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O sistema endocanabinóide como alvo terapêutico para os desfechos metabólicos, neuroinflamatórios e comportamentais da obesidade materna

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SUMÁRIO

LISTA DE ABREVIATURAS, SÍMBOLOS E UNIDADES

AC: Adenilato Ciclase
AEA: Anandamida
AIM: Ativação Imune Materna
AMPc: Adenosina Monofosfato Cíclico
AP-1: Proteína de Ativação 1
ASC: Proteína Semelhante a Speck Associada à Apoptose Contendo um Domínio Recrutador de Caspase
BDNF: Fator Neurotrófico Derivado do Cérebro
CAF : Dieta de cafeteria
CB1R: Receptor Canabinóide do Tipo 1
CB2R: Receptor Canabinóide do Tipo 2
CBD: Canabidiol
CD: Cluster de Diferenciação
COVID-19: Doença por Coronavírus 2019
CREB: Proteína de Ligação ao Elemento de Resposta ao AMPc
DAG: Diacilglicerol
DAGL: Diacilglicerol Lipase
DAMPs: Padrões Moleculares Associados a Danos
DNA: Ácido Desoxirribonucleico
DOHaD: Development Origins of Health and Disease (Origem da Saúde e da Doença durante o Desenvolvimento)
DRD2: Receptor Dopaminérgico D2
ERK1/2: cinase Regulada por Sinal Extracelular 1/2
FAAH: Hidrolase de Amida de Ácido Graxo
FADD: Proteína Associada ao Fas com Domínio de Morte
GABA: Ácido Gama-Aminobutírico
GFAP: Proteína Ácida Fibrilar Glial
GPR: Receptor Acoplado à Proteína G
GPR55: Receptor Acoplado à Proteína G55
GSDMD: Gasdermina D
HFD: High Fat Diet (dieta rica em gorduras)
HOMA-IR: Índice de Resistência à Insulina
IBA-1: Antígeno 1 Associado a Macrófagos
IL-1β: Interleucina 1 Beta
IL-6: Interleucina 6
iNOS: Óxido Nítrico Sintase Induzível
IP3: Inositol Trifosfato
KO: *Knockout* (nocaute genético)
LPS: Lipopolissacarídeo
LRR: Repetições Ricas em Leucina
MAGL: Monoacilglicerol Lipase\
MC4R: Receptor Melanocortina 4
MEK1/2: Cinase de Sinalização Extracelular Regulada por Mitógeno 1/2
mtDNA: DNA Mitocondrial
MyD88: Resposta Primária de Diferenciação Mieloide 88
NAT: N-Aciltransferase
NAc: Núcleo Acúmbens
NADA: N-Arachidonil-Dopamina
NAPE-PLD: Fosfolipase D Hidrolisante de N-Acilfosfatidiletanolamina
NFkB: Fator Nuclear Kappa B
NLRP3: Proteína Contendo Domínio NOD-, LRR- e Pirina-3
NO: Óxido Nítrico
NOS: Óxido Nítrico Sintase
NPY/AgRP: Neuropeptídeo Y/Proteína Relacionada ao Agouti
Nrf2: Fator Nuclear Eritroide 2-Relacionado ao Fator 2
OMS: Organização Mundial da Saúde
P450: Citocromo P450

PAMPs: Padrões Moleculares Associados a Patógenos
PEA: Palmitoiletanolamida
PKA: Proteína cinase A
PKC: Proteína cinase C
PLC: Fosfolipase C
PLCβ: Fosfolipase C Beta
PPAR: Receptor Ativado por Proliferadores de Peroxissomos
PPARγ: Receptor Ativado por Proliferadores de Peroxissomos Gama
PRR: Receptores de Reconhecimento de Padrões
RIPK1: Proteína Quinase 1 que Interage com Receptor
ROS: Espécies Reativas de Oxigênio
SCD1: Estearoil-CoA desaturase 1
SEc: Sistema Endocanabinóide
SNC: Sistema Nervoso Central
STATs: Transdutores de Sinal e Ativadores de Transcrição
TDAH: Transtorno do Déficit de Atenção com Hiperatividade
TEA: Transtorno do Espectro Autista
THC: Tetrahidrocanabinol
TLR4: Receptor do Tipo Toll 4
TNF: Fator de Necrose Tumoral
TRPV: Receptor Vanilóide de Potencial Transitório
UCP1: Proteína Desacopladora 1
VIGITEL: Vigilância de Fatores de Risco e Proteção para Doenças Crônicas por Inquérito Telefônico
VPA: Ácido Valpróico
VTA: Tegmento Ventral

α: Alfa
β: Beta
γ: Gama
μ: Micro
mL: Mililitro
L: Litro
μL: Microlitro
nm: Nanômetro

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RESUMO

Evidências crescentes sugerem que a obesidade materna e os maus hábitos alimentares durante a gestação e a lactação interferem no desenvolvimento neuroimune da prole, podendo aumentar o risco de desregulação metabólica central e periférica, além de favorecer o desenvolvimento de transtornos psiquiátricos, como ansiedade, depressão, esquizofrenia e transtorno do espectro autista. Embora medidas preventivas direcionadas à mãe sejam bem documentadas, as abordagens práticas para lidar com os danos uma vez que já estão estabelecidos ainda são limitadas. Portanto, este trabalho é composto por duas vertentes: (i) experimentos *in vivo* em ratos Wistar, onde investigamos a interação entre a obesidade materna induzida por dieta de cafeteria e os desfechos metabólicos, neuroinflamatórios, neuroquímicos e comportamentais na prole juvenil e adulta, assim como o potencial do Canabidiol (CBD) para mitigar esses possíveis distúrbios; e (ii) experimentos *in vitro* em células microgliais estimuladas com lipopolissacarídeo (LPS), onde investigamos as vias de sinalização através das quais o CBD exerce seu efeito neuroprotetor nessas células. Para os experimentos *in vivo*, ratas Wistar fêmeas foram alimentadas com uma dieta de cafeteria por 12 semanas antes do acasalamento, e durante toda a gestação e lactação. A prole foi tratada com CBD (50 mg/kg) por 3 semanas a partir do 21º ou 70º dia de vida. Testes comportamentais foram realizados ao longo da última semana de tratamento a fim de avaliar comportamentos do tipo ansioso e exploratório, assim como a interação e a memória social da prole. Marcadores neuroinflamatórios e neuroquímicos foram avaliados no hipotálamo, córtex pré-frontal e hipocampo, e o perfil bioquímico foi avaliado no plasma. O tratamento com CBD foi capaz de atenuar diversas das alterações comportamentais induzidas pela obesidade materna, restaurando pelo menos parcialmente os desbalanços neuroinflamatórios e neuroquímicos, bem como as alterações no perfil metabólico da prole. Notavelmente, pudemos demonstrar uma importante influência da idade e do sexo da prole tanto nos efeitos da obesidade materna quanto na capacidade do CBD de reverter ou atenuar essas alterações. Já no estudo *in vitro*, demonstramos que o CBD atenuou a polarização microglial pró-inflamatória causada pelo LPS através dos receptores CB2 e PPARγ, reduzindo a ativação do inflamassoma NLRP3 e da oxido nítrico sintase induzível (iNOS). Esses achados destacam a influência persistente da obesidade materna na saúde dos descendentes, bem como o papel do sistema endocanabinoide na mediação desses resultados ao longo da vida, sugerindo a manipulação desse sistema através do tratamento com CBD como uma abordagem promissora para atenuar os desfechos neuroinflamatórios, metabólicos e neuroquímicos na prole.

ABSTRACT

Growing evidence suggests that maternal obesity and poor dietary habits during pregnancy and lactation interfere with the offspring's neuroimmune development, potentially increasing the risk of central and peripheral metabolic dysregulation and promoting the development of psychiatric disorders such as anxiety, depression, schizophrenia, and autism spectrum disorder. Although preventive measures targeted at the mother are well-documented, practical approaches to address the damages once they are already established remain limited. Therefore, this study is composed of two main components: (i) *in vivo* experiments in Wistar rats, where we investigated the interaction between maternal obesity induced by a cafeteria diet and the metabolic, neuroinflammatory, neurochemical, and behavioral outcomes in juvenile and adult offspring, as well as the potential of Cannabidiol (CBD) to mitigate these possible disturbances; and (ii) *in vitro* experiments in microglial cells stimulated with lipopolysaccharide (LPS), where we explored the signaling pathways through which CBD exerts its neuroprotective effect on these cells. For the *in vivo* experiments, female Wistar rats were fed a cafeteria diet for 12 weeks before mating and throughout gestation and lactation. The offspring were treated with CBD (50 mg/kg) for 3 weeks starting from either the 21st or the 70th day of life. Behavioral tests were conducted during the last week of treatment to assess anxiety-like and exploratory behaviors, as well as social interaction and memory in the offspring. Neuroinflammatory and neurochemical markers were evaluated in the hypothalamus, prefrontal cortex, and hippocampus, and the biochemical profile was assessed in the plasma. CBD treatment was able to attenuate several of the behavioral alterations induced by maternal obesity, restoring neuroinflammatory and neurochemical imbalances at least partially, as well as changes in the offspring's metabolic profile. Notably, we demonstrated a significant influence of the offspring's age and sex on both the effects of maternal obesity and the ability of CBD to reverse or attenuate these alterations. In the *in vitro* study, we showed that CBD attenuates LPS-induced pro-inflammatory microglial polarization via CB2 and PPARγ receptors, reducing the activation of the NLRP3 inflammasome and inducible nitric oxide synthase (iNOS). These findings underscore the persistent influence of maternal obesity on offspring health and highlight the role of the endocannabinoid system in mediating these outcomes throughout life, suggesting the manipulation of this system with CBD as a promising approach to mitigate neuroinflammatory, metabolic, and neurochemical outcomes in the offspring.

1. INTRODUÇÃO

1.1. Obesidade

A obesidade é uma doença crônica caracterizada pelo acúmulo excessivo de tecido adiposo que apresenta um papel etiológico nas principais causas de morte por fatores não-comunicáveis (CENSIN et al., 2019), uma vez que está fortemente associada a diversas comorbidades, como o diabetes mellitus tipo II (BROWN et al., 2017; TOPLAK et al., 2019), doenças cardiovasculares (POWELL-WILEY et al., 2021) e vários tipos de câncer (AVGERINOS et al., 2019; LEGA; LIPSCOMBE, 2020). A etiologia da obesidade não é de fácil identificação, uma vez que esta é caracterizada como uma doença multifatorial, ou seja, é resultado de uma complexa interação entre fatores comportamentais, culturais, genéticos, fisiológicos e psicológicos (MAYORAL et al., 2020). De acordo com a Organização Mundial da Saúde (OMS), no ano de 2022 2,5 bilhões de adultos (43%) apresentavam sobrepeso. Destes, 890 milhões vivem com obesidade (16%) (OKUNOGBE et al., 2022; WHO, 2024). No Brasil, dados do Ministério da Saúde apontam que, em 2023, 61,4% dos adultos apresentaram sobrepeso, dentre os quais 24,3% eram obesos (VIGITEL, 2023). Além dos danos inerentes à condição em si, as comorbidades associadas à obesidade acabam sendo muito onerosas para o sistema de saúde, devido aos altos custos de tratamento (SWINBURN et al., 2019).

A epidemiologia da obesidade tem sofrido uma grande influência da transição nutricional oriunda das alterações no padrão dietético da população, fazendo com que o número de indivíduos obesos cresça de forma pandêmica (BLÜHER, 2019; BAKER et al., 2020). Além de diversos fatores relacionados aos níveis crescentes de urbanização e mudanças de renda (REARDON et al., 2019), o consumo alimentar é muito afetado pela disponibilidade física dos alimentos, os preços destes e sua aceitabilidade social e cultural, influenciando diretamente no estado nutricional do indivíduo (SAGAR; GUPTA, 2018). Ainda, devido aos avanços tecnológicos e à liberalização do mercado, produtos alimentícios processados, hiper-palatáveis, densos em energia e reduzidos em vitaminas e minerais estão mais prontamente disponíveis e geralmente são mais baratos do que alimentos

mais nutritivos (FINKELSTEIN et al., 2014; ZORBAS; GRIGSBY-DUFFY; BACKHOLER, 2020). Nesse contexto, é importante destacar que a obesidade e o consumo desses alimentos ultraprocessados ocorrem frequentemente de forma concomitante, tornando difícil discernir os efeitos da obesidade e seu perfil metabólico em si dos relacionados aos aspectos nutricionais dos alimentos consumidos (CIRULLI et al., 2022). Ainda, concomitantemente ao aumento do consumo de alimentos ultraprocessados, a prática de exercícios físicos também foi reduzida durante o processo de urbanização, o que agravou consideravelmente a epidemia de obesidade (CONGDON, 2019; LIN; LI, 2021).

É importante ressaltar que a recente pandemia de COVID-19 contribuiu de forma significativa para o aumento do consumo de alimentos ultraprocessados e para a redução na atividade física. As restrições de movimento e medidas de distanciamento social, muitas vezes resultaram em mudanças nos padrões alimentares e comportamentais. O estresse emocional e a incerteza associados à pandemia também contribuíram para escolhas alimentares menos saudáveis, como alimentos ricos em açúcares, gorduras saturadas, conservantes e outros aditivos. Além disso, o fechamento de academias, parques e espaços públicos de recreação limitou as oportunidades de exercício físico, levando a um estilo de vida mais sedentário (APERRIBAI et al., 2020; MEIER; VAN HOEKEN; HOEK, 2022). Ainda, a experiência prolongada com o trabalho remoto durante a pandemia acelerou a adoção dessa prática por empresas e trabalhadores em todo o mundo. No entanto, essa nova realidade pode contribuir para uma redução significativa na atividade física devido à natureza sedentária do trabalho remoto (WILMS et al., 2022).

1.2. Obesidade gestacional

A obesidade está presente em taxas alarmantes em mulheres em idade reprodutiva, com estudos mostrando que mais da metade das mulheres em países desenvolvidos têm um índice de massa corporal acima do recomendado pela Organização Mundial da Saúde (HILLEMEIER et al., 2011; STRAUSS et al., 2021). A nutrição materna inadequada antes e durante a gravidez é uma preocupação crescente em todo o mundo, conhecida por ter várias implicações para o desenvolvimento fetal que levam a consequências de longo prazo para a saúde e

o bem-estar da mãe e da prole (NELSON et al., 2020). Desde os estudos pioneiros sobre os efeitos do ambiente perinatal sobre o desenvolvimento fetal, como a teoria da Origem do Desenvolvimento da Saúde e da Doença (Development Origins of Health and Disease - DOHaD), o contexto passou da falta de nutrientes devido à fome e à desnutrição para o excesso devido à pandemia global de obesidade (BARKER, 1986).

A obesidade é conhecida por perturbar o eixo hipotálamo-hipofisário-gonadal, sendo que mulheres com sobrepeso apresentam fases lúteas mais curtas e níveis mais baixos de hormônio folículo estimulante, hormônio luteinizante e progesterona, o que pode tornar o processo de concepção mais difícil (SNIDER; WOOD, 2019; BASILE et al., 2022). Além disso, causa problemas de curto e longo prazo para a gestante, como aumento do risco de diabetes gestacional (ZEHRAVI; MAQBOOL; ARA, 2021; SONG et al., 2023), pré-eclâmpsia (ABRAHAM; ROMANI, 2022; VENKATESH et al., 2022), aborto espontâneo (GONÇALVES et al., 2023) e parto prematuro (DINSMOOR; UGWU, 2023). Além disso, mulheres obesas estão em maior risco de gravidez afetada por anomalias congênitas (HELLE; PRIEST, 2020; WU et al., 2020). A obesidade materna, resistência à insulina e hiperinsulinemia juntas prejudicam o perfil global de genes relacionados à disfunção mitocondrial e ao metabolismo energético. No início da gravidez, a placenta humana é responsiva às altas concentrações de insulina materna encontradas em mulheres obesas, o que resulta em expressão gênica alterada em relação à produção mitocondrial de hormônios esteróides e metabolismo energético (LASSANCE et al., 2015; KELLY; POWELL; JANSSON, 2020). Por exemplo, a resposta secretora de insulina materna no início da gravidez está fortemente correlacionada com o peso placentário; como resultado, as placentas de mulheres obesas são significativamente mais pesadas no momento do parto (HIVERT et al., 2020; BEAUMONT et al., 2023) o que apresenta uma forte correlação com o peso ao nascer e adiposidade neonatal (CATALANO; SHANKAR, 2017; SATHASIVAM et al., 2023).

1.3. Efeitos da obesidade materna perinatal sobre o desenvolvimento da prole

As potenciais vias subjacentes aos efeitos da obesidade materna sobre a prole incluem inflamação sustentada aumentada, mudanças no transporte e armazenamento de lipídios, desregulação do metabolismo da glicose e modificações no microbioma, o que desencadeia aumento da translocação de lipopolissacarídeos (LPS) para a corrente sanguínea e dos níveis circulantes de citocinas pró-inflamatórias (SEGOVIA; VICKERS; REYNOLDS, 2017; REEMST et al., 2022). Esses mediadores inflamatórios são capazes de atravessar a barreira placentária e criar um ambiente prejudicial para os tecidos fetais em desenvolvimento (YOCKEY; IWASAKI, 2018; SOTIROS et al., 2021). Além disso, a resistência à insulina, os níveis de glicose e lipídios, com um elevado fornecimento de nutrientes de forma desequilibrada para o feto, contribuem para estabelecer mudanças persistentes no seu desenvolvimento (GOMES et al., 2022; KERELIUK; DOLINSKY, 2022). Essa inflamação prolongada promovida pela obesidade pode resultar na chamada "ativação imune materna" (AIM), que é caracterizada por uma série de mecanismos pelos quais o estresse sofrido pela mãe pode afetar criticamente a programação genética da prole (OSBORNE et al., 2019; MAO et al., 2022). Os modelos consolidados de AIM em animais envolvem a ativação do sistema imunológico materno durante o desenvolvimento fetal usando uma variedade de imunógenos, como LPS e ácido polinossínico:policitidílico (poli I:C) (SIMÕES et al., 2018; KENTNER et al., 2019; KOWASH et al., 2019), entretanto, outros fatores são passíveis de promover alterações no desenvolvimento da prole como, por exemplo, estresse, trauma, dieta e a microbiota materna (WANG et al., 2019).

Estudos experimentais e clínicos têm destacado a importância da nutrição materna durante as fases iniciais do desenvolvimento do feto e do neonato (SURESHCHANDRA et al., 2022; FERNANDO; CRAIG; DAWSON, 2023), evidenciando a dieta da gestante como fator determinante para as condições de vitalidade do recém-nascido, as alterações do ambiente intrauterino e os padrões de crescimento com desfechos metabólicos na vida adulta (CARROLL et al., 2023). O aumento da ingesta energética durante a gestação e lactação pode predispor o

feto ao desenvolvimento de doenças metabólicas crônicas, fato que pode ser parcialmente explicado pela hipótese da “programação fetal precoce”, caracterizada por um processo adaptativo a um ambiente intrauterino adverso que resulta na redefinição dos processos de desenvolvimento, a fim de garantir a sobrevivência fetal (ŞANLI; KABARAN, 2019). Tal adaptação provoca alterações morfológicas, fisiológicas, metabólicas e epigenéticas, que propiciam o desenvolvimento de diversas doenças na vida adulta da prole (KWON; KIM, 2017). Perifericamente, como resultado, a obesidade materna aumenta substancialmente o risco da prole desenvolver obesidade, resistência à insulina, diabetes tipo 2, pressão arterial elevada e perfil lipídico alterado ao longo da vida (GOMES et al., 2022; KERELIUK; DOLINSKY, 2022).

Em relação ao sistema nervoso central (SNC), a exposição a um ambiente obesogênico durante o desenvolvimento pré-natal tem demonstrado efeitos sobre a formação e maturação nervosa, promovendo mudanças cerebrais já evidentes no início do período intrauterino (JAIS; BRÜNING, 2017). Consequentemente, estudos epidemiológicos têm mostrado evidências de que o estado pró-inflamatório sustentado durante o desenvolvimento perinatal aumenta o risco de transtornos neurológicos na prole (OSBORNE et al., 2019; SALAS-VENEGAS et al., 2022), incluindo esquizofrenia (KHANDAKER et al., 2013), transtorno do espectro autista (TEA), depressão e ansiedade (ZHU et al., 2020) e transtorno bipolar tardio (CARON; JANE MICHAEL, 2021). Entre as regiões cerebrais, o hipotálamo, o córtex cerebral e o hipocampo têm sido mostrados como afetados de forma notável, com perturbações nas células progenitoras neurais, aumento do estresse oxidativo, diminuição das projeções dendríticas e, no geral, uma maturação sináptica prejudicada (GLENDINING et al., 2020; NORR et al., 2021). O neurodesenvolvimento alterado nessas estruturas pode originar distúrbios comportamentais que se manifestam ao longo da vida, como comportamentos do tipo ansioso, depressivo e hiperativo, além de prejuízos no comportamento social (LEE et al., 2023; MUSILLO et al., 2023; WU et al., 2023).

O hipotálamo é a principal área do cérebro que controla o equilíbrio energético, integrando informações do corpo e iniciando respostas comportamentais, humorais e neurais apropriadas. Sabe-se que a inflamação

hipotalâmica durante o neurodesenvolvimento é um mecanismo provável para a desregulação do controle homeostático do equilíbrio energético, o que pode levar a uma maior suscetibilidade a alterações metabólicas e obesidade (SEONG et al., 2019). A sinalização anormal de insulina durante o neurodesenvolvimento está relacionada à má formação de projeções neurais que afetam a função e plasticidade hipotalâmica, resultando em um equilíbrio energético alterado (POIZAT et al., 2019). Esses efeitos também podem ser atribuídos à ativação aumentada de astrócitos e microglia, com a subsequente secreção de citocinas inflamatórias, como fator de necrose tumoral-alfa (TNFα), interleucina (IL)-6 e IL-1β (TITO et al., 2021).

Em relação ao circuito mesocorticolímbico, o qual inclui, entre outras estruturas, o córtex pré-frontal e o hipocampo, diversas alterações neuronais foram identificadas como resultado de um ambiente intrauterino desfavorável, incluindo anormalidades proliferativas, morfológicas e sinápticas (BAINES et al., 2020), o que sugere uma rota mecanística para estudos recentes que mostraram que desenvolvimento e maturação sináptica aberrantes podem permear a fisiopatologia de transtornos do neurodesenvolvimento, como a esquizofrenia (GAO et al., 2022). Além disso, a obesidade materna foi especificamente relacionada com distúrbios na transmissão dopaminérgica, o que pode estar atrelado às alterações presentes no TEA, uma vez que defeitos na via de recompensa mesocorticolímbica resultam em déficits de sociabilidade e comportamentos repetitivos observados nos fenótipos clássicos (RIVERA et al., 2020; TRUJILLO VILLARREAL et al., 2021). De acordo com a hipótese da motivação social, o TEA é um transtorno de motivação no qual os indivíduos preferem explorar o ambiente não social em detrimento do social (KOHLS et al., 2018). Ainda, modelos animais confirmam que a função deficiente do circuito mesocorticolímbico favorece ao isolamento social. Por exemplo, em ratos, os níveis de interação social mostram expressão gênica diferencial de genes relacionados à recompensa, incluindo os sistemas opióides, serotoninérgico e dopaminérgico (ALUGUBELLY et al., 2019; DICARLO et al., 2019). Ademais, especificamente no hipocampo, estudos demonstram que a obesidade materna é capaz de interferir na regulação epigenética da expressão gênica no desenvolvimento do hipocampo (GLENDINING et al., 2020), causando

redução na expressão de Fator Neurotrófico Derivado do Cérebro (BDNF) (MUSILLO et al., 2023) e consequente perda de volume da estrutura, redução da mielinização e prejuízos na transmissão sináptica (SHIADEH et al., 2024).

Notavelmente, a via de recompensa mesocorticolímbica é modulada pela inflamação sistêmica e central que ocorre durante a obesidade materna. Portanto, é proposto que um balanço energético positivo durante a gestação gera uma resposta inflamatória que modula a neurotransmissão dopaminérgica em áreas cerebrais seletivas da via de recompensa, sugerindo um possível papel das ativação inflamatória na geração de transtornos do neurodesenvolvimento (TRUJILLO VILLARREAL et al., 2021). Várias citocinas pró-inflamatórias estão associadas aos distúrbios do desenvolvimento intrauterino, com IL-6 e TNFα (VELMESHEV et al., 2019; KWON et al., 2021; URBONAITE et al., 2022). A despeito de as citocinas desempenharem papéis críticos no desenvolvimento normal do feto, incluindo proliferação de neurônios e sinaptogênese, níveis elevados de citocinas pró-inflamatórias maternas alteram o curso normal desses processos e são associados a um neurodesenvolvimento alterado (BAI et al., 2023). Por exemplo, altas concentrações de IL-6 materna já foram associadas a mudanças maturacionais da substância branca frontolímbica da prole nos primeiros 12 meses pós-parto e ao desenvolvimento anormal de regiões encefálicas associadas ao processamento sensorial e controle de impulso aos 2 anos de idade (RASMUSSEN et al., 2019). Supõe-se que esse “desafio” imunológico pré-natal atue como um "*primer*" que, quando combinado com outros fatores ambientais, genéticos e epigenéticos, altera a trajetória do neurodesenvolvimento fetal e pode favorecer o surgimento de uma série de distúrbios do SNC (ESSHILI et al., 2020), como a esquizofrenia (DE CARVALHO LIMA et al., 2020), TEA (TRUJILLO VILLARREAL et al., 2021), transtorno bipolar (LEE et al., 2020; CARON; JANE MICHAEL, 2021), transtorno de déficit de atenção/hiperatividade (TDAH) (HAN et al., 2021; QUAGLIATO; DE MATOS; NARDI, 2021), depressão e ansiedade (ZHU et al., 2020) na prole (BERGDOLT; DUNAEVSKY, 2019).

1.4. Neuroinflamação

A neuroinflamação é definida como uma resposta inflamatória dentro do SNC e é amplamente mediada pelas células da glia, principalmente astrócitos e microglia, as quais desempenham um papel crucial na manutenção da homeostase cerebral e na resposta a diversos insultos, como infecções e lesões traumáticas (KEANE et al., 2021). As células microgliais são células dinâmicas que podem adotar diferentes fenótipos ou estados funcionais em resposta ao seu microambiente. Os principais fenótipos são frequentemente descritos como pró-inflamatório (M1) e anti-inflamatório (M2), dependendo do contexto fisiológico (WOODBURN; BOLLINGER; WOHLEB, 2021). O fenótipo pró-inflamatório é principalmente induzido através do reconhecimento de padrões moleculares associados a patógenos ("Pathogen-associated molecular patterns", PAMPs) pelos receptores de reconhecimento de padrões (PRR), ou padrões moleculares associados a danos ("Damage-associated molecular patterns", DAMPs) em células hospedeiras danificadas. A ativação do PRR desencadeia uma resposta imune através da secreção de citocinas pró-inflamatórias como o TNF-α, IL-1β, IL-6, quimiocinas, espécies reativas de oxigênio e óxido nítrico (nitric oxide/NO) (KWON; KOH, 2020).

Um dos principais PRRs na microglia envolvido no estado pró-inflamatório é a proteína contendo domínio NOD-, LRR- e pírin-3 (NLRP3). O complexo NLRP3 é um PRR intracelular encontrado em macrófagos, neutrófilos, linfócitos, células epiteliais e dendríticas, osteoblastos e neurônios (BLEVINS et al., 2022). É ativado como resultado da transcrição do fator nuclear kappa B (NFkB) por uma variedade de estímulos, incluindo infecções, irritantes exógenos ou DAMPs endógenos. Ao reconhecer esses sinais, o NLRP3 (sensor) recruta a ASC (apoptosis-associated-speck-like protein containing a CARD) e a pró-enzima pró-caspase-1 (efetor) para formar o complexo inflamassoma NLRP3 no citosol (SWANSON; DENG; TING, 2019). A ativação da caspase-1 desencadeia a maturação e liberação das citocinas pró-inflamatórias IL-1β e IL-18, que funcionam tanto de forma parácrina quanto autócrina para desencadear respostas imunes inatas e adaptativas (MOLLA et al., 2020) (Figura 1).

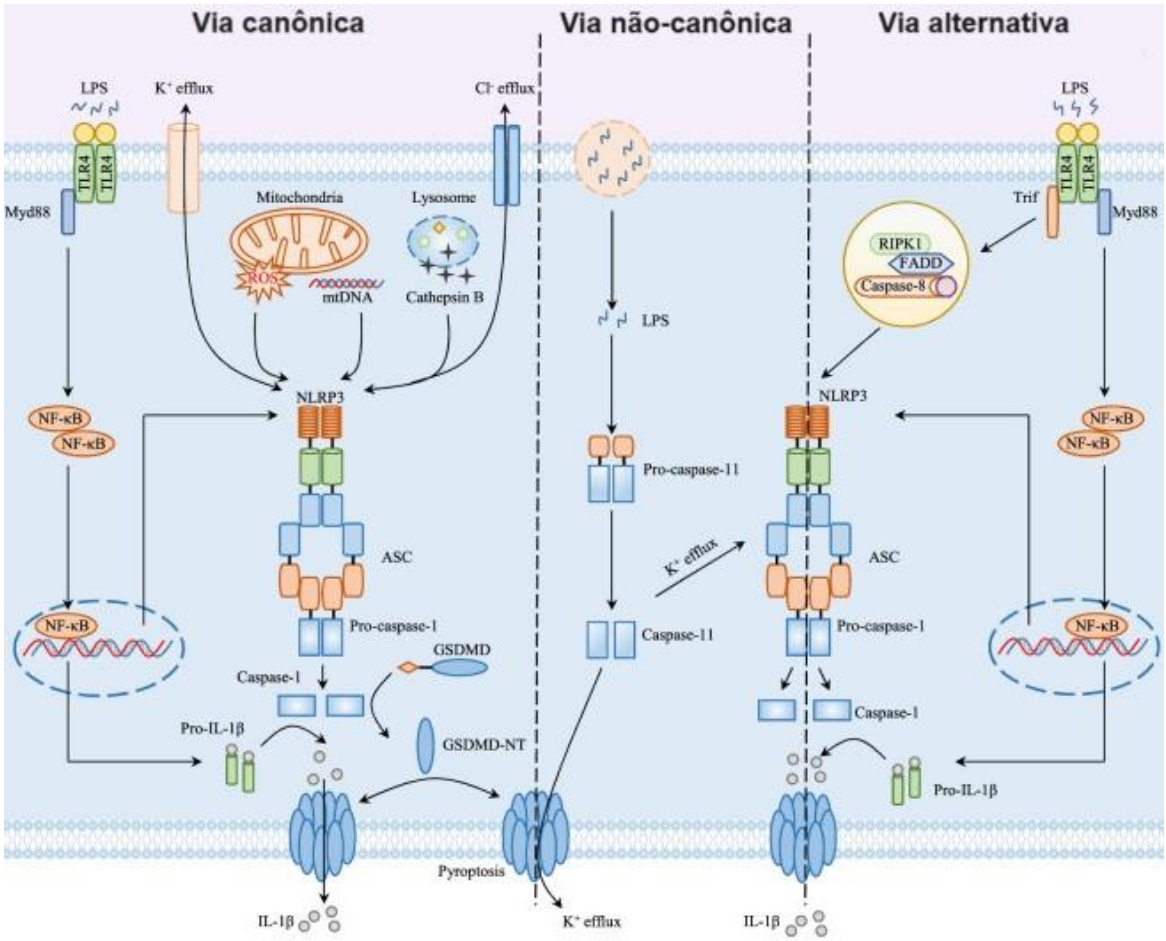

Figura 1. Mecanismos de ativação do Inflamassoma NLRP3. ASC, Proteína Semelhante a Speck Associada à Apoptose Contendo um CARD; FADD, Proteína Associada ao Fas com Domínio de Morte; GSDMD, Gasdermina D; IL-18, Interleucina 18; IL-1β, Interleucina 1 beta; LPS, Lipopolissacarídeo; mtDNA, DNA mitocondrial; MyD88, Resposta Primária de Diferenciação Mieloide 88; NFkB, Fator Nuclear Kappa B; NLRP3, Proteína Contendo Domínio NOD-, LRR- e Pirina-3; RIPK1, Proteína cinase 1 que interage com receptor; ROS, Espécies Reativas de Oxigênio; TLR4, Receptor do Tipo Toll 4; Trif, Adaptador Contendo o Domínio TIR Induzindo Interferon-beta (Adaptado de HUANG; XU; ZHOU, 2021).

Outro produto bem conhecido da sinalização do PRR e ativação do NFkB é a enzima óxido nítrico sintase induzível (iNOS). A iNOS não é expressa constitutivamente na maioria das células, mas pode ser induzida em resposta a sinais inflamatórios. Ao contrário das outras isoformas de NOS (nNOS e eNOS), que produzem NO de maneira regulada e localizada para sinalização fisiológica, a

iNOS gera grandes quantidades de NO por períodos prolongados em resposta a estímulos inflamatórios (CINELLI et al., 2020; MATHY et al., 2021). Enquanto a produção de NO mediada pela iNOS é crucial para a defesa do hospedeiro, a ativação excessiva ou prolongada da iNOS e a subsequente produção de NO contribuem para a cascata inflamatória que sustenta danos teciduais crônicos (FORSTERMANN; SESSA, 2012; CINELLI et al., 2020).

Durante o desenvolvimento perinatal, a polarização microglial em direção a um fenótipo pró-inflamatório é um fator etiológico para as alterações do neurodesenvolvimento associadas à AIM (CHAMERA et al., 2020; LOAYZA et al., 2023), uma vez que esse contexto altamente inflamatório resulta em alterações na diferenciação e maturação das células neuronais e do SNC como um todo (BAINES et al., 2020; CIEŚLIK et al., 2020). Em modelos *in vivo* de AIM, os fetos de mães expostas a um desafio inflamatório durante a gestação apresentaram um aumento da densidade microglial, assim como uma maior proporção de células microgliais CD86+, um marcador para o estado pró-inflamatório, associado a um aumento da expressão de citocinas pró-inflamatórias (LOAYZA et al., 2023). Embora crítica para a manutenção da homeostase do SNC, a ativação excessiva ou prolongada das células microgliais, especialmente durante o neurodesenvolvimento, pode contribuir para uma neuroinflamação sustentada e resultar danos teciduais permanentes, o que está associado a várias condições, como esclerose múltipla, doença de Alzheimer e TEA (DONG; YONG, 2019; BAI; XUE; YONG, 2020).

1.5. O sistema endocanabinóide (SEc)

O potencial de intervenções ao longo da vida serem capazes de mitigar os efeitos da obesidade materna perinatal ainda não é claro. No entanto, algumas estratégias anti-inflamatórias parecem promissoras. O sistema endocanabinóide (SEc) tem sido extensivamente estudado no contexto da obesidade e inflamação, mostrando uma relação próxima com o metabolismo energético e o controle alimentar (DI MARZO; SILVESTRI, 2019). Além disso, a modulação do SEc surge como uma possível intervenção uma vez que intersecta tanto a resposta inflamatória quanto a regulação sináptica no SNC (CASTILLO et al., 2012).

O SEc é uma rede complexa e difusa, com uma ampla variedade de funções específicas em tecidos diversos. É composto por seus dois principais receptores, os receptores de canabinóides do tipo 1 e 2 (CB1R, CB2R), e por dois ligantes, 2-araquidonilglicerol (2-AG) e anandamida (AEA), além de outros receptores putativos, como os Receptores Ativados por Proliferadores de Peroxissomos (PPARs), o Receptor acoplado à Proteína G55 (GPR55) e Receptores Vanilóides de Potencial Transitório (TRPVs) (JOSHI; ONAIVI, 2019). Ainda, possui um conjunto de enzimas de síntese e degradação de seus ligantes, sendo as principais Fosfolipase D hidrolisante de N-acilfosfatidiletanolamina (NAPE-PLD) e N-aciltransferase (NAT), responsáveis pela síntese de AEA, Fosfolipase C (PLC) e Diacilglicerol Lipase (DAGL), responsáveis pela síntese de 2-AG, Hidrolase de Amida de Ácido Graxo (FAAH), responsável pela degradação de AEA e Monoacilglicerol Lipase (MAGL), responsável pela degradação de 2-AG (Figura 2). Assim, as funções gerais do SEc podem estar relacionadas com os papéis destes componentes, que incluem cognição, liberação de insulina e imunorregulação (AN et al., 2020). O foco principal deste trabalho será nos receptores endocanabinóides 1 e 2 e o Receptor Ativado por Proliferadores de Peroxissomos Gama (PPARγ).

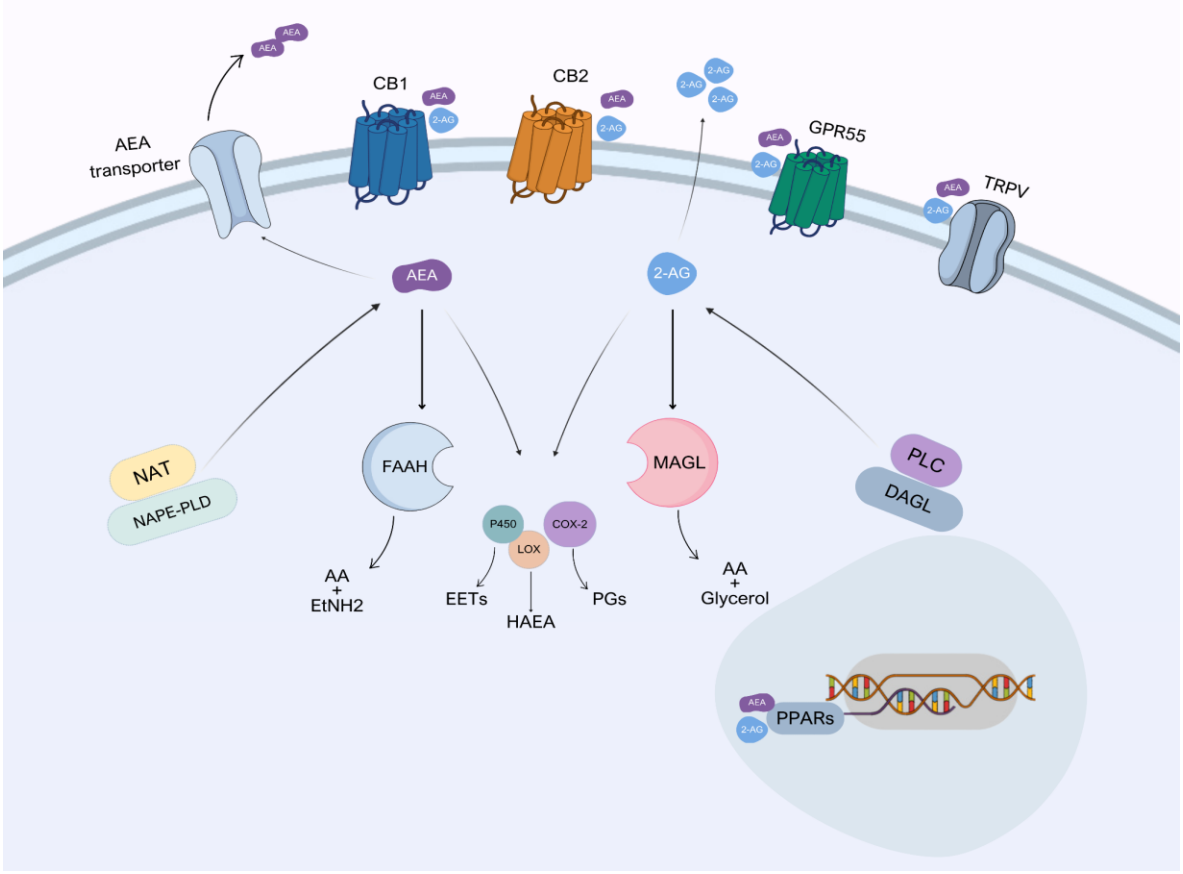

Figura 2. Visão geral dos principais componentes do sistema endocanabinóide. AEA, Anandamida; CB1, receptor canabinóide do tipo 1; CB2, receptor canabinóide do tipo 1; COX-2, Ciclooxigenase-2; DAGL, Diacilglicerol Lipase; EET, Ácidos Epoxyeicosatrienoicos; EtNH2, Etanolamina; FAAH, Hidrolase de Amida de Ácido Graxo; GPR55, Receptor acoplado à Proteína G55; HAEA, Hidroxietanolamida araquidônica; LOX, Lipoxigenase; MAGL, Monoacilglicerol Lipase; NAPE-PLD, Fosfolipase D hidrolisante de N-acilfosfatidiletanolamina; NAT, N-aciltransferase; P450, Citocromo P450; PG, Prostaglandinas; PLC, Fosfolipase C; PPAR, Receptor Ativado por Proliferadores de Peroxissomos; TRPV, Receptor Vanilóide de Potencial Transitório (Figura elaborada pela autora).

1.5.1. Receptores canabinóides do tipo 1 e 2

Tanto CB1R quanto CB2R são receptores acoplados à proteína G (GPCRs), o que significa que a ligação do ligante permite sinalização por meio da dissociação da proteína G ou recrutamento de β-arrestina (IBSEN; CONNOR; GLASS, 2017). No CB1R, a sinalização ocorre via subunidade Gα-inibitória (Gαi) dissociada; o que inibe a adenilato ciclase (AC), reduz os níveis de Adenosina Monofosfato Cíclico (AMPc), reduzindo subsequentemente a atividade da proteína cinase A (PKA). Além disso, causa ativação parcial da cinase regulada por sinal extracelular 1/2 (ERK1/2). Simultaneamente, o complexo da subunidade β/γ dissociado fecha os canais de cálcio do tipo L e ativa os canais de potássio retificados internamente acoplados à proteína G, enquanto também ativa parcialmente a ERK1/2. Posteriormente, o recrutamento da β-arrestina para atenuar a ativação do CB1R permite a ativação completa da ERK1/2, ativando assim essa via de sinalização (PATEL et al., 2020) (Figura 3).

Os CB1R são os receptores acoplados à proteína G mais comuns no SNC, majoritariamente encontrados em neurônios pré-sinápticos dopaminérgicos, gabaérgicos e glutamatérgicos de diversas regiões, como neocórtex, estriado e hipocampo (BUSQUETS GARCIA et al., 2016). Sua ampla distribuição permite que coordenem uma série de funções, variando de cognição, memória, humor e respostas sensoriais até a regulação do apetite (KRUK-SLOMKA et al., 2017), mostrando uma relação estreita com respostas comportamentais relacionadas à ansiedade, sociabilidade e memória (WANG; HAN, 2020; PETERS; CHEER; TONINI, 2021). No hipotálamo, são expressos nos neurônios que expressam pró-opiomelanocortina (POMC) e neuropeptídeo Y/proteína relacionada ao agouti (NPY/AgRP), desempenhando um papel importante na sinalização dos hormônios leptina e grelina (MAZIER et al., 2019). Sua ativação é geralmente considerada um poderoso sinal orexigênico; assim, sua inibição pode apresentar benefícios para o tratamento da obesidade e doenças metabólicas relacionadas (BELALI et al., 2023).

