UNIVERSIDADE FEDERAL DE MINAS GERAIS

Instituto de ciências biológicas

Programa de pós-graduação em neurociências

Roberta dos Santos Ribeiro

AVALIAÇÃO CRÔNICA DE ASPECTOS NEUROINFLAMATÓRIOS, COGNITIVOS E DE MEMÓRIA EM MODELO MURINO TRATADO COM DIETA HIPERPALATÁVEL.

Belo Horizonte

2022

Roberta dos Santos Ribeiro

AVALIAÇÃO CRÔNICA DE ASPECTOS NEUROINFLAMATÓRIOS, COGNITIVOS E DE MEMÓRIA EM MODELO MURINO TRATADO COM DIETA HIPERPALATÁVEL.

Tese apresentada ao Programa de Pós-Graduação em Neurociências da Universidade Federal de Minas Gerais, como requisito parcial à obtenção do título de Doutor em Neurociências.

Área de concentração: Neurociência Básica

Orientadora: Prof. Dra. Luciene Bruno Vieira

Belo Horizonte

2022

043 Ribeiro, Roberta dos Santos.
Avaliação crônica de aspectos neuroinflamatórios, cognitivos e de memória em modelo murino tratado com dieta hiperpalatável [manuscrito] / Roberta dos Santos Ribeiro. – 2022.
104 f. : il. ; 29,5 cm.

Orientadora: Prof. Dra. Luciene Bruno Vieira.
Tese (doutorado) – Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas. Programa de Pós-Graduação em Neurociências.

1. Neurociências. 2. Obesidade. 3. Memória. 4. Inflamação. 5. Transmissão Sináptica. 6. Dieta Hiperlipídica. I. Vieira, Luciene Bruno. II. Universidade Federal de Minas Gerais. Instituto de Ciências Biológicas. III. Título.

CDU: 612.8

Ficha catalografica elaborada pela bibliotecária Fabiane C. M. Reis – CRB: 6/2680

UNIVERSIDADE FEDERAL DE MINAS GERAIS
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM NEUROCIÊNCIAS

FOLHA DE APROVAÇÃO

AVALIAÇÃO CRÔNICA DE ASPECTOS NEUROINFLAMATÓRIOS, COGNITIVOS E DE MEMORIA EM MODELO MURINO TRATADO COM DIETA HIPERPALATÁVEL

ROBERTA DOS SANTOS RIBEIRO

Tese submetida à Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em NEUROCIÊNCIAS, como requisito para obtenção do grau de Doutor em NEUROCIÊNCIAS, área de concentração NEUROCIÊNCIAS BÁSICAS

Aprovada em 27 de dezembro de 2022, pela banca constituída pelos membros:

Prof(a). Paula Maria Quaglio Bellozi
UNB

Prof(a). Nancy Scardua Binda
UFOP

Prof(a). Diogo de Barros Peruchetti
UFMG

Prof(a). Victor Rodrigues Santos
UFMG

Prof(a). Luciene Vieira - Orientador
UFMG

Belo Horizonte, 27 de dezembro de 2022.

Documento assinado eletronicamente por **Nancy Scardua Binda**, **Usuária Externa**, em 27/12/2022, às 18:11, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

Documento assinado eletronicamente por **Paula Maria Quaglio Bellozi**, **Usuária Externa**, em 27/12/2022, às 18:17, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

Documento assinado eletronicamente por **Victor Rodrigues Santos**, **Professor do Magistério Superior**, em 27/12/2022, às 18:26, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

Documento assinado eletronicamente por **Luciene Bruno Vieira**, **Professora do Magistério Superior**, em 27/12/2022, às 18:26, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

Documento assinado eletronicamente por **Diogo de Barros Peruchetti**, **Professor do Magistério Superior**, em 27/12/2022, às 18:29, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

A autenticidade deste documento pode ser conferida no site https://sei.ufmg.br/sei/controlador_externo.php?acao=documento_conferir&id_orgao_acesso_externo=0, informando o código verificador **1964217** e o código CRC **577E4152**.

Referência: Processo nº 23072.273181/2022-67 SEI nº 1964217

AGRADECIMENTOS

À Universidade Federal de Minas Gerais, instituição pública, gratuita e de excelência, onde concluo o doutorado em neurociências e na qual adquiri grande conhecimento profissional e científico.

À CAPES, pela concessão da bolsa de doutorado.

À CAPES, ao CNPq e à FAPEMIG, pelo suporte financeiro para realização dessa pesquisa.

Aos meus amados familiares, especialmente os meus pais, Roberto e Núbia, minha irmã, Valéria, minha avó, Maria, meu avô, Luis (Seu Franço) (*In memorian*), primos (as) e tios (as) queridos. Obrigada por todo amor, apoio e compreensão. Foram anos difíceis, mas esta conquista é um presente de dias melhores para todos nós!

À minha Orientadora, Prof. Dra Luciene Bruno Vieira, pela orientação, colaboração, compreensão, incentivo, otimismo e paciência. Obrigada pela acolhida, por todos os ensinamentos, oportunidades e confiança depositada em mim.

Aos professores Dr. Antônio Carlos Pinheiro de Oliveira e Dr. Helton José dos Reis por todo direcionamento, confiança, profissionalismo, oportunidades e acolhida ao longo destes anos.

À toda família presente e egressa do laboratório de Neurofarmacologia (nosso NEUROFAR). Agradeço aos amigos Maria Carolina, Suélyn, Gabriela Lopes, Anna Luíza, Giovanni, Carla, Vivian, Carolina Ferrari, Gabriela Oliveira, Douglas, Érica, Wellerson, Idiongo, Ana Luísa, Larissa e Thainã. Obrigada por toda contribuição técnica, científica, confiança, suporte e amizade construída, fundamentais para meu crescimento como profissional e como pessoa.

Um agradecimento especial as bolsistas de iniciação científica Bárbara, Carolina Sena e Gabriela Reis. Minhas queridas meninas! Obrigada por toda troca de conhecimento, trabalho duro e por estarem comigo. Obrigada por todo carinho recíproco. Com seus sorrisos, vocês me ensinaram muito mais do que ser alguém que orienta.

Aos camundongos e todos os animais que doaram suas vidas ao desenvolvimento e progresso da ciência, todo o meu respeito. Obrigada!

Aos professores Dr. Fabrício Araújo e Dra. Daniele Aguiar, bem como a todos os membros amigos do laboratório de Neuropsicofarmacologia (LNP), sobretudo a Lia, Raíssa, Nícia, Aline e Ana Paula. Obrigada pelo suporte técnico, científico, convivência diária, acolhida, amizade e pelos bons cafés (Cujos quais apreciei com grande felicidade, mesmo não podendo beber, devido a enxaqueca).

À toda equipe do laboratório RNAi, em especial a professora Dra Fabíola Mara Ribeiro e a Dra Emanuele Guimarães, por sua contribuição, gentileza e colaboração para as análises de RT-PCR presentes neste trabalho.

À toda equipe docente e discente do Núcleo de neurociências (NNC), que gentilmente me forneceram suporte técnico e permitiram utilizar a estrutura do NNC. Agradeço especialmente à professora Dra Grace Schenatto Pereira Moraes e ao Dr. Leonardo Guarnieri, pela paciência e colaboração para as análises comportamentais de medo condicionado ao contexto e interação social.

As Professoras Dra Tatiani Maioli, Dra Ana Maria Caetano e toda sua equipe, em especial Msc. Felipe Caixeta, pela disponibilidade e colaboração técnica-científica nas análises de RT-PCR relacionadas à inflamação.

À Professora Dra Aline Miranda e a equipe do Laboratório de Neurobiologia, especialmente a Dra Heliana Fernandes, pela gentileza e colaboração com o presente trabalho, fazendo possível a realização de ELISAs para dosagem de leptina, fatores neurotróficos e citocinas.

À Professora Dra Cláudia Ferreira e a Victoria Lopes, pela colaboração com o presente trabalho na realização de dosagem de colesterol dos grupos experimentais.

Às professoras Dra. Daniele Bonaventura e Dra. Sílvia Guatimosim, bem como a Dra. Nathália Araújo e aos Msc. Naiara de Assis e Mário Morais, por toda gentileza e colaboração no projeto paralelo relacionado a esta tese.

A equipe do laboratório de neurodesenvolvimento (NEURODEV), especialmente a Dra. Muíara Moraes, bem como a Msc. Laila Blanc e a Bsc Bruna Resende, por toda gentileza, aprendizado e auxílio em minha tentativa de realizar a técnica de coloração de Golgi.

À todo corpo técnico, docente e discente do programa de pós-graduação em Neurociências (UFMG).

Ao professor Dr. Stêfany Cau e as amigas do laboratório de Farmacologia Cardiovascular e da Inflamação (LAFACI), Bárbara (Berg), Karla e Ana Flávia, por todo suporte, gentileza, apoio e amizade.

À Professora Dra Jane Lima dos Santos, minha orientadora de mestrado, e todos os colegas do Laboratório de Imunobiologia (LIBI) da Universidade Estadual de Santa Cruz (UESC-BA). Profa. Jane, em especial, obrigada pela confiança e por me incentivar a nunca desistir.

Ao professor Dr. Stêfany Cau e as amigas do laboratório de Farmacologia Cardiovascular e da Inflamação (LAFACI), Bárbara (Berg), Karla e Ana Flávia, por todo suporte, gentileza, apoio e amizade.

Aos professores Dr. Jacques Robert Nicoli, Dr. Flavíano Martins, Dra Elizabeth Neumann, Dra Sílvia de Moura, Dr. Daniel Assis, Dra Maria Aparecida Stoianoff e a todos os membros dos Laboratórios de Ecologia e Fisiologia de microorganismos (LEFM), Agentes terapêuticos (LABIo) e Micologia, em especial aos amigos: Karen, Gabrielle, Samantha, Débora, César e Gustavo. Obrigada por esta terem me acolhido tão bem durante o período de mestrado e doutorado na UFMG e por todo o carinho e amizade cultivado ao longo destes anos.

À todo o corpo técnico, administrativo e de serviços gerais do Instituto de Ciências Biológicas (ICB), especialmente aos senhores (as) Rinaldo, Webster, Gislene, Kátia, Aline, Samuel, Gerson, Serafim, Cíntia, Jefferson e Alexandre. Ouvir as palavras e ver os gestos de gentileza de cada um de vocês, durante estes mais de quatro anos, deixou o peso dos dias mais leve e feliz.

Aos colegas de curso e amigos Bruno Monteiro de Sousa e Victor Ulysses Matos. Obrigada por toda contribuição mútua para nosso crescimento científico, por meio de discussões instigantes sobre a ciência e a vida.

Á Nazaré Lúcio, minha querida amiga, que me abrigou fraternalmente em seu lar na minha investida inicial rumo ao êxito no ingresso do doutorado.

Aos meus amigos, Daniel e Edivan (Ed) que desde a graduação me acompanham. Obrigada por todo carinho e cuidado! Vocês são pessoas incríveis.

Ao meu amigo Filipe, cujo qual tenho profundo carinho. Obrigada por compartilhar comigo tão boas conversas sobre a vida, pela confiança e alegria que esta amizade proporciona.

Aos meus velhos amigos, Joane e Ricardo. Meus queridos irmãos que me acompanham desde a infância. A felicidade de nossa amizade transborda minha alma de alegria. Ter vocês em minha vida é fundamental para ser feliz sendo quem sou.

Aos meus queridos amigos, Edie e Gabryelle. Migos! Vocês tornam os dias mais felizes com sua leveza e espontaneidade. Obrigada por todos os momentos felizes de hoje e de sempre. O carinho e a felicidade de tê-los em minha vida é gigante.

E por fim, agradeço ao meu companheiro de vida, Joedison, por todo amor e companheirismo. Obrigada por caminhar junto comigo, com este sorriso, alegria e força. Eu te amo muito!

COLABORADORES

Prof. Dr. Antônio Oliveira, Departamento de Farmacologia, ICB/UFMG

Prof. Dra. Fabíola Ribeiro, Departamento de Bioquímica e Imunologia, ICB/UFMG

Prof. Dra. Grace Schenatto Pereira Moraes, Departamento de Fisiologia e Biofísica, ICB/UFMG

Prof. Dra. Aline Miranda, Departamento de Morfologia, ICB/UFMG

Prof. Dra. Tatiani Maioli, Departamento de Bioquímica e imunologia, ICB/UFMG

Prof. Dra. Ana Maria Caetano, Departamento de Bioquímica e imunologia, ICB/UFMG

Prof. Dra. Claudia Ferreira, Colégio técnico, COLTEC/UFMG

Dra. Emanuele Guimarães, Departamento de Bioquímica e imunologia, ICB/UFMG

Dr. Giovanni Gomes, Faculdade de medicina de Ribeirão preto, FMRP/USP

Dra. Heliana Fernandes, Departamento de Morfologia, ICB/UFMG

Dr. Leonardo Guarnieri, Departamento de Fisiologia e Biofísica, ICB/UFMG

Msc Maria Carolina Machado da Silva, Departamento de Farmacologia, ICB/UFMG

Msc Felipe Caixeta Moreira, Departamento de Bioquímica e imunologia, ICB/UFMG

SUPORTE FINANCEIRO

Fundação de Amparo à Pesquisa do Estado de Minas Gerais - FAPEMIG

Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES

Programa de apoio a pós graduação (PROAP)- CAPES

"O homem muitas vezes se torna o que acredita ser. Se continuo dizendo a mim mesmo que não posso fazer uma determinada coisa, é possível que acabe me tornando realmente incapaz de fazê-lo. Pelo contrário, se tenho a convicção de que posso fazê-lo, certamente adquirirei a capacidade de fazê-lo, mesmo que não o tenha no início."

Mahatma Gandhi

RESUMO

O consumo excessivo de alimentos hiperpalatáveis e hipercalóricos tem sido apontado como um fator associado ao declínio cognitivo e ao comprometimento da memória na obesidade. Neste contexto, sabe-se que a integração entre inflamação periférica e central pode atuar como um importante catalisador dos impactos danosos da obesidade sobre a memória, especialmente em regiões vulneráveis ao estresse nutricional, como o hipocampo. Todavia, pouco se sabe sobre como os mecanismos inflamatórios poderiam impactar diretamente a regulação de sistemas específicos de neurotransmissão associados à regulação da memória. Desta forma, o objetivo deste trabalho foi testar a hipótese de que a obesidade murina, desencadeada pela exposição crônica a uma dieta hiperpalatável, poderia induzir neuroinflamação hipocampal, em um mecanismo dependente da inflamação periférica, levando a hiperativação da neurotransmissão glutamatérgica e ao comprometimento da memória. Para tal, camundongos C57BL/6J machos (3-4 semanas de idade) foram expostos a uma dieta isocalórica ou rica em gordura e açúcar (High sugar and Butter diet (HSB)) por 12 semanas. Os testes comportamentais foram realizados entre a 11-12ª semana. Ao fim do protocolo, foram avaliados o padrão sérico e hipocampal de citocinas pró-inflamatórias, a expressão de proteínas associadas à permeabilidade da barreira hematoencefálica, os níveis de glutamato e receptores glutamatérgicos, de fatores neurotróficos, bem como do eixo fractalquina-CX3CR1. Foi observado que os animais obesos apresentaram um menor tempo de investigação no objeto novo ou nova localização, bem como anormalidades na extinção da memória. Além disso, em nível molecular, o consumo crônico da dieta obesogênica foi capaz de promover disfunção metabólica, aumentando os níveis hipocampais de glutamato. Curiosamente, embora tenha sido observado um perfil pró-inflamatório sérico, o modelo proposto não apresentou alterações neuroinflamatórias, sugerindo que o estabelecimento da disfunção glutamatérgica parece ser independente da inflamação. Todavia, o consumo crônico da dieta obesogênica foi capaz de aumentar as concentrações de fractalquina, uma quimiocina chave associada à comunicação entre neurônios e microglia. Assim, considerando o papel neuroprotetor e pró-inflamatório da fractalquina, os seguintes dados embasam a hipótese de que o aumento dos níveis de glutamato poderia estar associado à saturação da comunicação sináptica, limitando parcialmente a plasticidade. Em contrapartida, o aumento dos níveis de fractalquina poderia ser uma estratégia para diminuir o potencial efeito excitotóxico mediado pelo aumento de glutamato. Entretanto, também é possível que a manutenção deste mecanismo possa predispor a um ambiente neuroinflamatório em longo prazo, onde os níveis

elevados de glutamato poderiam ser uma das causas, mas não uma consequência, do processo neuroinflamatório associado à obesidade.

Palavras-chave: Obesidade, memória, inflamação, neurotransmissão glutamatérgica, dieta hiperpalatável.

ABSTRACT

Excessive consumption of hyperpalatable and hypercaloric food has been pointed out as a factor associated with cognitive decline and memory impairment in obesity. In this context, it is known that the integration between peripheral-central inflammation may act as an important catalyst for the negative impacts of obesity on memory, especially in key areas which are vulnerable to nutritional stress, such as the hippocampus. However, little is known about how the inflammatory state generated by obesity may impact specific neurotransmission systems associated with memory regulation, such as the glutamatergic system. Here, we tested the hypothesis that chronic obesity exposure to a highly palatable diet in a murine model may induce neuroinflammation, glutamatergic dysfunction, and memory impairment. For that, we exposed 3-4 weeks old C57BL/6J male mice to an isocaloric or a high sugar and butter diet (HSB) for 12 weeks. Behavioral tests, hippocampal and serum pro-inflammatory cytokines pattern, blood-brain barrier permeability proteins, as well the levels of glutamate, glutamatergic receptors, neurotrophic factors and fractalkine-CX3CR1 axis were evaluated. Our results showed that chronic consumption of the HSB diet was able to promote metabolic dysfunction, increasing the hippocampal glutamate levels, as well as inducing a decrease in memory reconsolidation and extinction. Although our data indicated a peripheral pro-inflammatory profile, we did not observe neuroinflammatory features in our model, suggesting that the establishment of glutamatergic dysfunction appears to be independent of inflammation, and likely modulated by metabolic dysfunctions. Interestingly, we also observed that the HSB diet also increased hippocampal fractalkine levels, a key chemokine associated with neuroprotection and pro-inflammatory conditions. Then, we hypothesized that increased glutamate levels may saturate synaptic communication, partially limiting plasticity, and that increased levels of fractalkine could be a strategy to decrease glutamatergic damage. However, we also speculate that in the long term, this mechanism may predispose to a neuroinflammatory environment.

Keywords: Obesity, memory impairment, inflammation, glutamatergic neurotransmission, hyperpalatable diet.

LISTA DE FIGURAS

LISTA DE TABELAS

LISTA DE ABREVIATURAS

AgRP -Agouti-related protein

AIN93G- American Institute of Nutrition 93-Growth diet

AM- Amygdala

AMPAR- Amino-3-hydroxy-5-methyl-4 isoxazolproprionate receptor

ARC- Arcuate nucleus

AT- Adipose tissue

BBB- Blood-brain barrier

BDNF- Brain Derived Neurotrophic Factor

CA - Cornu Ammonis

CART - Cocaine-amphetamine regulated transcript

CCK- Cholecystokinin

CNS- Central nervous system

CX3CL1- Fractalkine

CX3CR1- Fractalkine receptor

DG- Dentate gyrus

DIO- Diet-induced obesity models

EAT- Epididymal adipose tissue

ENS- Enteric nervous system

GDNF- Glial derived neurotrophic factor

GFAP- Glial fibrillary acidic protein

GI- Gastrointestinal tract

GLT-1- Glutamate transporter-1 (GLT-1)

HFD- High-fat diet

HSB- High Butter and Sugar diet

Iba-1- ionized calcium-binding adaptor molecule-1

iGluR- Ionotropic glutamate receptors

IL-10- Interleukin 10

IL-1β- Interleukin 1 beta

IL-2- Interleukin 2

IL-4- Interleukin 4

IL-6- Interleukin 6

INFɣ- Interferon-Gamma

LTD- Long-term depression

LTM- Long-term social memory

LTP- Long Term Potentiation

MAT- mesenteric adipose tissue

MCP-1- Monocyte chemoattractant protein type 1

mGluRs- Metabotropic glutamate receptors

NAc- Nucleus accumbens

NF-κβ -Nuclear factor kappa beta

NGF- Nerve growth factor

NMDA-R- N-Methyl-D- Aspartate receptor

NPY- Neuropeptide Y

NTS- Solitary nucleus

OF- Open Field test

oGTT- Oral glucose tolerance test

OLT- Object Location Test

ORT-Object Recognition Test

POMC- Pro-opiomelanocortin

PPY- Peptide YY

PVN-Paraventricular nucleus

RPAT- Retroperitoneal adipose tissue

STM- Short-term social memory

STMo- Short-term working memory

Th- T helper cell

TNF-α- Tumor necrosis factor-alpha

Treg- T regulatory cell

TRL4- Toll-like receptor-4

VAT- Visceral adipose tissue

VTA- Ventral tegumentary area

ZO-1- Zonula occludens-1

SUMÁRIO

RESUMO EXPANDIDO

1.0- INTRODUÇÃO

Embora a associação entre obesidade e dietas hiperpalatáveis esteja classicamente relacionada ao desenvolvimento de disfunções metabólicas no sistema cardiovascular, trabalhos demonstraram que os efeitos dessa associação poderiam impactar o sistema nervoso central (SNC), predispondo a alterações em diversas funções superiores, incluindo as ligadas à memória e cognição (CHEKE et al., 2017; DE PAULA et al., 2021; MELO et al., 2020; PRICKETT; BRENNAN; STOLWYK, 2015; TAKASE et al., 2016). Neste sentido, estudos demonstraram que o consumo crônico de alimentos hiperpalatáveis poderia ocasionar alterações em memórias de curto e longo prazo, bem como em subtipos específicos de memória, como a operacional, episódica e aversiva, impactando assim o comportamento alimentar, a tomada de decisões, atenção, habilidades executivas e o comportamento social (BOCARSLY et al., 2015; FORTE et al., 2021; HAYASHI et al., 2020; PRICKETT; BRENNAN; STOLWYK, 2015; REICHELT et al., 2015; SPYRIDAKI; AVGOUSTINAKI; MARGIORIS, 2016; TAKASE et al., 2016; YAMADA-GOTO et al., 2012). Todavia, a compreensão dos mecanismos moleculares que regulam essas associações ainda não são completamente elucidados.

A neuroinflamação patológica é apontada como uma condição intimamente relacionada à obesidade e potencialmente nociva à função cognitiva e à memória (BUTLER, 2021; DISABATO; QUAN; GODBOUT, 2016; RANSOHOFF et al., 2015). Interessantemente, estudos em modelos de obesidade induzida por dieta (DIO) demonstraram que a inflamação periférica gerada pela obesidade poderia ser um catalisador para a neuroinflamação, tendo em vista que estão hiper-regulados os níveis séricos de imunomoduladores, como IL-1β, IL-6, TNF-α, IFNγ, leptina e ácidos graxos livres, e bem como ocorre redução da permeabilidade da barreira hematoencefálica (BBB) (MAIOLI et al., 2016; MELO et al., 2020; OGATA et al., 2019; RHEA et al., 2017; STRANAHAN et al., 2016). Adicionalmente, a presença destas alterações levantou a hipótese de que o influxo de imunomoduladores periféricos em algumas regiões susceptíveis do SNC, como hipocampo e hipotálamo, poderia desencadear a ativação/ produção de citocinas pró-inflamatórias por células gliais, incluindo astrócitos e microglia (ALEXAKI, 2021a; MILLER; SPENCER, 2014; RHEA et al., 2017). Vale lembrar que o estado clássico de polarização da microglia (M1), caracterizado pela produção de IL-1β, IL-6, TNF-α e óxido nítrico, está associado à diminuição da produção de fatores neurotróficos,

engolfamento sináptico anormal e disfunção em sistemas de comunicação entre neurônios e glia, a exemplo da via mediada pela fractalquina e seu receptor (CX3CR1), o que poderia levar a redução na complexidade e no número de espinhas dendríticas, além de comprometimento na formação no potencial de longa duração (*Long Term Potentiation* - LTP) em modelos do tipo DIO (CAVALIERE et al., 2019; COPE et al., 2018; HAO et al., 2016; KAWAMURA et al., 2021; SOBESKY et al., 2014). No entanto, é importante salientar que, embora a neuroinflamação possa impactar diretamente os mecanismos relacionados à memória, seus efeitos sobre sistemas específicos de neurotransmissão potencialmente afetados pela obesidade ainda não são conhecidos.

O L-glutamato é o principal eurotransmissor excitatório do SNC dos mamíferos, cuja sinalização depende da ação dos receptores ionotrópicos (iGluR) e metabótropicos e (mGluR). Dentre os iGluRs, temos: ácido propiônico a-amino-3-hidroxi-metil-4-isoxazol (AMPA-R), receptores de Kainato (GluK1–GluK5), N-metil-D-aspartato (NMDA-R), e dentre os mGluR (mGluR1-8) (ABEL; LATTAL, 2001; RIEDEL; PLATT; MICHEAU, 2003). Neste contexto, disfunções na regulação dos níveis de glutamato, ou na atividade de seus receptores, estão associadas a impactos nocivos na neuroplasticidade, incluindo redução na produção de fatores neurotróficos, declínio cognitivo e de memória, e efeitos neurotóxicos (ABEL; LATTAL, 2001; CHOI, 1985; KATAGIRI; TANAKA; MANABE, 2001; MATTSON, 2008; RIEDEL; PLATT; MICHEAU, 2003; RIEDEL; REYMANN, 1996). Embora ainda seja pouco compreendido, evidências apontam que alguns eventos imunomoduladores podem estar envolvidos na regulação central da dinâmica glutamatérgica. Por exemplo, experimentos demonstraram que a estimulação de fatias de hipocampo murino com INF-ɣ, TNF ou IL-1β foi capaz de aumentar a expressão, atividade, e o recrutamento de subunidades pós-sinápticas AMPA-R e NMDA-R (GluR1, NR2A/NR2B, respectivamente), sugerindo a influência destas citocinas na sinalização fisiopatológica glutamatérgica (MIZUNO et al., 2008; STELLWAGEN et al., 2005; VIVIANI et al., 2003). Além disso, um ambiente pró-inflamatório pode induzir a produção de glutamato pelas células gliais, aumentando assim a concentração de glutamato na fenda sináptica, bem como suprimindo a expressão de transportadores de glutamato, a exemplo do transportador glial de glutamato tipo 1 (GLT-1), o que poderia estar associado à neurotoxicidade e danos na plasticidade neuronal (BARGER et al., 2007; HAROON; MILLER; SANACORA, 2017; YE; SONTHEIMER, 1996). Assim, levando em consideração que poucos estudos investigaram o papel da sinalização glutamatérgica na relação entre memória e obesidade induzida por dieta

(LABBAN et al., 2020; MARTÍNEZ-OROZCO et al., 2021; TSAI et al., 2018; VALLADOLID-ACEBES et al., 2012), bem como o fato de que o impacto da neuroinflamação neste contexto precisa ser explorado, foi testada a hipótese de que a exposição crônica a uma dieta hiperpalatável poderia induzir neuroinflamação hipocampal, em um mecanismo desencadeado por inflamação periférica, o que poderia levar a uma desregulação da neurotransmissão glutamatérgica, e consequente comprometimento da memória em um modelo murino de obesidade.

2.0 – OBJETIVO GERAL

- Investigar se a exposição crônica de murinos a uma dieta obesogênica poderia induzir déficits de memória, neuroinflamação e alterações na neurotransmissão glutamatérgica em nível hipocampal.

3.0- MATERIAL E MÉTODOS

Aspectos éticos e obtenção do modelo: Todos os experimentos foram realizados com aprovação prévia da comissão de ética no uso de animais (CEUA) da UFMG (protocolo nº 217/200 (**Anexo 1**)). Foram utilizados camundongos C57BL/6J machos (3-4 semanas de idade), oriundos do biotério central da UFMG. Para a obtenção do modelo experimental, estes animais foram expostos a uma dieta isocalórica ou rica em gordura e açúcar (High sugar and Butter diet (HSB)) por 12 semanas. A mensuração do peso corpóreo e do consumo alimentar, para verificação do consumo cumulativo de calorias, foi realizada semanalmente para validar o modelo (NASCIMENTO et al., 2008). **Experimentos comportamentais:** Entre a 11ª e a 12ª semana, foram realizados os experimentos comportamentais. Todos os experimentos foram executados durante o ciclo claro, com água e comida *ad libitum* e condições controladas de temperatura e luminosidade (24 ± 2 ºC, 70-80 lux). Trinta minutos antes dos testes, os animais foram aclimatados à sala. Blocos de 2 experimentos diferentes foram executados em diferentes coortes de animais, sendo os mesmos ordenados conforme o grau crescente de potencial estressor. Além disso, foi concedido um intervalo de 2 a 3 dias entre os testes comportamentais. Assim, visando avaliar parâmetros exploratórios, de memória de curta e longa duração, foram realizados os seguintes experimentos, cuja descrição metodológica pode ser consultada em trabalhos prévios: Campo aberto (Open Field (OF)), labirinto em Y, reconhecimento de objeto novo (RON), localização de objetos (RLO), medo condicionado ao contexto, e interação e memória social (ALMEIDA-SANTOS et al., 2019;

DENNINGER; SMITH; KIRBY, 2019; LAZARONI et al., 2016; RADYUSHKIN et al., 2009). Por fim, os testes comportamentais foram analisados através dos softwares *Any-maze Video Tracking System* (*version 5.26, Stoelting*®) e X-Plot-Rat ® 2005 (Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, USP, Brasil).

Mensuração dos níveis de adiposidade e alterações metabólicas: Na 12ª semana, uma coorte de animais foi submetida ao teste de tolerância à glicose, conforme o protocolo de Pedro et al (2020). Posteriormente, um subgrupo dos animais empregados nas análises comportamentais foi eutanasiado e utilizado para a avaliação das alterações na composição corpórea, indicada pelo índice de adiposidade e de Lee (ROGERS; WEBB, 1980; SANTOS et al., 2022), e dos níveis séricos de colesterol por meio de ensaio colorimétrico comercial (Bioclin, MG, Brazil).

