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Efeitos Neuroprotetores da Exendina-4 na Demência Esporádica do tipo Alzheimer induzido por Estreptozotocina em Ratos

Porto Alegre
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Tese submetida ao Programa de Pós-Graduação em Patologia da Universidade Federal de Ciências da Saúde de Porto Alegre como requisito para a obtenção do grau de Doutora.

Orientador: Dr.ª Marilda da Cruz Fernandes
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“Dai-me, Senhor, a perseverança das ondas do mar, que fazem de cada recuo um ponto de partida para um novo avanço”.

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Resumo

Introdução: A doença de Alzheimer (DA) é responsável por aproximadamente 60 a 70% de todos os casos de demência no mundo. A demência é uma patologia neurodegenerativa e irreversível que ocasiona declínio progressivo da memória, comprometimento cognitivo, alterações comportamentais, afetando severamente as atividades diárias e levando à morte. **Objetivos:** Investigar os efeitos neuroprotetores da exendina-4 (EXE-4) sobre alterações cognitivas e morfológicas no hipocampo de ratos submetidos a um modelo experimental de demência esporádica do tipo Alzheimer induzido por estreptozotocina (STZ). **Material e Métodos:** Foram utilizados 40 ratos *Wistar* machos, que receberam estreptozotocina (STZ-Intracerebroventricular, 10 µl, 3 mg/kg, e líquido cefalorraquidiano artificial como veículo) e tratados com EXE-4 (10 µg/kg, via intraperitoneal, solução salina como veículo), durante 21 dias. Após, os animais foram submetidos aos testes para avaliar a memória, a atividade locomotora e exploratória. Ao final, os animais foram submetidos à eutanásia, e seus cérebros foram removidos para análises teciduais das regiões do Corno de Ammon 1 e 3 (CA1 e CA3) e giro denteado no hipocampo, pelas técnicas de imunofluorescência e imuno-histoquímica. **Resultados:** Verificou-se que a EXE-4 apresentou efeitos benéficos sobre os neurônios piramidais e granulares que constituem o hipocampo, protegeu a memória de curta e longa duração, melhorou a proliferação celular e reduziu a neuroinflamação. **Conclusão:** Concluiu-se que a EXE-4 pode ser um adjuvante no tratamento da DA, melhorando a qualidade de vida das pessoas acometidas por essa patologia.

Palavras-chave: doença de Alzheimer; exendina-4; memória; neuroinflamação.

Abstract

Introduction: Alzheimer's disease (AD) is responsible for approximately 60 to 70% of all cases of dementia worldwide. Dementia is a neurodegenerative and irreversible pathology that causes progressive memory decline, cognitive impairment, behavioral changes, severely affecting daily activities and leading to death. **Aim of study:** To investigate the neuroprotective effects of exendin-4 (EXE-4) on cognitive and morphological alterations in the hippocampus of rats submitted to an experimental model of sporadic dementia of the Alzheimer type, induced by streptozotocin (STZ). **Material and Methods**: Forty male Wistar rats were used, which received streptozotocin (STZ-Intracerebroventricular, 10 µl, 3 mg/kg, and artificial cerebrospinal fluid as vehicle) and treated with EXE-4 (10 µg/kg, intraperitoneally, saline solution as a vehicle) for 21 days. Afterwards, the animals were submitted to tests to assess memory, locomotor and exploratory activity. At the end, the animals were euthanized, and their brains were removed for tissue analysis of the Horn of Ammon regions 1 and 3 (CA1 and CA3) and dentate gyrus in the hippocampus, using immunofluorescence and immunohistochemistry techniques. **Results**: EXE-4 was found to have beneficial effects on pyramidal and granular neurons that make up the hippocampus, protect short- and long-term memory, improve cell proliferation, and reduce neuroinflammation. **Conclusion**: It was concluded that EXE-4 can be an adjuvant in the treatment of AD, improving the quality of life of people affected by this pathology.

Keywords: Alzheimer's disease; exendin-4; memory; neuroinflammation.

Lista de siglas

8-OHdG: 8-Hidroxidesoxiguanosina

AC: Adenilato ciclase

ACh: Acetilcolina

AChE: Acetilcolinesterase

ADP: Adenosina difosfato

AKt: Proteína quinase b, do inglês *protein kinase B*

AMPA: Ácido-α-amino-3-hidroxi-4-isoxazolpropiônico

AMPc: Adenosina 3,5-monofosfato cíclico

AP: Área postrema

APOE: Apolipoproteína E

APP: Proteína precursora amiloide, do inglês *amyloid precursor protein*

ATP: Adenosina trifosfato

Aβ: β-amiloide, do inglês *amyloid beta*

BACE-1: Beta secretase

BDNF: Fator neurotrófico derivado do cérebro, do inglês *brain-derived neurotrophic factor*

BHE: Barreira hematoencefálica

BrdU: 5-bromo-2'-deoxiuridina, do inglês *5-bromo-2'-deoxyuridine*

CA: Corno de Ammon

CA1: Região 1 do Corno de Ammon

CA2: Região 2 do Corno de Ammon

CA3: Região 3 do Corno de Ammon

CA4 - Região 4 do Corno de Ammon

CDK5: Quinase 5 dependentes de ciclina, do inglês *cyclin-dependent kinase 5*

ChAT: Colina acetil-transferase

CREB: Proteína de ligação ao elemento de resposta da adenosina 3,5-monofosfato cíclico, do inglês c*yclic adenosine 3',5'-monophosphate response element-binding protein*

DA: Doença de Alzheimer

DCX: Doublecortina

DG: Giro denteado, do inglês *dentate gyrus*

DM2: Diabetes mellitus tipo 2

DM3: Diabetes mellitus tipo 3

DPP-4: Dipeptidil peptidase-4

DRG: Gânglio da raiz dorsal, do inglês *dorsal root ganglion*

EAE: Encefalomielite autoimune experimental

EGFR: Receptor do fator de crescimento epidérmico, do inglês *epidermal growth factor receptor*

EM: Esclerose múltipla

ENFs: Emaranhados neurofibrilares

Epac: proteína de troca diretamente ativada por AMPc do inglês *exchange protein directly activated by cAMP*

ERK: Quinase regulada por sinal extracelular, do inglês *extracellular signal-related kinases*

EXE-4: Exendina-4

FDA: Food and Drug Administration

FoxO: Proteínas de fatores de transcrição, do inglês *transcription factors*

GABA: Ácido gama-aminobutírico, do inglês *Gamma-AminoButyric Acid*

GEFs: Fatores de troca de nucleotídeos de guanina, do inglês *guanine nucleotide exchange factors*

GIP: Peptídeo inibidor gástrico ou polipeptídeo insulinotrópico dependente de glicose, do inglês *glucose-dependent insulinotropic polypeptide*

GIPR: Receptor do peptídeo inibidor gástrico, do inglês *glucose-dependent insulinotropic polypeptide recptor*

GLP-1: Peptídeo semelhante ao glucagon-1, do inglês *Glucagon-like peptide-1*

GLP-1RA: Agonista do receptor de GLP-1, do inglês *GLP-1 receptor agonists*

GLP-2: Peptídeo semelhante ao glucagon-2, do inglês do inglês *Glucagon-like peptide-2*

GLUT 1-3: Transportadores de glicose 1-3, do inglês *insulin dependent glucose transporters 1-3*

GLUT 2: Transportador de glicose 2, do inglês *insulin dependente glucose transporters 2*

GLUT 4: Transportador de glicose insulina-dependente, do inglês *Insulin dependent glucose transporter 4*

GRPP: Polipeptídeo pancreático relacionado com glicina, do inglês *glicentin-related pancreatic polypeptide*

GSK3β: Glicogênio sintase quinase 3 beta

HNE: 4-Hidroxi-2-nonenal

i.p: Intraperitoneal

ICV ou i.c.v.: Intracerebroventricular

IGF: Fator de crescimento semelhantes à insulina, do inglês *insulin-like growth factor*

IGF-1: Fator de crescimento semelhante à insulina 1, do inglês *insulin like growth factor-1*

IGF-2: Fator de crescimento semelhante à insulina 2, do inglês *insulin like growth factor-2*

IL: Interleucina

IL-17: Interleucina-17

IL-1β: Interleucina- 1beta

IL-6: Interleucina-6

IP-1: Peptídeo interveniente-1, do inglês *intervening peptide-1*

IP-2: Peptídeo interveniente-2, do inglês *intervening peptide-2*

IRS: Substratos do receptor de insulina, do inglês *insulin receptor substrate*

IRS-1: Substrato do receptor de insulina -1, do inglês *insulin receptor substrate-1*

IRS-2: Substrato do receptor de insulina -2, do inglês *insulin receptor substrate-2*

KATP: Canais de potássio sensíveis ao ATP, do inglês *ATP-sensitive potassium channels*

LEC: Córtex entorrinal lateral, do inglês *lateral entorhinal cortex*

LPS: Lipopolissacarídeo

LTD: Depressão de longo prazo, do inglês *long -term depression*

LTM: Memória de longo prazo, do inglês *long-lerm memory*

LTP: Potenciação de longo prazo, do inglês *long-term potentiation*

MAPK: Proteína quinase ativada por mitógeno, do inglês *mitogen activated protein kinase*

MEC: Córtex entorrinal medial, do inglês *medial entorhinal cortex*

MEK: quinase ativadora da MAP quinase, do inglês *MAP kinase activating kinase*

mTOR: Alvo em mamíferos da rapamicina, do inglês *mammalian target of rapamycin*

NF-kB: Fator nuclear kappa beta, do inglês *factor nuclear kappa B*

NMDA: N-metil-D-aspartato

NOS: óxido nítrico-sintase, do inglês *nitric oxide synthase*

NOX-1: NADPH oxidase-1

Nrf2: Fator nuclear eritroide 2 relacionado ao fator 2, do inglês *nuclear factor erythroid factor 2*

NTS: Núcleo do Trato Solitário

OMS: Organização Mundial de Saúde

OXM: Oxintomodulina

p53: Gene pró-apoptose

PC: Pró-hormônio convertase

PC1 ou PCSK1: Pró-hormônio convertase 1

PC2 ou PCSK2: Pró-hormônio convertase 2

PDK: Quinase dependente de fosfatidilinositol, do inglês *phosphatidylinositol-dependent kinase*

PDK1: Proteína quinase-1 dependente de fosfoinositídeo, do inglês *phosphatidylinositol-dependent kinase 1*

PG: Pró-glucagon

PI3K: Fosfatidilinositol-3-quinase, do inglês *phosphatidylinositol 3-kinase*

PI3Ka: Fosfatidilinositol-4,5 bifosfato 3-quinase, do inglês *phosphatidylinositol-4,5-bisphosphate 3-kinase*

PIP2: Fosfatidilinositol 4,5 bifosfato

PIP3: Fosfatidilinositol 3,4,5 trifosfato

PKA: Proteína quinase A

PKB ou Akt: Proteína quinase B

PKC: Proteína quinase C

PS1: Presenilina 1

PS2: Presenilina 2

PSD: Densidade pós-sináptica, do inglês *postsynaptic density*

Rap1A: Proteína membro da família de oncogenes RAS, do inglês *member of the Ras oncogene family*

RE: Retículo endoplasmático

Rho A: Membro da família de genes homólogos Ras A, do inglês *member of RAS oncogene family*

RI: Receptor de insulina

SAD: Demência esporádica do tipo Alzheimer, do inglês *Sporadic dementia of the Alzheimer type*

SFO: Órgão subfornical, do inglês *subfornical organ*

SNC: Sistema Nervoso Central

STM: Memória de curto prazo, do inglês *short-term memory*

STZ: Estreptozotocina, do inglês *streptozotocin*

TBARS: Ácido tiobarbitúrico, do inglês *thiobarbituric acid reactive substances*

TCA: Ácido tricarboxílico, do inglês t*ricarboxylic acid*

TNF-α: Fator de necrose tumoral alfa, do inglês t*umour necrosis factor alpha*

VDCC: Canais de cálcio dependentes de voltagem, do inglês *Voltage-gated calcium channels*

ZSG: Zona subgranular

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SUMÁRIO

1 REFERENCIAL TEÓRICO

1.1 HIPOCAMPO

O hipocampo é uma das estruturas cerebrais mais investigadas. Desde o relatório de 1957, do estudo de caso do paciente H.M., notoriamente, ele perdeu a capacidade de formar novas memórias após a remoção cirúrgica do hipocampo e das estruturas próximas do lobo temporal para tratar a epilepsia intratável. O hipocampo tem estado na vanguarda da pesquisa sobre as bases neurobiológicas da memória[1]. O termo é derivado das palavras gregas *hippos*, cavalo; *kampos*, monstro do mar, uma referência à semelhança com “cavalo-marinho”. É uma estrutura que circunda o ventrículo lateral do lobo temporal do encéfalo e faz parte do sistema límbico [2, 3, 4].

O sistema límbico (Figura 1) é responsável pelo impacto emocional que as ações, os comportamentos e as situações exercem sobre os indivíduos. Ele direciona as respostas para essas emoções e atua na criação, no armazenamento e na recuperação das memórias, particularmente as que despertam emoções fortes [2, 3, 4].

O hipocampo consiste em um grupo de estruturas localizadas na face medial de cada hemisfério cerebral e no diencéfalo, sendo as principais: giro do cíngulo, formação hipocampal (hipocampo e giro para-hipocampal) e amígdala. O giro do cíngulo e o parahipocampal são normalmente chamados de “lobo límbico”, que não se trata de um lobo verdadeiro como os outros, mas que abrange partes dos lobos frontal, parietal e temporal (Figura 2) [2, 3, 4].

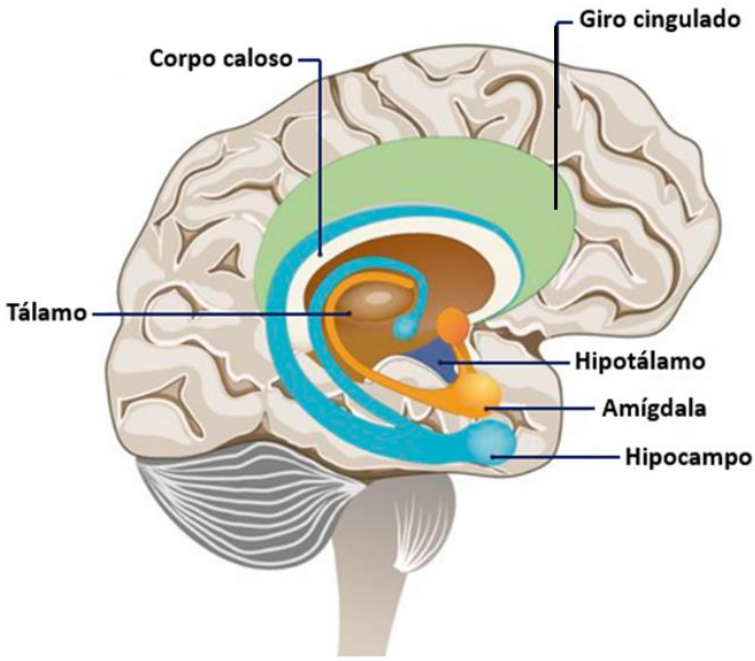

Figura 1: Sistema Límbico.
Legenda: Em branco, o corpo caloso; verde o giro cingulado; marrom claro, o tálamo; azul escuro, o hipotálamo; laranja, a amígdala e, em azul claro, o hipocampo. Extraído de um banco de imagens sem direitos autorais (https://pixabay.com).

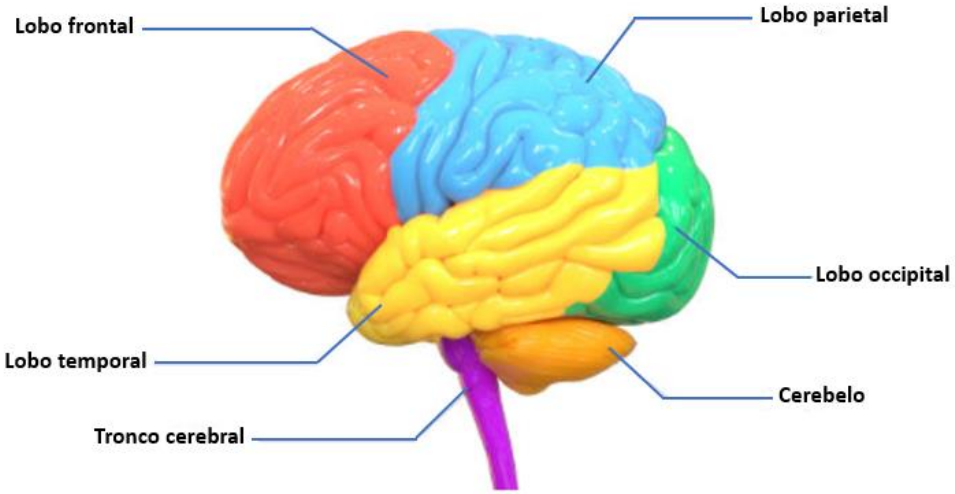

Figura 2: Lobos cerebrais.
Legenda: vermelho, o lobo frontal; azul, o lobo parietal; amarelo, o lobo temporal; verde, o lobo occipital; laranja, o cerebelo e, em roxo, o tronco cerebral. Extraído de um banco de imagens sem direitos autorais (https://pixabay.com).

Ventralmente ao hipocampo, estão três importantes regiões corticais que cercam o sulco rinal: o córtex entorrinal, o córtex perirrinal e o córtex para-hipocampal.

As aferências alcançam, primeiramente, os córtices rinal (córtex entorrinal e o perirrinal) e para-hipocampal, antes de serem passados para o hipocampo (Figura 3). Uma das principais vias eferentes do hipocampo é o fórnix, que circunda o tálamo antes de terminar no hipotálamo [2].

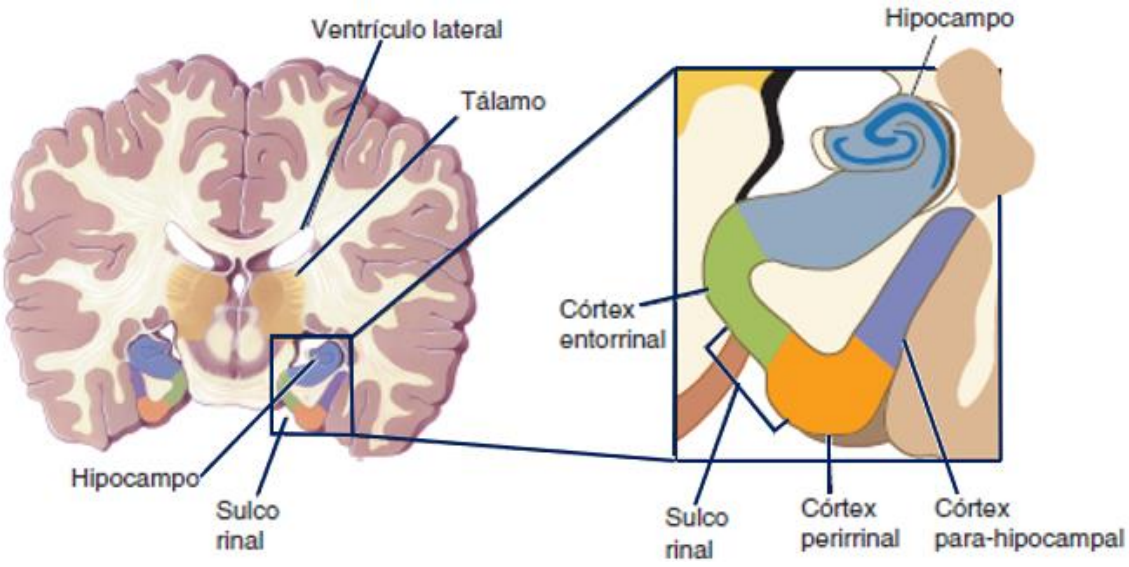

Figura 3: O encéfalo em secção coronal para mostrar o hipocampo e o córtex entorrinal no lobo temporal medial.
Legenda: Em azul, o hipocampo; verde, o córtex entorrinal; laranja, o córtex perirrinal e violeta, o córtex para-hipocampal (Adaptado de Bear, Connors, Paradiso e Jorge Alberto, 2017) [2].

O hipocampo consiste em finas camadas de neurônios, dobradas uma sobre a outra. Uma é chamada de giro denteado (DG), composta por neurônios granulados, e a outra de Corno de Ammon (CA, do latim "*Cornu Ammonis*"), composta por neurônios piramidais e formada por quatro regiões (CA1, CA2, CA3 e CA4), que, junto com o córtex entorrinal e o complexo subicular, integram a formação hipocampal (Figuras 4 A e 4 B) [2, 5].

O hipocampo desempenha um papel importante no aprendizado, na formação, armazenamento e evocação de memórias, nos processos emocionais e cognitivos [1, 6]. Além disso, o hipocampo está envolvido no processo de neurogênese adulta, que ocorre na zona subgranular (ZSG) do giro denteado (Figura 4 A) [7].

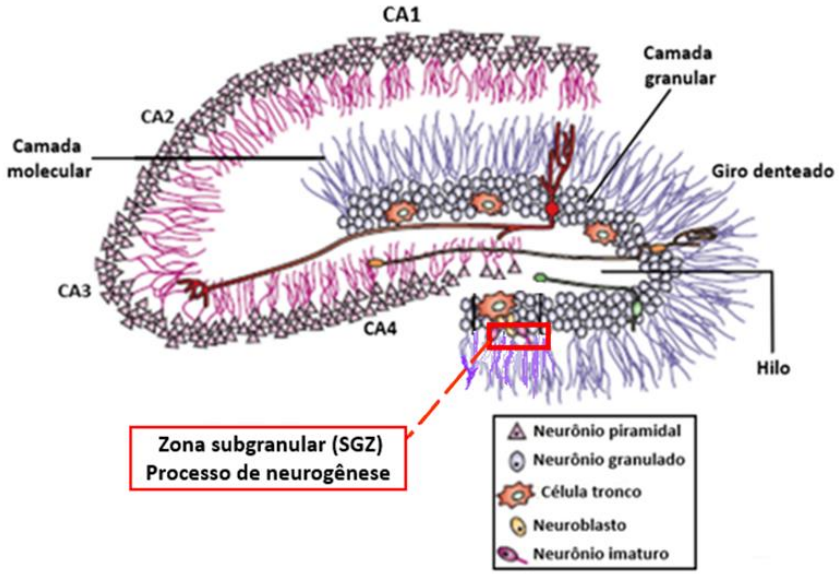

Figura 4 A: Representação do hipocampo, suas respectivas regiões, principais tipos celulares e o processo de neurogênese, começando na zona subgranular (SGZ) da camada granular do giro denteado.
Legenda: CA1, CA2, CA3 e CA4 – Regiões 1, 2, 3 e 4 do Corno de Ammon (Adaptado de Vescovi, Galli e Reynolds, 2006) [8].