Dentre as diversas funções exercidas, no contexto deste trabalho destaca-se sua influência no circuito de recompensa do cérebro, particularmente em

resposta a substâncias de abuso, mas também em resposta a certos grupos alimentares, em especial os altamente palatáveis (CUNHA et al., 2020). Estes alimentos estão associados a um estado de conforto e recompensa devido à ativação do sistema dopaminérgico, em especial o eixo amígdala – área tegmental ventral (VTA) – núcleo accumbens (NAc), modulando o controle hedônico do comportamento alimentar (KOEKKOEK; MUL; LA FLEUR, 2017). O circuito dopaminérgico, portanto, está intrinsecamente ligado à atividade canabinóide uma vez que os CB1Rs estão densamente localizados nas regiões estriadas que medeiam a função de recompensa (NAc e VTA) (WENZEL; CHEER, 2018).

A sinalização via CB2R é um pouco mais complexa, pois acontece através dos mesmos mecanismos do CB1R, mas pode liberar uma subunidade Gαi ou Gα estimulatória (Gαs). A liberação de subunidades opostas acontece simultaneamente, no entanto, ambas resultam na fosforilação e ativação da Proteína de Ligação ao Elemento de Resposta ao AMPc (CREB). O Gαi sinaliza via p38 para induzir a ativação de CREB, enquanto o Gαs estimula a AC, aumentando assim os níveis de AMPc e a ativação de PKA. PKA então fosforila diretamente e ativa CREB, levando a mudanças transcricionais (SAROZ et al., 2019; TURU et al., 2021) (Figura 3).

No SNC, os CB2R são principalmente expressos em células da glia e são conhecidos por modular a ativação microglial e astrocitária (LI et al., 2023; ZHANG et al., 2022; ZHU et al., 2023). Diversos estudos demonstraram efeitos neuroprotetores associados ao CB2R, especificamente através da redução de moléculas de sinalização pró-inflamatórias e da transição das células microgliais do estado pró-inflamatório (M1) para anti-inflamatório (M2) (KOMOROWSKA-MÜLLER; SCHMÖLE, 2020). Animais *knockout* para CB2R (CB2R-/-) demonstram aumento na produção das citocinas pró-inflamatórias TNFα e IL-6 por células da microglia, além de um elevado estresse celular. Interessantemente, o *knockout* de CB2R também prejudicou a aprendizagem social em ratos jovens (KOMOROWSKA-MÜLLER et al., 2021; TAO et al., 2022).

Além disso, os CB2Rs estão amplamente presentes em tecidos metabólicos e imunes periféricos. Por exemplo, sabe-se que a presença de uma variante menos funcional desses receptores (CB2 Q63R), está relacionada com uma maior

propensão ao desenvolvimento de doenças autoimunes (ARGENZIANO et al., 2020). Já no tecido adiposo, a estimulação do CB2R leva a um efeito protetor importante em adipócitos de indivíduos obesos, aumentando a expressão de proteína desacopladora 1 (UCP1) e, consequentemente, a geração de calor e do gasto energético (ROSSI et al., 2016).

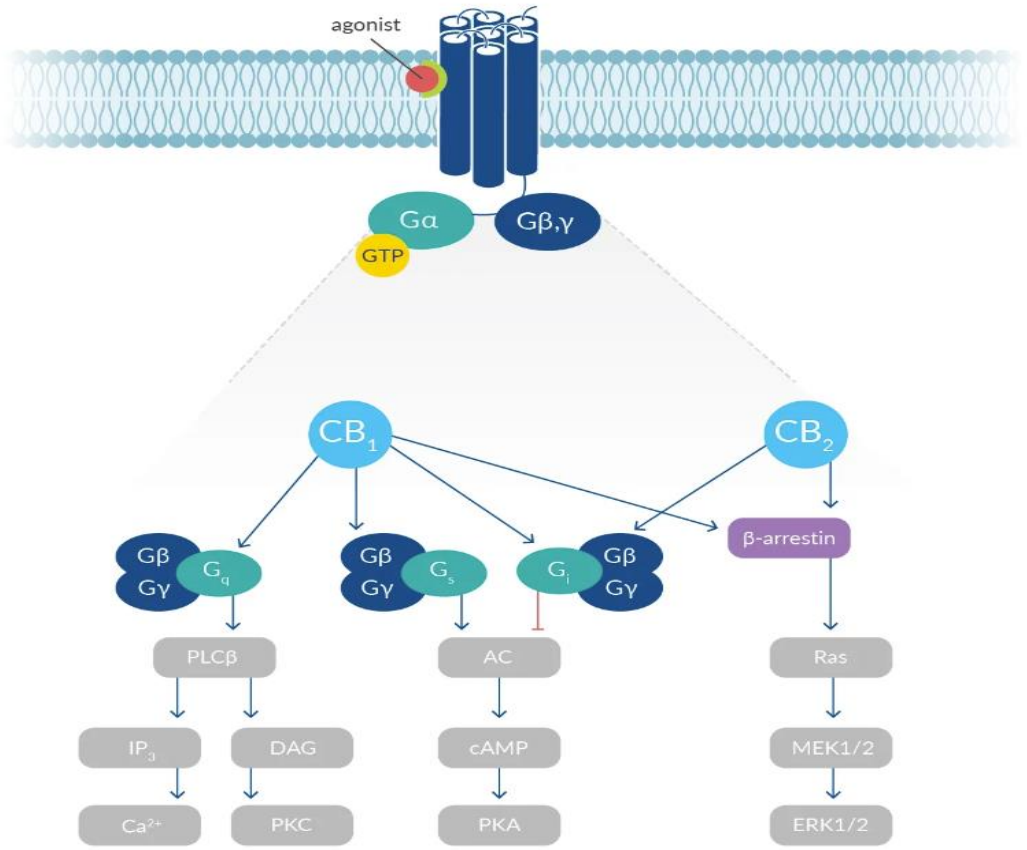

Figura 3. Vias de sinalização dos receptores CB1 e CB2. AC, Adenilato Ciclase; CB1, Receptor Canabinóide do Tipo 1; CB2, Receptor Canabinóide do Tipo 2; AMPc, Adenosina Monofosfato Cíclico; DAG, Diacilglicerol; ERK1/2, cinase Regulada por Sinal Extracelular 1/2; IP3, Inositol Trifosfato; MEK1/2, cinase de Sinalização Extracelular Regulada por Mitógeno 1/2; PKA, Proteína cinase A; PKC, Proteína cinase C; PLCβ, Fosfolipase C Beta; Ras, Proteína Ras (Cayman, 2020).

1.5.2. Receptor Ativado por Proliferadores de Peroxissomos Gama (PPARγ)

Os PPARs são uma família de três receptores nucleares hormonais: PPARα, PPARγ e PPARβ/δ. Eles apresentam várias funções celulares, incluindo homeostase energética, metabolismo da glicose e ácidos graxos e resposta à insulina (TYAGI et al., 2011). Embora co-expressos em vários tipos diferentes de órgãos e tecidos, cada isoforma apresenta características funcionais distintas e especificidade de ligante (KLIEWER et al., 1994; CHRISTOFIDES et al., 2021). Desde sua identificação, os PPARs têm sido reconhecidos como receptores para uma variedade de lípidos endógenos (como ácidos graxos insaturados, monoinsaturados e poli-insaturados) e compostos exógenos naturais (IANNOTTI; VITALE, 2021).

O PPARγ é considerado um regulador mestre da adipogênese e é abundantemente expresso no tecido adiposo, onde está principalmente envolvido no metabolismo de gorduras e carboidratos (LIU et al., 2022). Além disso, exerce efeitos anti-inflamatórios em vários tecidos, incluindo o SNC, reprimindo a expressão e a função de fatores pró-inflamatórios, como NF-κB, Nrf2/CREB, AP-1, STATs e iNOS (MAHUNG et al., 2022; LI et al., 2023; WEI et al., 2023). A sua ativação tem sido demonstrada como capaz de reduzir a hiper-ativação de astrócitos e microglia, diminuindo marcadores pró-inflamatórios e aumentando marcadores anti-inflamatórios (WEN et al., 2018; MACHADO et al., 2019). Diversos estudos apoiam essas descobertas usando diferentes intervenções farmacológicas, como o antioxidante e agonista do PPARγ Malibatol A, que foi capaz de diminuir os marcadores de fenótipo pró-inflamatório CD16, CD32 e CD86 e aumentar a expressão dos marcadores de fenótipo anti-inflamatório CD206 e YM-1 na microglia (PAN et al., 2015).

1.5.3. Ligantes Endocanabinóides

O SEc transmite sinais através de ligantes lipídicos, com os dois principais sendo o 2-AG e a AEA. Embora não seletiva, AEA é a mais versátil, ligando-se à maioria dos receptores do SEc, incluindo os CBRs, PPARs, outros GPRs e TRPV1 (MIRALPEIX et al., 2021). Além disso, existem outros ligantes putativos não

ligantes aos CBRs, como a palmitoiletanolamida (PEA) e a oleoiletanolamida (OEA), que ativam PPARs, a N-arachidonil-dopamina (NADA), que ativa o TRPV1, e a NAGly, que ativa o GPR55 (CONSOLE-BRAM et al., 2017; LAWTON et al., 2017).

Diversas enzimas e transportadores estão envolvidos na regulação do SEc através da síntese e degradação de 2-AG e da AEA, conforme anteriormente mostrado na Figura 2. Uma molécula central nessa rede é o ácido araquidônico (ARA), que atua como precursor e produto de degradação para ambos os ligantes. A AEA é principalmente sintetizada a partir do ARA e da fosfatidiletanolamina pela N-acetiltransferase, resultando em N-arachidonoyl-fosfatidiletanolamina, que é então combinada com ácido fosfatídico via fosfolipase D específica para N-acil-fosfatidiletanolamina para produzir AEA. Por outro lado, o 2-AG é principalmente sintetizado a partir de fosfatidilinositol, que é convertido em 1,2-diacilglicerol pela fosfolipase C, sendo então convertido em 2-AG pela DAGL-α/β (MIRALPEIX et al., 2021).

A degradação de AEA e 2-AG é essencial para a atenuação da sinalização endocanabinóide. Várias enzimas catalisam esse processo, sendo as principais a FAAH e MAGL, para AEA e 2-AG, respectivamente (REN et al., 2020). Além da degradação dos ligantes, a sinalização endocanabinóide também pode ser atenuada através da recaptura de AEA e 2-AG na fenda sináptica (REYNOSO-MORENO et al., 2018; PAREDES-RUIZ et al., 2021).

Na fenda sináptica, os ligantes canabinóides endógenos, são liberados por neurônios pós-sinápticos e funcionam como moduladores, ligando-se aos CB1R pré-sinápticos para modular a liberação de neurotransmissores, como o ácido gama-aminobutírico (GABA), glutamato e dopamina (COVEY et al., 2017; KÖFALVI et al., 2020). Ainda, no contexto da neuroinflamação, é importante destacar que o metabolismo do 2-AG leva à produção de ácido araquidônico, que é um precursor de efetores pró-inflamatórios, como prostaglandinas e leucotrienos (ALHOUAYEK; MASQUELIER; MUCCIOLI, 2014). Com isso, observou-se que a inibição da MAGL acarreta na redução na produção de prostaglandinas e leucotrienos, com consequente efeito anti-inflamatório (MECHA et al., 2019; ARGENZIANO et al., 2020; WANG; HAN, 2020).

1.6. Canabidiol

O canabidiol (CBD) é um terpenofenol não psicotrópico isolado da *Cannabis sativa* com efeitos anti-inflamatórios e antioxidantes benéficos para diversos estados imunológicos (ATALAY; JAROCKA-KARPOWICZ; SKRZYDLEWSKA, 2019). Estudos demonstram que os grupos hidroxila do anel fenólico na estrutura do CBD interferem nas reações em cadeia de radicais livres, conferindo ao CBD sua atividade antioxidante (BORGES et al., 2013; ATALAY; JAROCKA-KARPOWICZ; SKRZYDLEWSKA, 2019). Sabe-se que o CBD é capaz de inibir a sinalização endocanabinoide via CB1R de forma dose-dependente, ligando-se ao sítio alostérico desses receptores e alterando a potência de outros ligantes primários como, os endocanabinóides ou o tetrahidrocanabinol (THC), sem os efeitos colaterais relacionados aos agonistas inversos ou antagonistas dos CB1R, como o rimonabant e AM251 (STRAIKER et al., 2018; THAM et al., 2019). Além disso, o CBD também age como um agonista de CB2R, resultando na redução da produção de espécies reativas de oxigênio e de moléculas pró-inflamatórias (DOS-SANTOS-PEREIRA et al., 2020).

Além dos receptores CB1R e CB2R, o CBD também é conhecido por interagir com outros receptores endocanabinóides putativos relevantes para a homeostase metabólica, como os PPARs, GPR55, e TRPVs (ROSSI et al., 2018; MULLER; MORALES; REGGIO, 2019; LAGO-FERNANDEZ et al., 2021). Esse amplo espectro de comunicação entre os sistemas se traduz em papéis modulatórios em diversos aspectos metabólicos em todo o organismo, desde o metabolismo e armazenamento de lipídios no fígado até a atividade mitocondrial e o gasto de energia no tecido adiposo (LIU et al., 2012; PARRAY; YUN, 2016). Além disso, o CBD também interage com o metabolismo da glicose, melhorando a tolerância à glicose (BIELAWIEC et al., 2020; GORELICK et al., 2022), o eixo cérebro-intestino, mitigando a disbiose (CLUNY; REIMER; SHARKEY, 2012; DI MARZO, 2020; GORELICK et al., 2022) e com os neuromoduladores anorexígenos hipotalâmicos (DI GIACOMO et al., 2020). Nesse sentido, além de melhorar diretamente uma série de aspectos fisiológicos relacionados à obesidade, a modulação do SEc parece também ser eficaz na moderação dos comportamentos alimentares, como a ingestão de alimentos ricos em gordura e açúcares

(WIERUCKA-RYBAK; WOLAK; BOJANOWSKA, 2014), hiperfagia (SCOPINHO et al., 2011), autoadministração de sacarose (BI et al., 2020) e compulsão alimentar (PUCCI et al., 2022), o que abre caminho para sua utilização como uma potencial intervenção nas disfunções metabólicas relacionadas à obesidade materna.

Além dos receptores endocanabinóides, estudos demonstram que o CBD é capaz de interagir com receptores serotoninérgicos, dopaminérgicos e glutamatérgicos (RENARD et al., 2017; MARTÍNEZ-AGUIRRE et al., 2023). Consequentemente, foi proposto como benéfico no contexto de uma série de condições neurológicas, como doença de Parkinson e Alzheimer, epilepsia, esquizofrenia e TDAH (BRIGIDA et al., 2017; BAR-LEV SCHLEIDER et al., 2019). Notavelmente, o tratamento com CBD já demonstrou potencial de melhorar o comportamento social, diminuir a ansiedade e estimular a comunicação em pacientes com TEA (ARAN et al., 2019), o que abre caminho para sua utilização como uma potencial intervenção em disfunções neurológicas e comportamentais decorrentes da exposição perinatal a contexto imunológico aversivo.

Nas células da microglia, o CBD exerce efeitos anti-inflamatórios ao suprimir a produção de citocinas pró-inflamatórias, inibir a ativação e proliferação de células imunes e reduzir espécies reativas de oxigênio (ATALAY; JAROCKA-KARPOWICZ; SKRZYDLEWSKA, 2019; DOS-SANTOS-PEREIRA et al., 2020). Ainda, estudos anteriores apontam CB2 e PPARγ como alvos promissores para o CBD em macrófagos (PRETEROTI et al., 2023), células epiteliais intestinais (CORPETTI et al., 2021), neurônios sensoriais (SILVA et al., 2022) e células supressoras derivadas de mieloides (KHOSROPOOR et al., 2023).

Considerando os efeitos já conhecidos do CBD em diversas disfunções neurológicas, assim como o envolvimento do sistema endocanabinóide na modulação sináptica e imune tanto do SNC quanto em órgãos periféricos, o estudo do seu potencial como intervenção direcionada aos efeitos da obesidade materna perinatal na prole pode trazer contribuições importantes no manejo das alterações inflamatórias/comportamentais induzidas pelo ambiente intrauterino desfavorável durante o desenvolvimento.

2. OBJETIVOS

2.1. Objetivo geral

Avaliar os efeitos do tratamento com canabidiol sobre aspectos metabólicos, comportamentais, neuroinflamatórios e neuroquímicos da prole de ratas Wistar obesas durante a adolescência e a vida adulta (Figura 4).

2.2. Objetivos específicos

- Determinar o perfil metabólico das mães obesas por meio do ganho de peso corporal e consumo calórico, assim como por dosagens plasmáticas de glicemia, triglicerídeos, colesterol total, e insulina;
- Identificar os efeitos da dieta materna e do tratamento com canabidiol (CBD) sobre comportamentos do tipo ansioso e alterações no comportamento social na prole juvenil (35-42 dias) e na prole adulta (84-90 dias) de ratas Wistar obesas;
- Determinar o perfil metabólico da prole por meio do ganho de peso corporal, consumo calórico e peso da gordura visceral, assim como dosagens plasmáticas de glicemia, triglicerídeos, colesterol total e insulina;
- Avaliar os efeitos da dieta materna e do tratamento sobre a expressão de marcadores neuroinflamatórios (IBA-1, GFAP, TNFα, IL-6 e IL-1β) no cérebro da prole;
- Investigar o efeito da dieta materna e do tratamento sobre componentes do SEc (expressão de CB1R e CB2R e dosagem de 2-AG e AEA) no cérebro da prole;
- Investigar o efeito da dieta materna e do tratamento sobre a transmissão dopaminérgica através da expressão do Receptor Dopaminérgico D2 (DRD2) e dosagem de Ácido Homovanílico (HVA), oxitocinérgica através da expressão do Receptor de Ocitocina (OXTR), GABAérgica através da dosagem de GABA e Glutamatérgica através da dosagem de Glutamato no córtex pré-frontal e hipocampo da prole;
- Realizar ensaios *in vitro* com cultura de células microgliais para investigação dos mecanismos moleculares através dos quais o tratamento com CBD exerce seus efeitos neuroprotetores.

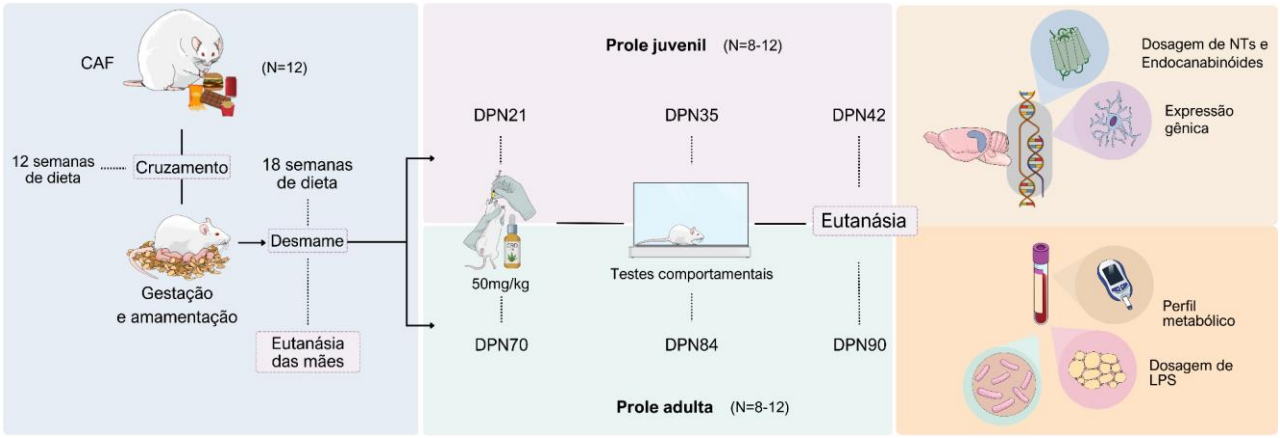

Figura 4. Desenho experimental do estudo. A obesidade foi induzida nas mães através de uma dieta de cafeteria (CAF) mantida por 12 semanas antes do cruzamento e durante toda a gestação e amamentação. A prole foi tratada com óleo de canabidiol (50mg/kg) por 3 semanas a partir dos 21° ou 70° dia pós-natal (DPN). A análise comportamental foi realizada na última semana de tratamento e a eutanásia e coleta tecidual no 42° ou 90° DPN. No tecido cerebral foi avaliada a expressão de receptores e marcadores inflamatórios, assim como o conteúdo de neurotransmissores e endocanabinóides. No plasma foi analisado o perfil bioquímico e a dosagem de lipopolissacarídeos (LPS).

3. ARTIGOS CIENTÍFICOS

3.1. Artigo científico 1

Cannabidiol treatment improves metabolic profile and decreases hypothalamic inflammation caused by maternal obesity

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Cannabidiol treatment improves metabolic profile and decreases hypothalamic inflammation caused by maternal obesity

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Introduction: The implications of maternal overnutrition on offspring metabolic and neuroimmune development are well-known. Increasing evidence now suggests that maternal obesity and poor dietary habits during pregnancy and lactation can increase the risk of central and peripheral metabolic dysregulation in the offspring, but the mechanisms are not sufficiently established. Furthermore, despite many studies addressing preventive measures targeted at the mother, very few propose practical approaches to treat the damages when they are already installed.

Methods: Here we investigated the potential of cannabidiol (CBD) treatment to attenuate the effects of maternal obesity induced by a cafeteria diet on hypothalamic inflammation and the peripheral metabolic profile of the offspring in Wistar rats.

Results: We have observed that maternal obesity induced a range of metabolic imbalances in the offspring in a sex-dependant manner, with higher deposition of visceral white adipose tissue, increased plasma fasting glucose and lipopolysaccharides (LPS) levels in both sexes, but the increase in serum cholesterol and triglycerides only occurred in females, while the increase in plasma insulin and the homeostatic model assessment index (HOMA-IR) was only observed in male offspring. We also found an overexpression of the pro-inflammatory cytokines tumor necrosis factor-alpha (TNFα), interleukin (IL) 6, and interleukin (IL) 1β in the hypothalamus, a trademark of neuroinflammation. Interestingly, the expression of GFAP, a marker for astrogliosis, was reduced in the offspring of obese mothers, indicating an adaptive mechanism to *in utero* neuroinflammation. Treatment with 50 mg/kg CBD oil by oral gavage was able to reduce white adipose tissue and revert insulin resistance in males, reduce plasma triglycerides in females, and attenuate plasma LPS levels and overexpression of TNFα and IL6 in the hypothalamus of both sexes.

Discussion: Together, these results indicate an intricate interplay between peripheral and central counterparts in both the pathogenicity of maternal obesity and the therapeutic effects of CBD. In this context, the impairment of internal

hypothalamic circuitry caused by neuroinflammation runs in tandem with the disruptions of important metabolic processes, which can be attenuated by CBD treatment in both ends.

KEYWORDS

maternal obesity, neuroinflammation, cannabidiol, hypothalamus, insulin resistance

1. Introduction

Maternal malnourishment before and during pregnancy is a growing worldwide concern known to bear several implications for fetal development that lead to long-term consequences on offspring health and well-being (1, 2). Interestingly, since the pioneering studies on the effects of the perinatal environment, such as the Developmental Origin of Health and Disease (DOHaD) theory, the context transitioned from the lack of nutrients due to hunger and starvation to excess due to the global obesity pandemic (3). In fact, globalization and urbanization have gradually led to an increase in the consumption of "junk food" (i.e., ultra-processed, rich in fats, and sugar) associated with the reduction of physical activity, a phenomenon called "nutritional transition" (4, 5). In this context, it is important to highlight that obesity and the consumption of mentioned "junk food" most often co-occur, making it difficult to discriminate the effects of obesity and its metabolic profile *per se* from those related to nutritional aspects of the foods consumed (6).

A broad range of studies has demonstrated that an abnormal inflammatory milieu during *in utero* development triggers the so-called "early-life programming" of the offspring metabolism (3, 7). A number of potential pathways underlying the effects of maternal obesity include increased sustained inflammation, changes in lipid transport and storage, dysregulation of glucose metabolism, and modifications to the microbiome, which triggers increased translocation of lipopolysaccharides (LPS) to the bloodstream and circulating levels of pro-inflammatory cytokines (8–10). These inflammatory mediators are able to cross the placental barrier and create a harmful environment for developing fetal tissues (11, 12). Other than that, the increased insulin resistance, glucose levels, and lipids, with a potentially elevated supply of nutrients to the developing fetus, contribute to setting persistent changes in the offspring's energy balance, appetite regulation, lipid and glucose homeostasis, and gut dysbiosis. Overall, as a result, maternal obesity substantially raises the risk of offspring obesity, insulin resistance, type 2 diabetes, high blood pressure, and adverse lipid profile (7, 10, 13).

The hypothalamus is the predominant brain area that controls energy balance by integrating information from the body and initiating appropriate behavioral, humoral, and neural outputs. Current evidence indicates hypothalamic inflammation as a likely mechanism for the dysregulation of the homeostatic control of energy balance, which might lead to an increased susceptibility to metabolic alterations and obesity in the offspring (14, 15). Abnormal insulin signaling during neurodevelopment leads to malformation of neural projections that affect hypothalamic function and plasticity, resulting in altered energy homeostasis in the offspring (16, 17). These effects can also be attributed to enhanced activation of resident immune cells, such as astrocytes and microglia, with the consequent secretion of inflammatory cytokines, such as tumor necrosis factor-alpha (TNFα), interleukin (IL)-6, and IL-1β (18).

The extent to which changes in the offspring's habits and resolutive approaches throughout life can modify the effects of perinatal maternal obesity is yet not known, but a few anti-inflammatory approaches seem to exert positive effects. The endocannabinoid system has been extensively studied in the context of obesity and inflammation, showing a close relationship with energy metabolism and the feeding circuitry (19). Cannabidiol (CBD) is a non-psychotropic terpenophenol isolated from *Cannabis sativa* with anti-inflammatory and antioxidant effects discussed to be beneficial for diverse immunological states (20). It has been suggested that the hydroxyl groups of the phenol ring in CBD structure interfere with free radical chain reactions, which confers CBD its antioxidant activity (20, 21). Furthermore, the modulation of endocannabinoid signaling *via* downregulation of CB1 receptor activity and upregulation of CB2 receptor activity results in reduced reactive oxygen species (ROS) production and reduced pro-inflammatory signaling (22, 23).

Besides the endocannabinoid receptors CB1 and CB2, CBD is also known to interact with other systems and receptors relevant to metabolic homeostasis, such as the G protein-coupled receptor 55 (GPR55), Transient Receptor Potential Vanilloid (TRVP) channel and nuclear peroxisome proliferator-activated receptors (PPARs) (24–27). This broad spectrum of communication among systems translates into modulatory roles in diverse metabolic aspects throughout the entire body, from lipid metabolism and storage in the liver to mitochondrial activity and energy expenditure in the adipose tissue (28, 29). Furthermore, CBD was also shown to interact with glucose metabolism by improving glucose tolerance (30, 31), the brain-gut axis by mitigating microbiome dysbiosis (30, 32, 33), and hypothalamic anorexigenic neuromodulators (34). In this sense, beyond directly improving a range of physiological aspects related to obesity, modulation of the endocannabinoid system seems to also be effective on tempering eating behaviors, such as high-fat and high-sucrose food intake (35), hyperphagia (36), sucrose self-administration (37) and binge eating (38), which sets the stage for it as a potential intervention on maternal obesity-related metabolic dysfunctions.

Here, we investigated the effects of CBD treatment on maternal obesity-induced hypothalamic inflammation and metabolic outcomes on the early-adulthood of the offspring.

2. Materials and methods

2.1. Animals

Eighteen female Wistar rats (3-weeks-old) were obtained from the animal facility of the Federal University of Health Sciences of Porto Alegre (UFCSPA). The animals were group-housed (3 animals per cage) under standard laboratory conditions at a controlled temperature (23 ± 1°C) and 12-h light:dark cycle. This study was approved by UFCSPA Institutional Animal Care and Use Committee under protocol N° 751/21. All experiments were designed and performed to minimize the number and suffering of subjects, following the international laws that regulate the care of laboratory animals.

2.2. Experimental groups and diet

Three-week-old female breeders ($N = 9$) were placed on either control diet (CT) composed by standard chow (Nuvilab® CR-1 Nuvital®, Curitiba, PR, Brazil) (3.4 kcal/g, 63% carbohydrates, 26% protein, and 11% fat) or a Cafeteria Diet (CAF) composed by standard chow plus bacon mortadella (Perdigão®), strawberry wafers (Isabela®), chocolate cookies (Isabela®), pizza-flavored crackers (Parati®), white chocolate (Harald®), sausage (Alibem®), and orange-flavored soda (Sukita®) (4.3 kcal/g, 43% carbohydrates, 14% protein, and 43% fat) with water *ad libitum*. CAF group was fed with three menus with different combinations among the foods mentioned interchanged every 2 days, to maintain novelty and stimulate consumption. CAF was chosen as an obesogenic diet since it mimics the Western dietary habits in a more translational manner than regular high-fat and/or high-sugar diets, once CAF provides the variety of textures, options and palatability that contribute to hedonic eating and are not present on manufactured chows (39, 40). The diets were maintained for 12 weeks prior to and during mating with a 3-months-old male, throughout gestation, lactation, and until weaning. Dam weight and the weight of consumed diets were recorded weekly. Day of parturition was considered postnatal day zero (PND0).

To reduce the impact of litter effects, litters were adjusted to seven to nine pups per dam with an equal proportion of males to females when possible. All offspring were weaned at PND21, placed on standard chow and weighed weekly. Litters were divided equally among treatment groups and by sex, which created four groups per sex: CT mother + Vehicle (CT-Veh), CT mother + Cannabidiol (CT-CBD), CAF mother + Vehicle (CAF-Veh), and CAF mother + Cannabidiol (CAF-CBD).

Treatment started at the same day of weaning (PND21) and consisted of CBD oil diluted in corn oil for a dose of 50 mg/kg (Prati-Donaduzzi®, Toledo, PR, Brazil) or corn oil (vehicle), both in a volume of 1 mL/kg by oral gavage. The treatment was administered 7 days a week for 3 weeks (**Figure 1**). Treatment dose and duration were chosen based on previous studies on different cognitive-assessment models with oral administration of CBD (41–45). Furthermore, we have performed a pilot study assessing doses of 2,5 mg/kg, 10 mg/kg, and 50 mg/kg, to which 50 mg/kg showed most significant positive results (data not shown).

2.3. Tissue processing

At the end of the 3 weeks of treatment, on PND42, animals were euthanized by decapitation. The gonadal visceral adipose tissue was weighed, truncal blood was centrifuged, and plasma was separated. The brain was dissected immediately and all tissues were snap-frozen in liquid nitrogen and stored in −80°C for further processing and analysis.

2.4. Biochemical analysis

Fasting plasma levels of glucose, total cholesterol and triglycerides were quantified using enzymatic colorimetric kits (Labtest, Lagoa Santa, Brazil). Insulin levels in the plasma were determined by enzyme-linked immunosorbent assay (ELISA) (Insulin ELISA kit, Cat# RAB0904; Sigma-Aldrich, St. Louis, MO, USA). Subsequently, the homeostatic model assessment (HOMA-IR) index was calculated to determine insulin resistance through the following formula: glucose (mg/dL) × insulin (uU/mL)/22.5.

2.5. LPS quantification

Plasma (150 μL) was hydrolyzed with 75 μL of NaCl 150 mM and 300 μL of HCl 8M and then incubated for 4 h at 90°C. Afterward, 3 mL of hexane were added, and samples were centrifuged at 3,500 rpm for 10 min. The upper organic phase was withdrawn, and the residue was reconstituted in 50 μL of methanol, transferred to a vial, and an aliquot of 3 μL was injected into the analytical system. A Nexera-i LC-2040C Plus system coupled to a LCMS-8045 triple quadrupole mass spectrometer (Shimadzu, Kyoto, Japan) was used for the analysis.

2.6. RT-qPCR

Total RNA was isolated from the hypothalamus using TRIzol® (Invitrogen, Brazil) according to the manufacturer's instructions. The quantification of total RNA was done by spectrometry BioSpec-nano® (Shimazu, Kioto, Japan) at 260 and 280 nm. For cDNA synthesis, 1,000 ng of RNA were reverse transcribed according to the manufacturer's instructions (GoScript Reverse Transcription Kit, Promega, Brazil). To conduct real time quantitative polymerase chain reaction (RT-qPCR), cDNA was added to a reaction mix (10 μL final volume) containing 100 nM gene-specific primers and universal SYBR green supermix (Applied Biosystems, Thermo Fisher Scientific CA, USA). All samples were run in duplicate and were analyzed on an QuantStudio Real-Time PCR instrument (Applied Biosystems, Thermo Fisher Scientific CA, USA) for quantitative monitoring of PCR product formation. Relative gene expression was normalized to β-Actin controls and assessed using the 2-ΔΔCT method. Primer sequences are as follows: β-Actin: F: TATGCCAACACAGTGCTGTCTGG; β-Actin: R: TACTCCTGCTTGCTGATCCACAT; Iba1: F: GCAAGGATTTGCAGGGAGGA; Iba1: R: CGTCTTGAAGGCCTCCAGTT; GFAP: F: CGAAGAAAACCGCATCACCA; GFAP: R: CCGCATCTCCACCGTCTTTA; TNFα: F: TGGCGTGTTCATCCG

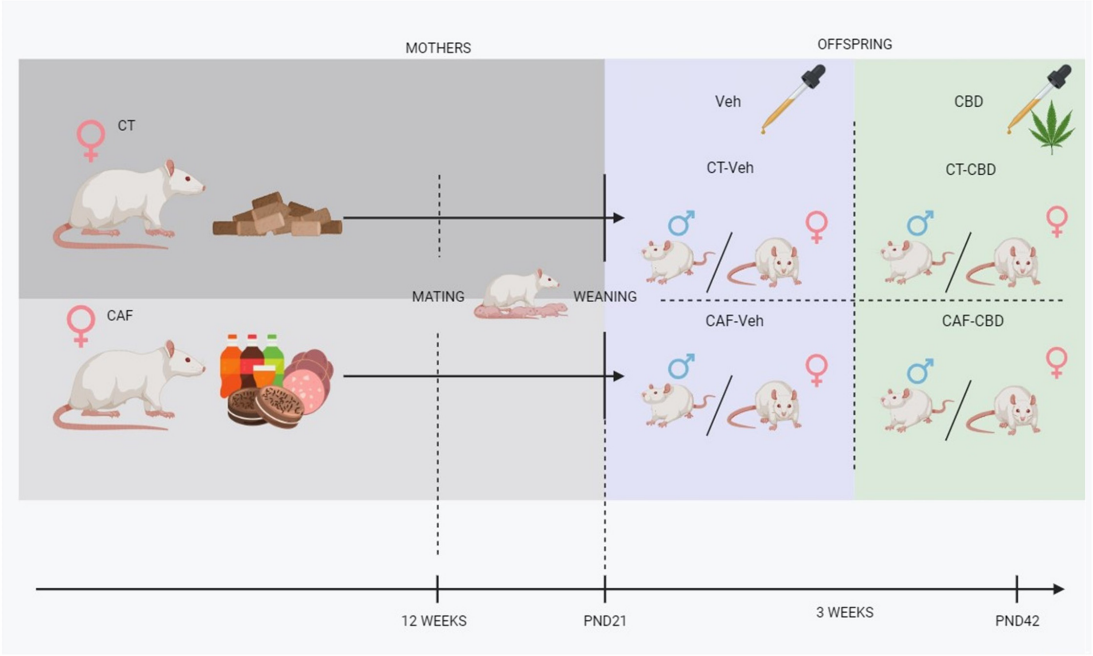

FIGURE 1
Experimental design. CT, control chow-fed dam; CAF, cafeteria diet-fed dam; Veh, offspring treated with vehicle (corn oil); CBD, offspring treated with cannabidiol (50 mg/kg); PND, post-natal day.

TTCTCTACC; TNFα: R: CCCGCAATCCAGGCCACTACTT; IL6: F: GACCAAGACCATCCAACTCATC; IL6: R: GCTTAG GCATAGCACACTAGG; IL1β: F: TGAGGCTGACAGACCCCAA AAGAT; IL1β: R: GCTCCACGGGCAAGACATAGGTAG.

2.7. Data analysis and statistics

Data were analyzed using Graphpad Prism 9 statistical software (GraphPad Software, San Diego, CA, USA). Two-way ANOVA with a Bonferroni *post hoc* analysis was performed within sexes. The main effects were: maternal diet and CBD treatment. The interaction between these two factors was also analyzed. The results were expressed as the mean ± standard error of the mean (SEM). Outliers were removed using the ROUT test, and statistically significant differences were considered at $p < 0.05$.

3. Results

3.1. Cafeteria diet induces obesity in female Wistar rats after 9 weeks of diet

The dams from both CT and CAF groups were weighed every week throughout the experiment to assess the impact of the diets on weight gain. Repeated measures two-way ANOVA has shown a significant diet effect ($F_{1,16} = 8.705$; $p = 0.0094$). From the 9th week of diet, CAF-fed female Wistar rats presented significantly higher body weight than the CT group ($p = 0.0349$), which persisted until mating in the 12th week ($p = 0.0062$). Despite no differences in body weight being shown during most of gestational and lactational

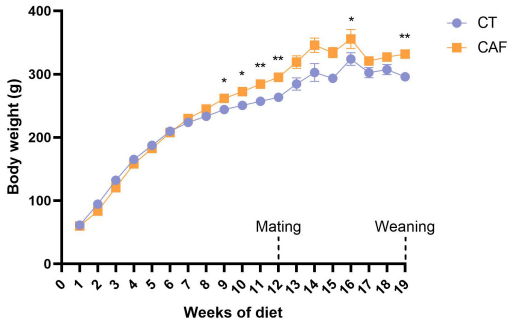

FIGURE 2
Dams' body weight throughout the experiment. Cafeteria diet-fed (CAF) dams presented significantly higher body weight when compared to control diet (CT) from the 9th week of diet, which was consistent until mating (12th week of diet) and after weaning of the offspring (19th week of diet). Data are presented as mean ± SEM. $n = 9$/group. *$p < 0.05$ **$p < 0.01$.

time, except for the 15th week ($p = 0.0298$), the difference became significant again right after weaning of the offspring on the 19th week ($p = 0.0012$) (Figure 2).

3.2. Cafeteria-induced maternal obesity increases visceral white adipose tissue deposits even though it does not affect offspring total body weight

The offspring was weighed weekly from weaning (PND21) to euthanasia (PND42) to determine weight gain in early-life and

throughout treatment and visceral white adipose tissue (WAT) was weighed at euthanasia. No groups showed any differences in weight gain related to neither maternal diet nor CBD treatment (Figure 3A), however, both male and female offspring of CAF-fed dams, presented an increase in WAT (maternal diet effect: $F_{1,32} = 17.02$; $p = 0.0002$ and $F_{1,31} = 24.92$; $p < 0.0001$ respectively). Indeed, untreated male offspring of obese dams (CAF-Veh) showed heavier visceral WAT when compared to the offspring of control dams (CT-Veh) ($p = 0.0005$). Also, both female CAF-Veh and CAF-CBD had more visceral fat than CT ones ($p = 0.0008$ and $p = 0.0084$). However, CBD treatment was able to reduce the deposition of visceral fat on male CAF-CBD when compared to CAF-Veh ($p = 0.0256$) (Figure 3B).

These data suggest a complex energy-balance disruption on the offspring of obese mothers, with an increase in the accumulation of visceral fat while maintaining total body weight. Also, CBD treatment seems to exert a positive effect in a sex-dependent manner.

3.3. Female offspring lipid profile is more affected by CAF-induced maternal obesity with partial effects of cannabidiol treatment

Plasma cholesterol and triglyceride levels were assessed in order to evaluate the biochemical profile of the offspring. On female offspring, there was a maternal diet effect ($F_{1,31} = 11.29$; $p = 0.0023$) and an interaction between diet and CBD treatment ($F_{1,31} = 6.027$; $p = 0.0208$) on total cholesterol levels. CAF-CBD had higher levels of plasma cholesterol than CT-CBD ($P = 0.0008$) (Figure 4A). Regarding triglycerides, there was a maternal diet effect ($F_{1,32} = 4.997$; $p = 0.0325$). CAF-Veh presented higher levels of triglycerides when compared to CT-Veh ($P = 0.0166$), while CAF-CBD showed lower levels when compared to CAF-Veh ($P = 0.0395$) (Figure 4B). There were no significant differences among male groups.

Together, these data suggest that CAF-induced maternal obesity affects lipid metabolism in the offspring in a sex dependent manner, with apparently more severe effects in females. However, even though CBD treatment did not exert any effects on total cholesterol, it was able to revert the increased triglyceride levels in females.

3.4. Cannabidiol treatment reverts insulin resistance caused by maternal obesity in male offspring

Plasma levels of fasting glucose and insulin were evaluated, and the HOMA-IR was determined in order to assess glucose metabolism and insulin resistance in the offspring. In male offspring we found a maternal diet effect on glucose levels ($F_{1,33} = 10.60$; $p = 0.0026$). CAF-Veh had higher fasting glucose than CT-Veh ($p = 0.0072$), with no effect of CBD (Figure 5A). On insulin, there was an interaction between maternal diet and CBD treatment ($F_{1,34} = 4.916$; $p = 0.0334$). CAF-Veh showed higher insulin levels than CT-Veh ($p = 0.0458$), while CAF-CBD had lower insulin levels than CAF-Veh ($p = 0.0354$) (Figure 5B). Consequently, on the HOMA-IR there was an interaction between maternal diet and CBD treatment ($F_{1,32} = 7.674$; $p = 0.0093$). CAF-Veh showed an increased HOMA-IR when compared to CT-Veh ($p = 0.0068$), while CAF-CBD had a lower index than CAF-Veh ($p = 0.0029$) (Figure 5C).

In female offspring, fasting glucose levels showed a maternal diet effect ($F_{1,30} = 15.30$; $p = 0.0005$). Both female CAF-Veh and CAF-CBD showed higher levels of plasma glucose when compared to their CT ($p = 0.0377$ and $p = 0.0099$) (Figure 5A). However, we did not find differences regarding insulin levels (Figure 5B) and HOMA-IR (Figure 5C) in female offspring.

These findings suggest that CAF-induced maternal obesity affects glucose metabolism and promotes insulin resistance in the offspring in a sex-dependent manner. Opposed to what was observed in lipid metabolism, glucose disturbances appear to be more severe in males. On the other hand, CBD treatment was able to reduce plasma insulin in male offspring of obese dams to control levels, which led to an improved HOMA-IR in this group as well.

3.5. Cannabidiol treatment reverts LPS-induced endotoxemia caused by maternal obesity

Plasma levels of LPS were measured to evaluate metabolic endotoxemia. In male offspring, we found a maternal diet effect ($F_{1,28} = 7.215$; $p = 0.0120$). Male CAF-Veh showed a higher concentration of LPS than CT-Veh ($p = 0.0148$), while CAF-CBD had lower levels when compared to CAF-Veh ($p = 0.0470$) (Figure 6). In female offspring, there were maternal diet ($F_{1,28} = 32.46$; $p < 0.0001$) and treatment ($F_{1,28} = 15.81$; $p = 0.0004$) effects and an interaction between both ($F_{1,28} = 14.88$; $p = 0.0006$). Female CAF-Veh presented higher levels of plasma LPS than CT-Veh ($P < 0.0001$), while CAF-CBD had lower levels than CAF-Veh ($p < 0.0001$) (Figure 6). Thus, CBD treatment seems to be effective to reduce plasma levels of LPS in the offspring of obese dams.