Avaliação do perfil neuroinflamatório: O bloco seguinte de experimentos buscou investigar a hipótese de que a dieta HSB poderia induzir o aumento na permeabilidade hematoencefálica (BBB), permitindo a passagem de imunomoduladores periféricos, ativação de células da glia e a produção de citocinas pró-inflamatórias em nível central. Para tal, amostras de hipocampo e plasma dos animais submetidos aos experimentos comportamentais foram obtidas por meio de decapitação e dissecação a fresco. As amostras foram estocadas a -80ºC até o momento de uso. Para a verificação de alterações na expressão de proteínas de integridade de barreira (Claudina-5, Zonula Occludens e Ocludina) e citocinas pró-inflamatórias no hipocampo (IL-1β, IL-6, INF-ɣ, TNF-α), foi realizada a técnica de PCR em tempo real (RT-PCR). Além disso, para a mensuração dos níveis destas mesmas citocinas no soro e no hipocampo, bem como de leptina sérica, foi executado um ensaio imunoenzimático (ELISA), através de kits comerciais, conforme instruções do fabricante (R&D system (DuoSet, Minneapolis, MN)). Por fim, para a avaliação da densidade microglial, bem como da ativação de microglia e astrócitos, foram realizadas imunohistoquímicas e imunofluorescências. Brevemente, a coorte restante de animais foi anestesiada (cetamina 80mg/Kg, xilazina 8mg/Kg, I.P) e submetida à perfusão transcardíaca com solução fosfato salina (PBS, pH 7.4), seguida de solução de paraformaldeído (PFA) 4% (pH 7.4). Após a perfusão transcardíaca, o cérebro foi obtido, fixado em PFA 4% (overnight), crioprotegido em sacarose e congelado em isopentano. Para a imunomarcação, o protocolo foi realizado de acordo com Silva et al (2021), sendo utilizados o marcador microglial Iba-1 (Rabbit anti-Iba-1, 1:500; Wako Chemicals, Osaka, Japan) e GFAP, para os astrócitos (mouse anti-GFAP, 1:500; Millipore, Darmstadt, Germany) (DA

SILVA et al., 2021). Para as análises de densidade microglial, e ativação microglial e astrocitária, foram respectivamente, utilizadas as mensurações de densidade óptica de Iba-1 e a razão do corpo sobre a área total da célula (índice de ativação microglial), bem como a avaliação da área ocupada e a intensidade de fluorescência de células GFAP positivas (DA SILVA et al., 2021; GOMES et al., 2020).

Avaliação de possíveis alterações na neurotransmissão glutamatérgica: Este bloco de experimentos buscou investigar a hipótese de que a dieta HSB poderia aumentar os níveis de glutamato no hipocampo, bem como induzir alterações na expressão gênica de receptores do tipo iGluR e mGluR e do Transportador glial de glutamato (GLT-1). Para a mensuração da concentração de glutamato, amostras de hipocampo, obtidas a fresco, foram imediatamente submetidas à preparação de sinaptossomas (DUNKLEY et al., 1986). Adicionalmente, a mensuração da expressão de subunidades funcionais de AMPA e NMDA, respectivamente: GLUR2, NR1, NR2A-B, bem como de mGluR5 e GLT-1 foi realizada por RT-PCR.

Mensuração dos níveis de fatores neurotróficos e do eixo fractalquina-CX3CR1: A fim de verificar se a dieta HSB estaria associada à modulação dos níveis de fatores neurotróficos e do eixo fractalquina-CX3CR1, foi realizada a mensuração dos níveis de do fator de crescimento neuronal (NGF), fator de crescimento derivado do cérebro (BDNF), fator de crescimento derivado da glia (GDNF) e de fractalquilna (CX3CL1). Para isto, a técnica de ELISA foi realizada pela utilização de kits comerciais (R&D system (DuoSet, Minneapolis, MN)). Adicionalmente, a mensuração dos níveis de expressão gênica de fractalquina e de seu receptor (CX3CR1) foi realizada por RT-PCR.

Análises estatísticas: Todas as análises foram realizadas utilizando-se o software *graphpad prism* 8 (San diego, USA). A normalidade e a homocedasticidade dos dados foram testadas usando os testes de Shapiro-Wilk e Levene. Os outliers foram detectados através do método de interpolação via box-plot. Quando apropriado foram empregados os testes de t-student, Mann-Whitney, ANOVA de duas vias ou ANOVA de medidas repetidas. O nível de significância adotado foi de $p<0,05$.

4.0- RESULTADOS E DISCUSSÃO

Foi observado um efeito de interação entre o tempo e o tipo de dieta no aumento do peso corporal dos animais analisados (ANOVA de duas vias, $F_{11,\ 456} = 15,42$, $p<0,0001$) (**Figura 12A-B**). Além disso, animais expostos à dieta HSB também apresentaram aumento nos

índices de adiposidade e de Lee, bem como no consumo cumulativo de calorias (Teste t não pareado, índice de adiposidade, t_{18}=5,832, p<0,0001; Índice de Lee, t_{18}=2,330, p=0,0316; consumo cumulativo de calorias, t_{10}=7,639, p<0,0001) (**Figura 12C-F**). Adicionalmente, o grupo obeso apresentou níveis mais elevados de glicose em jejum, aumento da tolerância oral à glicose e do colesterol total (Teste t não pareado, glicose em jejum, t_{18}=3,548, p=0,0023; área sob curva (AUC) t_{18}=3,117, p=0,0052; teste de Mann-Whitney, colesterol total, p=0,0002) (**Figura 12G-I**), sugerindo que, assim como em trabalhos prévios, a exposição à dieta HSB foi efetiva na indução do fenótipo obesogênico (MAIOLI et al., 2016; MOREIRA JÚNIOR et al., 2021). Quanto às alterações comportamentais, não foram observadas mudanças comportamentais significativas nos testes do campo aberto, *Y maze*, evocação da memória aversiva e de interação e memória social (**Figuras 13, 14A, 15A, 16**). Entretanto, os animais obesos apresentaram um menor tempo de investigação nos testes de reconhecimento e localização de objetos (Teste t não pareado, índice de reconhecimento do objeto novo t_{18}=2,731, p=0,0137; índice de reconhecimento de nova localização de objetos t_{16}=2,315, p=0,0342), bem como anormalidades na curva e no teste de extinção da memória associado ao medo condicionado ao contexto (Teste t não pareado, teste de extinção da memória t_{15}=4,417, p=0,005) (**Figuras 14D-E, 15B-C**). Isto poderia sugerir que a dieta HSB pode induzir alterações em processos ligados à reconsolidação e extinção da memória. Assim, considerando a presente hipótese de que um aumento do ambiente inflamatório periférico poderia aumentar a permeabilidade da BBB, contribuindo assim para a ativação glial, disfunção na neurotransmissão glutamatérgica e declínio da memória da obesidade, foi investigado se alterações neuroinflamatórias hipocampais poderiam estar associadas aos déficits de memória observados no modelo proposto. Apesar dos níveis plasmáticos de IL-1β e TNF-α não terem sido alterados, foi observado um aumento dos níveis de leptina, INF-ɣ e uma tendência de aumento para IL-6, o que poderia sugerir inflamação em sistêmica periférica (Teste t não pareado, IL-1β t_{15}=0,9297, p=0,3672; TNF-α t_{10}=1,843, p=0,0951; leptina t_9=12,01, p<0,0001; INF-ɣ t_{18}=2,334, p=0,0314; e IL-6 t_{16}=2,066, p=0,0593) (**Figura 17**). Todavia, não foram observadas alterações sugestivas de neuroinflamação, tendo em vista que os níveis de citocinas pró-inflamatórias centrais, ativação glial e expressão gênica de junções comunicantes associadas à regulação da permeabilidade da BBB permaneceram inalteradas (**Figuras 18-21**). Quanto às alterações associadas à neurotransmissão, foi observado o aumento nos níveis de glutamato no hipocampo dos animais expostos a dieta HSB (Teste t não pareado, glutamato hipocampal t_{17}=4,003, p=0,0009) (**Figura 22B**). Este

dado derrubou a hipótese levantada pelo trabalho, indicando que, possivelmente, o aumento dos níveis de glutamato independe da presença da neuroinflamação. Neste sentido, trabalhos prévios em modelos DIO demonstraram que a exposição a dietas do tipo hiperpalatáveis (8-10 semanas) ou a presença de alterações no metabolismo da glicose poderiam estar associadas à redução da atividade bioquímica para a produção de glutamato, bem como ao aumento da atividade e da expressão de GLT-1 (MARTÍNEZ-OROZCO et al., 2021; SICKMANN et al., 2010; VALLADOLID-ACEBES et al., 2012). Embora o modelo proposto neste trabalho não tenha apresentado alterações na expressão gênica de GLT-1 (**Figura 22I**), e levando em consideração que o tempo de exposição às dietas hiperpalatáveis nos estudos anteriores foi menor do que o empregado neste estudo, bem como o fato de que a atividade do GLT-1 tende a decair com a persistência da falência metabólica predispondo à excitotoxicidade glutamatérgica e ao declínio na memória (ANDERSEN et al., 2021; TSAI et al., 2018), é possível que o aumento dos níveis de glutamato possa ser resultado de um mecanismo inicial compensatório do GLT-1. Além disso, tomando como base as alterações comportamentais observadas, é possível que o aumento dos níveis de glutamato possa estar relacionado à redução de mecanismos plásticos mediados pelo glutamato no hipocampo. Embora isto seja especulativo, estudos em DIO demonstraram redução da LTP e LTD em regiões CA1-CA3, além da redução da expressão de NR2B e da LTP no hipocampo, sugerindo que o ambiente obesogênico poderia, por meio de alterações na sinalização glutamatérgica, aumentar a vulnerabilidade para disfunções plásticas (HWANG et al., 2010; PORTER et al., 2010; SPINELLI et al., 2017). Embora no modelo do presente trabalho não se tenha observado alterações na expressão gênica de subunidades dos receptores AMPA, NMDA e mGluR5 (**Figura 12C-G**), não é possível descartar a hipótese de que alterações funcionais estejam presentes no modelo investigado. Assim, embora especulativo, duas alternativas poderiam ser propostas para uma possível elucidação dos fenômenos comportamentais e moleculares observados até então: I) Tendo em vista que o excesso de glutamato poderia levar à saturação da atividade sináptica de iGluR, disfunção na formação de LTP e LTD e ao enfraquecimento de engramas consolidados e da aprendizagem (MARTIN; GRIMWOOD; MORRIS, 2000; MOSER; MOSER, 1999) é possível que o aumento dos níveis de glutamato levem a saturação sináptica, limitando a capacidade plástica do hipocampo, bem como a déficits na reconsolidação e extinção da memória. II) É possível que algum sistema compensatório possa estar atuando de forma neuroprotetora, minimizando parcialmente os potenciais efeitos danosos promovidos pelo excesso de glutamato. Assim, foi verificado, *a posteriori*, esta

segunda hipótese, através da mensuração dos níveis de fractalquina e fatores neurotróficos. Foi observado que o grupo de animais obesos apresentou um aumento dos níveis hipocampais de fractalquina (Teste t não pareado, t_{16}=2,451, p=0,0399), sem alterar os níveis de expressão gênica da fractalquina e do CX3CR1 (Teste t não pareado, Fractalquina t_{18}=0,6510, p=0,5233; Teste de Mann-Whitney, CX3CR1, p=0,6842) (**Figura 23B-D**). Além disso, os níveis de fatores neurotróficos permaneceram inalterados em ambos os grupos (Teste t não pareado, BDNF t_8=0,7803, p=0,4577; teste de Mann-Whitney, NGF, p=0,5476; and GDNF, p=0,4857) (**Figura 23E-G**), O eixo fractalquina/CX3CR1 tem sido associado à comunicação entre neurônios e microglia, regulando elementos da plasticidade sináptica e neurotransmissão glutamatérgica (LUO et al., 2019). Adicionalmente, trabalhos *in vitro* apontam possíveis efeitos neuroprotetores da fractalquina contra a excitotoxicidade mediada por glutamato, incluindo a redução da atividade dos iGluR e o aumento da produção de fatores neurotróficos (LAURO et al., 2015a; LIMATOLA et al., 2005; MIZUNO et al., 2003; RAGOZZINO et al., 2006). Assim, é possível que estes eventos estejam associados a uma possível neuroproteção mediada pela fractalquina no modelo proposto, o que poderia atuar na manutenção dos níveis de fatores neurotróficos. Todavia, é importante salientar que o aumento dos níveis de fractalquina também pode indicar uma piora do ambiente obesôgenico em longo prazo. Embora não tenham sido observadas alterações na expressão de CX3CR1 e na ativação microglial, as ações neuroprotetoras desta quimiocina dependem do *background* ambiental (LAURO et al., 2015b). Então levando em consideração que a manutenção de altos níveis de fractalquina poderia recrutar a microglia e que um ambiente potencialmente excitotóxico poderia levar a um perfil neuroinflamatório (BARGER et al., 2007; CHAPMAN et al., 2000; COPE et al., 2018; WARD et al., 2009), a permanência do atual ambiente no modelo proposto poderia representar um fator de risco para o desenvolvimento da neuroinflamação e piora dos déficits de memória atrelados à dieta HSB. Todavia, maiores investigações são necessárias para compreender os efeitos em longo prazo desta relação.

5.0-CONSIDERAÇÕES FINAIS

As alterações nos níveis de glutamato parecem ser independentes da neuroinflamação no modelo de obesidade proposto. Entretanto, especulamos que os níveis elevados de glutamato possam atuar nas alterações comportamentais observadas, e que o aumento nos níveis de CX3CL1 poderiam ser uma estratégia para diminuir possíveis efeitos neurotóxicos deste

neurotransmissor. Contudo, em longo prazo, tal aumento poderia representar um fator de susceptibilidade ao desenvolvimento de eventos neuroinflamatórios.

1.0- GENERAL INTRODUCTION

1.1 - General and epidemiological aspects associated with obesity

Obesity, a chronic non communicable disease characterized by excessive accumulation of adipose tissue and a body mass index (BMI) of 30 kg/m^2, affects over 677 million adults worldwide (WORLD HEALTH ORGANIZATION-WHO, 2021; GLOBAL NUTRITION REPORT; 2020; LEE; MATTSON, 2014). In this context, except for a few Asian and sub-Saharan African countries, the global obesity prevalence has tripled over the last four decades and is expected to rise further this century (KELLY et al., 2008; WHO, 2020). In 2016, the number of overweight or obese adults was 1.9 billion and 650 million, respectively (WHO, 2020). However, it is already estimated that by 2030, overweight and obesity will reach 2.16 and 1.12 billion people, respectively, representing 38% and 20% of the world's adult population (WHO, 2020; KELLY et al., 2008). Given that the obesity epidemic is also affecting children, where the prevalence of childhood obesity has risen from 4% to 18% since 1975, it is clear that obesity is one of the most serious global public health challenges of the 21st century (WHO, 2021; GLOBAL NUTRITION REPORT; 2020). Notably, although it involves genetic mechanisms, the increase in the prevalence of overweight and obesity in recent decades may be associated with several environmental components, including socio demographic, economic, cultural, and urbanization factors, as well as the individual lifestyle (BOUCHARD, 2021; ENDALIFER; DIRESS, 2020; FOX; FENG; ASAL, 2019; NEWTON; BRAITHWAITE; AKINYEMIJU, 2017). Under this perspective, the change in feeding behavior, promoted by the nutritional transition over the last century, was pointed out along with sedentarism, as one of the main environmental triggers for obesity development (CORDAIN et al., 2005; KOPP, 2019; POPKIN; GORDON-LARSEN, 2004). In addition, there has being a reduced consumption of fresh or minimally processed foods, associated with the increased consumption of ultra-processed meals, which present a nutritional composition poor in fiber, and rich in simple carbohydrates, salt, and fats (EGGER; DIXON, 2014; FOX; FENG; ASAL, 2019; GORYAKIN et al., 2015). This may be related to the modulation of regulatory crucial neuroendocrine circuits associated with the balance of consumption, energy expenditure and weight control, providing a fertile ground for obesogenic behaviors and chronic non-communicable diseases (EGGER; DIXON, 2014; MORTON; MEEK; SCHWARTZ, 2014).

1.2 - Physiological mechanisms involved in appetite regulation

The control of appetite and weight is mediated by the integration between the central nervous system (CNS), the gastrointestinal tract (GI), and the adipose tissue (AT) (PIAGGI et al., 2018; VALASSI; SCACCHI; CAVAGNINI, 2008). Furthermore, humoral factors produced by the TGI or white AT, such as leptin, resistin, adiponectin, ghrelin, insulin, peptide YY (PPY), and cholecystokinin (CCK), act as mediators in this equation, reporting the status of available energy stores to the CNS (GIBBS; YOUNG; SMITH, 1973; KENNEDY, 1953) (**Figure 1**). In this context, hypothalamic regions as the lateral hypothalamic area, arcuate nucleus (ARC), paraventricular nucleus (PVN), ventromedial and dorsomedial hypothalamus process peripheral signals and transmit them, at a secondary level, to mesolimbic, hippocampal and cortical areas (ANAND; BROBECK, 1951; BROBECK; TEPPERMAN; LONG, 1943; MORTON et al., 2006; STELLAR, 1954). For the occurrence of satiety, anorexigenic hormones produced by the GI, such as CCK, signal via vagal afferent fibers the replacement of energy reserves to the solitary nucleus (NTS), in order to reduce food intake (GIBBS; YOUNG; SMITH, 1973). In this milieu, projections from the NTS transmit information to the ventral tegumental area (VTA) and limbic structures, such as the amygdala (AM) and the nucleus accumbens (NAc). In these regions, the activation of the dopaminergic and opioidergic systems stimulates the synthesis of leptin in adipocytes and insulin in the pancreas, and these hormones are transported to the ARC, where they inhibit the activity of orexigenic neurons, especially those expressing Neuropeptide Y (NPY) and Agouti-related protein (AgRP). Concomitantly, this process increases the frequency of firing of anorexigenic neurons, which express pro-opiomelanocortin (POMC) and cocaine-amphetamine regulated transcript (CART), which results in activation of catabolic processes, reduced appetite, and increased energy expenditure (COWLEY et al., 2001; ELIAS et al., 2001; KLOK; JAKOBSDOTTIR; DRENT, 2007; MORTON et al., 2006). On the other hand, the process of increased appetite starts with a gradual decrease in leptin and insulin levels, which causes the disinhibition of ARC neurons that co-express NPY and AgRP, resulting in the inhibition of POMC neurons. Thus, orexigenic neuronal projections from the ARC to mesolimbic areas, as well as the enteric nervous system (ENS), stimulate ghrelin production by the stomach, leading to resource-seeking behavior and food intake (**Figure 1**) (MORTON et al., 2006; VALASSI; SCACCHI; CAVAGNINI, 2008). Additionally, studies show that other CNS regions, such as the hippocampus, also act as regulators of energetic balance (DAVIDSON et al., 2007; HANNAPEL et al., 2019). Although poorly elucidated, the

expression of receptors for adipogenic hormones and ghrelin, as well as the integration of this area with other CNS regions, such as visuospatial cortex, olfactory and reward system structures, are associated with hedonic value processing, learning/memory regarding food, and decision making for calorie intake, even without an organic increase in appetite, especially when the food is considered palatable (KANOSKI; GRILL, 2017; KENNY, 2011; MORTON; MEEK; SCHWARTZ, 2014).

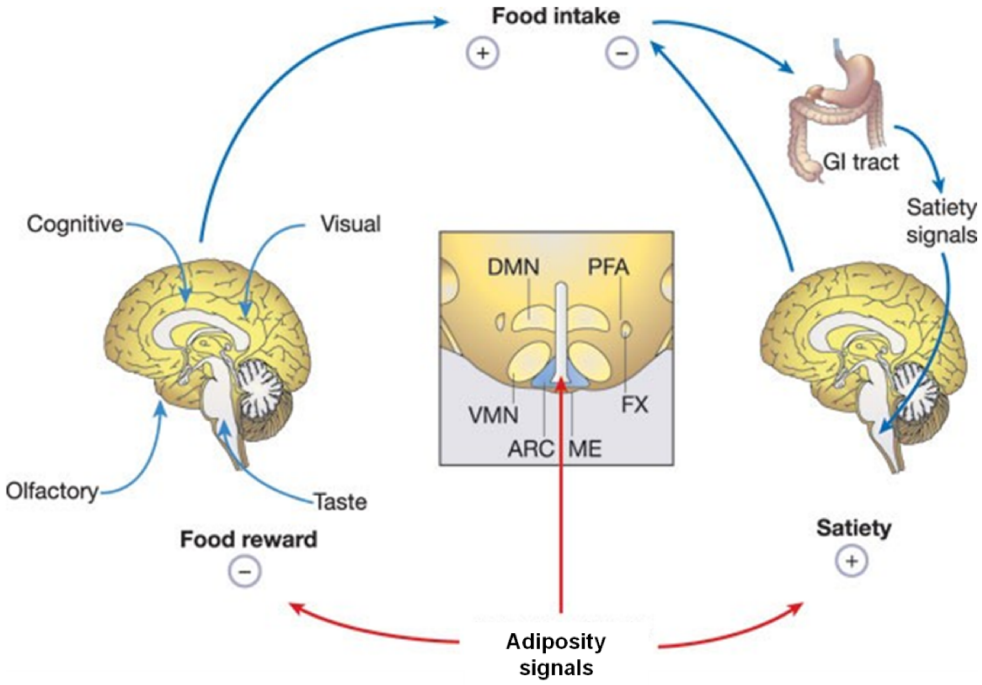


Figure 1. Simplified model of homeostatic food intake regulation in the face of physiological energy status. Adipogenic hormones, such as leptin, act in hypothalamic regions, such as t h e ARC, mediating effects associated with food intake control. At low leptin concentrations, natural reward mechanisms are stimulated, resulting in motivated search behavior and food intake by activation of cortical, limbic, and sensory centers . Subsequently, food intake stimulates the production of anorexigenic hormones by the GI , which in turn, signals satiety to the CNS, stimulating the production of leptin by adipocytes, which reinforces the inhibition of food intake. ARC: Arcuate nucleus, DMV: Dorsal motor nucleus of the vagus, FX: Fornix, ME: Median eminence, PFA: Perifornical area, VMN: Ventro-medial hypothalamic nucleus; CNS: Central nervous system. Source: Morton et al (2006) (Adapted).

1.3- Hyperpalatable diets as possible inducers of the obesogenic environment

1.3.1 - Hyperpalatable diets as modulators of neuroendocrine circuits associated with food intake and weight control.

Although there is no consensual definition of what is a hyperpalatable food, Fazzino et al (2019), suggest that foods that combine different ingredients that enhance palatability, such as sugars, fat, and salt, but have a low nutritional content, high caloric value and have the potential of delaying central satiety mechanisms, prolonging the activity of the reward system, could fall into this category, as do some ultra-processed foods (FAZZINO; ROHDE; SULLIVAN, 2019). Additionally, chronic consumption of highly palatable and energy-dense foods may be related to pathological neuroendocrine modulation, which could predispose to obesity (MORTON; MEEK; SCHWARTZ, 2014). In this regard, the development of Diet-induced obesity models (DIO) using high-sugar, , and high-fat diets (HFD) represented a breakthrough in understanding the pathophysiology of obesity, exhibiting that chronic consumption of highly palatable and energy-dense foods affects not only peripheral body weight control mechanisms, but also the CNS, contributing to the increased expression of NPY and AgRP, altered excitability of orexigenic neurons in different hypothalamic nuclei, induction of apoptosis in anorexigenic neurons in the ARC, impairment in glutamatergic, gabaergic and dopaminergic signaling in the NTS, reduced expression of endogenous opioids in the VTA and induction of central and peripheral resistance to insulin and leptin (MARTIRE et al., 2013; SOUZA et al., 2016; WEI et al., 2015). Nevertheless, due to the heterogeneity of the cafeteria diet in inducing weight gain and its deficiency in micronutrients, as well as the growing criticism regarding the use of HFD as a translational model of obesity, some authors have pointed out the need to develop pre-clinical diets with the nutritional composition closer to the ultra-processed ingested by humans, in order to generate more reliable pre-clinical models of obesity (HINTZE et al., 2018; MAIOLI et al., 2016; PINI et al., 2017; PRINZ, 2019; SPEAKMAN, 2019). Recently, some in vivo studies have used a second generation of highly palatable and energy-dense foods with lipids, carbohydrates, and micronutrient compositions more similar to obesogenic human diets. (HASEGAWA et al., 2020; MAIOLI et al., 2016). An example of these diets is High Sugar and Butter diet (HSB), a diet rich in commercial fat and sugar, but without micronutrients deficiency, developed at the biochemistry department of the Federal University of Minas Gerais (UFMG) (MAIOLI et al., 2016). However, although studies with these new hyperpalatable diets have demonstrated a robust obesity phenotype at peripheral level, the

characterization of central modulation aspects is still poorly elucidated, revealing the need for further investigations associated with this second generation of diets (HASEGAWA et al., 2020; MAIOLI et al., 2016).

1.3.2 - Obesity, hyperpalatable diets and their association with a systemic proinflammatory environment.

The mechanisms associated with the installation of neurometabolic dysfunctions in obesity are not yet fully elucidated. However, highlights have linked nutritional excess to the establishment of a systemic pro-inflammatory environment that could culminate in energetic homeostatic dysfunction (BORST; CONOVER, 2005; FANTUZZI, 2005; MAIOLI et al., 2016; PERMANA; MENGE; REAVEN, 2006). Notably, some studies in DIO models have demonstrated that the excess of lipidic stores may be associated with adipocyte hyperplasia, hypertrophy, hypoxia, as well AT necrosis, which may predispose to the establishment of inflammatory mechanisms through the local, and systemic dissemination of free fatty acids (ALVAREZ-CURTO; MILLIGAN, 2016; AMANO et al., 2014; YE et al., 2007). It is worth remembering that the AT is composed of distinct cell populations, including cells of innate and adaptive immunity, such as neutrophils, macrophages, T helper (Th), and T regulatory (Treg) lymphocytes, among other populations. Under physiological conditions, they produce regulatory cytokines crucial for the balance of adipokine levels, such as IL-10, IL-2, IL-4 (CHOE et al., 2016; FEUERER et al., 2009). However, AT remodeling, promoted by chronic ingestion of highly palatable and energy-dense foods, may increase the expression of chemokines by adipocytes, as monocyte chemoattractant protein type 1 (MCP-1), as well as promote Th1-like responses, associated with elevated INF- levels, decreased number of Treg cells, and leptin resistance (FEUERER et al., 2009; MAIOLI et al., 2016). Furthermore, these pro-inflammatory conditions may lead to the recruitment of circulating monocytes to the AT and their local differentiation into classically activated macrophages (M1), resulting in the establishment of chronic low-grade inflammation, which is characterized by the production of inflammation mediators such as IL-1, IL-6, and TNF- by immune cells and adipocytes (**Figure 2**) (BORST; CONOVER, 2005; FANTUZZI, 2005; FEUERER et al., 2009; MAIOLI et al., 2016; PERMANA; MENGE; REAVEN, 2006; WANG; WU, 2018). Moreover, the transport of pro-inflammatory cytokines that reduce insulin sensitivity, such as IL-6 and TNF-α, from visceral adipose tissue (VAT) to the portal-hepatic circulation is pointed out as a possible contributor to the dysfunction of carbohydrate and lipid metabolism. This could contribute to the establishment of low-grade systemic inflammation and the development of

obesity-associated comorbidities, such as type 2 diabetes mellitus, dyslipidemia and metabolic syndrome (CHOE et al., 2016; COTTAM et al., 2004; FANTUZZI, 2005; FRANSSEN et al., 2011; REVELO et al., 2014). Although the chronology of low-grade systemic inflammation in obesity is still unclear, AT is suggested as a primary site in inflammatory genesis (CHOE et al., 2016; FANTUZZI, 2005). However, similar immune responses have also been observed, in a proximal or delayed manner, in other tissues, which suggests a gradual interdependence of the dissemination of inflammatory mediators from the AT, via the systemic circulation, to other systems, such as the cardiovascular system, GI, and the CNS (CHEN et al., 2021; CHOE et al., 2016; REVELO et al., 2014).

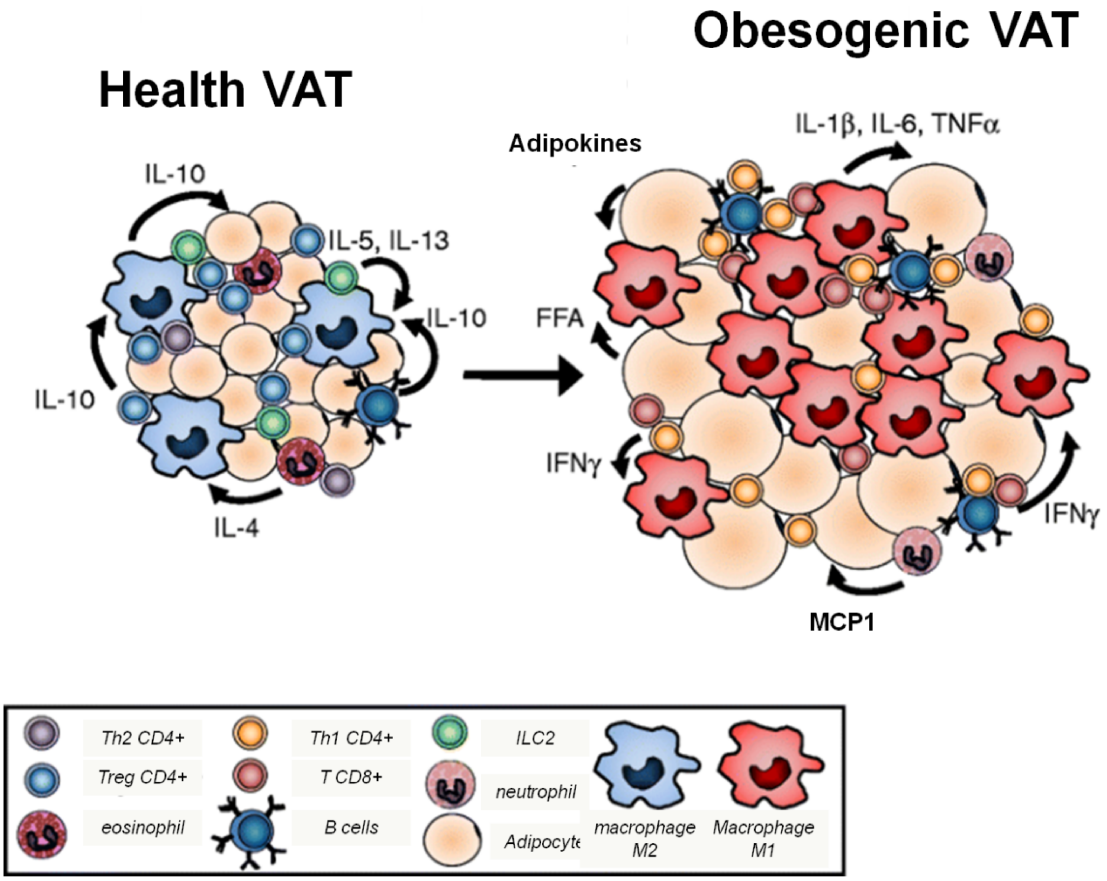


Figure 2. Interactions between visceral adipose tissue (VAT) and immune cells under physiological and pathological conditions. Under homeostatic conditions, the diversity of innate and adaptive immune cells encompasses Th2-type responses, characterized by the action of regulatory T cells (Tregs), B lymphocytes, Th2 CD4+, eosinophils, and type 2 innate lymphoid cells (ILC2s). These populations secrete IL-4, IL-5, IL-10, and IL-13 promoting macrophage polarization to the alternative (M2, anti-inflammatory) profile, which is associated with the regulation of adipokine levels. However, in an obesogenic environment, VAT is remodeled , and also an excess of free fatty acids (FFA), followed by hypoxia and tissue necrosis, is associated with a pro-inflammatory profile, starring the action of CD8+ T cells and Th1 CD4+ lymphocytes. The increased secretion of IFNγ could favor the polarization of M1 macrophages and the production of pro-inflammatory cytokines,

including IL-1β, IL-6, and TNF-α. In addition, expression of monocyte chemoattractant protein type 1 (MCP-1) by adipocytes may recruit circulating monocytes, where the obesogenic environment could mediate their activation in M1 macrophages. Taken together, these mechanisms could contribute to altered leptin production, dysregulation of adipokine levels, and insulin resistance. Source: Revelo et al (2014) (Adapted).

