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Gestational THC exposure perturbates hippocampal mitochondrial respiration in the memory-impaired adolescent progeny: Is there a role for mitochondrial CB1 receptor?

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ABSTRACT

Mitochondria are central to cellular energy metabolism, contributing to synaptic transmission and plasticity. The mitochondrial membranes present the cannabinoid type-1 receptor (mito-CB1R), which has been functionally linked to neuronal energy supply and cognitive processing. Prenatal exposure to Δ9-tetrahydrocannabinol (pTHC) has been associated with cognitive impairments associated with molecular cellular and functional abnormalities in several brain regions, including the hippocampus. This study aims at assessing whether, besides the memory impairment, pTHC exposure may result in mitochondrial molecular and functional alterations in the hippocampus of the offspring. Moreover, the assessment of CB1R expression is also carried out as a proxy of CB1 signalling in pTHC-exposed offspring. THC (2 mg/Kg), or vehicle, was administered to the dams from gestational day (GD) 5 to GD20, and the offspring were tested for declarative memory using the Novel Object Recognition test in the L-maze. We also assessed: mitochondrial respiration by high-resolution respirometry; mitochondrial respiratory complex-I subunit NDUFS1 protein levels, and mito-CB1R expression by ELISA. Our results revealed: significant memory impairment in pTHC-exposed offspring; attenuated mitochondrial respiration in the hippocampus alongside a marked reduction in complex-I-subunit NDUFS1; a significant increase in mito-CB1R expression. This is the first evidence of pTHC exposure-induced impairment in memory processing in the offspring that suggests a functional link between an attenuation in mitochondrial bioenergetics and abnormal CB1R signalling in the hippocampus.

1. Introduction

Mitochondria play a central role in cellular bioenergetics through oxidative metabolism and the synthesis of adenosine triphosphate (ATP) [1]. They efficiently meet the energy demand of the brain, which actually requires 20 % of the body's oxygen consumption [2,3]. In addition, brain mitochondria participate in Ca2 + homeostasis, the production of reactive oxygen species (ROS), and the synthesis and

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metabolism of neurotransmitters, thus regulating synaptic transmission and neuroplasticity [4–8].

During the 1970s, several studies highlighted the impact of exogenous cannabinoids on mitochondrial function [9–11], traditionally attributing these effects to cannabinoid type-1 receptors (CB1Rs) on the plasma membrane [12,13].

However, functional CB1Rs have recently been identified on the mitochondrial membrane (mito-CB1Rs) of neuronal and astroglial cells, where they modulate the bioenergetic processes of high brain functions [1,14–18]. Indeed, the acute activation of mito-CB1Rs, a seven-transmembrane G protein-coupled receptor, by exogenous cannabinoids in the hippocampus modulated memory processes by regulating mitochondrial energy metabolism. This effect was reported to be correlated to mito-Gαi/PKA-dependent phosphorylation of complex-I NADH:ubiquinone oxidoreductase subunit S2, NDUFS2, in hippocampal neurons [14] and the mitochondrial complex-I subunit NDUFS4 in astrocytes [16]. Besides those subunits of mitochondrial complex-I, the core subunit NDUFS1 represents a crucial complex-I enzymatic activity as it catalyzes the oxidation of NADH and carries out the first steps of the electron transfer chain [19]. Despite its functional relevance, little is known about its involvement in pathophysiological processes involving mitochondrial dysfunction.

While the functional role of mito-CB1Rs in the acute regulation of mitochondrial activity is increasingly explored [1,16–18,20], the consequences of a chronic modulation of mito-CB1R on mitochondrial bioenergetics are still unknown. Filling this gap is particularly relevant in the context of chronic exposure to cannabis, especially during critical neurodevelopmental periods, and could help in understanding the long-term functional and metabolic outcomes.

Indeed, THC, the primary psychoactive compound of cannabis, crosses the placenta and interacts with the endocannabinoid system (ECS) from the early stages of embryogenesis [21]. Notably, endocannabinoids, by activating CB1R, modulate cell survival, migration, neuronal and glial differentiation, synaptic functionality, and metabolic support [22].