3.6. Cannabidiol treatment rescues hypothalamic neuroinflammation resulted from maternal obesity

Real time quantitative polymerase chain reaction (RT-qPCR) was conducted to evaluate gene expression of TNFα, IL6, IL1β, GFAP, and IBA-1 in the hypothalamus.

In male offspring, the gene expression of TNFα showed a maternal diet ($F_{1,27} = 4.939$; $p = 0.0348$) and CBD treatment ($F_{1,27} = 6.035$; $p = 0.0207$) effects, and also an interaction between both factors ($F_{1,27} = 16.34$; $p = 0.0004$). Male CAF-Veh showed higher levels of TNFα mRNA than CT-Veh ($p = 0.0002$), while CAF-CBD had lower levels than CAF-Veh ($p = 0.0001$) (Figure 7A). Regarding IL6 gene expression, there was an interaction between maternal diet and CBD treatment ($F_{1,26} = 20.20$; $p = 0.0001$). Male CAF-Veh showed higher levels of IL6 mRNA than CT-Veh ($p = 0.0054$), while CAF-CBD had lower levels than CAF-Veh ($p = 0.0003$) (Figure 7B). Regarding

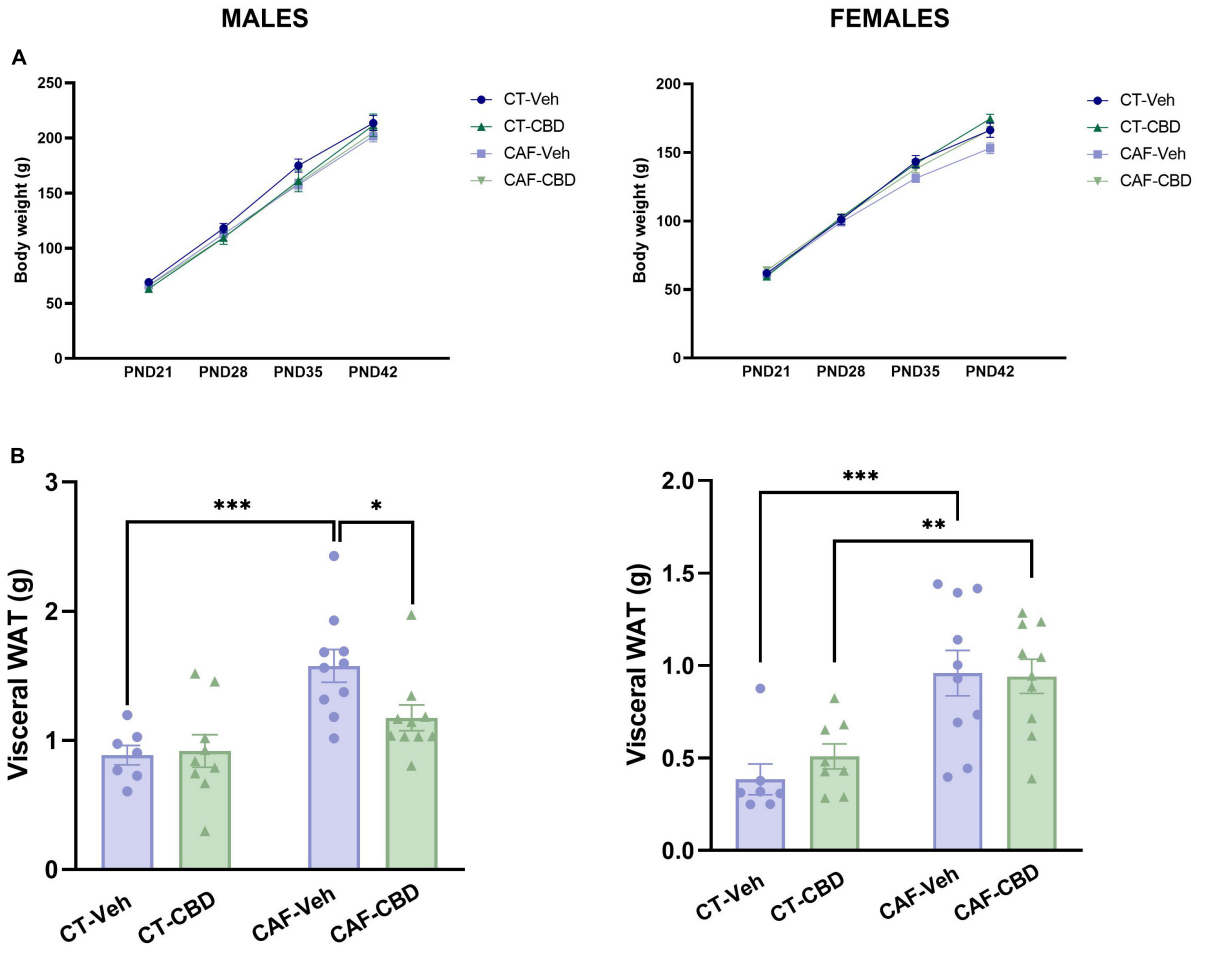

FIGURE 3
Offspring's body weight and visceral white adipose tissue (WAT). Neither male nor female offspring showed influences of maternal diet or cannabidiol (CBD) treatment in weight at weaning (PND21) and the following 3 weeks of treatment **(A)**. Maternal diet increased visceral fat deposit in both male and female cafeteria diet (CAF)-Veh offspring with CBD effect only in males **(B)**. Data are presented as mean ± SEM. n = 8–10/group. $^{*}p < 0.05$ $^{**}p < 0.01$ $^{***}p < 0.001$.

IL1β expression, there was a maternal diet effect ($F_{1,26}$ = 4.919; p = 0.0355), with no differences among groups on the *post-hoc* test (Figure 7C). Regarding GFAP expression, there were maternal diet ($F_{1,27}$ = 6.816; p = 0.0146) and CBD treatment ($F_{1,27}$ = 7.402; p = 0.0113) effects. CAF-Veh presented much lower levels of GFAP mRNA than CT-Veh (p = 0.0055), while CT-CBD had lower levels than CT-Veh as well (p = 0.0045) (Figure 7D). There were no differences in IBA-1 expression (Figure 7E).

In female offspring, there was an interaction between maternal diet and CBD treatment ($F_{1,28}$ = 4.250; p = 0.0486) regarding the gene expression of TNFα. Female CAF-Veh presented higher levels of TNFα mRNA than CT-Veh (p = 0.0156), while CAF-CBD had lower levels than CAF-Veh (p = 0.0351) (Figure 7A). Regarding IL6 expression, there was a maternal diet effect ($F_{1,28}$ = 5.637; p = 0.0247), a treatment effect ($F_{1,28}$ = 6.014; p = 0.0207) and an interaction ($F_{1,28}$ = 6.057; p = 0.0203). CAF-Veh presented higher levels of IL6 mRNA than CT-Veh (p = 0.0039), while CAF-CBD had lower levels than CAF-Veh (p = 0.0034) (Figure 7B). There was a maternal diet effect ($F_{1,25}$ = 15.62; p = 0.0006) on IL1β gene expression. Both CAF-Veh and CAF-CBD showed higher levels of IL1β mRNA than their CT (p = 0.0150 and p = 0.0255), with no effect of CBD treatment (Figure 7C). Regarding GFAP expression, there was an interaction between maternal diet and CBD treatment ($F_{1,27}$ = 8.541; p = 0.0069). CAF-Veh showed lower levels of GFAP mRNA than CT-Veh (p = 0.0123), while CT-CBD had lower levels than CT-Veh as well (p = 0.0068) (Figure 7D). No differences were found in IBA-1 expression among groups (Figure 7E).

These findings indicate that maternal obesity leads to hypothalamic inflammation in the offspring. Nonetheless, treatment with CBD reduced the gene expression of the proinflammatory cytokines.

4. Discussion

Genetic and epidemiological studies provide evidence supporting the contribution of a transgenerational background of parental obesity to the development of obesity itself and further metabolic risks in the offspring (13, 46–48). Other than understanding the underlying mechanisms through which parental obesity takes its toll on the offspring's health, an increasing body of research has been raising resolutive approaches. However, most of

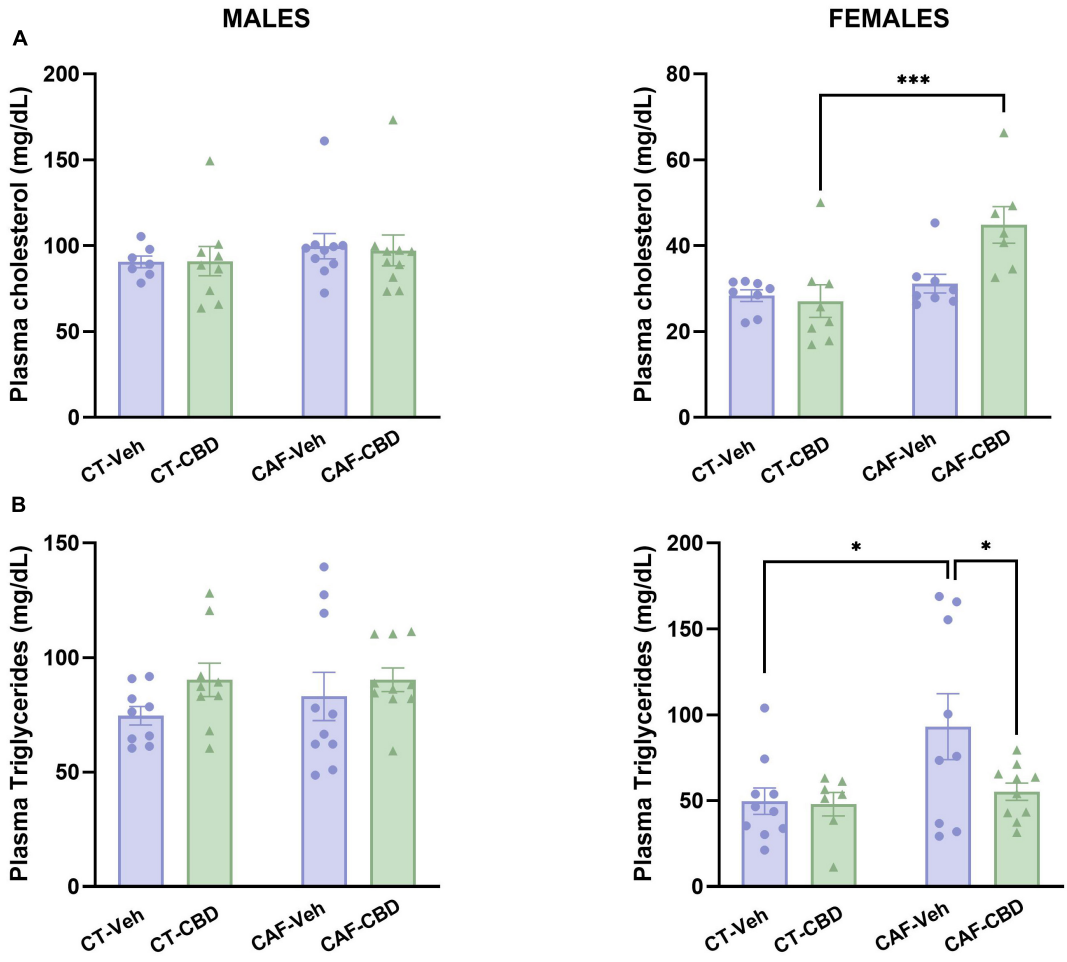

FIGURE 4
Offspring's plasma levels of total cholesterol and triglycerides. Female cafeteria diet (CAF)-cannabidiol (CBD) presented higher levels of plasma cholesterol than control diet (CT)-CBD **(A)**. Maternal diet increased triglyceride levels in females, but CBD treatment was able to revert this effect **(B)**. No differences were seen in males. Data are presented as mean ± SEM. n = 8–10/group. $^{*}p < 0.05$ $^{***}p < 0.001$.

them rely on preventive measures targeted at the pre-conception and/or gestational period (49). Here, to address the problem once the damage is already set, we investigated the effects of CBD treatment on the offspring as a way to attenuate the negative outcomes of maternal obesity.

An increasing number of studies have addressed the pleiotropic role of the endocannabinoid system on metabolic regulation at the central and peripheral levels. Endocannabinoid regulation of metabolism is extremely relevant to the central nervous system (CNS), especially in the hypothalamus where it plays a pivotal role on energy balance and feeding behaviors, contributing not only for the pathogenicity of obesity but also the development of eating disorders (34, 36–38, 50, 51). Nonetheless its receptors are also expressed in peripheral organs such as the adipose tissue, liver, skeletal muscle, pancreas, kidney, and gastrointestinal tract (52), hence its particularly promising modulation in the context of obesity and metabolic disorders (53, 54). Here, we show that CBD treatment is able to revert a number of metabolic dysfunctions and neuroinflammation arising from maternal consumption of CAF during pregnancy and lactation, including higher visceral adiposity, insulin resistance, endotoxemia, and overexpression of inflammatory markers in the hypothalamus of the offspring.

Besides obesity itself, the consumption of a diet rich in saturated fats and carbohydrates is also associated with the development of metainflammation, a chronic and self-sustained state of low-grade inflammation (55). In this study, we demonstrated that both male and female offspring of obese mothers had higher visceral WAT deposits and fasting glucose levels, followed by elevated plasma cholesterol and triglycerides levels in females and insulin in males. Previous studies with different models of maternal obesity have established that both the gestation and the lactation-suckling periods are critical for WAT development, impacting epigenetic regulation of key genes for energy metabolism–such as dopamine and opioid genes related to food behavior (56) and hypothalamic nutrient sensors (46)–and altering long-term adiposity set points (57, 58). In a model of maternal high-fat diet (HFD), the offspring of obese mothers showed an increased expression and activity of stearoyl-CoA desaturase-1 (SCD1), a key enzyme of fatty acid (FA) metabolism. SCD1 converts saturated FAs, such as palmitate and stearate, to monounsaturated FAs, the predominant substrates for triglyceride synthesis (59). It is important to highlight that CAF,

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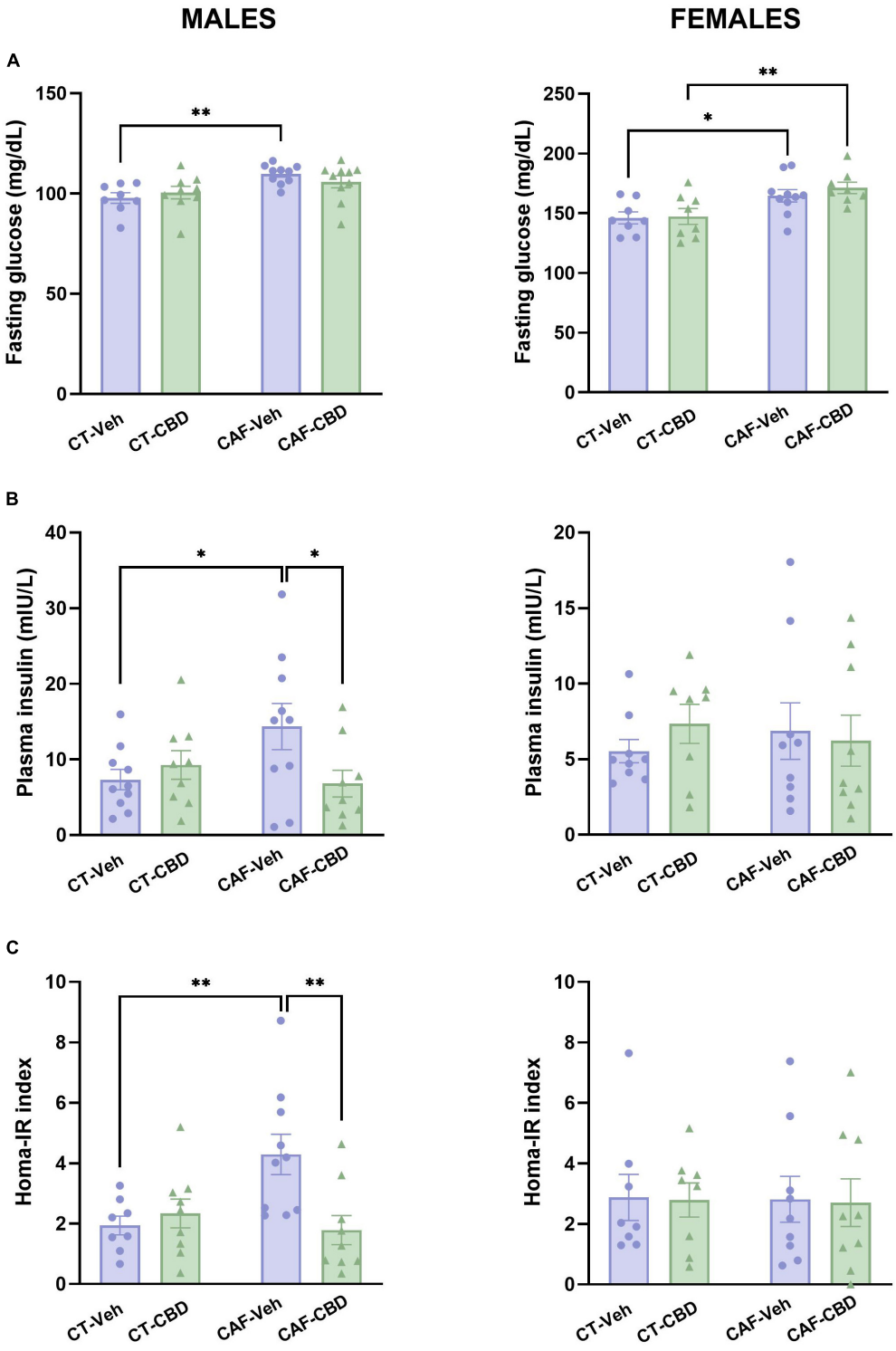

FIGURE 5
Plasma levels of glucose and insulin and calculated homeostatic model assessment (HOMA-IR) index of the offspring. Maternal obesity increased fasting glucose levels in both male and female offspring with no cannabidiol (CBD) effect **(A)**. Maternal obesity increased levels of plasma insulin in males, but CBD treatment was able to revert this damage **(B)**. Male cafeteria (CAF)-vehicle (Veh) presented an elevated HOMA-IR index, which was alleviated by CBD treatment **(C)**. Data are presented as mean ± SEM. n = 8–10/group. $^{*}p < 0.05$ $^{***}p < 0.01$.

which closely mimics the Western urban eating patterns, is not only high in sugar but also saturated fats, with palmitate being the most predominant FA, hence the prominent impact of this dietary pattern on lipid profile (60, 61).

Interestingly, no effect of maternal obesity was found on total body weight, even though WAT deposition was altered. This finding could be due to a diminished muscle mass that may have compensated for the heavier adiposity. In previous studies, both

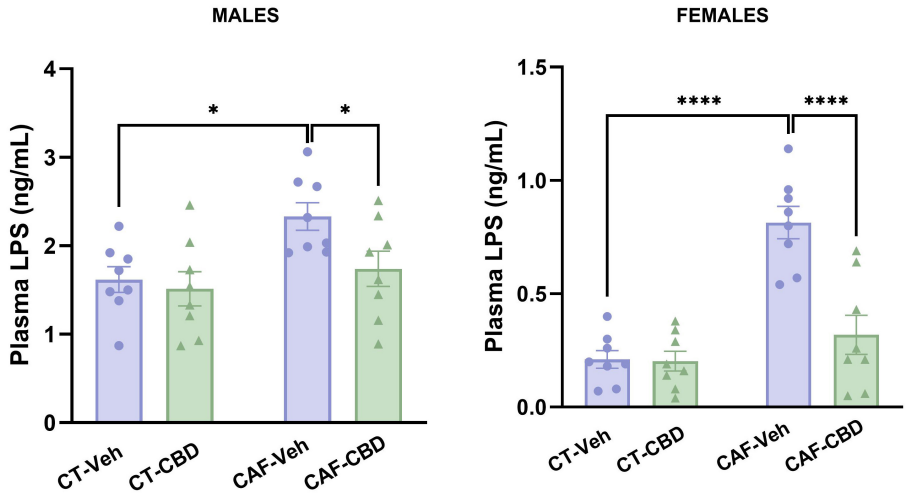

FIGURE 6
Plasma levels of lipopolysaccharides (LPS) of the offspring. Maternal obesity increased circulating LPS in both males and females, but cannabidiol (CBD) treatment was able to decrease its levels. Data are presented as mean ± SEM. n = 8–10/group. *$p < 0.05$ ****$p < 0.0001$.

3- and 12-weeks old pups from CAF-fed mothers showed equal or lower body weight and lean mass but greater fat accumulation than controls, which has been described as the thin-outside-fat-inside phenotype (62, 63). Also, since in our study the offspring were fed a normal diet after weaning, we were able to show that the metabolic impairments observed were independent of the offspring's own diet. However, in previous studies, when offspring of CAF-fed dams were given CAF after weaning, there was no increase in body weight at puberty (4 weeks of life), but animals had higher weight at adulthood (16 weeks of life), and no difference in visceral adiposity was reported in male offspring. Thus, differences in body composition seem to be dependent on the post-weaning diet as well as the sex of the offspring (64, 65).

Cannabidiol (CBD) treatment was able to mitigate most of the metabolic dysfunctions caused by maternal obesity, reducing visceral fat content and IR in males, plasma triglyceride levels in females, and plasma LPS in both sexes. CB1 receptor activation is generally considered a powerful orexigenic signal; thus, the endocannabinoid system's inhibition is beneficial for treating obesity and related metabolic diseases. Since CBD is an allosteric modulator of CB1 receptors, inhibiting its activation by endogenous ligands or exogenous agonists might trace the pathway through which CBD attenuates peripheral disturbances arising from maternal obesity (66). CB1-KO mice maintained on a normocaloric, standard diet have been shown to have a decreased body weight gain over time, which was associated with increased energy expenditure and elevated β(3)-adrenergic receptor and uncoupling protein-1 (UCP1) mRNA levels in the brown adipose tissue, suggestive of enhanced peripheral sympathetic activation and thermogenesis (66).

Diets high in saturated FAs, such as the Western diet, increase the uptake and storage of sphingolipids and their essential fractions, such as ceramide, sphingosine, sphinganine, and sphingomyelin. Interestingly, previous data suggest that phytocannabinoids and other agonists of CB1 or CB2 receptors can modulate sphingolipid concentrations in specific organs under the increased availability of FAs in the diet. CBD significantly lowered the concentration of sphingolipids in the adipose tissue (67), the skeletal muscle (31), and the brain by increasing catabolism, inhibiting salvage and/or *de novo* synthesis, which restores the tissue's insulin sensitivity and, therefore, attenuates IR (68). Other than that, other mechanisms are proposed for the beneficial effects of CBD on metabolic disorders in peripheral organs, such as the protective effect of CBD on adipose-derived stem cells against endoplasmic reticulum stress and its complications related to IR and diabetes (69) and attenuation of oxidative stress and inflammatory response, associated with an improved n-6/n-3 polyunsaturated fatty acids (PUFAs) ratio in the white and red skeletal muscle (70), indicating a narrow relationship between the endocannabinoid system and hormonal and energetic balance.

It is worth noting that the sexual dimorphisms observed in this study regarding glucose metabolism and IR corroborate with what has been demonstrated in different models of obesity and maternal obesity (71). Female sex hormones play a fundamental role in dimorphic insulin signaling since estrogens increase insulin sensitivity in metabolic tissues and upregulate insulin transcription in pancreatic beta cells as well as GLUT4 synthesis in adipose tissue and muscle (72). Female mice fed an HFD showed reduced susceptibility to developing obesity-induced IR and WAT inflammation when compared to HFD-fed males. Meanwhile, HFD-fed males treated with estradiol presented the same protective effect as females, indicating that the dimorphic effects of obesity on IR may be due to estrogen-mediated reductions in WAT inflammation (73). Furthermore, a recent study has shown that an androgen-driven gut microbiome may also be responsible for the increased susceptibility to IR in males since gut microbiome depletion abolishes sex-biased glucose metabolism in HFD-fed mice (74).

A growing body of evidence suggests that males are more sensitive to intrauterine hyperglycemia as well; hence both animal and human studies show the same pattern of higher risk for obesity and IR in male offspring of obese/diabetic mothers. In a model of maternal high-sucrose diet (HSD), female HSD offspring were shown to be more glucose intolerant, while male

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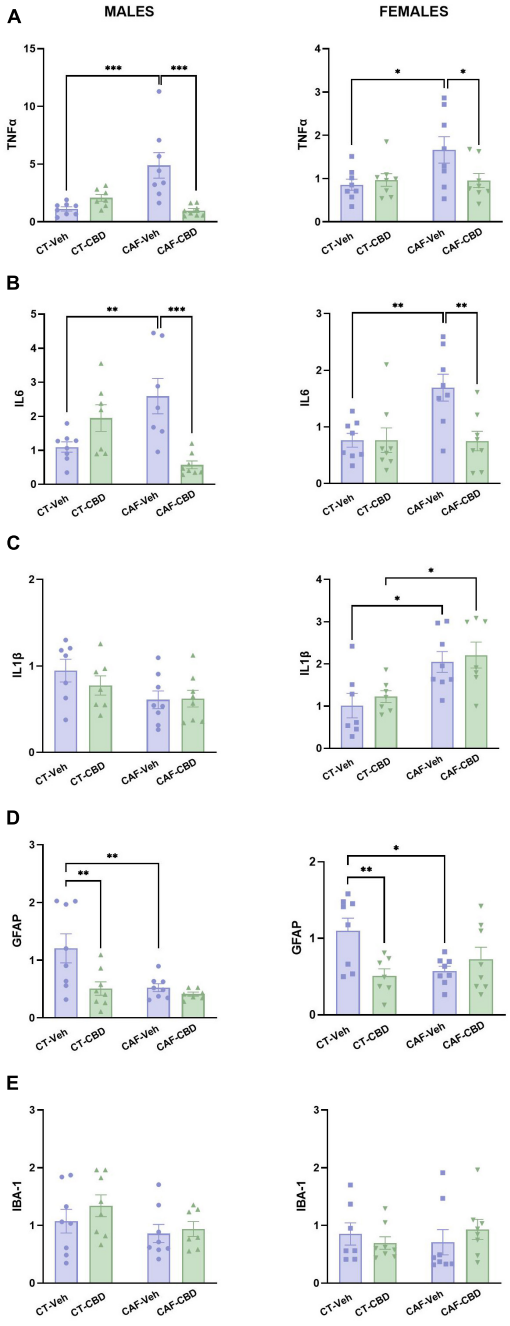

FIGURE 7

Relative gene expression of tumor necrosis factor-alpha (TNFα), interleukin 6 (IL6), interleukin 1β (IL1β), glial fibrillary acidic protein (GFAP), and ionized calcium-binding adapter molecule 1 (IBA1) in the hypothalamus of the offspring. Maternal obesity increased the expression of TNFα **(A)** and IL6 **(B)** in both males and females, with a positive effect of cannabidiol (CBD) treatment. Maternal obesity increased the expression of IL1β only in females, with no effect of CBD **(C)**. Both maternal obesity and CBD treatment decreased GFAP expression in males and females when compared to control diet (CT) **(D)**. No difference was found in IBA1 expression **(E)**. Data are presented as mean ± SEM. n = 6–8/group. $^{*}p < 0.05$ $^{**}p < 0.01$ $^{***}p < 0.001$.

counterparts were more insulin resistant (75). Furthermore, human cohort and prospective studies have shown a strong correlation between offspring metabolic impairments and maternal diabetes for males but not for females (76–78). This relationship may be explained by the fact that, during preimplantation, the male embryo is believed to have a greater ability to adapt to the adverse environment and, as a result, has a higher sensitivity to programming influences (79).

In addition, our results showed that CBD effectively reverted the increase in plasma LPS levels in both male and female CAF offspring. Obesogenic diets change the gut microbiota composition by altering the Firmicutes: Bacteroidetes ratio, the two most detected bacterial phyla in rodents as well as humans. In normal-weight animals this relation is characterized by a high ratio of Bacteroidetes to Firmicutes, while the opposite is found in obese counterparts (80, 81). It has been proposed that Firmicutes bacteria are more effective in extracting energy from food than Bacteroidetes, thus promoting a more efficient absorption of calories and boosting weight gain (82, 83). This imbalance resulting from obesogenic diets can be traced back to the overabundance of refined sugars and fats as well as the low intake of vegetables, fruits and dietary fibers (84–87). The simultaneous collapse of the gut barrier with increased permeability allows high levels of LPS, Gram negative bacteria's most potent immunogenic component, to reach the bloodstream and initiate a diffuse inflammatory process named endotoxemia (88). The structural components of LPS are recognized by B cells *via* cluster of differentiation 14 (CD14) and toll-like receptor 4 (TLR4), thus leading to nuclear factor kappa-B (NFkB) activation and the release of pro-inflammatory cytokines, such as TNFα and IL1β (89). During pregnancy, these pro-inflammatory mediators, together with LPS itself, can interact with the placenta and cause a range of disturbances, including immature blood vessels, hypoxia, increased inflammation, autophagy, and altered stress markers (90, 91). In that sense, several models of maternal immune activation rely on prenatal exposure to LPS, resulting in a myriad of altered physiological and neurological outcomes in the offspring (92, 93). The data seen here indicates that the endotoxemia caused by obesogenic diets affects not only the pregnant mother but can be seen in the offspring later on, independently of the offspring's own diet. These findings corroborate with previous studies demonstrating that maternal obesity during gestation and/or lactation negatively impacts the offspring's gut microbiota (94). On the other hand, CBD has rescued this damage on the offspring, lowering plasma LPS to control levels. Even though we have not performed gut-specific analysis, previous studies in different pre-clinical and clinical models lead us to infer that the effects observed may be due to the influence of CBD on gut microbiota composition (95–97) and/or a protective effect on maintaining gut barrier integrity (98–102).

Regarding the alterations provoked by maternal overnutrition on CNS neuroinflammation, here we show that the treatment with CBD was able to rescue hypothalamic inflammation by reducing gene expression of TNFα and IL6 in the offspring of obese dams. The hypothalamus is one of the main homeostatic centers of the CNS and, therefore, needs to be effectively responsive to fluctuations in peripheral systems. However, due to its naturally increased permeability in order to better receive and respond to signals coming from metabolic organs, the hypothalamus is also one of the first brain regions to suffer with systemic disruptions, resulting in neuroinflammation (103, 104). In the present study, we have demonstrated molecular alterations that

are trademarks of neuroinflammation in the hypothalamus of the offspring born from CAF-fed dams. In both sexes, the expressions of TNFα and IL6 were increased in CAF-Veh animals, while the expression of IL1β was increased only in females. These findings corroborate with previous data from a different model of maternal obesity that demonstrates that mice born from mothers fed a HFD diet have increased expression of these inflammatory markers in the hypothalamus compared to the offspring of lean parents (105).

Tumor necrosis factor-alpha (TNFα), IL6, and IL1β are well-known pro-inflammatory cytokines involved in microglial and astrocytic activation in the entire nervous tissue. However, especially in the hypothalamus, they have a remarkable role in the modulation of hypothalamic feeding circuits. It has been previously demonstrated that HFD and high-carbohydrate diets stimulate orexigenic neuropeptide Y/agouti-related peptide (NPY/AgRP) neurons to produce advanced glycation end products, which activate TNFα, enhancing microglia reactivity. This scenario results in the dysfunction of anorexigenic neurons, altering the appetite-regulatory circuits (106). In addition, Proopiomelanocortin (POMC) neurons, which present anorexigenic activities, also suffer a significant impact from maternal obesity (107). The melanocortin system plays an important role on the regulation of appetite, energy expenditure, and metabolism, therefore, impairments in the POMC and melanocortin 4 receptor (MC4R) pre- and post-translational processing are forerunners for the development of obesity (108, 109). Decreased activity in POMC cells has been shown to be associated with increased food intake and obesity (107) and has been demonstrated in the offspring of obese mothers (110–113). When observing the precise localization of NPY and POMC in the hypothalamus of the offspring of obese mothers, Ornellas and collaborators found that NPY nerve fibers from the ARC to the periventricular nucleus and around the third ventricle were increased, while POMC were diminished in the same areas (105). Variations in the reactivity and/or distribution of hypothalamic astrocytes also seem to affect synaptic organization and POMC responsiveness to glucose, which is associated with energy and metabolic imbalances (114, 115).

Although endocannabinoid signaling has been implicated in the modulation of both food intake and energy expenditure, a complete understanding of its role in the hypothalamus is still lacking. A recent study demonstrated that a HFD diet in CB1 receptor-deficient mice contributes to the offspring's nutritional programming, resulting in increased susceptibility to metabolic challenges both perinatally and during adulthood (116). Additionally, maternal HFD has been shown to upregulate CB1 hypothalamic expression in the offspring, which was associated with leptin pathway impairment and increased susceptibility to obesity (117–119). Other than that, few studies have evaluated cannabinoid modulation in the context of parental obesity, however, the findings shown here are still in line with different models that show the anti-inflammatory effects of CBD on other neuroinflammatory conditions (95, 120–122). Elevated hypothalamic endocannabinoid content has been associated with higher orexigenic signaling of ghrelin (123–125) and defective leptin signaling, observed in genetic models of obesity such as obese Zucker rats and *db/db* and *ob/ob* mice (126, 127). These findings suggest that endocannabinoid mediators contribute to hyperphagia and obesity, which also supports the restorative effects of CBD treatment, once it reduces endocannabinoid signaling, especially through CB1 receptors (128). When it comes to inflammation, effects of CBD *via* CB2 receptor are more distinguished, since this receptor is more predominantly expressed on immune cells, including glial cells. CB2 expression is upregulated in microglia stimulated with pro-inflammatory cytokines, indicating a significant role of CB2 in the regulation of neuroinflammatory states (129). In line with this, CBD has been shown to exert a CB2-dependant anti-inflammatory effect on microglial inflammation (23, 130).

Astrogliosis is a very well-established marker for obesity-related neuroinflammation (16, 131). Variations in the reactivity and/or distribution of hypothalamic astrocytes seem to affect synaptic organization and responsiveness to peripheral fluctuations, which is associated with energy and metabolic imbalances (114, 115). In animal models of obesity, gene and protein expression of the glial fibrillary acidic protein (GFAP), an astrocyte marker, are commonly higher in obese groups when compared to control animals (131–134). Interestingly, we have found that GFAP gene expression was reduced in the hypothalamus of the offspring of obese mothers. This result may have been induced by an adaptative reprogramming mechanism in response to the exposure to a harmful intrauterine environment during neurodevelopment, indicating that molecular mechanisms that rule maternal obesity-induced neuroinflammation may differ from the ones associated with obesity in the individual itself (135–137). Reduction in astrocyte expression can be deleterious during neurodevelopment since these cells play a pivotal role in synapse maturation, and their reduced expression is related to a range of neurological disorders (138–140). We have observed the same reduction of GFAP expression in CBD-treated CT offspring, however, we cannot affirm that the same detrimental effect applies. The reduction in GFAP expression of CAF offspring is a response to a prenatal immune challenge, while the reduction seen in CT-CBD is more likely to be the result of the anti-inflammatory activity of CBD (141, 142).

Regarding microglial activation, unlike previous studies (143, 144), we have not found any differences in the gene expression of ionized calcium-binding adapter molecule 1 (IBA1) in the hypothalamus of the offspring of obese mothers. However, the expression of IBA1 is related to the proliferation and distribution of microglial cells and not the polarization toward a pro-inflammatory state (145). Furthermore, it has recently been described that prenatal immune stress blunts microglia reactivity throughout life (146), which means that the expression levels of microglial cells may remain at control levels, but their innate reactivity to immune stressors can be defective.

These gene expression patterns are consistent with impaired energy and metabolic regulation in the hypothalamus, which might have originated the peripheral deficits observed in the offspring of obese mothers. Together, these results indicate an intricate interplay between peripheral and central counterparts in both the pathogenicity of maternal obesity and the modulation of the endocannabinoid system by CBD. In this context, the impairment of internal hypothalamic circuitry caused by neuroinflammation runs in tandem with the disruptions of important metabolic processes, which can be attenuated by CBD treatment in both ends.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by Comissão de Ética no Uso de Animais–Universidade Federal de Ciências da Saúde de Porto Alegre.

Author contributions

FR, MG, and RG: conceptualization. FR, JJ, GF, VD, SE, and TD: *in vivo* experimental procedures and *ex vivo* tissue and sample analysis. FR, JJ, and RG: statistical analysis. MG and RG: critical revision of the manuscript. All authors have writing, read, edited, and approved the final version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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3.2. Artigo científico 2

Cannabidiol improves maternal obesity-induced behavioral, neuroinflammatory and neurochemical dysfunctions in the juvenile offspring

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Cannabidiol improves maternal obesity-induced behavioral, neuroinflammatory and neurochemical dysfunctions in the juvenile offspring

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ABSTRACT

Maternal obesity is associated with an increased risk of psychiatric disorders such as anxiety, depression, schizophrenia and autism spectrum disorder in the offspring. While numerous studies focus on preventive measures targeting the mothers, only a limited number provide practical approaches for addressing the damages once they are already established. We have recently demonstrated the interplay between maternal obesity and treatment with cannabidiol (CBD) on hypothalamic inflammation and metabolic disturbances, however, little is known about this relationship on behavioral manifestations and neurochemical imbalances in other brain regions. Therefore, here we tested whether CBD treatment could mitigate anxiety-like and social behavioral alterations, as well as neurochemical disruptions in both male and female offspring of obese dams. Female Wistar rats were fed a cafeteria diet for 12 weeks prior to mating, and during gestation and lactation. Offspring received CBD (50 mg/kg) from weaning for 3 weeks. Behavioral tests assessed anxiety-like manifestations and social behavior, while neuroinflammatory and neurochemical markers were evaluated in the prefrontal cortex (PFC) and hippocampus. CBD treatment attenuated maternal obesity-induced anxiety-like and social behavioral alterations, followed by rescuing effects on imbalanced neurotransmitter and endocannabinoid concentrations and altered expression of glial markers, CB1, oxytocin and dopamine receptors, with important differences between sexes. Overall, the findings of this study provide insight into the signaling pathways for the therapeutic benefits of CBD on neuroinflammation and neurochemical imbalances caused by perinatal maternal obesity in the PFC and the hippocampus, which translates into the behavioral manifestations, highlighting the sexual dimorphism encompassing both the transgenerational effect of obesity and the endocannabinoid system.

1. Introduction

The nutritional transition that has been established in recent decades, with increased consumption of highly palatable ultra-processed foods, combined with reduced physical activities, favors the worldwide epidemic of obesity (Urbonaite et al., 2022 Jul; Lin and Li, 2021 Sep; Hasebe et al., 2021 Jan 15). Obesity is present at alarming rates in women of reproductive age (Hillemeier et al., 2011 May; Strauss et al., 2021 Dec), with studies showing that more than half of women in developed countries have a body mass index above that recommended by the World Health Organization (Rind et al., 2014 Mar).

Maternal malnourishment, both before and during pregnancy, has emerged as a global concern, known for its profound impact on fetal development and the subsequent long-lasting effects on the health and well-being of offspring (Drake and Reynolds, 2010 Sep; Nelson et al., 2020 Oct). Through the prolonged inflammation promoted by obesity, pregnant women may develop the so-called “maternal immune activation” (MIA), which is characterized by a range of mechanisms through which the stress suffered by the mother can critically affect the genetic programming of the offspring (Lier et al., 2019 May; Matuszewska et al.,

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2021 Sep 17). Remarkably, exposure to an obesogenic environment during prenatal development has been shown to affect the formation and maturation of the central nervous system (CNS), promoting brain changes already evident early in the intrauterine period (Jais and Brüning, 2017 Jan 3). Among the brain regions, the cerebral cortex and the hippocampus have been shown noteworthy impairment, with disturbances in the neural progenitor cells, an increase in oxidative stress, a decrease in dendritic projections and overall hampered synapsis maturation (Miller and Spencer, 2014 Nov; Guillemot-Legris and Muccioli, 2017 Apr). Consequently, epidemiological studies have shown evidence that a hampered CNS development due to the sustained pro-inflammatory state during perinatal development increases the risk of development of neurological disorders in the offspring (Osborne et al., 2019 Dec; Salas-Venegas et al., 2022 Mar), including schizophrenia (Khandaker et al., 2013 Feb), autism spectrum disorder, depression and anxiety (Zhu et al., 2020 Dec) and late bipolar disorder (Caron and Jane, 2021).

The extent to which interventions throughout life can mitigate the effects of perinatal maternal obesity remains unclear. However, some anti-inflammatory strategies appear promising. The endocannabinoid system modulation emerges as a prospective intervention once it intersects both inflammatory response and synaptic regulation in the CNS (Castillo et al., 2012 Oct). For instance, Type 1 Cannabinoid Receptors (CB1R) are present in the presynaptic membrane of dopaminergic, gabaergic, and glutamatergic neurons in the prefrontal cortex (PFC) and the hippocampus, showing a tight relationship with anxiety-related behavioral responses, sociability and memory (Peters et al., 2021 Jun; Patricio et al., 2020 Dec; Wang and Han, 2020). Moreover, Type 2 Cannabinoid Receptors (CB2R) are mostly expressed on glial cells and are known to modulate microglial and astrocytic activation (Zhang et al., 2022 Mar; Zhu et al., 2023 Dec 19; Li et al., 2023 May).

Cannabidiol (CBD), a non-psychotropic phytocannabinoid derived from *Cannabis sativa*, is recognized for its anti-inflammatory and antioxidant properties and is believed to be advantageous in various immunological states (Atalay et al., 2019 Dec 25; Borges et al., 2013 Oct 14; Fiani et al., 2020 Nov). In addition to acting as CB1R negative allosteric modulator and a CB2R agonist, CBD has been shown to interact with serotoninergic, dopaminergic, and glutamatergic receptors (Montoya-Alatriste and Alarcon-Aguilar, 2022; Martínez-Aguirre et al., 2023 Dec; Renard et al., 2017 Apr). Accordingly, it has been demonstrated to benefit a handful of neurological conditions, such as Parkinson's and Azheimer's disease, epilepsy, schizophrenia, and attention deficit hyperactivity disorder (Ibeas Bih et al., 2015 Oct; Brigida et al., 2017 Jul 3; Bar-Lev Schleider et al., 2019 Jan 17). Notably, CBD was also found to improve social behavior, decrease anxiety, and stimulate communication in ASD patients (Aran et al., 2019 Mar), which sets the stage for it as a potential intervention on neurological and behavioral dysfunctions stemming from perinatal exposure to an aversive context.

Importantly, although maternal obesity and the endocannabinoid system have been extensively explored in a myriad of models, most studies focus on the male offspring. However, both perinatal exposure outcomes and the endocannabinoid system show sexually dimorphic interactions (Rubino and Parolaro, 2011; Osborne et al., 2019 Oct). Thus, here we investigated the effects of CBD treatment on maternal obesity-related anxiety-like behaviors, social interaction and memory, glial markers, neurotransmitters and endocannabinoids in the PFC and hippocampus of both male and female offspring.