1.3.3 - Obesity, hyperpalatable diets, and neuroinflammation

By definition, neuroinflammation comprises the central elevation of inflammatory mediators produced by glia, endothelial cells, and peripheral immunity (DISABATO; QUAN; GODBOUT, 2016). Interestingly, the permanence of the triggering stimulus, and the nature of the immune response are crucial to determine whether these events will be protective or harmful to neural tissue (DISABATO; QUAN; GODBOUT, 2016; RANSOHOFF et al., 2015). Interestingly, obesity has previously been linked to neuroinflammation in humans, which is consistent with the findings in animals chronically exposed to hypercaloric diets such as HFD (BUCKMAN et al., 2013; GUILLEMOT-LEGRIS et al., 2016; SAMARA et al., 2020). Furthermore, studies in DIO suggest that obesity could trigger neuroinflammation by several mechanisms, including dysfunction in the regulation of the blood-brain barrier (BBB), and the stimulation of cellular immune responses in the CNS, especially innate responses modulated by the microglia (**Figure 3**) (ANDRÉ et al., 2014; BUCKMAN et al., 2013; GUILLEMOT-LEGRIS et al., 2016; OGATA et al., 2019; YI et al., 2017). The BBB is composed of tissue basement membranes, endothelial microvasculature, astrocytes, pericytes, neurons that are in physical proximity to endothelium and are connected by occlusive proteins, and tight junctions, as claudins, occludins, zonula occludens, cadherins, and vinculin (ABBOTT et al., 2010; PERSIDSKY et al., 2006). Functionally, the BBB controls the traffic of ions, nutrients, neurotransmitters, hormones, signaling molecules, cytokines, and immune cells from the periphery to the CNS (ABBOTT et al., 2010). Although the exact mechanism remains unknown, it has been proposed that under conditions of chronic exposure to highly palatable and energy-dense foods, the impairment of the BBB, could make some CNS regions more vulnerable to peripheral immunomodulators originated from the obesogenic environment (MILLER; SPENCER, 2014; RHEA et al., 2017). Indeed, the establishment of dysfunctions in the transporter system for leptin and cytokines, as well as the reduction in the expression of ZO-1, claudin-5, claudin-12, and occludin, have emerged as potential alterations observed in DIO models. This might be associated with altered BBB permeability at multiple sites, allowing exacerbated entry of pro-inflammatory cytokines and free fatty acids in the CNS, which might trigger neuroinflammation (MILLER; SPENCER, 2014; OGATA et al.,

2019; RHEA et al., 2017; STRANAHAN et al., 2016). In this context, it has been shown that the microglia, an innate phagocytic cell resident in the CNS, acts as a key for neuroinflammation processes in numerous pathologies (DISABATO; QUAN; GODBOUT, 2016; MARIN; KIPNIS, 2017; RANSOHOFF et al., 2015). Physiologically, the microglia perform not only immune vigilance and pathogen protection functions, but also support neurotransmission, synapse formation, plasticity, and vascular remodeling (BUTLER, 2021; MARIN; KIPNIS, 2017). Nevertheless, in obesity, increased central levels of free fatty acids and peripheral cytokines, such as TNF-α and INF-ɣ, could be associated with the activation of these cells, via Toll-like receptor-4 (TRL4) (BUTLER, 2021; MILLER; SPENCER, 2014). Consequently, in DIO models, it has already been shown that these mechanisms might polarize microglia into a M1 profile, characterized by signature markers such as CD11b and CD68, as well as by activation of the nuclear factor kappa B (NF-κB) pathway, which culminates in the production of IL-1β, IL-6, TNF-α, and nitric oxide; mediators that when in excess, are associated with dysfunction in synaptic regulation, neurodegeneration, and oxidative stress (ALEXAKI, 2021b; GUILLEMOT-LEGRIS; MUCCIOLI, 2017; VALDEARCOS et al., 2014, 2017). Furthermore, under these conditions, microglial activation may contribute to the release of proinflammatory cytokines by astrocytes, as well as the recruitment of peripheral myeloid cells to the CNS, which may contribute to the inflammatory process. (LIDDELOW et al., 2017; VALDEARCOS et al., 2014, 2017). The chronology of neuroinflammation in obesity is still not well understood. However, it is hypothesized that the hypothalamus may be the first affected site, leading to the reinforcement of neuroendocrine dysfunction and the maintenance of the obesogenic environment (**Figure 3**) (ALEXAKI, 2021a; LÉON; NADJAR; QUARTA, 2021; MILLER; SPENCER, 2014). However, other structures such as the cortex, cerebellum, amygdala, and hippocampus also present neuroinflammation states, but with some peculiarities that depend on the tissue microglial diversity, the time of dietary exposure, age, as well the diet composition (GUILLEMOT-LEGRIS et al., 2016; GUILLEMOT-LEGRIS; MUCCIOLI, 2017). Notably, the possible association between neuroinflammation at multiple sites and metabolic dysfunction might predispose to deficits in CNS higher functions, leading to behavioral alterations and decline in cognition and memory (CHUNCHAI; CHATTIPAKORN; CHATTIPAKORN, 2018; HAO et al., 2016; MELO et al., 2020).

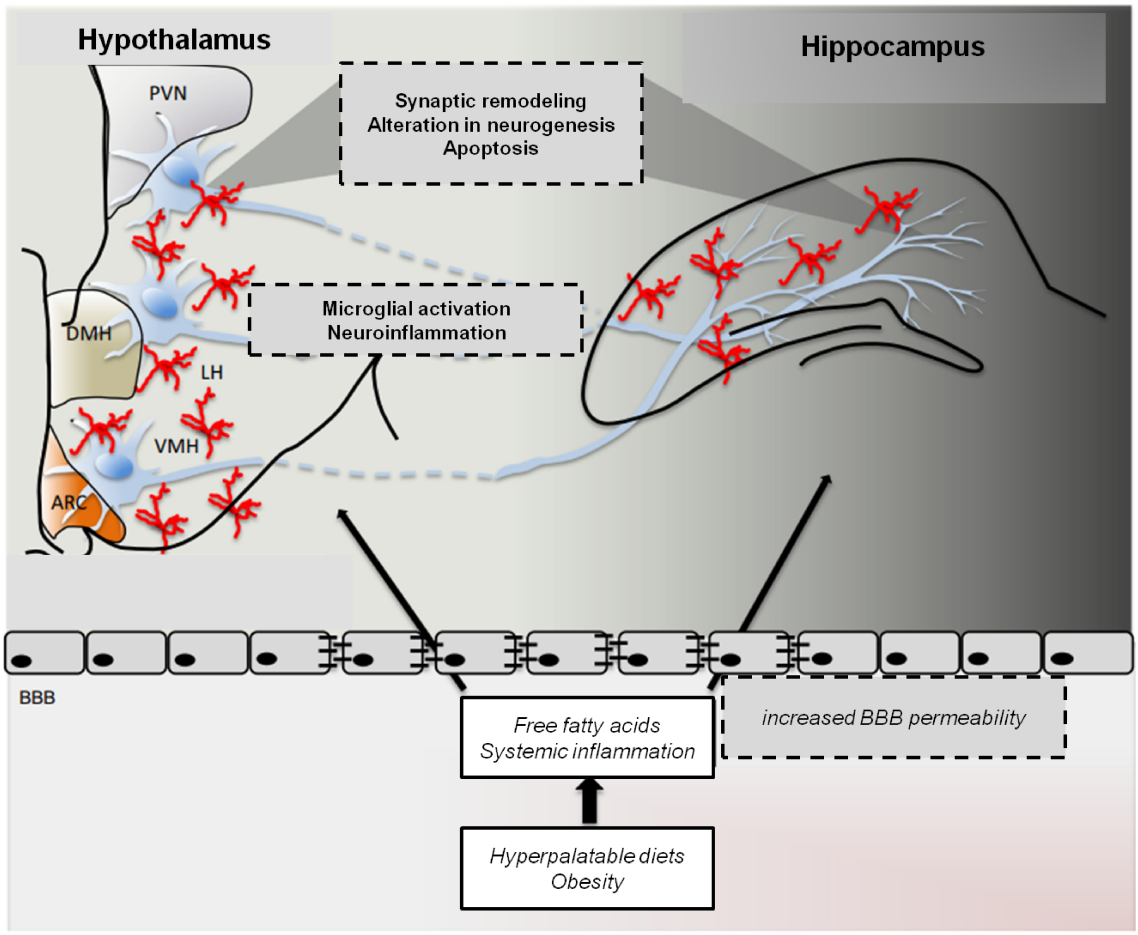


Figure 3. Postulated mechanisms to demonstrate the relationship between obesity and neuroinflammation. Obesogenic environment and chronic exposure to hyperpalatable type diets could increase circulating levels of free fatty acids, pro-inflammatory cytokines, and chemokines that may infiltrate the CNS by increasing BBB permeability and/or through its absence in some sites (e.g. ARC). This could initiate a process of central inflammation, first in the hypothalamus, causing microglial activation, as well as increased expression of pro-inflammatory mediators, which may affect regions whose hypothalamus has projections, such as the hippocampus. Additionally, the temporal effect of hyperpalatable diets per se could trigger similar neuroinflammatory processes in various extra-hypothalamic areas, leading to a decline in neuronal communication, apoptosis, and hippocampal neurogenesis impairment , predisposing to a decline in memory and cognition. ARC: arcuate nucleus; BBB: blood-brain barrier; DMH: dorsomedial hypothalamus; LH: lateral hypothalamus; PVN: paraventricular nucleus of the hypothalamus; CNS: central nervous system; and VMH: ventromedial hypothalamus. Source: Miller & Spencer (2014) (Adapted).

1.4- Obesity, memory deficits, and cognitive decline

1.4.1- Influences of neuroinflammation on obesity-related memory decline

Studies in DIO have demonstrated the presence of neuroinflammation in several memory-related areas, including the hippocampus, a primary region for cognitive and learning processes. In obesity, hippocampal inflammation could be associated with dysfunction in short and long-term spatial memories, as well as in long-term aversive, episodic, and social memories (GUILLEMOT-LEGRIS et al., 2016; HAO et al., 2016; MELO et al., 2020; SPENCER et al., 2017; SUÁREZ et al., 2019). Although the mechanisms by which neuroinflammation might be related to memory dysfunction are not yet fully elucidated, communication between microglia and neurons has emerged as a key player in the relationship between obesity, neuroinflammation, and memory (COPE et al., 2018; HAO et al., 2016; KAWAMURA et al., 2021). For example, chronic exposure to the HFD diet is associated with increased expression of microglial and phagocytic markers, such as ionized calcium-binding adaptor molecule-1 (Iba-1) and CD68, synaptic engulfment by microglia, and reduced production of Brain Derived Neurotrophic Factor (BDNF), which could lead to reductions in the complexity and number of hippocampal dendritic spines, plastic changes, and cognitive decline (CAVALIERE et al., 2019; COPE et al., 2018; HAO et al., 2016). In addition, long exposition to HFD also may induce alterations in the expression of the microglial receptor for fractalkine (CX3CR1), a mediator in neuroglial communication associated with synaptic remodeling and Long Term Potentiation (LTP) formation, which may contribute to dysfunctions in short and long-term memories, as well as in a decline of synaptic plasticity (COPE et al., 2018; KAWAMURA et al., 2021). Interestingly, elevated levels of proinflammatory cytokines produced by the microglia, such as IL-1β and TNF-α, also appear to contribute, by themselves, to memory alterations in obesity (MELO et al., 2020; SOBESKY et al., 2014). In this context, the use of pharmacological inhibitors for IL-1RA and TNFR1 receptors (respectively activated by IL-1β and TNF-α) have been associated with restoration of episodic memory and contextual aversive memory consolidation in rodents chronically exposed to HFD (COPE et al., 2018; MELO et al., 2020; SOBESKY et al., 2014).

Somewhat less well known is the role of astrocytes in obesity-associated neuroinflammation. In this regard, these cells are associated with various functions such as support for neuronal metabolism, regulation of blood-brain barrier permeability, among others (COLOMBO; FARINA, 2016; HASEL et al., 2021). Interestingly, under conditions of

neuroinflammation, increased astrocyte reactivity, mediated by proinflammatory cytokines released by the microglia, may be associated with enhanced neuroinflammation and neurotoxicity by the activation of pro-inflammatory pathways, such as NF-κB, and production of pro-inflamatory cytokines (COLOMBO; FARINA, 2016; REID; KUIPERS, 2021). Additionally, glial fibrillary acidic protein (GFAP), a structural astrocyte marker, is upregulated in reactive astrocyte and are a commonly biomarker associated with these scenario (REID; KUIPERS, 2021). Notably, recent studies have demonstrated the increased expression of GFAP is associated with neurotoxicity and neuronal plasticity damage in DIO models although the mechanisms are not completely elucidated, these findings point to a possible contribution of astrocytes in the relationship between memory decline and neuroinflammation in obesity (CANO et al., 2014; LAU et al., 2021; TSAI et al., 2018).

Thus, the regulation of microglial phagocytic activity, astrocyte reactivity and the production of inflammation mediators seem, so far, to be the main pathway by which neuroinflammation could contribute to synaptic remodeling and memory decline in obesity. However, communication between glia and neurons is also associated with the regulation of the levels, production, and activity of different neurotransmitters that also contribute to physiological and pathological memory processes (ARAQUE et al., 1999; HAROON; MILLER; SANACORA, 2017; MAGISTRETTI, 2006). Importantly, although studies demonstrated that the decline in cognition and memory in DIO models may be associated with dysfunction in different neurotransmission systems at the hippocampal and cortical levels, such as the dopaminergic, serotoninergic, endocannabinoid, and glutamatergic systems, little is known about how the mechanisms of neuroinflammation in obesity could mediate alterations in specific neurotransmission systems to leading memory impairments (FORTE et al., 2021; HALEEM; MAHMOOD, 2021; KACZMARCZYK et al., 2013; SANDOVAL-SALAZAR et al., 2016).

1.3.3- Glutamatergic neurotransmission and memory deficits associated with obesity

L-glutamate is the major excitatory neurotransmitter in the mammalian CNS and it plays a key role in memory and learning processes (RIEDEL; PLATT; MICHEAU, 2003). Traditionally, this neurotransmitter is produced via the glutamine-glutamate cycle, in which glutamate is taken up by glial transporters expressed in astrocytes; converted into glutamine via α-ketoglutarate or glutamine synthetase, an enzyme exclusively present in glial cells; and released back to neurons, where it is converted into glutamate by phosphate-activated

glutaminase (**Figure 4**) (VAN DEN BERG; GARFINKEL, 1971; WALLS et al., 2015; WESTERGAARD; SONNEWALD; SCHOUSBOE, 1995).

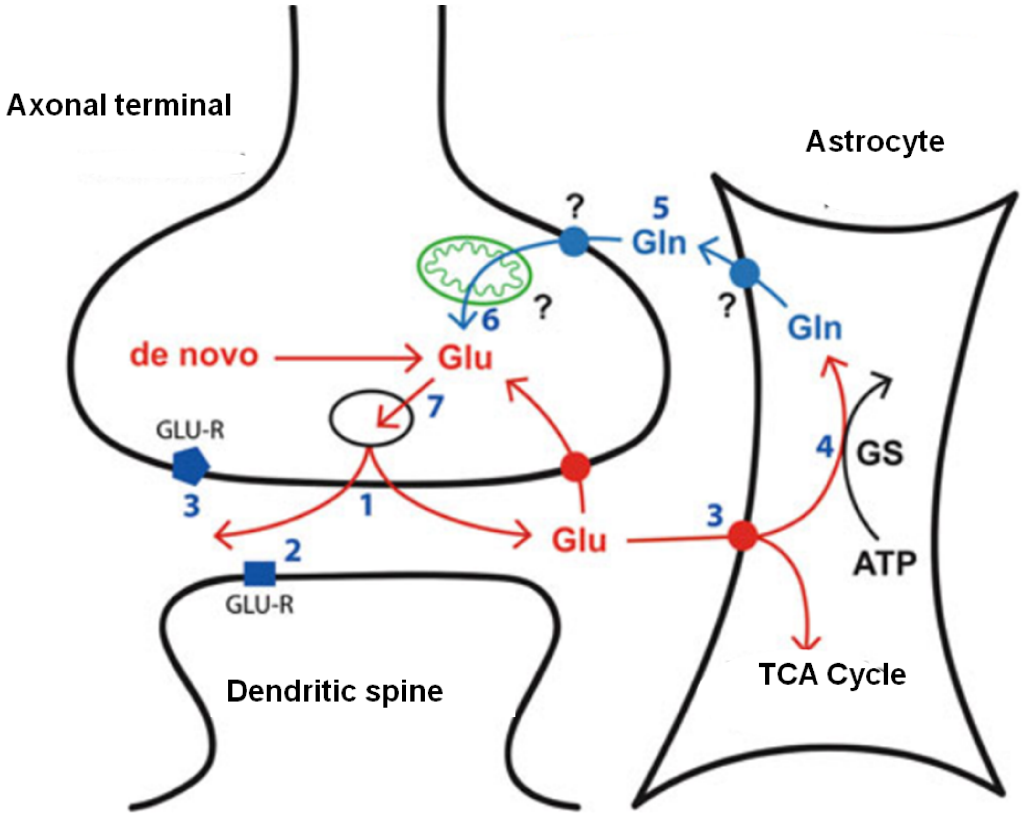


Figure 4. Simplified representation of the glutamate-glutamine cycle. (1) The presynaptic release of glutamate (Glu) stimulates the postsynaptic terminals (2), but this interaction is finalized by the uptake of glutamate in the synaptic cleft, promoted by the astrocytes, through Na-dependent excitatory amino acid transporters+ (EAATs) (3). In the astrocyte, the activity of EAATs alters the sodium (Na^+) gradient, activating the Na^+ /K^+ ATPase complex in order to restore ionic concentrations, which triggers the conversion of glutamate into (4) glutamine (Gln) via glutamine synthase (GS) for subsequent oxidative metabolism in the TCA cycle. Gln is thus transported to neurons (5 and 6), restoring glutamate production, by phosphate-activated glutaminase. Finally, the resulting glutamate is repackaged into vesicles for subsequent synaptic release. Source: Eid et al (2016) (Adapted).

Glutamate signaling is mediated by ionotropic and metabotropic glutamatergic receptors (iGluR and mGluR) that are differentially distributed in pre- and post-synaptic regions and contribute to trigger different functional roles of glutamate in neuronal memory networks (RIEDEL; PLATT; MICHEAU, 2003). In this sense, the formation of short and long-term potentials (STP and LTP, respectively) is traditionally mediated by different classes of iGluR,

such as Amino-3-hydroxy-5-methyl-4 isoxazolproprionate (AMPAR) and N-Methyl-D-Aspartate (NMDA-R), and mGluR, which includes especially the class I of mGluRs (mGluR1 and mGluR5) (RIEDEL; PLATT; MICHEAU, 2003; RIEDEL; REYMANN, 1996). Briefly, the plasticity mechanism might be modulated directly or indirectly by the NMDAR. In the NMDA-dependent plasticity, the activation of AMPAR activated by glutamate ligand could promote post-synaptic depolarization, leading to the unlocking of Mg^{+} ions in the postsynaptic NMDA-R receptors, allowing their activation, which in turn mediates the influx of Ca^{2+} ions, and activation of Ca^{2+} dependent intracellular pathways (ABEL et al., 1997; ABEL; LATTAL, 2001; RIEDEL; PLATT; MICHEAU, 2003). In the case LTP, the permanence and intensity of the excitatory stimulus could activate intracellular pathways mediated by adenylyl cyclase and phosphokinase, leading to the activation of nuclear transcription factors associated with neuronal growth, which can result in the creation of new synaptic connections (**Figure 5**) (ABEL; LATTAL, 2001; MILLER; MAYFORD, 1999). Finally, in the indirect NMDA activation, mGluR1 and mGluR5 receptors trigger activation of the phospholipase C/Inositol triphosphate (IP3) pathway, promoting an alternative and additional activation to NMDA receptors, since the activity pathway mobilizes intracellular calcium deposits, besides contributing to gene transcription processes, by phosphorylation of proteins associated to the diacylglycerol/protein kinase C pathway (RIEDEL; REYMANN, 1996).

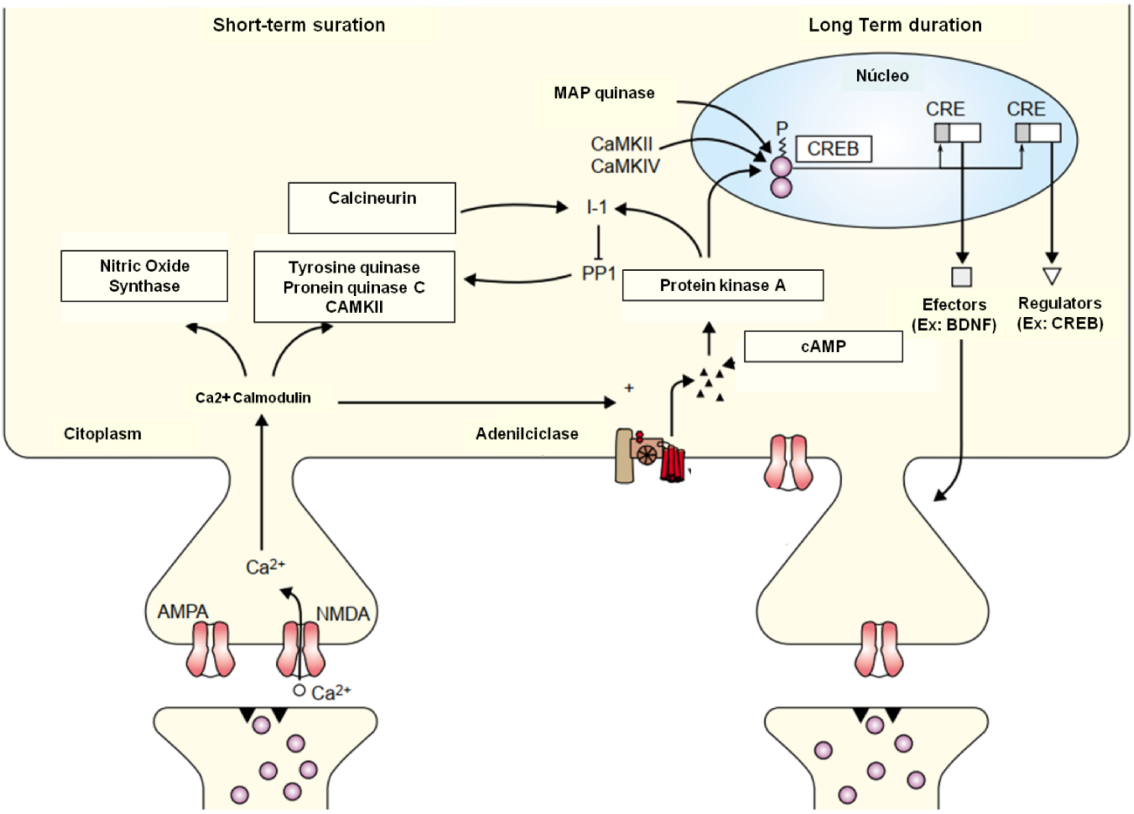


Figure 5. Molecular pathways associated with short and long term potentials. In events mediated by short term potentials, the postsynaptic activation of AMPA receptors allows the stimulation of NMDA receptors, causing an increase in Ca^{2+} conductance. The influx of Ca^{2+}in the postsynaptic neuron is associated with the activation of CaMKII, PKC and calcineurin. For the occurrence of the long term potentiation (LTP), similar mechanisms occur, but the adenylyl cyclase is activated via Ca or through G protein-coupled receptors. Alternatively, the activity of CaMKII, CaMKIV and MAP kinases could also lead to such phenomena, allowing LTP formation. BDNF: brain-derived neurotrophic factor; C/EBP, CCAAT: enhancer-binding protein; CaMK: Calmodulin; CRE: cAMP response element; I-1: protein phosphatase inhibitor-1; PP1: protein phosphatase type-1; tPA: tissue plasminogen activator. Source: Abel et al (1997) (Adapted).

The balance of available/released glutamate levels in the synaptic cleft is crucial for CNS, since reduced or increased gradients of this neurotransmitter may cause, respectively, impairment in neurotransmission and signal transduction via postsynaptic glutamatergic receptors, as well as mediate neurotoxic processes (CHOI, 1985; KATAGIRI; TANAKA; MANABE, 2001). In animal and human models, it has been suggested that imbalance in central glutamate levels could be related to alterations in mood, cognition, and dysfunction in memory processes (JAVITT, 2004; REVETT et al., 2013; SANACORA et al., 2003). Furthermore, blockade or changes in the expression of functional subunits of AMPA and NMDA receptors, as well as modulation of class I mGluRs, may be associated with decreased LTP, synaptic plasticity, and memory deficits (ABEL; LATTAL, 2001; RIEDEL; PLATT;

MICHEAU, 2003; RIEDEL; REYMANN, 1996). Although poorly understood, it is important to point out that some evidence also suggests that some immunomodulatory events may be involved in the regulation of CNS glutamatergic dynamics. For instance: in murine hippocampal slices, INF-ɣ, TNF, and IL-1β are able to, respectively, increase expression, activity, and recruitment of postsynaptic AMPA and NMDA subunits (GluR1, NR2A/NR2B, respectively), which may suggest the influence of these cytokines in physiological and pathological glutamate signaling (MIZUNO et al., 2008; STELLWAGEN et al., 2005; VIVIANI et al., 2003). Moreover, a pro-inflammatory environment may induce glutamate production by glial cells, increase glutamate concentration in the synaptic cleft, and suppress the expression of astrocyte glutamate transporters, such as glutamate transporter-1 (GLT-1), which may be associated with neurotoxicity and neuronal plasticity damage (BARGER et al., 2007; HAROON; MILLER; SANACORA, 2017; YE; SONTHEIMER, 1996). Nonetheless, the integration of inflammation and glutamate in memory remains understudied in metabolic dysfunction conditions, with most research focusing on the individual effect of glutamate in memory decline (KUMAR DATUSALIA; SUNDER SHARMA, 2016; MARTÍNEZ-OROZCO et al., 2021; TRUDEAU; GAGNON; MASSICOTTE, 2004; TSAI et al., 2018; VALLADOLID-ACEBES et al., 2012; VAN BUSSEL et al., 2016). In this milieu, dysregulation of glutamatergic neurotransmission is associated with memory alterations associated with metabolic-based diseases such as type 2 diabetes mellitus, however, this mechanism remains poorly explored in the case of obesity (KUMAR DATUSALIA; SUNDER SHARMA, 2016; TRUDEAU; GAGNON; MASSICOTTE, 2004; VAN BUSSEL et al., 2016). Notably, although deficits of hippocampus-dependent memories were observed, studies in DIO models demonstrated controversies regarding the increase or decrease of central glutamate levels associated with an obesogenic environment (LABBAN et al., 2020; MARTÍNEZ-OROZCO et al., 2021; TSAI et al., 2018). Furthermore, only two papers reported the investigation of the expression and functionality of NMDA and AMPA receptor subunits, as well as the action of GLT-1, promoted by highly palatable and energy-dense foods in DIO models during adulthood (TSAI et al., 2018; VALLADOLID-ACEBES et al., 2012). Finally, alterations in the expression of class 1 mGluRs triggered by highly palatable and energy-dense foods diets remain to be elucidated, which reveals the need for further basic investigations on how obesity could affect glutamatergic signaling in memory processes.

2.0-JUSTIFICATION AND THESIS PRESENTATION

Understanding the effects of highly palatable and energy-dense foods diets on the obesogenic environment and CNS functions is critical from a translational standpoint, given that global consumption and supply of ultra-processed foods has increased in recent decades. It is estimated that just in 2019, populations in developed and developing countries consumed the equivalent of 109.3 and 42 kg/per capita of ultra-processed foods, respectively (BAKER et al., 2020). This is a worrisome scenario because, as in DIO models, chronic exposure to ultra-processed foods is positively correlated with the development of overweight and obesity in humans (ASKARI et al., 2020). It is important to remember that the global increase of obesity incidence is associated not only with psychosocial effects on individuals affected by this disease, but also with a high economic cost, as obesity accounts for up to 2.8% of global health-care spending. Moreover, the costs employed in direct care of obese individuals may be up to 30% higher when compared to non-obese people (WITHROW; ALTER, 2011). Additionally, absenteeism and presenteeism rates tend to be higher in the obese population compared to non-obese individuals, which causes indirect costs due to this disease (LEHNERT et al., 2013; TROGDON et al., 2008). Impaired memory and cognition are thus important because these processes are closely linked to functional and social consequences, as well as the direct and indirect costs of obesity. However, although it has been demonstrated that chronic consumption of highly palatable and energy-dense foods could negatively influence cognition and memory processes in rodents, little is known about how the integration of immunological and neurotransmitter mechanisms could catalyze memory deficits associated with the obesogenic environment, which reveals the need for further basic elucidations regarding the theme. Thus, the current study sought to test the effects of neuroinflammatory regulation induced by hyperpalatable diets on memory, cognition, and neurotransmission processes in an obesity murine model. For that, we tested the hypothesis that chronic exposure to a highly palatable and energy-dense diet causes peripheral inflammation, which may induce hippocampal neuroinflammation, leading to dysfunctions in glutamatergic neurotransmission, which could lead to cognitive decline and memory changes.

3.0- GOALS

3.1- General goal

- To investigate whether mice exposed with a chronic highly palatable diet may present memory dysfunction, hippocampal neuroinflammation, and glutamatergic neurotransmission alterations.