Consistent with clinical observations [23–26], preclinical studies have demonstrated that gestational THC exposure results in dysregulation of emotional and cognitive processing, upregulation of CB1R gene expression, and altered expression of key markers of hippocampal synaptic plasticity in the adolescent offspring [27–30]. Indeed, adolescence is a period of rapid brain maturation in which threats to the developing brain are amplified by subsequent challenges, revealing functional impairments and risks for psychopathology [31,32].

Therefore, this study aims at assessing whether, besides exerting cognitive effects, gestational exposure to THC can involve disruption in mitochondrial homeostasis and alterations in mito-CB1R in the offspring's brain later in life. In detail, by a multifaceted approach, we investigated: (1) pTHC-induced perturbations on declarative memory in the Novel Object Recognition test in L-maze as a measure of hippocampal functioning; (2) mitochondrial respiration, (3) NDUFS1 protein level as indicator of respiratory chain complex-I activity, (4) cellular and mitochondrial CB1R protein levels as a proxy of THC exposure impact on the endocannabinoid signalling in the offspring later in life. The current results provide the first compelling evidence of significant alteration in CB1R signalling in pTHC exposure-induced detrimental consequences on hippocampal memory and bioenergetics.

2. Materials and methods

2.1. Animals and prenatal treatment with THC

Ten pregnant Sprague Dawley rats (Charles River, Italy), each weighing between 200 and 220 g, were individually housed in standard cages measuring 40 cm × 60 cm × 20 cm, with bedding. They had unrestricted access to water and food in a room maintained at a controlled temperature (22 ± 2 °C) and humidity (55 ± 5 %), with a 12-hour light/dark cycle. Starting from gestational day (GD) 5 and continuing until GD 20, the rats received daily subcutaneous injections of either a vehicle or THC at a dosage of 2 mg/kg. The THC concentration used in this study, as well as in previous research [30], corresponds to a level of mild THC consumption observed in humans.

After weaning, male rats were housed in pairs, with each experimental group consisting of one or two independent rats from each litter of dams treated with either vehicle or THC, resulting in a total of nine rats for each experimental group. All experiments were approved by the Italian Ministry of Health (819/2021-PR, Carla Cannizzaro) and conducted following animal protocols sanctioned by the Committee for the Protection and Use of Animals at the University of Palermo. The procedures adhered to the current Italian legislation on animal experimentation (D.L. 26/2014) and the European directives (2010/63/EU) governing the care and utilisation of laboratory animals. Rigorous efforts were made to minimise the number of animals used and to alleviate their suffering.

2.2. Drugs

The THC resin, obtained from the Forensic Laboratory of Biologically Active Substances at the University of Chemistry and Technology in Prague, Czech Republic, with a purity exceeding 97 % (verified by HPCL) [80], was dissolved in ethanol to achieve a final concentration of 20 %. Subsequently, the solution underwent sonication for 30 minutes. THC was then emulsified in 2 % Tween 80 and dissolved in sterile physiological saline. THC (2 mg/kg) or the vehicle was prepared in a 1 mL/kg volume and administered subcutaneously to rat dams.

2.3. Novel object recognition test

Male rat offspring, prenatally exposed to either vehicle (pCTRL) or THC (pTHC), were tested during adolescence, starting from postnatal day (PND) 28 onwards, during the light phase. The experiments were conducted in a sound-isolated room between 9:00 and 14:00. On the test days, rats were acclimatised to the testing room for 60 minutes before the experimental session. The rats' behaviour was recorded and scored by an automatic video-tracking system (AnyMaze, Stoelting Europe, Dublin, Ireland), controlled by an experimenter unaware of the experimental groups.