2. Materials and methods

2.1. Animals

Twenty four female Wistar rats (3-week-old), were acquired from the animal facility at the Federal University of Health Sciences of Porto Alegre (UFCSPA). These rats were accommodated in group housing, with three animals per cage, within standard laboratory settings, maintaining a controlled temperature of 23 ± 1 °C and a 12-hour light: dark cycle switching at 7 am and 7 pm. The study protocol received approval from the UFCSPA Institutional Animal Care and Use Committee (N° 751/21). All experimental procedures were executed according to international regulations governing the ethical treatment of laboratory animals, with a primary focus on minimizing both the number of animals involved and any potential suffering.

2.2. Experimental groups and diet

Three-week-old female breeders (N = 12) were randomly assigned to either a control diet (CT) comprising standard chow (Nuvilab® CR-1 Nuvital®, Curitiba, PR, Brazil) (3.4 kcal/g, 63 % carbohydrates, 26 % protein, and 11 % fat) or a Cafeteria Diet (CAF) incorporating standard chow along with bacon mortadella (Perdigão®), strawberry wafers (Isabela®), chocolate cookies (Isabela®), pizza-flavored crackers (Parati®), white chocolate (Harald®), sausage (Alibem®), and orange-flavored soda (Sukita®) (4.3 kcal/g, 43 % carbohydrates, 14 % protein, and 43 % fat), provided with *ad libitum* access to water. The CAF group received three menus featuring different combinations of the mentioned foods, rotated every 2 days to sustain novelty and encourage consumption. CAF was selected as an obesogenic diet due to its ability to emulate Western dietary habits more accurately than conventional high-fat and/or high-sugar diets, offering a variety of textures, options, and palatability conducive to hedonic eating, which are absent in manufactured chows (Johnson and Wardle, 2014; Reichelt et al., 2014). The diets were administered for 12 weeks leading up to and during mating with 3-month-old Wistar males, continuing through gestation, lactation, and until weaning. Weekly records were maintained for dam weight and the weight of consumed diets. The day of parturition was designated as postnatal day zero (PND0).

Litters were standardized to seven to nine pups per dam, maintaining an equal proportion of males to females whenever possible. Offspring were weaned at postnatal day 21 (PND21), transitioned to standard chow, and subjected to weekly weight monitoring. Litters were evenly distributed with equal numbers of littermates allocated to each treatment group, resulting in four groups per sex (N = 7–10): CT mother + Vehicle (CT-Veh), CT mother + Cannabidiol (CT-CBD), CAF mother + Vehicle (CAF-Veh), and CAF mother + Cannabidiol (CAF-CBD).

The treatment started on the same day as weaning (PND21) and consisted of CBD oil diluted in corn oil at a dose of 50 mg/kg (Prati-Donaduzzi®, Toledo, PR, Brazil) or corn oil (vehicle), both administered via oral gavage at a volume of 1 mL/kg. This treatment regimen was implemented seven days a week for a duration of 3 weeks (Fig. 1). Treatment was continued throughout behavioral testing but given at least 2 h after test completion, to avoid any potential acute effects (Deiana et al., 2012 Feb; Schwotzer et al., 2022 Oct 26;can.2022.0121.; Hložek et al., 2017 Dec). The choice of treatment dose and duration was based on prior studies involving different cognitive-assessment models with oral CBD administration (Millar et al., 2019 Sep; Watt et al., 2020 Apr 7; Trivedi et al., 2022 Feb; Witherspoon et al., 2022 Oct; Santiago-Castañeda et al., 2022 Aug).

2.3. Behavioral testing

Behavior was assessed between 9:00 am and 5:00 pm on the third week of treatment. For all behavioral tests, animals were acclimated in the room for an hour prior to the onset of testing. The apparatus were thoroughly cleaned with 70 % ethanol followed by distilled water between animals to remove any residual odors in the equipment. For all behavioral tests, data were recorded using a Sony Handycam camera mounted overhead. The analysis was made with ANY-maze tracking software (Stoelting Co.; Wood Dale, IL, USA). Specific regions of interest were defined digitally in the software and the center body and head of the animals were tracked for the duration of the tests in order to calculate behavioral measurements from the recorded video files.

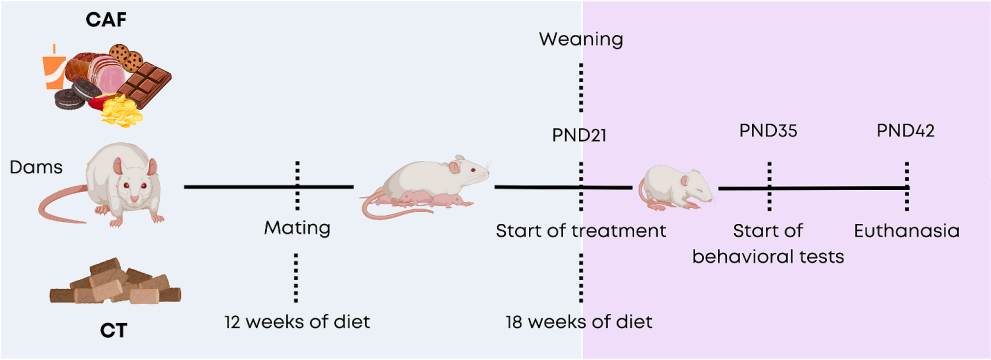

Fig. 1. Experimental design. CT, control chow-fed dam; CAF, cafeteria diet-fed dam; PND, post-natal day.

2.3.1. Elevated plus maze (EPM)

Animals were placed into the maze structure made of 2 open arms (25 x 5 x 0.5 cm) and 2 closed arms (25 × 5 × 16 cm), located 50 cm above the floor for 5 min to assess anxiety-like behavior. Primary measures of anxiety-like behavior were latency to first entry in the open arms, number of entries in the open arms and time spent in the open arms.

2.3.2. Open field (OF)

Open field was performed after a 48 h interval following EPM. Animals were placed into a black acrylic arena (60 × 60 × 40 cm) for 10 min to assess anxiety-like and exploratory behavior. The apparatus was virtually divided into quadrants delimiting a central field and lateral quadrants, closer to the walls. Locomotor activity was measured as the total distance traveled. Primary measures of anxiety-like behavior were latency to first entry in the center zone, number of entries in the center zone, time spent in the center zone, number of freezing episodes, total freezing time and number of fecal boli expelled during the test.

2.3.3. Social preference

Social behavior was assessed on the next day after OF on the same apparatus, as the previous task would serve as habituation to the chamber. Two clear perforated acrylic cylinders were placed in opposing sides of the arena and animals were initially acclimated to the box and empty cylinders for five minutes and then placed in temporary holding cages. A sex-matched naive juvenile rat of the same strain (J1) was placed in one of the inverted cylinders and a neutral object (OBJ) was placed in the other. The perforations in the cylinders enabled visual, olfactory, and auditory interactions between J1 and experimental animals. Animals were subsequently monitored for five minutes in the presence of the encaged conspecific. Virtual circular regions encompassing the cylinders were defined using ANY-maze software and the head of the test animal was tracked to determine olfactory exploration of the juvenile or the object. Social preference was expressed as the percentage of exploration of J1 of total exploration time, as follows:

[(Time exploring J1 / (Time exploring J1 + Time exploring OBJ)] x100.

2.3.4. Social recognition

Social recognition test was conducted on the day following the social preference test, to evaluate if the animals were able to distinguish between familiar and unfamiliar conspecifics. Animals were placed in the same chamber containing the same juvenile (J1) in its original cylinder and a novel juvenile (J2) in the cylinder previously containing the object. Test animals were monitored for five minutes in the presence of both familiar and new encaged conspecifics (Garrido Zinn et al., 2016 Aug 16 [cited 2024 Jan 18];113(33).). Social recognition memory was expressed as the percentage of exploration of J2 of total exploration time, as follows:

[(Time exploring J2 / (Time exploring J1 + Time exploring J2)] x100.

2.4. Tissue processing

After behavioral tests, on PND42, animals were euthanized by decapitation. The brain was dissected immediately and snap-frozen in liquid nitrogen and stored in − 80 °C for further processing and analysis. The entire hippocampus of a hemisphere was used for RNA extraction. The prefrontal cortex was determined as the first 3 mm of the frontal portion of the cortex, which was divided between RNA extraction and neurotransmitter/endocannabinoid analysis. Euthanasia was performed without administration of treatment on that day, in order to minimize acute effects.

2.5. Maternal biochemical profiling

Dams' plasma levels of fasting glucose, total cholesterol and triglycerides were quantified using enzymatic colorimetric kits (Labtest, Lagoa Santa, Brazil). Insulin levels in the plasma were determined by enzyme-linked immunosorbent assay (ELISA) (Insulin ELISA kit, Cat# RAB0904; Sigma-Aldrich, St. Louis, MO, USA). Subsequently, the homeostatic model assessment (HOMA-IR) index was calculated to determine insulin resistance through the following formula: glucose (mg/dL) × insulin (uU/mL)/22.5.

For LPS quantification, plasma (150 µL) was hydrolyzed with 75 µL of NaCl 150 mM and 300 µL of HCl 8 M and then incubated for 4 h at 90 °C. Afterward, 3 mL of hexane were added, and samples were centrifuged at 3,500 rpm for 10 min. The upper organic phase was withdrawn, and the residue was reconstituted in 50 µL of methanol, transferred to a vial, and an aliquot of 3 µL was injected into the analytical system. A Nexera-i LC-2040C Plus system coupled to a LCMS-8045 triple quadrupole mass spectrometer (Shimadzu, Kyoto, Japan) was used for the analysis.

2.6. RT-qPCR

Total RNA was isolated from the PFC and the hippocampus using TRIzol® (Invitrogen, Brazil) according to the manufacturer's instructions. The quantification of total RNA was done by spectrometry BioSpec-nano® (Shimazu, Kioto, Japan) at 260 and 280 nm. For cDNA synthesis, 1000 ng of RNA were reverse transcribed according to the manufacturer's instructions (GoScript Reverse Transcription Kit, Promega, Brazil). To conduct real time quantitative polymerase chain reaction (RT-qPCR), cDNA was added to a reaction mix (10 µL final volume) containing 100 nM gene-specific primers and universal SYBR green supermix (Applied Biosystems, Thermo Fisher Scientific CA, USA). All samples were run in duplicate and were analyzed on an QuantStudio Real-Time PCR instrument (Applied Biosystems, Thermo Fisher Scientific CA, USA) for quantitative monitoring of PCR product formation.

Relative gene expression was normalized to β-Actin controls and assessed using the 2-ΔΔCT method taking CT-Veh groups as the reference group within sexes. Primer sequences are as follows: β-Actin: F: TATGCCAACACAGTGCTGTCTGG; β-Actin: R: TACTCCTGCTTGCTGATCCACAT; IBA-1: F: GCAAGGATTTGCAGGGAGGA; IBA-1: R: CGTCTTGAAGGCCTCCAG TT; GFAP: F: CGAAGAAAACCGCATCACCA; GFAP: R: CC GCATCTCCACCGTCTTTA; CB1R: F: CACTCTGGCATCAGGGTTATC; CB1R: R: CATCTGGTAGTTGGGCCTATTT. DRD2: F: CTCAGGAGCTGGAAATGGAG; DRD2: R: CATGCCCATTCTTTTCTGGT; OXTR: F: CGATTGCTGGGCGGTCTTCA; OXTR: R: CCGCCGCTGCCGTCTTGAG.

2.7. Neurotransmitter quantification

An aliquot of 50 mg of PFC tissue was homogenized in 500 μL of 2 % formic acid. An aliquot of 50 μL of the homogenate was transferred to plastic centrifuge microtubes, followed by the addition of 930 μL of acetone and 20 μL of analyte internal standard (250 ng/mL). The sample was vortexed for 30 s, centrifuged for 6 min at 10,000 g and 880 μL of the supernatant was collected, dried under nitrogen flow at room temperature, and then resuspended in 50 μL of ultrapure water. 20 μL were injected into the liquid chromatograph (Nexera UFLC) coupled to an LCMS-8045 triple quadrupole mass spectrometer (Shimadzu, Japan). Mass spectrometer optimization was performed by evaluating source temperature, capillary energy, nebulization flow and collision-induced dissociation gas flow, in addition to MRM transitions optimizations for the following analytes: gamma-aminobutyric acid (GABA), glutamate and homovanillic acid (HVA).

2.8. Endocannabinoid quantification

Endocannabinoid quantification was performed as previously described (Lehtonen et al., 2011 Apr). Briefly, 20 mg of tissue was homogenized in 500 μL ice-cold methanol. Lipids were extracted by adding chloroform and water to yield a methanol/chloroform/water ratio of 1:2:1 (v/v/v), and centrifuged at 1500 × g for 10 min at 10 ∘C to achieve a sharp phase separation. The lower organic layer was transferred to a new centrifuge tube. The liquid extraction was repeated once by adding 1 mL chloroform to the aqueous layer that was left in the original tube followed by a second centrifugation, and the organic layers were combined. The samples were then evaporated to dryness under forced airflow at room temperature and the residue was reconstituted in 50 μL of ice-cold acetonitrile. The sample was allowed to dissolve for 5 min and then 20 μL of water was added. After another centrifugation at 12,000 × g for 10 min at 10 ∘C, the supernatants of the samples were then transferred to an HPLC sample vials.

A Nexera-i LC-2040C Plus system coupled to a LCMS-8045 triple quadrupole mass spectrometer (Shimadzu, Kyoto, Japan) was used for the analysis. 10 μL of the sample solution was injected onto a reverse-phase HPLC column (Shim-pack gist C18 2.1 mm x 100 mm, 1.9 μm) (Shimadzu, Kyoto, Japan), using an isocratic mobile phase consisting of H2O/ACN/formic acid (33:67:0.1, v/v/v), delivered at 150 μL/min. Standard curves were performed using 2-Arachidonoyl glycerol (2-AG) – (Avanti Polar Lipids #870450) and Anandamide (AEA) – (Avanti Polar Lipids #870430).

2.9. Data analysis and statistics

Data were analyzed using Graphpad Prism 9 statistical software (GraphPad Software, San Diego, CA, USA). Unpaired t tests were used to compare dam's variables, between CT and CAF groups. Two-way ANOVA with a Bonferroni post hoc analysis was performed on the offspring's data. The main effects analyzed were maternal diet and CBD treatment. The interaction between these two factors was also analyzed. The results were expressed as the mean ± standard error of the mean (SEM). Outliers were removed using the ROUT test, and statistically significant differences were considered at $p < 0.05$.

3. Results

3.1. Cafeteria diet induces obese phenotype and hyperphagia in female Wistar rats

CAF-fed dams' weight was significantly higher than CT group from the 9th week of diet, as previously reported (da Rodrigues et al., 2023 Mar), which was maintained until the last week of gestation ($P = 0.0012$) (Fig. 2A), together with a higher weekly caloric intake from the second week of diet (Fig. 2B). CAF dams also presented increased plasma concentrations of fasting glucose ($P = 0.0459$) (Fig. 2C) and insulin ($P = 0.0456$) (Fig. 2D), resulting in an elevated HOMA insulin resistance index ($P = 0.0293$) (Fig. 2E). Additionally, CAF diet increased plasma concentrations of total cholesterol ($P = 0.05$) (Fig. 2F), triglycerides ($P = 0.0494$) (Fig. 2G) and lipopolysaccharides ($P = 0.0295$) (Fig. 2H). These parameters are a consistent indication that the CAF-fed dams presented an obese phenotype.

3.2. Cannabidiol treatment reverses increased anxiety-like behavior in the offspring of obese dams

To determine if maternal obesity induced by a Cafeteria diet (CAF) contributed to increased anxiety-like behavior and if the treatment with Cannabidiol (CBD) had any effects on this parameter, we performed the open field (OF) and the elevated plus maze (EPM) tests in the offspring.

In male offspring, we did not find differences in the latency to enter the center zone of the OF (Fig. 3A). However, there was an increased number of freezing episodes in the offspring of obese dams, which was reduced with CBD treatment (interaction $F_{1,32} = 5.781$; $p = 0.00222$; maternal diet effect $F_{1,32} = 5.301$; $p = 0.00280$; CT-Veh vs. CAF-Veh $p = 0.0045$; CAF-Veh vs. CAF-CBD $p = 0.0280$) (Fig. 3B). Freezing time (interaction $F_{1,32} = 8.728$; $p = 0.0058$; CBD effect $F_{1,32} = 5.614$; $p = 0.0240$; CT-Veh vs. CAF-Veh $p = 0.038$; CAF-Veh vs. CAF-CBD $p = 0.001$) (Fig. 3C) as well as the number of fecal boli expelled during the test (interaction $F_{1,32} = 4.998p = 0.0325$; CT-Veh vs. CAF-Veh $p = 0.0438$; CAF-Veh vs. CAF-CBD $p = 0.0377$) (Fig. 3D) showed a similar pattern, demonstrating the effect of CDB treatment in reducing anxiety-like behavior. In female offspring, CAF-Veh presented an elevated latency to enter the center zone of the OF for the first time (interaction $F_{1,28} = 8.171$; $p = 0.0079$; CT-Veh vs. CAF-Veh $p = 0.0315$; CAF-Veh vs. CAF-CBD $p = 0.0386$) (Fig. 3A), as well as an increase in the number of freezing episodes (interaction $F_{1,31} = 6.042$; $p = 0.0198$; CT-Veh vs. CAF-Veh $p = 0.0372$; CAF-Veh vs. CAF-CBD $p = 0.0324$) (Fig. 3B) and freezing time (interaction $F_{1,30} = 9.269$; $p = 0.0048$; CT-Veh vs. CAF-Veh $p = 0.0074$; CAF-Veh vs. CAF-CBD $p = 0.0056$) (Fig. 3C). Again, CBD treatment exerted a positive effect, reducing all three alterations in female CAF-CBD. No differences were found in the number of fecal boli in female offspring (Fig. 3D). No differences were seen for total distance traveled, number of entries in the center zone and the time spent in the center by both males and females (data not shown).

In the EPM, male CAF-Veh showed fewer entries (interaction $F_{1,32} = 7.348$; $p = 0.0107$; CT-Veh vs. CAF-Veh $p = 0.0437$; CAF-Veh vs. CAF-CBD $p = 0.0289$) (Fig. 4B) and reduced permanence (interaction $F_{1,31} = 9.960$; $p = 0.0035$; CT-Veh vs. CAF-Veh $p = 0.0433$; CAF-Veh vs. CAF-CBD $p = 0.0033$) (Fig. 4C) in the open arms of the apparatus when compared to CT-Veh, which was restored to control levels in CAF-CBD animals when compared to CAF-Veh. Female CAF offspring presented an elevated latency to enter the open arms for the first time (maternal diet effect $F_{1,27} = 11.56$; $p = 0.0021$; CBD effect $F_{1,27} = 6.144$; $p = 0.0197$; CT-Veh vs. CAF-Veh $p = 0.0219$), with no post hoc differences between CAF-Veh and CAF-CBD (Fig. 4A), and no differences in the number of entries (Fig. 4B) or permanence (Fig. 4C) in the open arms.

Together, these data suggest that maternal obesity leads to exacerbated anxiety-like behavior in the offspring, and that treatment with

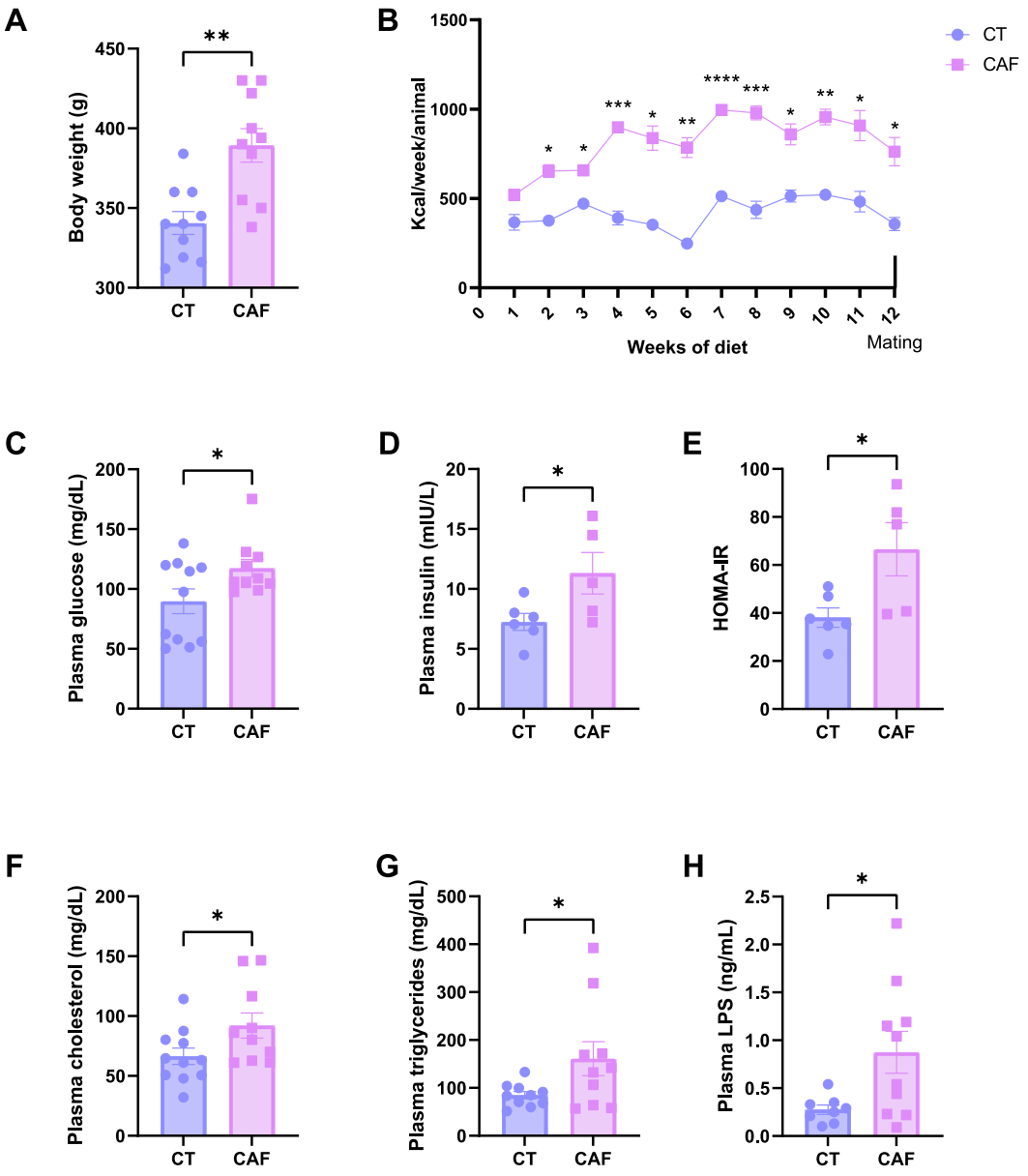

Fig. 2. Effects of CAF diet on body weight, metabolic phenotype and caloric intake of dams. CAF dams presented increased body weight at the end of gestation (A), increased weekly caloric intake (B), increased concentrations of plasma glucose (C) and insulin (D), with a higher resulting HOMA-IR (E), as well as increased cholesterol (F), triglycerides (G), and LPS (H) when compared to CT. n = 10–12/group. *p < 0.05 **p < 0.01 ***p < 0.001 ****p < 0.0001.

cannabidiol is able to mitigate most of the behavioral alterations.

3.3. Cannabidiol treatment reverses sex-specific social alterations caused by maternal obesity

To determine if CAF-induced maternal obesity contributed to decreased social interaction and/or social memory and if CBD treatment exerted any behavioral effect, the offspring were analyzed in the social preference and memory tests. Regarding social preference, no significant differences were found in males (Fig. 5A). However, in female CAF-Veh there was a lower preference for exploring the juvenile (J1) in detriment of the object (OBJ) and CBD treatment was able to improve social preference (maternal diet effect $F_{1,29} = 6.186$; $p = 0.0189$; CBD effect $F_{1,29} = 4.701$; $p = 0.0385$; CT-Veh vs. CAF-Veh $p = 0.0167$; CAF-Veh vs. CAF-CBD $p = 0.0184$) (Fig. 5A).

In social memory, on the other hand, a difference was found only in male offspring, with CAF-Veh showing a lower preference for exploring the novel juvenile (J2) in detriment of the familiar one (J1) when compared to CT-veh. Again, exploration levels were restored in CAF-CBD males (CBD effect $F_{1,31} = 4.317$; $p = 0.0461$; interaction $F_{1,32} = 6.697$; $p = 0.0128$; CT-Veh vs. CAF-Veh $p = 0.0275$; CAF-Veh vs. CAF-CBD $p = 0.0040$) (Fig. 5B). No differences were found in female offspring regarding social memory.

These results indicate that maternal obesity promotes a reduced sociability in female offspring, as well as impaired social memory and ability to recognize familiar individuals in male offspring. In both cases, CBD treatment was able to reverse both the interaction and memory deficits caused by maternal diet.

3.4. Cannabidiol treatment partially reverts neuroinflammatory and neurochemical changes associated with maternal obesity in the offspring's prefrontal cortex and hippocampus

RT-qPCR was conducted to evaluate gene expression of the glial markers Ionized calcium binding adaptor molecule 1 (IBA-1) and Glial fibrillary acidic protein (GFAP), as well as cannabinoid receptor 1

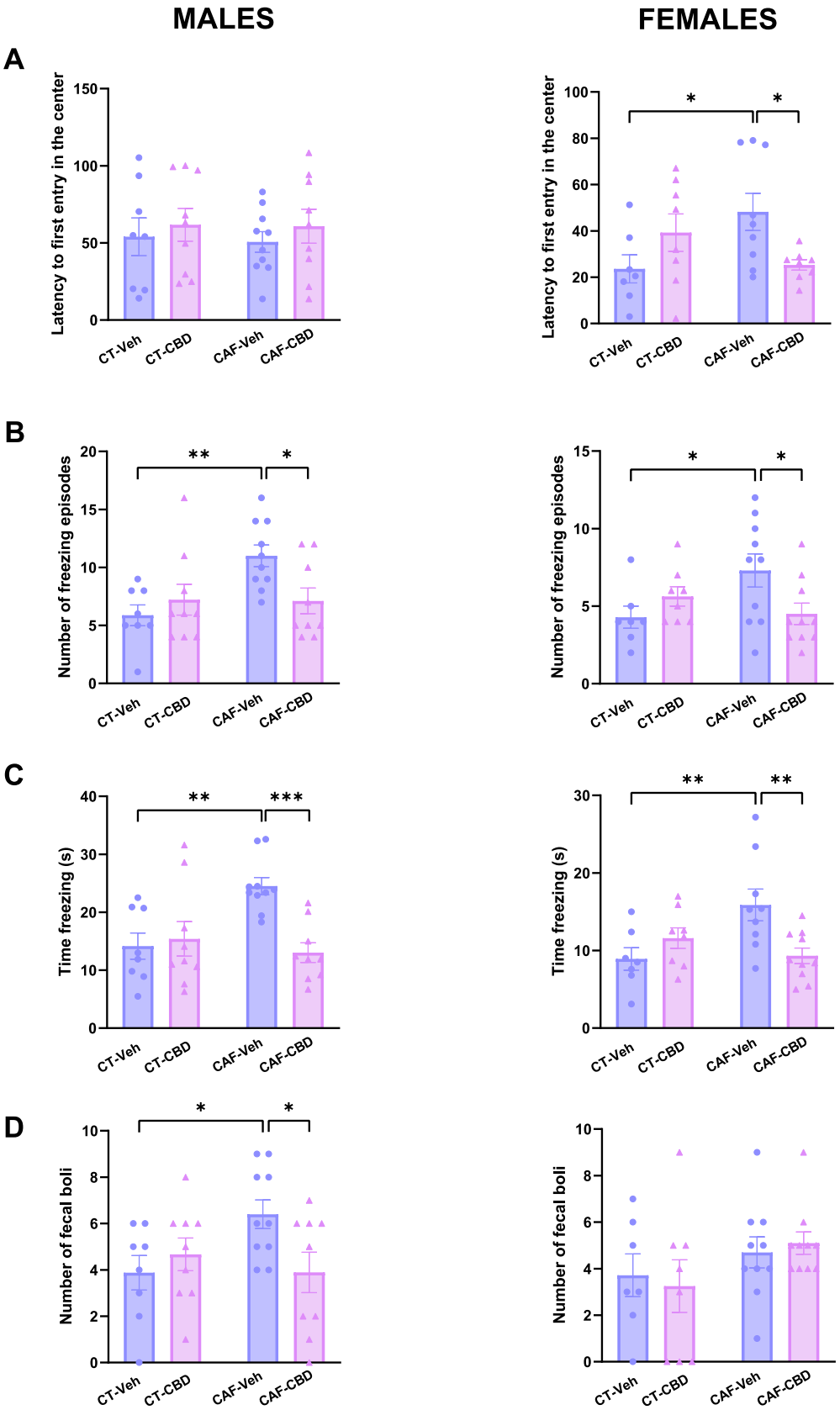

Fig. 3. Effects of CBD treatment on anxiety-like behaviors in the Open Field in the offspring of obese dams. Male CAF-Veh offspring showed increased number of freezing episodes (B), time freezing (C) and number of fecal boli (D), while female CAF-Veh offspring showed increased latency to first entry in the center (A), freezing episodes (B) and time freezing (C). All of which was attenuated by CBD treatment on the CAF-CBD group. Data are presented as mean ± SEM. n = 8–10/group. *p < 0.05 **p < 0.01 ***p < 0.001.

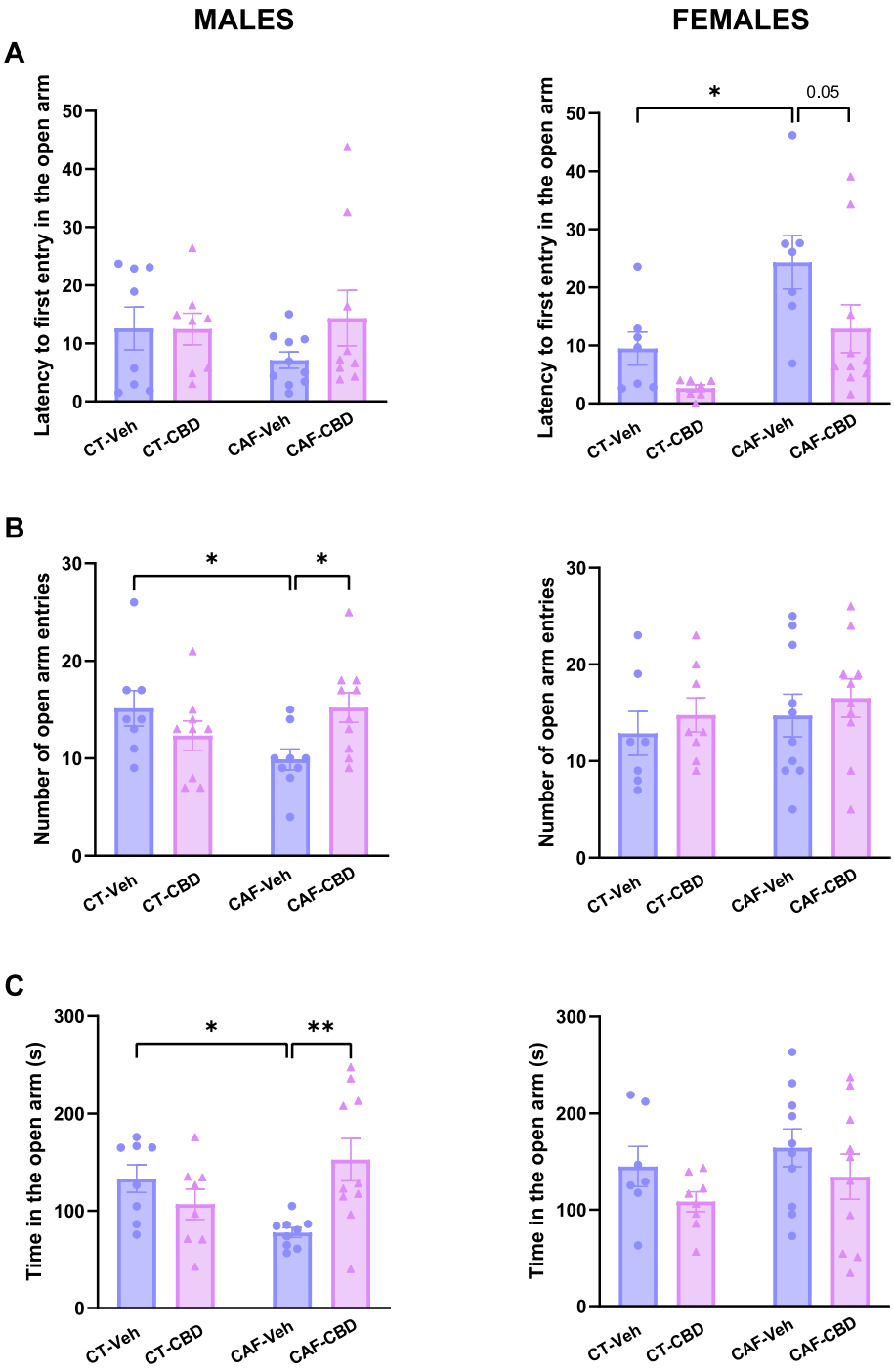

Fig. 4. Effects of CBD treatment on anxiety-like behaviors in the Elevated Plus Maze in the offspring of obese dams. Male CAF-Veh offspring showed reduced number of open arm entries (B) as well as reduced time spent in the open arms (C), while female CAF-Veh offspring showed increased latency to first entry in the open arm (A). CBD treatment was able to attenuate the behavioral disturbances on male CAF-CBD. Data are presented as mean ± SEM. n = 8–10/group. *p < 0.05 **p < 0.01.

(CB1R), dopamine receptor 2 (DRD2) and oxytocin receptor (OXTR) in the PFC and hippocampus of the offspring. In the PFC of male offspring, CBD treatment reduced the overexpression of IBA-1 in the offspring of obese dams when compared to CAF-Veh animals (interaction $F_{1,23}$ = 6.142; p = 0.0210; CBD effect $F_{1,23}$ = 8.202; p = 0.0088; CAF-Veh vs. CAF-CBD p = 0.0016) (Fig. 6A). Male CAF-Veh also presented increased gene expression of GFAP (interaction $F_{1,23}$ = 27.35; p < 0.0001; maternal diet effect $F_{1,23}$ = 14.54; p = 0.0009; CBD effect $F_{1,23}$ = 8.041; p = 0.0094; CT-Veh vs. CAF-Veh p < 0.0001; CAF-Veh vs. CAF-CBD p < 0.0001) (Fig. 6B), CB1R (interaction $F_{1,23}$ = 10.07; p = 0.0041; maternal diet effect $F_{1,23}$ = 5.457; p = 0.0286; CBD effect $F_{1,23}$ = 7.030; p = 0.0143; CT-Veh vs. CAF-Veh p = 0.0012; CAF-Veh vs. CAF-CBD p = 0.0007) (Fig. 6C), DRD2 (maternal diet effect $F_{1,21}$ = 5.396; p = 0.0303; CT-Veh vs. CAF-Veh p = 0.0319) (Fig. 6D) and OXTR (interaction $F_{1,22}$ = 8.652; p = 0.0076; CT-Veh vs. CAF-Veh p = 0.0029; CAF-Veh vs. CAF-CBD p = 0.0077) (Fig. 6E) when compared to CT-Veh, while CBD treatment attenuated alterations on GFAP, CB1R and OXTR, but not DRD2 expression.

In the PFC of female offspring, CAF-CBD animals presented higher levels of IBA-1 mRNA when compared to CT-CBD, however no

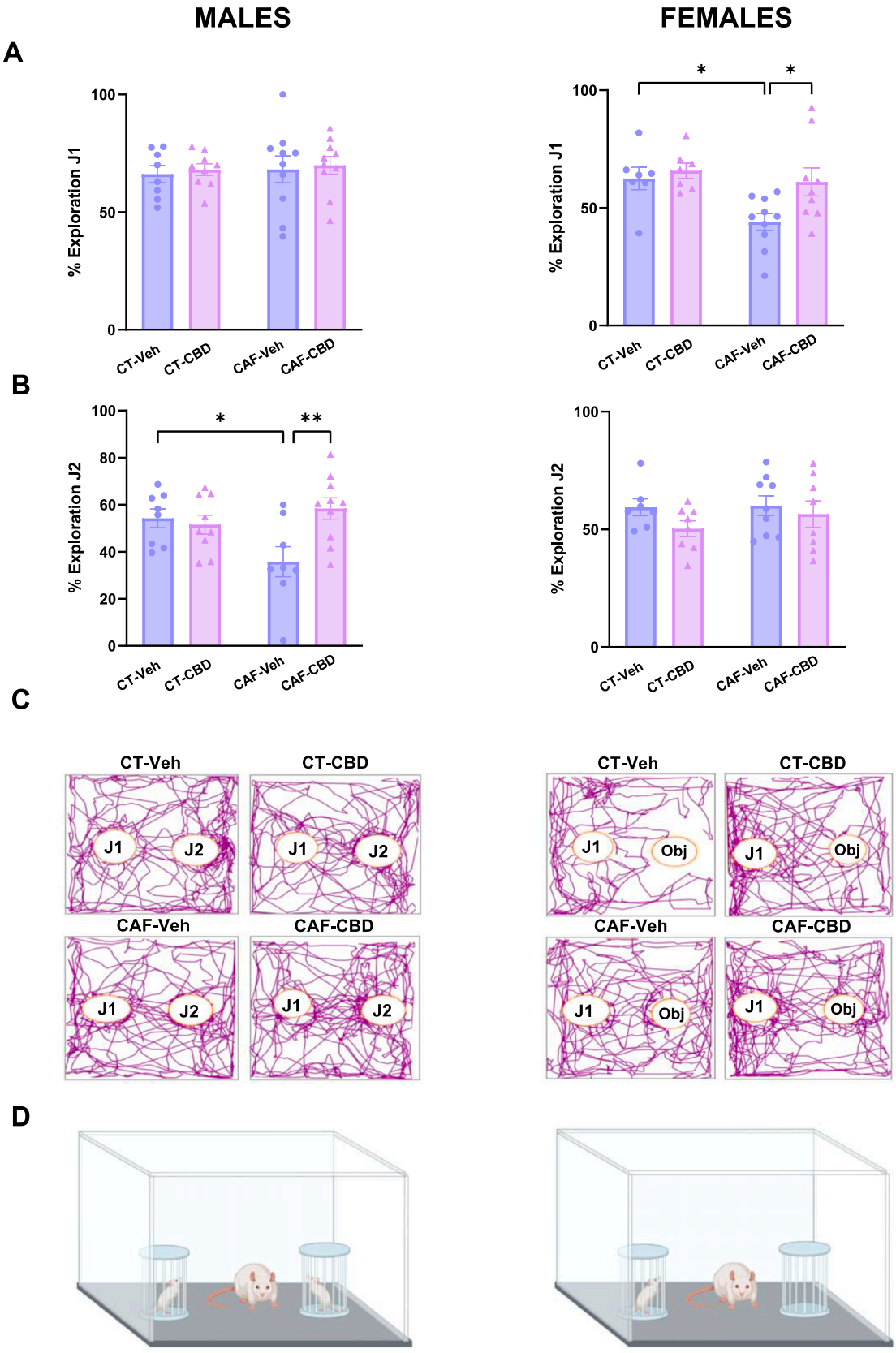

Fig. 5. Effects of CBD treatment on social behaviors in the offspring of obese dams. Female CAF-Veh offspring showed reduced preference for exploring the conspecific compared to the object (A), while male CAF-Veh offspring showed reduced exploration of the new juvenile compared to the familiar one (B). CBD treatment was able to attenuate the behavioral disturbances in both cases. Track plot showing animals exploration during social memory (males) and social preference (females) tests (C). Visual schematics of the tests represented on the track plots (D). Data are presented as mean ± SEM. n = 8–10/group. *p < 0.05 **p < 0.01.

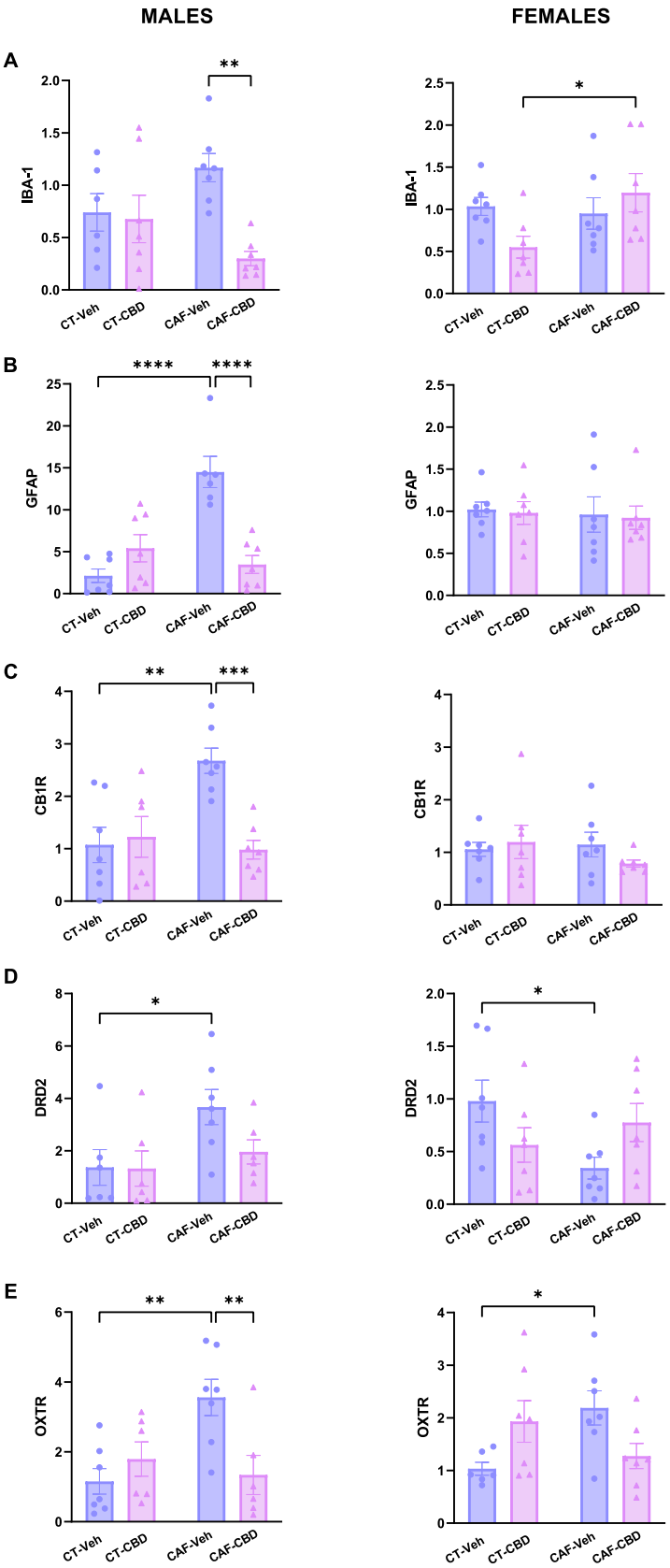

Fig. 6. Effects of CBD treatment on gene expression in the PFC of the offspring of obese dams. Male CAF-Veh showed increased expression of GFAP (B), CB1R (C), DRD2 (D) and OXTR (E) when compared to CT-Veh. CBD treatment attenuated the effects of maternal obesity on all markers, except DRD2. Female CAF-CBD presented higher expression of IBA-1 when compared to CT-CBD (A), CAF-Veh presented reduced DRD2 expression (D) and higher gene expression of OXTR (E) when compared to CT-Veh, with no effects of CBD. Data are presented as mean ± SEM. n = 5–7/group. *p < 0.05 **p < 0.01 ***p < 0.001 ****p < 0.0001.

differences were found among Veh groups (interaction $F_{1,24} = 4.628$; $p = 0.0417$; CT-CBD vs. CAF-CBD $p = 0.0253$) (Fig. 6A). Female CAF-Veh also showed decreased DRD2 (interaction $F_{1,24} = 6.581$; $p = 0.0170$; CT-Veh vs. CAF-Veh $p = 0.0242$) (Fig. 6D) and increased OXTR (interaction $F_{1,23} = 9.201$; $p = 0.0059$; CT-Veh vs. CAF-Veh $p = 0.0267$) (Fig. 6E) gene expression than CT-Veh, with no effect of CBD. No differences were observed in the expression of GFAP (Fig. 6B) and CB1R (Fig. 6C) in the PFC of female offspring.