3.2- Specific goals

- To evaluate the effects of chronic high palatable diet exposure in short-and long term memory.
- To evaluate whether chronic hyperpalatable diet exposure modulates inflammation, through microglial, and astrocytic activation in the hippocampus, as well as the pattern of pro-inflammatory cytokine levels in the hippocampus and peripheral serum.
- To determine the effects of chronic hyperpalatable diet exposure on blood-brain barrier permeability.
- To verify the effects of chronic hyperpalatable diet exposure on the central glutamatergic transmission;
- To verify the effects of chronic hyperpalatable diet exposure on the expression of ionotropic (AMPA, NMDA), metabotropic glutamate receptor (mGluR5), and glutamate transporter (GLT-1) in the hippocampus.
- To determine the effects of chronic hyperpalatable diet exposure on hippocampal levels of neurotrophic factors.
- To evaluate whether chronic hyperpalatable diet exposure and obesogenic environment are able to modulate the hippocampal levels of fractalkine (CX3CL1) and microglial receptor (CX3CR1).

4.0- MATERIAL AND METHODS

4.1- Animals and ethical aspects

Three-four weeks old C57BL/6J male mice were purchased from the animal facility of Universidade Federal de Minas Gerais (UFMG). These animals were housed five per cage, in the animal care facility of the Neuropharmacology Laboratory, under the following environmental conditions: 12 h light/12 h dark cycle, temperature at 24 ± 2 ºC with food and water provided *ad libitum*. All experiments were carried out with prior approval of the local ethical committee of animal use (CEUA), under protocol number 217/200 (Appendix 1).

4.2- Diets and Experimental design

After one week of habituation, mice were divided into two groups, fed for 12 weeks with an isocaloric diet (American Institute of Nutrition 93-Growth diet (AIN93G, (Carbohydrates: 64%, Proteins: 20%; Lipids: 16%; 3.9 Kcal/g) or a highly palatable and energy-dense diet (High Butter and Sugar diet (HSB, (Carbohydrate: 36%, Proteins: 16%; Lipids: 48%; 4.9 Kcal/g) (MAIOLI et al., 2016) (**Table 1**). Mice were weighed once a week. In addition, the cumulative energy intake was calculated by summing the weekly values of the food amount eaten per cage multiplied by the caloric value of each diet (NASCIMENTO et al., 2008). Behavioral analysis was performed between the 11th and 12th weeks of the diets exposure protocol to evaluate memory and cognitive performance. A separate group of animals was subjected to the oral glucose tolerance test at the same time. To obtain tissues for biochemical and histological assays, euthanasia was performed at week 12 of diet exposition (**Figure 6**).

Table 1: Ingredients and nutritional composition of AIN93G and HSB diet. Source: Maioli et al., 2016 (Adapted).

Ingredients/Diets (g)	AIN93G	HSB
Sugar	100	232
Cornstarch	397,5	208,6
Dextrinized starch	132	0
BHT (Tert-butylhydroquinone)	0,014	0,014
Choline bitartrate	2,5	2,5
Casein	200	200
Cellulose	50	50
L-cystine	3	3
Commercial butter	0	189
Vitamin Mix	10	10
Mineral mix	35	35
Soybean oil	70	70
Total	**1000**	**1000**
Centesimal composition (%)		
Carbohydrate	64	36
Lipid	16	48
Protein	20	16
Kcal/g	3,9	4,9

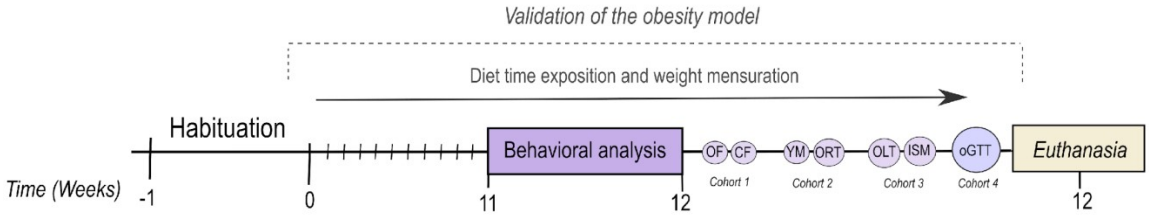


Figure 6: Experimental design. C57BL/6J male mice with ages between 3 to 4 weeks are habituated for one week and divided into two groups-AIN93G or HSB diet group-. Diet time exposition was 12 weeks and mice were weighed once a week. Between 11-12th weeks, behavioral analysis or oral glucose tolerance test (oGTT) were performed in different animal cohorts. In the 12th week, mice were euthanized. OF: Open field test, CF: Contextual conditioned fear test, YM: Y maze test, ORT: Object Location Test (ORT), ORT: Object Recognition Test, ISM: Interaction and social memory test. Source: The author.

4.3- Behavioral assessment

All behavioral tests were performed during the light cycle, under controlled conditions of temperature (24 ± 2 ºC), and brightness (70-80 lux). Furthermore, a 30 min acclimation period was given to the animals before starting the experiments. All apparatus were cleaned with ethanol 70% between the experimental sessions to avoid olfactory bias. None of the mice cohorts used were subjected to more than two different behavioral tests (**Figure 6**). The experimental tasks were ordered so the most stressful behavioral experiments were performed at last. Intervals between 1-3 days were given between experimental tasks. In addition, the animals were not deprived of food or water during experimentation. All procedures were recorded for afterward evaluation, and analyzes were performed blindly. Moreover, all behavioral memory experiments were manually analyzed with the support of the software X-Plot-Rat ® 2005 (developed by the research team of Dr. Morato, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, USP, Brazil).

4.3.1 – Open Field

To evaluate locomotor and exploratory behavior the open field test was performed. Mice were randomly placed in a cylindrical and circular acrylic apparatus (30 cm diameter x 40 cm height), where free exploration was allowed for 30 minutes. Total distance traveled (cm), the average speed (m/s), as well as the time spent in the center of the arena (s) of the interesting groups were analyzed by Any-maze Video Tracking System software (*version 5.26, Stoelting*®).

4.3.2 – Y maze test

The evaluation of short-term operational memory was performed using the Y maze test. For that, a grey plastic Y maze with three arms (A, B, C) (30 cm length, 6 cm width, 20 cm height) arranged at a 60° angle was used (**Figure 7**). In addition, different visual clues were used in each arm to help the mice to recognize them. The animals were placed individually at the distal part of A-arm and were let free to explore the apparatus for 8 minutes. An arm entry was considered only when both hind paws were placed completely inside an arm. The percent of alternation was calculated using the following formula: [Number of alternations/ (Total number of arm entries-2)] x 100.

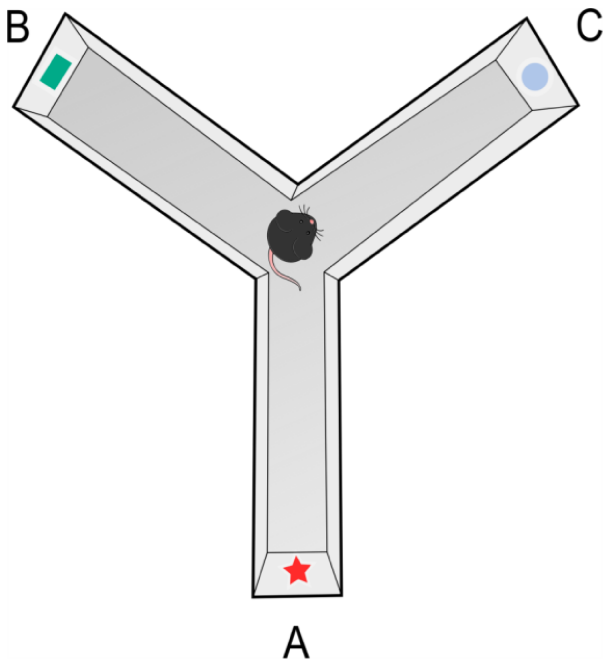


Figure 7: Representation of the apparatus used in the Y maze test. For the behavioral test, the animals were placed individually at the distal part of A-arm and free to explore the apparatus for 8 minutes. Percent alternation was calculated based on the formula: [Number of alternations/ (Total number of arm entries-2)] x 100. In addition, an arm entry was considered only when both hind paws were placed completely inside an arm. Source: The author.

4.3.3 - Object Location Test (OLT) and Object Recognition Test (ORT)

Two different cohorts of mice were submitted to the OLT and ORT. A white square acrylic open field (38 x 38 x 15 cm) was utilized for both tests. At the first day, mice were habituated to the apparatus for 5 minutes (**Figure 8**). Twenty-four and 48 hours later, mice were re-

exposed to the open field containing two identical objects, located symmetrically at 80 mm of the apparatus wall and vertically separated from each other by 6 cm. In addition, free exploration was measured for 10 and 5 minutes, respectively (**Figure 8**). Finally, at the OLT test day, one of the objects was displaced 6 cm away from the original position (novel position). In the ORT, one of the objects was substituted for a new object, without altering the original localization. In both tests, mice were allowed to freely explore for 5 minutes (**Figure 8**). Interaction with the objects was defined as sniffing until 2 cm of distance from the object, as well as touching the objects with the nose or forepaws. Turning around or sitting next to the object was not considered exploratory behavior. The percent of total investigated time was used for measuring the long-term memory and was calculated in accordance with previous literature reports (DENNINGER; SMITH; KIRBY, 2019): Total investigation time (%): [Time $_{\text{with novel location or object}}$]/ (Time $_{\text{with novel location or object}}$ + Time $_{\text{with familiar location or object}}$)] x 100.

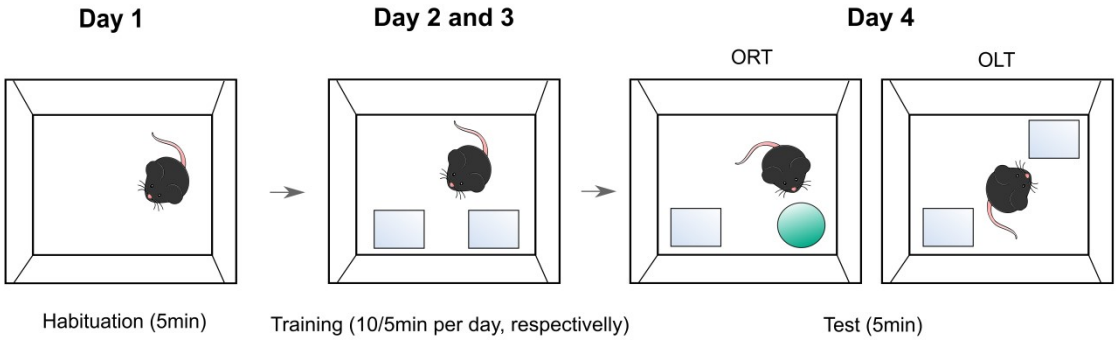


Figure 8: Stages of the object location test (OLT) and object recognition test (ORT). This behavioral test was performed over four days, consisting of habituation (day 1, 5 minutes), training with two identical objects (day 2 and 3, 10 and 5 minutes, respectively), and testing with the insertion of a new or familiar object moved to a new position (day 4, 5 minutes). Source: The author.

4.3.4 – Contextual conditioned fear test

A conditioning chamber with a grid floor (39 × 47 × 32 cm; Insight Ltda, Brazil) was used for mice conditioning. On the first day of the experimental procedure, a trained session was initiated before 120 seconds of context habituation. Subsequently, a foot shock (Unconditioned stimulus (US), 0.7mA) was delivered. In total, three US were performed with a 60 s interval between them (**Figure 9**). The evocation memory test was executed 24 hours after the training session. For this, mice were exposed to context for 5 min. Furthermore, in order to induce extinction of fear memory, mice remained in exposition to context for more

than 15 minutes (**Figure 9**). Finally, on the third day, mice were again exposed to context for the evaluation of memory extinction (**Figure 9**). For analysis, freezing was considered as the entire absence of movement, excluding breathing (ANAGNOSTARAS et al., 2000; VALENTINUZZI et al., 1998). The computation of freezing was performed every minute during the entire memory evocation and extinction tests sessions. The results were expressed in percent of time freezing.

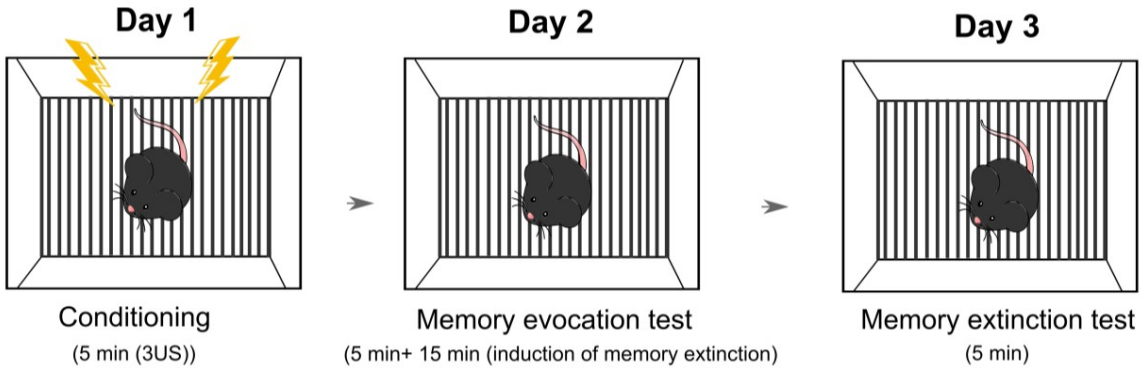


Figure 9: Stages of the context-conditioned fear test. This behavioral test was conducted over three days, consisting of conditioning with 3 shocks (unconditioned stimuli (US)) (day 1), memory test and memory extinction induction (day 2, 5 and 15 minutes, respectively), and memory extinction test (day 3, 5 minutes). Freezing computation was used as a behavioral measure. Source: The author.

4.3.5– Social interaction and social memory test

A screening of social interaction and social memory skills was conducted in a transparent and rectangular acrylic box (60 × 40 cm) divided into three compartments (20 × 40 × 22 cm) separated by two interior doors (**Figure 10A**). Adult mice were individually habituated for 5min in the central compartment. Subsequently, for the evaluation of social interaction, a plastic transparent cylinder (10 cm diameter with distributed holes) containing an unfamiliar male juvenile mouse (Stranger 1) was introduced into one of the chambers. An identical empty plastic cylinder was introduced in the remaining chamber. After 3 minutes, inner doors were opened and the adult mice were free to explore the social apparatus for 10 minutes (**Figure 10B**). Additionally, the location chamber of the plastic cylinder containing the juvenile mouse or the empty cylinder was alternated between experimental sessions. Then, a second unfamiliar mouse (Stranger 2) was placed in the previously empty cylinder for the evaluation of social memory. Thus, the adult mice freely explored the apparatus for another 10 minutes (**Figure 10C**).

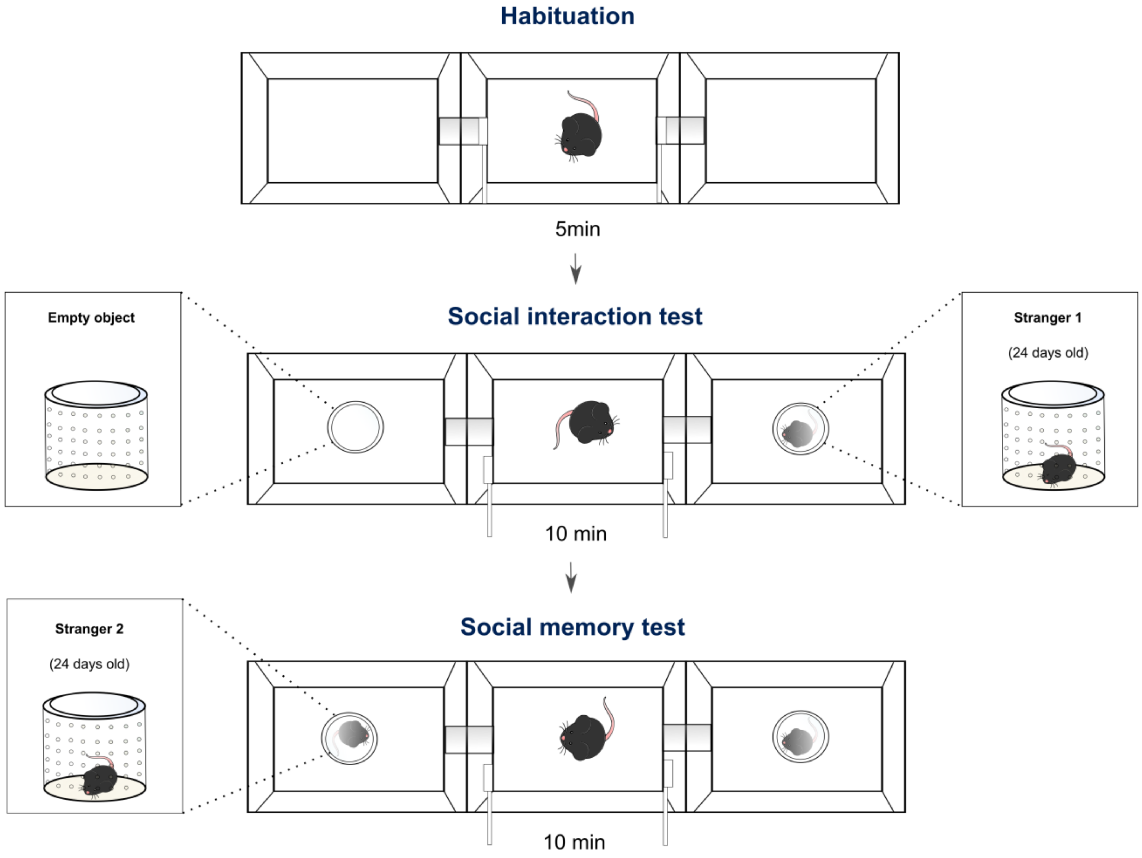


Figure 10: Stages of the classic social interaction and social memory test. Animals were allocated individually in the central chamber for 5 minutes. After this period, for the social interaction test, one juvenile mouse (Stranger 1) or an empty cylinder were placed in each of the chambers (10 minutes). Subsequently, to assess social memory, another juvenile rat (Stranger 2) was placed in the chamber containing the cylinder (10 minutes). For both tests, the adult's preference was computed by the time of interaction with the juvenile mouse. Source: The author.

Furthermore, an additional single-chamber protocol was performed to assess short- and long-term social memory (ALMEIDA-SANTOS et al., 2019; THOR; HOLLOWAY, 1982). In summary, the training session consisted of habituating the adult mice for 15 minutes in a standard cage. After, a plastic transparent cylinder (10 cm diameter with distributed holes) containing an unfamiliar male juvenile mouse was presented to an adult mouse for 5 minutes (**Figure 11**). For the evaluation of short-term memory (STM) and long-term memory (LTM), the same experimental procedures, and the same juvenile mouse were reintroduced to the groups of interest 1.5 h and 24 h after the training session, respectively (**Figure 11**).

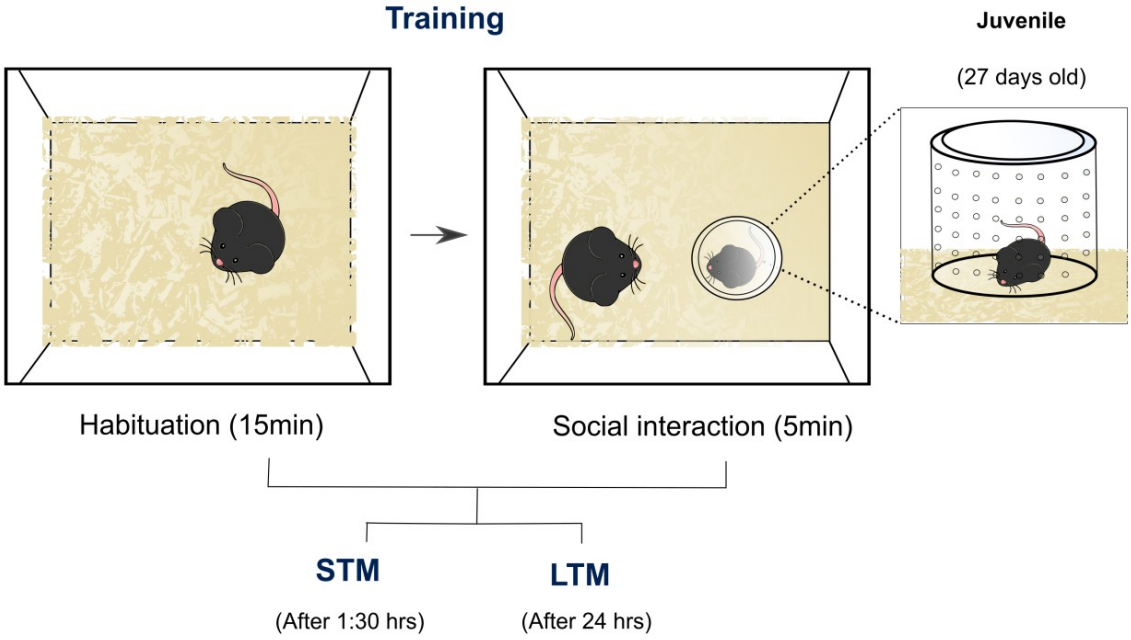


Figure 11: Steps of a single-chamber social memory test. For the training, animals were allocated individually in a cage per 15 minutes. After this period, one juvenile mouse was placed in the cage and computation of social interaction (5 minutes). After one hour and a half and 24 hours the same procedure was repeated to assess short and long-term social memory, respectively. Source: The author.

Importantly, in three and one chamber paradigms, none of the juvenile mice were used more than four times. Furthermore, in both protocols, the social investigation was computed as the time that the resident mice spent introducing nose or whiskers inside any of the cylinder's holes (ALMEIDA-SANTOS et al., 2019). Additionally, for the 3-chamber protocol, the number of transitions between chambers and the time exploring empty cylinder was also measured. The social interaction and social memory index were calculated according to the following formulas:

Social interaction (%): [Time $_{\text{with stranger 1}}$/ Time $_{\text{with stranger 1}}$+ Time $_{\text{with empty object}}$] x 100;
Social memory (%): [Time $_{\text{with stranger 2}}$/ Time $_{\text{with stranger 2}}$+ Time $_{\text{with stranger1}}$] x 100.

STM and LTM were expressed by the raw interaction time with juvenile mice, in comparison to training, as well by the percentage time of interaction with the juvenile mice considering the total test time.

4.4 – Oral glucose tolerance test

The oral glucose tolerance test (oGTT) was adapted from Pedro et al (2020) (PEDRO; TSAKMAKI; BEWICK, 2020). In the 12th week, a cohort of mice was submitted to fast for 6 hours (07 AM to 01 PM). Glucose (30%) was administered by gavage (2g/kg). Blood sampling was obtained by the tip of the tail, using a scissor, and glucose concentration (mg/dL) was measured with a glucometer (On call plus II®), before glucose gavage, and 15, 30, 60, 90, and 120 min thereafter. The area under the curve (AUC) was calculated to assess differences in glucose metabolism between the groups of interest (VIRTUE; VIDAL-PUIG, 2021).

4.5 – Adiposity and lee index calculation

Alterations in body composition caused by diet induction of obesity (DIO) and cardiometabolic risk were evaluated through adiposity measurements and Lee Indexes. The sum of the weight of visceral adipose tissue fat pads (VAT) (Epididymal (EAT), mesenteric (MAT), and retroperitoneal (RPAT) fat pad) was used for the calculation of visceral adipose tissue index: [VAT (g) /Body weight (g)] x 100. Lee index was calculated by the following formula: [body weight (g)$^{0.33}$/naso-anal length (cm)] (ROGERS; WEBB, 1980).

4.6 – Intracardiac perfusion and brain slices

In the 12th week, animals were anesthetized with ketamine (80 mg/Kg, i.p) and xylazine (8 mg/Kg, i.p), and then submitted to a thoracotomy for heart exposition. A needle coupled to a peristaltic pump infusion system was inserted in the left ventricle and a small incision was made in the right atrium, allowing the withdrawal of blood by infusion of phosphate saline buffer (pH 7.4; 5 mL/min; 30-100 mL per animal). After, animals were perfused with a paraformaldehyde solution (PFA 4%, pH 7.4, 20-30 mL per animal), decapitated, and their brains were stored overnight in PFA 4%. Subsequently, brains were cryoprotected, before being frozen in ice-cold isopentane (99%, 20s), by gradual dehydration in a 15% sucrose buffer (pH 7.4), and then in a 30% sucrose buffer (pH 7.4,) until complete saturation. Brains were sliced into 30µm sections in a cryostat at -25ºC (Leica Biosystems, Buffalo Grove, USA).

4.7 - Evaluation of astrocytic activation

Immunofluorescence analysis was executed to evaluate astrocytic activation. Hippocampal brain slices were incubated with citrate buffer (pH 6.0) at 70ºC for 60 minutes, washed with TBS (3x, 5min), and blocked overnight with 4% BSA in 0.5% TBS triton (TBSt), before incubation with primary antibody (mouse anti-GFAP (1:500; Millipore, Darmstadt, Germany) for 48 hours at 4ºC. Afterwards, slices were washed with TBS (3x, 5min) and incubated, at dark, with secondary antibody (120 minutes), (goat anti-mouse, 1:1000; Alexa Fluor 488, Life Technologies, Carlsbad, USA), Finally, after the washing (TBS, 3x, 5min), 1µg/mL of DAPI was added (30 min). Sections were washed (TBS, 3x, 5min), mounted in histological slices, and after drying, coverslipped with Fluoromount (Sigma-Aldrich, St. Louis, USA). Images were acquired with a fluorescence microscopy (AxioCam M2, Carl Zeiss, Jena, Germany) in 20X magnification. The quantification of cell bodies was performed using FIJI software (NIH, Bethesda MD, USA), and the results represented the percentage of area stained for GFAP and the mean fluorescence intensity of the cells.

4.8- Evaluation of microglial activation

Microglial morphological characteristics might provide information about cellular functional phenotypes (HOVENS; NYAKAS; SCHOEMAKER, 2014). Thus, in order to evaluate whether an obesogenic diet affects the microglial densitometry and morphological parameters, immunohistochemistry analyses were performed. Slices were incubated with citrate buffer (pH 6.0, 70 ºC, 1h), washed with TBS (3x, 5min), pretreated with 1% of H_2O_2 (15 minutes), washed (TBS, 3x, 5min) and blocked with BSA 4% in TBS triton 0.5% for 1 h at room temperature followed by primary antibody incubation (rabbit anti-Iba-1 (1:500; Wako Chemicals, Osaka, Japan) for 48 h at 4ºC). After washing (TBS, 3x, 5min), slices were incubated with biotinylated secondary antibody (goat anti-rabbit (1:500 in TBS; Vector Laboratories, Burlingame, USA; overnight at 4º C)). Posteriorly, slices were incubated with Avidin-Biotin solution (ABC) (VECTASTAIN ABC kit, Vector Laboratories, Burlingame, USA) for 1 h. For revealing the staining, DAB (Sigma-Aldrich, St. Louis, USA) diluted in TBS and activated with H_2O_2 0.04% was utilized in accordance with manufacturer instructions. Sections were washed (TBS, 3x, 5min) and mounted in histological slices. After drying, slices were sequentially dehydrated in ethanol 70, 80, 95 % (3 min), and 100% (twice, 4min), and cleaned in xylene (two times, 5 min) before coverslipped with DPX (Sigma-Aldrich, St. Louis, USA). Images were acquired in a light microscopy (APOTOME.2, Carl

Zeiss, Jena, Germany) in 20X and 40X magnification, for densitometry and morphological analysis, respectively. The quantification of optical density and measurement of activation index was performed using FIJI software (NIH, Bethesda MD, USA) in accordance with previously published methods (GOMES et al., 2020; HOVENS; NYAKAS; SCHOEMAKER, 2014).

4.11 – Fresh tissue isolation

For all other experiments, euthanasia was performed via decapitation. The brain was isolated and the hippocampus was dissected for molecular measurements. For obtaining the plasma, cervical blood samples were collected in heparinized tubes and centrifuged at 1,500 x g, 4ºC, for 15 min. In addition, all samples were stocked at −70ºC until use.

4.12– Total cholesterol levels

The total cholesterol levels were measured in serum by a commercial colorimetric enzymatic test (Bioclin, MG, Brazil) in accordance with manufacturer instructions. A spectrophotometer at 500 nm was utilized for the quantification of the results. The following formula was utilized for final concentration determination: Total Cholesterol (mg/dL): [Sample absorbance x 200]/standard absorbance.

4.13- Measurement of cortical and hippocampal glutamate release

4.13.1 – Purification of synaptosomes

The cortical and hippocampal pools of synaptosomes (3 animals per pool) were obtained in accordance with Dunkley et al (1986) (DUNKLEY et al., 1986). After decapitation, tissues were immediately homogenized in an ice-cold gradient solution (320 mM sucrose, 0.25 mM dithiothreitol, 1 mM EDTA), centrifuged (1000g, 10min, 4ºC) and the resultant supernatant was purified by separation in a discontinuous percoll gradient (Sigma Aldrich®) at concentrations of 23%,15%,10%, and 3% (pH 7.4). After centrifugation (6000g, 15 min, 4ºC), the isolated synaptosomes were re-suspended in a Krebs–Ringer–HEPES solution (KRH) without $CaCl_2$ (124mM NaCl, 4mM KCl, 1.2mM $MgSO_4$, 10mM glucose, 25mM HEPES, pH 7.4, 10mg/mL), and centrifuged (6000g, 15 min, 4ºC). The obtained Pellet was centrifuged (3.333g, 60s), re-suspended in KRH, and incubated at 37ºC for 30 min. This process was repeated once more and synaptosome aliquots were immediately led for reading and quantification.

4.13.2 – Measurements of glutamate release

Glutamate release was indirectly quantified by the fluorescent method (NICHOLLS; SIHRA; SANCHEZ-PRIETO, 1987). Fluorescence quantification was measured using a fluorimeter (Synergy TM2, Biotek®). Excitation and emission wavelengths were recorded at 360 nm and 450 nm, respectively. Isolated nerve terminals were incubated with $CaCl_2$ (1 mM), and $NADP^+$ (1 mM) in KRH medium (2 min). Glutamate dehydrogenase (Sigma Aldrich®; 50 units per well) was added to each well, and readings were performed until the fluorescence reached balance (5min). As a depolarizing stimulus, KCl 33mM was utilized (10 minutes). Finally, calibration curves were obtained with the last reading, when a known amount of glutamate (Sigma Aldrich®; 5nM/µL) was added to the medium (5 minutes). Glutamate levels were normalized to the total amount of synaptosomal protein that was obtained through Bradford assay.