The male offspring were tested for declarative memory in the novel object recognition test in the L-maze (adapted from [14,33–37], Fig. 1 A). The task occurred in an L-shaped maze made of grey polyvinyl chloride made by two identical perpendicular arms (60 cm and 50 cm long, respectively, for external and internal L walls, 10 cm wide and 40 cm high walls) placed on a dark background. The task occurred in a room adjacent to the animal house with a light intensity fixed at 45–55 lux range. The task consisted of 3 sequential daily trials of 5 minutes each. During the habituation phase (day 1), rats were placed in the maze's centre and allowed to explore the arms without any objects freely. The training phase (day 2) consisted of putting the rats back in the corner of the maze in the presence of two identical objects positioned at the extremities of each arm and then leaving them to explore the labyrinth and the objects freely. The testing phase occurred 24 hours later (day 3): one of the familiar objects was replaced by a novel object different in shape, colour, and texture, and rats were left to explore both objects. The position of the novel object and the associations of novel and familiar were randomised. All objects were previously tested to avoid biased preference. Memory performance was assessed by the discrimination index (DI). The DI was calculated as the difference between the time spent exploring the novel (TN) and the familiar object (TF) divided by the total exploration time (TN+TF): DI = [TN-TF]/[TN+TF]. Memory was also evaluated by directly comparing the exploration time of novel and familiar objects. Object exploration was defined as the orientation of the nose to the object at less than 2 cm.

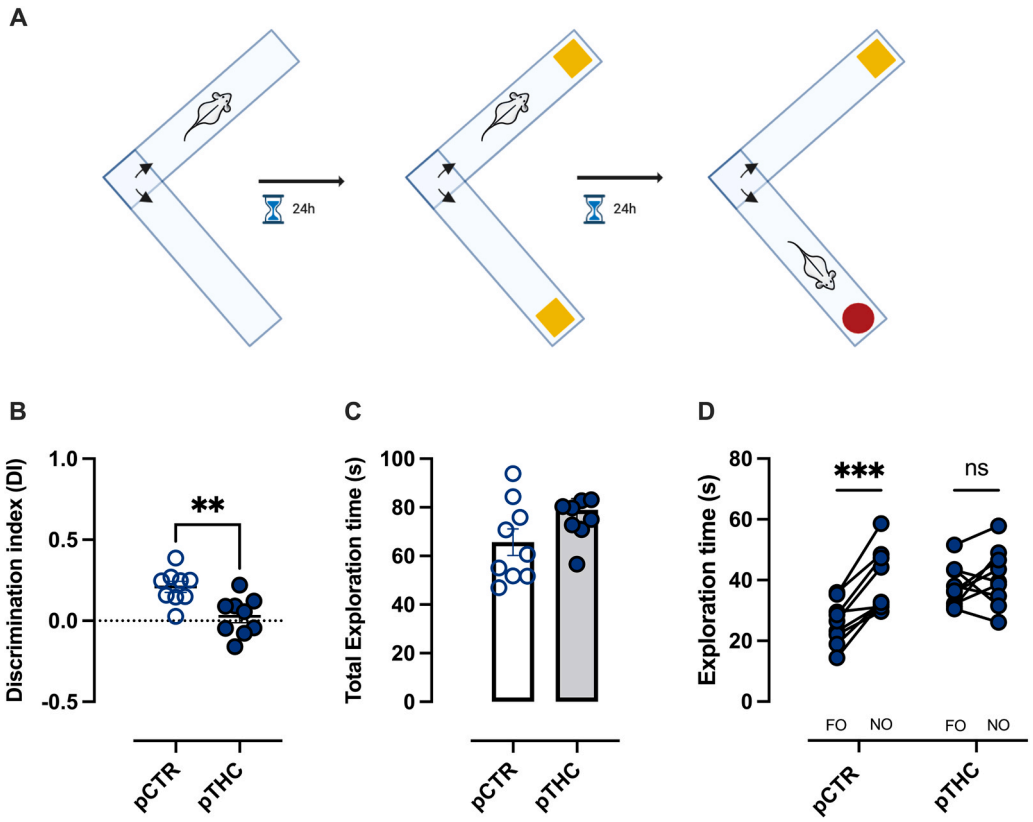

Fig. 1. Prenatal THC exposure affected declarative memory in the Novel Object Recognition task. The L-maze allows the assessment of memory formation in the Novel Object Recognition memory (A). A reduced discrimination index is shown in pTHC male offspring (B). No effect was observed in the total exploration time (C). Increased novel object exploration was observed in pCTR offspring compared to the pTHC group, with no exploration difference (D). Each bar and dot represent the mean of n = 9 control- and n = 9 pTHC rats; error plots indicate SEM. ***p < 0.001, **p < 0.01, *p < 0.05; pCTRL: offspring prenatally exposed to vehicle; pTHC: offspring prenatally exposed to THC; FO: Familiar object; NO: Novel object; ns: non significant.