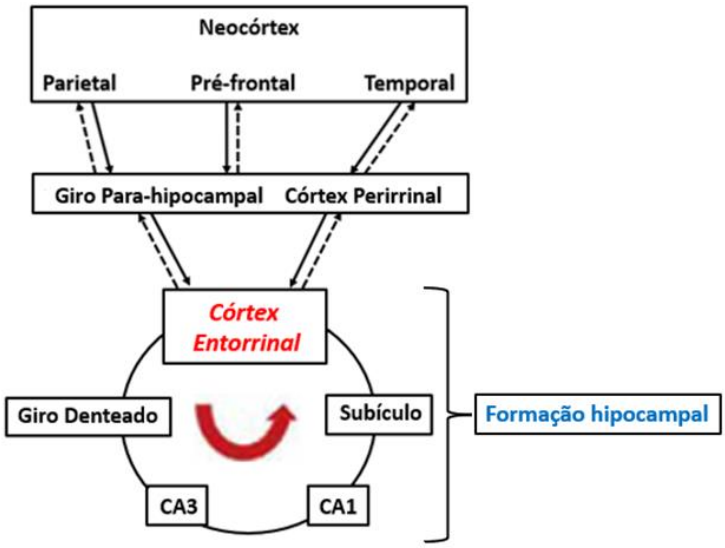

Figura 4 B: Padrão de conectividade cortical e intrínseca da formação hipocampal. Note o fluxo recíproco de informações corticais convergindo via giro para-hipocampal e córtex perirrinal sobre o córtex entorrinal.
Legenda: Corno de Ammon 1 e 3 (CA1 e CA3) (Adaptado de Schultz e Engelhardt, 2014) [5].

No hipocampo, as conexões neuronais são altamente ordenadas. Por isso, é fácil identificar populações específicas de neurônios e sinapses. O córtex entorrinal fornece a

principal aferência cortical para o hipocampo. Os axônios dos neurônios do córtex entorrinal atravessam o subículo e entram no giro denteado por meio da via perfurante, e formam sinapses com células granulares do giro denteado e os axônios das células granulares. As fibras musgosas ligam-se aos dendritos apicais dos neurônios piramidais da região CA3, que, por sua vez, através da via colateral de Schaffer, as conduz para a região CA1. Na sequência, os axônios dos neurônios piramidais da região CA1 projetam-se para o subículo, via regressa ao córtex entorrinal. Todo esse circuito é chamado de "alça trissináptica" (Figura 5), e as sinapses são todas excitatórias e utilizam glutamato como neurotransmissor [6, 1].

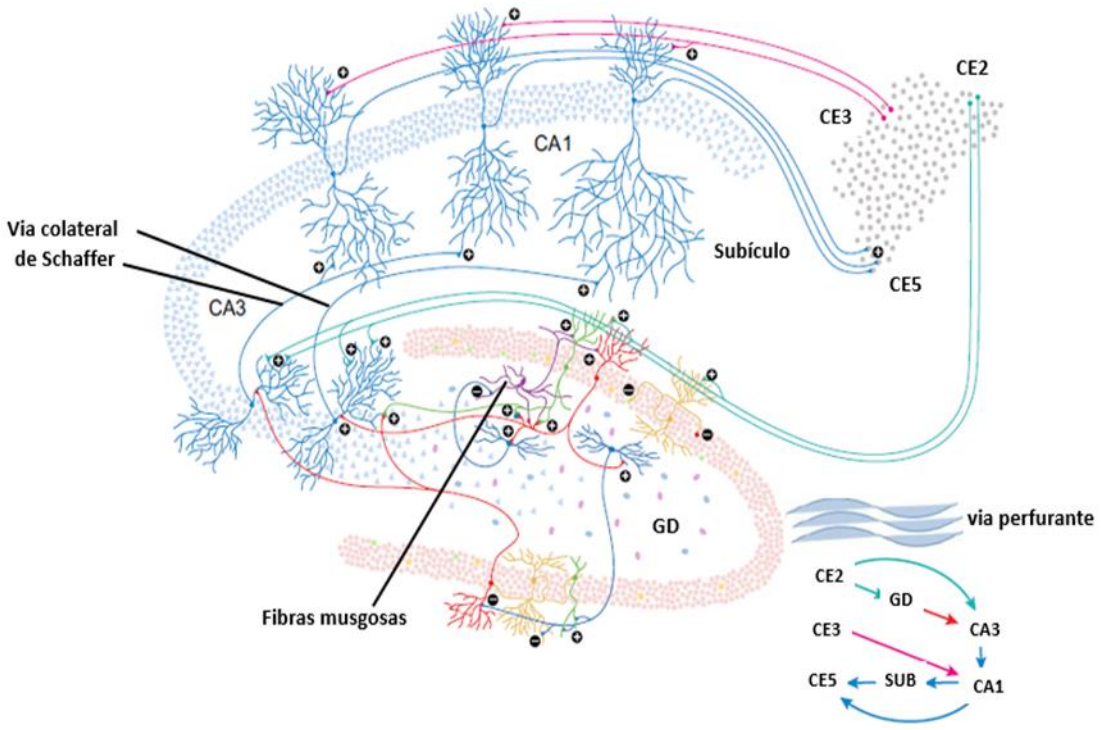

Figura 5: Circuitos e conexões do hipocampo.
Legenda: CA1 e CA3: Regiões 1 e 3 do Corno Ammon; CE2, 3 e 5: Córtex entorrinal 2, 3 e 5; GD: Giro denteado (Adaptado de Aimone, Li, Lee, Clemenson, Deng e Gage, 2014) [9].

As estruturas que constituem a formação hipocampal são as primeiras estruturas cerebrais a serem afetadas pela Doença de Alzheimer, que desencadeia

déficits de aprendizado, memória e outras funções cognitivas, como percepção, atenção e linguagem, ocasionando também deterioração do tecido nervoso [10].

1.2 MEMÓRIA

Conceitualmente, “memória é a aquisição, a formação, a conservação e a evocação de informações”. Podemos afirmar que “somos aquilo que recordamos”. Portanto, não podemos fazer, nem comunicar nada que desconheçamos, isto é, nada que não esteja na nossa memória. O acervo de nossas memórias faz com que cada um de nós seja o que é, cada indivíduo, um ser para o qual não existe outro idêntico [11].

O aprendizado é a aquisição de novas informações ou conhecimentos, que conduz ao armazenamento de informação como consequência da prática, da observação, do estudo, da experiência e ou da introspecção [2].

A memória pode ser classificada em dois tipos, de acordo com seu conteúdo. Ela pode ser declarativa ou explícita e não declarativa ou implícita.

A memória declarativa ou explícita é uma memória de fatos e eventos que ocorreram em nossa vida, como um casamento, uma viagem. É aquela evocada pelo consciente e a qual verbalizamos, são mais fáceis de formar e, também, facilmente esquecidas [2].

A memória não declarativa ou implícita, também chamada de memória procedural, é uma memória de habilidades, hábitos e comportamentos, como andar de bicicleta, tocar piano, jogar bola ou dirigir um automóvel. É evocada pelo inconsciente e que não verbalizamos, sua formação exige repetição e prática durante um período mais longo, mas essas memórias são menos prováveis de serem esquecidas [2].

A memória também pode ser classificada conforme o tempo de duração. Nessa classificação, estão a memória de trabalho, a memória de curto prazo e a memória de longo prazo.

A Memória de trabalho basicamente armazena as informações por um período muito curto de tempo (segundos a minutos) e não deixa nenhum registro duradouro de qualquer tipo. Memória de curto prazo (STM, do inglês *short-term memory*): que dura algumas horas, ou seja, informações recém aprendidas são temporariamente armazenadas. A Memória de longo prazo (LTM, *long-term memory*): que dura dias, meses ou anos. As memórias de longo prazo não ficam determinadas em sua forma permanente imediatamente depois de sua aquisição. O processo que leva à fixação definitiva das memórias de longo prazo de maneira que mais tarde possam ser evocadas nos dias ou anos seguintes chama-se consolidação [2, 12, 11].

Em estudos experimentais, é possível avaliar esses tipos de memória. Quillfeldt [12] propôs uma classificação para os tipos de memória e as tarefas comportamentais que podem ser realizadas (Figura 6).

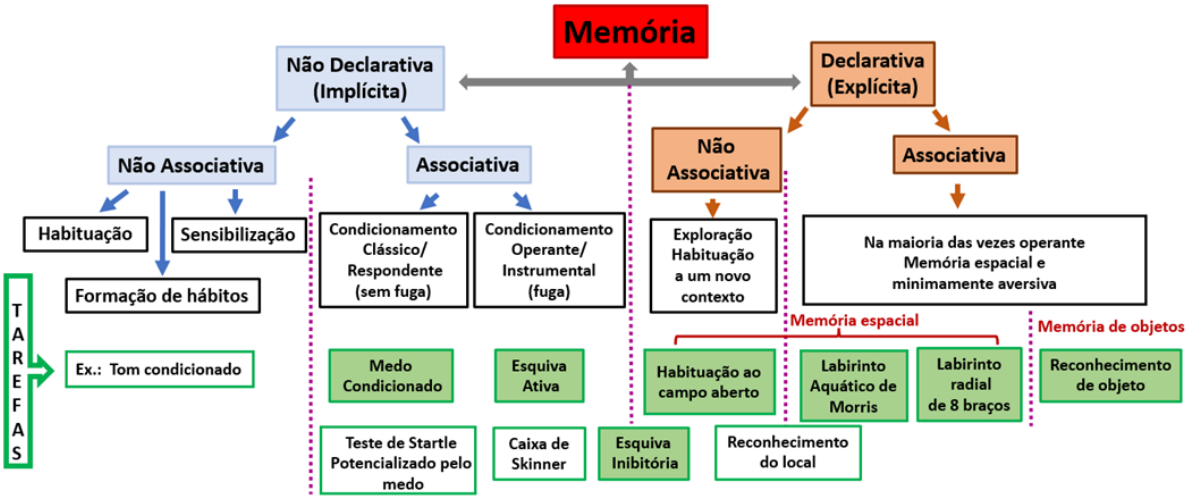

Figura 6: Classificação das memórias segundo o conteúdo em declarativa e não declarativa e tarefas comportamentais (Adaptado de Quillfeldt, 2016)[12].

Em algumas espécies de mamíferos, basicamente, como os sistemas neuronais são muito semelhantes (o homem, o rato e o camundongo possuem lobos cerebrais), podemos fazer inferências sensatas de achados em uma dessas espécies menores e extrapolá-la aos humanos. Por exemplo, uma lesão do lobo temporal produz alterações da memória semelhantes no homem e no camundongo. A interferência no cérebro do rato e do homem em determinado passo de uma rota bioquímica tem efeitos parecidos sobre a memória de ambas as espécies [11].

Muitos estudos em humanos e modelos animais estabelecem que o hipocampo executa um papel crítico na combinação de diferentes tipos de informações (“o que”, “onde” e “quando”), características da memória episódica. Segundo os autores dessa revisão, essa teoria propõe que esses tipos de informações são transmitidos ao hipocampo por diferentes circuitos neurais, envolvendo regiões parahipocampais circundantes [13].

Na memória episódica, “o que” envolve o córtex perirrinal. Este se projeta para o córtex entorrinal lateral (LEC), que, por sua vez, possui conexões mútuas com o hipocampo. Diferentemente, a informação “onde” envolve atividade no córtex parahipocampal (conhecido como córtex pós-rinal em roedores), que se projeta para o córtex entorrinal medial (MEC), que, por sua vez, tem conexões mútuas com o hipocampo. A informação “quando” permanece amplamente separada pelas vias LEC e MEC até ser combinada por projeções entorrinais no hipocampo. Considera-se que a atividade neural do hipocampo codifique, entre outras coisas, a identidade de itens e estímulos, a posição do animal no espaço, e o tempo decorrido em intervalos relevantes para a tarefa, propondo que o hipocampo processa diferentes tipos de informação [13].

Durante a codificação da memória, as informações sobre o contexto espacial ativam a região parahipocampal, que fornece informações ao córtex entorrinal medial (MEC), que, por sua vez, projeta-se para o hipocampo, no qual o ambiente espacial é parcialmente codificado (ou seja, o “onde”). Ao mesmo tempo, a informação sobre o conteúdo de um evento envolve o circuito do córtex perirrinal, córtex entorrinal lateral e hipocampo (“o que” e possivelmente “quando”). Essas duas vias convergem para as mesmas células da região do DG e CA3 do hipocampo, permitindo uma combinação das informações “o que”, “onde” e “quando” em representações conjunturais. Já neurônios da região CA1 do hipocampo recebem entradas de cada via, permitindo a separação entre informações espaciais de não espaciais [13].

A consolidação da memória envolve processos que ocorrem tanto durante a vigília como durante o sono em vários níveis de organização e função no cérebro, do molecular ao comportamental, e varia de segundos a meses e anos. Os mecanismos moleculares, sinápticos e celulares relativamente rápidos, provavelmente servem como procedimentos repetitivos nos mecanismos que incorporam consolidações mais lentas, nas quais as informações dependentes da experiência são redistribuídas pelos circuitos cerebrais [14].

Sabe-se que o hipocampo é uma estrutura cerebral responsável pelo aprendizado e pela memória, atuando especialmente na aquisição e consolidação de informações [15], e que o SNC com o envelhecimento natural e outras causas, como disfunções no funcionamento da insulina cerebral, síndrome metabólica, má alimentação, obesidade, diabetes mellitus, doença hepatica gordurosa não alcoólica e sedentarismo, pode desenvolver vários graus de disfunções e uma variedade de doenças neurodegenerativas, entre elas, Parkinson e Alzheimer [16, 17, 18].

1.3. DEMÊNCIA

A demência é caracterizada pela perda ou declínio da memória e outras habilidades cognitivas. É causada por diversas doenças, que resultam em danos nas células cerebrais, comprometendo as atividades da vida diária, sendo uma das principais causas de dependência, incapacidade e mortalidade [19, 20]. É atualmente a sétima principal causa de morte entre todas as doenças e tem impactos físicos, psicológicos, sociais e econômicos, não apenas para as pessoas que vivem com demência, mas também para seus cuidadores, famílias e sociedade [21].

Atualmente, cerca de 55 milhões de pessoas têm demência em todo o mundo, e há quase 10 milhões de novos casos a cada ano, com mais de 60% vivendo em países de baixa e média renda. Como a população de idosos está aumentando em quase todos os países, espera-se que esse número aumente para 78 milhões em 2030 e 139 milhões em 2050 [21].

1.4 DOENÇA DE ALZHEIMER

A Doença de Alzheimer (DA) é a principal causa de demência em todo o mundo, totalizando 60 a 70% dos casos. Segundo a Organização Mundial de Saúde (OMS), estima-se que existam 35,6 milhões de pessoas com DA no mundo, sendo que o número tende a dobrar até o ano de 2030 e triplicar até 2050 [21]. No Brasil, há cerca de 1,2 milhão de casos, a maior parte deles sem diagnóstico [22].

A DA foi primeiramente descrita em 1907 pelo médico alemão Alois Alzheimer, que relatou em seu artigo intitulado “Uma doença peculiar do córtex cerebral”, o caso de uma paciente de 51 anos de idade, que apresentou como primeiro sintoma dessa doença um forte sentimento de ciúmes pelo seu marido. Em seguida, ela passou a apresentar prejuízos crescentes de memória, delírios, desorientação, déficits de

linguagem e distúrbios comportamentais, continuou a declinar até ficar acamada e morrer. Após sua morte, a autópsia revelou um cérebro atrófico difuso, e o exame microscópio mostrou perda neuronal e alterações histológicas, hoje conhecidas como placas senis e emaranhados neurofibrilares, que se tornaram os principais indicativos patológicos da DA [2, 23, 19].

A DA é uma patologia neurodegenerativa e irreversível que ocasiona perda progressiva de memória, comprometimento cognitivo e alterações comportamentais, o que afeta severamente as atividades diárias, levando à morte [19].

A DA é classificada em esporádica ou familiar. A esporádica ocorre geralmente após os 65 anos de idade, sendo a forma mais comum da doença, responsável por 95% do total de casos, com etiologia complexa devido a interações entre componentes ambientais e genéticos, principalmente na presença do alelo E4 da Apolipoproteína E (APOE). A forma familiar normalmente se apresenta na meia-idade entre os 30 e 50 anos, representando os 5% restantes do total de casos, uma condição genética muito rara causada por uma mutação nos genes da Proteína Precursora Amiloide (APP) e Presenilina 1 e 2 (PS1 e PS2) [24, 25, 26].

As características neuropatológicas comumente encontradas no encéfalo de pacientes com DA são a presença de placas amiloides extracelulares compostas por peptídeo β-amiloide (Aβ), emaranhados neurofibrilares intracelulares e morte de neurônios [27].

As placas amiloides são produtos do metabolismo da APP. O domínio extracelular da APP é clivado pelas enzimas secretases do tipo α ou β, e a porção transmembrana remanescente é clivada pela secretase do tipo γ. A clivagem da APP pela α-secretase libera produtos solúveis, chamada de via não amiloidogênica, já na clivagem pela β-secretase (BACE 1), inicia um processo patogênico e amiloidogênico

da APP. Após a ação da β-secretase, o fragmento remanescente no interior do domínio transmembrana sofre ação da γ-secretase. A clivagem da γ-secretase formará peptídeos, que variam de 38 a 42 resíduos de aminoácidos (Aβ1-38, Aβ1-40 e Aβ1-42). O fragmento de 42 resíduos de aminoácidos (Aβ-42) é neurotóxico, agrega-se progressivamente em oligômeros, fibrilas, formando as placas Aβ insolúveis (placas senis), associada à doença de Alzheimer (Figura 7) [28, 29].

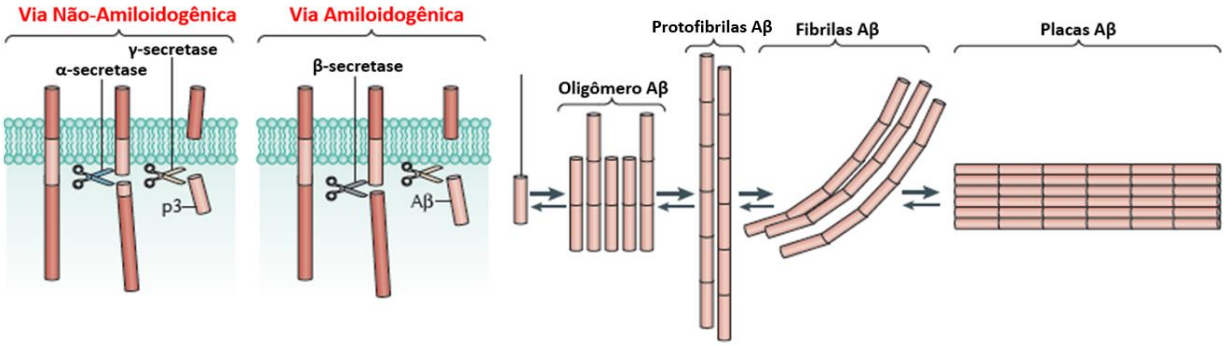

Figura 7: A clivagem da APP ocorre de forma não amiloidogênica (fisiológica) ou amiloidogênica (patológica) que inicia por ação da enzima β-secretase, o fragmento remanescente no interior do domínio transmembrana sofre ação da γ-secretase formando peptídeos de Aβ1-38 a Aβ1-42. O Aβ1-42 agrega-se progressivamente em oligômeros, fibrilas e posteriormente nas placas Aβ (Adaptado de Heppner, Ransohoff e Becher, 2015) [30].

Os emaranhados neurofibrilares (ENFs), outro agregado proteico, são constituídos por proteína tau hiperfosforilada. Em condições normais, a tau promove a montagem e a estabilização dos microtúbulos presentes no citoesqueleto dos neurônios que define a morfologia/estrutura normal dos neurônios e serve como trilhas para o transporte axonal. Além disso, as proteínas tau interagem com outros elementos do citoesqueleto para permitir o espaçamento entre os microtúbulos e serve como âncora para outras proteínas ou enzimas envolvidas na sinalização celular. Estudos recentes demonstraram que a tau também está ativamente envolvida na regulação da viabilidade e atividade celular [31, 32].

A hiperfosforilação da tau faz com que essa proteína se desprenda dos microtúbulos, provocando a sua desestabilização. Com isso, ocorre o sequestro de proteínas normais associadas aos microtúbulos, o que interrompe a dinâmica dos microtúbulos e compromete o transporte axonal (anterógrado e retrógrado). A tau hiperfosforilada, após se desprender dos microtúbulos, forma filamentos helicoidais pareados que se agregam, formando os emaranhados neurofibrilares, que contribuem para a disfunção sináptica e morte celular, observadas na DA (Figura 8) [32].

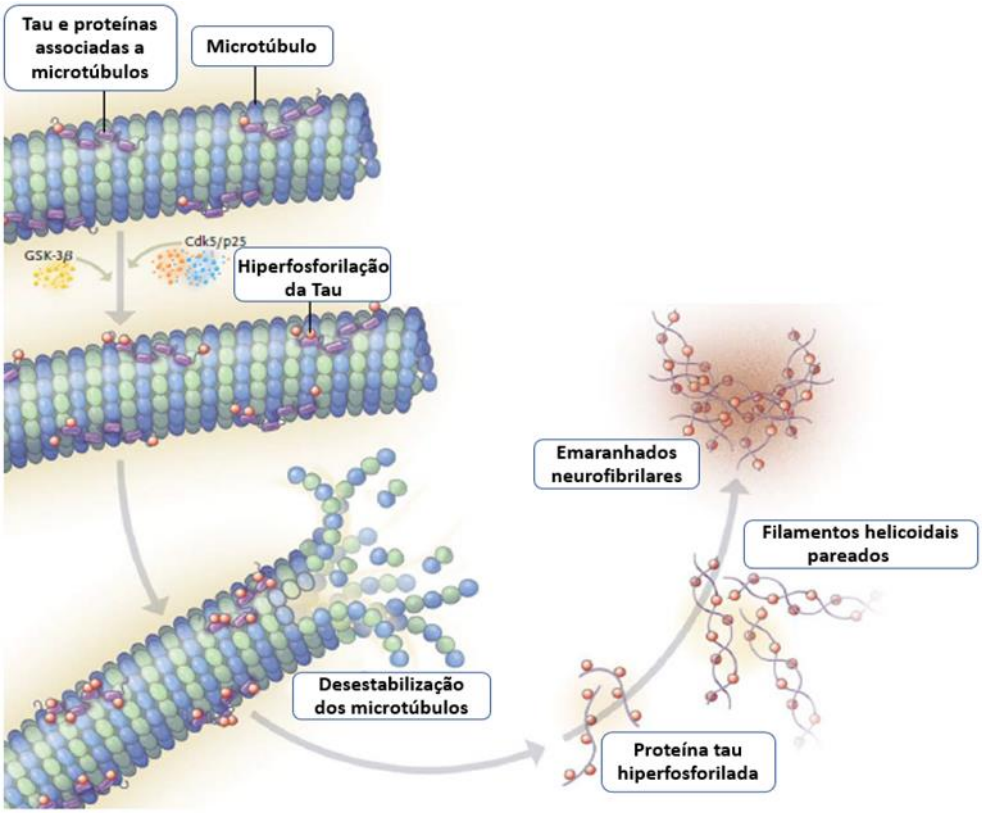

Figura 8: Hiperfosforilação da proteína tau (Adaptado de Querfurth e Laferla, 2010) [33].