4.15- Real-Time PCR

For evaluating whether the HSB diet might induce alterations in hippocampal genes associated with fractalkine/CX3CR1 axis, real time-PCR (RT-PCR) was performed for genes involved in glutamatergic neurotransmission (AMPA: GLUR2; NMDA: NR1, NR2A, NR2B; mGluR5: Grim5, GLT-1:SCLA2), BBB permeability (Claudin-5, Occludin, Zoocludin-1), and inflammation (IL-1β, IL-6, IL-10, TNF-α, INF-ɣ, TGF-β),. RNA was isolated with TRIZOL reagent® (Invitrogen, Burlington, EUA) in accordance with manufacturer instructions. The RNA concentration and quality weredetermined by spectrophotometer lecture (NanoDropTM (Thermo Scientific, Wilmington, USA)), and electrophoresis in agarose gel, respectively. cDNA was prepared from 400ng (2µl) of the isolated RNA through reverse transcriptase reaction. Quantitative PCR was performed with Power SYBR® Green PCR Master Mix kit in accordance with manufacturer instructions. StepOnePlus™ Real-Time PCR Systems and QuantStudio™ 6 & 7 Flex Real-Time PCR System were employed for execution of the qPCR, as well as data analysis. The nucleotide sequence of the target genes was designed with Primer3plus software and validated with NCBI Primer-BLAST. The sequence of the target genes can be consulted in table 2. Samples were prepared in triplicate and gene expression variations were determined by the ΔCt method using the RPL32, a lysosomal protein, as a constitutive normalizing gene. In the case of cytokines, the mean of two normalizing genes was used by the ΔCt calculation: GAPDH and HPRT.

Table 2- Primers sequences utilized for RT-PCR Source: The author.

Targets	Sequence
GLUR2 (AMPA2)-F	*ATGCGACCTGACCTCAAAGG*
GLUR2 (AMPA2)-R	*AGCAGAATCCAGCACAGCTT*
NR1(NMDA) - F	*ACTCCCAACGACCACTTCAC*
NR1(NMDA) – R	*GTAGACGCGCATCATCTCAA*
NR2A (NMDA) - F	*TACTCCAGCGCTGAACATTG*
NR2A (NMDA) – R	*CATGCGTGATGAGGCTCTTA*
NR2B (NMDA) – F	*GTGAGAGCTCCTTTGCCAAC*
NR2B (NMDA) - R	*ATGAAAGGGTTTTGCGTGAC*
mGluR5 - F	*TGTAACTCAAGACCTGAATCTATGG*
mGluR5- R	*GCTGGGCCAACTGAACTTTA*
Slc1a2 (GLT-1)-F	*ATTGGTGCAGCCAGTATTCC*
Slc1a2 (GLT-1)-R	*CCAGCTCAGACTTGGAAAGG*
Cx3CL1-F	*CGACAAGATGACCTCACGAA*
Cx3CL1-R	*CTGTGTCGTCTCCAGGACAA*
Cx3CR1-F	*TGCCTTCTTCCTCTTCTGGA*
Cx3CR1-R	*TAAAGGGGTTGAGGCAACAG*
Claudin-5-F	*GTTAAGGCACGGGTAGCACT*
Claudin-5-R	*GACAACGATGTTGGCGAACC*
ZO-1-F	*CAACAGGTACAGGCCAGAGG*

ZO-1-R	*ACTGCTTGGGCTCAGATGAC*
Occludin-F	*ATGTCCGGCCGATGCTCTC*
Occludin-R	*TTTGGCTGCTCTTGGGTCTGTAT*
IL-1β- F	*GGGCCTCAAAGGAAAGAATC*
IL-1β- R	*TACCAGTTGGGGAACTCTGC*
IL-6- F	*AACGCTACACACTGCATCTTGG*
IL-6- R	*GCCGTGGCAGTAACAGCC*
TNF-α- F	*TTCTGGCTCAAAAAGAGAATT*
TNF-α- R	*TGGTGGTCTTGTTGCTTAAGG*
INF-ɣ- F	*TCAAGTGGCGATGTGGAAGAA*
INF-ɣ- R	*TGGCTCTGCAGATTTTCATG*
Rpl32-R	*GCTGCCATCTGTTTTACGG*
Rpl32-R	*TGACTGGTGCCTGATGAACT*
GAPDH - F	*GGTGAAGGTCGGTGTGAACT*
GAPDH - R	*CTCGCTCCTGGAAGATGGTG*
HPRT- F	*GTTAAGCAGTACAGCCCCAAA*
HPRT- R	*AGGGCATATCCAACAACAAACTT*

4.16- Evaluation of neurotrophic factors, fractalkine, and cytokine levels

In order to measure hippocampal levels of neurotrophic factors and fractalkine (BDNF, NGF, GDNF, and CX3CL1), serum leptin, and inflammatory cytokines (IL-1β, IL-6, TNF-α, INF-ɣ), in serum and hippocampus, ELISA assay was performed. An extraction solution (100 mg of tissue per milliliter), containing Tris-HCl (20 mM); NaCl (137 mM); NP40 (1%); Glycerol (10%); phenyl methyl sulfonylfluoride (1 mM) or aprotinin A (0,5 µg/mL), Pesptatin A (1 µM), EDTA (10 mM), E-64 (10 µM), sodium vanadate (0,5 mM), and deionized water was added to hippocampus samples. Samples were mechanically processed, and centrifuged (14,000 rpm, 4ºC, 20 min). The hippocampal supernatant and serum samples were stocked at -80ºC until use. The levels of all interesting targets were measured using commercial kits from R&D system (DuoSet, Minneapolis, MN) in accordance with manufacturer instructions. Results were acquired on a spectrophotometer and expressed in pg/mL or pg/mg of protein.

4.17 – Statistical analysis

Statistical analyses were performed using the graph pad prism software 8 (San diego, USA). The normality and homoscedasticity of the data were tested using Shapiro-Wilk and Levene's test, respectively. Outliers were detected via the box-plot interpolation method. Data were expressed by mean ± SEM. Comparison between two experimental groups was assessed by a two-tailed t-student test or Mann-Whitney test. In addition, in ORT and OLT we also used a one-sample T-test for comparing the experimental control means with a hypothetical mean of 0.5 (different from random) (DENNINGER; SMITH; KIRBY, 2019). Furthermore, as appropriated, unpaired t-test, Mann-Whitney test, repeated measures ANOVA, two-way ANOVA, and mixed effect model, followed by Tukey post hoc test were employed. The level of statistical significance adopted was $p<0.05$.

5.0- RESULTS

5.1- HSB diet induces obesogenic phenotype and metabolic abnormalities

Our results showed a significant time x diet interaction effect, associated with a gradual increase of body weight in our DIO model (Two-way ANOVA, $F_{11, 456} = 15.42$, $p<0.0001$) (**Figure 1A-B**). Furthermore, HSB mice presented a higher adiposity index (unpaired T student-test, adiposity index $t_{18}=5.832$, $p<0.0001$), increased lee index (unpaired T student-test, lee index $t_{18}=2.330$, $p=0.0316$), and elevated cumulative energy intake (unpaired T student-test, cumulative energy intake $t_{10}=7.639$, $p<0.0001$) (**Figure 1C-F**), all pointing out to a possible alteration in body composition caused by the increase of palatable diet consumption. Regarding metabolic dysfunction, chronic exposure to the HSB diet induced higher fasting glucose levels (unpaired T student-test, fasting glucose $t_{18}=3.548$, $p=0.0023$) (**Figure 1G**), and increased oral glucose tolerance (unpaired T student-test, area under curve (AUC) $t_{18}=3.117$, $p=0.0052$) (**Figure 12G-I**). Additionally, elevated levels of total cholesterol were observed (Mann-Whitney test, total cholesterol, $p=0.0002$) (**Figure 1J**). Taken together, these data suggest that the HSB diet was effective in inducing obesity features, which is in accordance with previous literature (MAIOLI et al., 2016; MOREIRA JÚNIOR et al., 2021).

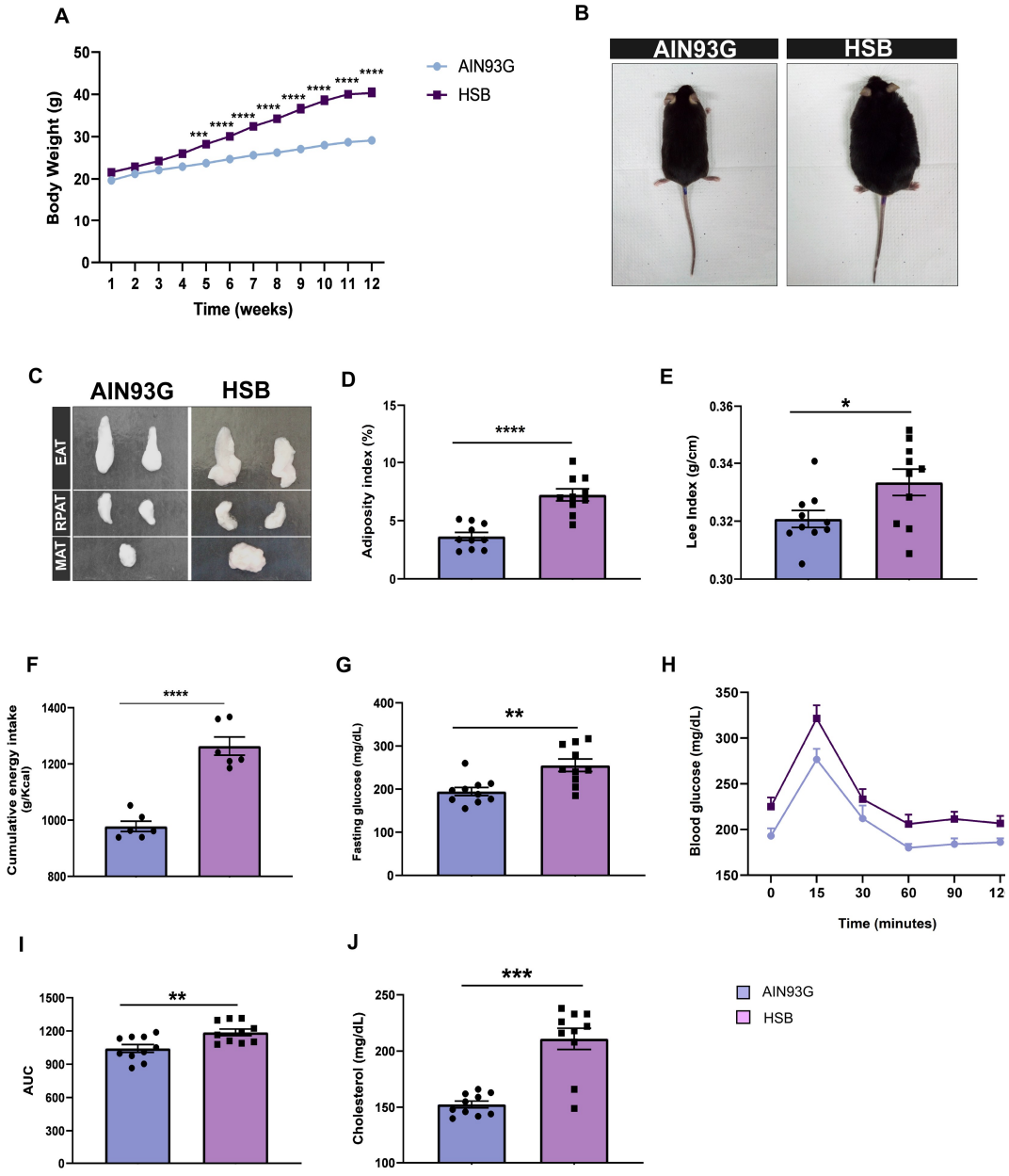


Figure 12. Chronic consumption of HSB diet induces an increase in body weight and metabolic disturbances in C57BL/6J mice. (**A**) Body weight measurements of AIN93G and HSB groups showed a significant time x diet interaction effect ($F_{(11, 456)} = 15{,}42$). (**B-C**) The representative phenotype of interest groups and isolated visceral adipose tissues (epididymal (**EAT**), mesenteric (**MAT**) and retroperitoneal (**RPAT**)) at 12th week of the protocol. (**D-E**) Measurements of the adiposity (%) and Lee index (g/cm). (**F**) Cumulative energy intake (g/Kcal) per cage. (**G**) Evaluation of the glucose levels (mg/dL) after 6 h of fasting. (**H-I**) Representative oGTT curve and evaluation of the Area under curve (AUC) of blood glucose. (**J**) Total cholesterol levels. Error bars represent the mean ± SEM; n = 40 (**A**), 10 (**D,E,G,H,I,J**), and 6 (**F**). Two-way ANOVA followed by Tukey's post-test (**A**), Unpaired two tail T-student test (**D**,**E**,**F**,**G,I**), Mann Whitney test (**J**). *p <0.05; **p <0.01; ***p <0.001; ****p <0.0001.

5.2 – HSB diet impairs the process of memory reconsolidation and extinction

To investigate possible memory impairment in our DIO model, we first assessed whether the HSB diet-induced changes in exploratory behavior, and short-term working memory (STMo). For this, the open field and Y maze test were performed, respectively. We observed that in both behavioral tests, none of the investigated parameters were affected by the obesogenic diet (Open field: unpaired T student-test, Total traveled distance t_{16}=0.4205, p=0.6797, mean speed t_{16}=0.4873, p=0.6327; Mann-Whitney test, time in center, p= 0.1359. Y maze: unpaired T student-test, alternation index t_{18}=0.06189, p=0.9513) (**Figure 13 and 14B**). Next, in order to evaluate the impact of obesity in events associated with hippocampus-dependent long-term memories, mice were subjected to the ORT, OLT, or contextual conditioned fear test (**Figure 14D-E and 15**). Notably, in ORT and OLT test, HSB diet reduced the total investigation time in the new and moved object, respectively, which may suggest a decline in episodic memory (Unpaired t-test, total investigation time, ORT t_{18}=2.731, p=0.0137; and OLT t_{16}=2.315, p=0.0342) (**Figure 14D-E**). Moreover, although memory evocation associated with the contextual aversive environment remained unchanged in the HSB group (Unpaired t-test, memory evocation test, t_{14}=1.612, p=0.1292) (**Figure 15**), we observed that the HSB group presented an abnormal extinction curve and an increased freezing percentage in the extinction test as compared to the lean group (Two-way ANOVA, extinction curve $F_{3, 56}$ = 30.06, p<0.0001; Unpaired t-test, extinction test t_{15}=4.417, p=0.005) (**Figure 15B-C**). Therefore, this dataset may suggest that HSB diet is able to impact specific memory events, such as reconsolidation and extinction. Finally, to determine whether the long-term memory impairment promoted by the HSB diet might impact social skills, we conducted some assays to verify social interaction and social memory. First, we evaluate general social interaction and social memory in the classical three-chamber social interaction paradigm (**Figure 16A-B**). Our trial data showed that HSB diet was not able to decrease social interaction and social memory index (Mann-Whitney test, social interaction Index, p=0.1481; Unpaired t-test, social memory Index t_{18}=0.3997, p=0.6941) (**Figure 16A-B**). Based on this, we decided to investigate whether short-term and long-term social memory (STM and LTM) could be affected by the obesogenic environment using these animals to perform a single-chamber social interaction test (**Figure 16 C-E**). In this context, as compared to their performance in the training session, we observed that the HSB group showed a decrease in the raw interaction time as compared to the familiar juvenile mice in the STM, and a tendency to decrease in the LTM assessment tests (One-way repeated measure, STM

p=0.0094; LTM p=0.0678) (**Figure 16 C**). This pattern was also corroborated by the comparison between the lean and obese group, where the STM and LTM indexes remained unchanged in the HSB group, suggesting that social skills and social memory were not affected by the obesogenic environment (Unpaired t-test, STM index t_{16}=0.08577, p=0.9327; LTM index t_{16}=1.378, p=0.1872) (**Figure 16D-E**).

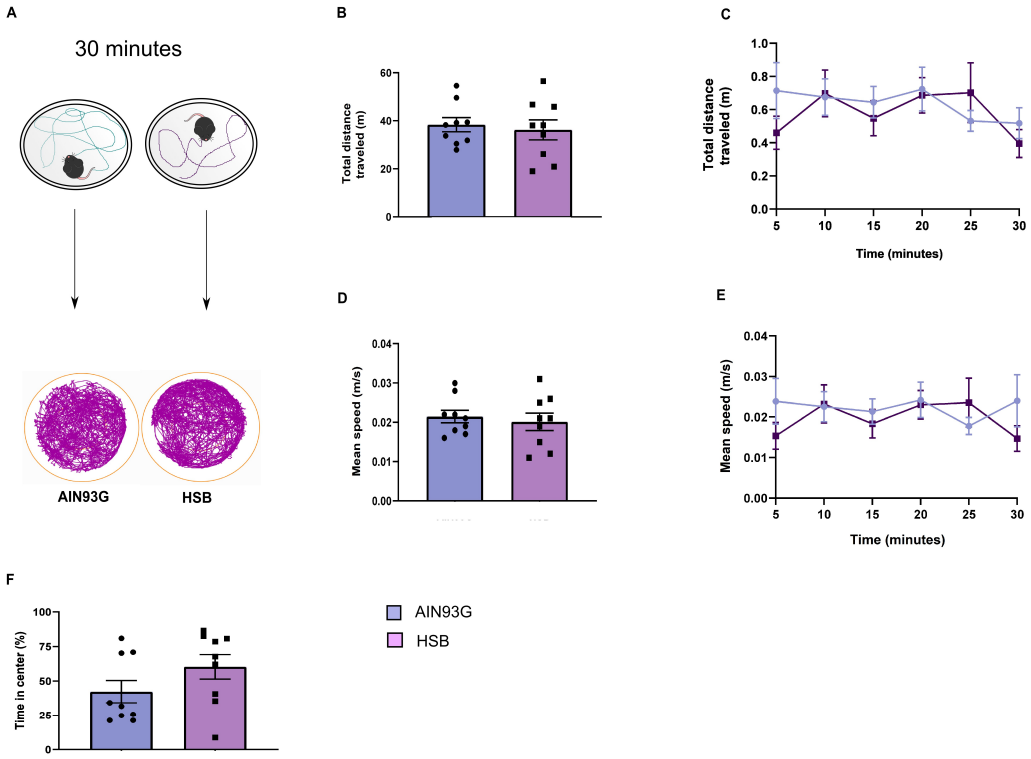


Figure 13. Chronic consumption of HSB does not alter locomotor and exploratory behaviors. (A-F) Evaluation of locomotor and exploratory behavior in Open field test: **(A)** Schematic representation of method used in the open field test and representative track plot of exploratory behavior in all behavioral test time (30 minutes). **(B-C)** Computation and representative curve of total traveled distance (m), respectively. **(D-E).** Computation, and representative curve of the mean of speed (m/s) of the interesting groups. **(F)** Evaluation of the percentage time at the center of open field apparatus. Error bars represent the mean ± SEM; n =9-10. Unpaired two tail T-student test (**B, D,** and **F**).

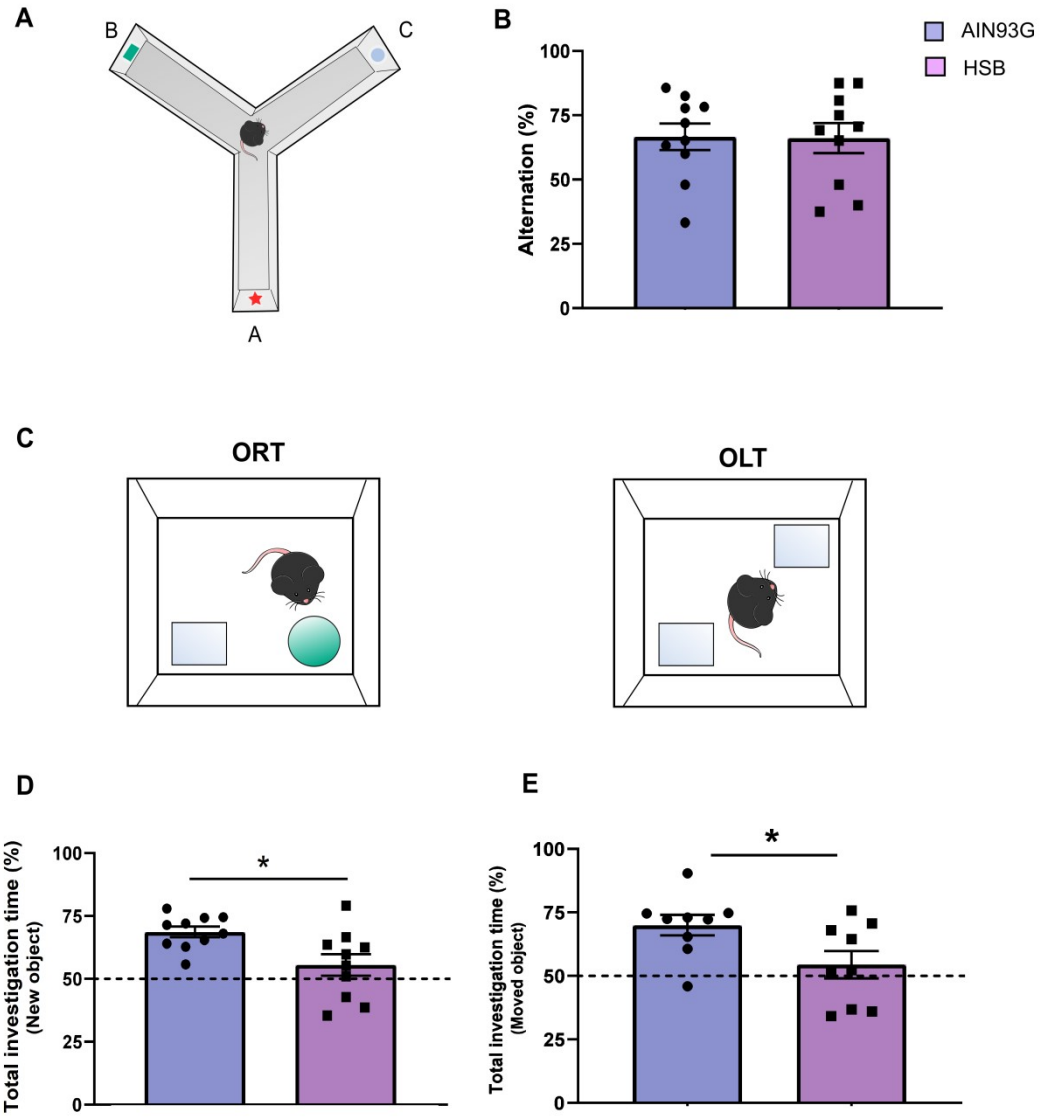


Figure 14. Chronic consumption of HSB does not alter operational short-term memory behavior, but induces alterations in memory reconsolidation. (A) Schematic representation of methodology used in the Y maze test. **(B)** Evaluation of operational short-term memory by the Y maze test. The figure represents the calculation of arms alternation index (%) in the apparatus. (**C**) Representations of the last day (test day) of the novel object recognition and novel object localization test (ORT and OLT). (**D-E**) Measurement of total investigation time index (%) for ORT and OLT tests, respectively. Error bars represent the mean ± SEM; n =10. Unpaired two tail T-student test (**B, D-E**). *p <0.05.

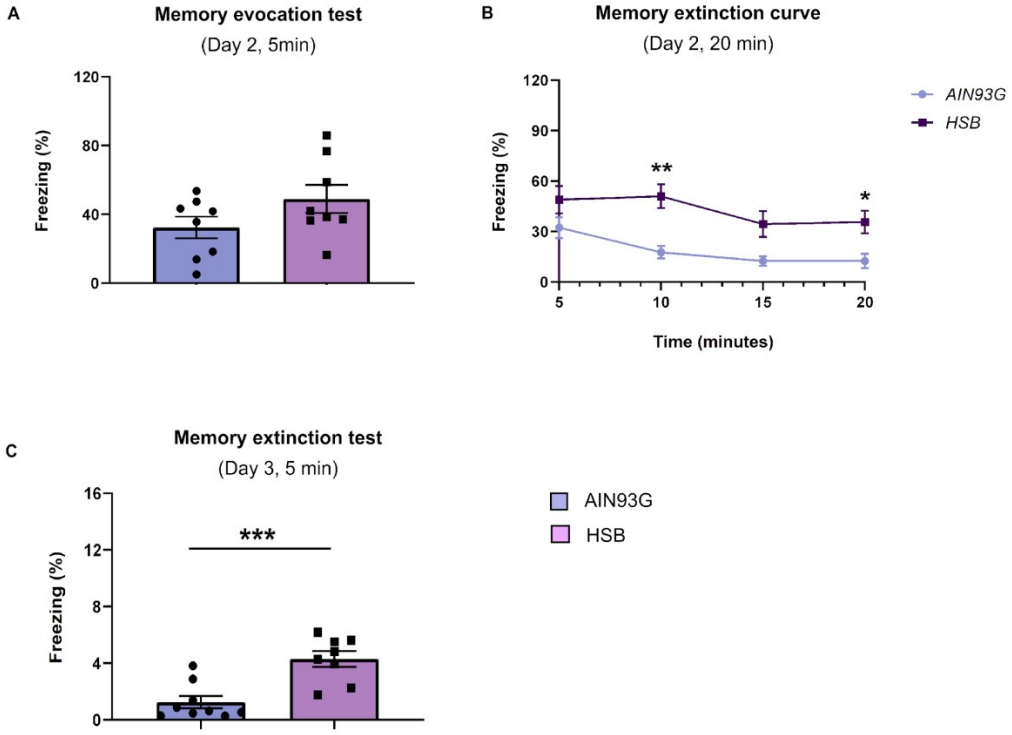


Figure 15. Chronic consumption of HSB impairs the extinction process in contextual conditioned fear evaluation. After 24hrs of conditioning**,** mice were re-submitted to context for 20 minutes, and the freezing percentage was quantified per minute. **(A)** Evaluation of memory evocation during the first 5 minutes of re-exposure to the context. **(B)** Representative curve of extinction during the entire re-exposure of the context memory evocation session. **(C)** Measurement of memory extinction, 24 hrs after the re-exposure session, during a 5 min duration session. Error bars represent the mean ± SEM; n =8-9. Unpaired two-tail T-student test (**A and C**), and Two-way ANOVA followed by Tukey's post-test (**B**). *p <0.05; **p <0.01; ***p <0.001.

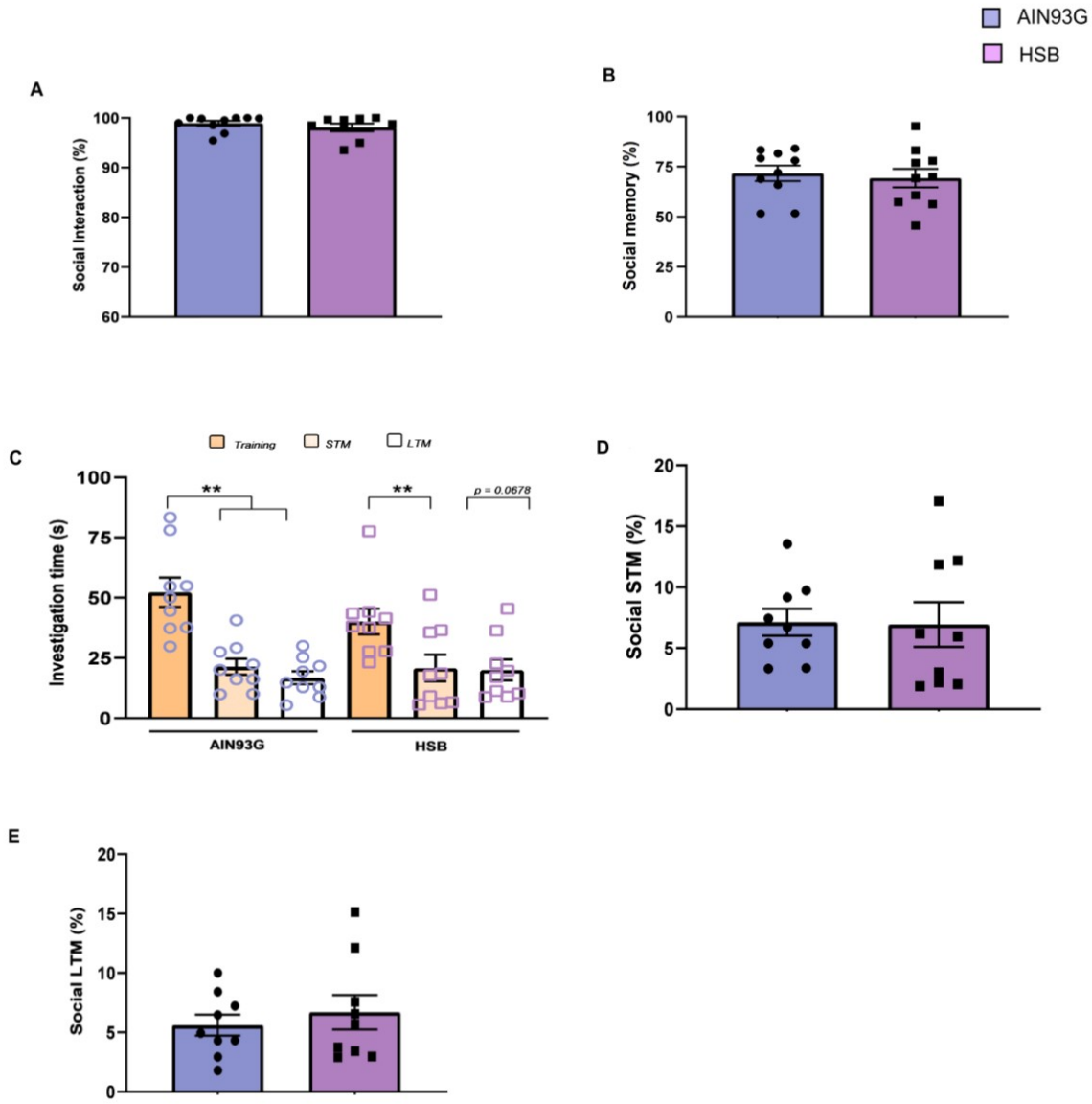


Figure 16. Chronic consumption of HSB does not alter social interaction and social memory. (A-B) Representation of general social interaction and social memory index, respectively, obtained from a three-chamber social paradigm. **(C-E)** In the one-chamber social memory test, short and long-term social memory (STM and LTM) were respectively evaluated after 1 hour and 30 minutes and 24 hours after the training session. **(C)** Computation of raw interaction time with juvenile mice in training and experimental sessions. **(D-E)** Calculation of STM and LTM index. Error bars represent the mean ± SEM; n =9-10. Mann-Whitney test (**A**), Unpaired two tail T-student test (**B, D,** and **E**), and One-way repeated measures-ANOVA, followed by Tukey post-test (**C**). **p <0.01.