2.4. Tissue collection

24 hours following the end of the behavioural procedures, hippocampus samples were collected as previously described, with minor modifications [38,39]. Briefly, rats were anaesthetised and sacrificed in the early afternoon. Brains were rapidly collected on ice, and the whole hippocampus was dissected according to the brain atlas [40], flash-frozen in liquid nitrogen, and stored at −80°C until used for further processing.

2.5. Cellular CB1R protein analysis

Frozen hippocampal tissue was suspended in fixed volumes of lysis buffer containing Tissue protein extraction reagent (T-PER; Thermo Scientific, Rockford, IL, USA) and complete protease inhibitor cocktail (Sigma, P8340) [41], homogenised with a Polytron for 10 s in ice and centrifuged at 10,000 x g for 20 min. The supernatant was collected for protein analysis. The total amount of proteins was measured using the Bradford assay (Quick Start™ Bradford Protein Assay Kit, BIO-RAD). Lysate samples were processed for quantification of target protein by sensitive and specific two-site enzyme-linked immunoassays. In detail, CB1R levels were assessed by the LS-F7052–1 LSBio rat CNR1/CB1 ELISA Kit (assay range 0.156–10 ng/mL, intra-assay CV 8.7 %). Standards and samples were assayed in duplicate according to the manufacturer's instructions. The optical density absorbance was read at a Multiskan FC Microplate Photometer (ThermoFisher). Results were expressed in pg/mg total protein amount.

2.6. Mitochondria fractionation

Frozen hippocampal tissues were homogenized in lysis buffer (20 mM pH 7.6 Tris HCl, 150 mM NaCl, 124 0.5 % NP-40, 2 mM EDTA) containing protease and phosphatase inhibitor cocktail. The Mitochondria Isolation Kit for Tissue (ab110168, Abcam, Cambridge, England) was used to separate the mitochondria fraction according to the manufacturer's protocol. Briefly, the minced tissue suspended in the isolation buffer was homogenised with a manual potter, sonicated for one minute, and then centrifuged at 1000 g for 10 min. The supernatant was recovered and then centrifuged at 12,000 g for 15 min. The pellet is recovered and resuspended in an isolation buffer, centrifuged at 12,000 g for 15 min. The pellet is again collected, resuspended, and re-centrifuged. Finally, the purified mitochondria pellet is recovered and resuspended in the isolation buffer [42].

2.7. Mitochondrial CB1R assay concentration

To dose mito-CB1R, the resulting mitochondrial pellet from mitochondria fractionation was solubilized in a mitochondrial extraction buffer mixture.

All assays were performed on mitochondria normalized for protein quantity (Biorad Assay Protein, Hercules, CA, USA).

The CB1 assay was determined in mitochondria and standards using the ELISA (enzyme-linked immunosorbent assay) kit, following the manufacturer's protocols (LSBio LS-234259). Briefly, CB1 standards (at a concentration of 1.25,2.5,5,10 ng/mL) or mitochondria were added, in triplicate, to wells pre-coated with CB1 antibody. After adding the antibody cocktail, the plate was read at 450 nm with a microplate reader. The concentration of mito-CB1R in the samples was calculated in

ng/mL by interpolating the absorbance values of the standard curve.

2.8. NADH-ubiquinone oxidoreductase subunit 1 (NDUFS1) concentration assay

The assay for NDUFS1 protein levels was also determined in mitochondria and standards using the ELISA kit (MyBioSource MBS 9922427). Briefly, the standards (at a concentration of 1.25, 2.5, 10 and 20 ng/mL) and mitochondria were added in triplicate to the wells precoated with the NDUFS1 antibody. After adding the antibody cocktail, the plate was read at 450 nm. The protein concentration in the mitochondria was calculated in ng/mL by interpolation of the absorbance values of the standard curve.

