes, again diminishing production of the pro-inflammatory PGE$_2$, and leukotriene B$_4$, in both primary human macrophages and rodent models [47, 207]. Critically, these mediators also enhance placental antioxidant defenses by upregulating Nrf2 target genes (e.g., HO-1, NQO1), which may neutralize ROS generated by THC smoke inhalation (e.g. cannabis smoke) or potentially through downstream signaling [208, 209]. In vitro, DHA supplementation of RAW264.7 macrophages reduces lipopolysaccharide-induced IL-1β and TNF-α secretion via NFκB inhibition, while EPA decreases IL-6 production through STAT3 modulation [47, 207, 210]. These anti-inflammatory shifts potentially restore placental expression of FATP2 and MFSD2A (lipid transporters critical for DHA and AA transfer), thus preserving fetal PUFA supply and preventing the placental labyrinth zone's vascular rarefaction observed in THC-exposed rats [5, 11, 42, 125, 153]. The timing and duration of supplementation are pivotal: continuous DHA/EPA provision from GD7 through PD21 overlaps with trophoblast invasion, placental angiogenesis, and peak CRH expression, ensuring DHA/EPA-derived mediator levels remain sufficient to offset any potential THC-induced inflammatory response. Comparable perinatal

omega-3 regimens have rescued placental cytokine profiles and fetal growth in models of maternal high-fat diet, gestational famine, alcohol exposure, and poly(I:C)–induced immune activation, each demonstrating positive outcomes, and, when assessed, reduced placental inflammatory activity [54, 123, 211–214]. Moreover, a recent study examining the cardiometabolic outcomes following PTE (3mg/kg i.p. in Wistar rats) with this same perinatal omega-3 supplementation model exhibited a promising prevention of both FGR and cardiac outcomes in the omega-3 supplemented groups, reinforcing placental immunomodulation as a potential mechanism [44]. Translating these insights to humans will require defining optimal dosing, formulation, and intervention windows. Nonetheless, the capacity of omega-3–derived mediators to curb placental NFκB activation and restore fatty acid transport emphasizes their potential as an adjunctive strategy—recognizing, however, that cessation of cannabis use remains the most direct and feasible approach to protect fetal development.

It is also important to consider that THC displays a canonical biphasic profile in rodents, with opposite behavioral and neurobiological consequences at the low and high ends of the dose–response curve [12, 215, 216]. Our choice of 3 mg/kg, situated in the midrange of published prenatal exposure paradigms [5, 6, 11, 26, 28, 153, 216], was intended to balance detectability of disruptions with preservation of fetal viability. Lower doses (≤ 1 mg/kg) preferentially engage CB1R on GABAergic interneurons, leaving glutamatergic pyramidal cells relatively unaffected [217, 218]; this selective engagement has been linked to enhanced neurogenesis and synaptic plasticity in the hippocampus [216, 219, 220]. In contrast, doses ≥ 4 mg/kg saturate CB1R on both interneurons and excitatory neurons, prolonging Gi/o coupled suppression of adenylate cyclase, dampening cAMP–PKA pathways, and downregulating CREB-dependent transcription [215–218]. As placental CB1R and FAAH are also dose-responsive, higher maternal doses intensify placental disruptions, alter ECS tone, and ultimately increase indices of fetal demise or growth restriction [32, 221, 222]. Dose (and method of adminstration) is likewise central when reconciling divergent findings across PTE models, and the potential for the omega-3 supplementation to limit the impact of PTE. The extent of placental labyrinth disruptions in other models are poorly characterized, but without such data, it will be difficult to assert that similar lipidomic disruptions occur within the fetal/adult brain. Defining a therapeutic window for any cotreatment therefore requires systematic dose–response work that integrates lipidomics, endocannabinoid quantification, and pathway specific readouts in both the placenta and fetal brain.

Our exploratory study highlights multiple divergent impacts of PTE on developing male and female brains, with differential treatment responses and long-term effects following dietary omega-3 intervention. However, several translational gaps must be resolved before recommending this strategy in obstetric practice. Foremost, the human-equivalent dose, optimal formulation (triglyceride versus ethyl ester), and developmental window for supplementation remain undefined, and extrapolation from rodents is complicated by species differences in placental fatty acid transporter expression and fetal accretion of DHA [133, 223]. Moreover, the striking sex-specificity of both the pathological and protective effects observed highlights the necessity of integrating fetal sex as a variable in future intervention studies. Equally important, the incomplete rescue achieved in our model reinforces that omega-3 s cannot replace cannabis cessation and should, at best, be integrated into a broader harm-reduction framework that includes counselling, potency reduction, and monitoring of maternal cannabinoid biomarkers. Carefully powered prospective cohorts and ultimately randomized controlled trials will be required to link neonatal omega-3 indices (for example, the erythrocyte DHA + EPA to total fatty acid ratio) with

long-term cognitive, emotional, and circuit-level outcomes in cannabis-exposed children. Only with such evidence can public-health recommendations move beyond abstinence messaging to include targeted nutritional modulation for those pregnancies at highest risk.

DATA AVAILABILITY
All data is available in the main text or the Supplementary Materials and Tables.

CODE AVAILABILITY
MATLAB code used to calculate Area's Under the Curve (AUC) for MALDI IMS is available at https://github.com/MHSarikahya/MALDI-IMS-Script.

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