1.5 MODELO ANIMAL DE DOENÇA DE ALZHEIMER INDUZIDA POR ESTREPTOZOTOCINA (STZ) INTRACEREBROVENTRICULAR

A STZ foi isolada de uma cepa de bactéria do solo *Streptomyces achromogenes* no final da década de 1950. É um composto glucosamina-nitrosoureia

inicialmente desenvolvido como antibiótico e posteriormente como um agente anticancerígeno. Em 1963, foi descoberto como diabetogênico em animais experimentais. Desde então, a aplicação sistêmica (via intraperitoneal) de STZ tornou-se o modelo experimental mais estudado de diabetes insulino-dependente (tipo 1) [34]. O STZ apresenta caráter hidrofílico devido à presença do grupamento hexose na sua estrutura química, que é de grande importância para ser internalizado pelas células, conforme Figura 9 [35].

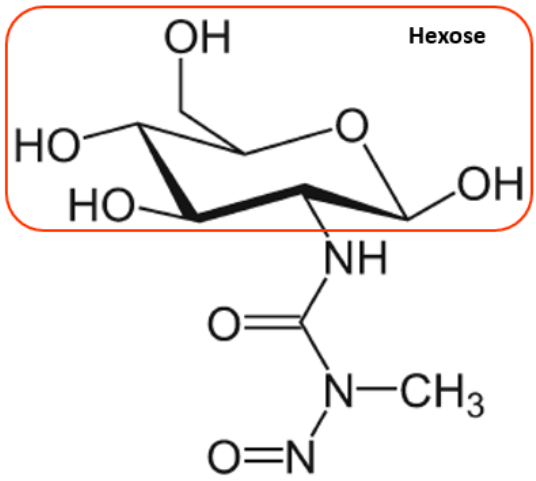

Figura 9: Fórmula estrutural da estreptozotocina (STZ) (Adaptado de Grieb, 2016) [36].

A injeção de STZ intracerobroventricular (ICV) altera o metabolismo cerebral de glicose [37] e piora a via insulina/ IGF (fatores de crescimento semelhantes à insulina), simulando algumas das alterações cerebrais observadas durante a DA [38]. Também induz à disfunção do receptor de insulina cerebral, um dos primeiros eventos observados na doença. Além disso, causa neuroinflamação, estresse oxidativo com expressão aumentada do gene pró-apoptose (p53), NOS e NOX-1, aumento da imunorreatividade de 4-Hidroxi-2-nonenal (HNE) e 8-hidroxidesoxiguanosina (8-OHdG), como ocorre na DA [18, 39]. Também ocorre aumento da população microglial e de astrócitos, aumento da atividade glicogênio sintase quinase 3 (GSK3β) e quinase

5 dependentes de ciclina (CDK5), déficits na função colinérgica, deficiências na aprendizagem espacial, memória e lesões histopatológicas, como na DA [18, 39, 40].

Levando em consideração que o hipometabolismo da glicose é um sinal precoce e persistente da DA e que os cérebros de pacientes com Alzheimer apresentam características de sinalização prejudicada da insulina, as injeções ICV de STZ são exploradas como um modelo de demência esporádica do tipo Alzheimer (SAD) (Figura 10) [36].

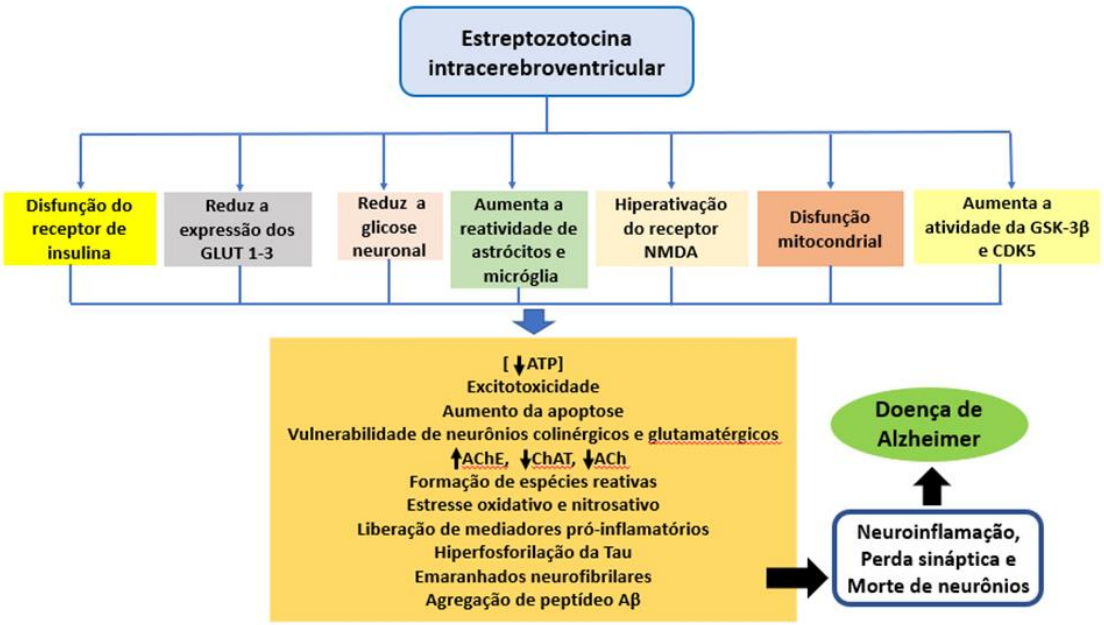

Figura 10: Mecanismo central de ação da estreptozotocina intracerebroventricular administrado em ratos.
Legenda: GLUT 1-3 (Transportadores de glicose 1-3); NMDA (N-metil-D-aspartato); GSK-3β (glicogênio sintase quinase 3 beta); CDK5 (quinase 5 dependente de ciclina); ATP (Adenosina trifosfato); AChE (acetilcolinesterase); ChAT (colina acetiltransferase) e ACh (acetilcolina) (Adaptado de Mehan, Arora, Sehgal, Sharma, Sharma, 2012; De La Monte e Tong, 2014; De La Monte, Tong, Lester-Coll, Plater e Wands, 2006; Mishra, Singh, Shukla e Shukla, 2018 e Yuliani, Lobentanzer e Klein, 2020)[41, 18, 39, 40, 42].

1.6 SINALIZAÇÃO DA INSULINA NO SNC

A insulina é um hormônio polipeptídico produzido e liberado pelas células β pancreáticas, que age tanto no cérebro como nos tecidos periféricos do nosso organismo [43]. Seu transporte para o cérebro é feito por um processo mediado por

receptores, por meio da barreira hematoencefálica (BHE) e pela insulina produzida pelos neurônios piramidais presentes no hipocampo, córtex pré-frontal e entorrinal [44, 45].

A insulina no SNC regula o desenvolvimento neuronal, modula vias de sinalização dos neurotransmissores e participa do aprendizado e da memória [46]. Em relação à memória, a insulina ativa cascatas de sinalização no hipocampo que afetam a plasticidade sináptica [47]. A insulina também regula a utilização de energia, função mitocondrial, autofagia, estresse oxidativo e função cognitiva [48].

A insulina liga-se ao seu receptor (RI), que está distribuído por todo o SNC e em maior densidade no hipocampo, amígdala, hipotálamo, córtex, córtex entorrinal, bulbo olfatório e cerebelo [49, 50]. O RI está, na sua maioria, localizado nos neurônios e concentrado nas sinapses em forma de componente da densidade pós-sináptica (PSD). A sinapse é um local importante na sinalização de insulina no cérebro [50]. Esse receptor é formado por uma proteína transmembrana de tirosina quinase, é composto por dímeros da subunidade α/β, que estão ligados por pontes dissulfeto, compostas por duas subunidades α extracelulares e duas subunidades β [48]. O local de ligação da insulina é com a subunidade α extracelular que ainda se liga à subunidade β. Cada subunidade β possui um domínio transmembranar e uma esfera de tirosina quinase intracelular que regula a autofosforilação. A ligação da insulina à subunidade α traz mudanças conformacionais indispensáveis para a autofosforilação de resíduos tirosina na região citosólica da subunidade β [51]. Os substratos autofosforilados da subunidade β são então identificados pelos substratos do receptor de insulina (IRS) IRS-1 e IRS-2, que são necessários para a propagação precisa da sinalização da insulina [52].

Igualmente à insulina, o fator de crescimento semelhante à insulina 1 (do inglês: *insulin-like growth factor-1*, IGF-1) também atravessa a BHE e se encontra presente no SNC [45]. Os fatores de crescimento semelhantes à insulina (IGFs) são proteínas

que se ligam aos receptores de insulina além dos receptores de IGF. Existem dois subtipos de IGF: IGF-1 e IGF-2. A insulina e os fatores de crescimento semelhantes à insulina (IGF-1) se conectam aos receptores de tirosina quinase [52]. A sinalização de insulina/IGF desempenha um papel crucial na regulação de diferentes funções do SNC [53], como cognitivas e comportamental (córtex cerebral e hipocampo), ligando a insulina ao domínio da cognição [49] (Figura 11).

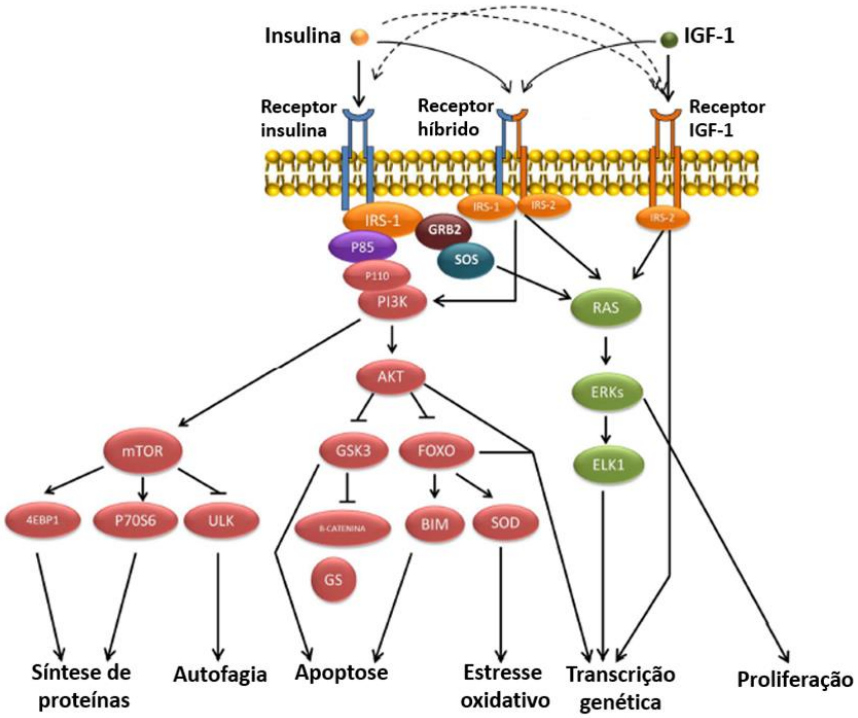

Figura 11: Transdução de sinais e ações biológicas induzidas por insulina ou IGF-1 por meio de seu receptor ou de seus receptores híbridos (Adaptado de Blázquez, Velázquez, Hurtado-Carneiro e Ruiz-Albusac, 2014) [54].

A insulina liga-se à subunidade-α do RI e ativa a tirosina quinase da subunidade-β, estimulando a auto fosforilação do seu receptor [46]. Os RIs fosforilados fosforilam, por sua vez, os respectivos substratos 1 e 2 (substratos do RI, RIS-1 e RIS-2), desencadeando a ativação da via fosfatidilinositol-3-quinase (PI3K). A ligação da PI3K aos RIS-1 e RIS-2 recruta a proteína quinase B (PKB ou Akt) para a membrana, na qual é fosforilada e ativada por proteínas quinases específicas (proteína quinase-

1 dependente de fosfoinositídeo ou PDK1). A PKB/Akt encontra-se envolvida na proliferação, crescimento e sobrevivência celular por meio da inibição de agentes pró-apoptóticos, como a FoxO (proteínas de fatores de transcrição) e a GSK3β (glicogênio sintase quinase 3β), protegendo assim a atividade neuronal [55, 56].

A proteína quinase ativada por mitógeno (MAPK) envolve a ativação da proteína Ras, que, consequentemente, ativa a Raf quinase *(*proteína quinase serina*/*treonina), que fosforila a MEK (proteína quinase ativada por mitógeno), que ativa a ERK *(*quinase regulada por sinal extracelular), induzindo a expressão genética e a proliferação, regeneração e reparo neuronal, (Figura 12) [56].

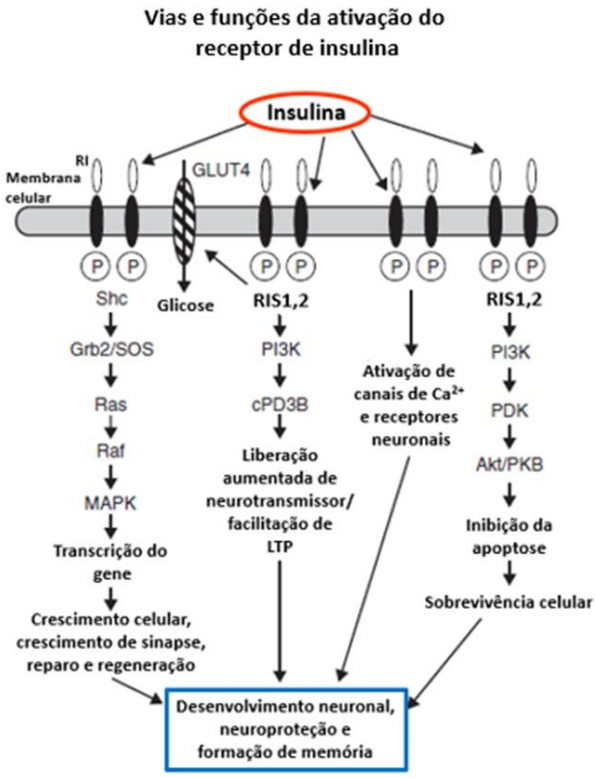

Figura 12: Sinalização da insulina nos neurônios. Legenda: RIS1,2- substrato do receptor de insulina 1 e 2; GLUT 4- transportador de glicose insulina-dependente; MAPK- proteínas quinases ativadas por mitógeno; PI3K- fosfatidilinositol-3 quinase (Adaptado de Hölscher, 2014) [57].

A insulina também regula a expressão e os níveis dos mecanismos mediados pelos receptores glutamatérgicos NMDA (N-metil-D-aspartato), AMPA (ácido-α-

amino-3-hidroxi-4-isoxazolpropiônico e GABA (ácido gama-aminobutírico), que têm influência sobre a potenciação de longo prazo (*long-term potentiation,* LTP) e depressão de longo prazo (*long -term depression*, LTD). Ainda, a insulina está crucialmente envolvida na expansão e preservação de sinapses excitatórias [58] e formação de espinhas dendríticas por meio da ativação de PI3K/Akt/mTOR e vias relacionadas a Ras [59, 60], que são essenciais para a sinalização da insulina [61]. A insulina também influencia a sobrevivência celular, modulando as vias apoptóticas e os intermediários envolvidos na cascata apoptótica [62].

1.7 SINALIZAÇÃO DA INSULINA NA DOENÇA DE ALZHEIMER

Muitas pessoas estão familiarizadas com o diabetes mellitus tipo 1 e 2, no entanto, existe outra forma de diabetes que foi identificada, conhecida como diabetes mellitus tipo 3 (DM3), que se manifesta como resistência à insulina no cérebro e tem grande potencial para impactar a neurocognição e contribui para a etiologia da doença de Alzheimer [63].

Nas últimas décadas, a descoberta da expressão do receptor de insulina (RI) em áreas do cérebro envolvidas em funções diferentes do controle da alimentação, como aprendizado e memória, revolucionou essa ideia e abriu caminho para a compreensão de como o cérebro é um órgão sensível à insulina [64, 65]. A plasticidade cerebral, a capacidade desse órgão de sofrer mudanças estruturais e funcionais em resposta a estímulos ambientais, é finamente modulada pela dieta e por hormônios dependentes de nutrientes, como a insulina [66].

A glicose é o combustível obrigatório para o cérebro e é crucial para a resistência neuronal, conexões sinápticas, produção de ATP e integridade da BHE.

Todas essas funções dependem da disponibilidade ótima de glicose, que depende de mecanismos de transporte regulados pela insulina e pelos receptores de insulina [67, 68]. Assim, a alteração na sinalização da insulina no SNC pode acelerar o envelhecimento, prejudicar a plasticidade cerebral e promover a neurodegeneração [69].

Na DA, as principais moléculas envolvidas na sinalização prejudicada da insulina são: RIS, PI3K, Akt e GSK-3β. A ativação ou inativação dessas moléculas por meio da fosforilação aumentada ou diminuída desempenha um papel nas anormalidades de sinalização ou na resistência à insulina [53, 70]. A resistência à insulina é a principal responsável pelas características da DA decorrentes de neuroinflamação e estresse oxidativo. Além disso, caspases, fator nuclear eritroide *2* relacionado ao fator *2* (Nrf2) e factor nuclear kappa B (NF-kB) influenciam essa via de forma indireta [53].

A resistência à insulina pode afetar as funções da memória ao limitar a captação de glicose por meio de níveis reduzidos de GLUT-1 e 3, e essas alterações são detectáveis na DA e se correlacionam com a densidade de emaranhados neurofibrilares e atrofia hipocampal [71, 49]. Além disso, a resistência à insulina regula negativamente a expressão dos receptores de insulina no endotélio dos capilares cerebrais, que, por sua vez, afetam a permeabilidade seletiva da BHE, levando a níveis diminuídos de insulina, o que afeta a atividade neuronal e glial [72, 73, 49].

A disfunção da sinalização de PI3K caracterizada pela resistência à insulina na DA ativa a via do GSK-3β que causa hiperfosforilação da proteína tau, e formação de ENFs. Além disso, a GSK-3β ativada também abre caminho para o aumento da liberação e acúmulo de placas amiloides, neuroinflamação, síntese reduzida de acetilcolina, o que, finalmente, leva aos sintomas patológicos de demência e comprometimento cognitivo da DA.

Além disso, descobriu-se que o Aβ ativa o GSK-3β, o que leva ainda à hiperfosforilação da proteína tau, ativação de caspase-3, fragmentação do DNA de neurônios e gliose, sendo que esses fenômenos agravam o processo neurodegenerativo [53] (Figura 13).

Também a desregulação da sinalização do mTOR (alvo em mamíferos da rapamicina) é responsável pelo envelhecimento, diabetes, câncer, obesidade, complicações cardiovasculares e neurodegenerativas, incluindo a DA. A ativação do mTOR aumenta a geração e a deposição de Aβ e contribui para a hiperfosforilação da tau [74].

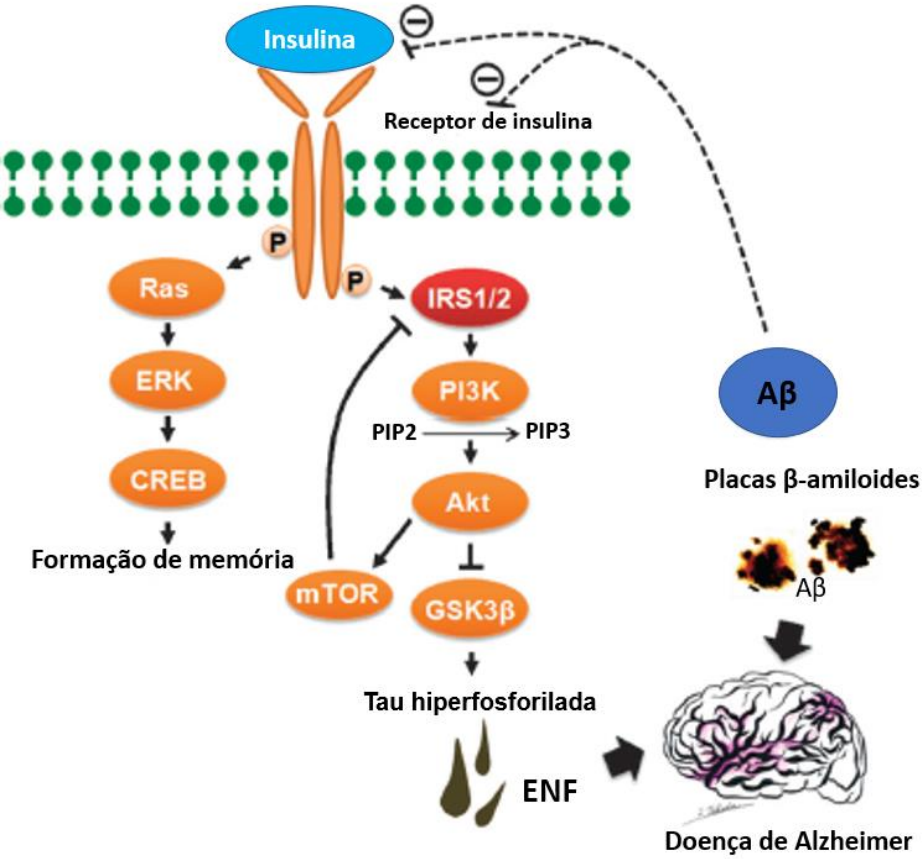

Figura 13: Sinalização da insulina, memória e DA. A sinalização da insulina modula a memória e a patologia da DA. A GSK-3β é um regulador chave da fosforilação da proteína tau. A tau hiperfosforilada é o principal componente dos emaranhados neurofibrilares (ENF). O peptídeo β-amiloide (Aβ) modula a sinalização da insulina e pode prejudicar o aprendizado e a memória.
Legenda: IRS – substrato do receptor de insulina; PI3K – fosfatidilinositol-3-quinase; ERK- quinase regulada por sinal extracelular; CREB - proteína de ligação ao elemento

de resposta do AMPc; PIP2 - fosfatidilinositol 4,5 bifosfato); PIP3 – fosfatidilinositol 3,4,5 trifosfatos; mTOR – alvo em mamíferos da rapamicina (Adaptado de Takeda, Sato, Rakugi e Morishita, 2011) [75].