2.9. High-resolution respirometry

Respiration in isolated mitochondria was monitored with high-resolution respirometry (OROBOROS Oxygraph-2 k, Innsbruck, Austria) operating at 37 °C with a 2 mL chamber volume in order to investigate functional differences between controls and their treated counterparts. Mitochondria respiration experiments were carried out in two O2k chambers operated in parallel after calibration of the oxygen sensors at air saturation and an instrumental background correction. An air-saturated medium was calibrated immediately before the oxygen flux measurement was taken. The data acquisition and analysis were carried out using Datlab 4.2 software (OROBOROS Instruments). Respiration in mitochondrial preparations was investigated in mitochondrial respiration medium (MIR05) containing 0.5 mM EGTA, 3 mM MgCl2, 60 mM potassium lactobionate, 20 mM taurine, 10 mM KH2PO4, 20 mM HEPES, 110 mM sucrose and 1 g/L fatty-acid-free BSA, pH 7.1 [43]. Briefly, respiring mitochondria were first supplemented with respiratory substrates (5 mM glutamate, 5 mM malate), followed by the addition of ADP (100–150 mM) to the respiration medium, thus resulting in maximal, ADP-stimulated respiration (state 3) and ATP synthesis by the mitochondrial F0/F1-ATP synthase. The respiration that slows down and returns to a rate comparable to that before the addition of ADP, following ADP exhaustion, is classically referred to as ‘‘state 4’’ respiration. Then, the uncoupler FCCP (to a final concentration of 60 nM) was added into the oxygraph chambers to follow, for a further 5 minutes, the stimulated respiration, which reached values higher than those observed during the recording of state-3 respiration [44]. In this set of experiments, the ratio state3/state4 (RCR) was calculated to study the tight coupling between respiration and phosphorylation.

2.10. Statistical analysis

Data were analyzed using a two-tailed *t*-test (paired, unpaired). All data were tested for normality and equal variances. When data exhibited normality and equal variances, differences between groups were determined using an unpaired parametric Student’s *t*-test. Data that did not display normal distribution or equal variance were analysed using the nonparametric Mann–Whitney U-test. Grubb’s test was employed to identify outliers.

Statistical analysis was done using GraphPad Prism v10.2.0 (GraphPad Software, Inc., San Diego, CA, USA). All data are presented as mean ± SEM. The statistical significance was set at alpha = 0.05.

3. Results

3.1. Gestational THC exposure induced an impairment in the NOR memory task in the adolescent offspring

Offspring underwent the Novel Object Recognition test in the L-maze to evaluate the effect of gestational exposure to THC on declarative learning and memory (Fig. 1 A). A Student’s *t*-test performed on the discrimination index revealed a significant effect of pTHC in adolescent offspring ($t = 3.49$, df=16, $p = 0.003$) compared to controls (Fig. 1 B), with no changes in total exploration time (Student’s *t*-test, $t = 1.84$, df=16, $p = 0.08$, Fig. 1 C). Additionally, when exploration time for familiar versus novel objects in the test session was evaluated, a two-tailed paired *t*-test showed a significant difference in the pCTR group ($t = 6.08$, df=8, $p < 0.001$), while no difference was shown in the pTHC group ($t = 0.880$, df=8, $p = 0.40$; Fig. 1 D).

3.2. Gestational THC exposure induced an increase of cellular- and mito-CB1R expression in the hippocampus of the adolescent offspring

When hippocampal levels of CB1R were assessed, the Student’s *t*-test revealed that rats prenatally exposed to THC displayed increased CB1R levels in the hippocampus with respect to pCTRL offspring ($t = 12.6$, df=15, $p < 0.001$) (Fig. 2 A). As to CB1R levels, Grubb’s test identified one outlier in the pCTRL group that was removed from the analysis.

No significant effect was observed in the levels of total proteins in the hippocampus (Student’s *t*-test, $t = 1.16$, df=16, $p = 0.26$).