As for the hippocampus, male CAF-Veh presented increased gene expression of IBA-1 (interaction $F_{1,23} = 8.967$; $p = 0.0065$; maternal diet effect $F_{1,23} = 21.86$; $p = 0.0001$; CT-Veh vs. CAF-Veh $p < 0.0001$; CAF-Veh vs. CAF-CBD $p = 0.0399$) (Fig. 7A) and GFAP (maternal diet effect $F_{1,24} = 28.38$; $p < 0.0001$; CT-Veh vs. CAF-Veh $p < 0.0001$; CT-CBD vs. CAF-CBD $p = 0.0326$) (Fig. 7B) and a reduced expression of OXTR (maternal diet effect $F_{1,23} = 6.079$; $p = 0.0216$; CT-Veh vs. CAF-Veh $p = 0.0159$; CAF-Veh vs. CAF-CBD $p = 0.0289$) (Fig. 7E) when compared to CT-Veh, while treatment was able to attenuate IBA-1 and OXTR mRNA levels on CAF-CBD compared to CAF-Veh. No differences were found in the expression of CB1R (Fig. 7C) and DRD2 (Fig. 7D).

In female offspring, CAF-Veh showed higher levels of IBA-1 (maternal diet effect $F_{1,23} = 21.34$; $p = 0.0001$; CT-Veh vs. CAF-Veh $p = 0.0024$; CT-CBD vs. CAF-CBD $p = 0.0178$) (Fig. 7A), GFAP (interaction $F_{1,22} = 18.82$; $p = 0.0003$; maternal diet effect $F_{1,22} = 27.67$; $p < 0.0001$; CBD effect $F_{1,22} = 19.02$; $p = 0.0002$; CT-Veh vs. CAF-Veh $p < 0.0001$; CAF-Veh vs. CAF-CBD $p < 0.0001$) (Fig. 7B) and CB1R (interaction $F_{1,24} = 33.03$; $p < 0.0001$; maternal diet effect $F_{1,24} = 18.87$; $p = 0.0002$; CBD effect $F_{1,24} = 37.68$; $p < 0.0001$; CT-Veh vs. CAF-Veh $p < 0.0001$; CAF-Veh vs. CAF-CBD $p < 0.0001$) (Fig. 7C) mRNA when compared to CT-Veh, however CBD treatment was only able to restore GFAP and CB1R gene expression on CAF-CBD. Female CAF-Veh also presented a reduced expression of DRD2 (maternal diet effect $F_{1,23} = 36.26$; $p < 0.0001$; CT-Veh vs. CAF-Veh $p < 0.0001$; CT-CBD vs. CAF-CBD $p = 0.0053$) (Fig. 7D) and OXTR (maternal diet effect $F_{1,23} = 94.12$; $p < 0.0001$; CT-Veh vs. CAF-Veh $p < 0.0001$; CT-CBD vs. CAF-CBD $p < 0.0001$) (Fig. 7E), when compared to CT-Veh, however CBD treatment did not exert any effects.

3.5. *Cannabidiol treatment partially attenuates neurotransmitter and endocannabinoid changes caused by maternal obesity in the offspring's prefrontal cortex*

Quantification of neurotransmitters and endocannabinoids in the PFC was performed by LC-MS/MS. Male CAF-Veh presented an increased concentration of glutamate, which was decreased by CBD treatment (interaction $F_{1,27} = 14.85$; $p = 0.0007$; maternal diet effect $F_{1,27} = 5.407$; $p = 0.0278$; CT-Veh vs. CAF-Veh $p = 0.0003$; CAF-Veh vs. CAF-CBD $p = 0.0012$) (Fig. 8B), and a reduced concentration of AEA (maternal diet effect $F_{1,26} = 7.794$; $p = 0.0097$; CT-Veh vs. CAF-Veh $p = 0.0213$), with no CBD effect (Fig. 8E). Furthermore, CAF-CBD presented lower concentrations of 2-AG when compared to CT-CBD (maternal diet effect $F_{1,25} = 12.40$; $p = 0.0017$; CT-CBD vs. CAF-CBD $p = 0.0066$), with no post hoc differences among Veh groups (Fig. 8D). No differences were found for GABA (Fig. 8A) and HVA (Fig. 8C) concentrations.

CAF-Veh female offspring presented increased concentrations of glutamate (interaction $F_{1,26} = 6.983$; $p = 0.0138$; CBD effect $F_{1,26} = 8.573$; $p = 0.0070$; CT-Veh vs. CAF-Veh $p = 0.0187$; CAF-Veh vs. CAF-CBD $p = 0.0011$) (Fig. 8B) and HVA (maternal diet effect $F_{1,26} = 16.35$; $p = 0.0004$; CBD effect $F_{1,26} = 4.395$; $p = 0.0459$; CT-Veh vs. CAF-Veh $p = 0.0022$) (Fig. 8C), and reduced concentrations of 2-AG (maternal diet effect $F_{1,22} = 35.49$; $p < 0.0001$; CBD effect $F_{1,22} = 5.842$; $p = 0.0244$; CT-Veh vs. CAF-Veh $p < 0.0001$; CAF-Veh vs. CAF-CBD $p = 0.0167$) (Fig. 8D) and AEA (maternal diet effect $F_{1,26} = 20.95$; $p = 0.0001$; CBD effect $F_{1,26} = 5.614$; $p = 0.0255$; CT-Veh vs. CAF-Veh $p = 0.0004$; CAF-Veh vs. CAF-CBD $p = 0.0257$) (Fig. 8E). CBD treatment attenuated these effects on Glutamate, 2-AG and AEA, but not HVA. No differences were observed for GABA concentrations (Fig. 8A).

4. Discussion

Our results confirm that maternal obesity disturbs the complex interplay between neuroinflammation, neurotransmission and the endocannabinoid system in the offspring, and that might translate into behavioral disturbances. Our previous research (Jantsch et al., 2023 Sep; Neto et al., 2023 Mar; González, et al., 2023), along with numerous studies in the field (Matuszewska et al., 2021 Sep 17; Reichelt et al., 2014; Lalanza and Snoeren, 2021 Mar; Leigh et al., 2020 Jan 27), supports the characterization of the CAF diet as a robust model of diet-induced obesity. The present study reinforces this characterization by demonstrating increased weight gain, dyslipidemia, and disturbances in glycemic control following CAF exposure in the dams. As for the effects of maternal obesity, we have previously reported the effects of maternal CAF diet on the offspring body composition and metabolism as well, with a positive effect of CBD treatment (da Rodrigues et al., 2023 Mar). Additionally, since in the present study the offspring were fed a control diet after weaning, we were able to show that the alterations seen in the offspring behavior and tissue composition were independent of the offspring's own diet, but rather an outcome of perinatal programing.

Previous work using models of maternal immune activation (MIA) and maternal obesity show similar findings, such as glial hyperactivation (Zhang et al., 2019 Feb; Mao et al., 2022 Sep), as well as impaired dopaminergic (Luchicchi et al., 2016), glutamatergic (Southey et al., 2023 Jul 25) and oxytocinergic (Buffington et al., 2016 Jun) signaling. Noteworthy, Santoni et al have recently demonstrated alterations in the dopaminergic and endocannabinoid systems, followed by behavioral disturbances in a MIA model of schizophrenia (Santoni et al., 2023 Mar). Therefore, here we focused on the specific interplay between maternal obesity and CBD treatment in the neurological and behavioral outcomes in the offspring. Considering the well-established connection between maternal obesity and an elevated risk of neurodevelopmental disorders, particularly autism spectrum disorder (ASD) (Moss and Chugani, 2014 Jan; Li et al., 2017 Oct 1; Chao et al., 2018 Jul) and schizophrenia (Urbonaite et al., 2022 Jul; Khandaker et al., 2012 Jun; Rivera et al., 2015 Nov) in the offspring, and given that recent clinical data further strengthens the correlation between impaired social function and anxiety, as individuals with high-functioning autism have been reported to exhibit heightened levels of anxiety compared to their healthy counterparts (Top et al., 2016 Jul; Perdikaris and Dermon, 2023 May), our research aimed to investigate both anxiety-like and social behavior in the offspring from mothers fed a cafeteria diet (CAF).

We hypothesized that the sustained neuroinflammation caused by perinatal maternal obesity could interfere with the functioning of the endocannabinoid system, which connects different neurotransmission systems and leads to increased anxiety-like behavior and aberrant social responses. Thus, treatment with CBD could help mitigate these harmful effects. Moreover, we have recently demonstrated that CBD can attenuate hypothalamic inflammation and peripheral metabolic disturbances in the offspring of obese dams (da Rodrigues et al., 2023 Mar). In line with our hypothesis, the present findings revealed that CBD treatment indeed attenuated anxiety-like behaviors in both open field and elevated plus maze tests, as well as the sexually dimorphic social shortcomings in CAF offspring. While MIA and maternal obesity models are well established, most studies still focus on the male progeny, lacking a comprehensive insight on how these perinatal exposures affect the female offspring. Here we have seen increased anxiety-like behaviors in both sexes, while social manifestations were heterogeneous, with males showing a social memory deficit, while females presented a social interaction/preference deficit. Corroborating with our findings, a study using a mouse model of maternal high-fat diet (HFD) has demonstrated social interaction differences exclusively on females (Kang et al., 2014 Dec). In contrast, other studies with mice showed both social interaction and social memory alterations in males, while female offspring were not assessed (Buffington et al., 2016 Jun; Liu et al., 2021 May). Additionally, a study using a non-human primate model of maternal Western diet

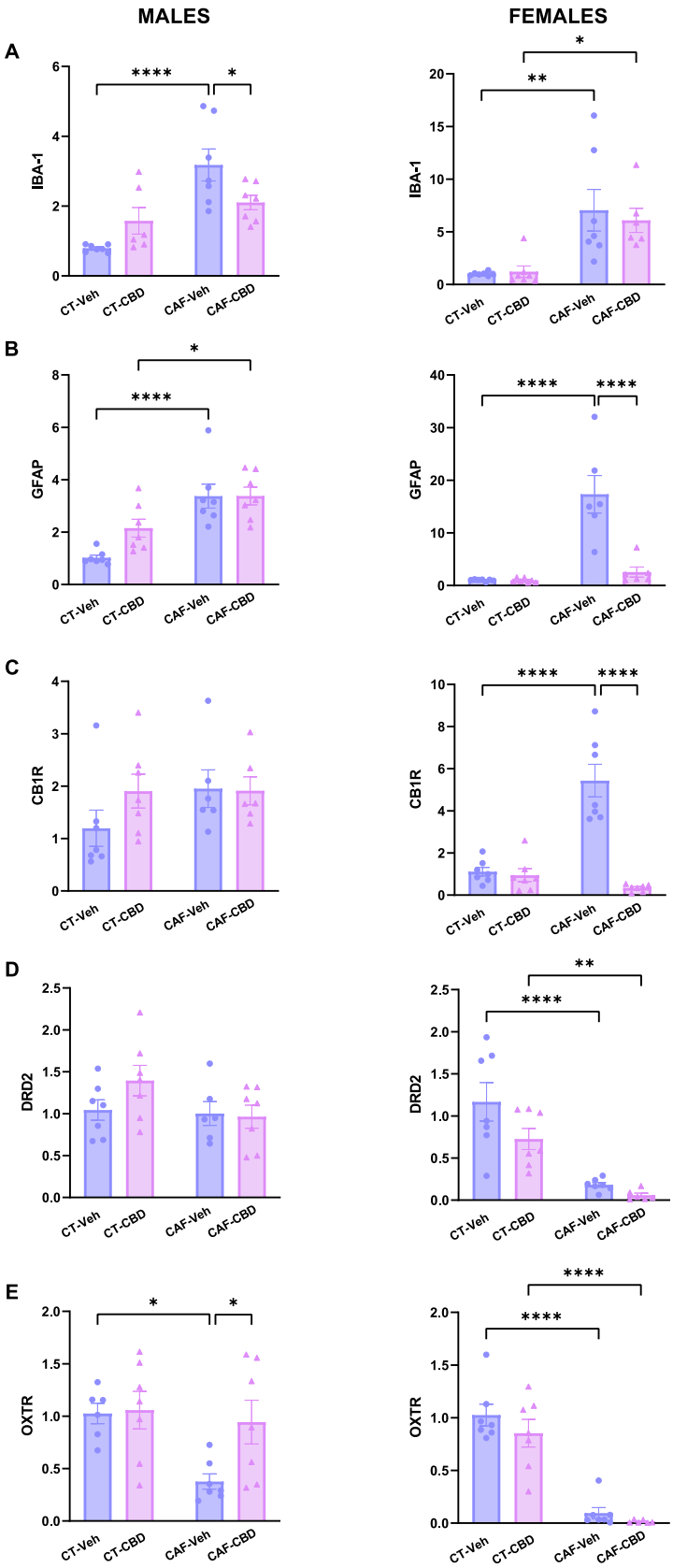

Fig. 7. Effects of CBD treatment on gene expression in the hippocampus of the offspring of obese dams. Male CAF-Veh showed increased expression of IBA-1 (A) and GFAP (B), and reduced expression of OXTR (E) when compared to CT-Veh. CBD treatment attenuated the effects of maternal obesity on IBA-1 and OXTR, but not on GFAP. Female CAF-Veh presented higher expression of IBA-1 (A), GFAP (B) and CB1R (C), and reduced expression of DRD2 and OXTR. CBD treatment was only able to attenuate these effects on GFAP and CB1R. Data are presented as mean ± SEM. n = 5–7/group. *p < 0.05 **p < 0.01 ****p < 0.0001.

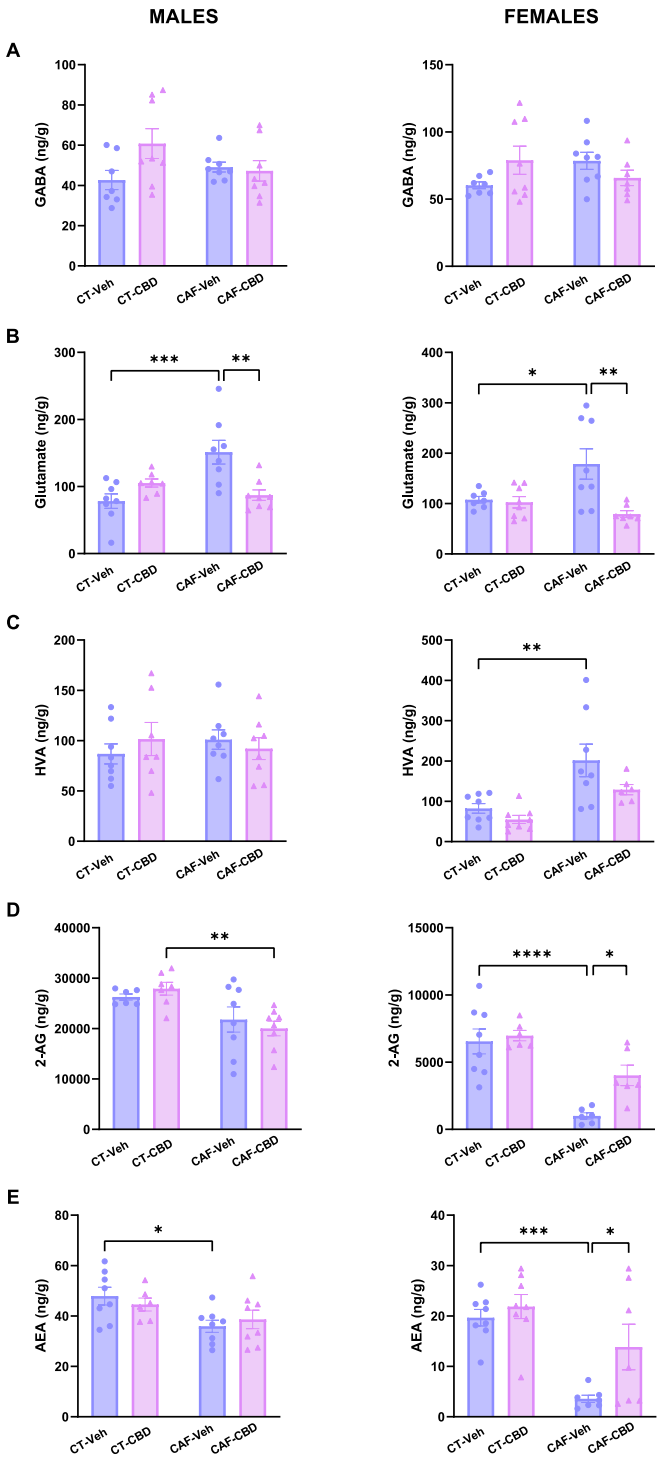

Fig. 8. Effects of CBD treatment on neurotransmitter and endocannabinoid concentrations in the PFC of the offspring of obese dams. Male CAF-Veh showed increased concentration of Glutamate (B) and reduced concentration of AEA (E). CBD treatment attenuated the effect on Glutamate. In female offspring, CAF-Veh demonstrated increased concentrations of Glutamate (B) and HVA (C), and reduced concentrations of 2-AG (D) and AEA (E). CBD treatment attenuated the effects on Glutamate, 2-AG and AEA, but not on HVA. Data are presented as mean ± SEM. $n = 6–8$/group. *p < 0.05 **p < 0.01 ***p < 0.001 ****p < 0.0001.

demonstrated social interaction disturbances in both males and females, while social memory was not included (Mitchell et al., 2022 Oct). We believe these discrepancies might be due to species and diet related differences, once in our study we used Wistar rats fed a CAF diet, as well as diverging test protocols. In sum, CAF-induced maternal obesity causes anxiety-like behavior and sexually dimorphic social manifestations in the offspring, and CBD is helpful to revert these behavioral alterations.

We observed an increased expression of IBA-1 in the PFC and hippocampus of male and female offspring, while GFAP was increased on male PFC and in both male and female hippocampus. Glial reactivity has already been demonstrated on models of MIA, being associated with significant anxiety-like behavioral manifestations and impairments in memory and learning tasks (Ratnayake et al., 2012 Nov; Antonson et al., 2018; Esshili et al., 2020 Jun; Savareh and Davoodian, 2022), and show an important sex-dependency (de Souza et al., 2015). Our data corroborates with a similar study using a CAF-induced maternal obesity that showed increased GFAP expression in the hippocampus of the offspring (Trujillo-Villarreal et al., 2021 Jan 14), however, regarding the PFC, reports are controversial. For instance, while some studies show a reduced GFAP expression in the cerebral cortex (Ogassawara et al., 2018 Feb; Contu et al., 2019 Oct), others show there are no differences (White et al., 2009 Jul; Teo et al., 2017 Jul; Rivera et al., 2020 Jun 19). However, two important factors may play a role in these differences: 1) with exception of Rivera et al, all the studies were conducted on maternal HFD and not CAF/Western/High palatable diet, and 2) most of the studies evaluated the offspring during adulthood (over 20 weeks of age). Since maternal diet effects have been shown to change throughout offspring's life (Contu et al., 2022 Dec 4), we believe that the overexpression of GFAP in the PFC seen here might be a transient response specifically to that period of life (6 weeks). Similarly, studies show both increased and equal IBA-1 expression in the offspring's PFC and hippocampus (White et al., 2009 Jul; Teo et al., 2017 Jul; Rivera et al., 2020 Jun 19). For both markers, CBD treatment has been shown to downregulate their expression, correlated with reduced glial reactivity (Schiavon et al., 2014 Nov; Gomes et al., 2015 May; Silva-Cardoso et al., 2023 Aug; dos Santos et al., 2024 Mar). This downregulation has been hypothesized to also be related to the protective effect of CBD against the pro-inflammatory profile induced by systemic LPS (Trujillo-Villarreal et al., 2021 Jan 14). Indeed, our previous study showed that male and female offspring of obese rats exhibited elevated concentrations of LPS in the plasma, however, CBD treatment reverted these concentrations to control levels (da Rodrigues et al., 2023 Mar). This could indicate a remarkable intersection between peripheral and central inflammation, where attenuation of peripheral pro-inflammatory markers accompany the attenuation seen in the brain. Interestingly, in the hippocampus of male offspring, a dietary effect on GFAP levels persisted despite CBD treatment, indicating that the reactivity of astrocytes cannot be completely regulated by this treatment throughout the brain.

Regarding CB1R, our results demonstrate that CB1R expression is influenced not only by maternal diet but also by CBD treatment in the PFC of males and in the hippocampus of females, presenting increased expression in CAF-Veh animals and regular levels in CAF-CBD. CB1R activation has been shown to modulate the balance between excitation and inhibition of layer II/III pyramidal neurons towards excitation (den Boon et al., 2015 Jul), and increased CB1R expression has been correlated with altered GABAergic and glutamatergic signaling in the telencephalon of a zebrafish model of social withdrawal (Perdikaris and Dermon, 2023 May). Consequently, we have also seen increased cortical glutamate concentrations in both male and female offspring, in accordance with other studies with maternal obesity models (Mizera et al., 2021; Wolfrum and Peleg-Raibstein, 2019 Oct 3). In the hippocampus, CB1R mediates the activity of dopaminergic neurons and dopamine levels in reward-related brain regions, and its upregulation plays a pivotal role in the development of reward and social-related behavioral abnormalities (Subbanna et al., 2015; Loureiro et al., 2015 May).

Interestingly, both human and animal studies of schizophrenia have shown increased expression of CB1R in the PFC, indicating its relationship with cognitive and affective disorders (Dean et al., 2001 Feb; Dalton et al., 2011 Jul; Verdurand et al., 2014 Jul; Chou et al., 2023 Sep). CBD has been shown to downregulate CB1R signaling by inhibiting its activation by endogenous ligands or exogenous agonists (Laprairie et al., 2015 Oct), as well as directly reduce short- and long-term glutamate exacerbated release (Santiago-Castañeda et al., 2022 Aug), which is in agreement with the effects seen here. Furthermore, it is noteworthy that CB1R levels correlate with GFAP levels in the PFC of males and in the hippocampus of females. This association between astrogliosis and CB1R expression and/or stimulation has been previously demonstrated in different models of neuroinflammation-related conditions (Ahluwalia et al., 2023 Mar; Blanco-Calvo et al., 2014; Bembrick et al., 2020 May; Shinjyo et al., 2013 Jul). Additionally, it has been shown that CB1R are expressed in astrocytes, therefore, alterations in astrocyte proliferation could reflect in CB1R expression as well, which agrees with the patterns seen here (Achicallende et al., 2022 Dec; Gutiérrez-Rodríguez et al., 2018 Jul).

On that same line, we have observed a maternal diet effect on endocannabinoid levels, where CAF offspring showed reduced concentrations of both 2-AG and AEA in the PFC. It has been demonstrated that 2-AG exerts anti-inflammatory effects through CB1R/CB2R and, possibly, via peroxisome proliferator-activated receptors (PPARs) (Alhouayek et al., 2014 Mar; Mecha et al., 2018 Jul). Considering the known role of 2-AG as a potential 'reservoir' for arachidonic acid production during inflammatory conditions, it has been proposed that reduced levels of 2-AG may be correlated to a deficient immune response (Alhouayek et al., 2014 Mar). In fact, evidence shows that inhibition of 2-AG degradation enhances glial immunity (Zhu et al., 2023 Dec 19; Wang et al., 2018 Dec; Mecha et al., 2019 Mar). As for AEA, similar reductions have been demonstrated in models of prenatal exposure to valproic acid (VPA), which relates to ASD, together with increased expression of fatty acid amide hydrolase (FAAH), followed by abnormal social behavior (Servadio et al., 2016 Sep 27; Melancia et al., 2018 Sep). Thus, upregulation of endocannabinoids has been proposed to ameliorate social impairments (Manduca et al., 2015 Aug; Schiavi et al., 2023 May). CBD has been shown to act as an inhibitor of AEA hydrolysis and uptake, which in turn increases AEA concentrations, as we have seen in female CAF offspring treated with CBD (De Petrocellis et al., 2011 Aug; Bisogno et al., 2001 Oct).

As for dopamine, MIA and maternal obesity have been demonstrated to alter dopaminergic signaling in the offspring (Santoni et al., 2023 Mar; Rivera et al., 2015 Nov; Dias-Rocha et al., 2018 Jan; Ruegsegger et al., 2017 Dec). Interestingly, we have seen an increase in DRD2 expression in the PFC of the male CAF offspring, with no changes in HVA concentrations, a dopamine metabolite, while female CAF showed a downregulation of DRD2 expression, accompanied by higher concentrations of HVA, indicative of increased dopamine metabolism and reduced dopamine signaling (Van Der Heyden et al., 2003 Jan). In the hippocampus, there was a decrease in DRD2 expression only on females. While a positive tendency can be observed mainly on the PFC, CBD did not exert any significant effects on receptor expression or HVA concentrations on both males and females.

Lastly, we have observed an increased gene expression of OXTR in the PFC, and reduced expression in the hippocampus on both male and female offspring. Corroborating with our findings, most *in vivo* studies on affective disorders correlate a reduced OXTR expression in the hippocampus with social and anxiety-like behavioral alterations (Tsai et al., 2022 Dec; Cymerblit-Sabba et al., 2023), However, little is known about the PFC (Morel et al., 2023 May; Pierzynowska et al., 2023 Feb 15). Interestingly, recent post-mortem studies indicated that childhood abuse (Almeida et al., 2022 Feb) and diagnoses of major depressive disorder and bipolar disorder (Lee et al., 2018 Oct) were associated with significantly increased OXTR mRNA levels in the PFC. Additionally, epigenetic studies have correlated reduced OXTR DNA methylation with perinatal stress, postnatal depression, social anxiety and autism in children (Maud et al., 2018 Dec). Taken together, these findings might indicate that oxytocin signaling differs among brain structures, where upregulation in the PFC and downregulation in the hippocampus set the stage for neurological disorders related to depression, anxiety and impaired sociability, as we have seen in the behavioral assessments. Very little is known about CBD effects on oxytocin signaling, but evidence suggests that CBD can modulate OXTR expression (Kozela et al., 2020 Mar). In our study, CBD was able to attenuate the alterations on the PFC and hippocampus of males, but not females.

It is important to highlight that, while interrelated, maternal obesity and MIA represent two distinct factors. Obesity-induced inflammation stems from chronic low-grade activation of the innate immune system in response to excessive adipose tissue accumulation (González, et al., 2023; de Oliveira et al., 2021 Jan; Radford-Smith and Anthony, 2023 Mar 20). In contrast, inflammation resulting from infection – such as the case for MIA – arises from the activation of the innate and adaptive immune responses to combat invading pathogens (Kalish et al., 2021 Feb; Estes and McAllister, 2016 Aug 19; Kim et al., 2022 Jan). While both obesity-induced and infection-induced inflammation involve cytokine-mediated immune responses and can affect the developing fetus similarly, as discussed here, their temporal dynamics, triggers, and consequences differ fundamentally in the individual and, therefore, in the exposed offspring as well.

5. Conclusion

Overall, our data suggests that maternal obesity induces a complex neurochemical and neuroinflammatory phenotype that can be correlated with different models of prenatal challenge and psychiatric conditions, but with certain particularities specific to the CAF-induced model. These findings also provide insight into the signaling pathways for the therapeutic benefits of CBD on behavioral dysfunctions stemming from neuroinflammation and neurochemical imbalances caused by perinatal maternal obesity, highlighting the sexual dimorphism encompassing both the transgenerational effect of obesity and the endocannabinoid system. Further studies assessing the endocannabinoid system as a modulator of neurotransmission and neuroinflammation in other brain structures are necessary to better understand its therapeutic prospects.

CRediT authorship contribution statement

Fernanda da Silva Rodrigues: Writing – original draft, Investigation, Formal analysis, Conceptualization. **Jeferson Jantsch:** Writing – original draft, Investigation, Formal analysis. **Gabriel de Farias Fraga:** Writing – original draft, Investigation. **Vitória Luiza de Camargo Milczarski:** Investigation, Writing – original draft. **Victor Silva Dias:** Writing – original draft, Investigation. **Camila Scheid:** Investigation. **Josias de Oliveira Merib:** Investigation. **Marcia Giovernardi:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Formal analysis, Conceptualization. **Renata Padilha Guedes:** Conceptualization, Formal analysis, Project administration, Supervision, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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3.3. Artigo científico 3

Cannabidiol partially rescues behavioral, neuroinflammatory and endocannabinoid dysfunctions stemming from maternal obesity in the adult offspring

Artigo submetido à revista Neuropharmacology (Fator de impacto 4.7)

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3.4. Artigo científico 4

Cannabidiol prevents LPS-induced inflammation by inhibiting the NLRP3 inflammasome and iNOS activity in BV2 microglia cells via CB2 receptors and PPARγ

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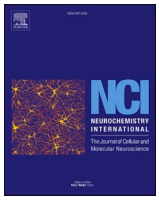

Cannabidiol prevents LPS-induced inflammation by inhibiting the NLRP3 inflammasome and iNOS activity in BV2 microglia cells via CB2 receptors and PPARγ

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ABSTRACT

Neuroinflammation stands as a critical player in the pathogenesis of diverse neurological disorders, with microglial cells playing a central role in orchestrating the inflammatory landscape within the central nervous system. Cannabidiol (CBD) has gained attention for its potential to elicit anti-inflammatory responses in microglia, offering promising perspectives for conditions associated with neuroinflammation. Here we investigated whether the NLRP3 inflammasome and inducible nitric oxide synthase (iNOS) are involved in the protective effects of CBD, and if their modulation is dependent on cannabinoid receptor 2 (CB2) and PPARγ signalling pathways. We found that treatment with CBD attenuated pro-inflammatory markers in lipopolysaccharide (LPS)-challenged BV2 microglia in a CB2- and PPARγ-dependent manner. At a molecular level, CBD inhibited the LPS-induced pro-inflammatory responses by suppressing iNOS and NLRP3/Caspase-1-dependent signalling cascades, resulting in reduced nitric oxide (NO), interleukin-1β (IL-1β), and tumour necrosis factor-alpha (TNF-α) concentrations. Notably, the protective effects of CBD on NLRP3 expression, Caspase-1 activity, and IL-1β concentration were partially hindered by the antagonism of both CB2 receptors and PPARγ, while iNOS expression and NO secretion were dependent exclusively on PPARγ activation, with no CB2 involvement. Interestingly, CBD exhibited a protective effect against TNF-α increase, regardless of CB2 or PPARγ activation. Altogether, these findings indicate that CB2 receptors and PPARγ mediate the anti-inflammatory effects of CBD on the NLRP3 inflammasome complex, iNOS activity and, ultimately, on microglial phenotype. Our results highlight the specific components responsible for the potential therapeutic applications of CBD on neuroinflammatory conditions.

1. Introduction

Neuroinflammation is defined as an inflammatory response within the central nervous system (CNS) and it is largely mediated by microglia, the primary innate immune cells of the CNS. They play a crucial role in maintaining the brain's homeostasis and responding to various challenges, such as infection and traumatic injury (Lily et al., 2021). Microglia are dynamic cells that can adopt different phenotypes or functional states in response to their microenvironment. The main phenotypes are often described as pro-inflammatory and anti-inflammatory, depending on the physiological context (Paolicelli et al., 2022; Woodburn et al., 2021). The pro-inflammatory state is mainly induced through the recognition of pathogen-associated

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Abbreviations

AM630	CB2 receptor antagonist
ASC	Apoptosis-associated speck-like protein containing a CARD
BV2	Immortalised murine microglial cell line
cAMP	Cyclic AMP
CB2	Cannabinoid receptor 2
CBD	Cannabidiol
CD86	Cluster of differentiation 86
COX2	Cyclooxygenase-2
DAMPs	Damage-associated molecular patterns
DAPI	4′,6-Diamidino-2-phenylindole
DMEM	Dulbecco's Modified Eagle Medium
ELISA	Enzyme-linked immunosorbent assay
ERK	Extracellular signal-regulated kinase
F4/80	Epidermal growth factor (EGF)-like module-containing mucin-like hormone receptor-like 1
FAAH	Fatty acid amide hydrolase
FBS	Foetal bovine serum
GPR55	G protein-coupled receptor 55
GW9662	PPARγ antagonist
IL-1β	Interleukin 1β
iNOS	Inducible nitric oxide synthase
JNK	Jun N-terminal kinase
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinase
MTT	3-(4,5-Dimethylthiazol-2-yl) −2,5-diphenyltetrazolium bromide
NF-κB	Nuclear factor kappa B
NLRP3	Nod-like receptor protein 3
NO	Nitric oxide
Nrf2	Nuclear factor erythroid 2–related factor 2
P/S	Penicillin/streptomycin
PAMPs	Pathogen-associated molecular patterns
PCA	Principal Components Analysis
PKA	Protein kinase A
PBS	Phosphate-buffered saline
PPARγ	Nuclear peroxisome proliferator-activated receptor gamma
PRR	Pattern recognition receptors
ROS	Reactive oxygen species
RT	Room temperature
TLR4	Toll-like receptor 4
t-SNE	T-distributed stochastic neighbour embedding
TNF-α	tumour necrosis factor-alpha
TRPV	Transient Receptor Potential Vanilloid

molecular patterns (PAMPs) by pattern recognition receptors (PRR), or damage-associated molecular patterns (DAMPs) on damaged host cells. PRR activation triggers an immune response via the secretion of pro-inflammatory cytokines such as tumour necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, chemokines, reactive oxygen species (ROS) and nitric oxide (NO) (Kwon and Koh, 2020).

One of the main PRRs in microglia involved in the pro-inflammatory state is the Nod-like receptor protein 3 (NLRP3). It is an intracellular PRR found in macrophages, neutrophils, lymphocytes, epithelial and dendritic cells, osteoblasts and neurons (Blevins et al., 2022). NLRP3 is activated as a result of nuclear factor kappa B (NFkB) transcription by a variety of stimuli, including infections, exogenous irritants or endogenous DAMPs. Upon recognising these signals, NLRP3 (sensor) recruits the apoptosis-associated speck-like protein containing a CARD (ASC, adaptor) and pro-Caspase-1 (effector) to form the large cytosolic NLRP3 inflammasome complex (Swanson et al., 2019). Activation of Caspase-1 triggers the maturation and release of pro-inflammatory cytokines IL-1β and IL-18 which function in both paracrine and autocrine manners to trigger innate and adaptive immune responses (Derbew Molla et al., 2020). For experimental purposes, microglial inflammation via NLRP3 activation is often induced by treatment with lipopolysaccharide (LPS), a main component of Gram-negative bacterial cell wall, as a direct connection between LPS-triggered inflammation and NLRP3 activation has been shown both *in vitro* and *in vivo* (Riester et al., 2020; Tejera et al., 2019).

Another well known downstream product of PRR signalling and NFkB activation is the inducible nitric oxide synthase (iNOS). iNOS is not constitutively expressed in most cells but can be induced in response to inflammatory signals. Unlike the other NOS isoforms (nNOS and eNOS), which produce NO in a regulated and localised manner for physiological signalling, iNOS generates large amounts of NO for extended periods in response to inflammatory stimuli (Cinelli et al., 2020; Mathy et al., 2021). While iNOS-mediated NO production is crucial for host defence, excessive or prolonged activation of iNOS and the subsequent production of NO contributes to the inflammatory cascade which sustains chronic tissue damage (Cinelli et al., 2020; Forstermann and Sessa, 2012).

While crucial for maintaining CNS homeostasis, excessive or prolonged microglial activation can contribute to neuroinflammation and tissue damage, which is associated with several conditions, such as multiple sclerosis, Alzheimer's disease and autism spectrum disorder (Qian et al., 2020; DiSabato et al., 2016; Dong and Wee Yong, 2019). Therefore, the suppression of the excessive inflammatory responses from hyperactivated microglia is expected to limit the production of pro-inflammatory cytokines and ROS, which can be a promising strategy for the treatment of neurological disorders (Bagheri-Mohammadi, 2021; Yuan et al., 2023).

Cannabidiol (CBD), a non-psychotropic terpenophenol derived from Cannabis sativa, is known for having anti-inflammatory and antioxidant properties that have been suggested to benefit a range of immunomediated conditions (Atalay et al., 2019). Studies indicate that CBD can attenuate symptoms associated with epilepsy, anxiety, chronic pain, and cognitive dysfunctions (Rodrigues et al., 2023; Vitale et al., 2021). CBD exerts anti-inflammatory effects by suppressing the production of pro-inflammatory cytokines, inhibiting the activation and proliferation of immune cells, and reducing reactive oxygen species (Atalay et al., 2019; dos-Santos-Pereira et al., 2020; Han et al., 2009). However, although the anti-inflammatory effects of CBD have been well documented, the signalling pathways through which such effects take place are still not well understood. In addition to its interactions with the cannabinoid receptors-1 (CB1) and 2 (CB2), CBD engages with other systems and receptors crucial for immunological response. These include the G protein-coupled receptor 55 (GPR55), Transient Receptor Potential Vanilloid (TRVP) channel, and nuclear peroxisome proliferator-activated receptors (PPARs) (Landucci et al., 2022). Previous studies have pointed towards CB2 and PPARγ as promising targets for CBD in macrophages (Preteroti et al., 2023), intestinal epithelial cells (Corpetti et al., 2021), sensory neurons (Rodrigues Silva et al., 2022) and myeloid-derived suppressor cells (Khosropoor et al., 2023). However, in microglia, the role of CB2 receptors and PPARγ on the effects of CBD is under explored.

In the present study, we aimed to investigate whether CBD exerts its protective effects on BV2 microglia cells via NLRP3 inflammasome and iNOS and if such modulation is dependent on CB2 receptors and/or PPARγ activation.

2. Materials and methods

2.1. Chemicals and reagents

Cannabidiol (CBD) (ab120448), anti-NLRP3 (ab4207), anti-inducible nitric oxide synthase (iNOS) (ab204017), Donkey Anti-Rabbit IgG H&L Alexa Fluor 647 (ab150075), Goat Anti-Rabbit IgG H&L Alexa Fluor 488 (ab150077), Goat Anti-Rat IgG H&L Alexa Fluor 555 (ab150158), Donkey Anti-Goat IgG H&L Alexa Fluor 488 (ab150129) and Mounting medium with 4′,6-diamidino-2-phenylindole (DAPI) (ab104135) were purchased from Abcam (Cambridge, UK). AM630 (1120) and GW9662 (1508) were purchased from Tocris (Bristol, UK). Mouse tumour necrosis factor alpha (TNF-α) (88-7324-86) and mouse Interleukin- 1β (IL-1 β) (88-7013A-88) ELISA Kits, anti-CD86 (14-0862-82), Goat anti-Rat IgG H&L Alexa Fluor 647 (A-21247), eBioscience™ Foxp3/Transcription Factor Staining Buffer Set (00-5523-00) and Fixable Viability Dye eFluor 450 (65-0863-14) were purchased from Invitrogen (Waltham, MA, USA). High glucose Dulbecco's modified Eagle medium + GlutaMAX (DMEM + GlutaMAX) (10569010), Fetal bovine serum (FBS) (A5256801), Penicillin-Streptomycin (15140122), and Trypsin-EDTA 0.05% Cell Dissociation Reagent (25300062) were purchased from Gibco (Carlsbad, CA, USA). Lipopolysaccharides from Escherichia coli O111:B4 (L4391), N-(1-Naphthyl)ethylenediamine dihydrochloride (33461), Sulfanilamide (S9251), Sodium Nitrite (S2252), Phosphoric Acid (695017) Ammonium Chloride (213330), Triton X-100 (X100), 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide (MTT) and anti-α-Tubulin (MAB1864) were obtained from Sigma-Aldrich (Saint Louis, MO, USA). PE/Cyanine7 anti-F4/80 (123114) was obtained from BioLegend (San Diego, CA, USA). Anti-ASC/TMS1 (67824) was purchased from Cell signalling Technology Inc. (Danvers, MA, USA). FAM-FLICA® Intracellular Caspase-1 Detection Probe (YVAD) was purchased from Immunochemistry Technologies (Davis, California, USA).

2.2. Cell culture and treatments

BV2 microglia cells were provided by Oswaldo Cruz Foundation (FIOCRUZ - Brazil) (ICLC Cat# ATL03001, RRID:CVCL_0182) and were cultured in DMEM + GlutaMAX supplemented with 10% heat-inactivated FBS and 1% Penicillin-Streptomycin at 37 °C in a constant temperature incubator with an atmosphere of 95% humidity, and 5% CO_2. Cells were cultured at a density of 1×10^5 cells/mL in T75 flasks until 80% confluence was achieved and were used between 15 and 25 passages. Cultures were tested for mycoplasma contamination regularly.

Drugs were freshly diluted in Dimethyl sulfoxide (DMSO) and then further diluted into serum-free cell culture media to the established doses. Final concentrations of DMSO in culture wells were equal to or below 0.01%. Cells were changed to serum-free media and treated with either 1 μM AM630 (CB2R inverse agonist) (Oh et al., 2010) or 10 μM GW9662 (PPARγ antagonist) (Zhang and Chen, 2015; Zhao et al., 2022) for 30 min before treatment with 1 μM CBD (dos-Santos-Pereira et al., 2020; Li et al., 2022) for 1 h, and then stimulated with 1 μg/mL of LPS for 24 h in serum-free cell culture media (Fig. 1). Selection of doses and treatment time was done based on previous studies on similar models. Additionally, we have tested CBD concentrations of 1, 5 and 10 μM, with 1 μM presenting the best results without inducing cell toxicity (data not shown). Control groups for the treatments received DMSO in the same volume used for the drugs and control groups for LPS received deionized water in the same volume used for LPS.