5.3– Hippocampal neuroinflammation was not associated with chronic consumption of HSB diet

Considering the hypothesis that an increased peripheral inflammatory environment may impair BBB permeability, contributing to glial activation, and consequently to memory decline in obesity through a neuroinflammatory mechanism (MILLER; SPENCER, 2014) , we decided to investigate whether HSB diet could modulate serum leptin levels, also the pattern of peripheral and hippocampal pro-inflammatory cytokines, microglia and astrocyte activation, as well as the expression of proteins associated with BBB integrity. The results showed that in the periphery, HSB diet did not impairs the plasmatic levels of IL-1β, and TNF-α, but increased leptin levels, INF-ɣ, and also showed a tendency to elevate IL-6 concentrations (Unpaired t-test test, IL-1β t_{15}=0.9297, p=0.3672; TNF-α t_{10}=1.843, p=0.0951; leptin t_{9}=12.01, p<0.0001; INF-ɣ t_{18}=2.334, p=0.0314; and IL-6 t_{16}=2.066, p=0.0593) (**Figure 17**). However, this fact was not observed in the hippocampus, where the levels of cytokines evaluated, and the mRNA expression of inflammatory genes associated remained unchanged in the HSB group (RT-PCR: Unpaired t-test test, IL-1β t_{11}=0.007761, p=0.9939; IL-6 t_{12}=0.2917, p=0.7755; TNF-α t_{12}=0.8585, p=0.4074; Mann-Whitney test, INF-ɣ, p=0.2593; ELISA: IL-1β t_{8}=0.0004175, p=0.9997; IL-6 t_{8}=0.4157, p=0.6885; TNF-α t_{8}=1.587, p=0.1511; and INF-ɣ t_{8}=1.985, p=0.0824) (**Figure 18 B-H**). In accordance with these data, the comparison between Iba-1 optical density, microglia activation index, as well the GFAP fluorescence intensity and percentage of GFAP stained area did not reveal significant differences between the lean and obese mouse groups (Microglial optical density: Unpaired t-test test, DG t_{14}=0.1013, p=0.9192; CA1 t_{14}=0.1171, p=0.9084; CA2-CA3 t_{13}=0.6522, p=0.5257; Microglial body to cell size ratio: Unpaired t-test test, DG t_{14}=0.08705, p=0.9319; Mann-Whitney test, CA1, p=0.9329; CA2-CA3, p=0.3282; GFAP fluorescence intensity: Mann-Whitney test, DG, p=0.6991; Unpaired t-test test, CA1 t_{10}=0.3422, p=0.7393; CA2-CA3 t_{10}=0.7844, p=0.4510; GFAP stained area: Mann-Whitney test, DG, p=0.3095; Unpaired t-test test, CA1 t_{10}=0.3420, p=0.7394; and CA2-CA3 t_{10}=0.7843, p=0.4510) (**Figure 19 and 20**). Finally, HSB diet was not able to modulate the mRNA levels of Claudin-5, ZO-1, and Occludin levels, which may indicate maintenance of BBB integrity in the obese mice group (Unpaired t-test test, Claudin-5 t_{17}=0.7456, p=0.4661; ZO-1 t_{18}=0.9340, p=0.3627; and Occludin t_{17}=0.2230, p=0.8262) (**Figure 21 B-D**). Thus, this data set may suggest that the inflammatory features in our DIO model are present only in the periphery, but are not associated with the development of a neuroinflammatory process.

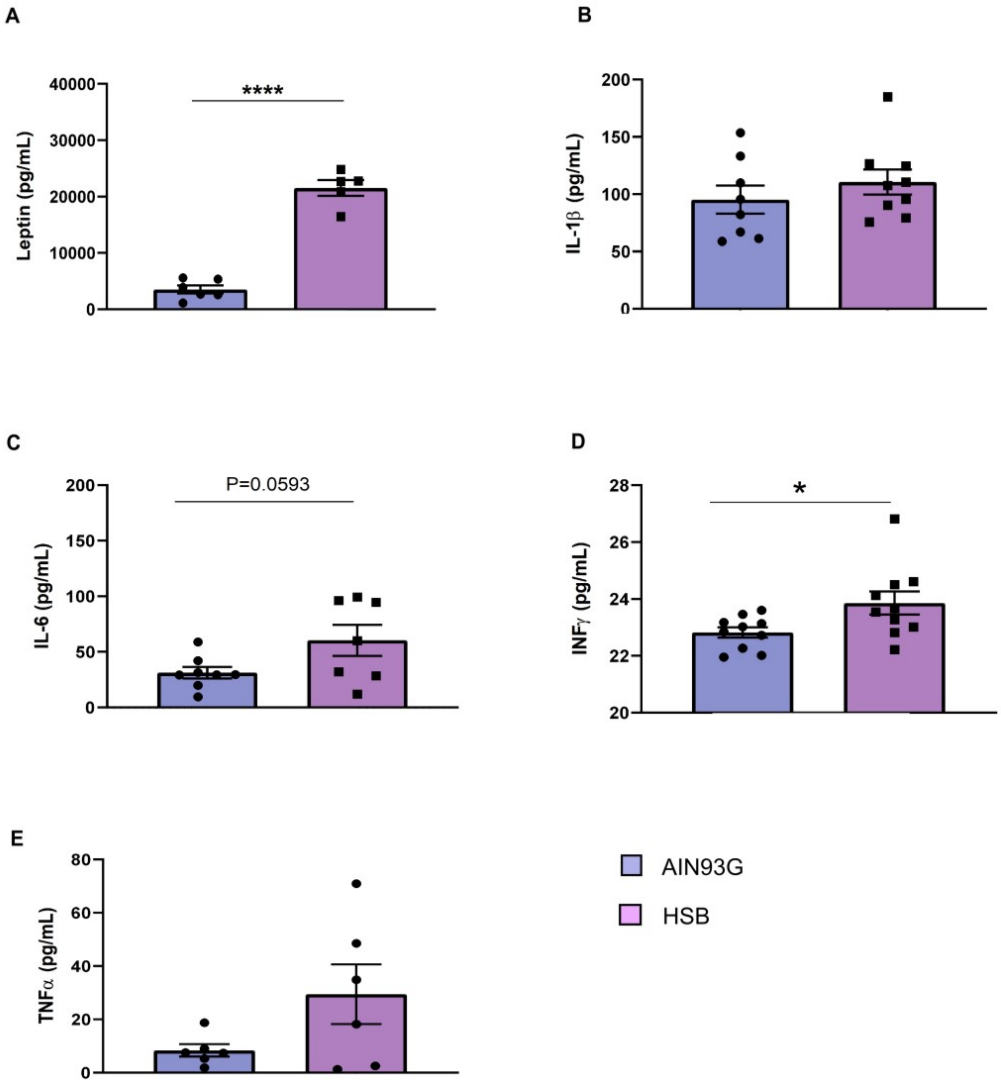


Figure 17. Chronic consumption of HSB induces a peripheral pro-inflammatory environment. (A-F) Measurements of the protein levels of serum leptin (**A**), IL-1β (**B**), IL-6 (**C**), INF-ɣ (**D**), and TNF-α (**E**). Error bars represent the mean ± SEM; n =5-10. Unpaired two tail Student t-test. *p <0.05, ****p <0.0001.

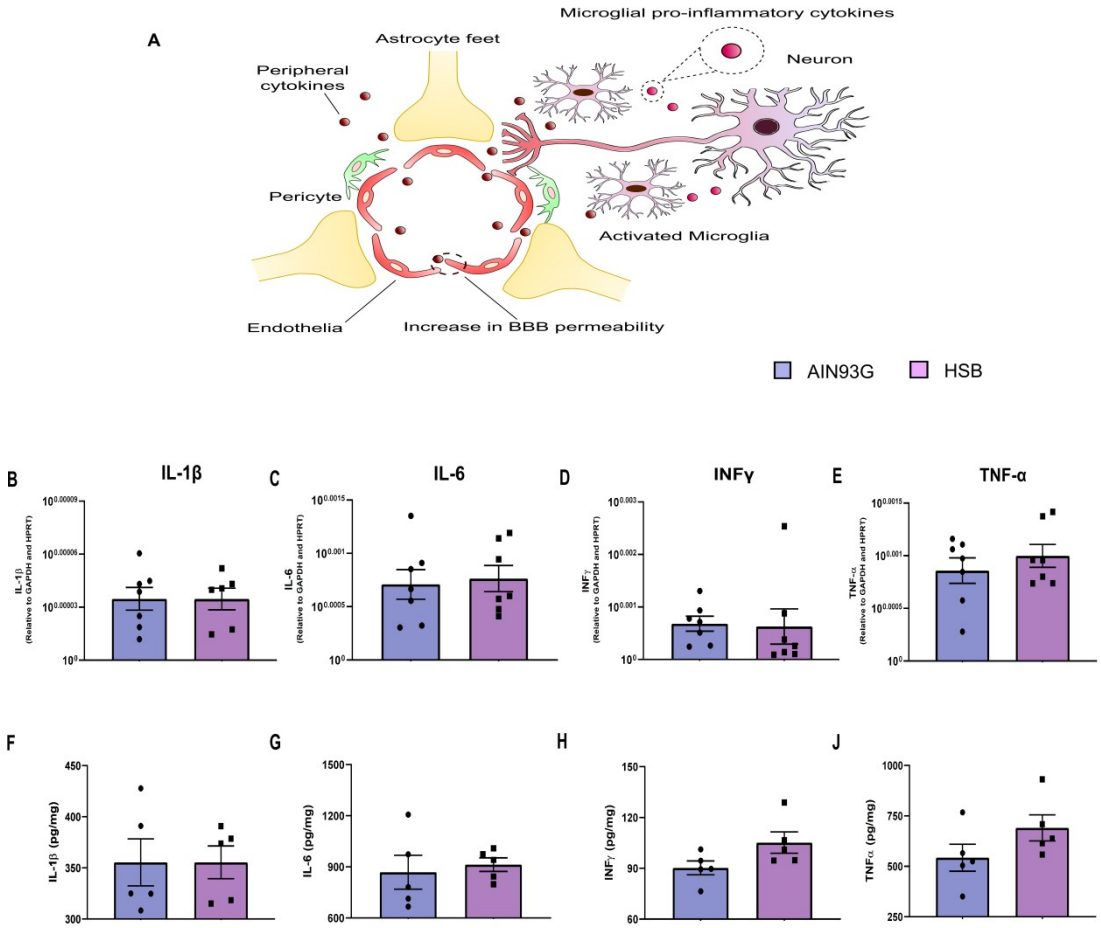


Figure 18. Chronic consumption of HSB is not able to modulate cytokine levels in the hippocampus. (A) Schematic representation of postulated mechanism associated with BBB breakdown, due to the presence of proinflammatory peripheral cytokines, and neuroinflammation establishment in an obesity condition. (**B-E**) Quantification of pro-inflammatory cytokine mRNA levels (IL-1β, IL-6, INF-ɣ, and TNF-α). (**F-J**) Measurements of protein levels of IL-1β, IL-6, INF-ɣ, and TNF-α. Error bars represent the mean ± SEM; n =5-10. Unpaired two tail T-student test (**B, D, E-J)** and Mann-Whitney test (**C**).

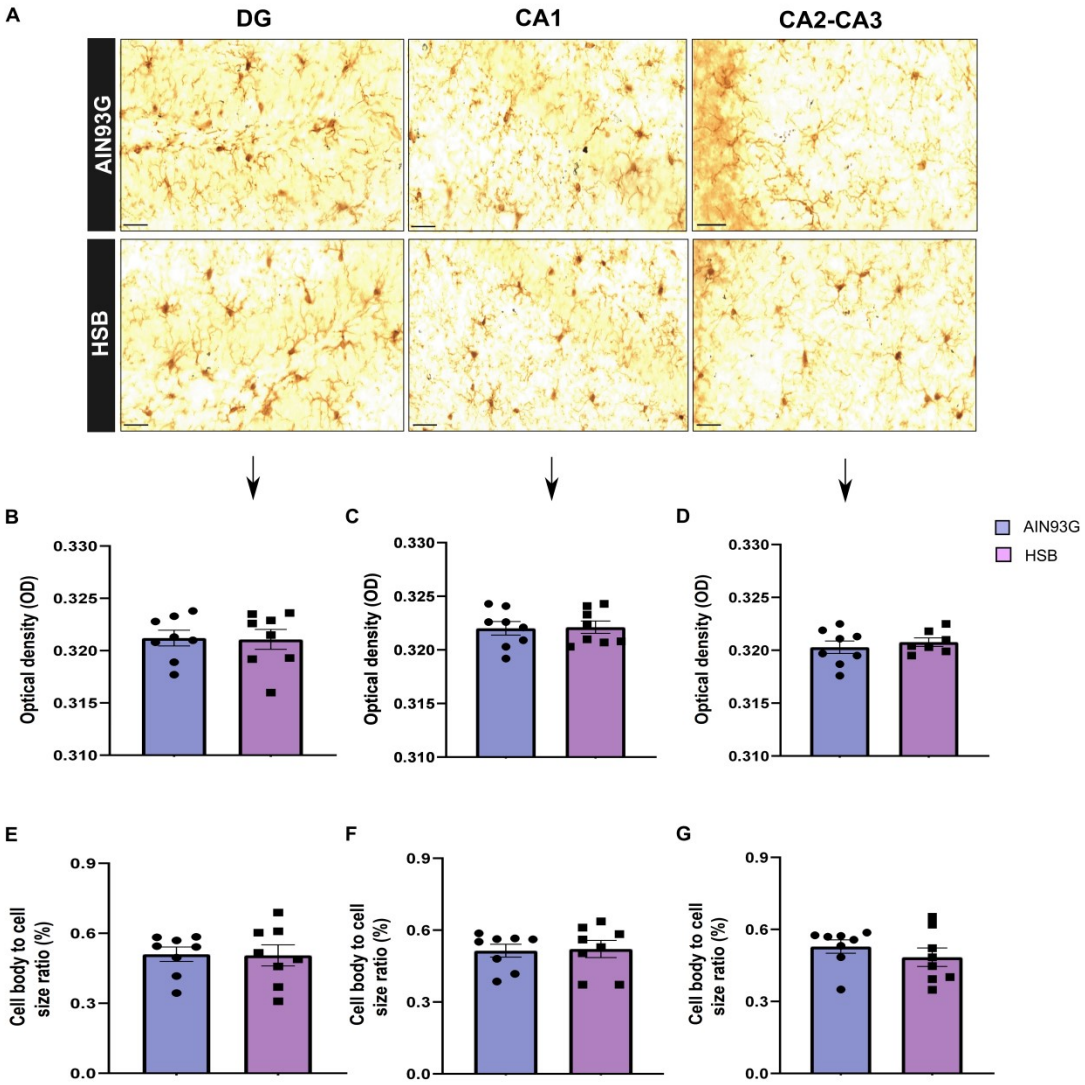


Figure 19. Chronic consumption of HSB did not alter densitometric and morphological parameters in hippocampal microglia. (A) Representative photomicrography of Iba-1 stained cells in dentate gyrus (DG), CA1, and CA2-3 of lean (AIN93G) and obese (HSB) groups. **(B-D)** Mensuration of microglial optical densitometry in DG (**B**), CA1 (**C**), and CA2-3 (**D**). **(E-G)** Cell body to cell size ratio (Activation index) of microglia in DG (**E**), CA1 (**F**), and CA2-3 (**G**).Microscope lens 40 × and 50 µm scale bars for the images. Error bars represent the mean ± SEM; n =8. Unpaired two tail Student t-test (**B-E**), and Mann-Whitney test (**F-G**).

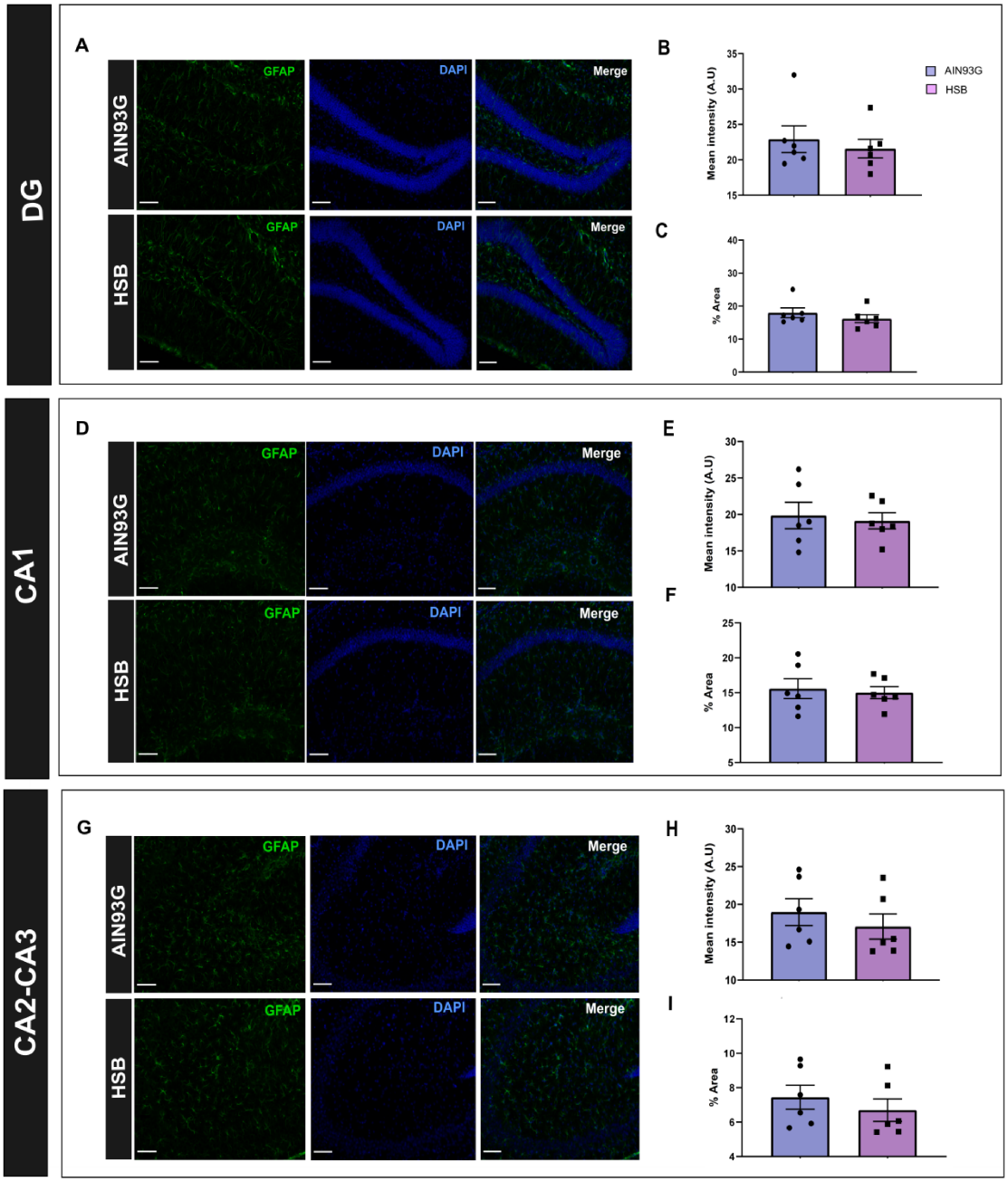


Figure 20. HSB does not alter hippocampal astrocyte activation. (A, D, G) Representative photomicrography of GFAP positive cells in the dentate gyrus (DG), CA1, and CA2-3. **(B, E, H)** Mean intensity fluorescence of GFAP stained for $GFAP^{+}$ cells in DG, CA1, and CA2-3. (**C, F, I)** Percentage area stained for $GFAP^{+}$ cells in DG, CA1, and CA2-3. Microscope lens 20 × and 80 µm scale bar for the image representation. Error bars represent the mean ± SEM; n =6. Mann-Whitney test (**B-C**), and Unpaired two tail Student t-test (**E,F,H,I**).

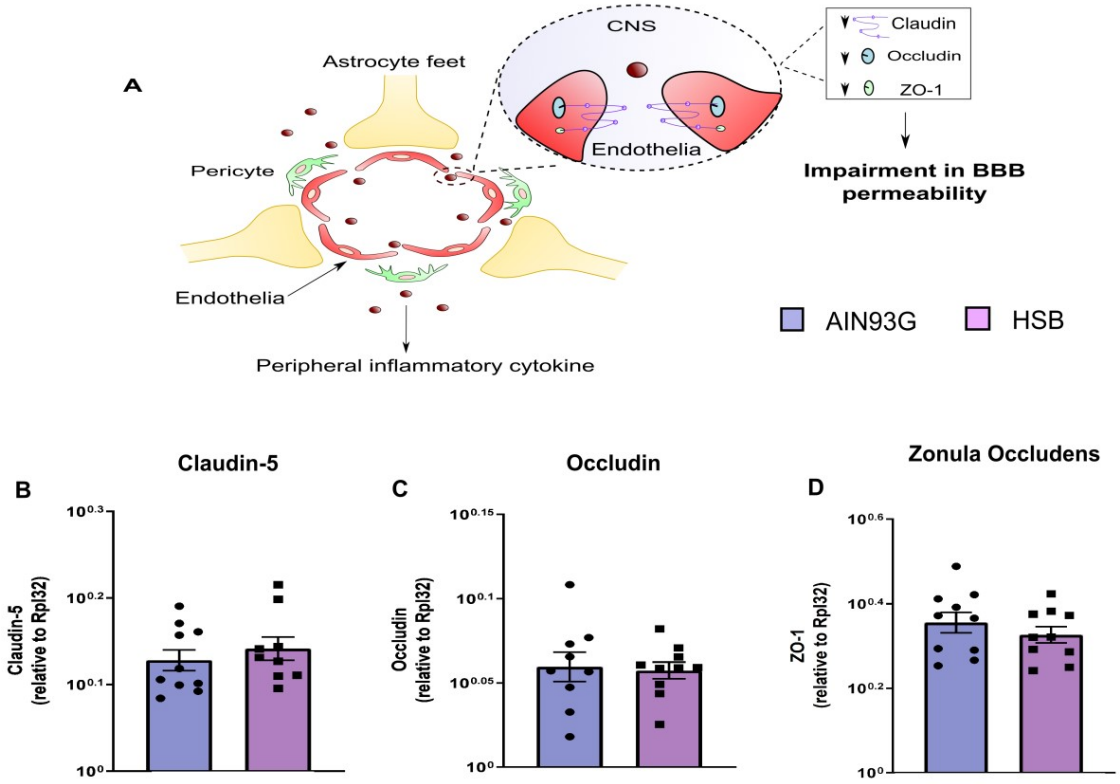


Figure 21. Chronic consumption of the HSB diet is not able to decrease the expression of blood-brain barrier (BBB) tight junctions proteins. (A) Schematic representation of BBB increases permeability associated with the decrease of tight junctions proteins due to a chronic peripheral inflammation environment. **(B-C)** Quantification of hippocampal claudin-5, and Zonula occludens-1 (ZO-1) mRNA levels. Error bars represent the mean ± SEM; n =5-10. Unpaired two tail Student t-test.

5.4 – Chronic consumption of HSB diet promotes an increase in cortical and hippocampal glutamate release

In order to verify the impact of the obesogenic environment on glutamatergic neurotransmission, we started our investigation by measuring hippocampal glutamate levels (**Figure 22**). Notably, HSB diet increases hippocampal glutamate levels (Unpaired t-test test, hippocampal glutamate t_{17}=4.003, p=0.0009) (**Figure 22B)**. Considering this, we decided to evaluate whether the increase of glutamate levels in the obese group might be associated with dysfunctional expression of AMPA and NMDA receptors by the quantification of functional subunits, respectively, GluR2, NR1, and NR2A/NR2B, as well the mRNA expression for mGluR5. Interestingly, our findings did not show remarkable differences between the diets (Unpaired t-test test, GluR2 t_{18}=0.9338, p=0.3627; NR1 t_{17}=0.3773, p=0.7106; NR2A t_{17}=0.8998, p=0.3808; mGluR5 t_{18}=1.102, p=0.2851; Mann-Whitney test, NR2B, p=0.6305) (**Figure 22C-G**). Finally, in order to evaluate whether excess of glutamate promoted by HSB diet consumption was able to impair glutamatergic clearance in the synaptic cleft, we also quantify the mRNA levels of astrocyte glutamate transporter (GLT-1). Interestingly, we did not find alterations in the comparison between groups (Unpaired t-test test, GLT-1 t_{17}=0.01760, p=0.9862) (**Figure 22H**).

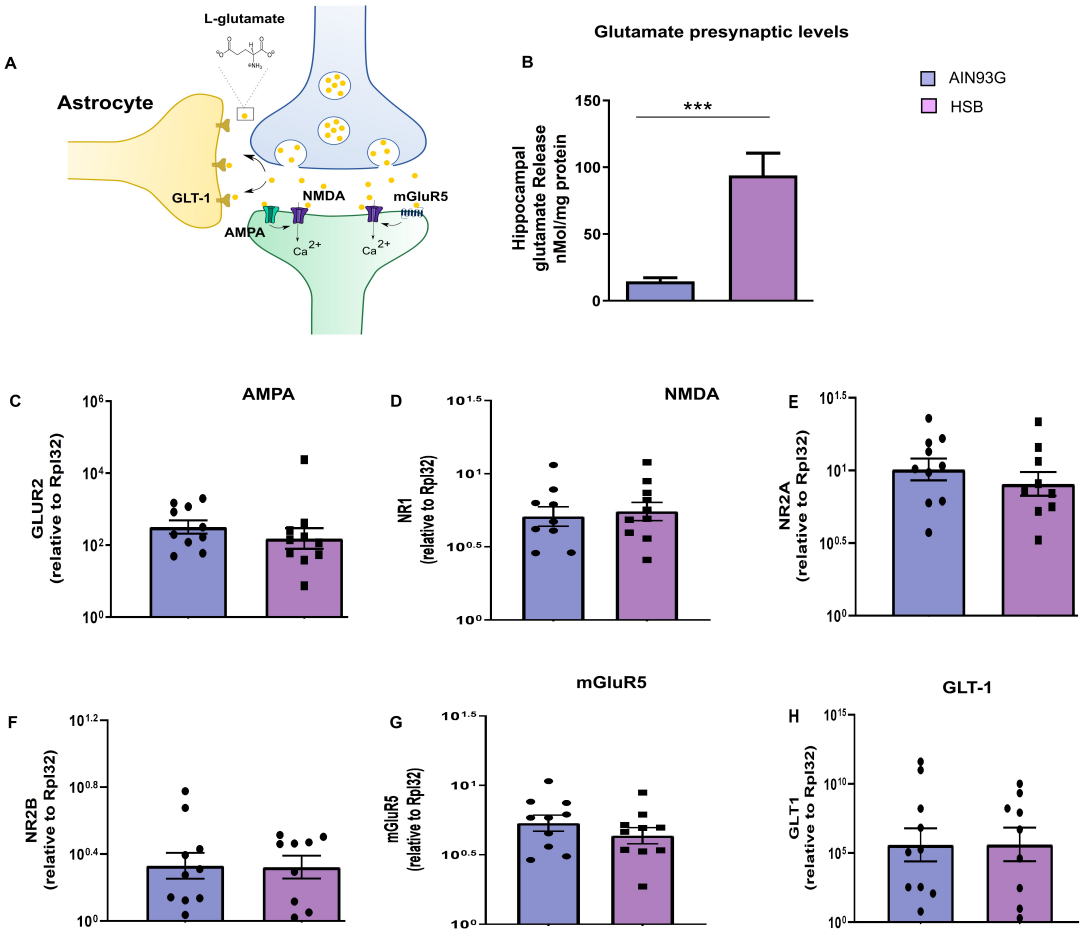


Figure 22. Chronic consumption of HSB diet increases hippocampal glutamate levels. (A) Schematic representation of tripartite glutamatergic neurotransmission. **(B)** Quantification of hippocampal synaptosomal glutamate levels. (**C**) mRNA levels for GLUR2 AMPA subunit. (**D-F**) mRNA levels for NR1, NR2A, and NR2B NMDA subunit. (**G**) mRNA levels for mGluR5. (**H**) Evaluation of mRNA levels from glial glutamate transporter (GLT-1). Error bars represent the mean ± SEM; n =4 (**B**), and 9-10 (**C-H**). Unpaired two tail T-student test (**B, C, D, E, G ,H)**, and Mann-Whitney test (**F**). ***p <0.001.

5.5 – HSB diet up-regulates fractalkine, but does not alter neurotrophic factors levels in the hippocampus

The Fractalkine/CX3CR1 axis is associated with microglia and neuron crosstalk by regulating synaptic plasticity and glutamatergic neurotransmission, which provides implications in various pathological conditions of memory (LUO et al., 2019). In addition, neurotrophic factors and glutamate levels interact with each other to regulate neuroplasticity, providing a key role in memory regulation (MATTSON, 2008). Thus, we decided to conduct a preliminary investigation, *a posteriori*, to elucidate whether the obesogenic environment might cause alterations in the Fractalkine/CX3CR1 axis and the levels of BDNF, NGF, and GDNF, the main neurotrophic factors associated with memory regulation (**Figura 23A**). Our results showed that mRNA levels of fractalkine and CX3CR1 were unaffected by HSB diet (Unpaired t-test test, Fractalkine t_{18}=0.6510, p=0.5233; Mann-Whitney test, CX3CR1 p=0.6842) (**Figure 23B-C**). However, we observed that hippocampal fractalkine protein levels were up-regulated in obese mice (Unpaired t-test, t_{16}=2.451, p=0.0399) (**Figure 23D**). In regard to neurotrophic factors, our preliminary data does not reveal alterations in any of neurotrophic factors evaluated (Unpaired t-test test, BDNF t_8=0.7803, p=0.4577; Mann-Whitney test, NGF, p=0.5476; and GDNF, p=04857) (**Figure 23E-G**).