After mitochondrial extraction from the hippocampal homogenates, the effects of pTHC on mito-CB1R protein levels were evaluated as a marker of the mitochondria-specific ECS signalling in the hippocampus. The Student’s *t*-test showed that pTHC-exposed offspring displayed increased levels of the receptors expressed in the hippocampal mitochondria compared to the pCTR counterpart ($t = 3.48$, df=16, $p = 0.003$) (Fig. 2 B).

3.3. Gestational THC exposure decreased mitochondrial respiration and altered the complex-I subunit NDUFS1 in the adolescent offspring

When assessing the pTHC exposure effects on mitochondrial respiration in homogenized hippocampi, our statistical analysis revealed a reduction in respiration in purified brain mitochondria in the adolescent offspring (Student’s *t*-test, $t = 9.93$, df=16, $p < 0.001$; Fig. 3 A). Additionally, our evaluation of the protein abundance of NDUFS1, a key subunit involved in NADH oxidation to NAD+ by complex-I of the respiratory chain, showed a reduction in its hippocampal mitochondrial protein levels in pTHC-exposed offspring compared to pCTR counterpart (Student’s *t*-test, $t = 12.3$, df=16, $p < 0.001$; Fig. 3 B).

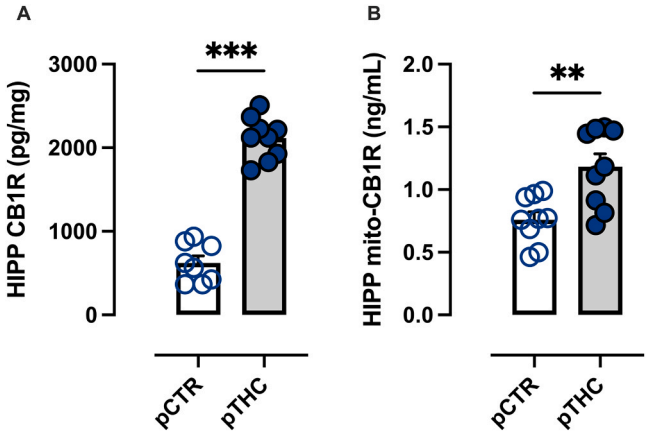

Fig. 2. Prenatal THC exposure increased the protein expression of hippocampal CB1R and mito-CB1R in the adolescent offspring. The offspring exposed to pTHC showed increased CB1R (A) and mito-CB1R levels in the hippocampus than control counterparts (B). Bars represent the mean values of each group; error plots refer to SEM. ***$p < 0.001$, **$p < 0.01$; pCTRL: offspring prenatally exposed to vehicle; pTHC: offspring prenatally exposed to THC. HIPP: hippocampus; CB1R: cannabinoid receptor type 1; mito-CB1R: mitochondrial cannabinoid receptor type 1.

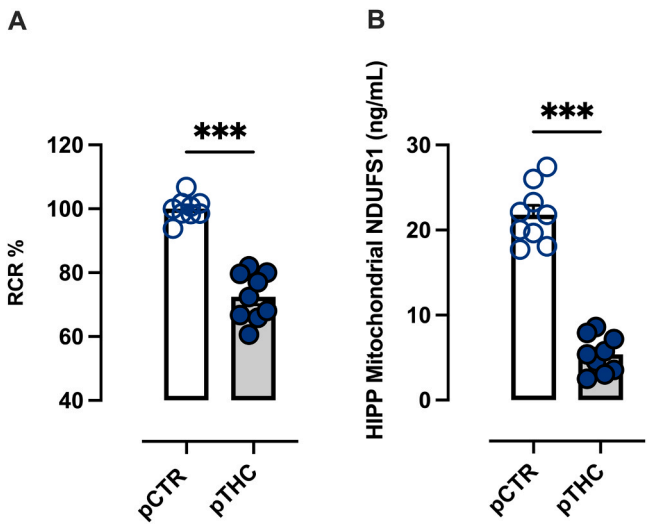

Fig. 3. Prenatal THC exposure reduced the mitochondrial respiration and protein abundance of complex-I subunit NDUFS1 in the hippocampus of the adolescent offspring. The offspring exposed to pTHC showed reduced mitochondrial respiration (A) and complex-I NDUFS1 subunit protein abundance (B) in the hippocampus than control counterparts. pCTR: offspring prenatally exposed to vehicle; pTHC: offspring prenatally exposed to THC; RCR: Respiratory Control Ratio; HIPP: hippocampus; NDUFS1: NADH-ubiquinone oxidoreductase subunit 1. Bars represent the mean values of each group; error plots refer to SEM; ***$p < 0.001$.