2.3. Cytotoxicity assay

The 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide (MTT) assay was used to determine BV2 cells proliferation and survival in response to LPS and the drugs (Mosmann, 1983). For that, BV2 cells were seeded in a 96-well plate (1,5 x 10^4 cells/well) and cultured overnight. After treatment with the drugs and/or CBD and stimulation with LPS for 24 h, 10 μL of MTT stock solution in PBS (5 mg/mL) was added to the wells and incubated at 37 °C for 2h. Subsequently, the cells were lysed, and the dark blue crystals were solubilized in 100 μL of acidified isopropanol (2% HCl 2M). The optical density of each well was determined using a Tecan Infinite M200 Pro® plate reader at 570 nm.

2.4. Flow cytometry

BV2 cells were seeded in a 96-well plate (2 x10^5 cells/well) and cultured overnight. After treatment with the drugs and/or CBD and stimulation with LPS for 24 h, the plates were centrifuged at 1000 rpm for 5 min and the supernatant was removed. For regular immunodetection, cells were marked with a fixable viability dye (eFluor 450) for 30 min at 4 °C, then fixed and permeabilized using a Transcription Factor Staining Buffer Set according to the manufacturer's instructions. Cells were then incubated with primary antibodies against CD86 (1:250), NLRP3 (1:100), ASC (1:250) and iNOS (1:250) for 30 min at 4 °C, followed by incubation with secondary antibodies marked with Alexa Fluor 488 and 647 (1:500) for 30 min at 4 °C. Lastly, cells were incubated with a PE/Cyanine7 anti-F4/80 conjugated antibody (1:250) for another 30 min at 4 °C. All the washes in between were performed at

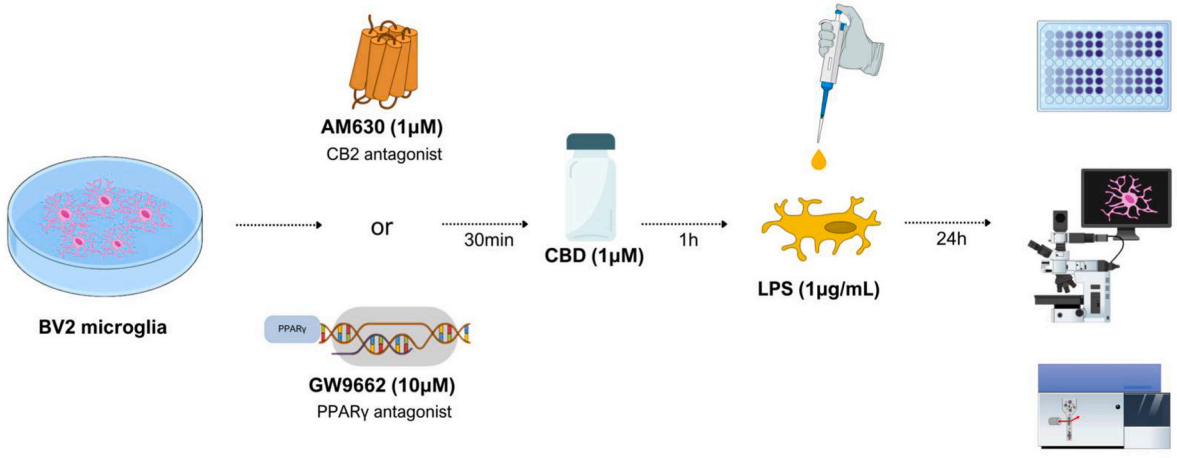

Fig. 1. A schematic description of the experimental design and groups. BV2 microglial cells were treated with CB2 antagonist (AM630) or PPARγ antagonist (GW9662) for 30 min. Then, Cannabidiol (CBD) was added to the cell culture medium for 1 h. Lastly, lipopolysaccharide (LPS) was added and cells were incubated for 24 h. Assays, flow cytometry and confocal microscopy were performed after the 24 h incubation period.

400g for 5 min using a Blocking solution (Permeabilization buffer + 2% FBS).

For Caspase-1 activity assessment, after treatments the plates were centrifuged at 1000 rpm for 5 min and the supernatant was removed. Intracellular Caspase-1 detection probe was added to the wells (1:100 in PBS) and incubated for 30 min at 37°. The cells were washed once with PBS then resuspended in PBS for analysis.

All the flow cytometry analyses were performed at a Beckman Coulter CytoFlex® and data were analysed using FlowJo software. Data were expressed as Mean Fluorescence Intensity (MFI) for cell surface markers and as Stimulation Index (SI) (frequency of each well/average frequency of control wells within each experiment) for the intracellular markers.

2.4.1. Clustering and dimensionality analysis

Mean Fluorescence Intensity (MFI) data obtained from flow cytometry were utilised for dimensionality reduction and clustering analysis. These data were normalised per experimental day. The clustering and dimensionality reduction analyses were conducted in R (version 4.2.2). Principal Components Analysis (PCA) and k-means analysis were performed using the base R package. T-distributed stochastic neighbour embedding (t-SNE) analysis was carried out using the "Rtsne" package (version 0.16).

2.5. Immunofluorescence

BV2 cells were seeded in 12-well plates on glass coverslips (10^5 cells/well) and cultured overnight. After treatment with the drugs and/or CBD and stimulation with LPS for 24 h, media was removed and the cells were washed with PBS 2 times and fixed with 4% paraformaldehyde at room temperature (RT) for 15 min. Next, the cells were washed again three times with PBS, permeabilized with Ammonium Chloride 50 mM for 10min, and 0.2% Triton X-100 for 5 min at RT, then washed again another three times with PBS, and 5% goat serum was added to block the non-specific binding at RT for 30 min. The cells were incubated with primary antibodies against CD86 (1:250) and α-Tubulin (1:1000) at RT for 2 h, followed by incubation with secondary antibodies labelled with Alexa Fluor 555 and 647 at RT for 1 h. Finally, the coverslips were transferred to glass slides with a mounting medium containing DAPI and imaged by confocal microscopy (ZEISS LSM 880®). At least one hundred cells were imaged from each slide. Images were analysed using Fiji ImageJ software.

2.6. Determination of NO in cell supernatant

NO concentration on cell supernatant was assessed by Griess assay. BV2 cells were seeded in a 96-well plate (1,5 x 10^4 cells/well) and cultured overnight. After treatment with the drugs and/or CBD and stimulation with LPS for 24 h, the plates were centrifuged at 1000 rpm for 5 min and the supernatant was collected. Griess reagent was prepared mixing 1,45 mL Phosphoric acid, 0,25g Sulfanilamide and 0,025g N-(1-Naphthyl)ethylenediamine dihydrochloride to a total of 25 mL in dH2O. Standard curve was generated by serial dilution of Sodium Nitrite 50 mM for 8 points starting at 200uM in cell culture medium. In a 96-well plate, equal volumes of cell supernatant and Griess reagent were added to each well. The plate was incubated at room temperature for 5 min and absorbance was measured using a Tecan Infinite M200 Pro® plate reader at 540 nm.

2.7. Determination of TNF-α and IL-1β concentrations

Concentrations of TNF-α and IL-1β were measured by enzyme-linked immunosorbent assay (ELISA) kits according to instructions from the manufacturer. Briefly, BV2 cells were seeded in a 96-well plate (1,5 x 10^4 cells/well) and cultured overnight. After treatment with the drugs and/or CBD and stimulation with LPS for 24 h, the plates were centrifuged at 1000 rpm for 5 min and the supernatant was collected for detection of the aforementioned inflammatory cytokines. Absorbance was measured using a Tecan Infinite Pro ® plate reader at 450 nm.

2.8. Data analysis and statistics

Data were analysed using Graphpad Prism 9 statistical software (GraphPad Software, San Diego, CA, USA), submitted to the Kolmogorov–Smirnov test to assess normality and then to one-way ANOVA with Bonferroni post hoc. The results were expressed as the mean ± standard error of the mean (SEM). All analyses were based on three to four experiments consisting of an independent cell culture preparation, with duplicates or triplicates within each experiment. Each point represents the one replicate within each individual experiment. Outliers were removed using the ROUT test, and statistically significant differences were considered at $p < 0.05$.

3. Results

3.1. Cannabidiol does not prevent cellular viability loss caused by LPS in BV2 microglia cells

We firstly assessed cell viability by MTT assay to evaluate the effects of LPS and the treatments on BV2 microglial cells (Fig. 2a). We confirmed the viability by the Fixable viability dye (FVD) by flow cytometry (Fig. 2b). LPS exposure significantly reduced cell viability after 24 h compared with CT on both MTT (ANOVA: $F_{7,102} = 28.41$; $P < 0.0001$. LPS vs CT: $P < 0.0001$) and FVD (ANOVA: $F_{5,83} = 17.59$; $P < 0.0001$. LPS vs CT: $P < 0.0001$). Pre-treatment with CBD and/or AM630 and GW9662 did not exert any effects on cell survival after LPS stimulation. These findings indicate a lack of protective effect of CBD against LPS-induced cell death. Additionally, GW9662 and AM630 by themselves or as CBD co-treatments showed no effects on control cells (data not shown).

3.2. Cannabidiol attenuates microglial polarisation towards a pro-inflammatory phenotype induced by LPS through CB2 and PPARγ activation

Microglial polarisation in BV2 cells was assessed by CD86 and F4/80 markers - both constitutive microglial markers whose increased expression is associated with a pro-inflammatory phenotype. LPS induced a significantly increased expression of CD86, seen on both immunofluorescence (ANOVA: $F_{5,3380} = 25.31$; $P < 0.0001$. CT vs LPS: $P < 0.0001$) (Fig. 3a and b) and flow cytometry (ANOVA: $F_{5,45} = 17.71$; $P < 0.0001$. CT vs LPS: $P < 0.0001$) (Fig. 3c), presenting cells with a more activated morphology as well as higher fluorescence intensity. However, CBD was able to significantly attenuate this overexpression (LPS vs. CBD-LPS: $P = 0.0351$ and 0.0208, respectively). Interestingly, CB2 receptor antagonism completely inhibited CBD's effect on CD86 expression as seen on immunofluorescence (CBD-LPS vs AM630/CBD-LPS: $P < 0.0001$). However, on flow cytometry, it only marginally blunted this effect, since the group was different from neither LPS nor CBD-LPS. PPARγ antagonism partially attenuated CBD's effect on CD86 expression as well.

Regarding F4/80, LPS insult increased its expression (ANOVA: $F_{5,48} = 11.69$; $P < 0.0001$. CT vs LPS: $P = 0.0410$), while CBD also attenuated this effect (LPS vs CBD-LPS: $P = 0.0107$) (Fig. 3d and e). Antagonism of both CB2 receptors and PPARγ before CBD partially prevented this effect, suggesting that the protective effect exerted by CBD is dependent on these pathways at least to some extent.

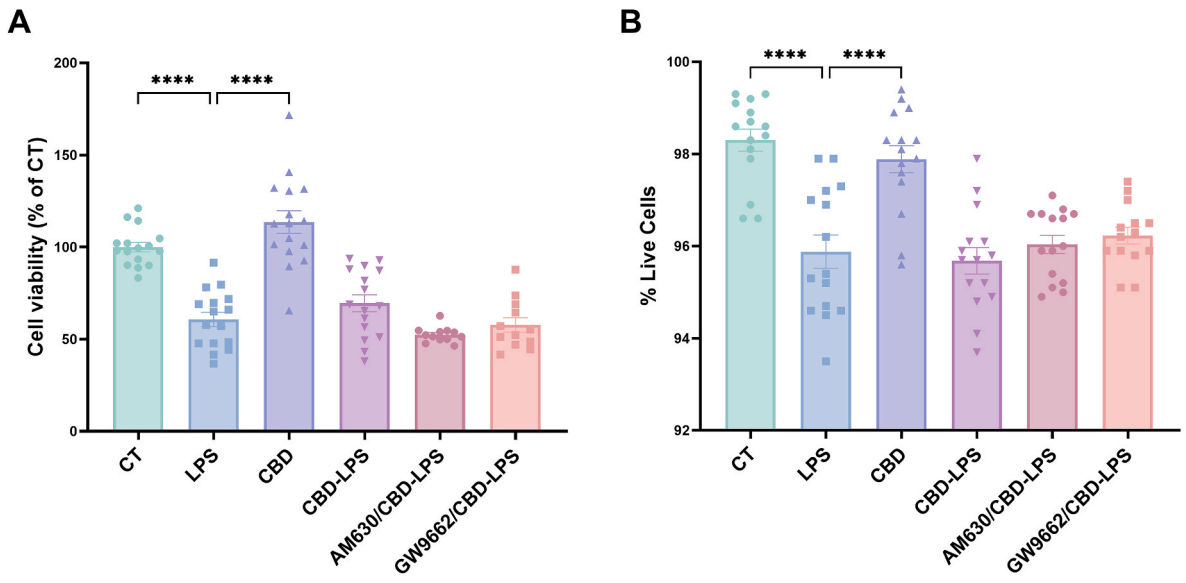

Fig. 2. Effects of CBD, AM630 and GW9662 on BV2 microglial cells viability assessed by MTT (a) and Fixable viability dye (b). LPS reduced cell viability compared to CT regardless of previous treatment. CBD, AM630 or GW9662 did not exert any effects on cell viability. ****P < 0.0001.

3.3. Cannabidiol reduces inducible nitric oxide synthase (iNOS) expression and nitric oxide (NO) concentration in a PPARγ-dependent manner

Expression of iNOS in BV2 microglial cells was assessed by flow cytometry and NO concentration in cell supernatant was quantified by the Griess assay. LPS induced an increased expression of iNOS (ANOVA: $F_{5,50} = 16.30$; $P < 0.0001$. CT vs LPS: $P < 0.0001$) (Fig. 4a) along with elevated concentration of secreted NO (ANOVA: $F_{5,17} = 8.428$; $P = 0.0004$. LPS vs CT: $P = 0.0088$) (Fig. 4b), while CBD was able to significantly reduce this effect on both ends (LPS vs CBD-LPS: $P = 0.0046$ and 0.0060, respectively). However, the antagonism of PPARγ before CBD prevented these effects (CBD-LPS vs GW9662/CBD-LPS: $P = 0.0039$ and 0.0211), suggesting that the protective effect of CBD on the production of NO is dependent on this signalling pathway. On the other hand, antagonism of CB2 receptors partially interfered with the effects of CBD on iNOS expression and did not influence its effects on NO concentrations (LPS vs AM630/CBD-LPS: $P = 0.0045$).

3.4. Cannabidiol reduces inflammasome activation and Caspase-1 activity in a CB2 and PPARγ-dependent manner

To assess if CBD anti-inflammatory effects were mediated by the NLRP3 Inflammasome in BV2 microglial cells, we measured NLRP3 and ASC expression and Caspase-1 activity by flow cytometry (Fig. 5). As expected, LPS induced an increase in all the aforementioned markers (NLRP3 - ANOVA: $F_{5,60} = 11.04$; $P < 0.0001$. CT vs LPS: $P = 0.0002$. ASC - ANOVA: $F_{5,40} = 6.141$; $P = 0.0003$. CT vs LPS: $P = 0.0003$. Caspase-1 - ANOVA: $F_{5,52} = 7.874$; $P < 0.0001$. CT vs LPS: $P = 0.0002$), while CBD significantly reduced this effect on NLRP3 (LPS vs CBD-LPS: $P = 0.0181$) and Caspase-1 (LPS vs CBD-LPS: $P = 0.0439$). The antagonism of both CB2 receptors and PPARγ slightly impaired CBD effects on the expression of NLRP3 (Fig. 5a) as well as the percentage of active Caspase-1 (Fig. 5c and d), since AM630/CBD-LPS and GW9662/CBD-LPS groups are not different from LPS nor CBD-LPS. Interestingly, the effect of CBD on ASC expression was not significant, once CBD-LPS is not different from LPS ($P = 0.0813$), however CBD-LPS is not different from CT as well and an overall tendency similar to NLRP3 can be observed (Fig. 5b). These results suggest that the activation of the NLRP3 Inflammasome by LPS can be prevented by CBD and it is partly mediated by CB2 receptors and PPARγ.

3.5. Cannabidiol modulates overall clustering and dimensionality away from LPS in a CB2 and PPARγ-dependent manner

PCA and t-SNE analysis were conducted to explore the underlying structure and patterns within the groups using the MFI from all the markers analysed by flow cytometry. The goal was to visualise high-dimensional data in a lower-dimensional space while preserving local structures. Each point in the plot represents an observation, and the proximity of points reflects their similarity. Both PCA (Fig. 6a) and t-SNE (Fig. 6b) visualisations reveal distinct clusters and patterns within the data, consistent with experimental groups. For instance, the cluster representing the CBD-LPS group overlaps with the one for the CT group, suggesting tight inter-cluster similarities. However, when CB2 receptors (AM630/CBD-LPS) and PPARγ (GW9662/CBD-LPS) are inhibited, the samples tend to cluster closer to the LPS group.

3.6. Cannabidiol differentially attenuates the effects of LPS on TNF-α and IL-1β concentrations

Lastly, to assess the effects of CBD on the secretion of pro-inflammatory cytokines in response to LPS, we dosed the concentrations of TNF-α and IL-1β on the BV2 cells' supernatants by ELISA (Fig. 7). LPS exposure induced an increase in the concentrations of both cytokines (TNF-α - ANOVA: $F_{5,36} = 13.27$; $P < 0.0001$. CT vs LPS: $P < 0.0001$. IL-1β - ANOVA: $F_{5,42} = 2.626$; $P = 0.0370$. CT vs LPS: $P = 0.0241$), while CBD was able to reduce this effect (LPS vs CBD-LPS: $P = 0.0057$ and 0.0432, respectively). The antagonism of both CB2 receptors and PPARγ marginally impaired CBD effects on IL-1β (Fig. 7a), however, neither CB2 nor PPARγ interfered with the effects of CBD on TNF-α concentration (LPS vs AM630/CBD-LPS: $P = 0.0052$; LPS vs. GW9662/CBD-LPS: $P = 0.0171$) (Fig. 7b). These results suggest that the secretion of IL-1β induced by LPS can be attenuated by CBD partially through its activation of CB2 receptors and PPARγ, but its effects on TNF-α do not seem to be mediated by these signalling pathways.

4. Discussion

Neuroinflammation has emerged as a focal point when delving into the intricate cellular dynamics underlying various neurological disorders. Among the diverse cell populations contributing to the neuroinflammatory milieu, microglial cells have drawn significant attention

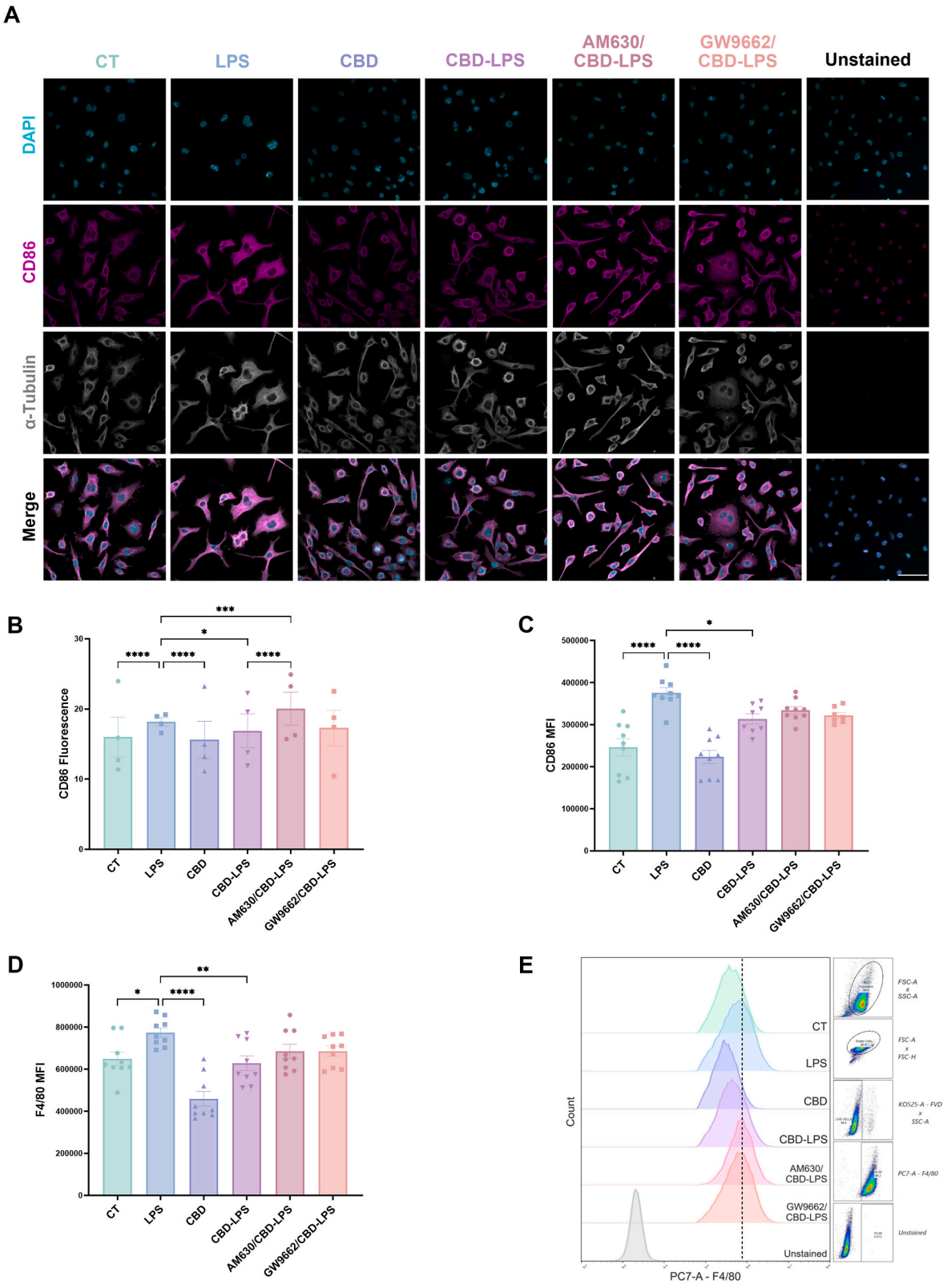

Fig. 3. Effects of CBD on microglial polarisation in response to LPS in BV2 cells. CBD suppressed the pro-inflammatory phenotype induced by LPS as shown by CD86 (a, b and c) and F4/80 (d and e) expression. CB2 and PPARγ antagonism by AM630 and GW9662, respectively, partially attenuated the effects of CBD. Gating strategy (E). Scale bar is 50μm. *P < 0.05, **P < 0.01, ****P < 0.0001.

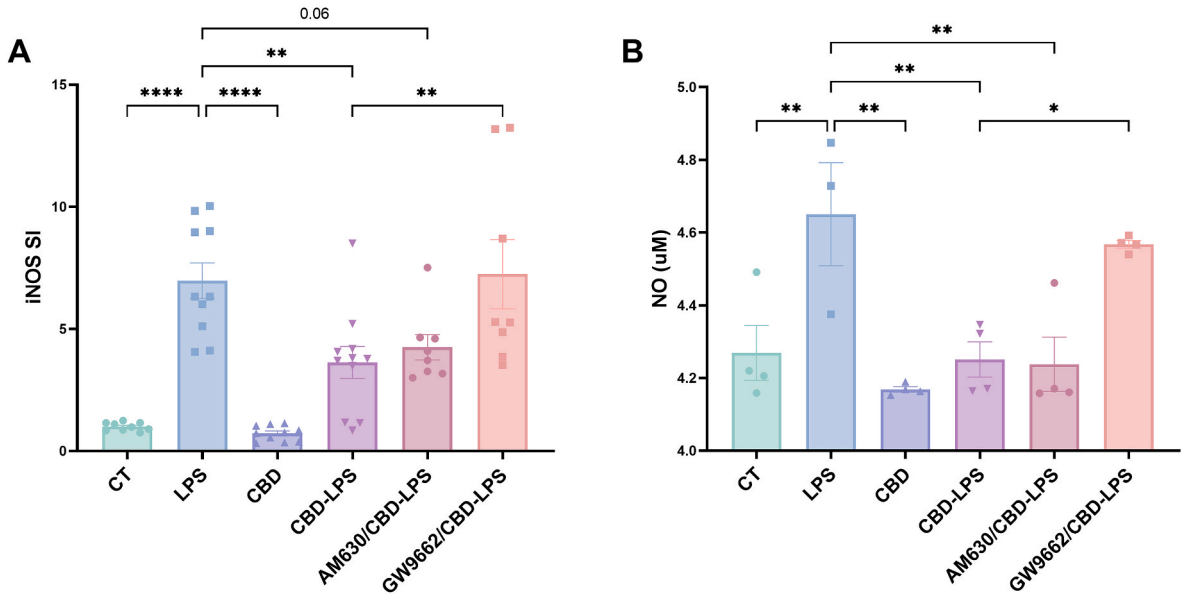

Fig. 4. Effects of CBD on iNOS expression and NO concentration in BV2 microglial cells in response to LPS. CBD suppressed the overexpression of iNOS (a) and the elevated concentration of NO in the cell supernatant (b) induced by LPS. PPARγ antagonism by GW9662 hampers the effects of CBD, but blockage of CB2 receptors by AM630 exerts no effects. The percentage of cells expressing iNOS was calculated as a relative value to an average percentage of CT. *P < 0.05, **P < 0.01, ****P < 0.0001.

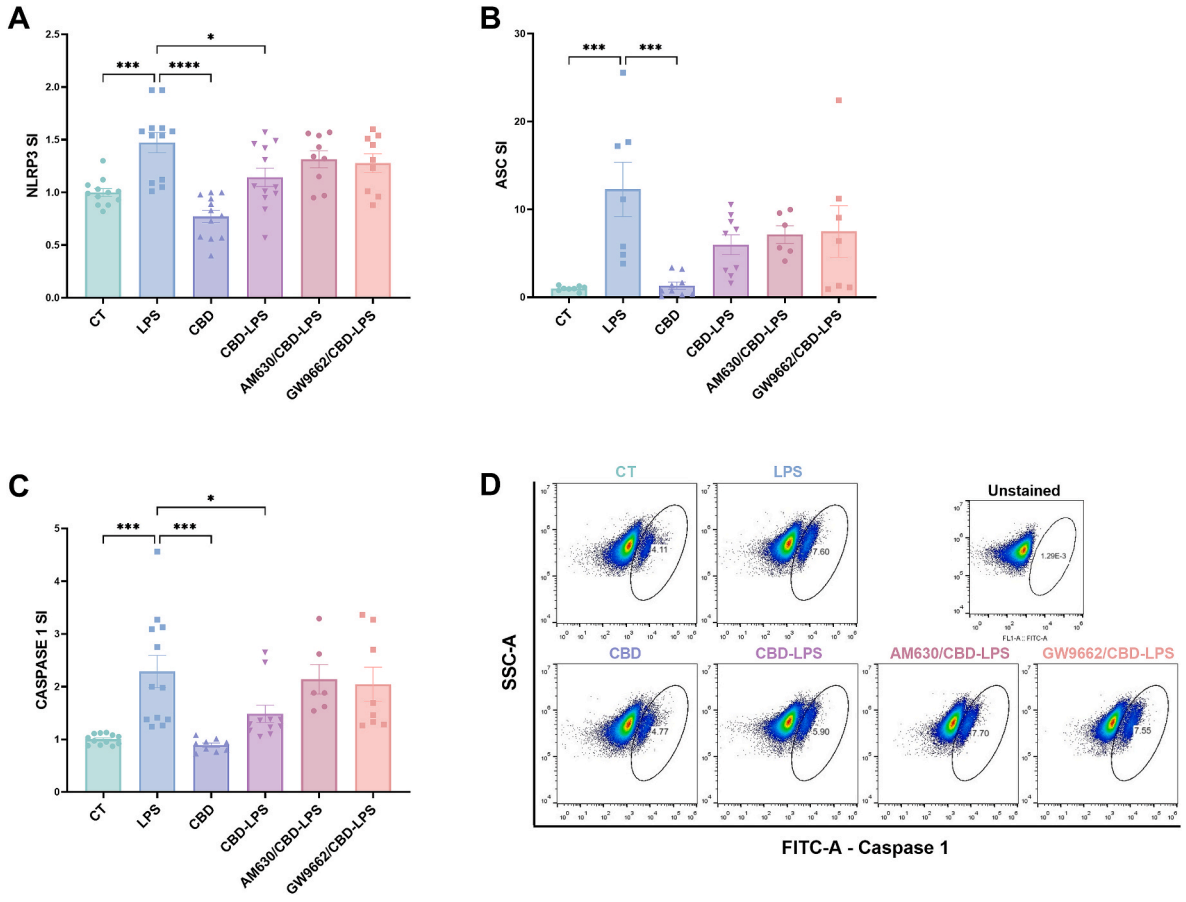

Fig. 5. Effects of CBD on NLRP3 and ASC expression and Caspase-1 activity in response to LPS. CBD suppressed the overexpression of NLRP3 (a), and attenuated Caspase-1 activity (c) induced by LPS. CBD effects were partially blunted by CB2 and PPARγ antagonism by AM630 and GW9662, respectively. The percentage of cells expressing NLRP3 and ASC and with active Caspase-1 was calculated as a relative value to an average percentage of CT. *P < 0.05, ***P < 0.001 ****P < 0.0001.

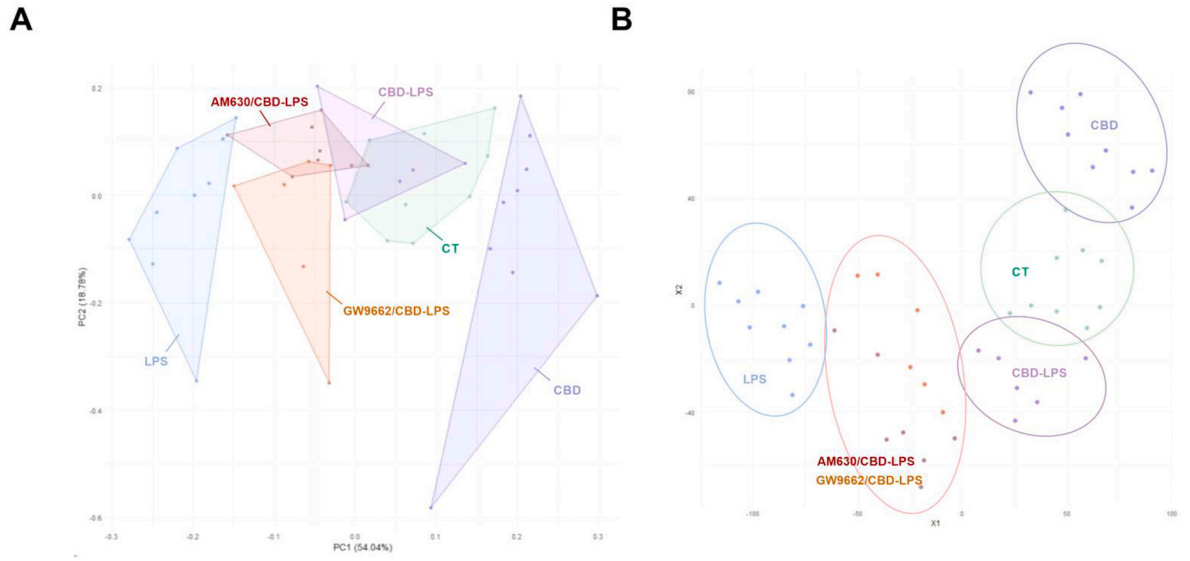

Fig. 6. Effects of CBD on experimental clustering and dimensionality. CBD treatment deviates clustering towards CT and away from LPS, while CB2 and PPARγ antagonism deviates the groups towards LPS on both PCA (a) and t-SNE (b).

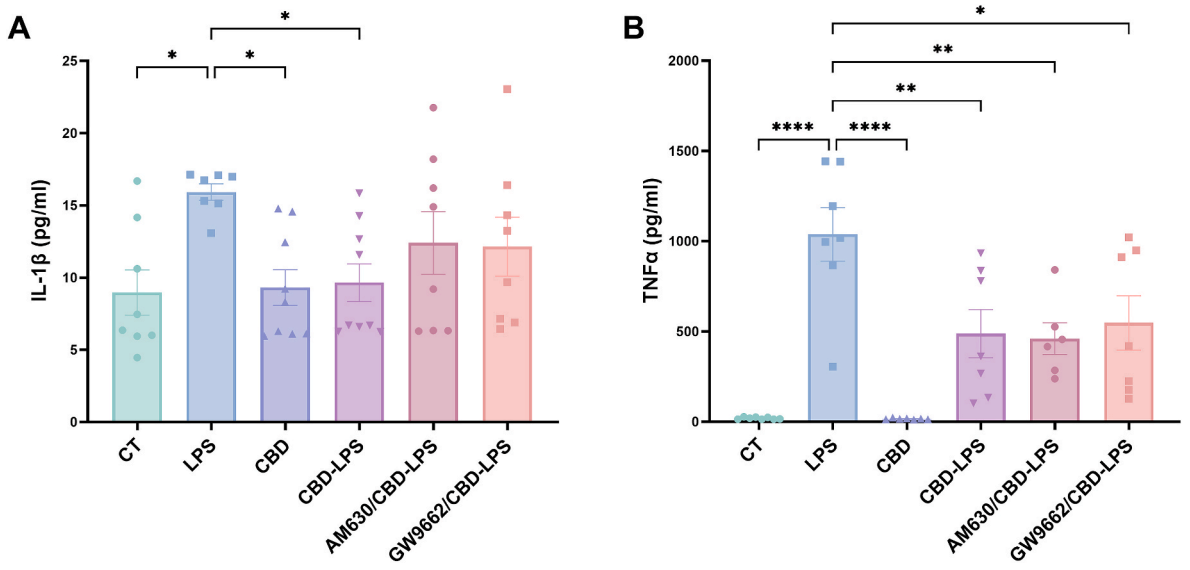

Fig. 7. Effects of CBD on IL-1β and TNF-α secretion by BV2 cells in response to LPS. CBD suppresses the overproduction of both IL-1β and TNF-α induced by LPS. CBD effects on IL-1β were marginally blocked by AM630 and GW9662 (a), while the effects on TNF-α were not affected by CB2 or PPARγ antagonism (b). *P < 0.05, **P < 0.01, ****P < 0.0001.

due to their central role in immune surveillance within the central nervous system. Cannabidiol (CBD) has been shown to promote anti-inflammatory responses in these cells, which could be beneficial for a series of conditions stemming from neuroinflammation (dos-Santos-Pereira et al., 2020; Lana et al., 2022; Landucci et al., 2022; Wang et al., 2023b). Here we have studied the signalling pathways of the anti-inflammatory potential of CBD on LPS-triggered BV2 microglial cells through the NLRP3 inflammasome and iNOS activity.

One of the first telltale signs of microglial immune response is the shift towards a pro-inflammatory phenotype, characterised by higher production and release of pro-inflammatory cytokines, ROS and NO (Plastira et al., 2016; Wang et al., 2023a). Here we observed an increase in CD86 and F4/80 surface markers in BV2 cells exposed to LPS, indicating a pro-inflammatory polarisation. Pre-treatment with CBD was able to prevent this transition. Furthermore, its effects on CD86 expression seem to be highly CB2-dependent and partially PPARγ-dependent, while the effects on F4/80 seem to be only marginally blunted by CB2 and PPARγ antagonism. Reflecting the surface findings on an internal level, we found that LPS stimulation caused a substantial increase in NLRP3 and ASC speck expression, indicative of inflammasome assembly. The mechanisms of NLRP3 inflammasome activation in macrophages have been extensively investigated, showing a strong correlation with microglia-mediated neuroinflammation in response to various insults, such as LPS (Arioz et al., 2019; Han et al., 2021; Ugur Tufekci et al., 2021), amyloid beta peptides (Fan et al., 2022; Jin et al., 2019) and mitochondrial complex inhibitors (Ahmed et al., 2021). Furthermore, LPS-stimulated cells presented a higher percentage of active Caspase-1, with a consequential increase in IL-1β concentration in cell supernatant. CBD treatment attenuated the upregulation of NLRP3 expression, Caspase-1 activity and IL-1β concentration, while the antagonism of CB2 receptors or PPARγ partially hindered this effect. Similarly, corroborating with previous studies (Alonso et al., 2023; Li

et al., 2023; Wang et al., 2021), we have also shown that CBD is able to reduce iNOS overexpression and subsequent NO secretion through PPARγ activation, but not CB2.

CB2 receptors are coupled primarily to inhibitory G proteins, leading to the suppression of adenylate cyclase and subsequent reduction in cyclic AMP (cAMP) levels. This signalling route can modulate various downstream effectors, including protein kinase A (PKA) and mitogen-activated protein kinase (MAPK) pathways, including extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 MAPK. These pathways play crucial roles in regulating inflammatory responses, cell proliferation, and cell survival in microglia, therefore, CB2 activation is known to result in an anti-inflammatory shift in microglial cells (López et al., 2018; Maddukuri et al., 2022; Tanaka et al., 2020; Young and Denovan-Wright, 2022). Likewise, PPARγ is a ligand-activated transcription factor that regulates the expression of genes involved in inflammation, lipid metabolism, and cell differentiation. Its upregulation has been shown to exert anti-inflammatory effects, upregulating nuclear factor erythroid 2–related factor 2 (Nrf2) transcription, inhibiting nuclear factor kappa B (NF-κB) activity, and promoting a shift towards the so-called M2 phenotype (Dhapola et al., 2021; Hang et al., 2020; He et al., 2022; Arturo Iannotti and Maria Vitale, 2021; Thawkar and Kaur, 2019). Accordingly, the effects of CBD on different models of neuroinflammatory conditions have been shown to be at least partially dependent on its activation of CB2 receptors and/or PPARγ (Borgonetti et al., 2022; Silva Dos Santos et al., 2023; Mori et al., 2021; Perez et al., 2021), resulting in downregulation of JNK and p38 MAPK (Li et al., 2023), reduced expression of cyclooxygenase-2 (COX-2) (Ruiz-Valdepeñas et al., 2011), and suppression of production of pro-inflammatory cytokines (Nagarkatti et al., 2009).

It is worth noting that one study by dos-Santos-Pereira and collaborators has demonstrated protective effects of CBD similar to the ones seen here on primary microglia culture, however, such effects were only marginally blunted by CB2 receptor antagonism and completely independent of PPARγ activation. Nevertheless in the present study we have used an immortalised microglial cell line, which may present distinct responses from primary cells (dos-Santos-Pereira et al., 2020).

Interestingly, CBD also exerted a protective effect against the increase in TNF-α concentration, however, the blockage of neither CB2 nor PPARγ reverted these results. While the most known targets for CBD are cannabinoid receptors 1 (CB1) and 2 (CB2), some of the biological effects of CBD are mediated through other putative candidates, including serotonergic, adenosine, α1 adrenergic, μ-opioid receptors, and transient-receptor-potential channels (Gregory Biringer, 2021; Douglas et al., 2020). CBD was also found to operate as an antagonist to the noncanonical G protein-coupled receptor 55 (GPR55) (Kaplan et al., 2017; Ryberg et al., 2007; Sharir and Abood, 2010). Evidence suggests GPR55 activation may aid effective TLR4 activation and/or signalling to induce inflammatory responses (Wilke Saliba et al., 2018; Wnorowski et al., 2021). Accordingly, GPR55 blockade can reduce activation of the arachidonic acid cascade in LPS-activated microglial cells, suggesting that CBD suppressive effects may also occur through inhibition of GPR55 (Kaplan et al., 2017; Wilke Saliba et al., 2018, 2021). Additionally, certain effects attributed to CBD through cannabinoid mediation might stem from its capacity to inhibit the degradation of endocannabinoids by the FAAH enzyme. Consequently, this leads to elevated concentrations of endocannabinoids, ultimately triggering receptor activation, mainly by anandamide (Bisogno et al., 2001; Douglas et al., 2020; Giacobbe et al., 2021). Lastly, CBD has been found to regulate mitochondrial energy metabolism, calcium concentrations, and oxidative stress in microglial cells (Li et al., 2023; Olivas-Aguirre et al., 2019; Wu et al., 2018), which would explain the partial effects seen in the presence of the antagonists, as well as persistence of its effects on TNF-α concentration even when CB2 or PPARγ were inactive.

Conversely, while NLRP3/Caspase-1 signalling is known for regulating apoptotic responses and, therefore, its downregulation should reflect on decreased cell death in response to LPS, we have not seen a recovery in viability on the CBD-LPS group. This corroborates with similar findings by Li et al., which showed that CBD improved inflammatory profile without rescuing cell viability on BV2 cells (Li et al., 2023). This raises the hypothesis that although CBD does not protect against LPS-triggered cell death, it may push the remaining cells towards an anti-inflammatory state, which can result in improved tissue repair over time, as shown in tissue regeneration studies (Kamali et al., 2019; Kumar Khajuria et al., 2023; Miller et al., 2021).

In summary, these findings bolster the anti-inflammatory role of CBD in BV2 microglial cells, primarily mediated through the inhibition of the NLRP3/Caspase-1 inflammasome complex and iNOS activity, with subsequent reduction of pro-inflammatory hallmarks. This knowledge can help narrow down targets for the treatment of neuroinflammatory conditions stemming from microglial hyperactivation.

5. Conclusions

Altogether, these findings indicate that CB2 receptors and PPARγ mediate the anti-inflammatory effects of CBD on the NLRP3 inflammasome complex, iNOS activity and, ultimately, on microglial phenotype. Our results highlight the specific components responsible for the potential therapeutic applications of CBD on neuroinflammatory conditions related to microglia function. More studies should be conducted on primary cultured cells and animal models in order to better explore these signalling pathways.

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Ethical approval

This is an in vitro study using immortalised cell lines. Lancaster University Research Ethics Committee has confirmed that no ethical approval is required.

CRediT authorship contribution statement

Fernanda da Silva Rodrigues: Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **William Robert Newton:** Investigation, Formal analysis. **Isadora D'Ávila Tassinari:** Investigation, Conceptualization. **Felipe Henrique da Cunha Xavier:** Formal analysis, Data curation. **Adél Marx:** Writing – original draft, Investigation. **Luciano Stürmer de Fraga:** Writing – review & editing, Supervision, Conceptualization. **Karen Wright:** Writing – review & editing, Supervision, Resources, Conceptualization. **Renata Padilha Guedes:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Victorio Bambini-Jr:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors have no relevant financial or non-financial interests to disclose.

Data availability

Data will be made available on request.

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4. DISCUSSÃO

4.1. Dieta de Cafeteria como indutora de obesidade nas mães

Estudos anteriores realizados pelo nosso grupo de pesquisa (GONZÁLEZ et al., 2023; JANTSCH et al., 2023; NETO et al., 2023), juntamente com inúmeros estudos no campo (LEIGH et al., 2020; LALANZA; SNOEREN, 2021; MATUSZEWSKA et al., 2021), apoiam a caracterização da CAF como um modelo robusto de obesidade induzida por dieta. O presente estudo reforça essa caracterização ao demonstrar aumento do peso corporal nas fêmeas a partir da nona semana de dieta até o fim da gestação, consumo calórico exacerbado desde a primeira semana de dieta, dislipidemia e distúrbios no controle glicêmico. Ainda, demonstramos um aumento na concentração de LPS circulante. Dietas obesogênicas alteram a composição da microbiota intestinal e diminuiem a diversidade bacteriana, o que pode ser atribuído à abundância de açúcares refinados e gorduras, bem como à baixa ingestão de vegetais, frutas e fibras dietéticas (DO et al., 2018; FAJSTOVA et al., 2020; SATOKARI, 2020).