A resistência à insulina é bem conhecida como uma característica essencial do DM3. Portanto, as estratégias de tratamento para DM3, particularmente aquelas destinadas a melhorar a sensibilidade à insulina, também podem beneficiar os pacientes com risco de DA nos estágios iniciais [63]. Devido às características patológicas entre diabetes mellitus, resistência à insulina e declínio cognitivo, terapias medicamentosas multidirecionadas, juntamente com intervenções no estilo de vida também são exploradas na perspectiva da pesquisa na indústria farmacêutica, incluindo atividade antioxidante, polifenóis, ácidos ômega-3, nutracêuticos, bem como as conexões cérebro-intestino com o uso dos agonistas do GLP-1 (peptídeo semelhante ao glucagon, uma incretina), como a exendina-4 (exenatida), liraglutida, semaglutida, entre outros [63, 76, 77, 78].

1.8 INCRETINAS

A palavra incretina é formada do inglês “**IN**testinal se**CRET**ion of **IN**sulin” [79]. As incretinas são hormônios peptídeos produzidos pelo trato gastrointestinal e pâncreas e que regulam o metabolismo da glicose. Essas moléculas são lançadas na circulação sanguínea em resposta à ingestão de nutrientes e aumentam a secreção de insulina, regulando o metabolismo glicolítico [80]. As incretinas foram identificadas na década de 30, mas suas propriedades insulinotrópicas foram reconhecidas nos anos 60 [81].

As principais incretinas são os peptídeos GLP-1 (peptídeo semelhante ao glucagon-1) e o peptídeo inibidor gástrico (GIP, também conhecido como: polipeptídeo

insulinotrópico dependente de glicose). Ambos são membros da superfamília de peptídeos do glucagon [82].

O GIP foi o primeiro hormônio incretina isolado do intestino delgado de suíno e, pela sua capacidade de inibir a secreção de ácido gástrico em cães, foi inicialmente chamado de polipeptídeo inibitório gástrico (GIP). Estudos posteriores revelaram que o GIP pode também estimular a secreção de insulina em animais e humanos [80]. Devido a sua ação incretina ocorrer em níveis fisiológicos, o hormônio foi então renomeado de polipeptídeo insulinotrópico dependente de glicose (do inglês *glucose-dependent insulinotropic polypeptide)* para refletir esta ação fisiológica, mantendo ainda sua sigla [80, 83, 84, 85, 86]. O GIP é secretado pelas células K, localizadas principalmente no duodeno e na parte proximal do jejuno [81].

O GLP-1 e GLP-2 foram descobertos a partir do precursor do glucagon, o pró-glucagon (PG), um polipeptídeo de 160 aminoácidos, codificado pelo gene do glucagon, produzido tanto nas células α das ilhotas de Langerhans como nas células L da mucosa intestinal. O PG possui três domínios importantes, ou seja, o próprio glucagon com resíduos de aminoácidos na sequência 33 e 61, e dois domínios com uma importante homologia (50%) de sequência com o glucagon, ambos cercados por pares de aminoácidos básicos (locais típicos de clivagem) ocupando posições 72-107/108 e 126-158 do PG, denominados, respectivamente, de GLP-1 e GLP-2 (Figura 14) [87, 88, 89, 90, 91].

Embora exista essa homologia, GLP-1 e GLP-2 foram testados quanto às suas ações insulinotrópicas, mas apenas o GLP-1 foi capaz de estimular a secreção de insulina [80, 85, 86]. Por esse motivo, o GLP-2 não é considerado um hormônio incretina [92]. O GLP-1 é secretado pelas células L, encontradas principalmente no íleo e no cólon [81].

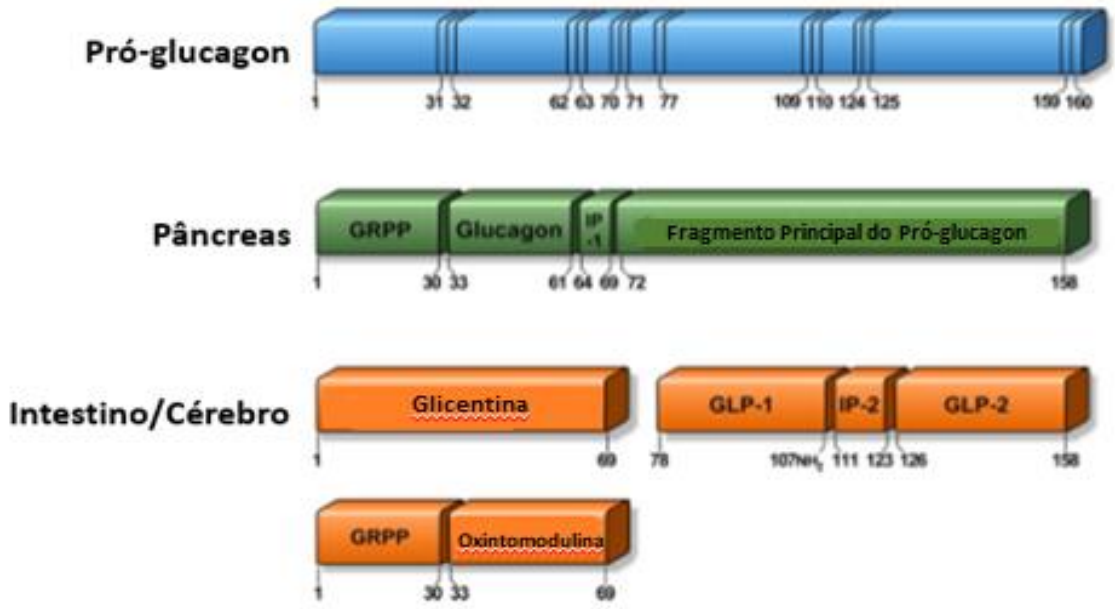

Figura 14: Processamento pós-traducional diferencial do pró-glucagon no pâncreas, estômago e cérebro. Os números indicam posições de aminoácidos na sequência de 160 aminoácidos do pró-glucagon, e as linhas verticais indicam as posições dos resíduos básicos de aminoácidos, locais típicos de clivagem.
Legenda: GRP- polipeptídeo pancreático relacionado com glicina; GLP-1 - peptídeo tipo glucagon-1; GLP-2 - peptídeo tipo glucagon-2; IP-1 -peptídeo interveniente-1 e IP-2 - peptídeo interveniente-2 (Adaptado de Holst, 2007) [87].

Como descrito anteriormente, o PG dá origem a uma série de hormônios peptídicos, como glucagon, GLP-1, GLP-2, glicentina, GRPP e OXM (Figura 13 e 14). Esses hormônios têm efeitos muito específicos e, em certa medida, opostos sobre a glicose e o metabolismo energético [93].

A especificidade tecidual da clivagem do PG é obtida pela expressão seletiva das enzimas PC. Assim, nas células α, o glucagon é clivado do PC2 ou PCSK2), enquanto o GLP-1, GLP-2, OXM e glicentina derivam do PG por meio da clivagem do PC1 ou PCSK1 mediada no cérebro e no intestino (Figura 15) [93, 80, 94, 95, 96].

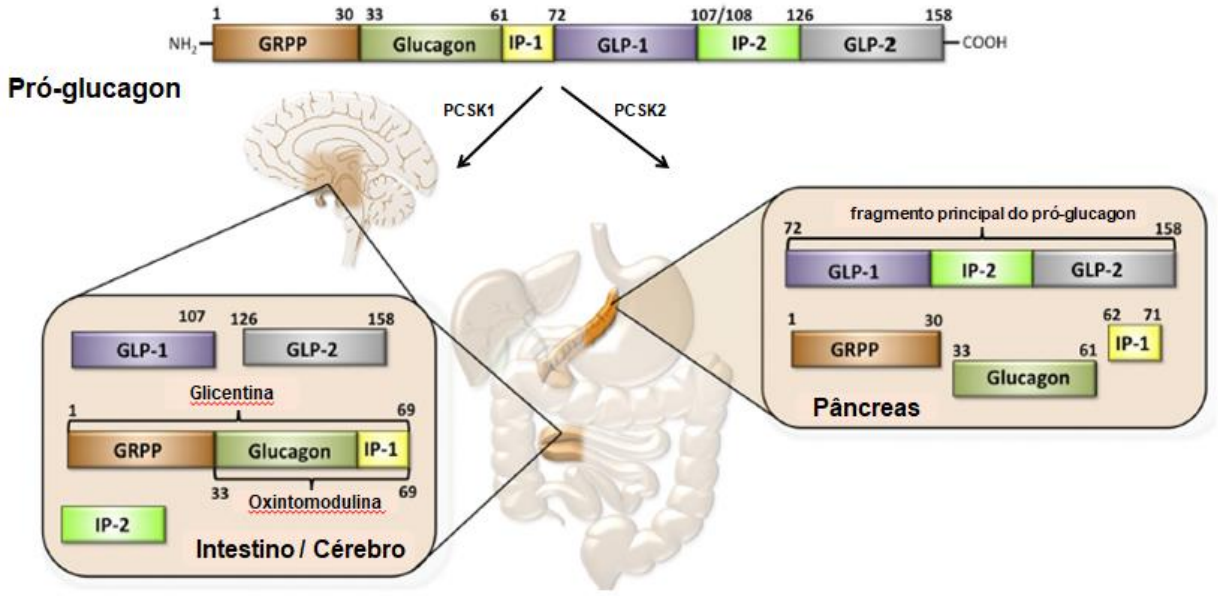

Figura 15: Esquema do processamento do pró-glucagon, por meio da ação de enzimas pró-hormônio convertases (PCs). No pâncreas, o pró-glucagon é clivado pelo pró-hormônio convertase 2 (PC2) em glucagon, polipeptídeo pancreático relacionado à glicentina (GRPP), o peptídeo interveniente 1 (IP-1) e um fragmento principal do pró-glucagon (MPGG). No intestino e no cérebro, o pró-glucagon é clivado pelo pró-hormônio convertase 1 (PC1) em glicentina, oxintomodulina (OXM), o peptídeo interveniente 2 (IP-2), GLP-1 e GLP-2 (Adaptado de Müller, Finan, Clemmensen, DiMarchi e Tschöp, 2017) [93].

Em relação aos aspectos moleculares, sabe-se que o gene do GIP está localizado no cromossomo humano 17q21.3-q22 e compreende seis exons [97]. O processamento proteolítico da pré-pró-GIP gera GIP secretado pelas células K. O gene do pró-glucagon está localizado no cromossomo humano 2q36-q37 e compreende seis exons. No intestino, o processamento proteolítico de PG gera GLP-1 e GLP-2, enquanto o glucagon é produzido no pâncreas [88, 89]. É importante ressaltar que mais de 70% do GLP-1 secretado pelas células L em humanos recebe uma amida na porção carboxila-terminal (Figura 16) [91]

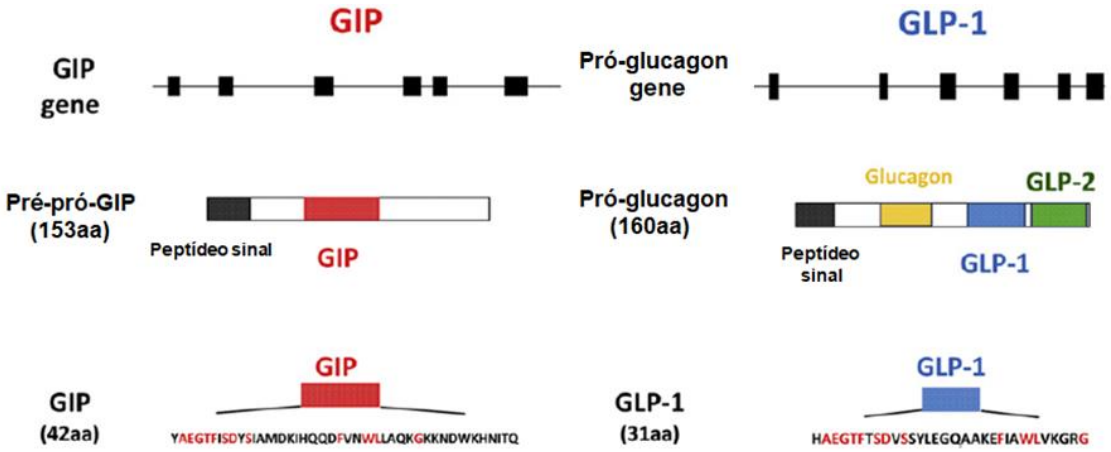

Figura 16: Os genes do GIP e do PG e suas localizações no cromossomo 17q21.3-q22 e 2q36-q37, respectivamente (adaptado de Yabe e Seino, 2011) [98].

O “efeito incretina" foi descrito em estudos que demonstraram que a glicose administrada por via oral resultou resposta insulínica (aumento de insulina sérica) maior do que a obtida com a administração intravenosa de glicose. Tal efeito ocorreu uma vez que a glicose via oral estimula as células L, presentes principalmente no íleo e no cólon, e as células K, presentes em regiões mais proximais (duodeno e jejuno) do intestino delgado. A ativação dessas células pela glicose aumenta os níveis de GLP-1, produzido pelas células L, e GIP, produzido pelas células K. Essas moléculas atuam nas células β-pancreáticas estimulando a produção e secreção de insulina, o que não ocorre quando a glicose é administrada via intravenosa [99, 100, 101, 102]. Nesse contexto, percebe-se o importante efeito das incretinas na resposta insulínica e estima-se que elas sejam responsáveis por aproximadamente 50 a 70% do total de insulina secretada após a ingestão oral de glicose [80]. Tal descoberta foi descrita por Nauck e colaboradores (1986)[103], que reportaram o efeito incretina após o consumo de glicose 25, 50 e 100g de glicose por via oral ou intravenosa sobre os níveis de insulina plasmática e peptídeo C, um produto formado pela clivagem da molécula pró-insulina para síntese de insulina. À medida que o tempo avançava, observaram um pico maior de insulina e peptídeo C séricos nos grupos “glicose oral” em relação ao grupo “glicose intravenosa” [103].

Os efeitos mediados pelas incretinas GIP e GLP-1 ocorrem por meio da interação hormônio-receptor. Os receptores específicos são: o receptor do peptídeo inibidor gástrico (GIPR) [104] e o receptor de GLP-1 (GLP-1R) [105]. Ambos pertencem à família de receptores acoplados à proteína G [106], que, por sua vez, ativam a enzima adenilato ciclase que eleva os níveis de AMPc citoplasmático nas células β pancreáticas. O aumento de AMPc engatilha a via de sinalização mediada pela proteína cinase dependente de AMP cíclico (PKA) e a proteína de troca ativada (Epac) pelo AMPc, o que leva a rápidos aumentos no cálcio intracelular e na liberação de insulina glicose-dependente via retículo endoplasmático rugoso [107]. Em paralelo, a PKA também ativa a via da MAPK, que tem papel central na proliferação de células beta pancreáticas, bem como na redução da apoptose celular (Figura 17) [107, 108].

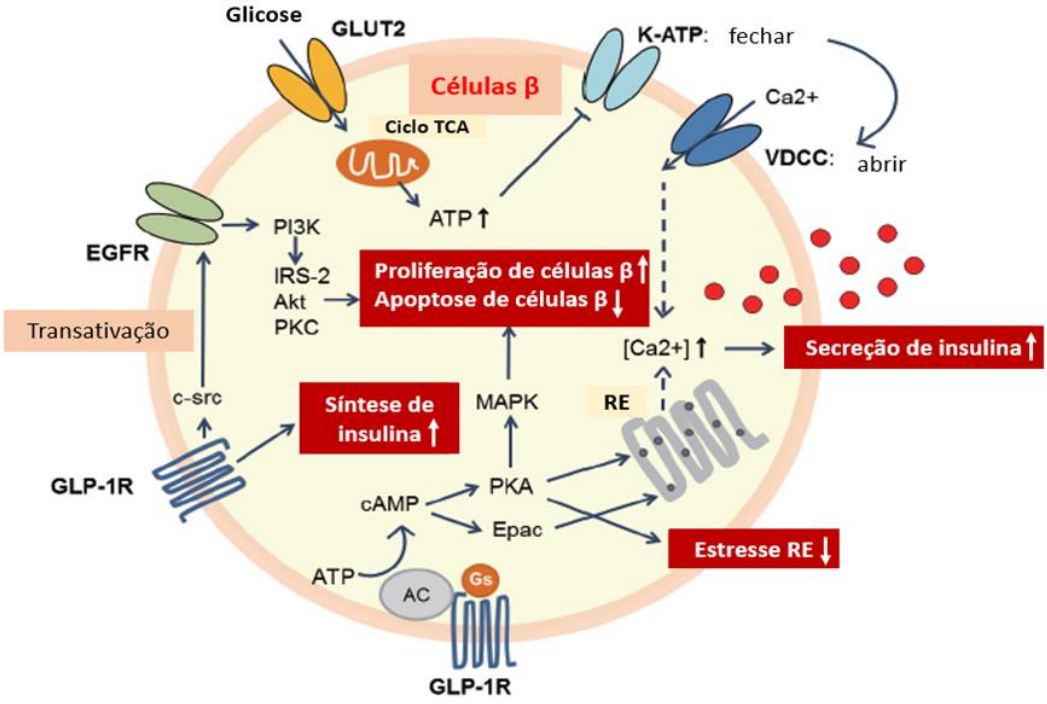

Figura 17: Efeito do GLP-1 e GLP-1R sobre a síntese e secreção de insulina.
Legenda: GLUT2, transportador de glicose 2; K-ATP, canal de potássio sensível a ATP; TCA, ácido tricarboxílico; EGFR, receptor do fator de crescimento epidérmico; VDCC, canais de cálcio dependentes de voltagem; PI3K, fosfatidilinositol-3 quinase; IRS, substrato do receptor de insulina; PKC, proteína quinase C; MAPK, proteína quinase ativada por mitógeno; RE, retículo endoplasmático; cAMP, monofosfato de adenosina cíclico; PKA, proteína quinase A; AC, adenilato ciclase; Epac, proteína de troca ativada por cAMP (Adaptado de Jung, Kim e Lim, 2014) [109].

Os receptores GLP-1 (GLP-1Rs) podem ser encontrados não apenas nas células pancreáticas, mas também nas células miocárdicas, endotélio vascular e SNC. No SNC, os receptores do GLP-1 (GLP-1R) são expressos em várias regiões do cérebro de humanos, primatas e roedores, incluindo o hipocampo, córtex, hipotálamo, cerebelo, células de Purkinje, núcleo do trato solitário (NTS), neocórtex, gânglios da base, axônios e dendritos. Existem evidências crescentes de que os astrócitos e micróglia expressam GLP-1Rs e que, em lesão e neuroinflamação, elevam sua expressão [110, 111, 112, 113].

Os miméticos de GLP-1 podem proteger neurônios cultivados de estressores, reduzir a apoptose e aumentar a divisão celular [57]. Eles também protegem as sinapses dos efeitos prejudiciais de Aβ na plasticidade sináptica no hipocampo. Além disso, podem atravessar a barreira hematoencefálica, uma propriedade importante para o tratamento de distúrbios neurodegenerativos do SNC [79].

O GLP-1, assim como a insulina e o IGF-1, ativa as vias de sinalização de segundo mensageiro, que são comumente ligadas à sinalização do fator de crescimento nos neurônios [110]. Os efeitos neuroprotetores da ligação entre o GLP-1 e seu receptor (GLP-1R), mediado pelas vias PKB/Akt e MAPK/ERK, ativa uma proteína G/ adenilato ciclase (AC), o que resulta aumento no AMPc, levando a eventos intracelulares, como sobrevivência celular, inibição da apoptose, ativação de canais de Ca^{2+}, crescimento celular, reparo e regeneração, liberação aumentada de neurotransmissor/facilitação de potenciação a longo prazo (*long-term potentation*, LTP). Dessa forma, resulta em desenvolvimento neuronal, neuroproteção e formação de memória (Figura 18) [57].

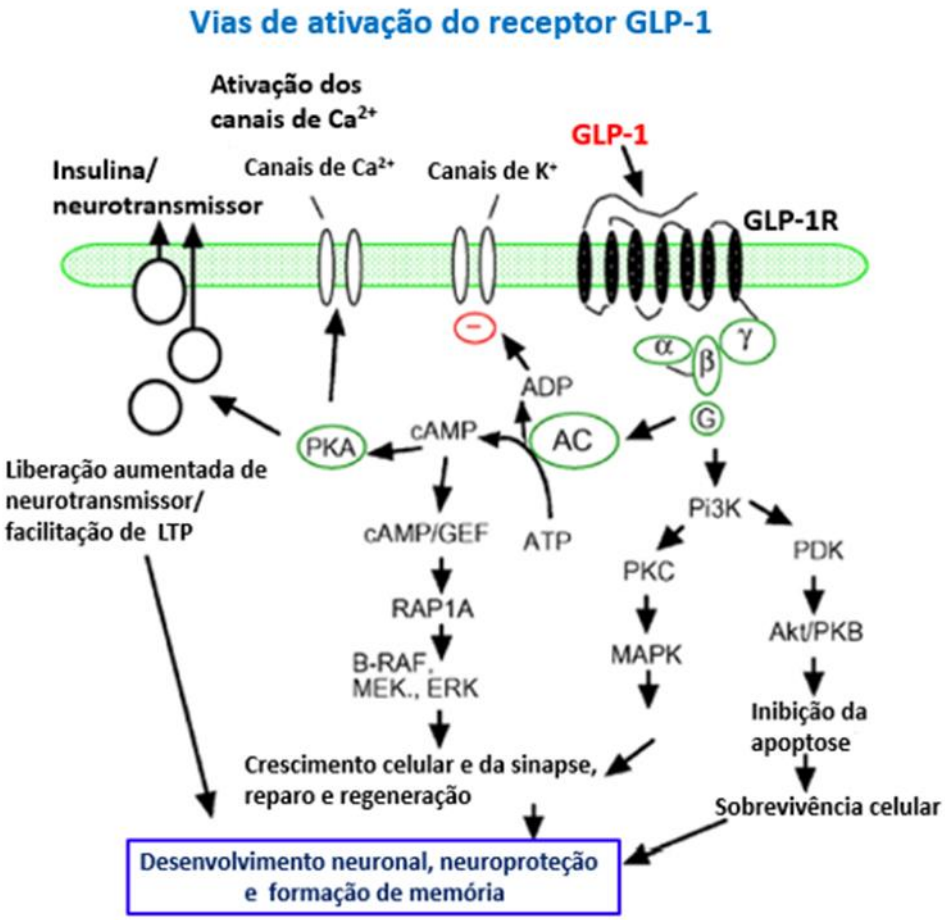

Figura 18: Sinalização celular relacionada ao fator de crescimento ativada por receptores GLP-1 em neurônios.
Legenda: adenil- ciclase (AC); adenosina difosfato (ADP); proteína quinase b (AKt); regulação B-Raf B de alfa-fetoproteína; monofosfato de adenosina cíclico (cAMP); quinase regulada por sinal extracelular (ERK); fatores de troca de nucleotídeos de guanina (GEFs); peptídeo-1 semelhante ao glucagon (GLP-1); receptor do peptídeo 1 semelhante ao glucagon (GLP-1R); potenciação a longo prazo (LTP); proteínas quinases ativadas por mitógeno (MAPK); quinase (MEK); quinase dependente de fosfatidilinositol (PDK); fosfatidilinositol - 4,5-bifosfato 3-quinase alfa (Pi3Ka); proteína quinase A (PKA); proteína quinase B (PKB); proteína quinase C (PKC); proteína membro da família de oncogenes RAS (Rap1A) (adapatado de Calsolaro e Edison, 2015)[110.]