4. Discussion

This study provides the first evidence of the association between memory impairment, mitochondrial dysfunction, and altered mito-CB1R expression in the offspring following gestational exposure to THC. We discuss the hypothesis that the early modulation of the endocannabinoid system by pTHC exposure intersects with mitochondrial functioning, thus affecting cellular metabolism and hippocampal memory in adolescent offspring.

Our results reveal a specific memory deficit in pTHC-exposed male adolescent offspring in the NOR task. This deficit is shown by a significantly reduced discrimination index and, thus, by a reduced exploration time of the novel object compared to the familiar one, which indicates impaired declarative memory. Importantly, the absence of differences in total exploration time between groups suggests that the memory impairment is not confounded by variation in basic exploratory behaviour, motivation or motor skills [45].

Interestingly, previous studies in rats [30,46] and mice [47] offspring reported no significant deficits in declarative memory in the NOR task following gestational exposure to CB1R agonists. The significant memory impairment observed in our study was revealed by the alternative maze utilised, the L-maze, which differs from the most popular tool used for NOR investigation, i.e., the open field. However, despite its large diffusion, the open arena suffers a main drawback: the unprotected open space is generally perceived as hostile by the rats and can generate anxiety [48,49]. This increase in the emotional state may inhibit exploratory behaviour and lately affect rats' performance in the cognitive task [50,51]. Accordingly, when the animals are housed in high-stress conditions, they explore the surroundings less or are more prone to avoid open space instead of interacting with new objects [52]. In contrast, the L-maze helps reduce this unwanted increase in emotionality as it provides an isolated and partitioned environment; therefore, the measurement of memory performance and novelty preference is more accurate and liberated from the confounding emotional factor [20,35,37,53]. This important methodological refinement likely enhanced the sensitivity to detect differences in the memory task performed [54,55].

Efficient mitochondrial support in the hippocampus is critical for maintaining ion flow, neuronal excitability and synaptic plasticity, thus allowing normative cognitive processing [2,56]. In particular, mitochondrial calcium buffering and ATP production are essential for maintaining synaptic stability and adaptability [57–60]. Moreover, impairment in mitochondrial function can precipitate cellular oxidative stress and as shown by many reports, also compromise synaptic plasticity and memory formation [1,6,61,62].

Our findings reveal, for the first time, that gestational exposure to THC is associated with reduced mitochondrial respiration in the hippocampus, indicating a compromised energy supply during adolescence. Specifically, we observed a significant reduction in oxygen consumption and a downregulation of the NDUFS1 protein subunit of complex-I in the respiratory chain. Complex-I dysfunction, a common cause of mitochondrial diseases, could result from abnormalities in the structural subunits or assembly factors [63–65]. Mutations or alterations in NDUFS1 and other subunits (e.g., NDUFS4, NDUFS2) are known to disrupt complex-I assembly and function, leading to impaired NADH dehydrogenation, reduced electron transport into the respiratory chain and, consequently, ATP production [66–71]. Intriguingly, there is a close functional relationship between the endocannabinoid signalling through the CB1 receptor and energy production: the acute stimulation of mito-CB1Rs by exogenous cannabinoids in the hippocampus by decreasing cAMP transduction cascade is able to impair both mitochondrial energy metabolism and memory processes [14]. More than an association, this seems to be a causal relationship given that transgenic mice lacking mitochondrial-associated CB1R do not show impairment in bioenergetics and memory processing [14,72]. By reducing cAMP, CB1R signalling interferes with PKA/CREB pathways that noteworthy critically regulate NDUFS1 expression and phosphorylation [73,74]. Therefore, it is reasonable to hypothesize that the dysregulation of CB1R signalling, as a consequence of the gestational THC exposure, may alter CREB-mediated NDUFS1 expression (and phosphorylation), leading lately to mitochondrial dysfunction. Accordingly, our findings show that gestational exposure to THC significantly elevates mito-CB1R protein levels in the hippocampus of the exposed offspring, highlighting a critical dysregulation in its normative signalling. Such an imbalance is poised to disrupt energy-demanding processes integral to synaptic plasticity, learning, and memory functions deeply reliant on efficient mitochondrial bioenergetics (1,14). This aligns with evidence from Beiersdorf et al. [75], which underscores the pivotal role of mitochondrial complex-I in neuronal bioenergetics during critical development period, potentially leading to long-lasting impairments in brain function.