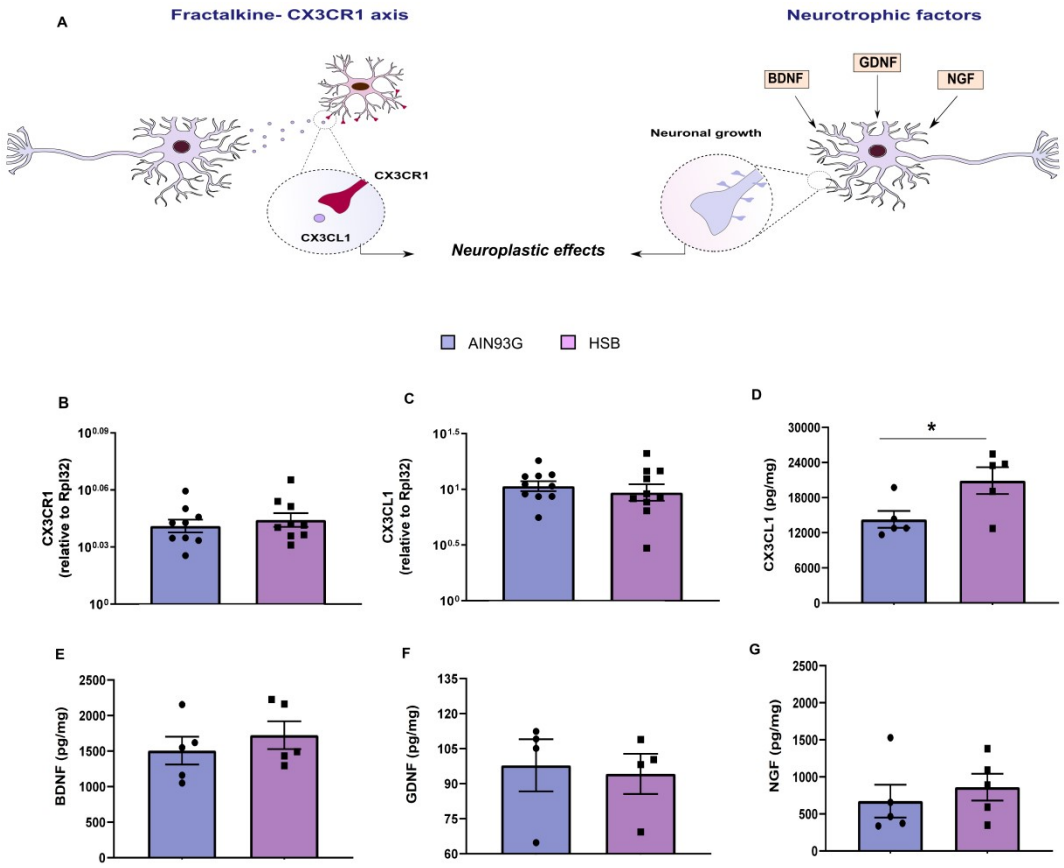


Figure 23. Chronic consumption of HSB increases hippocampal fractalkine levels. (A) Schematic representation of fractalkine neuron-glial communication and the control effect of neurotrophic factors in neuronal synaptic plasticity. **(B-C)** Quantification of mRNA levels of fractalkine receptor (CX3CR1), and fractalkine (CX3CL1). (**D-E**) Measurements of protein levels of CX3CL1, Brain Derived Neurotrophic Factor (BDNF), Nerve growth factor (NGF), and Glial derived neurotrophic factor (GDNF). Error bars represent the mean ± SEM; n =4-9. Unpaired two tail Student t-test (**B-E)**, and Mann-Whitney test (**F-G**). *p <0.05.

6.0- DISCUSSION

The main idea of this work was to understand how an obesogenic inflammatory environment, associated with prolonged consumption of a highly palatable and energy-dense diet, may impact central neurotransmission and its functionality. For this, we have demonstrated that chronic exposure to the HSB diet promotes hippocampus-dependent memories impairment and increases glutamate levels in the cortex and hippocampus of obese mice. Although we observed elevated levels of peripheral inflammatory markers typical of obesity, such as leptin, IL-6, and INF-ɣ, We did not observe a damage into BBB integrity, neither neuroinflammatory features associated with the exposure of obesogenic diet, which overturned our hypothesis that increased central glutamate levels could be associated with neuroinflammatory mechanisms triggered by obesogenic peripheral inflammation (**Figures 17-21**). In this milieu, highly palatable and energy-dense foods are strongly associated with glucose metabolism impairment and diabetes mellitus type 2 (TDM2) (MAIOLI et al., 2016; SICKMANN et al., 2010). An interesting study demonstrated that the co-association between obesity and TDM2 was able to reduce the molecular carbon labeling of glutamate in the hippocampus and cortex, which could be associated with decrease in glucose metabolism in the tricarboxylic acid (TCA) cycle, generating biochemical dysfunctions in glutamate production (SICKMANN et al., 2010). In accordance with that, exposure to a high-fat or high fat-sucrose diet may decrease glutamine synthase activity, glutamate/glutamine levels, and up-regulate GLT-1 expression and activity, which may suggest that these dietary components, by themselves, impact glutamate metabolism (MARTÍNEZ-OROZCO et al., 2021; VALLADOLID-ACEBES et al., 2012). Considering that glutamatergic recycling is a process that requires a large energy input, it is possible that the increase of GLT-1 expression and/or activity acts as a compensatory mechanism in order to improve the glutamatergic recycling efficiency and also vesicular glutamate levels in a metabolic failure scenario (ANDERSEN et al., 2021; HARRIS; JOLIVET; ATTWELL, 2012; MARTÍNEZ-OROZCO et al., 2021; VALLADOLID-ACEBES et al., 2012). However, it is important to note that it has been suggested that the GLT-1 clearance efficiency tends to decrease gradually after persistent metabolic dysfunctions or increased neuroinflammation, which may predispose to glutamatergic excitotoxicity and memory decline (ANDERSEN et al., 2021; TSAI et al., 2018). Therefore, considering that the similar DIO studies with a shorter diet exposure time, as well as the fact that the HSB diet induces a metabolic impairment without affecting GLT-1 expression (**Figures 12 and 22 H**), we hypothesized that the increase in cortical and

hippocampal glutamate levels was the result of an earlier GLT-1 compensatory mechanism due to the persistence of metabolic failure (**Figure 24**).

It has been reported that increased glutamate levels are associated with impairment in synaptic plasticity, including abnormal formation of LTP and long-term depression (LTD), in a mechanism involving over activation of ionotropic (iGluR) and metabotropic (mGluR) receptors, increased Ca^{+2} signaling, decreased production of neurotrophic factors, among others (BARNES et al., 2020; KATAGIRI; TANAKA; MANABE, 2001; MATTSON, 2008). Interestingly, in DIO models, LTP and LTD phenomena are decreased in CA1-CA3 region, which may be associated with acquisition and reconsolidation dysfunctions of aversive and episodic memory, suggesting that an obesogenic environment may increase the vulnerability to a poor synaptic plasticity regulation and cognitive impairment (HWANG et al., 2010; PORTER et al., 2010; SPINELLI et al., 2017). Although none of these studies evaluated whether decrease in synaptic plasticity and memory were associated with glutamatergic neurotransmission, it has been demonstrated that HFD promotes a downregulation of NMDA hippocampal NR2B subunit, which may lead to dysfunctions in synaptic plasticity dependent on LTD, likely contributing to plastic decline regulation in obesity environment (VALLADOLID-ACEBES et al., 2012). Here, we did not observe changes in the levels of expression of NMDA and AMPA subunits, as well as other classical receptors that also may contribute to the regulation of LTP and LTD, such as mGluR5. (**Figure 22 C-G**). However, we cannot rule out the possibility that the functional pattern of these receptors may be altered. In this context, it is well known that the saturation of iGluR activity, due to high glutamate levels, can disrupt the formation of new LTP and LTD patterns, contributing to the weakening of reinforcing traces of memories, new memory encoding, and learning (MARTIN; GRIMWOOD; MORRIS, 2000; MOSER; MOSER, 1999). Interestingly, our behavioral dataset suggests that the main deleterious effects of the HSB diet and obesogenic environment are concentrated on processes associated with the reinforcement of consolidated memories (reconsolidation) and new learning (extinction) (**Figures 14 D-E, and 15 B-C**). Thus, although we did not assess the hippocampal pattern of LTP/LTD, which is an important limitation of our work, we hypothesized that increased glutamate levels might induce the saturation of glutamatergic receptor activity, promoting an impairment of synaptic plasticity, as well as limiting the plastic capacity of the hippocampal network, which could impair signal transduction associated with refinement and extinction of an already acquired memory. Nevertheless, the absence of behavioral changes observed in the Y maze test, aversive

memory evocation, and social recognition suggests that possibly primary memory processes such as acquisition, consolidation, and retrieval could still be preserved (**Figures 14 B, 15A, 16**). Although speculative, we believe that two alternatives might support this phenomenon: I) Impairment of synaptic plasticity, promoted by the elevation of glutamate levels is not strong enough to saturate the signal transduction associated with acquisition and consolidation. However, increased glutamate levels can limit consolidated circuits and the full acquisition of new information relative to a familiar context. II) Compensatory systems may be attempting to partially protect hippocampal circuitry from the potentially deleterious effects of increased glutamate levels on plasticity (**Figure 24**).

Fractalkine, a chemokine abundantly produced by neurons, belonging to the CX3C chemokine family, acts in several synaptic plastic processes and also contributes to memory and learning in physiological conditions (LAURO et al., 2015b; ROGERS et al., 2011). Furthermore, *in vitro* experiments showed that increased hippocampal fractalkine levels are associated with neuroprotective effects against glutamatergic excitotoxicity in a mechanism that involves the reduction of postsynaptic AMPA-evoked currents, increased expression of synaptic NR2A/NMDA, activation of survivor pathways, and production of neurotrophic factors, such as BDNF (LAURO et al., 2015a; LIMATOLA et al., 2005; MIZUNO et al., 2003; RAGOZZINO et al., 2006). Importantly, we observed that the HSB diet can increase hippocampal fractalkine levels, but did not change the concentration of neurotrophic factors (**Figure 24**). Perhaps this fact is due to neuroprotective fractalkine effects in response to increased glutamate levels, which could be evidence in favor of the compensatory neuroprotective hypothesis previously raised. However, further experiments, such as electrophysiological data and molecular investigation of survival and cell growth pathways modulated by fractalkine may help to support the hypothesis. On the other hand, it is important to note that an increase in fractalkine levels may also indicate a possible detrimental progression of an obesogenic environment in the hippocampus. In this context, it was hypothesized that fractalkine may have a neuroprotective or neurotoxic effect depending on microglial activation state (LAURO et al., 2015b). Notably, although fractalkine mRNA expression remains unchanged following a glutamatergic neurotoxic stimulus, free fractalkine levels increase as a result of cleavage from neuronal membrane, potentially triggered by the recruitment of immune effectors, such as microglia, to initiate a neuroinflammatory response (CHAPMAN et al., 2000). Interestingly, the partial knockdown of CX3CR1 in a DIO mice model was able to protect against microglial activation and memory decline, which might

suggest that deregulation of fractalkine/CX3CR1 is closely associated with the maintenance of a neuroinflammatory environment and memory impairment in obesity (COPE et al., 2018). Considering that glutamate may act as a pro-inflammatory stimulus for several immune cells population, including microglia (BARGER et al., 2007; WARD et al., 2009), and that we did not observe microglial activation and increase in mRNA CX3CR1 expression (**Figures 19 and 23B**), it is possible that the combination of the high levels of glutamate and fractalkine may represent the first stage in the development of a future neuroinflammatory process. Therefore, we hypothesize that the increased levels of fractalkine may be an attempt to limit a possible hippocampal synaptic damage promoted by glutamate at the first moment. Nonetheless, with the maintenance of elevated glutamate levels, high fractalkine levels might induce microglial chemoattraction and microglia activation, through the action of CX3CR1, and glutamatergic overstimulation, which may establish a classic process of neuroinflammation associated with obesity (**Figure 24**).

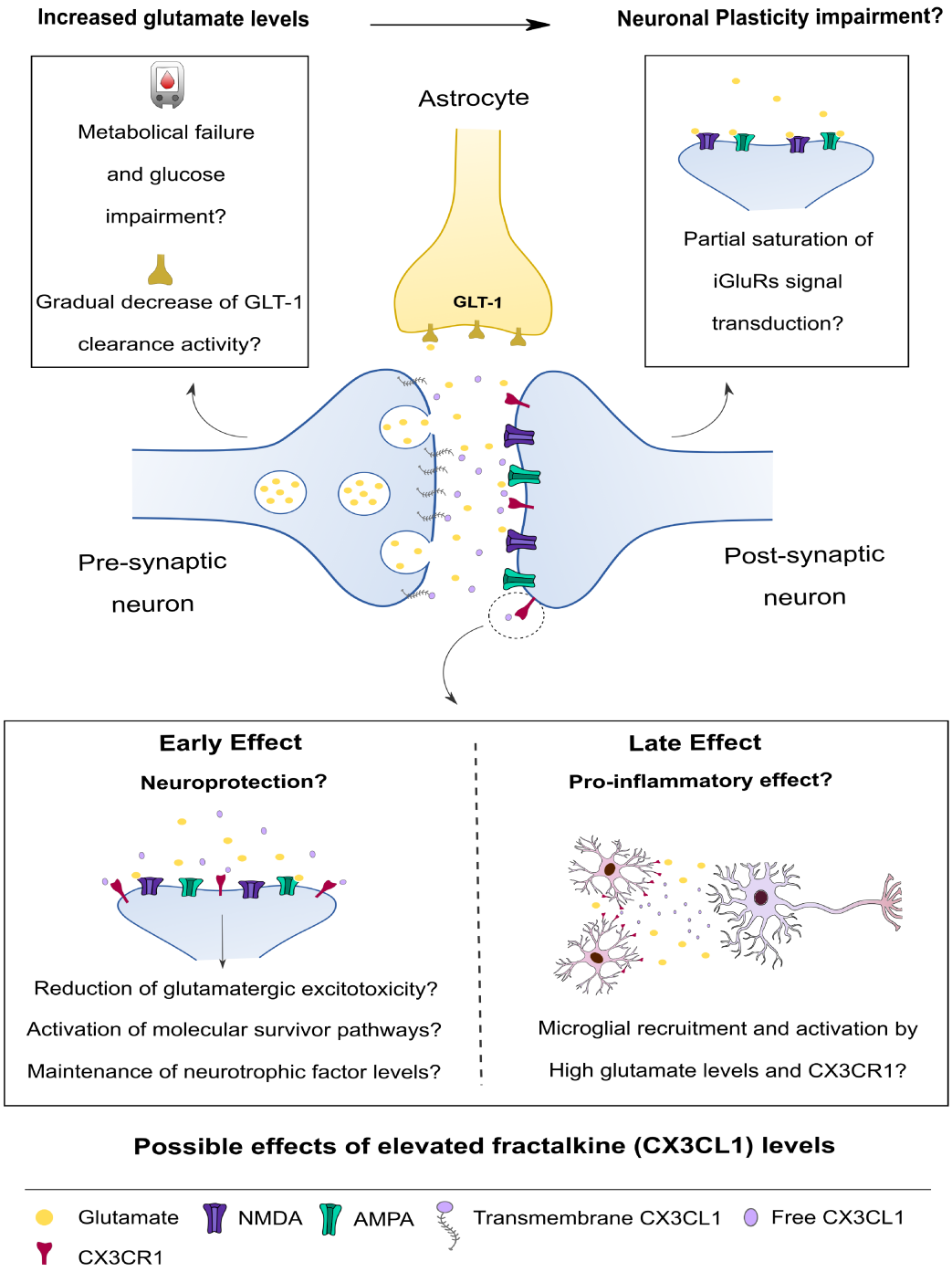


Figure 24. Possible mechanisms associated with hippocampal glutamatergic neurotransmission dysfunction triggered by diet-induced obesity. We hypothesized that an earlier GLT-1 compensatory mechanism, associated with glucose metabolic failure, increases cortical and hippocampal glutamatergic levels. In this context, we believe that the persistence of metabolic abnormalities might reduce GLT-1 clearance efficiency which might increase glutamate levels, and may induce the saturation of postsynaptic glutamatergic receptor activity, leading to an impairment of synaptic plasticity, and a dysfunction in the plastic capacity of the hippocampal network. In a short term, we hypothesized that these mechanisms may increase the levels of fractalkine, in order to limit a possible hippocampal synaptic damage promoted by glutamatergic toxicity.

However, it is also possible that the maintenance of glutamatergic overstimulation, and high fractalkine levels may induce microglial recruitment and activation, through the action of CX3CR1 which may establish a classic process of neuroinflammation associated with obesity.

7.0- CONCLUDING REMARKS

Our findings suggest that the co-association between a highly palatable, energy-dense diet in murine model of DIO may trigger impairments in the reinforcement and extinction of consolidated memories. In this context, we believe that these findings may be associated with the increase of hippocampal glutamate levels in a mechanism that possibly involves the impoverishment of synaptic plasticity. Furthermore, it is important to point out that, in contrary to our initial hypothesis, the memory impairment and increased glutamate levels appear to be independent of a neuroinflammatory process mediated by peripheral inflammation generated by the obesogenic environment. Although the absence of neuroinflammatory features, we hypothesized that the increased of hippocampal glutamate and fractalkine levels may represent the first stage in the development of a neuroinflammatory process, which may suggest that dysfunctional glutamate production might be a cause, but not a consequence of neuroinflammation. Although speculative, these data are interesting not only because of the aspect of abnormal synaptic plasticity but also by their implications involved in the establishment of hippocampus-dependent memory alterations and neuroinflammation.

8.0- REFERENCES

ABBOTT, N. J. et al. Structure and function of the blood-brain barrier. **Neurobiology of Disease**, v. 37, n. 1, p. 13–25, 2010.

ABEL, T. et al. Genetic demonstration of a role for PKA in the late phase of LTP and in hippocampus-based long-term memory. **Cell**, v. 88, n. 5, p. 615–626, 1997.

ABEL, T.; LATTAL, K. M. Molecular mechanisms of memory acquisition, consolidation and retrieval. **Current Opinion in Neurobiology**, v. 11, n. 2, p. 180–187, 2001.

ALEXAKI, V. I. The impact of obesity on microglial function: Immune, metabolic and endocrine perspectives. **Cells**, v. 10, n. 1584, p. 1–34, 2021a.

ALEXAKI, V. I. The impact of obesity on microglial function: Immune, metabolic and endocrine perspectives. **Cells**, v. 10, n. 7, 2021b.

ALMEIDA-SANTOS, A. F. et al. Social isolation impairs the persistence of social recognition memory by disturbing the glutamatergic tonus and the olfactory bulb-dorsal hippocampus coupling. **Scientific Reports**, v. 9, n. 1, p. 1–13, 2019.

ALVAREZ-CURTO, E.; MILLIGAN, G. Metabolism meets immunity: The role of free fatty acid receptors in the immune system. **Biochemical Pharmacology**, v. 114, p. 3–13, 2016.

AMANO, S. U. et al. Local proliferation of macrophages contributes to obesity-associated adipose tissue inflammation. **Cell Metabolism**, v. 19, n. 1, p. 162–171, 2014.

ANAGNOSTARAS, S. G. et al. Computer-assisted behavioral assessment of Pavlovian fear conditioning in mice. **Learning and Memory**, v. 7, n. 1, p. 58–72, 2000.

ANAND, B. K.; BROBECK, J. R. Localization of a “Feeding Center” in the Hypothalamus of the Rat. **Proceedings of the Society for Experimental Biology and Medicine**, v. 77, n. 2, p. 323–325, 1951.

ANDERSEN, J. V. et al. Glutamate metabolism and recycling at the excitatory synapse in health and neurodegeneration. **Neuropharmacology**, v. 196, p. 108719, 2021.

ANDRÉ, C. et al. Diet-induced obesity progressively alters cognition, anxiety-like behavior and lipopolysaccharide-induced depressive-like behavior: Focus on brain indoleamine 2,3-dioxygenase activation. **Brain, Behavior, and Immunity**, v. 41, n. 1, p. 10–21, 2014.

ARAQUE, A. et al. Tripartite synapses: Glia, the unacknowledged partner. **Trends in Neurosciences**, v. 22, n. 5, p. 208–215, 1999.

ASKARI, M. et al. Ultra-processed food and the risk of overweight and obesity: a systematic review and meta-analysis of observational studies. **International Journal of Obesity**, v. 44, n. 10, p. 2080–2091, 2020.

BAKER, P. et al. Ultra-processed foods and the nutrition transition: Global, regional and national trends, food systems transformations and political economy drivers. **Obesity Reviews**, v. 21, n. 12, p. 1–22, 2020.

BARGER, S. W. et al. Glutamate release from activated microglia requires the oxidative burst and lipid peroxidation. **Journal of Neurochemistry**, v. 101, n. 5, p. 1205–1213, 2007.

BARNES, J. R. et al. The relationship between glutamate dynamics and activity-dependent synaptic plasticity. **Journal of Neuroscience**, v. 40, n. 14, p. 2793–2807, 2020.

BOCARSLY, M. E. et al. Obesity diminishes synaptic markers, alters Microglial morphology, and impairs cognitive function. **PNAS**, v. 112, n. 51, p. 15731–15736, 2015.

BORST, S. E.; CONOVER, C. F. High-fat diet induces increased tissue expression of TNF-α. **Life Sciences**, v. 77, n. 17, p. 2156–2165, 2005.

BOUCHARD, C. Genetics of Obesity: What We Have Learned Over Decades of Research. **Obesity**, v. 29, n. 5, p. 802–820, 2021.

BROBECK, J. R.; TEPPERMAN, J.; LONG, C. N. Experimental Hypothalamic Hyperphagia in the Albino Rat. **The Yale journal of biology and medicine**, v. 15, n. 6, p. 831–53, 1943.

BUCKMAN, L. B. et al. Obesity induced by a high-fat diet is associated with increased immune cell entry into the central nervous system. **Brain, Behavior, and Immunity**, v. 35, p. 33–42, 2013.

BUTLER, M. J. The role of Western diets and obesity in peripheral immune cell recruitment and inflammation in the central nervous system. **Brain, Behavior, & Immunity - Health**, v. 16, n. May, p. 100298, 2021.

CANO, V. et al. Morphological changes in glial fibrillary acidic protein immunopositive astrocytes in the hippocampus of dietary-induced obese mice. **Neuroreport**, v. 25, n. 11, p. 819-822, 2014.

CAVALIERE, G. et al. High-Fat Diet Induces neuroinflammation and mitochondrial impairment in Mice cerebral cortex and synaptic fraction. **Frontiers in Cellular Neuroscience**, v. 13, n. November, p. 1–13, 2019.

CHAPMAN, G. A. et al. Fractalkine cleavage from neuronal membranes represents an acute event in the inflammatory response to excitotoxic brain damage. **The Journal of Neuroscience**, v. 20, p. 1–5, 2000.

CHEKE, L. G. et al. Obesity and insulin resistance are associated with reduced activity in core memory regions of the brain. **Neuropsychologia**, v. 96, p. 137–149, 2017.

CHEN, K. H. E. et al. Visceral adipose tissue imparts peripheral macrophage influx into the hypothalamus. **Journal of Neuroinflammation**, v. 18, n. 140, p. 1–19, 2021.

CHOE, S. S. et al. Adipose tissue remodeling: Its role in energy metabolism and metabolic disorders. **Fronties in Endocrinology**, v. 7, n. 30, p. 1–16, 2016.

CHOI, D. W. Glutamate neurotoxicity in cortical cell culture is calcium dependent. **Neuroscience Letters**, v. 58, n. 3, p. 293–297, 1985.

CHUNCHAI, T.; CHATTIPAKORN, N.; CHATTIPAKORN, S. C. The possible factors affecting microglial activation in cases of obesity with cognitive dysfunction. **Metabolic Brain Disease**, v. 33, n. 3, p. 615–635, 2018.

COPE, E. C. et al. Microglia play an active role in obesity-associated cognitive decline. **Journal of Neuroscience**, v. 38, n. 41, p. 8889–8904, 2018.

COLOMBO, E.; FARINA, C. Astrocytes: key regulators of neuroinflammation. Trends **in immunology**, v. 37, n. 9, p. 608-620, 2016.

CORDAIN, L. et al. Origins and evolution of the Western diet: Health implications for the 21st century. **American Journal of Clinical Nutrition**, v. 81, n. 2, p. 341–354, 2005.

COTTAM, D. R. et al. The chronic inflammatory hypothesis for the morbidity associated with morbid obesity: Implications and effect of weight loss. **Obesity Surgery**, v. 14, n. 5, p. 589–600, 2004.

COWLEY, M. A. et al. Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. **Nature**, v. 411, n. 6836, p. 480–484, 2001.

DA SILVA, M. C. M. et al. Inhibition of CSF1R, a receptor involved in microglia viability, alters behavioral and molecular changes induced by cocaine. **Scientific Reports**, v. 11, n. 1, p. 1–16, 2021.

DAVIDSON, T. et al. A potential role for the hippocampus in energy intake and body weight regulation. **Current Opinion in Pharmacology**, v. 7, n. 6, p. 613–616, 2007.

DE PAULA, G. C. et al. Hippocampal Function Is Impaired by a Short-Term High-Fat Diet in Mice: Increased Blood–Brain Barrier Permeability and Neuroinflammation as Triggering Events. **Frontiers in Neuroscience**, v. 15, n. November, p. 1–12, 2021.

DENNINGER, J. K.; SMITH, B. M.; KIRBY, E. D. Novel Object Recognition and Object Location Behavioral Testing in Mice on a Budget. **Journal of Visualized Experiments**, v. 20, n. 141, p. 1–20, 2019.

DISABATO, D.; QUAN, N.; GODBOUT, J. P. Neuroinflammation: The Devil Is in the Details. **Journal of neurochemistry**, v. 139, n. SUPPL2, p. 136–153, 2016.

DUNKLEY, P. R. et al. A rapid method for isolation of synaptosomes on Percoll gradients. **Brain Research**, v. 372, n. 1, p. 115–129, 1986.

EGGER, G.; DIXON, J. Beyond obesity and lifestyle: A review of 21st century chronic disease determinants. **BioMed Research International**, v. 2014, p. 1–12, 2014.

ELIAS, C. F. et al. Characterization of CART neurons in the rat and human hypothalamus. **Journal of Comparative Neurology**, v. 432, n. 1, p. 1–19, 2001.

ENDALIFER, M. L.; DIRESS, G. Epidemiology, Predisposing Factors, Biomarkers, and Prevention Mechanism of Obesity: A Systematic Review. **Journal of Obesity**, v. 2020, p. 1–8, 2020.

FANTUZZI, G. Adipose tissue, adipokines, and inflammation. **Journal of Allergy and Clinical Immunology**, v. 115, n. 5, p. 911–919, 2005.

FAZZINO, T. L.; ROHDE, K.; SULLIVAN, D. K. Hyper-Palatable Foods: Development of a Quantitative Definition and Application to the US Food System Database. **Obesity**, v. 27, n. 11, p. 1761–1768, 2019.

FEUERER, M. et al. Lean , but not obese , fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. **Nature Medicine**, v. 15, n. 8, p. 930–939, 2009.

FORTE, N. et al. Orexin-A and endocannabinoids are involved in obesity-associated alteration of hippocampal neurogenesis, plasticity, and episodic memory in mice. **Nature Communications**, v. 12, n. 1, p. 1–20, 2021.

FOX, A.; FENG, W.; ASAL, V. What is driving global obesity trends? Globalization or "modernization"? **Globalization and Health**, v. 15, n. 1, p. 1–16, 2019.

FRANSSEN, R. et al. Obesity and Dyslipidemia. **Medical Clinics of North America**, v. 95, n. 5, p. 893–902, 2011.

GIBBS, J.; YOUNG, R. C.; SMITH, G. P. Cholecystokinin decreases food intake in rats. **Obesity Research**, v. 5, n. 3, p. 284–290, 1973.

GOMES, J. A. S. et al. High-refined carbohydrate diet consumption induces neuroinflammation and anxiety-like behavior in mice. **Journal of Nutritional Biochemistry**, v. 77, p. 108317, 2020.

GORYAKIN, Y. et al. The impact of economic, political and social globalization on overweight and obesity in the 56 low and middle income countries. **Social Science and Medicine**, v. 133, p. 67–76, 2015.

GUILLEMOT-LEGRIS, O. et al. High-fat diet feeding differentially affects the development of inflammation in the central nervous system. **Journal of Neuroinflammation**, v. 13, n. 206, p. 1–11, 2016.

GUILLEMOT-LEGRIS, O.; MUCCIOLI, G. G. Obesity-Induced Neuroinflammation: Beyond the Hypothalamus. **Trends in Neurosciences**, v. 40, n. 4, p. 237–253, 2017.

HALEEM, D. J.; MAHMOOD, K. Brain serotonin in high-fat diet-induced weight gain, anxiety and spatial memory in rats. **Nutritional Neuroscience**, v. 24, n. 3, p. 226–235, 2021.

HANNAPEL, R. et al. Postmeal optogenetic inhibition of dorsal or ventral hippocampal pyramidal neurons increases future intake. **eNeuro**, v. 6, n. 1, 2019.

HAO, S. et al. Dietary obesity reversibly induces synaptic stripping by microglia and impairs hippocampal plasticity. **Brain, Behavior, and Immunity**, v. 51, p. 230–239, 2016.

HAROON, E.; MILLER, A. H.; SANACORA, G. Inflammation, Glutamate, and Glia: A Trio of Trouble in Mood Disorders. **Neuropsychopharmacology**, v. 42, n. 1, p. 193–215, 2017.

HARRIS, J. J.; JOLIVET, R.; ATTWELL, D. Synaptic Energy Use and Supply. **Neuron**, v. 75, n. 5, p. 762–777, 2012.

HASEL, P. et al. Neuroinflammatory astrocyte subtypes in the mouse brain. **Nature neuroscience**, v. 24, n. 10, p. 1475-1487, 2021.

HASEGAWA, Y. et al. Long-term effects of western diet consumption in male and female mice. **Scientific Reports**, 2020.

HAYASHI, R. et al. Oxytocin Ameliorates Impaired Behaviors of High Fat Diet-Induced Obese Mice. **Frontiers in Endocrinology**, v. 11, n. July, p. 1–13, 2020.

HINTZE, K. J. et al. Modeling the western diet for preclinical investigations. **Advances in Nutrition**, v. 9, n. 3, p. 263–271, 2018.

HOVENS, I.; NYAKAS, C.; SCHOEMAKER, R. A novel method for evaluating microglial activation using ionized calcium-binding adaptor protein-1 staining: cell body to cell size ratio. **Neuroimmunology and Neuroinflammation**, v. 1, n. 2, p. 82, 2014.

HWANG, L. L. et al. Sex differences in high-fat diet-induced obesity, metabolic alterations and learning, and synaptic plasticity deficits in mice. **Obesity**, v. 18, n. 3, p. 463–469, 2010.

JAVITT, D. C. Glutamate as a therapeutic target in psychiatric disorders. **Molecular Psychiatry**, v. 9, n. 11, p. 984–997, 2004.

KACZMARCZYK, M. M. et al. Methylphenidate prevents high-fat diet (HFD)-induced learning/memory impairment in juvenile mice. **Psychoneuroendocrinology**, v. 38, n. 9, p. 1553–1564, 2013.

KANOSKI, S.; GRILL, H. Hippocampus contributions to food intake control: mnemonic, neuroanatomical, and endocrine mechanisms. **Biological psychiatry**, v. 81, n. 9, p. 748–756, 2017.

KATAGIRI, H.; TANAKA, K.; MANABE, T. Requirement of appropriate glutamate concentrations in the synaptic cleft for hippocampal LTP induction. **European Journal of Neuroscience**, v. 14, n. 3, p. 547–553, 2001.

KAWAMURA, N. et al. Impaired brain fractalkine-CX3CR1 signaling is implicated in cognitive dysfunction in diet-induced obese mice. **BMJ Open Diabetes Research and Care**, v. 9, n. 1, p. 1–10, 2021.

KELLY, T. et al. Global burden of obesity in 2005 and projections to 2030. **International Journal of Obesity**, v. 32, n. 9, p. 1431–1437, 2008.

KENNEDY, G. The role of depot fat in the hypothalamic control of food intake in the rat. **Proceedings of the Royal Society B: Biological Sciences**, v. 140, n. 901, p. 578–592, 1953.