O GLP-1 exerce uma variedade de efeitos metabólicos em diferentes órgãos do corpo por meio do “tamponamento” glicêmico. No cérebro, atua reduzindo o apetite, o consumo alimentar e aumentando a neuroproteção (Figura 19) [114].

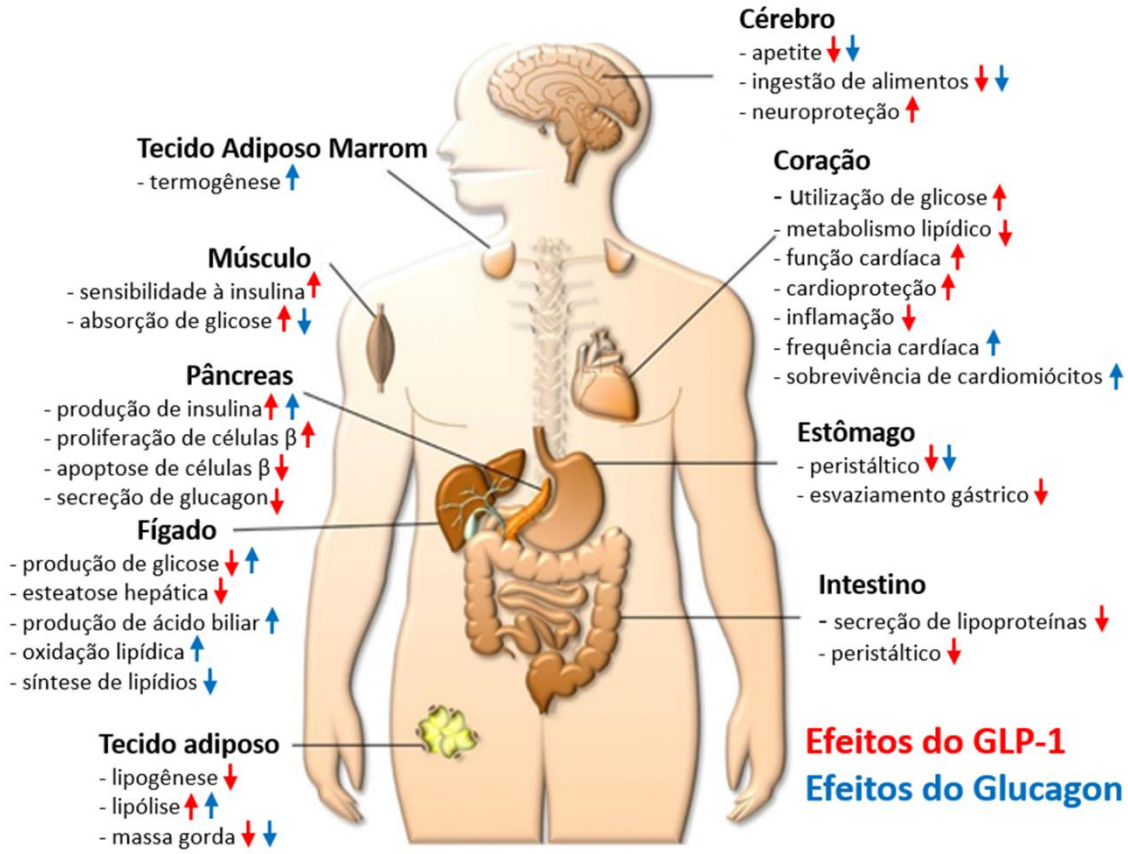

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Figura 19: Efeitos do GLP-1 e glucagon sobre os diferentes órgãos (Adaptado de Müller, Finan, Clemmensen, DiMarchi e Tschöp, 2017) [93].

Os efeitos neurotróficos e neuroprotetores do GLP-1 foram caracterizados em vários estudos pré-clínicos *in vitro* e *in vivo*, com o uso de agonistas do receptor de GLP-1 (GLP-1RA) e inibidores da dipeptidil peptidase-4 [115].

Em modelos experimentais da DA em camundongos, os análogos de GLP-1 mostraram melhora no aprendizado e na memória [116], atuar como fator de crescimento no cérebro, formando sinaptogênese, neurogênese e protegendo contra lesão oxidativa. Também foi observada redução dos níveis de marcadores patológicos da DA, incluindo Aβ oligomérico e placas Aβ, bem como diminuição da ativação microglial e melhora da memória [117].

Dois agonistas do GLP-1R, liraglutida e exenatida, em modelos de DA em camundongos, antagonizaram processos ligados à neurodegeneração e progressão da doença, mesmo na ausência de diabetes [117, 118].

Os efeitos positivos dos agonistas do receptor do GLP-1 em doenças neurodegenerativas se devem à melhora na sinalização da insulina, nas funções neurocognitivas, neuroproteção, neurogênese, proliferação, diferenciação e diminuição da neuroinflamação [79].

Análogos do GLP-1 administrados perifericamente, incluindo a EXE-4 e liraglutida, atravessam a BHE [119, 120] e, portanto, são capazes de se ligar a receptores de GLP-1 amplamente encontrados no cérebro, bem como nas células piramidais do córtex cerebral e formação hipocampal [112].

1.9 EXENDINA-4

Por meio do estudo da ecologia pôde-se obter pistas interessantes para a descoberta de novos medicamentos, como no caso da EXE-4, um fármaco derivado do veneno do monstro-de-gila (*Heloderma suspectum*) (Figura 20), um réptil venenoso nativo do deserto do sudoeste dos Estados Unidos e norte do México, que pode suportar longos períodos de jejum, comendo apenas três ou quatro vezes por ano. As bases fisiológicas para esse notável comportamento alimentar foram atribuídas à presença de um hormônio salivar EXE-4 que retarda a digestão e a absorção dos alimentos [121].

Uma forma sintética da EXE-4 (exenatida, Byetta®) foi aprovada pela FDA (Food and Drug Administration), em abril de 2005, para o controle do diabetes mellitus tipo II em pacientes cuja glicemia não pode ser controlada com agentes diabéticos orais (metformina, sulfonilureias ou tiazolidinedionas) isoladamente [122].

Figura 20: Monstro-de-Gila (*Heloderma suspectum*), extraído de um banco de imagens sem direitos autorais (https://pixabay.com/).

A EXE-4 é um polipeptídeo de 39 aminoácidos, de sequência homóloga em 50% ao GLP-1 e, devido a isso, exibe propriedades biológicas bem semelhantes (Figura 21). Eng *et al.*[121] fazem parte do grupo dos primeiros pesquisadores a elucidar o papel biológico das exendinas. Primeiramente, seu estudo investigou os peptídeos exendina-3 e EXE-4. Após o isolamento desses peptídeos, Eng relatou a presença de 39 aminoácidos contendo uma histidina amino-terminal e uma amida carboxi-terminal. Aplicando esses peptídeos em células acinares pancreáticas, a exendina-3 e a EXE-4 estimularam a liberação de amilase em concentrações crescentes e aumentaram os níveis de AMP cíclico celular. No entanto, a EXE-4 difere da exendina-3 por apenas duas substituições de aminoácidos nas posições 2 e 3 da porção N-terminal. A partir desse achado, Eng relatou a possibilidade de existir um suposto receptor para exendinas nas células acinares pancreáticas (modelo experimental *Cavia porcellus*) [121].

A presença desse receptor também foi determinada por Eng a partir do uso de um inibidor específico, a exendina (9-39) amida, molécula desenhada para ser um análogo das exendinas. Eng verificou que as exendinas (9-39) amida aboliram o aumento no AMPc induzido por exendina-3 e EXE-4. Após essa descoberta, o autor

prediz a existência de um análogo endógeno em mamíferos para os peptídeos de exendinas bem como um receptor para exendinas[121]. Em seguida, Raufman e colaboradores (1992)[123] descobriram que a exendina (9-39) amida era capaz de bloquear o efeito do GLP-1 humano em ácinos pancreáticos. Essa descoberta foi vital para compreender o local em que as exendinas atuariam. Os autores constataram que o GLP-1 pode atuar no mesmo receptor que a exendinas-3 e a EXE-4[121, 123].

Figura 21: A comparação de sequência entre EXE-4 e o GLP-1. Os asteriscos vermelhos indicam os resíduos idênticos compartilhados entre a EXE-4 e o GLP-1. EXE-4 também tem uma extensão C terminal que está ausente no GLP-1 (Adaptado de Yap e Misuan, 2019) [124].

A possibilidade de as exendinas atuarem no mesmo receptor que o GLP-1 veio a ser confirmada em seguida por Rai e colaboradores [125]. Experiências utilizando estômago de cobaias permitiram descobrir que GLP-1, exendinas-3 e 4 aumentavam as concentrações de AMPc celular e secreção de pepsinogênio. Essas ações foram progressivamente inibidas pelo aumento das concentrações do antagonista exendina-(9-39) amida. Esses achados clarificam o sítio de ação das exendinas, confirmando que estas compartilham o mesmo receptor que o GLP-1 [125].

Na busca para compreender melhor o sítio de ligação das exendinas, Goke e colaboradores [126] descobriram que o sítio de ligação da EXE-4 ocorre no receptor para GLP-1. Utilizando moléculas marcadas radioativamente, Goke e colaboradores (1993) [126] demonstraram que a EXE-4 e a exendina-(9-39) amida interagem especificamente com o receptor do GLP-1 nas células derivadas do insulinoma e em membranas pulmonares. Essa evidência aponta que a EXE-4 é um agonista, e a exendina (9-39)-amida é um

antagonista específico do receptor de GLP-1[126]. Posteriormente, Schepp e colaboradores confirmaram essa descoberta utilizando os peptídeos marcados radioativamente em células parietais gástricas de ratos [127].

Após as evidências de que EXE-4 atua sobre os receptores GLP-1, Kolligs e colaboradores [128] verificaram a possibilidade de um efeito incretina exercido por estes peptídeos. Administração de glicose intraduodenal e intravenosa foram realizadas em ratos e foi feita uma comparação sobre níveis de insulina. A glicose intraduodenal elevou os níveis de insulina numa concentração maior que a induzida pela via intravenosa, e esses efeitos foram mais pronunciados na ação do GLP-1 e da EXE-4. Após confirmado um efeito incretina, os autores verificaram também que a exendina (9-39) amida aboliu o efeito estimulante da insulina. Esses dados apoiam o conceito de que o GLP-1 e a EXE-4 são importantes hormônios incretina [128].

Uma vez estabelecido o papel da EXE-4 como um agonista dos receptores de GLP-1, Goke e colaboradores descobriram a presença de receptores para GLP-1 no sistema nervoso. Utilizando autorradiografia quantitativa *in vitro*, os pesquisadores detectaram uma alta densidade de locais de ligação específicas para o GLP-1 visto no lobo pituitário, lobos anterior e intermediário [129], septo lateral, no órgão subfornical (SFO), no tálamo, no hipotálamo, no núcleo interpenduncular, no núcleo tegmentar póstero-dorsal, na área postrema (AP), o NTS e regiões circunventriculares (2. Goke, et. al., 1995). Estudos de ligação com [125I] [Tyr 39] exendina-4 (uma exendina-4 marcada) mostraram um padrão de distribuição idêntico nos sítios de ligação para GLP-1 [130].

A EXE-4 é mais potente que o GLP-1 para aumentar a síntese de insulina dependente de glicose, diminuir a produção de glucagon e retardar o esvaziamento gástrico. Além disso, a exendina-4 tem maior duração de ação que o GLP-1, com

meia-vida superior a 2 horas versus 1-2 minutos para o GLP-1, sendo resistente à inativação enzimática pela dipeptidil peptidase-4 (DPP-4) [122]. A DPP-4 é a enzima que metaboliza e inativa o GLP-1 alguns minutos após sua secreção [110].

1.10 EXENDINA-4: EVIDÊNCIAS NO SNC

GLP-1 e a EXE-4 podem atuar como agentes neurotróficos regulando a proliferação celular, neurogênese, o crescimento neurítico e protegendo neuritos em condições patológicas [131, 132]. A EXE-4 na dose de 1 ug/kg administrada via i.p. em ratos machos durante 21 dias aumentou o número de células imunorreativas para Ki67 (1,51 vezes em relação ao controle), doublecortina (DCX, 2,5 vezes em relação ao controle) e 5-bromo-2-desoxiuridina (BrdU, 2,46 vezes em relação ao controle) na SGZ do DG [133.] Nesse estudo, fica claro que EXE-4 aumenta a diferenciação neuroblástica e a proliferação celular na SGZ do DG e sugere que a EXE-4 promove plasticidade estrutural no DG [133].

Em seguida, Hamilton e colaboradores [134] estimaram o número de células progenitoras ou de neurônios jovens positivos no DG para três modelos de ratos diabéticos. EXE-4 na dose de 25 nmol/Kg administrada via subcutânea durante quatro semanas também aumentou o número de células BrdU e DCX positivas em relação aos animais diabéticos e controle. Além disso, o antagonista exendina-9-36 (outro antagonista para exendina-4) reduziu a proliferação de células progenitoras nesses animais. Os resultados demonstram que os agonistas do GLP-1R são promissores como tratamento para doenças neurodegenerativas, uma vez que atravessam a BHE e aumentam a proliferação celular e neurogênese hipocampal [134]. Após verificar os efeitos da EXE-4 sobre a proliferação celular e neurogênese, novos estudos

mostraram o papel desse agonista do GLP1-R sobre parâmetros neurotróficos. EXE-4 foi incubada com neurônios do gânglio da raiz dorsal de ratos (DRG) adultos e células PC12. Nessa experiência, foi detectada a presença de GLP-1R, predominantemente localizado em neurônios peptidérgicos *in vivo* e *in vitro*, e viram que EXE-4 mostrou efeitos dose-dependentes (concentrações 1 ≤ 10 ≤ 100 nM), promovendo crescimento de neuritos e sobrevivência neuronal em dois e sete dias, respectivamente [135]. Além disso, o tratamento com 100 nM de EXE-4 restaurou o crescimento reduzido dos neuritos e aumentou a viabilidade de neurônios DRG causados pela remoção de insulina do meio. Buscando compreender melhor esse achado, os autores descobriram que a EXE-4 foi capaz de suprimir a atividade de RhoA, um regulador inibidor da regeneração de nervos periféricos, em células PC12. Além disso, os efeitos benéficos da EXE-4 foram atenuados quando ocorreu um co-tratamento com o inibidor (LY294002) da PI3K. Possivelmente, EXE-4 aumenta o crescimento de neuritos e a sobrevivência neuronal por meio da ativação da via de sinalização PI3K/RhoA. A EXE-4 e outros agonistas do GLP-1R podem compensar os efeitos reduzidos da insulina nos neurônios, sendo, portanto, benéficos para condições patológicas que incluem a redução de neuritos, como na neuropatia diabética [135].

A caracterizaram morfológica, estrutural e funcional das ações da EXE-4 usando um modelo de célula neuronal humana conhecida como células SH-SY5Y, e aumentou o número de neuritos paralelos por modificações na distribuição de actina e tubulina intracelular. Além disso, análises eletrofisiológicas mostraram um aumento dos canais iônicos na superfície da membrana celular, bem como sua sensibilidade. Em relação à atividade desses canais, foi encontrada uma condutância aumentada

dos canais de Na^+ e na amplitude das correntes de Ca^{2+} (tipos T e L, canais típicos de neurônios numa forma (ou fenótipo) mais madura) [131].

A EXE-4 administrada subcutaneamente foi benéfica em casos de traumatismo cranioencefálico leve. Quando administrada 48 horas antes ou 2 horas após o trauma, a EXE-4 impediu a indução de déficits cognitivos e as alterações induzidas pelo trauma na marcação para sinaptofisina no córtex cerebral e hipocampo. E *in vitro*, a EXE-4 protegeu neurônios do encolhimento neurítico e morte celular. O tratamento de culturas de HT22 com EXE-4 (25 a 100 nM), antes da lesão, atenuou os efeitos citotóxicos de lesões por estiramento biaxial e preservou o comprimento dos neuritos [132].

A literatura também aponta que a EXE-4 promove a regeneração em lesão de nervo periférico, porque pode atenuar a neuropatia sensorial periférica [136], além de acelerar o crescimento de neuritos em neurônios ganglionares cultivados, melhorar a percepção da dor e a condução neuronal motora e sensorial, mas seu mecanismo de ação não está elucidado [137]. Com isso, na tentativa de elucidar o mecanismo de ação da EXE-4, no tratamento de lesão de nervo periférico, e observou-se que houve regeneração dos nervos periféricos, proliferação e migração de células de Schwann por meio da via de sinalização Jak-STAT. Assim, esses resultados fornecem uma base e direção para melhor compreender os mecanismos da EXE-4 no reparo de lesão de nervo periférico e uma nova abordagem para o tratamento [138].

Na pesquisa dos efeitos do GLP-1R na nocicepção, cognição e neuroinflamação na dor crônica, foi utilizado um modelo de ligadura do nervo espinhal em ratos tratados com EXE-4 (10 μg/kg) injetada continuamente via subaracnóidea de 10 a 21 dias após a ligação do nervo espinhal. Com isso, observou-se que a EXE-4 atenuou o comprometimento cognitivo induzido pela dor ao aliviar a neuroinflamação do hipocampo. Assim, os resultados encontrados sugerem que a EXE-4 pode aliviar

as respostas neuroinflamatórias induzidas pela dor e promover a recuperação da função cognitiva por meio da via do GLP-1R[139].

Na investigação do efeito neuroprotetor de drogas hipoglicêmicas, como a insulina e EXE-4, foi usado um modelo *in vitro* da doença de Huntington, desenvolvido por neurônios dopaminérgicos diferenciados, tratados com o composto neurotóxico pró-oxidante 6-hidroxidopamina. Com essa investigação, os resultados demonstraram que insulina e EXE-4 reduziram a acúmulo de huntingtina mutante em células neuronais[140].

Para investigar os efeitos neuroprotetores do tratamento agudo com EXE-4, em um modelo animal de inflamação, utilizaram-se, no estudo, diferentes doses (0,1; 0,3 ou 0,5 µg/Kg) via intraperitoneal. Foram utilizados ratos *Wistar* machos, que receberam o lipopolissacarídeo (LPS) via intraperitoneal na dose de 0,25 mg/Kg e tratados com EXE-4. O fator neurotrófico derivado do cérebro (BDNF), as espécies reativas ao ácido tiobarbitúrico (TBARS) e a interleucina-6 (IL- 6) foram quantificados no soro e no hipocampo. A dose mais alta de EXE-4 reduziu a IL-6 no hipocampo dos animais que receberam LPS, e a EXE-4 *per se* reduziu os níveis séricos de TBARS com um efeito antioxidante moderado nos grupos LPS. Já os níveis de BDNF no hipocampo pareciam estar aumentados no grupo LPS+EXE-4 em comparação com o grupo LPS. Com isso, esse estudo fornece evidências sobre o efeito anti-inflamatório da EXE-4 no hipocampo de um modelo animal para inflamação[141].

Para verificar os efeitos do tratamento diário com EXE-4, na função cognitiva e plasticidade sináptica em um modelo de obesidade induzido por uma dieta rica em gordura, foram utilizados camundongos, que receberam a dieta e foram tratados com EXE-4 (25 nmol kg-1 de peso corporal; duas vezes ao dia) ou veículo (solução salina 0,9% p/v) durante 21 dias. Com isso, os autores do estudo observaram que, além de

melhorar o controle metabólico, os camundongos tratados com EXE-4 exibiram um aumento acentuado no índice de reconhecimento, destacando o aprendizado e a memória, e uma recuperação na eliminação da potenciação eletrofisiológica em longo prazo *in vivo*, causada pela dieta rica em gordura. Assim, a pesquisa mostra que a terapia com EXE-4 melhorou a função cognitiva e a plasticidade sináptica hipocampal, prejudicadas na obesidade [142].

Para investigar se a esclerose múltipla (EM) poderia ser um complemento para os distúrbios do SNC que estão associados ao eixo de sinalização GLP-1/GLP-1R, uma vez que as proteínas GLP-1 e GLP-1R estão expressas em neurônios, astrócitos e micróglia na medula espinhal de camundongos normais, Leo e colaboradores (2018) induziram encefalomielite autoimune experimental (EAE) em um modelo animal, e os camundongos com sintomas de EAE foram tratados diariamente com EXE-4 na dose de 5 µg / kg via intraperitoneal durante 13 dias. Com isso, observaram que a administração de EXE-4 melhorou significativamente os sinais clínicos da doença, juntamente com a reversão de sequelas histopatológicas, como acúmulo de células, desmielinização, astrogliose, ativação microglial e transformação morfológica da micróglia ativada na medula espinhal lesada. Essa melhora apresentada pela EXE-4 foi comparada à do fármaco utilizado para EM, o fingolimod (FTY720) na dose de 3mg/ kg, via intraperitoneal. Os efeitos neuroprotetores da EXE-4 contra a EAE também foram associados à diminuição da expressão de mRNA de citocinas pró-inflamatórias, como interleucina (IL) - 17, IL-1β, IL-6 e fator de necrose tumoral (TNF)-α. Assim, o estudo demonstrou que a ativação do eixo de sinalização GLP-1 / GLP-1R empregando EXE-4 tem potencial terapêutico contra a EAE monofásica, juntamente com respostas neuroinflamatórias reduzidas [142].