Overall, we suggest that as a consequence of pTHC exposure, an aberrant cellular- and mito-CB1R signalling (also [28]) can disrupt the regulation of cAMP-PKA-CREB pathway and its downstream targets, such as NDUFS1, thus leading to abnormal respiration. Such mitochondrial bioenergetic attenuation, especially in highly metabolically active regions like the hippocampus [76], can be functionally associated with a downscaled memory processing, as our experimental data in the NOR test have shown. Whether the abnormally upregulated mito-CB1R signalling can be directly ascribed to the exposure to THC during development rather than result from (mal)adaptive mechanisms during adolescence is hard to say. Likewise, whether the perturbed mito-CB1R signalling is causally linked to the attenuation in respiration and the downscaled memory processing is yet to be clarified: indeed, the reversal of mito-CB1R signalling by a chemogenetic strategy -currently under evaluation in our lab- will definitely answer the question posed in the title of this study.

To add complexity to the puzzle, mito-CB1R are expressed in astrocytes, where they regulate the balance between oxidative phosphorylation, glycolysis, and lactate production, ultimately governing the metabolic state of neurons [16,77]. Dysregulation of astrocytic mito-CB1R signalling could impair energy supply to neurons, thus, the interplay between neuronal and astrocytic bioenergetics is a factor that

should not be overlooked in contributing to the amnesic effects observed in pTHC-exposed offspring.

5. Conclusions

This research reveals for the first time that the exposure to THC during gestation significantly impairs declarative memory in the offspring and at the same time upregulates plasma membrane- and mito-CB1R signaling and perturb mitochondrial bioenergetics. Although currently, we can just speculate on a causal relationship between the molecular, the bioenergetic and the neurocognitive outcomes, our results prompt great caution in the use of THC during pregnancy and point to the ECS in the developmental programming of mitochondrial function. Notably, these findings have important implications expanding beyond phytocannabinoid research into the general role of mitochondrial regulation in neurodevelopmental disorders such as attention deficit hyperactivity disorder and autism spectrum disorders [78,79], in which aberrant endocannabinoidergic transmission has been reported. In a time when mitochondrial bioenergetics is turning from a theoretical concept into a real therapeutic target, this research provides a foundational innovative framework for: i) future studies into the pharmacological and pathophysiological roles of the endocannabinoid system and the bioenergetic mechanisms that control behaviour; ii) targeted approaches to mitigate the long-term neurocognitive consequences of early-life disruptions in mitochondrial signalling pathways.

CRediT authorship contribution statement

Palivec Petr: Resources, Methodology. **Engmann Olivia:** Methodology. **Bottoni Patrizia:** Investigation, Formal analysis. **Kuchar Martin:** Resources, Methodology. **Tringali Giuseppe:** Resources, Formal analysis. **Clementi Maria Elisabetta:** Investigation, Formal analysis. **D'Amico Cesare:** Formal analysis. **Vaccaro Francesca:** Formal analysis. **Cannizzaro Carla:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization. **Lavanco Gianluca:** Writing – review & editing, Writing – original draft, Resources, Investigation, Formal analysis. **Castelli Valentina:** Writing – review & editing, Investigation, Formal analysis. **Brancato Anna:** Writing – review & editing, Writing – original draft, Resources, Investigation, Formal analysis.

Declaration of Competing Interest

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Data availability

Data will be made available on request.

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