A translocação de LPS - o componente mais imunogênico das bactérias Gram negativas - para a corrente sanguínea é um indicador indireto de disbiose e dano à barreira intestinal, gerando um processo inflamatório difuso chamado endotoxemia (MOHAMMAD; THIEMERMANN, 2021). Os componentes estruturais do LPS são reconhecidos por células B via cluster de diferenciação 14 (CD14) e receptor tipo Toll 4 (TLR4), levando à ativação do NFkB e à liberação de citocinas pró-inflamatórias, como TNFα e IL1-β (VARGAS-CARAVEO et al., 2017). Durante a gestação, esses mediadores pró-inflamatórios, juntamente com o próprio LPS, podem interagir com a placenta e causar uma variedade de distúrbios, incluindo vasos sanguíneos imaturos, hipóxia, aumento da inflamação, autofagia e marcadores de estresse alterados (GOHIR et al., 2019; CSATLOSOVA et al., 2021), que contribuem para problemas de saúde na mãe, como diabetes gestacional, obesidade, pré-eclâmpsia, distúrbios digestivos e autoimunes, além de interferir no desenvolvimento do feto (SAJDEL-SULKOWSKA, 2023). Nesse sentido, vários modelos de AIM dependem da exposição pré-natal ao LPS,

resultando em uma infinidade de resultados fisiológicos e neurológicos alterados na prole (OSKVIG et al., 2012; SIMÕES et al., 2018).

Estudos genéticos e epidemiológicos demonstram a contribuição de um histórico transgeracional de obesidade parental para o desenvolvimento de obesidade em si, outros riscos metabólicos e distúrbios do neurodesenvolvimento na prole (RIVERA et al., 2020; TRUJILLO VILLARREAL et al., 2021; GOMES et al., 2022). Os dados apresentados neste trabalho corroboram com o conhecimento de que a obesidade materna perturba o desenvolvimento perinatal da prole, evidenciando a intrincada interação entre neuroinflamação, neurotransmissão e o SEc, potencialmente levando a distúrbios metabólicos e comportamentais. Além disso, neste estudo, a prole foi mantida em uma dieta padrão após o desmame, demonstrando que as alterações observadas no comportamento e na composição tecidual não são influenciadas pela dieta da prole, mas sim decorrem da programação perinatal relacionada à dieta e obesidade materna.

Além de desvendar os mecanismos pelos quais a obesidade materna afeta a saúde da prole, um número crescente de pesquisas tem explorado possíveis soluções. No entanto, muitas dessas soluções concentram-se em medidas preventivas direcionadas à mãe durante o período pré-concepção e/ou durante a gestação (BUFFINGTON et al., 2016). Ainda, a maioria dos estudos se concentra predominantemente na prole masculina, deixando uma lacuna na compreensão de como essas exposições perinatais afetam a prole feminina.

4.2. Alterações metabólicas periféricas na prole

Além da própria obesidade, o consumo de uma dieta rica em gorduras saturadas e carboidratos também está associado ao desenvolvimento de metainflamação, um estado crônico e auto-sustentável de inflamação de baixo grau (LAMA et al., 2022). Neste trabalho, demonstramos que, durante a adolescência, tanto os machos quanto as fêmeas filhotes de mães obesas apresentaram maiores depósitos de tecido adiposo visceral, assim como níveis elevados de glicose em jejum e de LPS circulante, enquanto somente as fêmeas apresentaram um aumento no colesterol e triglicerídeos plasmáticos, e somente os machos apresentaram

elevação da insulina e, consequentemente, do índice de resistência à insulina (HOMA-IR). Já durante a vida adulta, ambos apresentaram níveis elevados de LPS, enquanto apenas os machos apresentaram maiores depósitos de tecido adiposo visceral e apenas as fêmeas apresentaram maiores concentrações de glicose e triglicerídeos plasmáticos, indicando um importante efeito da idade e do sexo na gravidade dos impactos da obesidade materna.

Estudos anteriores com diferentes modelos de obesidade materna estabelecem que tanto a gestação quanto o período de lactação são críticos para o desenvolvimento do tecido adiposo branco, impactando a regulação epigenética de genes-chave para o metabolismo energético - como genes relacionados à transmissão dopaminérgica e opióide vinculados ao comportamento alimentar (VUCETIC et al., 2010) e sensores nutricionais hipotalâmicos (DESAI et al., 2020) - e alterando os *checkpoints* da homeostase do tecido adiposo a longo prazo (MINA et al., 2017; MENTING et al., 2019). Em um modelo de dieta materna rica em gordura (*high fat diet*, HFD), a prole de mães obesas mostrou uma elevação na expressão e na atividade da estearoil-CoA desaturase-1 (SCD1), uma enzima chave do metabolismo de ácidos graxos. A SCD1 converte AGs saturados, como palmitato e estearato, em AGs monoinsaturados, os substratos predominantes para a síntese de triglicerídeos (BUTRUILLE et al., 2019). É importante destacar que a CAF, que se assemelha aos padrões alimentares urbanos ocidentais, não é apenas rica em açúcar, mas também em gorduras saturadas, com o palmitato sendo o AG predominante, o que pode justificar o impacto proeminente desse padrão dietético no perfil lipídico (CHEN et al., 2018; YU et al., 2019).

Curiosamente, nenhum efeito da obesidade materna foi encontrado no peso corporal total tanto na adolescência quanto na vida adulta, mesmo que o acúmulo de tecido adiposo tenha sido alterado. Esse achado pode ser devido a uma redução na massa muscular esquelética que pode ter compensado a adiposidade aumentada. Em estudos anteriores, filhotes de mães alimentadas com CAF de 3 e 12 semanas de idade apresentaram peso corporal e massa magra iguais ou menores, mas com uma maior acúmulo de gordura, o que foi descrito como o fenótipo “magro por fora - gordo por dentro” (POMAR et al., 2017; VITHAYATHIL et al., 2018). No entanto, quando a prole de mães alimentadas com CAF recebeu CAF

após o desmame, também não houve aumento no peso corporal na puberdade (4 semanas de vida), mas sim na vida adulta (16 semanas de vida). Assim, as diferenças na composição corporal parecem depender também da dieta da prole ao longo da vida (MUCELLINI et al., 2014; MATUSZEWSKA et al., 2021).

Um número crescente de estudos tem abordado o papel pleiotrópico do SEc na regulação metabólica a nível central e periférico. A regulação endocanabinoide do metabolismo é extremamente relevante para o SNC, especialmente no hipotálamo, onde desempenha um papel fundamental no equilíbrio energético e nos comportamentos alimentares, contribuindo não apenas para a patogenicidade da obesidade, mas também para o desenvolvimento de transtornos alimentares (BI et al., 2020; DI GIACOMO et al., 2020; PUCCI et al., 2022). No entanto, seus receptores também são expressos em órgãos periféricos, como o tecido adiposo, fígado, músculo esquelético, pâncreas, rins e trato gastrointestinal (JOSHI; ONAIVI, 2019), daí seu potencial particularmente promissor no contexto da obesidade e distúrbios metabólicos (RAMÍREZ-OROZCO et al., 2019; MORENO; CAVIC; CANELA, 2021). Aqui, mostramos que o tratamento com CBD é capaz de reverter uma série de disfunções metabólicas decorrentes do consumo materno de CAF durante a gravidez e lactação principalmente na prole juvenil, incluindo redução da adiposidade visceral, resistência à insulina e endotoxemia. Entretanto, na prole adulta, o tratamento só foi capaz de reverter o aumento de glicose plasmática e LPS nas fêmeas.

A ativação dos receptores CB1 é geralmente considerada um poderoso sinal orexigênico; portanto, a inibição dessa via pode ser benéfica para o tratamento da obesidade e doenças metabólicas relacionadas. Uma vez que o CBD é um modulador alostérico dos receptores CB1, inibindo sua ativação por ligantes endógenos ou agonistas exógenos, isso pode sugerir o caminho pelo qual o CBD atenua as perturbações periféricas decorrentes da obesidade materna (LAPRAIRIE et al., 2015). Camundongos *knockout* para os receptores CB1 (CB1-KO) mantidos em uma dieta normocalórica padrão mostraram ganho de peso diminuído ao longo do tempo, o que foi associado a um aumento no gasto de energia e níveis elevados de mRNA do receptor β(3)-adrenérgico e da UCP1 no tecido adiposo marrom,

sugerindo uma ativação simpática periférica aumentada e maior termogênese (LAPRAIRIE et al., 2015).

Dietas ricas em ácidos graxos saturados, como a dieta ocidental, aumentam a absorção e o armazenamento de esfingolipídios e suas frações essenciais, como ceramida, esfingosina, esfinganina e esfingomielina. Curiosamente, estudos anteriores sugerem que fitocanabinoides e outros agonistas dos receptores CB1 ou CB2 podem modular as concentrações de esfingolipídios em órgãos específicos no contexto de um aumento da disponibilidade de ácidos graxos na dieta. Nestes estudos, o CBD reduziu significativamente a concentração de esfingolipídios no tecido adiposo (BERK et al., 2022), no músculo esquelético (BIELAWIEC et al., 2020) e no cérebro, aumentando o catabolismo, inibindo a recaptação e/ou a síntese *de novo*, o que restaura a sensibilidade à insulina do tecido (CHARYTONIUK et al., 2021). Além disso, outros mecanismos são propostos para os efeitos benéficos do CBD em distúrbios metabólicos nos órgãos periféricos, como o efeito protetor do CBD nas células-tronco derivadas do tecido adiposo contra o estresse do retículo endoplasmático e suas complicações relacionadas à resistência à insulina e diabetes (KOWALCZUK et al., 2022) e atenuação do estresse oxidativo e resposta inflamatória, associada a uma melhor relação de ácidos graxos poli-insaturados n-6/n-3 no músculo esquelético branco e vermelho (BIELAWIEC et al., 2021), indicando uma estreita relação entre o SEc e o equilíbrio hormonal e energético.

Ainda, os dados aqui observados indicam que a endotoxemia causada por dietas obesogênicas afeta não apenas a mãe, mas pode ser observada na prole ao longo da vida, independentemente da dieta da prole em si. Esses achados corroboram estudos anteriores que demonstraram que a obesidade materna durante a gestação e/ou lactação impacta negativamente a microbiota intestinal da prole (RUBINI et al., 2022). Por outro lado, o CBD foi capaz de resgatar esse dano em toda a prole adolescente e nas fêmeas adultas. Embora não tenhamos realizado análises específicas do intestino, estudos anteriores em diferentes modelos pré-clínicos e clínicos nos levam a inferir que os efeitos observados podem ser devido à influência do CBD na composição da microbiota intestinal (AL-GHEZI et al., 2019; SILVESTRI et al., 2020; GONG et al., 2022) e/ou um efeito protetor na manutenção

da integridade da barreira intestinal (ALHAMORUNI et al., 2010; DE FILIPPIS et al., 2011; KOAY; RIGBY; WRIGHT, 2014; PAGANO et al., 2016; COUCH et al., 2019).

4.3. Alterações comportamentais na prole

Considerando a conexão já conhecida entre obesidade materna e um risco elevado de distúrbios do neurodesenvolvimento, particularmente no TEA (MOSS; CHUGANI, 2014; LI et al., 2017; CHAO; YUNGER; YANG, 2018) e esquizofrenia (KHANDAKER; DIBBEN; JONES, 2012; RIVERA et al., 2015; URBONAITE et al., 2022) na prole, e que dados clínicos recentes fortalecem ainda mais a correlação entre função social prejudicada e ansiedade, uma vez que indivíduos com autismo de alto funcionamento exibem níveis elevados de ansiedade em comparação com indivíduos neurotípicos (TOP JR. et al., 2016; PERDIKARIS; DERMON, 2023), nossa pesquisa teve como objetivo investigar tanto o comportamento semelhante à ansiedade quanto o comportamento social na prole de mães alimentadas com CAF.

Aqui observamos aumento dos comportamentos do tipo ansioso em ambos os sexos, enquanto as manifestações sociais foram heterogêneas, com os machos apresentando déficit de memória social, enquanto as fêmeas apresentaram déficit de interação/preferência social, tanto na adolescência quanto na vida adulta. Corroborando com nossos achados, um estudo usando um modelo murino de HFD materna demonstrou diferenças na interação social exclusivamente em fêmeas (KANG et al., 2014). Em contraste, outros estudos com camundongos mostraram alterações tanto na interação social quanto na memória social em machos, porém as fêmeas não foram avaliadas (BUFFINGTON et al., 2016; LIU et al., 2021). Além disso, um estudo usando um modelo de dieta ocidental materna em primatas não humanos demonstrou distúrbios na interação social em ambos os machos e fêmeas, porém a memória social não foi incluída (MITCHELL et al., 2022). Acreditamos que essas discrepâncias podem ser devido a diferenças relacionadas às espécies e à dieta, uma vez que em nosso estudo usamos ratos Wistar alimentados com CAF, além de protocolos de teste divergentes.

Assim como outros distúrbios do neurodesenvolvimento, a obesidade materna está relacionada com o desenvolvimento de hiperatividade e/ou déficit de atenção (SANCHEZ et al., 2018; CHEN et al., 2021). Em consonância com estudos em humanos que associam a obesidade materna à hiperatividade, foi demonstrado que a HFD materna também leva à hiperatividade em modelos animais (LIPPERT et al., 2020; URBONAITE et al., 2022). Interessantemente, o comportamento do tipo hiperativo foi observado somente na prole adulta, a qual também apresentou um número menor de comportamentos relacionados à ansiedade, provavelmente devido à maior atividade exploratória dos animais.

De acordo com nossa hipótese, os resultados presentes revelaram que o tratamento com CBD de fato é capaz de atenuar os comportamentos semelhantes à ansiedade nas diferentes fases da vida, bem como as deficiências sociais sexualmente dimórficas na prole juvenil de mães CAF. Entretanto, tratando-se da prole adulta, o tratamento não exerceu efeito positivo nos comportamentos do tipo hiperativo e na preferência social alterada nas fêmeas.

4.4. Neuroinflamação na prole

O hipotálamo é um dos principais centros homeostáticos do SNC e, portanto, precisa responder de forma eficaz às flutuações nos sistemas periféricos. No entanto, devido à sua permeabilidade naturalmente aumentada para melhor receber e responder a sinais provenientes de órgãos metabólicos, o hipotálamo é também uma das primeiras regiões cerebrais a sofrer com perturbações sistêmicas, resultando em neuroinflamação (RODRÍGUEZ; BLÁZQUEZ; GUERRA, 2010; FORTE et al., 2020). No presente estudo, demonstramos alterações moleculares que são padrões típicos de neuroinflamação no hipotálamo da prole adolescente e/ou adulta nascida de mães alimentadas com CAF. TNFα, IL6 e IL1-β são citocinas pró-inflamatórias bem conhecidas envolvidas na ativação microglial e astrocitária em todo o tecido nervoso. No entanto, especialmente no hipotálamo, eles têm um papel notável na modulação dos circuitos alimentares hipotalâmicos. Foi demonstrado anteriormente que dietas ricas em gordura e carboidratos estimulam neurônios orexigênicos que expressam neuropeptídeo Y/peptídeo relacionado à

agouti (NPY/AgRP) a produzir produtos finais de glicação avançada, que ativam o TNFα, aumentando a reatividade da microglia. Este cenário resulta na disfunção de neurônios anorexígenos, alterando os circuitos reguladores do apetite (DEBÉDAT; CLÉMENT; ARON-WISNEWSKY, 2019). Além disso, neurônios que expressam proopiomelanocortina (POMC), que apresentam atividades anorexígena, também sofrem um impacto significativo da obesidade materna (RAU; HENTGES, 2019). O sistema melanocortina desempenha um papel importante na regulação do apetite, gasto energético e metabolismo, portanto, deficiências no processamento pré e pós-translacional da POMC e do receptor melanocortina 4 (MC4R) são precursores para o desenvolvimento da obesidade (TODA et al., 2017; MICIONI DI BONAVENTURA et al., 2020). A diminuição da atividade nas células POMC tem sido associada ao aumento da ingestão de alimentos e obesidade (RAU; HENTGES, 2019) e foi demonstrada na prole de mães obesas (DESAI et al., 2020; KULHANEK; WEIGEL; PAULSEN, 2020; ZHENG et al., 2021). Ao observar a localização precisa dos neurônios NPY e POMC no hipotálamo da prole de mães obesas, Ornellas e colaboradores descobriram que as fibras nervosas NPY do núcleo arqueado para o núcleo periventricular e ao redor do terceiro ventrículo aumentaram, enquanto aquelas positivas para POMC estavam diminuídas nas mesmas áreas (ORNELLAS et al., 2016). Variações na reatividade e/ou distribuição de astrócitos hipotalâmicos também parecem afetar a organização sináptica e a responsividade de POMC à glicose, o que está associado a desequilíbrios energéticos e metabólicos (VARELA et al., 2017; GARCÍA-CÁCERES et al., 2019). Esses achados corroboram com dados anteriores que demonstram que camundongos nascidos de mães alimentadas com HFD têm aumento da expressão desses marcadores inflamatórios no hipotálamo em comparação com a prole de pais magros (ORNELLAS et al., 2016).

A astrogliose é um marcador bem estabelecido para a neuroinflamação relacionada à obesidade (JAIS; BRÜNING, 2017; DE OLIVEIRA et al., 2021). Variações na reatividade e/ou distribuição de astrócitos hipotalâmicos parecem afetar a organização sináptica e a responsividade a flutuações periféricas, o que está associado a desequilíbrios energéticos e metabólicos (VARELA et al., 2017; GARCÍA-CÁCERES et al., 2019). Em modelos animais de obesidade, a expressão

gênica e proteica da proteína ácida fibrilar glial (GFAP), um marcador de astrócitos, é comumente maior nos grupos obesos em comparação com os animais controle (BONDAN et al., 2019; MICIONI DI BONAVENTURA et al., 2020; DE OLIVEIRA et al., 2021; GONZÁLEZ et al., 2023). Curiosamente, neste trabalho demonstramos que, como esperado, a expressão gênica de GFAP se encontra aumentada no hipotálamo de machos adultos filhotes de mães obesas, entretanto, encontra-se reduzida tanto em machos quanto em fêmeas na adolescência. Este resultado pode ter sido originado de um mecanismo adaptativo de reprogramação em resposta à exposição a um ambiente intrauterino prejudicial durante o neurodesenvolvimento, indicando que os mecanismos moleculares que regem a neuroinflamação induzida pela obesidade materna podem diferir daqueles associados à obesidade no próprio indivíduo (JOAQUIM et al., 2017; OGASSAWARA et al., 2018; MOLINA et al., 2020). Especialmente nos estágios iniciais da vida a redução na expressão de astrócitos pode ser prejudicial durante o neurodesenvolvimento, uma vez que essas células desempenham um papel fundamental na maturação da sinapse, e sua expressão reduzida está relacionada a uma série de distúrbios neurológicos (NAGY et al., 2015; COBB et al., 2016; TORRES-PLATAS et al., 2016).

Quanto à ativação microglial, ao contrário de estudos anteriores (BAE-GARTZ et al., 2019; MALDONADO-RUIZ et al., 2019), não encontramos diferenças na expressão gênica de antígeno 1 associado a aacrófagos (IBA-1) no hipotálamo da prole de mães obesas. No entanto, a expressão de IBA-1 está relacionada à proliferação e distribuição de células microgliais e não à polarização para um estado pró-inflamatório (JURGA; PALECZNA; KUTER, 2020). Além disso, recentemente foi descrito que o estresse imune pré-natal diminui a reatividade da microglia ao longo da vida (HAYES et al., 2022), o que significa que os níveis de expressão das células microgliais podem permanecer nos níveis de controle, mas sua reatividade inata a estressores imunes pode ser defeituosa. Interessantemente, nas fêmeas, a neuroinflamação hipotalâmica parece apresentar um padrão mais agressivo nas primeiras semanas de vida, com uma expressão gênica de citocinas pró-inflamatórias exacerbada que não é vista na vida adulta.

Além disso, observamos uma expressão aumentada de IBA-1 e GFAP no córtex prefrontal e hipocampo de forma dependente da idade e do sexo. No geral,

a prole adolescente demonstrou uma maior expressão desses marcadores do que a prole adulta. A reatividade glial já foi demonstrada em modelos de AIM, estando associada a manifestações comportamentais significativas semelhantes à ansiedade e prejuízos em tarefas de memória e aprendizado (RATNAYAKE et al., 2012; ANTONSON et al., 2018; ESSHILI et al., 2020), e mostra uma importante dependência sexual (DE SOUZA et al., 2015). Nossos dados corroboram com um estudo semelhante usando obesidade materna induzida por CAF que mostrou aumento na expressão de GFAP no hipocampo da prole (TRUJILLO VILLARREAL et al., 2021), no entanto, em relação ao córtex prefrontal, os relatos são controversos. Por exemplo, enquanto alguns estudos mostram uma expressão reduzida de GFAP no córtex cerebral (OGASSAWARA et al., 2018; CONTU et al., 2019), outros mostram que não há diferenças (WHITE et al., 2009; TEO; MORRIS; JONES, 2017; RIVERA et al., 2020). É importante ressaltar que a maioria dos estudos avaliou a prole em períodos mais avançados da vida (com mais de 20 semanas de idade), os quais concordam com os dados vistos neste trabalho na prole adulta, mas não na prole adolescente. Como os efeitos da dieta materna mudam ao longo da vida da prole (CONTU; HEATH; HAWKES, 2022), acreditamos que a hiperexpressão de GFAP no córtex prefrontal vista aqui na prole juvenil pode ser uma resposta transitória especificamente para esse período da vida (6 semanas). Da mesma forma, estudos mostram tanto a expressão aumentada quanto inalterada de IBA-1 no córtex prefrontal e hipocampo da prole (WHITE et al., 2009; TEO; MORRIS; JONES, 2017; RIVERA et al., 2020).

O tratamento com CBD mostrou-se capaz de reduzir a expressão desses marcadores pró-inflamatórios nas três estruturas analisadas, relacionando-se com uma redução da reatividade glial (SCHIAVON et al., 2014; GOMES et al., 2015; SILVA-CARDOSO et al., 2023; DOS SANTOS PEREIRA et al., 2024). Essa redução pode também estar relacionada ao efeito protetor do CBD contra o perfil pró-inflamatório induzido pelo LPS sistêmico, o que corrobora com os resultados observados na dosagem plasmática tanto na prole juvenil quanto adulta (TRUJILLO VILLARREAL et al., 2021). Isso poderia indicar uma notável interseção entre inflamação periférica e central, onde a atenuação dos marcadores pró-inflamatórios periféricos acompanha a atenuação observada no cérebro.

A sinalização endocanabinóide tem sido implicada na modulação tanto da ingestão de alimentos quanto do gasto energético, além de agir na neuroinflamação. Poucos estudos avaliaram a modulação canabinóide no contexto da obesidade parental, no entanto, os achados apresentados aqui ainda estão alinhados com diferentes modelos que mostram os efeitos anti-inflamatórios do CBD em outras condições neuroinflamatórias (RAMIREZ, 2005; DA SILVA et al., 2018; AL-GHEZI et al., 2019). O elevado conteúdo de ligantes endocanabinóides no hipotálamo tem sido associado a uma maior sinalização orexigênica da grelina (TUCCI et al., 2004; KOLA et al., 2008; CARDINAL et al., 2012), assim como uma sinalização defeituosa de leptina, observada em modelos genéticos de obesidade como ratos Zucker obesos e camundongos db/db e ob/ob (DI MARZO et al., 2001; BALSEVICH et al., 2018). Esses achados sugerem que os mediadores endocanabinoides contribuem para a hiperfagia e obesidade, o que também sustenta os efeitos restauradores do tratamento com CBD, uma vez que reduz a sinalização endocanabinoide via receptores CB1 (MIRALPEIX et al., 2021). Quando se trata de inflamação, os efeitos do CBD via receptor CB2 são mais notáveis, uma vez que esse receptor é predominantemente expresso em células imunes, incluindo células gliais. A expressão de CB2 é aumentada em células da microglia estimuladas com citocinas pró-inflamatórias, indicando um papel significativo do CB2 na regulação de estados neuroinflamatórios (KASATKINA; RITTCHEN; STURM, 2021). De acordo com isso, estudos demonstram que o CBD exerce um efeito anti-inflamatório dependente de CB2 na redução de marcadores inflamatórios (DOS-SANTOS-PEREIRA et al., 2020; BORGONETTI et al., 2022).

Esses padrões de expressão gênica são consistentes com uma regulação energética e metabólica prejudicada no hipotálamo, assim como uma neuroinflamação sustentada no córtex prefrontal e hipocampo, que pode ter originado os déficits metabólicos e comportamentais observados na prole de mães obesas. Juntos, esses resultados indicam uma interação intrincada entre contrapartes periféricas e centrais tanto na patogenicidade da obesidade materna quanto na modulação do SEc pelo CBD. Nesse contexto, o comprometimento do circuito de neurotransmissão associado à neuroinflamação caminha lado a lado

com as perturbações de processos metabólicos e mecanismos cognitivos, que podem ser atenuados pelo tratamento com CBD em ambos os extremos.

4.4.1. Ativação do complexo do Inflamassoma NLRP3 em células microgliais

Apesar de não termos avaliado a via do Inflamassoma NLRP3 no tecido cerebral da prole, pudemos correlacionar um modelo *in vitro* de inflamação microglial causada pela exposição ao LPS com os dados observados *in vivo*, levando em consideração as altas concentrações de LPS circulante vistas na prole de mães obesas, assim como outros marcadores de neuroinflamação associados à atividade microglial, como o IBA-1, TNFα e IL-1β. Com isso, estudamos as vias de sinalização através das quais o CBD exerce seus efeitos anti-inflamatórios em células microgliais BV2, demonstrando sua interação com a ativação do complexo do inflamassoma NLRP3 e da iNOS.

Um dos primeiros sinais da resposta imune microglial é a transição para um fenótipo pró-inflamatório, caracterizado pela maior produção e liberação de citocinas pró-inflamatórias, espécies reativas de oxigênio e NO (PLASTIRA et al., 2016; WANG; HE; ZHANG, 2023). Aqui, observamos um aumento nos marcadores de superfície CD86 e F4/80 nas células microgliais expostas ao LPS, indicando uma polarização pró-inflamatória que pode ser equivalente ao indicado pelo IBA-1 nos animais. Ainda, observamos que a estimulação com LPS causou um aumento substancial na expressão proteica de NLRP3 e ASC, indicativo do acoplamento do inflamassoma. Os mecanismos de ativação desse complexo em macrófagos têm sido amplamente investigados, mostrando uma forte correlação com a neuroinflamação mediada por microglia em resposta a vários insultos, especialmente ao LPS (ARIOZ et al., 2019; HAN et al., 2021; TUFEKCI et al., 2021). Além disso, as células estimuladas com LPS apresentaram uma porcentagem mais alta de caspase-1 ativa, com um consequente aumento na concentração de IL-1β no sobrenadante celular. O tratamento com CBD atenuou a expressão de NLRP3, a atividade da caspase-1 e da concentração de IL-1β, enquanto o antagonismo dos receptores CB2 ou PPARγ prejudicou parcialmente esse efeito. Da mesma forma, corroborando com estudos anteriores (WANG et al., 2021; ALONSO et al., 2023; LI

et al., 2023), também mostramos que o CBD é capaz de reduzir a superexpressão de iNOS e a subsequente secreção de NO através da ativação de PPARγ, mas não de CB2.

Assim, os efeitos do CBD em diferentes modelos de neuroinflamação demonstraram ser, pelo menos parcialmente, dependentes da ativação dos receptores CB2 e/ou PPARγ (MORI et al., 2021; PEREZ et al., 2021; BORGONETTI et al., 2022; DOS SANTOS et al., 2023), resultando na regulação negativa de JNK e p38 MAPK (LI et al., 2023), na redução da expressão da ciclooxigenase-2 (COX-2) (RUIZ-VALDEPEÑAS et al., 2011) e na supressão da produção de citocinas pró-inflamatórias (NAGARKATTI et al., 2009). Esses dados corroboram parcialmente com o que foi observado no hipotálamo da prole adulta, uma vez que a redução da expressão de receptores CB2 foi acompanhada da ausência de efeito do CBD na redução das citocinas pró-inflamatórias.

Estudos demonstram que o CBD também opera como um antagonista do receptor GPR55 (RYBERG et al., 2007; SHARIR; ABOOD, 2010; KAPLAN et al., 2017). A ativação do GPR55 pode auxiliar na sinalização do TLR4 - o principal receptor alvo do LPS (SALIBA et al., 2018; WNOROWSKI; WÓJCIK; MAJ, 2021). Assim, o bloqueio do GPR55 pode reduzir a ativação da cascata do ácido araquidônico em células microgliais ativadas por LPS, sugerindo que os efeitos supressores do CBD também podem ocorrer através da inibição do GPR55 (KAPLAN et al., 2017; SALIBA et al., 2018, 2021).

No geral, esses achados reforçam o papel anti-inflamatório do CBD em células microgliais, mediado principalmente através da inibição do complexo inflamassoma NLRP3/caspase-1 e da atividade da iNOS, com consequente redução dos marcadores pró-inflamatórios, o que reforça os dados observados *in vivo*.

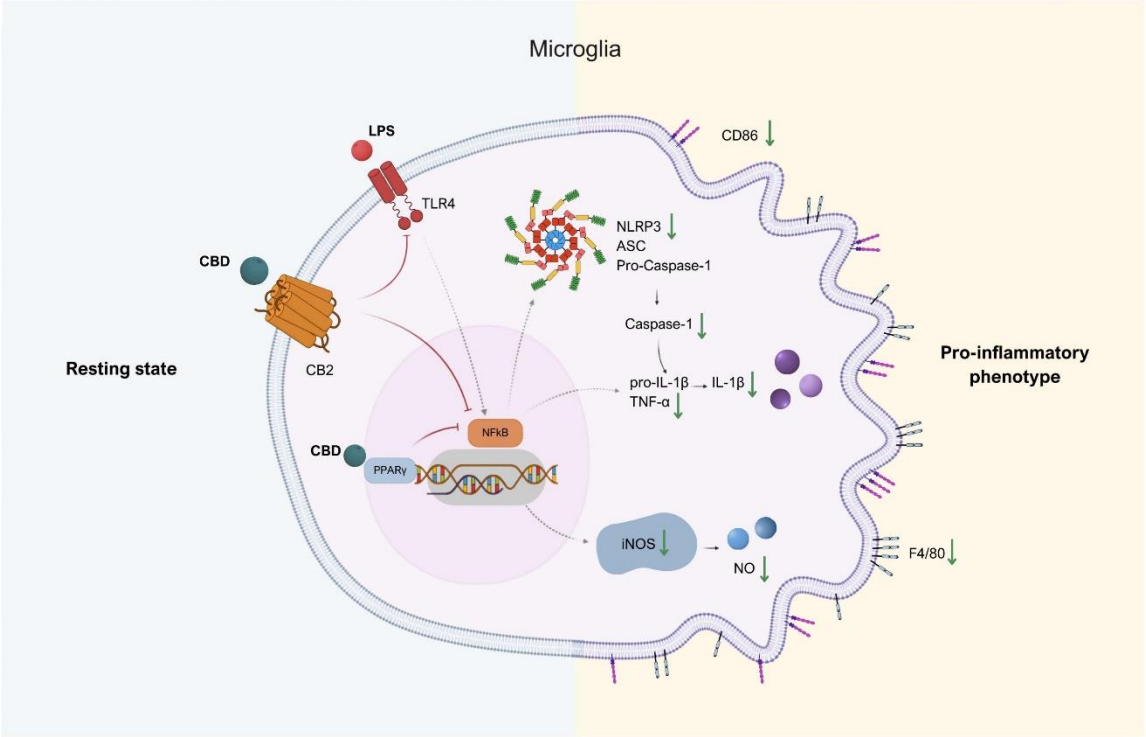

Figura 5. Mecanismos do efeito do CBD na polarização microglial via inflamassoma NLRP3. O tratamento com CBD em células microgliais BV2 inibe o complexo inflamassoma NLRP3/caspase-1 e a atividade da iNOS induzidos pela exposição ao LPS, com consequente redução dos marcadores pró-inflamatórios, via receptores CB2 e PPARγ.

4.5. Alterações no sistema endocanabinóide da prole

Embora a sinalização endocanabinoide tenha sido implicada em aspectos tanto metabólicos quanto comportamentais relacionados aos distúrbios perinatais, uma compreensão abrangente de seu papel nesse contexto ainda é elusiva. Aqui, observamos um aumento na expressão dos receptores CB1 no hipotálamo da prole adulta em resposta à obesidade materna, sem efeito do tratamento com CBD. Em concordância com nossos achados, foi constatado que a HFD materna aumenta a expressão hipotalâmica de CB1 nos descendentes, levando à disfunção da sinalização da leptina e a uma vulnerabilidade aumentada à obesidade (RAMÍREZ-LÓPEZ et al., 2016; DIAS-ROCHA et al., 2018).

Já em relação ao córtex prefrontal e hipocampo, nossos dados revelam influências significativas da dieta materna, do tratamento com CBD, do sexo e da idade da prole. No córtex prefrontal, a expressão de CB1R se encontrou aumentada nos grupos CAF-Veh nos machos adolescentes e adultos e nas fêmeas adultas, enquanto no hipocampo foi visto um aumento somente nas fêmeas adolescentes. Interessantemente, o tratamento com CBD foi capaz de reverter essa superexpressão somente na prole adolescente. A ativação do CB1R foi demonstrada como capaz de modular o equilíbrio entre excitação e inibição dos neurônios piramidais da camada II/III em direção à excitação (DEN BOON et al., 2015) e o aumento da expressão do CB1R tem sido correlacionado com a sinalização GABAérgica e glutamatérgica alterada no telencéfalo de um modelo de zebrafish com comportamento de retraimento social (PERDIKARIS; DERMON, 2023). Consequentemente, também observamos concentrações aumentadas de glutamato cortical na prole juvenil, em conformidade com outros estudos com modelos de obesidade materna (WOLFRUM; PELEG-RAIBSTEIN, 2019; MIZERA et al., 2021).

No hipocampo, o CB1R medeia a atividade dos neurônios dopaminérgicos e os níveis de dopamina em regiões do cérebro relacionadas à recompensa, e sua regulação positiva desempenha um papel crucial no desenvolvimento de anormalidades comportamentais relacionadas à recompensa e sociais (LOUREIRO et al., 2015; SUBBANNA et al., 2015). Curiosamente, tanto estudos clínicos quanto pré-clínicos de esquizofrenia têm reportado um aumento da expressão de CB1R no córtex prefrontal, indicando sua relação com distúrbios cognitivos e afetivos (DEAN et al., 2001; DALTON et al., 2011; VERDURAND et al., 2014). O CBD tem mostrado efeito repressor da sinalização do CB1R ao inibir sua ativação por ligantes endógenos ou agonistas exógenos (LAPRAIRIE et al., 2015), bem como redução direta da liberação exacerbada de glutamato a curto e longo prazo (SANTIAGO-CASTAÑEDA et al., 2022), o que está de acordo com os efeitos observados na prole juvenil. Além disso, é notável que os níveis de CB1R se correlacionam com os níveis de GFAP na adolescência. Esta associação entre astrogliose e expressão e/ou estimulação do CB1R foi previamente demonstrada em diferentes modelos de neuroinflamação (SHINJYO et al., 2013; BLANCO-CALVO et al., 2014;

BEMBRICK; BOORMAN; KEAY, 2020; AHLUWALIA et al., 2023). Adicionalmente, foi mostrado que os CB1R são expressos em astrócitos, portanto, alterações na proliferação de astrócitos poderiam refletir na expressão do CB1R também, o que concorda com os padrões observados aqui (GUTIÉRREZ-RODRÍGUEZ et al., 2018; ACHICALLENDE et al., 2022).

Em termos de inflamação, os efeitos do CBD via receptor CB2 são mais proeminentes, já que esse receptor é predominantemente expresso nas células da glia. Em estudos anteriores, o CBD demonstrou um efeito anti-inflamatório dependente do CB2 na inflamação microglial (DOS-SANTOS-PEREIRA et al., 2020; BORGONETTI et al., 2022). De fato, foi mostrado que a ativação do CB2R diminui os níveis de IL-1β e IL-6 e inibe a liberação de TNFα em células microgliais (AMENTA et al., 2014; ABD EL-RAHMAN; FAYED, 2022; ZHOU et al., 2023). TNFα e IL-6 são citocinas pró-inflamatórias bem descritas, implicadas na ativação da microglia e dos astrócitos em todo o tecido nervoso. No entanto, seu papel é particularmente notável no hipotálamo, onde exercem influência significativa sobre os circuitos hipotalâmicos de alimentação. Estudos anteriores demonstram o potencial do CBD de reduzir a produção de citocinas pró-inflamatórias (DOS-SANTOS-PEREIRA et al., 2020; GONG et al., 2022; WANG et al., 2023) e que alguns dos efeitos anti-inflamatórios do CBD dependem da ativação do CB2R (BORGONETTI et al., 2022; DOS SANTOS et al., 2023). No entanto, aqui observamos uma diminuição na expressão do CB2R no hipotálamo dos machos filhos de mães obesas, o que se correlaciona com o aumento de TNFα e IL-6. Assim, a diminuição da expressão do CB2R poderia explicar porque o tratamento não conseguiu reduzir a expressão das citocinas.

Por fim, em relação à concentração de ligantes endocanabinóides no córtex prefrontal, observamos um efeito antagônico tanto da obesidade materna quanto do tratamento com CBD entre as proles juvenil e adulta. Enquanto nos animais adolescentes foi observada uma redução nas concentrações de 2-AG e AEA na prole de mães obesas, nos machos adultos foi observado um aumento na concentração destes analitos. Foi demonstrado que o 2-AG exerce efeitos anti-inflamatórios através dos receptores CB1R/CB2R e, possivelmente, via receptores ativados por proliferadores de peroxissoma (PPARs) (ALHOUAYEK;

MASQUELIER; MUCCIOLI, 2014; MECHA et al., 2018). Considerando o papel conhecido do 2-AG como um potencial "reservatório" para a produção de ácido araquidônico durante condições inflamatórias, especula-se que níveis reduzidos de 2-AG - como visto na prole juvenil - podem estar correlacionados a uma resposta imunológica deficiente. Por outro lado, níveis aumentados de 2-AG - como visto nos adultos - podem ser uma resposta adaptativa ao aumento das citocinas pró-inflamatórias, de modo a frear a produção de eicosanóides (ALHOUAYEK; MASQUELIER; MUCCIOLI, 2014). Interessantemente, modelos de esquizofrenia induzidos por AIM apresentam uma redução nos níveis de MAGL, a enzima que hidrolisa o 2-AG, tanto no núcleo accumbens quanto no mesencéfalo. Adicionalmente, outros modelos animais de distúrbios psiquiátricos induzidos por diversos desafios pré-natais exibem uma plasticidade sináptica mediada por endocanabinoides modificada e uma sinalização aumentada de 2-AG. Isso implica em uma via compartilhada que, quando alterada, pode resultar em comprometimento do desenvolvimento neurológico (MELIS et al., 2014; FRAU et al., 2019; SANTONI et al., 2023).

Quanto ao AEA, reduções similares às vistas aqui na prole adolescente foram demonstradas em modelos de exposição pré-natal ao ácido valpróico (VPA), que se relaciona ao TEA, juntamente com o aumento da expressão da FAAH, seguido por comportamento social anormal (SERVADIO et al., 2016; MELANCIA et al., 2018). Assim, a regulação positiva do conteúdo de endocanabinoides tem sido proposta como uma alternativa para atenuar as alterações no comportamento social (MANDUCA et al., 2015; SCHIAVI et al., 2023). O CBD, por sua vez, foi descrito como um inibidor da hidrólise e captação de AEA, o que, por sua vez, aumenta as concentrações de AEA, como vimos nas fêmeas CAF tratadas com CBD (BISOGNO et al., 2001; DE PETROCELLIS et al., 2011).

Por outro lado, corroborando com o aumento da concentração de AEA vista na prole adulta, múltiplos estudos mostram níveis mais altos de AEA no plasma e no líquido cefalorraquidiano de pacientes com esquizofrenia em comparação com indivíduos saudáveis (KOETHE et al., 2009; REUTER et al., 2017; POTVIN et al., 2020). O aumento dos níveis de AEA na esquizofrenia tem sido proposto como um mecanismo homeostático para contrabalançar a neurotransmissão aumentada de

dopamina (GIUFFRIDA et al., 2004)). Consistente com esses achados, um estudo recente observou níveis mais altos de AEA em gêmeos discordantes para esquizofrenia em comparação com gêmeos saudáveis (KOETHE et al., 2009). Coletivamente, esses estudos destacam o AEA como um potencial biomarcador para avaliar o risco de desenvolvimento de psicose e para monitorar as respostas ao tratamento antipsicótico (SEABRA et al., 2018).

No geral, podemos concluir que a influência da obesidade materna sobre o SEc é variável ao longo da vida. Por exemplo, estudos recentes mostram que os níveis teciduais de 2-AG e AEA são dependentes do desenvolvimento, apresentando alterações relacionadas à idade na expressão de enzimas e receptores-chave envolvidos na função do SEc (KWOK et al., 2017). Durante a adolescência, o cérebro se encontra em uma fase crucial de desenvolvimento e remodelação sináptica, onde o SEc desempenha um papel vital na modulação da neuroplasticidade, da motivação e do comportamento emocional. Além disso, relatos sobre a ontogenia da sinalização do SEc apontam para as vastas mudanças neste sistema de maneira específica ao longo do desenvolvimento, especialmente durante a adolescência. Padrões precisos das funções do SEc ao longo do desenvolvimento caminham lado a lado com a reatividade ao estresse em períodos sensíveis, sugerindo que a sinalização corticolímbica do SEc pode estar envolvida na mediação da regulação fisiológica e comportamental específica da idade durante períodos sensíveis do desenvolvimento cerebral (GOLDSTEIN FERBER; TREZZA; WELLER, 2021).

Assim, é possível inferir que concentrações elevadas de endocanabinóides no início da vida podem estar relacionadas a respostas adaptativas mais severas e a resiliência neuronal em um período de intensa mudança cerebral. No entanto, à medida que a idade avança, a necessidade de alta plasticidade neuronal diminui, o que pode resultar na redução dos níveis de endocanabinóides na vida adulta, caracterizando uma adaptação fisiológica que reflete a transição do cérebro de um estado de desenvolvimento para um estado de manutenção e estabilidade funcional.

4.6. Alterações nas vias dopaminérgicas e oxitocinérgicas da prole

Em relação à dopamina, estudos anteriores demonstram que a AIM e a obesidade materna são capazes de alterar a sinalização dopaminérgica na prole (KHANDAKER; DIBBEN; JONES, 2012; RUEGSEGGER et al., 2017; DIAS-ROCHA et al., 2018; SANTONI et al., 2023). Curiosamente, observamos um aumento na expressão de DRD2 no córtex prefrontal dos machos CAF, sem alterações nas concentrações de HVA, um metabólito da dopamina, enquanto as fêmeas CAF mostraram uma regulação negativa da expressão de DRD2, acompanhada de concentrações mais altas de HVA, indicativas de aumento do metabolismo da dopamina e redução da sinalização dopaminérgica na prole adolescente (VAN DER HEYDEN; ROTTEVEEL; WEVERS, 2003). No hipocampo, houve uma diminuição na expressão de DRD2 apenas em fêmeas. Embora uma tendência positiva possa ser observada principalmente no córtex prefrontal, o CBD não exerceu efeitos significativos na expressão de receptores ou concentrações de HVA em ambos os machos e fêmeas.