Para analisar os efeitos e os mecanismos de GLP-1, e especialmente de seu análogo de longa ação, a EXE-4, na viabilidade de células neuronais, sob condições de hiperglicemia ou estresse oxidativo *in vitro* e os efeitos de EXE-4 sobre o déficit de memória, alterações histopatológicas, hiperfosforilação da tau e a principal atividade GSK-3β, em um modelo induzido por dano na sinalização de insulina cerebral *in vivo*, utilizaram ratos *Wistar* machos que receberam injeção STZ - ICV (3 mg/Kg de peso corporal, 6µl/ ventrículo) e tratados com EXE-4 (10µg/Kg de peso corporal) por via subcutânea duas vezes ao dia, durante 14 dias. Então, observaram que, tanto o GLP-1 como a EXE-4, melhoraram a lesão induzida pelo estresse oxidativo em células PC12 e exerceram um efeito protetor contra a redução da viabilidade das células PC12 causada pela hiperglicemia e que esse efeito foi mediado pela via PI3K. Além disso, a avaliação histopatológica confirmou os efeitos protetores do tratamento com EXE-4 nos neurônios do hipocampo. A EXE-4 também reverteu a hiperfosforilação da tau induzida por STZ- ICV por meio da regulação negativa da atividade de GSK-3β, uma quinase chave tanto na DM2 como na DA [143].

No tratamento da disfunção cognitiva, como a DA, utilizou-se o GLP-1, EXE-4 e L- penetratina para facilitar a entrada no cérebro de GLP-1 e EXE-4 (ambos 2,5 mg/mL). Para tal, os pesquisadores utilizaram camundongos ddY, SAMP8 (modelo de demência) e SAMR1 (controle normal para SAMP8) e observaram que a distribuição de EXE-4 no cérebro aumentou significativamente pela coadministração intranasal de L-penetratina, e a administração simultânea de EXE-4 por via intranasal ativou a sinalização de insulina no hipocampo. Com isso, um nível adequado de insulina foi confirmado pela análise da fosforilação de AKt. Além disso, após administração diária de EXE-4 com L-penetratina e insulina suplementar por quatro semanas, observaram uma melhora adicional da função cognitiva espacial, como mostrado no teste de

labirinto aquático de Morris. O estudo sugere que a administração intranasal de EXE-4 com insulina suplementar, mediada pela coadministração de L-penetratina, mostra-se promissora para o tratamento da disfunção cognitiva progressiva em SAMP8 (modelo de demência) [144].

Na busca do envolvimento do BNDF e visfatina cerebral nas alterações cognitivas no DM2, bem como os mecanismos subjacentes a essas alterações, foram utilizados ratos *Wistar* machos que receberam STZ na dose de 45mg/Kg via i.p. para induzir diabetes. Os ratos tratados receberam EXE-4, análogo do GLP-1, na dose de 3µg/kg administrada via subcutânea, durante 30 dias. Esse estudo fornece evidências do envolvimento do BDNF e da visfatina cerebral nas alterações cognitivas associadas ao DM2, além do efeito neuroprotetor da EXE-4 na sobrevivência neuronal e na integridade sináptica. O tratamento do DM2 com EXE-4 também melhorou significativamente o controle glicêmico e reduziu a resistência à insulina, dislipidemia, estado inflamatório, estresse oxidativo cerebral, área de neurônios degenerados e área de células gliais, o que pode contribuir para seu efeito benéfico nas funções cognitivas [145].

A EXE-4, utilizada como uma droga terapêutica para DM2, administrada por injeção intrahipocampal em ratos machos *Sprague-Dawley* (SD), na dose de 0,2nmol (1µL), apresentou um efeito antagônico na supressão da potenciação LTP induzida por Aβ1-42 no hipocampo de ratos na região CA1 *in vivo*. Essa neuroproteção pode ser mediada pela regulação da homeostase do cálcio. O pré-tratamento com EXE-4 administrada 15 minutos antes da injeção de Aβ1-42, na dose de 0,625 nmol (1µL), suprimiu a elevação induzida por Aβ1-42 na concentração de cálcio intracelular ([Ca2+] i) através de canais de cálcio dependentes de voltagem do tipo L (L-VDCCs) e receptores de N metil-D-aspartato (NMDARs). Além disso, a EXE-4 antagonizou a

diminuição da proteína quinase dependente de cálcio/calmodulina (p-CaMKII) induzida por Aβ1-42 na região CA1 no hipocampo de rato. Assim, os efeitos neuroprotetores da EXE-4, que provavelmente envolvem a regulação da homeostase do cálcio, fornecem suporte teórico para o uso da EXE-4 para tratar e prevenir a DA no futuro [146].

No estudo da atividade neuroprotetora da EXE-4, contra a toxicidade mitocondrial, aumento do nível de Aβ e disfunção colinérgica no hipocampo e córtex pré-frontal, foram utilizados ratos *Wistar* machos que receberam Aβ via i.c.v. para induzir manifestações semelhantes à DA. As atividades neuroprotetoras da EXE-4 administrada na dose de 5 μg/kg foram avaliadas na presença de LY294002, um inibidor de PI3K, para estabelecer a via do GLP-1R na modulação da bioenergética mitocondrial nas regiões do hipocampo e córtex pré-frontal. Logo, a EXE-4 atenuou a toxicidade mitocondrial por meio da via dependente de PI3K/Akt em ratos com déficit cognitivo induzido por Aβ1-42 nas regiões do hipocampo e córtex pré-frontal [147].

Com o objetivo de elucidar os mecanismos de hiperglicemia e/ou injeção intrahipocampal aguda de LPS na progressão da disfunção cognitiva e os efeitos neuroprotetores da EXE-4 contra hiperglicemia e/ou lesão aguda de LPS intrahipocampal, foram utilizados para o estudo camundongos que receberam STZ na dose de 200 mg/Kg via i.p. ou veículo (citrato de sódio). Após 10 dias da STZ, os camundongos foram tratados com EXE-4 na dose de 10mg/Kg/dia ou veículo (solução salina) via i.p. uma vez ao dia, durante 28 dias, e a injeção de LPS ou veículo via intrahipocampal na região CA1 no hipocampo foi realizada 30 minutos após a aplicação de EXE-4. Com isso, os achados revelaram que tanto a hiperglicemia como a injeção intrahipocampal de LPS induzem disfunção cognitiva por meio da ativação de respostas inflamatórias relacionadas ao NF-kB. No entanto, a injeção aguda de

LPS intrahipocampal exacerbou a progressão da disfunção cognitiva nos camundongos hiperglicêmicos por meio de um aumento nas respostas relacionadas à ativação dos astrócitos. Contudo, EXE-4 protegeu contra o comprometimento da cognição resultante da hiperglicemia e/ou injeção intrahipocampal de LPS. Assim, esses achados revelaram que EXE-4 pode ser um adjuvante para o tratamento de doenças neurodegenerativas [148].

Para explorar os mecanismos da EXE-4 na redução da fosforilação da proteína tau, foram utilizados ratos *Sprague-Dawley* machos adultos com DM2, que foram tratados com EXE-4 na dose de 3,2 μg / kg via i.p. e injeção ICV do inibidor de PI3K. Para tal fim, utilizaram uma linhagem de células neuronais-HT22, que foram tratadas com EXE-4 e/ou insulina. Essa pesquisa demonstrou um efeito crítico da via PI3K/AKT/GSK-3β na regulação da fosforilação da proteína tau no hipocampo de ratos e sugere que o efeito da EXE-4 na diminuição da fosforilação da tau é provavelmente devido ao aumento da insulina cerebral, que ativou a sinalização desta no cérebro. Assim sendo, são necessários mais estudos para explorar o efeito da EXE-4 nos níveis de insulina cerebral [149].

Para avaliar as mudanças nos níveis de proteínas específicas da BHE (occludina, claudina-5, ZO-1 e aquaporina-4), barreira sangue – líquido cefalorraquidiano (claudina-2 e aquaporina-1) e nas alterações funcionais dessas barreiras no hipocampo, foram utilizados ratos machos *Wistar Kyoto*, que receberam STZ via i.p. na dose de 75mg/Kg e tratados com EXE-4, na dose de 10 μg / kg via i.p. Essa avaliação demonstrou um efeito neuroprotetor da EXE-4 na reversão de alterações funcionais e estruturais nas barreiras cerebrais, bem como prejuízos cognitivos [150].

Na análise dos efeitos da injeção i.p. de EXE-4, na hiperfosforilação da proteína tau associada à DA e na via de sinalização da insulina no cérebro na DM2, foram utilizados ratos machos *Sprague-Dawley*, que receberam STZ uma vez na dose de 60 mg/Kg via i.p. Após, os ratos foram tratados com EXE-4 duas vezes ao dia, na dose de 3,2 µg/Kg via i.p., durante 28 dias. A EXE-4 reduziu o aumento dos níveis de proteína tau fosforilada nos locais Ser 199/202 e o nível de Thr 217 no hipocampo de ratos com DM2. Além disso, o tratamento com EXE-4 melhorou a via de sinalização da insulina no cérebro. Esse resultado foi demonstrado pelo aumento na atividade de PI3K/AKT e um declínio na atividade de GSK-β, após quatro semanas de intervenção da EXE-4. Os resultados do tratamento com EXE-4 por vários dias parecem prevenir a hiperfosforilação da proteína tau associada à DA, em razão do aumento da via de sinalização da insulina no cérebro. Esses achados apoiam o uso da EXE-4 para a prevenção e tratamento da DA em indivíduos com DM2 [151].

Nesse contexto, entre as possibilidades de atuação da EXE-4 sobre o SNC, torna-se relevante avaliar seu efeito em um protótipo que mimetize a DA, como o modelo experimental de demência esporádica do tipo Alzheimer induzido pela STZ. Dessa forma, é possível contribuir para a busca de novas terapias que possam beneficiar pacientes acometidos por essa patologia.

2 REFERÊNCIAS

1. Knierim JJ. The hippocampus. Curr Biol. 2015 dez;25(23):R1116-21.

2. Bear MF, Connors BW, Paradiso MA, Jorge Alberto CD. Neurociências: desvendando o sistema nervoso. 4. ed. Porto Alegre: Artmed; 2017. 974p.

3. Zieri R. Anatomia Humana I. Biblioteca Universitária Pearson. São Paulo: Pearson Education do Brasil; 2014.196p.

4. Krebs C, Weinberg J, Akesson E. Neurociências Ilustrada. Porto Alegre: Artmed; 2013. 433 p.

5. SCHULTZ C, ENGELHARDT M. Anatomy of the Hippocampal Formation. Front Neurol Neurosci. 2014;34:6-17.

6. Siegel GJ, Agranoff BW, Albers W, Fisher SK, Uhler MD, editores. Basic Neurochemistry: molecular, cellular and medical aspects. 7th. ed. London: Elsevier; 2006. 992 p.

7. Ming GL, Song H. Adult neurogenesis in the mammalian brain: significant answers and significant questions. Neuron. 2011 maio;70(4):687-702.

8. Vescovi AL, Galli R, Reynolds BA. Brain tumour stem cells. Nat Rev Cancer. 2006; 6(6):425-36.

9. Aimone JB, Li Y, Lee SW, Clemenson GD, Deng W, Gage FH. Regulation and function of adult neurogenesis: from genes to cognition. Physiol Rev. 2014;94(4):991-1026.

10. Ferreiro E, Lanzillo M, Canhoto D, Silva AMC da, Mota S, Dias IS, Ferreira IL, Fontes AR, Mastrela G, Pinheiro P, Valero J, Rego AC. Chronic hyperglycemia impairs hippocampal neurogenesis and memory in an Alzheimer's disease mouse model. Neurobiology Aging, 2020;92:98-113.

11. Izquierdo I. Memoria. Artmed: Porto Alegre; 2002. 95p.

12. Quillfeldt JA. Behavioral Methods to Study Learning and Memory in Rats. In: Andersen ML, Tufik S, editors. Rodent Model as Tools in Ethical Biomedical Research. Springer; 2016. Chapter 17: p. 271-311.

13. Souza, AF de; Chowdhury, A.; Silva, A. J. Dimensions and mechanisms of memory organization. Neuron. 2021;109(17):2649-2662.

14. Dudai Y, Karni A, Born J. The Consolidation and Transformation of Memory. Neuron. 2015;88(1):20-32.

15. Eichenbaum, H. "On the Integration of Space, Time, and Memory." Neuron. 2017; 95(5):1007-1018.

16. Morris GP, Clark IA, Zinn R, Vissel B. Microglia: a new frontier for synaptic plasticity, learning and memory, and neurodegenerative disease research. Neurobiol Learn Mem. 2013;105(3):40-53.

17. Pozueta J, Lefort R, Shelanski ML. Synaptic changes in Alzheimer's disease and its models. Neuroscience. 2013;251(5):51-65.

18. De La Monte SM, Tong M. Brain metabolic dysfunction at the core of Alzheimer's disease. Biochem Pharmacol. 2014;88(4): 548-59.

19. Alzheimer's Association. Alzheimer's disease facts and figures. Alzheimer’s & Dementia. 2010;6:58-194.

20. Lane C, Hardy J, Schott MJ. Alzheimer’s disease. European Journal of Neurology. 2018;25:59-70.

21. World Health Organization. Dementia. 2021.

22. Associação Brasileira de Alzheimer. O que é Alzheimer. 2019.

23. Hyman BT, Phelps CH, Beach TG, Bigio EH, Cairns NJ, Carrillo MC, Dickson DW, Duyckaerts C, Frosch MP, Masliah E, Mirra SS, Nelson PT, Schneider JA, Thal DR, Thies B, Trojanowski JQ, Vinters HV, Montine TJ. National Institute on Aging-Alzheimer’s Association guidelines for the neuropathologic assessment of Alzheimer’s disease. Alzheimers Dement. 2012;8(1):1-13.

24. Dorszewska J, Prendecki M, Oczkowska A, Dezor M, Kozubski W. Molecular basis of familial and sporadic Alzheimer’s disease. Curr. Alzheimer Res. 2016;13:952-963.

25. Dean CD, Jerskey BA, Chen K, Protas H, Thiyyagura P, Roontiva A, O'Muircheartaigh J, Dirks H, Waskiewicz N, Lehman K, Siniard AL, Turk MNN, Hua X, Madsen SK, Thompson PM, Fleisher AS, Huentelman MJ, Deoni SCL, Reiman EM. Brain Differences in Infants at Differential Genetic Risk for Late-Onset. Alzheimer Disease A Cross-sectional Imaging Study. JAMA Neurol. 2014 Jan;71(1):11-22.

26. Robakis KN. Molecular Neuropathology of Alzheimer Dementia and Therapeutic Approaches. Advances in Experimental Medicine and Biology. 2015;822.

27. Reitz C, Brayne C, MAYEUX R. Epidemiology of Alzheimer disease. Nat. Rev. Neurol. 2011;7:137-152.

28. Serrano-Pozo A, Frosch MP, Masliah E, Hyman BT Neuropathological Alterations in Alzheimer Disease. Cold Spring Harb Perspect Med. 2011;1:a006189.

29. Sosa LJ, Cáceres A, Dupraz S, Oksdath M, Quiroga S, Lorenzo A. The physiological role of the Amyloid Precursor Protein (APP) as an adhesion molecule in the developing nervous system. J Neurochem. 2017;143(1):11-29.

30. Heppner FL, Ransohoff RM, Becher B. Immune attack: the role of inflammation in Alzheimer disease. Nat. Ver Neurosci. 2015;16(6):358-72.

31. Cummingsa JL, Tongb G, Ballardc C. Treatment Combinations for Alzheimer's Disease: Current and Future Pharmacotherapy Options. Journal of Alzheimer's Disease. 2019;67:779-794.

32. Wang JZ, Gao X, Wang ZH. The physiology and pathology of microtubule-associated protein tau. Essays Biochem. 2014;(56):111-23.

33. Querfurth HW, Laferla FM. Mechanisms of Disease: Alzheimer's Disease. N Engl J Med. 2010;362:329-44.

34. Rahmeier FL, Zavalhia, LS, Tortorelli LS, Huf F, Gea LP, Meurer RT, Machado AC, Gomez R, Fernandes MC. The effect of taurine and enriched environment on behaviour, memory and hippocampus of diabetic rats. Neurosci Lett. 2016;630:84-92.

35. Chen S, Liu A-R, An F-M, Yao W-B, Gao X-D. Amelioration of neurodegenerative changes in cellular and rat models of diabetes-related Alzheimer's disease by exendina-4. Age (Dordr). 2012 Oct;34(5):1211-24.

36. Grieb, P. Intracerebroventricular Streptozotocin Injections as a Model of Alzheimer's Disease: in Search of a Relevant Mechanism. Mol Neurobiol. 2016;53:1741-1752.

37. HOYER S, LANNERT H. Long-term abnormalities in brain glucose energy metabolism after inhibition of the neuronal insulin receptor: implication of tau-protein. J Neural Transm. 2007;72:195-202.

38. Agrawal R, Tyagi E, Shukla R, Nath C. Insulin receptor signaling in rat hippocampus: A study in STZ (ICV) induced memory deficit model. Eur Neuropsicofarmacol. 2011 mar;21(3):261-73.

39. De La Monte SM, Tong M, Lester-Coll N, Plater Jr M, Wands JR. Therapeutic rescue of neurodegeneration in experimental type 3 diabetes: Relevance to Alzheimer's disease. J Alzheimers Dis. 2006 Sep;10(1):89-109.

40. Mishra SK, Singh S, Shukla S, Shukla R. Intracerebroventricular streptozotocin impairs adult neurogenesis and cognitive functions via regulating neuroinflammation and insulin signaling in adult rats. Neurochem Int. 2018 Feb;113:56-68.

41. Mehan S, Arora R, Sehgal V, Sharma D, Sharma G. Dementia – A Complete Literature Review on Various Mechanisms Involves in Pathogenesis and an Intracerebroventricular Streptozotocin Induced Alzheimer's Disease. 2012.

42. Yuliani T, Lobentanzer S, Klein J. Central cholinergic function and metabolic changes in streptozotocin-induced rat brain injury. Journal of Neurochemistry. 2020;00:1-13.

43. Nelson LD, Cox MM. Princípios de Bioquímica de Lehninger. 7. ed. Porto Alegre: Artmed; 2019.

44. Tyagi, A., Pugazhenthi, S. Targeting Insulin Resistance to Treat Cognitive Dysfunction. Mol. Neurobiol. 2021;58(6):2672-2691.

45. Duarte AI, Moreira PI, Oliveira CR. Insulin in central nervous system: more than just a peripheral hormone. J Aging Res. 2012;2012:384017.

46. González A, Calfío C, Churruca M, Maccioni RB. Glucose metabolism and AD: evidence for a potential diabetes type 3. Alzheimer's Research & Therapy. 2022;14:56.

47. Spinelli M, Fusco S, Grassi C. Brain Insulin Resistance and Hippocampal Plasticity: Mechanisms and Biomarkers of Cognitive Decline. Front Neurosci. 2019 Jul 31;13:788.

48. Akhtar A, Sah SP. Insulin signaling pathway and related molecules: role in neurodegeneration and Alzheimer's disease. Neurochemistry international. 2020;135:104707.

49. Sharma VK, Sing TG. Insulin resistance and bioenergetic manifestations: Targets and approaches in Alzheimer's disease. Life Sciences. 2020;262:118401.

50. Pomytkin I, Pinelis V. Brain Insulin Resistance: Focus on Insulin Receptor-Mitochondria Interactions. Life, 2021.

51. Hubbard SR. The insulin receptor: both a prototypical and atypical receptor tyrosine kinase. Cold Spring Harb Perspect Biol. 2013 Mar 1;5(3):a008946.

52. Bosco D, Fava A, Plastino M, Montalcini T, Pujia A. Possible implications of insulin resistance and glucose metabolism in Alzheimer's disease pathogenesis. J Cell Mol Med. 2011 Sep;15(9):1807-21.

53. Tumminia A, Vinciguerra F, Paris M, Frittitta L. Type 2 Diabetes Mellitus and Alzheimer's Disease: Role of Insulin Signalling and Therapeutic Implications. Int. J. Mol. Sci. 2018;19(11):3306.

54. Blázquez E, Velázquez E, Hurtado-Carneiro V, Ruiz-Albusac JM. Insulin in the brain: its pathophysiological implications for states related with central insulin resistance, type 2 diabetes and alzheimer's disease. Front Endocrinol (Lausanne). 2014 Oct 9;5:161

55. Kandimalla, R.; Thirumala, V.; Reddy, H. P. Is Alzheimer's disease a Type 3 Diabetes? A Critical Appraisal. Biochim Biophys Acta. 2017;1863(5):1078-1089.

56. Cunha S, Ribeiro CF, Cruz T, Silva S. Therapeutic potential of GLP-1R agonists in the Alzheimer Disease. Acta Farm Port. 2020;9(1):15-31.

57. Hölscher C. Central effects of GLP-1: new opportunities for treatments of neurodegenerative diseases. J Endocrinol. 2014;221:T31-T41.

58. Chiu SL, Chen CM, Cline HT. Insulin receptor signaling regulates synapse number, dendritic plasticity, and circuit function in vivo. Neuron. 2008;58(5):708-719.

59. Lee S-H, Zabolotny JM, Huang H, Lee H, Kim Y-B. Insulin in the nervous system and the mind: functions in metabolism, memory, and mood. Mol Metab. 2016;5(8):589-601.

60. Lee CC, Huang CC, Hsu KS. Insulin promotes dendritic spine and synapse formation by the PI3K/Akt/mTOR and Rac1 Signaling pathaways. Neuropharmacology. 2011;61(4):867-879.

61. Peineau S, Taghibiglou C, Bradley C, Wong TP, Liu L, Lu J, Lo E, Wu D, Saule E, Bouschet T, Matthews P, Isaac JTR, Bortolotto ZA, Wang YT, Collingridge GL. LTP inhibits LTD in the hippocampus via regulation of GSk3beta. Neuron. 2007 Mar 1;53(5):703-17.

62. Tomita T. Apoptosis in pancreatic β-islet cells in Type 2 diabetes. Bosn J Basic Med Sci. 2016;16(3):162-179.

63. Nguyen TT, Ta QTH, Nguyen TKO, Nguyen TTD, Giau VV. Type 3 Diabetes and Its Role Implications in Alzheimer's Disease. Int J Mol Sci. 2020 Apr;21(9):3165.

64. Hill JM, Lesniak MA, Pert CB, Roth J. Autoradiographic localization of insulin receptors in rat brain: prominence in olfactory and limbic areas. Neuroscience. 1986;17:1127-1138.