KENNY, P. J. Common cellular and molecular mechanisms in obesity and drug addiction. **Nature Reviews Neuroscience**, v. 12, n. 11, p. 638–651, 2011.

KLOK, M. D.; JAKOBSDOTTIR, S.; DRENT, M. L. The role of leptin and ghrelin in the regulation of food intake and body weight in humans: A review. **Obesity Reviews**, v. 8, n. 1, p. 21–34, 2007.

KOPP, W. How western diet and lifestyle drive the pandemic of obesity and civilization diseases. **Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy**, v. 12, p. 2221–2236, 2019.

KUMAR DATUSALIA, A.; SUNDER SHARMA, S. NF-κB Inhibition Resolves Cognitive Deficits in Experimental Type 2 Diabetes Mellitus through CREB and Glutamate/GABA Neurotransmitters Pathway. **Current Neurovascular Research**, 2016.

LABBAN, R. S. M. et al. High-fat diet-induced obesity and impairment of brain neurotransmitter pool. **Translational Neuroscience**, v. 11, n. 1, p. 147–160, 2020.

LAU, B. K. et al. Obesity-induced astrocyte dysfunction impairs heterosynaptic plasticity in the orbitofrontal cortex**. Cell reports**, v. 36, n. 7, p. 109563, 2021.

LAURO, C. et al. Fractalkine/CX3CL1 engages different neuroprotective responses upon

selective glutamate receptor overactivation. **Frontiers in Cellular Neuroscience**, v. 8, n. JAN, p. 1–8, 2015a.

LAURO, C. et al. Fractalkine in the nervous system: Neuroprotective or neurotoxic molecule? **Annals of the New York Academy of Sciences**, v. 1351, n. 1, p. 141–148, 2015b.

LAZARONI, T. L. DO N. et al. Angiotensin-(1-7)/Mas axis modulates fear memory and extinction in mice. **Neurobiology of Learning and Memory**, v. 127, p. 27–33, 2016.

LEE, E. B.; MATTSON, M. P. The Neuropathology of Obesity: Insights from Human Disease. **Acta neuropathologica**, v. 127, n. 1, p. 3–28, 2014.

LEHNERT, T. et al. Economic costs of overweight and obesity. **Best Practice and Research: Clinical Endocrinology and Metabolism**, v. 27, n. 2, p. 105–115, 2013.

LÉON, S.; NADJAR, A.; QUARTA, C. Microglia–neuron crosstalk in obesity: Melodious interaction or kiss of death? **International Journal of Molecular Sciences**, v. 22, n. 10, p. 1–16, 2021.

LIDDELOW, S. A. et al. Neurotoxic reactive astrocytes are induced by activated microglia. **Nature**, v. 541, n. 7638, p. 481- 487., 2017.

LIMATOLA, C. et al. Chemokine CX3CL1 protects rat hippocampal neurons against glutamate-mediated excitotoxicity. **Journal of Neuroimmunology**, v. 166, n. 1–2, p. 19–28, 2005.

LUO, P. et al. Fractalkine/CX3CR1 is involved in the cross-talk between neuron and glia in neurological diseases. **Brain Research Bulletin**, v. 146, n. November 2018, p. 12–21, 2019.

MAGISTRETTI, P. J. Neuron-glia metabolic coupling and plasticity. **Journal of Experimental Biology**, v. 209, n. 12, p. 2304–2311, 2006.

MAIOLI, T. U. et al. High sugar and butter (HSB) diet induces obesity and metabolic syndrome with decrease in regulatory T cells in adipose tissue of mice. **Inflammation Research**, v. 65, n. 2, p. 169–178, 2016.

MARIN, I. A.; KIPNIS, J. Central Nervous System: (Immunological) Ivory Tower or Not. **Neuropsychopharmacology**, v. 42, n. 1, p. 28–35, 2017.

MARTIN, S. J.; GRIMWOOD, P. D.; MORRIS, R. G. M. Synaptic plasticity and memory: An evaluation of the hypothesis. **Annual Review of Neuroscience**, v. 23, p. 649–711, 2000.

MARTÍNEZ-OROZCO, H. et al. High-fat and combined high-fat–high-fructose diets impair episodic-like memory and decrease glutamate and glutamine in the hippocampus of adult mice. **Nutritional Neuroscience**, p. 1–11, 2021.

MARTIRE, S. I. et al. Altered feeding patterns in rats exposed to a palatable cafeteria diet: Increased snacking and its implications for development of obesity. **PLoS ONE**, v. 8, n. 4, p. 1–10, 2013.

MATTSON, M. P. Glutamate and neurotrophic factors in neuronal plasticity and disease. **Annals of the New York Academy of Sciences**, v. 1144, p. 97–112, 2008.

MELO, H. M. et al. Palmitate Is Increased in the Cerebrospinal Fluid of Humans with Obesity

and Induces Memory Impairment in Mice via Pro-inflammatory TNF-α. **Cell Reports**, v. 30, n. 7, p. 2180- 2194.e8, 2020.

MILLER, A. A.; SPENCER, S. J. Obesity and neuroinflammation: A pathway to cognitive impairment. **Brain, Behavior, and Immunity**, v. 42, p. 10–21, 2014.

MILLER, S.; MAYFORD, M. Cellular and molecular mechanisms of memory: The LTP connection. **Current Opinion in Genetics and Development**, v. 9, n. 3, p. 333–337, 1999.

MIZUNO, T. et al. Production and neuroprotective functions of fractalkine in the central nervous system. **Brain Research**, v. 979, n. 1–2, p. 65–70, 2003.

MIZUNO, T. et al. Interferon-γ directly induces neurotoxicity through a neuron specific, calcium-permeable complex of IFN-γ receptor and AMPA GluRl receptor. **The FASEB Journal**, v. 22, n. 6, p. 1797–1806, 2008.

MOREIRA JÚNIOR, R. E. et al. Diet-induced obesity leads to alterations in behavior and gut microbiota composition in mice. **Journal of Nutritional Biochemistry**, v. 92, 2021.

MORTON, G. J. et al. Central nervous system control of food intake and body weight. **Nature**, v. 443, n. 7109, p. 289–295, 2006.

MORTON, G. J.; MEEK, T. H.; SCHWARTZ, M. W. Neurobiology of food intake in health and disease. **Nature Reviews Neuroscience**, v. 15, n. 6, p. 367–378, 2014.

MOSER, E. I.; MOSER, M. B. Is learning blocked by saturation of synaptic weights in the hippocampus? **Neuroscience and Biobehavioral Reviews**, v. 23, n. 5, p. 661–672, 1999.

NASCIMENTO, A. F. et al. A hypercaloric pellet-diet cycle induces obesity and co-morbidities in wistar rats. **Arquivos Brasileiros de Endocrinologia e Metabologia**, v. 52, n. 6, p. 968–974, 2008.

NEWTON, S.; BRAITHWAITE, D.; AKINYEMIJU, T. F. Socio-economic status over the life course and obesity: Systematic review and meta-analysis. **PLoS ONE**, v. 12, n. 5, p. 1–15, 2017.

NICHOLLS, D. G.; SIHRA, T. S.; SANCHEZ-PRIETO, J. Calcium-Dependent and-Independent Release of Glutamate from Synaptosomes Monitored by Continuous Fluorometry. **Journal of Neurochemistry**, v. 49, n. 1, p. 50–57, 1987.

OGATA, S. et al. Changes of Blood-Brain Barrier and Brain Parenchymal Protein Expression Levels of Mice under Different Insulin-Resistance Conditions Induced by High-Fat Diet. **Pharmaceutical Research**, v. 36, n. 141, p. 1–7, 2019.

PEDRO, P. F.; TSAKMAKI, A.; BEWICK, G. A. The glucose tolerance test in mice. In: **Animal Models of Diabetes.** Humana, New York, NY, 2020. p. 207-216.

PERMANA, P. A.; MENGE, C.; REAVEN, P. D. Macrophage-secreted factors induce adipocyte inflammation and insulin resistance. **Biochemical and Biophysical Research Communications**, v. 341, n. 2, p. 507–514, 2006.

PERSIDSKY, Y. et al. Blood-brain barrier: Structural components and function under

physiologic and pathologic conditions. **Journal of Neuroimmune Pharmacology**, v. 1, n. 3, p. 223–236, 2006.

PIAGGI, P. et al. Energy expenditure in the etiology of human obesity: spendthrift and thrifty metabolic phenotypes and energy-sensing mechanisms. **Journal of Endocrinological Investigation**, v. 41, n. 1, p. 83–89, 2018.

PINI, R. T. B. et al. Effects of cafeteria diet and high fat diet intake on anxiety, learning and memory in adult male rats. **Nutritional Neuroscience**, v. 20, n. 7, p. 396–408, 2017.

POPKIN, B. M.; GORDON-LARSEN, P. The nutrition transition: Worldwide obesity dynamics and their determinants. **International Journal of Obesity**, v. 28, p. S2–S9, 2004.

PORTER, D. W. et al. Four weeks administration of Liraglutide improves memory and learning as well as glycaemic control in mice with high fat dietary-induced obesity and insulin resistance. **Diabetes, Obesity and Metabolism**, v. 12, n. 10, p. 891–899, 2010.

PRICKETT, C.; BRENNAN, L.; STOLWYK, R. Examining the relationship between obesity and cognitive function: A systematic literature review. **Obesity Research and Clinical Practice**, v. 9, n. 2, p. 93–113, 2015.

PRINZ, P. The role of dietary sugars in health: molecular composition or just calories? **European Journal of Clinical Nutrition**, v. 73, p. 1216–1223, 2019.

RADYUSHKIN, K. et al. Neuroligin-3-deficient mice: Model of a monogenic heritable form of autism with an olfactory deficit. **Genes, Brain and Behavior**, v. 8, n. 4, p. 416–425, 2009.

RAGOZZINO, D. et al. Chemokine fractalkine/CX3CL1 negatively modulates active glutamatergic synapses in rat hippocampal neurons. **Journal of Neuroscience**, v. 26, n. 41, p. 10488–10498, 2006.

RANSOHOFF, R. M. et al. Neuroinflammation: Ways in Which the Immune System Affects the Brain. **Neurotherapeutics**, v. 12, n. 4, p. 896–909, 2015.

REICHELT, A. C. et al. Dietary-induced obesity disrupts trace fear conditioning and decreases hippocampal reelin expression. **Brain, Behavior, and Immunity**, v. 43, p. 68–75, 2015.

REID, J. K.; KUIPERS, H. She doesn't even go here: the role of inflammatory astrocytes in CNS disorders. **Frontiers in Cellular Neuroscience**, p. 332, 2021.

REVELO, X. S. et al. Morphological and inflammatory changes in visceral adipose tissue during obesity. **Endocrine Pathology**, v. 25, n. 1, p. 93–101, 2014.

REVETT, T. J. et al. Glutamate system, amyloid β peptides and tau protein: Functional interrelationships and relevance to Alzheimer disease pathology. **Journal of Psychiatry and Neuroscience**, v. 38, n. 1, p. 6–23, 2013.

RHEA, E. M. et al. Blood-Brain Barriers in Obesity. **AAPS Journal**, v. 19, n. 4, p. 921–930, 2017.

RIEDEL, G.; PLATT, B.; MICHEAU, J. Glutamate receptor function in learning and memory. **Behavioural Brain Research**, v. 140, n. 1–2, p. 1–47, 2003.

RIEDEL, G.; REYMANN, K. G. Metabotropic glutamate receptors in hippocampal long-term potentiation and learning and memory. **Acta Physiologica Scandinavica**, v. 157, n. 1, p. 1–19, 1996.

ROGERS, J. T. et al. CX3CR1 deficiency leads to impairment of hippocampal cognitive function and synaptic plasticity. **Journal of Neuroscience**, v. 31, n. 45, p. 16241–16250, 2011.

ROGERS, P.; WEBB, G. P. Estimation of body fat in normal and obese mice. **British Journal of Nutrition**, v. 43, n. 1, p. 83–86, 1980.

SAMARA, A. et al. Neuroinflammation and White Matter Alterations in Obesity Assessed by Diffusion Basis Spectrum Imaging. **Frontiers in Human Neuroscience**, v. 13, n. January, p. 1–15, 2020.

SANACORA, G. et al. Clinical studies implementing glutamate neurotransmission in mood disorders. **Annals of the New York Academy of Sciences**, v. 1003, p. 292–308, 2003.

SANDOVAL-SALAZAR, C. et al. A high-fat diet decreases GABA concentration in the frontal cortex and hippocampus of rats. **Biological Research**, v. 49, n. 15, p. 1–6, 2016.

SANTOS, R. P. M. et al. Metabotropic glutamate receptor 5 knockout rescues obesity phenotype in a mouse model of Huntington ' s disease. **Scientific Reports**, v. 12, n. 5621, p. 1–13, 2022.

SICKMANN, H. M. et al. Obesity and type 2 diabetes in rats are associated with altered brain glycogen and amino-acid homeostasis. **Journal of Cerebral Blood Flow and Metabolism**, v. 30, n. 8, p. 1527–1537, 2010.

SOBESKY, J. L. et al. High-fat diet consumption disrupts memory and primes elevations in hippocampal IL-1β, an effect that can be prevented with dietary reversal or IL-1 receptor antagonism. **Brain, Behavior, and Immunity**, v. 42, p. 22–32, 2014.

SOUZA, G. F. P. et al. Defective regulation of POMC precedes hypothalamic inflammation in diet-induced obesity. **Scientific Reports**, v. 6, n. 29290, p. 1–9, 2016.

SPEAKMAN, J. R. Use of high-fat diets to study rodent obesity as a model of human obesity. **International Journal of Obesity**, v. 43, n. 8, p. 1491–1492, 2019.

SPENCER, S. J. et al. High-fat diet and aging interact to produce neuroinflammation and impair hippocampal- and amygdalar-dependent memory. **Neurobiology of Aging**, v. 58, p. 88–101, 2017.

SPINELLI, M. et al. Brain insulin resistance impairs hippocampal synaptic plasticity and memory by increasing GluA1 palmitoylation through FoxO3a. **Nature communications**, v. 8, n. 1, p. 1-14, 2017.

SPYRIDAKI, E. C.; AVGOUSTINAKI, P. D.; MARGIORIS, A. N. Obesity, inflammation and cognition. **Current Opinion in Behavioral Sciences**, v. 9, n. Figure 1, p. 169–175, 2016.

STELLAR, E. The physiology of motivation. **Psychological Review**, v. 61, n. 1, p. 5–22, 1954.

STELLWAGEN, D. et al. Differential regulation of AMPA receptor and GABA receptor

trafficking by tumor necrosis factor-α. **Journal of Neuroscience**, v. 25, n. 12, p. 3219–3228, 2005.

STRANAHAN, A. M. et al. Blood-brain barrier breakdown promotes macrophage infiltration and cognitive impairment in leptin receptor-deficient mice. **Journal of Cerebral Blood Flow and Metabolism**, v. 36, n. 12, p. 2108–2121, 2016.

SUÁREZ, J. et al. Adipocyte cannabinoid CB1 receptor deficiency alleviates high fat diet-induced memory deficit, depressive-like behavior, neuroinflammation and impairment in adult neurogenesis. **Psychoneuroendocrinology**, v. 110, n. April, p. 104418, 2019.

TAKASE, K. et al. High-fat diet feeding alters olfactory-, social-, and reward-related behaviors of mice independent of obesity. **Obesity**, v. 24, n. 4, p. 886–894, 2016.

THOR, D. H.; HOLLOWAY, W. R. Social memory of the male laboratory rat. **Journal of Comparative and Physiological Psychology**, v. 96, n. 6, p. 1000–1006, 1982.

TROGDON, J. G. et al. Indirect costs of obesity: A review of the current literature. **Obesity Reviews**, v. 9, n. 5, p. 489–500, 2008.

TRUDEAU, F.; GAGNON, S.; MASSICOTTE, G. Hippocampal synaptic plasticity and glutamate receptor regulation: Influences of diabetes mellitus. **European Journal of Pharmacology**, v. 490, n. 1–3, p. 177–186, 2004.

TSAI, S. F. et al. High-fat diet suppresses the astrocytic process arborization and downregulates the glial glutamate transporters in the hippocampus of mice. **Brain Research**, v. 1700, p. 66–77, 2018.

VALASSI, E.; SCACCHI, M.; CAVAGNINI, F. Neuroendocrine control of food intake. **Nutrition, Metabolism and Cardiovascular Diseases**, v. 18, n. 2, p. 158–168, 2008.

VALDEARCOS, M. et al. Microglia dictate the impact of saturated fat consumption on hypothalamic inflammation and neuronal function. **Cell Reports**, v. 9, n. 6, p. 2124–2138, 2014.

VALDEARCOS, M. et al. Microglial inflammatory signaling orchestrates the hypothalamic immune response to dietary excess and mediates obesity susceptibility. **Cell Metabolism**, v. 26, n. 1, p. 185- 197.e3, 2017.

VALENTINUZZI, V. S. et al. Automated measurement of mouse freezing behavior and its use for quantitative trait locus analysis of contextual fear conditioning in (BALB/cJ x C57BL/6J)F2 mice. **Learning and Memory**, v. 5, n. 4–5, p. 391–403, 1998.

VALLADOLID-ACEBES, I. et al. High-fat diets induce changes in hippocampal glutamate metabolism and neurotransmission. **American Journal of Physiology - Endocrinology and Metabolism**, v. 302, n. 4, p. 396–402, 2012.

VAN BUSSEL, F. C. G. et al. Increased GABA concentrations in type 2 diabetes mellitus are related to lower cognitive functioning. **Medicine (United States)**, v. 95, n. 36, p. 1–6, 2016.

VAN DEN BERG, C. J.; GARFINKEL, D. A stimulation study of brain compartments. Metabolism of glutamate and related substances in mouse brain. **The Biochemical journal**, v. 123, n. 2, p. 211–218, 1971.

VIRTUE, S.; VIDAL-PUIG, A. GTTs and ITTs in mice: simple tests, complex answers. **Nature Metabolism**, v. 3, n. 7, p. 883–886, 2021.

VIVIANI, B. et al. Interleukin-1β enhances NMDA receptor-mediated intracellular calcium increase through activation of the Src family of kinases. **Journal of Neuroscience**, v. 23, n. 25, p. 8692–8700, 2003.

WALLS, A. B. et al. The Glutamine–Glutamate/GABA Cycle: Function, Regional Differences in Glutamate and GABA Production and Effects of Interference with GABA Metabolism. **Neurochemical Research**, v. 40, n. 2, p. 402–409, 2015.

WANG, Q.; WU, H. T cells in adipose tissue: Critical players in immunometabolism. **Frontiers in Immunology**, v. 9, n. 2509, p. 9–11, 2018.

WARD, R. J. et al. Neuro-inflammation induced in the hippocampus of "binge drinking" rats may be mediated by elevated extracellular glutamate content. **Journal of Neurochemistry**, v. 11, p. 1119–1128, 2009.

WEI, W. et al. Diet composition, not calorie intake, rapidly alters intrinsic excitability of hypothalamic AgRP/NPY neurons in mice. **Scientific Reports**, v. 5, n. February, p. 1–10, 2015.

WESTERGAARD, N.; SONNEWALD, U.; SCHOUSBOE, A. Metabolic trafficking between neurons and astrocytes: The glutamate/Glutamine cycle revised. **Developmental Neuroscience**, v. 17, p. 203–211, 1995.

WITHROW, D.; ALTER, D. A. The economic burden of obesity worldwide: A systematic review of the direct costs of obesity. **Obesity Reviews**, v. 12, n. 2, p. 131–141, 2011.

YAMADA-GOTO, N. et al. Impairment of Fear-Conditioning Responses and Changes of Brain Neurotrophic Factors in Diet-Induced Obese Mice. **Journal of Neuroendocrinology**, v. 24, n. 8, p. 1120–1125, 2012.

YE, J. et al. Hypoxia is a potential risk factor for chronic inflammation and adiponectin reduction in adipose tissue of ob/ob and dietary obese mice. **American Journal of Physiology - Endocrinology and Metabolism**, v. 293, n. 4, p. 1118–1128, 2007.

YE, Z.-C.; SONTHEIMER, H. Cytokine modulation of glial glutamate uptake: A possible involvement of nitric oxide. **Neuroreportport**, v. 7, p. 2181–2185, 1996.

YI, C. X. et al. TNFα drives mitochondrial stress in POMC neurons in obesity. **Nature Communications**, v. 8, n. 15143, 2017.

ANEXO

Anexo 1- Protocolo de aprovação da comissão de ética no uso de animais (CEUA)

UFMG

UNIVERSIDADE FEDERAL DE MINAS GERAIS

CEUA
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

Prezado(a):

Esta é uma mensagem automática do sistema Solicite CEUA que indica mudança na situação de uma solicitação.

Protocolo CEUA: 217/2019
Título do projeto: Avaliação dos mecanismos de ação dos receptores metabotrópicos de glutamato do subtipo 5 (mglur5) sobre regulação de parâmetros obesogênicos
Finalidade: Pesquisa
Pesquisador responsável: Luciene Bruno Vieira
Unidade: Instituto de Ciencias Biologicas
Departamento: Departamento de Farmacologia

Situação atual: Decisão Final - Aprovado

Aprovado na reunião do dia 30/09/2019. Validade: 30/09/2019 à 29/09/2024
Belo Horizonte, 30/09/2019.

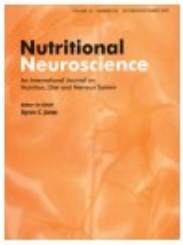
Atenciosamente,

Sistema Solicite CEUA UFMG
https://aplicativos.ufmg.br/solicite_ceua/

Universidade Federal de Minas Gerais
Avenida Antônio Carlos, 6627 – Campus Pampulha
Unidade Administrativa II – 2º Andar, Sala 2005
31270-901 – Belo Horizonte, MG – Brasil
Telefone: (31) 3409-4516
www.ufmg.br/bioetica/ceua - cetea@prpq.ufmg.br

APÊNDICES

Apêndice 1- Produtos gerados durante o período do doutorado



Nutritional Neuroscience

An International Journal on Nutrition, Diet and Nervous System

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ynns20

Is resveratrol a prospective therapeutic strategy in the co-association of glucose metabolism disorders and neurodegenerative diseases?

R. Ribeiro, A. C. Santos, M. O. Calazans, A. C. P. De Oliveira & L. B . Vieira

To cite this article: R. Ribeiro, A. C. Santos, M. O. Calazans, A. C. P. De Oliveira & L. B . Vieira (2021): Is resveratrol a prospective therapeutic strategy in the co-association of glucose metabolism disorders and neurodegenerative diseases?, Nutritional Neuroscience, DOI: 10.1080/1028415X.2021.1972514

To link to this article: https://doi.org/10.1080/1028415X.2021.1972514

Published online: 11 Sep 2021.

www.nature.com/scientificreports

scientific reports

Check for updates

OPEN

Metabotropic glutamate receptor 5 knockout rescues obesity phenotype in a mouse model of Huntington's disease

Rebeca P. M. Santos[1], Roberta Ribeiro[1], Talita H. Ferreira-Vieira[3,4], Rosaria D. Aires[1,4], Jessica M. de Souza[3], Bruna S. Oliveira[2], Anna Luiza D. Lima[1], Antônio Carlos P. de Oliveira[1], Helton J. Reis[1], Aline S. de Miranda[2], Erica M. L. Vieira[1], Fabiola M. Ribeiro[1,2]✉ & Luciene B. Vieira[1,2]✉

Obesity represents a global health problem and is characterized by metabolic dysfunctions and a low-grade chronic inflammatory state, which can increase the risk of comorbidities, such as atherosclerosis, diabetes and insulin resistance. Here we tested the hypothesis that the genetic deletion of metabotropic glutamate receptor 5 (mGluR5) may rescue metabolic and inflammatory features present in BACHD mice, a mouse model of Huntington's disease (HD) with an obese phenotype. For that, we crossed BACHD and mGluR5 knockout mice (mGluR5$^{-/-}$) in order to obtain the following groups: Wild type (WT), mGluR5$^{-/-}$, BACHD and BACHD/mGluR5$^{-/-}$ (double mutant mice). Our results showed that the double mutant mice present decreased body weight as compared to BACHD mice in all tested ages and reduced visceral adiposity as compared to BACHD at 6 months of age. Additionally, 12-month-old double mutant mice present increased adipose tissue levels of adiponectin, decreased leptin levels, and increased IL-10/TNF ratio as compared to BACHD mice. Taken together, our preliminary data propose that the absence of mGluR5 reduce weight gain and visceral adiposity in BACHD mice, along with a decrease in the inflammatory state in the visceral adipose tissue (VAT), which may indicate that mGluR5 may play a role in adiposity modulation.

Abbreviations

mGluR5	Metabotropic Glutamate Receptor 5
CNS	Central Nervous System
EAT	Epididymal adipose tissue
RPAT	Retroperitoneal adipose tissue
VAT	Visceral adipose tissues
DIO	Diet-Induced-Obesity
HFD	High-fat-diet
HD	Huntington's disease
TNF	Tumor Necrosis Factor
IFN-γ	Interferon gamma
IL	Interleukin
BACHD mice	Bacterial artificial chromosome (BAC)-mediated transgenic mouse model
mGluR5$^{-/-}$	Metabotropic Glutamate Receptor 5 knockout mice
BACHD/mGluR5$^{-/-}$	Double mutant mice
NAM	Negative allosteric modulator
KO	Knockout

[1]Departamento de Farmacologia, ICB, Universidade Federal de Minas Gerais, Ave. Antonio Carlos 6627, Belo Horizonte, MG CEP 31270-901, Brazil. [2]Departamento de Morfologia, ICB, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil. [3]Departamento de Bioquímica e Imunologia, ICB, Universidade Federal de Minas Gerais, Belo Horizonte CEP 31270-901, Brazil. [4]Faculdade Sete Lagoas, Sete Lagoas, Brazil. ✉email: fmribeiro@icb.ufmg.br; lubvieira@icb.ufmg.br

Neuropeptides 83 (2020) 102081

Contents lists available at ScienceDirect

Neuropeptides

journal homepage: www.elsevier.com/locate/npep

Gyroxin, a toxin from *Crotalus durissus terrificus* snake venom, induces a calcium dependent increase in glutamate release in mice brain cortical synaptosomes

C.Z. Ferrari[a], R. Ribeiro[a], A.M. Lima[b], A.M. Soares[b,c], W.L.G. Cavalcante[a,*], L.B. Vieira[a,*]

[a] Department of Pharmacology, ICB, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil
[b] Laboratório de Biotecnologia de Proteínas e Compostos Bioativos da Amazônia Ocidental, LaBioProt, Centro de Estudos de Biomoléculas Aplicadas à Saúde, CEBio, Fundação Oswaldo Cruz, FIOCRUZ, Fiocruz Rondônia e Universidade Federal de Rondônia, UNIR, Porto Velho, RO, Brazil
[c] Centro Universitário São Lucas, UniSL, Porto Velho, RO, Brazil

ARTICLE INFO

Keywords:
Snake toxin
Calcium channels
Glutamate release
Synaptosomes

ABSTRACT

Gyroxin is a thrombin-like toxin obtained from the venom of the South American rattlesnake, *Crotalus durissus terrificus*. Literature has reported "gyroxin syndrome" characterized, in mice, as series of aberrant motor behavior, known as barrel rotation, mainly after intraperitoneal administration. Despites several studies, a physiological mechanism of "gyroxin syndrome" are still not completely understood. In this context, alterations on the central nervous system (CNS), especially causing neurotoxic events, are pointed out as likely candidates. Then, we decided to investigate whether gyroxin induces alterations in glutamate release, one of the most important neurotransmitter involved in neurotoxicity. For that, we performed all experiments, in vitro, using a model of mice brain cortical synaptosomes. Notably, our results indicate that the administration of gyroxin on purified presynaptic brain cortical terminals resulted in an extracellular Ca^{2+}- dependent raise in glutamate release. Indeed, our results also showed that gyroxin increases intrasynaptosomal calcium (Ca^{2+}) levels through acting on voltage gated calcium channels (VGCC), specifically N and P/Q subtypes. Moreover, our data show that gyroxin increases exocytosis rate. Interestingly, these data suggest that gyroxin might induce neurotoxicity by increasing glutamate levels. However, future investigations are needed in order to elucidate the nature of the following events.

1. Introduction

Gyroxin (28 kDa) is a serine protease from the venom of the *Crotalus durissus terrificus* that corresponds to a fraction of 2.5% from the crude venom of the South American rattlesnake (da Silva et al., 2011). Although non-lethal, this toxin has thrombin like properties and also presents several actions, such as: decreasing blood pressure, pro-coagulating properties and neurotoxicity (Alexander et al., 1988; da Silva et al., 2012). Gyroxin syndrome or barrel rotation syndrome is a neurological condition induced by endovenous injection of gyroxin, characterized by opisthotonus and intermittent rotations around the long axis (Barrio, 1961; Alexander et al., 1988). Although, this behavior has been described likely, a toxic feat of this toxin, the precise mechanism leading to neurotoxicity is remaining unknown (Barrio, 1961; Alexander et al., 1988; Camillo et al., 2001). Importantly, literature described that gyroxin promoted increasing of blood-brain barrier (BBB) (da Silva et al., 2011, 2012) and alterations in behavioral, electroencephalographic, neuropathological effects, besides seizures after intrahippocampal injection in rats (Melo and Cavalheiro, 1989; Moreira, 1993). In contrast, gyroxin failed in modulating dopamine and acetylcholine release, suggesting that neurotoxic effects may involve hemodynamic disturbances (Camillo et al., 2001). Indeed, the effects of gyroxin in the release of other neurotransmitters are not fully described.

Glutamate is the major excitatory neurotransmitter in mammalian CNS and a key player in excitotoxicity (Nakanishi and Masu, 1994; Michaelis, 1998; Ferraguti et al., 2008). Glutamate binds to two types of glutamate receptors: ionotropic and metabotropic glutamate receptors (mGluRs) (Conn and Pin, 1997). Ionotropic glutamate receptors,

Abbreviations: ω-CgTX GVIA, ω-conotoxin GVIA; ω-CgTX MVIIC, ω-conotoxin MVIIC; ω-Aga IVA, ω-agatoxin IVA; VACC, voltage activated calcium channels; $[Ca^{2+}]_i$, intracellular calcium concentration; LDH, lactate dehydrogenase

* Corresponding authors at: Departamento de Farmacologia, ICB, Universidade Federal de Minas Gerais, Ave. Antonio Carlos 6627, Belo Horizonte, MG CEP: 31270-901, Brasil.
E-mail addresses: wcavacante@icb.ufmg.br (W.L.G. Cavalcante), lubvieira@icb.ufmg.br (L.B. Vieira).

https://doi.org/10.1016/j.npep.2020.102081
Received 2 May 2020; Received in revised form 10 August 2020; Accepted 10 August 2020
Available online 14 August 2020
0143-4179/