Sabe-se que a obesidade materna está relacionada com distúrbios na transmissão dopaminérgica, especialmente em áreas cerebrais seletivas da via de recompensa, sugerindo seu papel no desenvolvimento de transtornos do desenvolvimento (TRUJILLO VILLARREAL et al., 2021). Defeitos na via de recompensa mesocorticolímbica resultam em déficits de sociabilidade e comportamentos repetitivos, características observadas nos fenótipos clássicos do TEA, por exemplo (RIVERA et al., 2020; TRUJILLO VILLARREAL et al., 2021). Além disso, em modelos pré-clínicos, os níveis de interação social observados nos animais se correlacionam com uma expressão diferencial de genes relacionados à recompensa, incluindo os sistemas opióides, serotoninérgico e dopaminérgico (ALUGUBELLY et al., 2019; DICARLO et al., 2019).

O circuito dopaminérgico está intrinsecamente ligado à atividade canabinóide uma vez que os CB1R estão densamente localizados nas regiões estriadas que medeiam a função de recompensa (WENZEL; CHEER, 2018). Na fenda sináptica, os ligantes canabinóides endógenos, são liberados por neurônios pós-sinápticos e funcionam como moduladores, ligando-se aos CB1R pré-

sinápticos para modular a liberação de neurotransmissores, como a dopamina (COVEY et al., 2017; KÖFALVI et al., 2020). Além da modulação indireta via receptores endocanabinóides, estudos demonstram que o CBD é capaz de interagir diretamente com os receptores dopaminérgicos, especialmente DRD2 (RENARD et al., 2017; MARTÍNEZ-AGUIRRE et al., 2023), sendo capaz, inclusive, de reduzir a expressão exacerbada destes receptores (SZULC et al., 2023).

Por fim, observamos um aumento na expressão gênica de OXTR no córtex prefrontal e uma redução no hipocampo, tanto em machos quanto em fêmeas adolescentes. Corroborando com nossos achados, a maioria dos estudos *in vivo* sobre transtornos afetivos correlaciona uma redução na expressão de OXTR no hipocampo com alterações comportamentais sociais e de ansiedade (TSAI et al., 2022; CYMERBLIT-SABBA et al., 2023). No entanto, pouco se sabe sobre o córtex prefrontal (MOREL et al., 2023; PIERZYNOWSKA et al., 2023). Curiosamente, estudos *post mortem* recentes indicaram que abuso na infância (ALMEIDA et al., 2022) e diagnósticos de transtorno depressivo maior e transtorno bipolar (LEE et al., 2018) estão associados a níveis significativamente aumentados de mRNA de OXTR no córtex prefrontal. Além disso, estudos epigenéticos correlacionaram uma redução na metilação do DNA de OXTR com estresse perinatal, depressão pós-natal, ansiedade social e autismo em crianças (MAUD et al., 2018). Juntas, essas descobertas podem indicar que a sinalização de ocitocina difere entre estruturas cerebrais, onde a regulação positiva no córtex prefrontal e a regulação negativa no hipocampo criam condições para transtornos neurológicos relacionados à depressão, ansiedade e sociabilidade prejudicada, como vimos nas avaliações comportamentais tanto na prole juvenil quanto adulta. Sabe-se muito pouco sobre os efeitos do CBD na sinalização de ocitocina, mas evidências sugerem que o CBD pode modular a expressão de OXTR (KOZELA et al., 2020). Em nosso estudo, o CBD foi capaz de atenuar as alterações no córtex prefrontal e no hipocampo dos machos, mas não das fêmeas.

4.7. Limitações

Dentre os objetivos propostos neste trabalho, alguns não puderam ser alcançados nas duas vertentes do estudo (adolescência vs. vida adulta). Por exemplo, nos animais juvenis, nós não avaliamos a expressão de CB1R no hipotálamo, assim como a expressão de CB2R em nenhuma das três estruturas. Já nos animais adultos, não foram avaliadas a expressão de IL1-β no hipotálamo, DRD2 e OXTR no CPF e hipocampo, assim como os neurotransmissores no córtex prefrontal e a insulina no plasma.

Apesar das observações terem sido suficientes para uma análise robusta dos mecanismos propostos considerando as idades de forma separada, acreditamos que a caracterização destes dados ao longo da vida agregaria uma perspectiva importante para o contexto geral deste trabalho.

Ainda, a avaliação *in vivo* dos mecanismos avaliados no estudo *in vitro* também contribuiria para uma melhor compreensão tanto dos efeitos nocivos da obesidade materna quanto dos benefícios do tratamento com CBD.

5. CONCLUSÕES

No geral, os dados apresentados neste trabalho sugerem que a obesidade materna induz um fenótipo neuroquímico e neuroinflamatório complexo que pode ser correlacionado com diferentes modelos de distúrbios psiquiátricos oriundos de desafios pré-natais, mas com certas particularidades específicas ao modelo de obesidade materna induzida por CAF. Ainda, evidenciamos a influência persistente da obesidade materna na saúde da prole ao longo da vida, abrangendo irregularidades metabólicas, transtornos do neurodesenvolvimento e déficits comportamentais. Pudemos demonstrar a evolução dessas alterações, correlacionando com condições psiquiátricas já conhecidas com início precoce (nos primeiros anos de vida) ou de início tardio (na vida adulta), como o TEA, a hiperatividade e a esquizofrenia, por exemplo.

Essas descobertas também evidenciam as vias de sinalização através das quais o CBD exerce seus benefícios terapêuticos, destacando o dimorfismo sexual que engloba tanto o efeito transgeracional da obesidade quanto o SEc. Estudos adicionais avaliando o SEc como modulador da neurotransmissão e

neuroinflamação em outras estruturas cerebrais são necessários para entender melhor seu potencial terapêutico. Além disso, nossos resultados destacam a importância de compreender a influência da saúde e dieta materna sobre os riscos metabólicos e neurológicos na prole a longo prazo, evidenciando a necessidade de desvendar os mecanismos subjacentes a essas associações e desenvolver estratégias de tratamento eficazes adaptadas às distintas fases da vida (Figura 5).

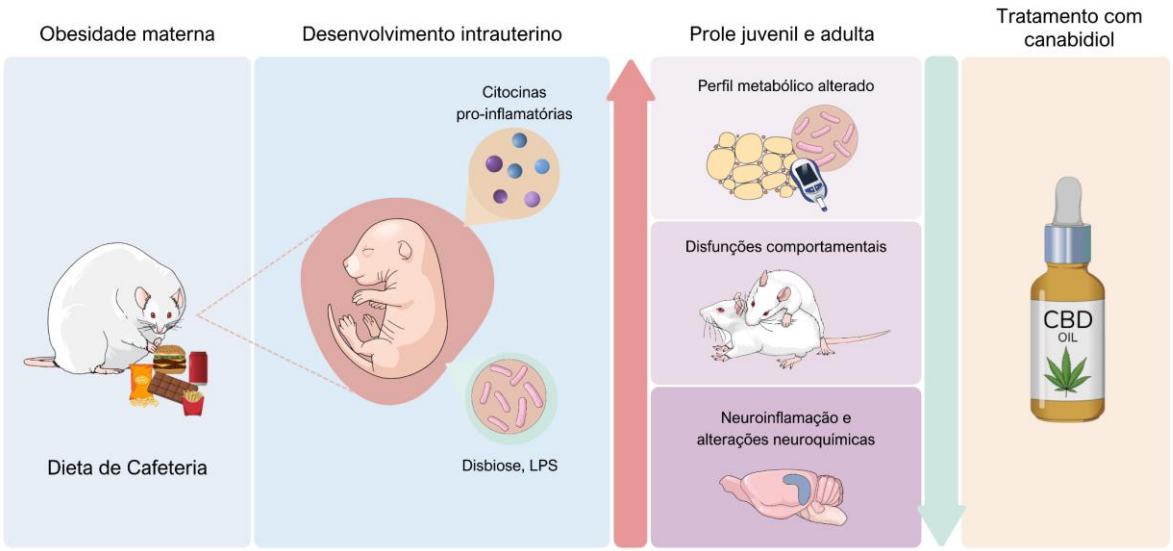

Figura 6. Conclusões gerais do estudo. A obesidade materna induzida por dieta de Cafeteria durante a gestação gera danos aos tecidos do feto em desenvolvimento, através de agentes imunogênicos, como citocinas pró-inflamatórias e lipopolissacarídeos (LPS). A prole tanto adolescente quanto adulta apresenta perfil metabólico alterado, disfunções comportamentais e alterações na sinalização endocanabinóide e nas respostas neuroinflamatórias, o que é atenuado pelo tratamento com canabidiol (CBD).

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ZHU, H. et al. Targeting CB2R in astrocytes for Parkinson’s disease therapy: unraveling the Foxg1-mediated neuroprotective mechanism through autophagy-mediated NLRP3 degradation. **Journal of Neuroinflammation**, v. 20, n. 1, p. 304, 19 dez. 2023.
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CEUA –COMISSÃO DE ÉTICA NO USO DE ANIMAIS

PARECER CONSUBSTANCIADO DE PROJETO DE PESQUISA E ENSINO

1) PROTOCOLO Nº: 282/21 versão 4

2) DATA DO PARECER:11 de agosto de 2021 Parecer 751/21

3) TÍTULO DO PROJETO:

Efeitos da manipulação do sistema endocanabinóide sobre prejuízos comportamentais e neuroinflamatórios da prole de ratas obesas

4) PESQUISADOR RESPONSÁVEL:

Renata Padilha Guedes

5) RESUMO DO PROJETO:

A obesidade ocasiona diversas alterações metabólicas, causando secreção crônica de moléculas pró-inflamatórias que podem desencadear morte celular em diferentes tecidos, incluindo o sistema nervoso central. Durante a gestação, essas citocinas pró-inflamatórias são capazes de ultrapassar a barreira placentária, podendo atingir os tecidos fetais e causar desde problemas no desenvolvimento intrauterino até desordens neurológicas que se manifestam após o nascimento, como autismo, depressão e esquizofrenia.
O projeto propõe avaliar os efeitos da obesidade materna induzida por uma dieta de cafeteria sobre parâmetros metabólicos, neuroinflamatórios e comportamentais da prole, além de aferir a capacidade do canabidiol (CBD) de atenuar essas consequências.
- hipótese: que o estado pró-inflamatório crônico desencadeado pela obesidade seja um fator de ativação imune materna (AIM), podendo favorecer o desenvolvimento de desordens neurológicas na prole; que a suplementação da prole com CBD seria capaz de atenuar esses prejuízos devido a sua capacidade de modular o sistema endocanabinóide, serotoninérgico e dopaminérgico, entre outros.
- proposta: indução de obesidade em ratas *Wistar* a partir da terceira semana de vida, utilizando uma dieta de cafeteria. A dieta será administrada por 16 semanas contando com o período de gestação e amamentação, sendo que entre a 9ª e a 10ª semana acontecerá o cruzamento, portanto a obesidade será mantida durante toda a gestação e amamentação. A prole será dividida em machos e fêmeas que passarão a receber a suplementação de CBD por gavagem a partir da 3ª ou 8ª semanas de vida, e serão submetidos aos testes comportamentais após 2 semanas de suplementação. Após os

testes, será realizada a eutanásia e coleta do material biológico para determinação do perfil metabólico, da microbiota intestinal, ácidos graxos de cadeia curta, níveis de neurotransmissores nos tecidos cerebrais, expressão dos receptores canabinóides nos tecidos cerebrais e metabólicos e ativação microglial e astrocitária.

Além disso, serão realizados ensaios *in vitro* com culturas de astrócitos e microglia para avaliação dos efeitos do canabidiol sobre mecanismos neuroinflamatórios.

6) OBJETIVOS DO PROJETO:

Objetivo geral:

Avaliar os efeitos da suplementação com canabidiol sobre aspectos metabólicos, comportamentais e neuroinflamatórios da prole gerados pela obesidade materna.

Objetivos específicos:

- Identificar comportamentos do tipo autista na prole juvenil e do tipo esquizofrênico na prole adulta de mães obesas.

- Avaliar o efeito da suplementação de canabidiol sobre parâmetros comportamentais da prole.

- Determinar o perfil metabólico e inflamatório das mães obesas por meio de dosagens plasmáticas de insulina, leptina, glicemia, triglicerídeos, HDL-colesterol, LDL-colesterol e citocinas inflamatórias.

- Caracterizar microbiota intestinal das mães e da prole.

- Determinar os níveis plasmáticos de ácidos graxos de cadeia curta das mães e os níveis plasmáticos e cerebrais na prole.

- Avaliar a ativação microglial e astrocitária na prole das mães obesas.

- Investigar o efeito da dieta e do tratamento com canabidiol sobre níveis de neurotransmissores (ocitocina, serotonina, dopamina e GABA) e de BDNF no córtex cerebral e hipocampo da prole.

- Avaliar a expressão dos receptores CB1R e CB2R no córtex cerebral, hipocampo, intestino e fígado.

- Realizar ensaios in vitro com culturas de astrócitos e microglia para avaliação dos efeitos do canabidiol sobre mecanismos neuroinflamatórios.

7) FINALIDADE DO PROJETO: [ ] Ensino [x] Pesquisa

8) ITENS METODOLÓGICOS E ÉTICOS DO PROJETO:

Título	[x]	Adequado	[ ]	Comentários
Introdução	[x]	Adequada	[ ]	Comentários
Objetivos	[x]	Adequados	[ ]	Comentários
Relevância e Justificativa	[x]	Adequados	[ ]	Comentários
Materiais e Métodos	[x]	Adequados	[ ]	Comentários
Cronograma para execução da pesquisa	[x]	Adequado	[ ]	Comentários
Orçamento e fonte financiadora	[x]	Adequados	[ ]	Comentários
Referências Bibliográficas	[x]	Adequadas	[ ]	Comentários

9) O PROJETO ESTÁ ADEQUADO À LEGISLAÇÃO VIGENTE:

[x] Sim [ ] Não

10) INFORMAÇÕES RELATIVAS AOS ANIMAIS:

Grau de dor/estresse: **B** | x **C** [ ] **D** [ ] **E** [ ]

Justifique:

Restrição hídrica e alimentar de 24h, restrição alimentar de 6h antes da eutanasia, imobilização para eutanasia, gavagem, testes comportamentais.

Grau de invasividade 1

Grau de severidade moderada.

Espécie: rato wistar **Número Amostral:** 159

Redução Amostral: [ ] Sim [x] Não

Justifique:

159 animais serão pedidos em diferentes momentos, ver item 11 (cronograma de utilização de animais).

Substituição de Metodologia: [ ] Sim [x] Não

Se achar necessário, justifique e sugira uma nova metodologia:

Aprimoramento da Metodologia: [ ] Sim [x] Não

Se achar necessário, justifique e sugira aprimoramentos da metodologia:

Acomodação e manutenção dos animais: [x] Adequada [ ] Inadequada

Se achar inadequada cite abaixo as melhorias necessárias:

Manipulação dos animais: [x] Adequada [ ] Inadequada

Se achar inadequada cite abaixo as melhorias necessárias:

Analgesia dos animais (se aplicável): [ ] Adequada [ ] Inadequada

Se achar inadequada cite abaixo as melhorias necessárias com analgésico substituto:

não aplicável

Anestesia dos animais (se aplicável): [ ] Adequada [ ] Inadequada

Se achar inadequada cite abaixo as melhorias necessárias com anestésico substituto:

não aplicável

Eutanásia dos animais (se aplicável): [x] Adequada [ ] Inadequada

Se achar inadequada cite abaixo as melhorias necessárias com metodologia substituta:

Faltou colocar a VIA de administração no formulário e no projeto

Local de Realização (Biotério/Labotarório):

Dieta - Biotério da UFCSPA,
Testes comportamentais - Lab. de fisiologia da UFCSPA,
Dosagens e western blot - Lab. de fisiologia comportamental e metabólica da UFCSPA,
Imunohistoquímica - Lab. de pesquisa em patologia da UFCSPA,
RT-qPCR - Lab. de biologia molecular da UFCSPA
Dosagem de neurotransmissor e acidos graxos - Central analitica da UFCSPA

Outra instituição. Qual?

11) CRONOGRAMA DE UTILIZAÇÃO DE ANIMAIS

Data	Espécie	Sexo	Quantidade
piloto: pedido 1	rato wistar (21 dias)	fêmea	6
piloto: pedido 2	rato wistar (cruzamento - 60d)	macho	3
resultado cruzamento	prole piloto		(48)
pedido 1	rato wistar (21 dias)	fêmea	24
pedido 2	rato wistar (cruzamento - 60d)	macho	12
resultado do cruzamento	prole (experimento)		(192)
	rato wistar (21 dias)		24
pedido - comportamento	rato wistar (1 a 3 dias)		90
pedido - estudo in vitro			

12) RECOMENDAÇÃO: As pendências deverão ser respondidas em uma carta, indicando as páginas do projeto que foram alteradas (nova versão), assinadas pelo pesquisador responsável.

[x] Aprovado

[ ] Com Pendência

[ ] Não aprovado

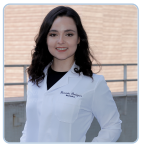
Data de início: 01 de junho de 2021 ou a partir da aprovação do CEUA Data de

Término: 31/03/2025

Comentários gerais sobre o projeto:

Currículo Lattes

Imprimir currículo



Fernanda da Silva Rodrigues

Endereço para acessar este CV: **https://lattes.cnpq.br/0001396283392703**

Última atualização do currículo em 09/06/2024

Biomédica formada pela Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA - 2020), com habilitação em Patologia Clínica (Hospital Materno Infantil Presidente Vargas - 2019) e Biologia Molecular (Laboratório Amplicon - 2020). Bolsista de doutorado-direto (CNPq) em Neurobiologia Celular e Molecular do PPG-Biociências da UFCSPA, sob orientação da Profa. Dra. Renata Padilha Guedes. Bolsista de doutorado sanduíche (CAPES) e pesquisadora visitante na Universidade de Lancaster - Reino Unido (11/2022 - 07/2023). Foi bolsista de Iniciação Científica no Centro de Memória do Instituto do Cérebro (PUCRS) orientada pelo Prof. Dr. Iván Izquierdo e pela Profa. Dra. Jociane de Carvalho Myskiw (2016-2018). Foi aluna de mobilidade acadêmica por um semestre em Tecnologia Biomédica no Instituto Politécnico de Setúbal - Portugal (2018). Possui experiência em neurofisiologia, comportamento e metabolismo. Atualmente dedica-se ao estudo dos mecanismos fisiopatológicos da obesidade materna perinatal e a integração com o sistema endocanabinóide, buscando elucidar seus efeitos sobre o neurodesenvolvimento e o metabolismo da prole. fernandargois@gmail.com ORCID: 0000-0002-1070-6379 **(Texto informado pelo autor)**

Identificação

Nome Fernanda da Silva Rodrigues

Filiação Janete Maria da Silva

Nascimento 13/01/1996 - Porto Alegre/RS - Brasil

Nome em citações bibliográficas RODRIGUES, F. S.;DA SILVA RODRIGUES, F.;DA SILVA RODRIGUES, FERNANDA;RODRIGUES, FERNANDA S.;RODRIGUES, FERNANDA DA SILVA;RODRIGUES, FERNANDA

Formação acadêmica/titulação

2020 Doutorado em BIOCIÊNCIAS.
Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, UFCSPA, Porto Alegre, Brasil
Título: EFEITOS DA MANIPULAÇÃO DO SISTEMA ENDOCANABINÓIDE SOBRE PREJUÍZOS COMPORTAMENTAIS E NEUROINFLAMATÓRIOS DA PROLE DE RATAS OBESAS
Orientador: Renata Padilha Guedes
Co-orientador: Marcia Giovenardi
Bolsista do(a): Conselho Nacional de Desenvolvimento Científico e Tecnológico, CNPq, Brasil.
Palavras-chave: Neurociência, obesidade, neuroinflamação.

2015 - 2020 Graduação em Biomedicina.
Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, UFCSPA, Porto Alegre, Brasil
Título: EFEITO DE UMA DIETA DE CAFETERIA ASSOCIADA À ADMINISTRAÇÃO DE ÔMEGA-3 SOBRE COMPORTAMENTOS RELACIONADOS À ANSIEDADE EM RATOS WISTAR
Orientador: Renata Padilha Guedes

Formação complementar

2016 - 2016 Extensão universitária em Curso de Extensão Teoria e Prática no Instituto de Pesquisas Biomédicas. (Carga horária: 480h).
Pontifícia Universidade Católica do Rio Grande do Sul, PUCRS, Porto Alegre, Brasil

2017 - 2017 Curso de curta duração em Curso Sobre Perícia Criminal. (Carga horária: 10h).
Instituto Galeno, IG, Brasil

2018 - 2018 Tecnologia Biomédica. . (Carga horária: 140h).
Instituto Politécnico de Setúba, ESCE, Setubal, Portugal

2020 - 2020 Curso de curta duração em XI Curso de Neurociências. (Carga horária: 42h).
Universidade Federal do Rio Grande do Sul, UFRGS, Porto Alegre, Brasil

Atuação profissional

Pontifícia Universidade Católica do Rio Grande do Sul - PUCRS

2016 - 2018 Vínculo: Bolsista , Enquadramento funcional: Iniciação Científica , Carga horária: 20, Regime: Pontifícia Universidade Católica do Rio Grande do SulParcial
Outras informações:
Pesquisa básica em Neurociências no Centro de Memória do Instituto do Cérebro sob orientação do Prof. Dr. Iván Antonio Izquierdo e Profa. Dra. Jociane de Carvalho Myskiw. Realização de cirurgia estereotáxica, tarefas comportamentais (Reconhecimento Social, Reconhecimento de Objetos, Esquiva Inibitória, Campo Aberto, Labirinto em Cruz Elevado, Medo Condicionado ao Contexto), eutanásia, histologia, infusão de fármacos intraestrutualmente e condução de experimentos em geral.

Fundação Universidade Federal de Ciências da Saúde de Porto Alegre - UFCSPA

2019 - 2019 Vínculo: Voluntário , Enquadramento funcional: Programa de Iniciação à Docência , Carga horária: 20, Regime: Fundação Universidade Federal de Ciências da Saúde de Porto AlegreParcial
Outras informações:
Desenvolvimento de Objetos de Aprendizagem para o Ensino de Fisiologia, sob orientação da Profa. Dra. Renata Padilha Guedes

2019 - 2020 Vínculo: Bolsista , Enquadramento funcional: Iniciação Científica , Carga horária: 20, Regime: Fundação Universidade Federal de Ciências da Saúde de Porto AlegreParcial
Outras informações:
Pesquisa em Neurociência na área da neuroinflamação sob orientação da Profa. Dra. Renata Padilha Guedes. Realização de tarefas comportamentais, preparação de reagentes e amostras biológicas,

administração de dietas e gavagem em modelos biológicos, realização de ensaios bioquímicos, histológicos, imunológicos e moleculares.

Amplicon Laboratório de Biologia Molecular - ABM

2020 - 2020 Vínculo: Estágio , Enquadramento funcional: Estágio curricular em Biologia Molecular , Carga horária: 30, Regime: Amplicon Laboratório de Biologia MolecularParcial
Outras informações:
Realização de análises em amostras biológicas para determinação de doenças genéticas, metabólicas e infecciosas, usando diversas técnicas automatizadas e in house, como PCR regular e em tempo real, eletroforese, sequenciamento e Array genômico. Período como voluntária no diagnóstico molecular de Sars-CoV-19. Carga horária total: 500 horas.

HOSPITAL MATERNO INFANTIL PRESIDENTE VARGAS - HMIPV

2019 - 2019 Vínculo: Estágio , Enquadramento funcional: Estágio curricular em Patologia Clínica , Carga horária: 30, Regime: HOSPITAL MATERNO INFANTIL PRESIDENTE VARGAS Parcial
Outras informações:
Recebimento, registro, preparo e encaminhamento de amostras biológicas (sangue, plasma, urina e secreções). Realização de análises hematológicas, bioquímicas, microbiológicas e urinálise por meios manuais e automatizados. Interpretação de resultados e elaboração de laudos. Experiência com sistemas CT/Wiener Lab, Sysmex, Vitek, Bactec, Madya, entre outros. Carga horária total: 500 horas.

Universidade Federal de Pelotas - UFPEL

2020 - 2021 Vínculo: Voluntário , Enquadramento funcional: Entrevistador , Carga horária: 60, Regime: Universidade Federal de PelotasIntegral
Outras informações:
Estudo Epidemiológico Populacional Sobre a Prevalência de Sars-CoV-2 no Rio Grande do Sul - EPICOVID.

Lancaster University - LANCASTER

2022 - 2023 Vínculo: Bolsista , Enquadramento funcional: Pesquisadora Visitante , Carga horária: 40, Regime: Lancaster UniversityDedicação exclusiva
Outras informações:
Bolsista de Doutorado Sanduíche - CAPES

Projetos

Projetos de pesquisa

2022 - Atual EFEITOS DA RESTRIÇÃO CALÓRICA E DO CONSUMO DE PREBIÓTICOS SOBRE O SISTEMA NERVOSO CENTRAL E O EIXO INTESTINO-CÉREBRO EM RATOS IDOSOS OBESOS

Descrição: O acúmulo de tecido adiposo e alterações no eixo intestino-cérebro desencadeiam um perfil inflamatório crônico e de baixo grau. Esse processo inflamatório também afeta o sistema nervoso central, causando neuroinflamação, o que pode acelerar e/ou intensificar condições patológicas relacionadas ao envelhecimento, incluindo doenças psiquiátricas e neurodegenerativas. Porém, as estratégias para impedir ou atenuar as consequências nocivas da obesidade durante o envelhecimento são escassas. Este projeto propõe testar os efeitos protetores da restrição calórica (RC) e da suplementação com prebióticos galacto-oligossacarídeos (GOS) sobre manifestações neuroinflamatórias, cognitivo comportamentais e alterações no eixo intestino-cérebro em um modelo de obesidade.
Situação: Em andamento Natureza: Projetos de pesquisa
Integrantes: Fernanda da Silva Rodrigues; Renata Padilha Guedes (Responsável); Jeferson Jantsch

2020 - Atual ANÁLISE DE INTERVENÇÕES NA DIETA, EM DIFERENTES FASES DO DESENVOLVIMENTO, ATRAVÉS DA AVALIAÇÃO DE PARÂMETROS COMPORTAMENTAIS, BIOQUÍMICOS, IMUNOLÓGICOS E GENÉTICOS

Descrição: Nas últimas décadas houve um aumento expressivo de estudos que demonstram o impacto nutricional sobre a saúde dos indivíduos. Contudo, ainda existem lacunas acerca dos mecanismos relacionados às alterações metabólicas da prole em decorrência do consumo alimentar inadequado das mães e de sua própria dieta ao longo da vida. Desta forma, o presente projeto tem como objetivo avaliar os efeitos de intervenções na dieta (restrição calórica, probióticos e dieta hipercalórica), em diferentes fases da vida do animal, através da análise de parâmetros comportamentais, bioquímicos, imunológicos e genéticos. A hipótese central deste estudo é que a restrição calórica e a administração de probióticos no período gestacional e lactacional poderão proteger a interface maternofetal, além de prevenir na vida adulta e na senescência, o aparecimento de alterações metabólicas, comportamentais, imunológicas e genéticas causadas pela alimentação hipercalórica ao longo da vida.
Situação: Em andamento Natureza: Projetos de pesquisa
Integrantes: Fernanda da Silva Rodrigues; Renata Padilha Guedes; Marcia Giovenardi (Responsável); Nicole Hiller Bondarczuk; Natalia Schmidt

2020 - Atual EFEITOS DA MANIPULAÇÃO DO SISTEMA ENDOCANABINÓIDE SOBRE PREJUÍZOS COMPORTAMENTAIS E NEUROINFLAMATÓRIOS DA PROLE DE RATAS OBESAS

Descrição: Nas últimas décadas, o número de indivíduos obesos tem crescido de forma exponencial, fazendo com que a obesidade já seja considerada uma pandemia mundial e um sério problema de saúde pública. Durante a gestação, as citocinas pró-inflamatórias secretadas no contexto da obesidade são capazes de ultrapassar a barreira placentária, podendo atingir os tecidos fetais e causar desde problemas no desenvolvimento intrauterino até desordens neurológicas que se manifestam após o nascimento, como autismo, depressão e esquizofrenia. Entretanto, ainda existem lacunas acerca dos mecanismos relacionados às alterações metabólicas e neurológicas da prole em decorrência do consumo alimentar inadequado das mães e possíveis intervenções que amenizem esses efeitos. Este projeto propõe avaliar os efeitos da obesidade materna induzida por uma dieta de cafeteria sobre parâmetros metabólicos, neuroinflamatórios e comportamentais da prole, além de aferir a capacidade do canabidiol (CBD) de atenuar essas consequências.
Situação: Em andamento Natureza: Projetos de pesquisa
Integrantes: Fernanda da Silva Rodrigues; Renata Padilha Guedes (Responsável); Jeferson Jantsch; Marcia Giovenardi
Financiador(es): Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq

2019 - 2023 EFEITOS METABÓLICOS E NEUROINFLAMATÓRIOS DA SUPLEMENTAÇÃO DE ÔMEGA-3 EM RATOS OBESOS

Descrição: Devido ao número crescente de indivíduos obesos, a obesidade tem sido considerada uma verdadeira epidemia mundial, tornando-se um sério problema de saúde pública. A obesidade ocasiona diversas alterações metabólicas, causando secreção crônica de moléculas pró-inflamatórias. Esse estado inflamatório sustentado desencadeia morte celular em diferentes tecidos do corpo, incluindo o sistema nervoso central (SNC). Desta forma, o presente projeto propõe avaliar o efeito da suplementação de ômega-3 sobre parâmetros metabólicos e neuroinflamatórios em ratos submetidos a uma dieta de cafeteria.
Situação: Concluído Natureza: Projetos de pesquisa
Integrantes: Fernanda da Silva Rodrigues; João Pereira Neto; Renata Padilha Guedes (Responsável)
Financiador(es): Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq

2017 - 2018 BLOQUEIO DOS RECEPTORES SEROTONINERGICOS 5-HT5A, 5-HT6 E 5-HT7 NA AMIGDALA BASOLATERAL E NA REGIÃO CA1 DO HIPOCAMPO

Descrição: A memória de medo está relacionada com nossa sobrevivência, a partir dela associamos uma situação de perigo com uma atitude de luta ou fuga. No entanto, as memórias de medo, quando disfuncionais, podem estar associadas a transtornos psiquiátricos, como transtornos de ansiedade, depressão e transtorno do estresse pós-traumático (TEPT). Esta pesquisa tem como objetivo verificar, em duas regiões cerebrais diferentes (região CA1 do hipocampo dorsal e amígdala basolateral), a participação dos receptores 5-HT5A, 5-HT6 e 5-HT7 serotoninérgicos na extinção da memória de medo

condicionado ao contexto.
Situação: Concluído Natureza: Projetos de pesquisa
Integrantes: Fernanda da Silva Rodrigues; JOCIANE DE CARVALHO MYSKIW; IVÁN ANTONIO IZQUIERDO (Responsável); EDUARDO DE ASSIS BRASIL
Financiador(es): Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-CAPES
Número de produções C,T & A: 1/

2015 - 2017 A INTERAÇÃO ENTRE: A EXPOSIÇÃO A UM CO-ESPECÍFICO, O RECEPTOR CB1 E A CONSOLIDAÇÃO E EXTINÇÃO DA MEMÓRIA DE MEDO CONDICIONADO AO CONTEXTO

Descrição: As memórias de medo são essenciais para a sobrevivência do indivíduo, pois levam a comportamentos defensivos frente a ameaças. Entretanto, quando expressas de forma desregulada e constante podem desencadear transtornos psiquiátricos incapacitantes, como ansiedade e transtorno do estresse pós-traumático (TEPT). Este estudo teve como objetivo verificar o efeito do suporte social sobre a aquisição, evocação e extinção das memórias de Medo Condicionado ao Contexto em ratos Wistar.
Situação: Concluído Natureza: Projetos de pesquisa
Integrantes: Fernanda da Silva Rodrigues; Flávia Fagundes Ferreira; JOCIANE DE CARVALHO MYSKIW; IVÁN ANTONIO IZQUIERDO (Responsável)
Financiador(es): Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul-FAPERGS
Número de produções C,T & A: 1/

Projeto de ensino

2022 - 2023 Prática Docente - Fisiologia Humana Geral

Descrição: Prática docente obrigatória parte do programa de Doutorado do PPG Biociências - UFCSPA (45h)
Situação: Concluído Natureza: Projeto de ensino
É um projeto em cooperação com: Instituição de Ensino.
Em relação a temática: Ensino e aprendizagem e Projetos de curso.
Objetivos e metas: Ministrar módulos de Fisiologia Humana Geral para os cursos de Biomedicina, Química Medicinal e Física Médica. O cursos consistiu em aulas presenciais e através de plataformas online nas áreas de Sistema Nervoso Central, Fisiologia Gastrointestinal e Respiratória.
Integrantes: Fernanda da Silva Rodrigues; Renata Padilha Guedes (Responsável)

2019 - 2019 Programa de Iniciação à Docência - Desenvolvimento de Objetos de Aprendizagem para o Ensino de Fisiologia

Situação: Concluído Natureza: Projeto de ensino
É um projeto em cooperação com: Instituição de Ensino.
O projeto possui ações inovadoras no(a): Graduação.
Em relação a temática: Ensino e aprendizagem, Aprendizagem por projetos e Inserção de tecnologias no ensino.
Objetivos e metas: Inserir acadêmicos de graduação no universo da docência, permitindo a criação de recursos complementares para o ensino de Fisiologia Humana.
Integrantes: Fernanda da Silva Rodrigues; Renata Padilha Guedes (Responsável); Jeferson Jantsch

Revisor de periódico

2024 - 2024 Clinical and Experimental Pharmacology and Physiology

Idiomas

Inglês Compreende Bem , Fala Bem , Escreve Bem , Lê Bem

Espanhol Compreende Razoavelmente , Fala Razoavelmente , Escreve Razoavelmente , Lê Razoavelmente

Prêmios e títulos

2022 Menção Honrosa na categoria Biologia do Comportamento, I Congresso em Biociências Aplicadas à Saúde

Producão

Produção bibliográfica

Artigos completos publicados em periódicos

1. **DA SILVA RODRIGUES, F.**; JANTSCH, J.; FARIAS, G. F.; MILCZARSKI, V. L. C.; DIAS, V. S.; SCHEID, C.; MERIB, J. O.; GIOVENARDI, M.; GUEDES, R.P.. Cannabidiol improves maternal obesity-induced behavioral, neuroinflammatory and neurochemical dysfunctions in the juvenile offspring. Brain Behavior And Immunity. JCR, v.119, p.301 - 316, 2024.

2. **RODRIGUES, F. S.**; JANTSCH, JEFERSON; FARIAS, G. F.; DIAS, V. S.; ELLER, SARAH; DE OLIVEIRA, TIAGO FRANCO; GIOVENARDI, M.; GUEDES, R.P.. Cannabidiol treatment improves metabolic profile and decreases hypothalamic inflammation caused by maternal obesity. FRONTIERS IN NUTRITION. JCR, v.10, p.1, 2023.

3. **RODRIGUES, FERNANDA DA SILVA**; NEWTON, WILLIAM ROBERT; TASSINARI, ISADORA D'ÁVILA; DA CUNHA XAVIER, FELIPE HENRIQUE; MARX, ADÉL; DE FRAGA, LUCIANO STÜRMER; WRIGHT, KAREN; GUEDES, RENATA PADILHA; BAMBINI-JR, VICTORIO. Cannabidiol prevents LPS-induced inflammation by inhibiting the NLRP3 inflammasome and iNOS activity in BV2 microglia cells via CB2 receptors and PPARγ. NEUROCHEMISTRY INTERNATIONAL. JCR, v.177, p.105769, 2024.

4. JANTSCH, JEFERSON; **RODRIGUES, FERNANDA DA SILVA**; FRAGA, GABRIEL DE FARIAS; ELLER, SARAH; SILVEIRA, ALEXANDRE KLEBER; MOREIRA, JOSÉ CLÁUDIO FONSECA; GIOVENARDI, MÁRCIA; GUEDES, RENATA PADILHA. Calorie restriction mitigates metabolic, behavioral and neurochemical effects of cafeteria diet in aged male rats. JOURNAL OF NUTRITIONAL BIOCHEMISTRY. JCR, v.119, p.109371, 2023.

5. TASSINARI, ISADORA D'ÁVILA; **RODRIGUES, FERNANDA DA SILVA**; BERTRAM, CRAIG; MENDES-DA-CRUZ, DANIELLA ARÊAS; GUEDES, RENATA PADILHA; PAZ, ANA HELENA; BAMBINI-JUNIOR, VICTORIO; DE FRAGA, LUCIANO STÜRMER. Lactate Protects Microglia and Neurons from Oxygen-Glucose Deprivation/Reoxygenation. NEUROCHEMICAL RESEARCH. JCR, v.49, p.1, 2024.

6. GONZÁLEZ, LUCÍA PAOLA FACCIOLA; **RODRIGUES, FERNANDA DA SILVA**; JANTSCH, JEFERSON; FRAGA, GABRIEL DE FARIAS; SQUIZANI, SAMIA; CASTRO, LUIS FELIPE DOS SANTOS; CORREIA, LÍDIA LUZ; NETO, JOÃO PEREIRA; GIOVENARDI, MÁRCIA; PORAWSKI, MARILENE; GUEDES, RENATA PADILHA. Effects of omega-3 supplementation on anxiety-like behaviors and neuroinflammation in Wistar rats following cafeteria diet-induced obesity. NUTRITIONAL NEUROSCIENCE. JCR, v.27, p.172 - 183, 2024. **Citações:** WEB OF SCIENCE™ 5 | SCOPUS 5

7. SQUIZANI, SAMIA; JANTSCH, JEFERSON; **RODRIGUES, FERNANDA DA SILVA**; BRAGA, MATHEUS FILIPE; ELLER, SARAH; DE OLIVEIRA, TIAGO FRANCO; SILVEIRA, ALEXANDRE KLEBER; MOREIRA, JOSÉ CLÁUDIO FONSECA; GIOVENARDI, MARCIA; PORAWSKI, MARILENE; GUEDES, RENATA PADILHA. Zinc Supplementation Partially Decreases the Harmful Effects of a Cafeteria Diet in Rats but Does Not Prevent Intestinal Dysbiosis. Nutrients. JCR, v.14, p.3921, 2022. **Citações:** WEB OF SCIENCE 8 | SCOPUS 8

8. NETO, JOÃO; JANTSCH, JEFERSON; **RODRIGUES, FERNANDA**; SQUIZANI, SAMIA; ELLER, SARAH; OLIVEIRA, TIAGO FRANCO; SILVEIRA, ALEXANDRE KLEBER; MOREIRA, JOSÉ CLÁUDIO FONSECA; GIOVENARDI, MARCIA; PORAWSKI, MARILENE; GUEDES, RENATA PADILHA. Impact of cafeteria diet and n3 supplementation on the intestinal microbiota, fatty acids levels, neuroinflammatory markers and social memory in male rats. PHYSIOLOGY & BEHAVIOR. JCR, v.260, p.114068, 2023. **Citações:** WEB OF SCIENCE 5 | SCOPUS 7

9. FERREIRA, FLÁVIA F.; **RODRIGUES, FERNANDA S.**; SCHMIDT, SCHEILA D.; CAVALCANTE, LORENA E.S.; ZINN, CAROLINA G.; FARIAS, CLARISSA P.; FURINI, CRISTIANE R.G.; MYSKIW, JOCIANE C.; IZQUIERDO, IVAN. SOCIAL SUPPORT FAVORS EXTINCTION AND IMPAIRS ACQUISITION OF BOTH SHORT- AND LONG-TERM CONTEXTUAL FEAR CONDITIONING MEMORY. NEUROSCIENCE LETTERS. JCR, v.712, p.134505, 2019. **Citações:** WEB OF SCIENCE 9 | SCOPUS 13

10. DE ASSIS BRASIL, EDUARDO SILVA; GUERINO FURINI, CRISTIANE REGINA; **DA SILVA RODRIGUES, FERNANDA**; NACHTIGALL, EDUARDA GODFRIED; KIELBOVICZ BEHLING, JONNY ANDERSON; SAENGER, BRUNA FREITAS; FARIAS, CLARISSA PENHA; DE CARVALHO MYSKIW, JOCIANE; IZQUIERDO, IVAN. The blockade of the serotoninergic receptors 5-HT5A, 5-HT6 and 5-HT7 in the basolateral amygdala, but not in the hippocampus facilitate the extinction of fear memory. BEHAVIOURAL BRAIN RESEARCH. JCR, v.372, p.112055, 2019. **Citações:** WEB OF SCIENCE 12 | SCOPUS 13

Orientações e Supervisões

Orientações e supervisões

Orientações e supervisões concluídas

Trabalhos de conclusão de curso de graduação

1. Gabriel de Farias Fraga. **Efeitos da suplementação de ômega-3 no metabolismo e sistema nervoso central de ratos obesos**. 2023. Curso (Biomedicina) - Fundação Universidade Federal de Ciências da Saúde de Porto Alegre

Iniciação científica

1. Victor Silva Dias. **O TRATAMENTO COM CANABIDIOL REVERTE PREJUÍZOS COMPORTAMENTAIS CAUSADOS PELA OBESIDADE MATERNA**. 2024. Iniciação científica (Biomedicina) - Fundação Universidade Federal de Ciências da Saúde de Porto Alegre. Inst. financiadora: Conselho Nacional de Desenvolvimento Científico e Tecnológico
2. Gabriel de Fraga Farias. **EFEITO DE UMA DIETA DE CAFETERIA ASSOCIADA À ADMINISTRAÇÃO DE ÔMEGA-3 SOBRE COMPORTAMENTOS RELACIONADOS À ANSIEDADE EM RATOS WISTAR**. 2023. Iniciação científica (Biomedicina) - Fundação Universidade Federal de Ciências da Saúde de Porto Alegre. Inst. financiadora: UFCSPA
3. Vitória Luiza de Camargo Milczarski. **EFEITOS DA SUPLEMENTAÇÃO DE CANABIDIOL NA PROLE DE RATAS OBESAS SOBRE A NEUROINFLAMAÇÃO HIPOTALÂMICA**. 2023. Iniciação científica (Biomedicina) - Fundação Universidade Federal de Ciências da Saúde de Porto Alegre. Inst. financiadora: Conselho Nacional de Desenvolvimento Científico e Tecnológico

Bancas

Bancas

Participação em banca de comissões julgadoras

Outra

1. **Mostra de Iniciação Científica - 3o Congresso UFCSPA**, 2023. Fundação Universidade Federal de Ciências da Saúde de Porto Alegre.

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