65. Zhao WQ, Alkon DL. Role of insulin and insulin receptor in learning and memory. Mol. Cell. Endocrinol. 2001;177,125-134.

66. Mainardi M, Fusco S, Grassi C. Modulation of hippocampal neural plasticity by glucose-related signaling. Neural Plast. 2015;2015:657928.

67.Winblad B, Amouyel P, Andrieu S, Ballard C, Brayne C, Brodaty H, Minguez AC, Dubois B, Edvardsson D, Feldman H, Fratiglioni L, Frisoni GB, Gauthier S, Georges J, Graff C, Iqbal K, Jessen F, Johansson G, Jonsson L, Kivipelto M, Knapp M, Mangialasche F, Melis R, Nordberg A, Rikkert MO, Qiu C, Sakmar TP, Scheltens P, Schneider LS, Sperling R, Tjernberg LO, Waldemar G, Wimo A, Zetterberg H. Defeating Alzheimer's disease and other dementias: a priority for European Science and Society. Lancet Neurol. 2016 Apr;(5):455-532.

68. Magistretti PJ, Allaman I. Lactate in the brain: from metabolic endproduct to signalling molecule. Nat Rev Neurosci. 2018;19:235-49.

69. Kullmann S, Heni M, Hallschmid M, Fritsche A, Preissl H, Häring H-U. Brain insulin resistance at the crossroads of metabolic and cognitive disorders in humans. Physiol. Rev. 2016;96:1169-1209.

70. Berlanga-Acosta J, Guillén-Nieto G, Rodríguez-Rodriguez N, Vega MLB, García-Del-Barco-Herrera D, Berlanga-Saez JO, García-Ojalvo A, Valdés-Sosa MJ, Valdés-Sosa PA. Afiliações expandir Insulin Resistance at the Crossroad of Alzheimer Disease Pathology: A Review. Front Endocrinol. 2020 Nov;11:560375.

71. Csajbók EA, Tamás G. Cerebral cortex: a target and source of insulin? Diabetologia. 2016;59:1609-1615.

72. Liu Y, Liu F, Iqbal K, Grundke-Iqbal I, Gong C-X.Decreased glucose transportes correlate to abnormal hyperphosphorylation of tau in Alzheimer disease. FEBS Lett. 2008;582(2):359-364.

73. Dienel GA. Brain glucose metabolism: integration of energetics with function. Physiol. Rev. 2019;99:949-1045.

74. Cai, Z, Chen G, He W, Xiao M, Yan L-J. Activation of mTOR: a culprit of Alzheimer's disease? Neuropsychiatr. Dis. Treat. 2015;11:1015-1030.

75. Takeda S, Sato N, Rakugi H, Morishita R. Molecular mechanisms linking diabetes mellitus and Alzheimer disease: beta-amyloid peptide, insulin signaling, and neuronal function. Mol. BioSyst. 2011;7:1822-1827.

76. Kandimalla, R., *et al.* Is Alzheimer’s disease a Type 3 Diabetes? A critical appraisal. Biochim. Biophys. Acta. Mol. Basis Dis.1863: 1078-1089, 2017.

77. Nguyen NH, Pham QT, Luong TNH, Le HK, Vo VG. Potential Antidiabetic Activity of Extracts and Isolated Compound from Adenosma bracteosum (Bonati). Biomolecul. 2020;10(2):201.

78. Giau, VV, Wu SY, Jamerlan A, Soo S, Kim SY, Hulme J. Microbiota and Their Neuroinflammatory Implications in Alzheimer’s Disease. Nutrient. 2018;10(11):1765.

79. Simsir IY, Soyaltin EU, Cetinkalp S. Glucagon like peptide-1 (GLP-1) likes Alzheimer’s disease. Diabetes Metab Syndr. 2018 maio;12(3):469-475.

80. Baggio LL, Drucker DJ. Biology of incretins: GLP-1 and GIP. Gastroenterology. 2007;132(6):2131-57.

81. Chacra AR. Efeito fisiológico das incretinas. Johns Hopkins: Advanced Studies in Medicine. 2006;6(7B):613-617.

82. Drucker DL, Nauck M. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidadese-4 inhibitors in type 2. Lancet. 2006 Nov 11;368(9548):1696-705.

83. Brown JC, Dryburgh JR, Ross SA. Dupre J. Identification and actions of gastric inhibitory polypeptide. Recent Prog Horm Res. 1975;31:487-532.

84. Lauritsen KB, Moody AJ, Christensen KC, Lindkaer Jensen S. Gastric inhibitory polypeptide (GIP) and insulin release after small-bowel resection in man. Scand J Gastroenterol. 1980;15:833–840.

85. Mojsov S, Weir GC, Habener JF. Insulinotropin: glucagon-like peptide I (7-37) co-encoded in the glucagon gene is a potent stimulator of insulin release in the perfused rat pancreas. J Clin Invest. 1987;79:616–619.

86. Kreymann B, Williams G, Ghatei MA, Bloom SR. Glucagon-like peptide-1 7-36: a physiological incretin in man. Lancet. 1987;2:1300–1304.

87. Holst JJ. The Physiology of glucagon-like peptide 1. Physiol Rev. 2007;87:1409-1439.

88. Bell GI, Santerre RF, Mullenbach GT. Hamster preproglucagon contains the sequence of glucagon and two related peptides. Nature.1983;302:716-718.

89. Bell GI, Sanchez-Pescador R, Laybourn PJ, Najarian RC. Exon Duplication and divergence in the human preproglucagon gene. Nature. 1983;304:368-371.

90. Novak U, Wilks A, Buell G, McEwen S. Identical mRNA for prepro glucagon in pancreas and gut. Eur J Biochem. 1987;164:553- 558.

91. Orskov C, Hoist JJ, Knuhtsen S, Baldissera FGA, Poulsen SS, Nielsen OV. Glucagon-like peptides GLP-1 and GLP-2, predicted products of the glucagon gene, are secreted separately from the pig small intestine, but not pancreas. Endocrinology. 1986; 119:1467-1475.

92. Kim W, Egan MJ. The Role of Incretins in Glucose Homeostasis and Diabetes Treatment. Pharmacol Rev. 2008 Dec;60(4):470-512.

93. Müller TD, Finan B, Clemmensen C, DiMarchi RD, Tschöp MH. The New Biology and Pharmacology of Glucagon. Physiol Ver. 2017;97(2):721-766.

94. Larsen PJ. Tang-Christensen M, Holst JJ, Orskov C. Distribution of glucagon-like peptide-1 and other preproglucagon-derived peptides in the rat hypothalamus and brainstem. Neuroscience. 1997;77:257-270.

95. Tucker JD, Dhanvantari S, Brubaker PL. Proglucagon processing in islet and intestinal cell lines. Regul Pept. 1996;62:29-35.

96. Vrang N, Hansen M, Larsen PJ, Tang-Christensen M. Characterization of brainstem preproglucagon projections to the paraventricular and dorsomedial hypothalamic nuclei. Brain Res. 2007;1149:118-126.

97. Inagaki N, Seino Y, Takeda J, Yano H, Yamada Y, Bell GI, Eddy RL, Fukushima Y, Byers MG. Gastric Inhibitory Polypeptide: structure and chromosomal localization of the human gene. Mol Endocrinol. 1989 Jun;3(6):1014-21.

98. Yabe D, Seino Y. Two incretin hormones GLP-1 and GIP: Comparison of their actions in insulin secretion and beta cell preservation. Prog Biophys Mol Biol. 2011 Nov;107(2):248-56

99. McIntyre N, Holsworth DC, Turner DS. New interpretation of oral glucose tolerance. Lancet. 1964 Jul 4;2(7349):20-1.

100. Elrick H, Stimmler L, Hlad CJ Jr, Arai Y. Plasma insulin response to oral and intravenous glucose administration. J Clin Invest. 1964;24:1076-1082.

101. Mortensen K, Christensen LL, Holst JJ, Orskov C. GLP-1 and GIP are colocalized in a subset of endocrine cells in the small intestine. Regul Pept. 2003;114:189-196.

102. Theodorakis MJ, Carlson O, Michopoulos S, Doyle ME, Juhaszova M, Petraki K, Egan JM. Human duodenal enteroendocrine cells: source of both incretin peptides, GLP-1 and GIP. Am J Physiol. 20060 290:E550–E559.

103. Nauck MA, Homberger E, Siegel EG, Allen RC, Eaton RP, Ebert R, Creutzfeldt W. Incretin Effects of Increasing Glucose Loads in Man Calculated from Venous Insulina and C-Peptide Responses. J Clin Endocrinol Metab. 1986 Aug;63(2):492-8.

104. Gremlich S, Porret A, Hani EH, Cherif D, Vionnet N, Froguel P, Thorens B. Cloning functional expression, and chromosomal localization of the human pancreatic islet glucose-dependent insulinotropic polypeptide receptor. Diabetes. 1995 Oct;44(10):1202-8.

105. Thorens, B. Expression cloning of the pancreatic beta cell receptor for the gluco-incretin hormone glucagon-like peptide 1. Proc Natl Acad Sci U S A. 1992 Sep 15;89(18):8641-5.

106. Brubaker PL, Drucker DJ. Structure-function of the glucagon receptor family of G protein-coupled receptors: the glucagon, GIP, GLP-1, and GLP-2 receptors. Receptors Channels. 2002;8:179-88.

107. Buteau J. GLP-1 receptor signaling: effects on pancreatic beta-cell proliferation and survival. Diabetes Metab. 2008;34 S73-S7.

108. Wang Q, Li L, Xu E, Wong V, Rhodes C, Brubaker PL. Glucagon-like peptide-1 regulates proliferation and apoptosis via activation of protein kinase B in pancreatic INS-1 beta cells. Diabetologia 2004 Mar;47(3):478-487.

109. Jung KY, Kim KM, Lim, S. Therapeutic Approaches for Preserving or Restoring Pancreatic beta-Cell Function and Mass. Diabetes Metab J. 2014;38(6):426-36.

110. Calsolaro V, Edison P. Novel GLP-1 (Glucagon-Like Peptide-1) Analogues and Insulin in the Treatment for Alzheimer's Disease and Other Neurodegenerative Diseases. CNS Drugs. 2015;29:1023-1039.

111. Fu Z, Gong L, Liu J, Wu J, Barrett EJ, Aylor KW, Liu Z. Brain Endothelial Cells Regulate Glucagon-Like Peptide 1 Entry Into the Brain via a Receptor-Mediated Process. Front Physiol. 2020 May;11:555.

112. Hamilton A, Hölscher C. Receptors for the incretin glucagon-like peptide-1 are expressed on neurons in the central nervous system. NeuroReport. 2009; 20(13):1161-1166.

113. Farkas E, Szilvásy-Szabó A, Ruska Y, Sinkó R, Rasch MG, Egebjerg T, Pyke C, Gereben B, Knudsen LB, Fekete C. Distribution and ultrastructural localization of the glucagon-like peptide-1 receptor (GLP-1R) in the rat brain. Brain Struct Funct. 2021 Jan;226(1):225-245.

114. Drucker DJ. The biology of incretin hormones. Cell Metab. 2006;3:153-165.

115. Kim DS, Choi H-I, Wang Y, Luo Y, Hoffer BJ, Nigel H. A New Treatment Strategy for Parkinson's Disease through the Gut-Brain Axis: The Glucagon-Like Peptide-1 Receptor Pathway. Cell Transplant. 2017 Sep; 26(9):1560-1571.

116. During MJ, Cao L, Zuzga DS, Francis JS, Fitzsimons HL, Jiao X, Bland RJ, Klugmann M, Banks WA, Drucker DJ, Haile CN. Glucagon-like peptide-1 receptor is involved in learning and neuroprotection. Nat Med, 9(9):1173–9, 2003.

117. McClean PL, Parthsarathy V, Faivre E, Hölscher, C. The diabetes drug liraglutide prevents degenerative processes in a mouse model of Alzheimer's disease. J Neurosci. 2011;31:6587-94.

118. Bomfim TR, Germano LF, Sathler LB, Brito-Moreira J, Houzel J-C, Decker H, Silverman MA, Kazi H, Melo HM , McClean PL, Holscher C, Arnold SE , Talbot K, Klein WL , Muñoz DP , Ferreira ST, De Felice FG. An anti-diabetes agent protects the mouse brain from defective insulin signaling caused by Alzheimer's disease-associated Ab oligomers. J Clin Invest. 2012;122:1339-53.

119. Hunter, K., Hölscher, C. Drugs developed to treat diabetes, liraglutide and lixesenatide, cross the blood brain barrier and enhance neurogenesis. BMC Neurosci. 2012;13:33.

120. Kastin AJ, Akerstrom V. Entry of exendin-4 into brain is rapid but may be limited at high doses. Int J Obes Rel Disord. 2003;27(3):313-318.

121. Eng J, Kleinman WA, Singh L, Singh G, Raufman JP. Isolation and characterization of exendin-4, an exendin-3 analogue, from Heloderma suspectum venom. Further evidence for na exendin receptor on dispersed acini from guinea pig pâncreas. J Biol Chem. 1992 Apr 15;267(11):7402-5.

122. Triplitt C, Chiquette E. Exenatide: From the Gila Monster to the Pharmacy. J Am Pharm Assoc. 2006 Jan-Feb;46(1):44-52.

123. Raufman JP, Singh L, Singh G, Eng J. Truncated glucagon-like peptide-1 interacts with exendin receptors on dispersed acini from guinea pig pancreas. Identification of a mammalian analogue of the reptilian peptide exendin-4. J Biol Chem. 1992 Oct 25;267(30):21432-7.

124. Yap MKK, Misuan N. Exendin-4 from Heloderma suspectum venom: From Discovery to its latest application as type II diabetes combatant. Basic Clin Pharmacol Toxicol. 2019;124:513-527.

125. Rai A, Singh G, Raffaniello R, Eng J, Raufman JP. Actions of Helodermatidae venom peptides and mammalian glucagon-like peptides on gastric chief cells. Am J Physiol. 1993 Jul;265(1 Pt 1):G118-25.

126. Goke R, Fehmann H-C, Linns T, Schmidt H, Krause M, Engt J, GokeII B. Exendin-4 is a high potency agonist and truncated exendin-(9-39) -amide an antagonist at the glucagon-like peptide 1-(7-36) -amide receptor of insulin-secreting beta-cells. J Biol Chem. 1993;268(26):19650-5.

127. Schepp W, Schmidtler J, Riedel T, Dehne K, Schusdziarra V, Holst JJ, Eng J, Raufman JP, Classen M Exendin-4 and exendin-(9-39) NH2: agonist and antagonist, respectively, at the rat parietal cell receptor for glucagon-like peptide-1-(7-36) NH2. Eur J Pharmacol. 1994 Oct 14;269(2):183-91.

128. Kolligs F, Fehmann HC, Göke R, Göke B. Reduction of the incretin effect in rats by the glucagon-like peptide 1 receptor antagonist exendin (9-39) amide. Diabetes. 1995 Jan;44(1):16-9.

129. Goke RP, Larsen J, Mikkelsen JD, Sheikh SP. Identification of specific binding sites for glucagon-like peptide-1 on the posterior lobe of the rat pituitary. Neuroendocrinology. 1995;62(2)130-4.

130. Goke RP, Larsen J, Mikkelsen JD, Sheikh SP. Distribution of GLP-1 binding sites in the rat brain: evidence that exendin-4 is a ligand of brain GLP-1 binding sites. Eur J Neurosci. 1995;7(11):2294-300.

131. Luciani P, Deledda C, Benvenuti S, Cellai I, Squecco R, Monici M, Cialdai F, Luciani G, Danza G, Di Stefano C, Francini F, Peri A. Affiliations expand Differentiating effects of the glucagon-like peptide-1 analogue exendin-4 in a human neuronal cell model. Cell Mol Life Sci. 2010;67(21):3711-23.

132. Rachmany L, Tweedie D, Rubovitch V, Li Y, Holloway H., Kim DS, Ratliff WA., Saykally JN, Citron BA, Hoffer BJ, Greig NH, Pick CG. Exendin-4 attenuates blast traumatic brain injury induced cognitive impairments, losses of synaptophysin and in vitro TBI-induced hippocampal cellular degeneration. Sci Rep, 2017;7(1):3735.

133. Li H, Lee CH, Yoo K-Y, Choi JH, Park OK, Yan BC, Byun K, Lee B, Hwang IK, Won M-H. Chronic treatment of exendin-4 affects cell proliferation and neuroblast differentiation in the adult mouse hippocampal dentate gyrus. Neurosci Lett. 2010; 486(1):38-42.

134. Hamilton A, Patterson S, Porter S, Gault VA, Holscher C. Novel GLP-1 mimetics developed to treat type 2 diabetes promote progenitor cell proliferation in the brain. J Neurosci Res. 2011;89(4):481-9.

135. Tsukamoto M, Niimi N, Sango K, Takaku S, Kanazawa Y. Neurotrophic and neuroprotective properties of exendin-4 in adult rat dorsal root ganglion neurons: involvement of insulin and RhoA. Histochem Cell Biol. 2015;44(3):249-59.

136. Perry, TA, Holloway HW, Weerasuriya A, Mouton PR, Duffy K, Mattison JA, Greig NH. Evidence of GLP-1-mediated neuroprotection in an animal model of pyridoxine-induced peripheral sensory neuropathy. Exp Neurol. 2007;203:293-301.

137. Hölscher C. New drug treatments show neuroprotective effects in Alzheimer's and Parkinson's diseases. Neural Regen. Res. 2014;9:1870-1873.

138. Pan B, Huo T, Hu Y, Cao M, Bu X, Li Z, Jing L, Luo X, Gao X, Feng H, Yuan F, Guo K. Exendin-4 Promotes Schwann Cell Proliferation and Migration via Activating the Jak-STAT Pathway after Peripheral Nerve Injury. Neurosciense. 2020;437:1-10.

139. Cui SS, Feng X-B, Zhang B-H, Xia Z-Y, Zhan LY. Exendin-4 attenuates pain-induced cognitive impairment by alleviating hippocampal neuroinflammation in a rat model of spinal nerve ligation Neural Regen Res.2020;15(7):1333-1339.

140. Rea S, Della-Morte D, Pacifici F, Capuani B, Pastore D, Coppola A, Arriga R, Andreadi A, Donadel G, Di Daniele N, Bellia A, Lauro, D. Insulin and Exendin-4 Reduced Mutated Huntingtin Accumulation in Neuronal Cell s. Front. Pharmacol. 2020 May; 28.

141. Géa LP, Rosa ED da, Panizzutti BS, Aguiar ÉZ de, Oliveira LF. de, Ferrari P, Piato A, Gomez R, Colombo R, Rosa AR. Reduction of hippocampal IL-6 levels in LPS-injected rats following acute exendin-4 treatment. Naunyn-Schmiedeberg's Archives of Pharmacology. 2020;393:1303-1311.

142. Gault VA, Porter WD, Flatt PR, Hölscher C. Actions of exendin-4 therapy on cognitive function and hippocampal synaptic plasticity in mice fed a high-fat diet. Int J Obes (Lond). 2010 Aug;34(8):1341-4.

143. Chen Y, Liang Z, Blanchard J, Dai C-L, Sun S, Lee MH, Grundke-Iqbal I, Iqbal K, Liu F, Gong C-X. Affiliations expand A Non-transgenic Mouse Model (icv-STZ Mouse) of Alzheimer's Disease: Similarities to and Differences from the Transgenic Model (3xTg-AD Mouse). Mol Neurobiol. 2013;47:711-725.

144. Kamei N, Okada N, Ikeda T, Choi H, Fujiwara Y, Okumura H, Takeda-Morishita M. Effective nose-to-brain delivery of exendin-4 via coadministration with cell-penetrating peptides for improving progressive cognitive dysfunction. Scientific Reports. 2018;8:17641.

145. Abdelwahed O, Rashed ML, Gamal el Din MM, Zickri M, Tork OM. Effect of glucagon-like peptide-1 analogue; Exendin-4, on cognitive functions in type 2 diabetes mellitus; possible modulation of brain derived neurotrophic factor and brain Visfatin. Brain Research Bulletin. 2018;139:67-80.

146. Wang X, Wang L, Jiang R, Yuan Y, Yu Q, Li Y. Exendin-4 antagonizes Aβ1-42-induced suppression of long-term potentiation by regulating intracellular calcium homeostasis in rat hippocampal neurons. Brain Research. 2015;1627:101-108.

147. Garabadu D, Verma J. Exendin-4 attenuates brain mitochondrial toxicity through PI3K/Akt dependent pathway in amyloid beta (1-42) -induced cognitive deficit rats. Neurochemistry International. 2019;128:39-49.

148. HUANG H, Chen Y-H, Liang K-C, Jheng Y-S, Jhao J-J, Su M-T, Lee-Chen G-J, Hsieh-Li HM. Exendin-4 Protected against Cognitive Dysfunction in Hyperglycemic Mice Receiving an Intrahippocampal Lipopolysaccharide Injection. PLoS ONE, 2012.

149. Yang Y, Ma D, Xu W, Chen F, Du T, Yue W, Shao S, Yuan G. Exendin-4 reduces tau hyperphosphorylation in type 2 diabetic rats via increasing brain insulin level. Mol Cell Neurosci. 2016 Jan;70:68-75.

150. Zanotto C, Simão F, Gasparin MS, Biasibetti R, Tortorelli LS, Nardin P, Gonçalves C-A. Affiliations expand Exendin-4 Reverses Biochemical and Functional Alterations in the Blood–Brain and Blood–CSF Barriers in Diabetic Rats. Mol Neurobiol. 2017 Apr;54(3):2154-2166.

151. Xu W, Yang Y, Yuan G, Zhu W, Ma D, Hu S. Affiliations expand Exendin-4, a Glucagon-Like Peptide-1 Receptor Agonist, Reduces Alzheimer Disease-Associated Tau Hyperphosphorylation in the Hippocampus of Rats With Type 2 Diabetes. J Investig Med. 2015;63:267-272.

3 OBJETIVOS

3.1 OBJETIVO GERAL

O objetivo desta tese foi investigar os efeitos neuroprotetores da exendina-4 sobre alterações cognitivas e morfológicas no hipocampo de ratos submetidos a um modelo experimental de demência esporádica do tipo Alzheimer.

3.2 OBJETIVOS ESPECÍFICOS

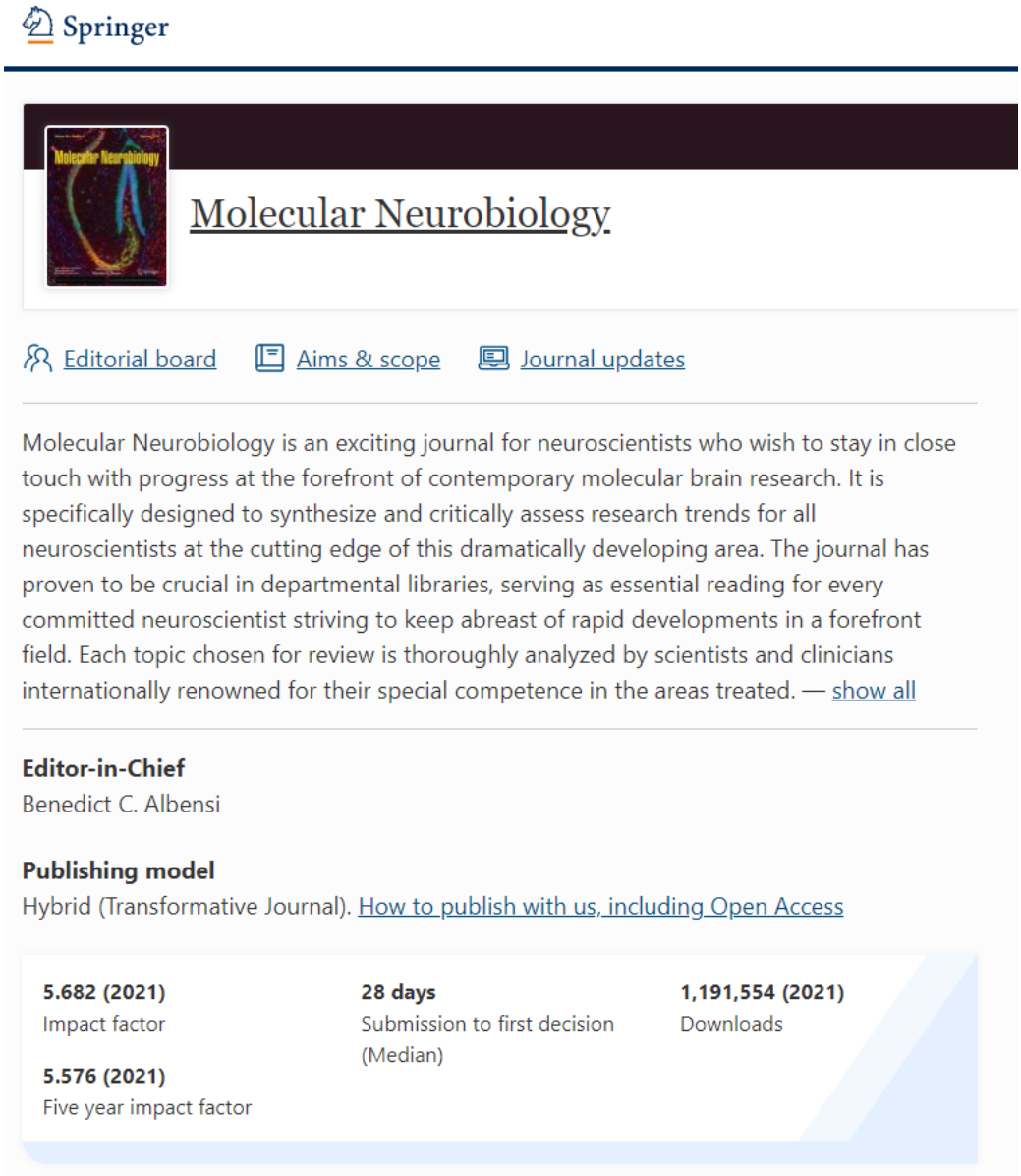
Em ratos submetidos ao modelo de demência esporádica do tipo Alzheimer e tratados com EXE-4 será avaliado o efeito da EXE-4 sobre:

- aprendizado e memória em tarefas comportamentais;
- marcadores neuronais, astrocitários e microgliais no hipocampo;
- apoptose no hipocampo;
- os marcadores de receptor de insulina e do antígeno nuclear de proliferação celular (PCNA).

4 MANUSCRITOS CIENTÍFICOS REDIGIDOS EM INGLÊS

4.1 MANUSCRITO 1

Dados do periódico



Qualis Capes do Periódico

Consultar Cancelar

Periódicos

ISSN	Título	Área de Avaliação	Classificação
0893-7648	MOLECULAR NEUROBIOLOGY	BIOTECNOLOGIA	A1
0893-7648	MOLECULAR NEUROBIOLOGY	CIÊNCIAS BIOLÓGICAS I	A1
0893-7648	MOLECULAR NEUROBIOLOGY	CIÊNCIAS BIOLÓGICAS II	A1
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FEDERAL UNIVERSITY OF HEALTH SCIENCES OF PORTO ALEGRE
PATHOLOGY RESEARCH LABORATORY
PORTO ALEGRE, RS, BRAZIL

To: Editor-in-chief of **Molecular Neurobiology**,
Benedict C. Albensi

December 2022

Dear Editor,

I would like to submit a research article for publication in the **Molecular Neurobiology** entitled "*Exendin-4 prevents memory loss and neuronal death in rats with sporadic Alzheimer-like disease*", (Total Words: ± 6.227, Figures: 9, References: 65).

The manuscript was co-authored by Adriana M. Zago (first author), Fabiano B. Carvalho, Francine L. Rahmeier, Marta Santin, Giuliano R. Guimarães, Jessié M. Gutierres and Marilda da C. Fernandes (corresponding author).

The main contribution of our work is that it outlines the nootropic effects of exendin-4 (EXE-4), an agonist of glucagon-like peptide 1 receptor (GLP1-R) and drug for the treatment of type 2 diabetes mellitus, in a model of sporadic dementia of the Alzheimer's type (SDAT). Through behavioral analyzes and morphology studies, we verified a protection in short and long-term memories present in SDAT. Furthermore, EXE-4 was shown to be effective in reducing apoptosis of pyramidal neurons in the CA1 and CA3 regions as well as granular neurons in the DG. EXE-4 increased the proliferation of neurons in the hippocampus and the expression of the insulin receptor in the CA1 region.

This study is of interest to neuroscience, nutrition, and metabolism professionals as these results may contribute to new insights into the role of incretin-mimetics against neuropathological conditions found in dementias such as Alzheimer's disease. In parallel, this study seeks to find new supporting tools in the protection of populations of hippocampal neurons against the neurotoxic effects present in SDAT.

We read and understand your journal's policies on copyright, ethics, etc., and we believe that neither the manuscript nor the study violates any of them.

Thanks for your consideration. We hope that our manuscript will be suitable for publication in your journal.

Sincerely,

Marilda da Cruz Fernandes
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Exendin-4 prevents memory loss and neuronal death in rats with sporadic Alzheimer-like disease

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Abstract:	This study investigated the neuroprotective effects of exendin-4 (EXE-4), an analogue of the glucagon-like peptide 1 receptor (GLP-1R) on memory and on the neuronal populations that constitute the hippocampus of rats submitted to a sporadic dementia of Alzheimer's type (SDAT). Male Wistar rats received streptozotocin (STZ icv, 3mg/kg diluted in ACFS, 5 µl/ventricle) and treated during 21 days with EXE-4 (10µg/kg, ip; saline as vehicle). Four groups were formed: Vehicle, EXE-4, STZ and STZ+EXE-4. The groups were submitted to Y-Maze (YM), Object Recognition (ORT) and Object Displacement (ODT) Tasks to assess learning and memory. The brains were used for immunohistochemical and immunofluorescent techniques with antibodies to NeuN, Cleaved Caspase-3 (CC-3), PCNA and Insulin Receptor (IR). STZ worsened spatial memory in the YMT, as well as short-term (STM) and long-term (LTM) memories in the ORT and ODT, respectively. EXE-4 protected against memory impairment in STZ animals. STZ reduced mature neurons density (NeuN) and increased cell apoptosis (CC-3) in the DG, CA1 and CA3. EXE-4 protected against neuronal death in all regions. EXE-4 increased PCNA+ cells in all regions of the hippocampus, and STZ attenuated this effect. EXE-4 increased immunoreactivity to IR in the CA1. From these findings, EXE-4 showed a beneficial effect on hippocampal pyramidal and granular neurons in the SDAT showing anti-apoptotic properties and promoting cell proliferation in. In parallel, EXE-4 preserved the memory of SDAT rats. In conclusion, these data indicate that agonists to GLP-1R have a beneficial effect on hippocampal neurons in AD.

Title Page

Exendin-4 prevents memory loss and neuronal death in rats with sporadic Alzheimer-like disease

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List of abbreviations: Alzheimer's Disease (AD); Amyloid beta (Aβ); cAMP-response binding protein element (CREB); Cleaved Caspase-3 (CC3); Exendin-4 (EXE-4); glucagon like peptide-1 (GLP-1); Glucagon like peptide-1 receptor (GLP-1R); Long-term memory (LTM); Long-term potentiation (LTP); Non-human primates (NHPs); Object Displacement Task (ODT); Object Recognition Task (ORT); Proliferating Cell Nuclear Antigen (PCNA); Short-term memory (STM); Sporadic Dementia of Alzheimer' Type (SDAT); Streptozotocin (STZ); Type 2 diabetes mellitus (T2DM).

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Data Availability Statement

The datasets generated during and/or analysed during the current study are not publicly available, but are perfectly available from the corresponding author on reasonable request.

Abstract

This study investigated the neuroprotective effects of exendin-4 (EXE-4), an analogue of the glucagon-like peptide 1 receptor (GLP-1R) on memory and on the neuronal populations that constitute the hippocampus of rats submitted to a sporadic dementia of Alzheimer's type (SDAT). Male *Wistar* rats received streptozotocin (STZ icv, 3mg/kg diluted in ACFS, 5 µl/ventricle) and treated during 21 days with EXE-4 (10µg/kg, ip; saline as vehicle). Four groups were formed: Vehicle, EXE-4, STZ and STZ+EXE-4. The groups were submitted to Y-Maze (YM), Object Recognition (ORT) and Object Displacement (ODT) Tasks to assess learning and memory. The brains were used for immunohistochemical and immunofluorescent techniques with antibodies to NeuN, Cleaved Caspase-3 (CC-3), PCNA and Insulin Receptor (IR). STZ worsened spatial memory in the YMT, as well as short-term (STM) and long-term (LTM) memories in the ORT and ODT, respectively. EXE-4 protected against memory impairment in STZ animals. STZ reduced mature neurons density (NeuN) and increased cell apoptosis (CC-3) in the DG, CA1 and CA3. EXE-4 protected against neuronal death in all regions. EXE-4 increased $PCNA^+$ cells in all regions of the hippocampus, and STZ attenuated this effect. EXE-4 increased immunoreactivity to IR in the CA1. From these findings, EXE-4 showed a beneficial effect on hippocampal pyramidal and granular neurons in the SDAT showing anti-apoptotic properties and promoting cell proliferation in. In parallel, EXE-4 preserved the memory of SDAT rats. In conclusion, these data indicate that agonists to GLP-1R have a beneficial effect on hippocampal neurons in AD.

Key-Words: SDAT; exendin-4; incretin mimetics; neurons; hippocampus; memory.

Introduction

Alzheimer's Disease (AD) is the most common form of dementia found and it is estimated that there are 35.6 million people with AD worldwide, a number that will double by 2030 and triple by 2050 [1,2]. AD is biologically defined by the presence of β-amyloid-containing plaques and tau-containing neurofibrillary tangles. AD is a genetic and sporadic neurodegenerative disease that causes an amnestic cognitive impairment in its prototypical presentation and non-amnestic cognitive impairment in its less common variants. The cognitive impairment is acquired in midlife and late-life but its clinical impact is modified by other neurodegenerative and cerebrovascular conditions, and a complex interplay of loss of synaptic homeostasis [3].

Dysfunctions in brain glucose metabolism and insulin signaling have been linked to a key role in the etiopathology of sporadic AD [4,5]. Glucose metabolism, as assessed by positron emission tomography, is substantially reduced in the hippocampus and brain cortex of familial AD subjects [6]. Furthermore, altered glucose uptake is also observed in the brain of patients with mild cognitive impairment, considered a prodromal stage of AD [7]. In this sense, the intracerebroventricular (icv) administration of streptozotocin (STZ), at a subdiabetogenic dose, has become an experimental model used to mimic Sporadic Dementia of Alzheimer Type (SDAT). STZ causes insulin signaling dysfunction by interfering with brain glucose metabolism, damages in cholinergic neurons, induces oxidative stress and neuroinflammation, culminates in tau hyperphosphorylation and amyloid beta (Aβ) accumulation, and results in deficits in learning and memory [8-12].

Exendin-4 (EXE-4) is a 39 amino acid polypeptide similar to glucagon like peptide-1 (GLP-1). It was isolated from the saliva of the Gila monster (*Heloderma suspectum*) and due to this similarity, it has biological properties analogue to GLP-1 acting as an agonist for its receptor [13]. Furthermore, EXE-4 has become a drug for the treatment of type 2 diabetes mellitus (T2DM) [13]. When administered peripherally, it crosses the blood-brain barrier (BBB) and acts as a neurotrophic agent regulating cell proliferation, neurogenesis, neuronal dendritic growth and protecting neurons in pathological conditions [14-17]. From this evidence, studies have shown the beneficial effect of EXE-4 and others GLP-1 mimetics on the CNS, stimulating neurogenesis, increasing cell repair and reducing pro-inflammatory responses, as well as improvements in cognitive impairment [18-21]. There is also evidences that EXE-4 promotes an increase in brain insulin synthesis in mice [22,23].

Finding a suitable molecular target and a successful multifaceted pharmacological approach to AD is a major challenge in drug development. Studies show a strong correlation between T2DM and AD [24,25]. Compared with the general population, the increased risk of dementia is 50%–150% in people with T2DM [26-28]. Subjects with T2DM have significant higher incidence of AD than those without diabetes, with a relative risk of 1.53 (1.42-1.63) [25]. This occurs due to glucose metabolism and insulin-mediated signaling are worsened in both pathologies [24].

In view of this, the present study sought to find a neuroprotective effect of EXE-4, a drug used in T2DM, in the face of worsening memory and neuronal loss found in SDAT. For this, spatial and discriminatory memory were investigated in rats treated with EXE-4 and induced to SDAT. Concomitantly, immunofluorescence and immunohistochemical markers for apoptosis and cell proliferation in the CA1, CA3 e DG. Based on these findings, this study seeks to find a nootropic and neuroprotective effect of EXE-4 in an

SDAT-model, and thereby contribute new information about the role of insulin signaling pathway and the use of incretin mimetics on neuronal survival and cell death present in sporadic dementia.

Materials and Methods

Chemicals used in treatments

Streptozotocin (STZ, Sigma-Aldrich®, Reference Code: S0130) and Exendin-4 (EXE-4 ≥ 97%, Sigma Aldrich®, Reference Code: E7144) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other reagents used in the experiments were of analytical grade and of highest purity.

Experiential model and ethical concepts

Adult male *Wistar* rats (n= 38) weighing 300g on average were obtained from the local breeding colony of Federal University of Health Sciences of Porto Alegre (UFCSPA, Brazil). The animals were maintained in the Central Animal House of the UFCSPA in colony cages at an ambient temperature of 23 ± 2°C and relative humidity of 45–55% with 12 h light/dark cycles and with free access to a standard rodent pellet diet and water *ad libitum.*

This study was approved by the Committee of Ethics and Animal Experimentation (CEUA/UFCSPA) of the Federal University of Health Sciences of Porto Alegre, Brazil, under protocol number 191/16, identification code: 488/16. The use of animals was in accordance with the Brazilian Guidelines for the Care and Use of Animals in Scientific Research Activities, which is in agreement with the National Council of Control of Animal Experimentation (CONCEA). The animals were handled in accordance with international and national laws for the ethical care and handling of laboratory animals (Guidelines of the Council of the European Communities of 22 September 2010, 2010/63/EU and Law 11.794/08).

Experimental Protocol

Treatment with Exendin-4

The rats received EXE-4 at a dose of 10µg/kg body weight, previously dissolved in saline via intraperitoneal (i.p.) (1ml/kg) during 21 days (a total of 21 administrations were performed, once a day, see figure **1A**). The dose was chose based on previous studies [29,30]**.** The control group received only the vehicle (1ml/kg of saline, i.p.). Rats were randomly distributed into four groups: Vehicle, EXE-4 10µg/kg (EXE-4), STZ, and STZ *plus* EXE-4 10µg/kg. More information about groups can be viewed in the experimental design (see figure **1B**).

Stereotactic Surgery and Induction of SDAT

SDAT-induction was performed as previously described [31]. The animals were anesthetized with ketamine (Dopalen® 10%, São Paulo/Brazil, 80-100 mg/kg i.p.) and xylazine (Anasedan® 2%, São Paulo/Brazil, 5-10 mg/kg i.p.) and then the animal's head hair was shaved before being placed in the stereotaxic apparatus. Asepsis was performed with 3% hydrogen peroxide for subsequent sagittal incision on the midline to expose the cranial scalp. Two bilateral holes were drilled with a drill, following the stereotaxic coordinates

of the lateral ventricles provided by Paxinos and Watson's atlas of stereotaxic coordinates (0.8 mm in the anteroposterior axis, 1.5 mm in the medial-lateral axis, and 4 mm in the dorsoventral axis) [32]. Through these orifices, with a microliter syringe (Hamilton®, 10µl, Reference Code: 80300), STZ dissolved in artificial cerebrospinal fluid (ACFS, 5 µl/ventricle) and injected at a dose of 3 mg/kg. This dose/volume has been pre-established in previous work by the project's collaborating group as well as described by other research groups and is shown to be effective in the development of dementia-related cognitive deficit [31,11]. Animals not induced to SDAT received ACFS only. After surgery, the animals were kept warm with the aid of an electric digital heater and latex hot water bag. After regaining consciousness, the rats were put back into the housing box with water and food *ad libitum*. Two days after surgery, all animals received a bottle with a watery solution of nest milk (20g/1000ml) renewed each day for five consecutive days to avoid body weight loss. The degree of invasiveness for this stereotactic procedure is considered moderate. More details can be found in figure **1A**.

Behavioral Tasks for Assessment of Learning and Memory

Y-Maze Task

The Y-Maze task occurred on the 21st day **(**figure **1A)**. The Y-Maze test consists of assessing the spatial short-term memory (STM) capacity. This test checks whether the rodent retains in its memory the space it has just explored. The experiments were conducted in an environment with sound attenuation and low intensity light. The rodent was positioned at the end of the Y-arm (START arm) with access to only one of the arms (FAMILIAR arm). During the first 5 minutes, the animals were allowed to explore these two arms (STAR and FAMILIAR arms). At the end of habituation, the animals were removed from the task. Four hours later, the animals returned for the test. Rats were placed in the START arm, but all arms were available for exploration. The NOVEL arm consists of the arm that was closed in the training session. The test session lasted five minutes. The number of entries and the time spent in the novel arm were used as a parameter for cognitive assessment as previously described [33].

Open-Field Task

The open-field task occurred on the 22nd day (figure **1A**), in a session of habituation to the box for the Object Tasks. The open-field allows observing how the animal behaves in a new and unfamiliar environment. For this, the animals were transferred to a square arena measuring 60 x 60x 60/cm (base x width x height), with the floor divided into quadrants. Each animal was placed in the center of this box, and given 10 minutes to explore the entire site. During that time, the number of crossings, rearing's and time spent in the center were recorded.

Object Recognition Task (ORT) to asses STM

The ORT occurred on day 23. It was performed in the open-field box. The objects used were pairs of glass bottles with different shapes and colors, but with the same size. The box and objects were cleaned after each animal tested with 30% ethanol. The ORT was performed as describe [34]. The animals were randomly selected to perform the behavioral task and the studies were conducted in a blinded manner. The task consisted of habituation, training, and testing sessions, each of them with duration of 10 min. Firstly, rats were habituated

to the apparatus, which stage consisted with open-field. Twenty-four hours later, the training session occurred. Animals were exposed to two equal objects (object A and B), and the exploration time (corresponding to animal's nose touch or get close the object less than 2 cm) was recorded. Climbing or sitting on the object was not considered exploration. The test session was carried out 4 h after training (STM). Rats were placed back in the box and one of the familiar objects (i.e., object A) was replaced by a novel object (i.e., object C). The time spent exploring the familiar and the novel objects were recorded. The discrimination index (INDEX) was then calculated, taking into account the difference of time spent exploring the novel (C) and the familiar (A or B) objects ×100 divided by the sum of time spent exploring the novel (C) and the familiar (A or B), and used as a cognitive parameter:

$$\boldsymbol{INDEX}: [(t.novel - t.familiar) \div (t.novel + t.familiar)]/100$$

Object Displacement Task (ODT) to asses Long Term-Memory (LTM)

The ODT was performed on the 24th. Twenty-four hours after performing the ORT, the animals were returned to the box in the presence of the same objects as the ORT (object A and C). However, object C was moved to another location in the box, and object A remained in the same location. In this situation, the rat's ability to discriminate against two familiar objects was observed, nonetheless one of them had its position in the box changed (object displaced). A time of 10 minutes was given to explore the objects. The time spent exploring the familiar object and the displaced object was recorded, and the INDEX was calculated following the same criteria and the same equation used for the ORT.

Fixation of the brain and preparation of cryosections

On day 24, the animals were deeply anesthetized by i.p. injection of with ketamine (Dopalen® 10%, São Paulo/Brazil, 80-100 mg/kg i. p.) and xylazine (Anasedan® 2%, São Paulo/Brazil, 5-10 mg/kg i. p.), and were euthanized by intracardic transfusion, heparinized with 0.1 ml of heparin (Heparin 5000UI®, Cristália®, São Paulo/Brazil) and perfused with 0.9% saline for 10 minutes, followed by paraformaldehyde (Sigma-Aldrich®, Reference Number:158127) 4% diluted in 0.1 M phosphate buffer (PBS at pH 7.4) for 30 minutes. The brains were post-fixed in 4% paraformaldehyde (24 hours), transferred to a solution of 30% sucrose in PBS-1% until total submersion [35]. Then, the fixed brains were sectioned (5 and 16 μm) following a stereotaxic coordinate atlas [32] and using a Cryostat Leica CM3050S (Leica Microsystem, German) at a temperature of -24 to -26ºC. Next, the sections were mounted on slides coated with 2% gelatin *plus* 0,08% chromalin (chromium and potassium sulfate, Sigma-Aldrich) and finally allowed to dry at room temperature during 24 hours. At last, all sections were stored at -20ºC until use.

Immunostaining for Cryosections

Immunohistochemistry (immunoperoxidase)

For the immunohistochemistry technique, the slides with the frozen samples were washed in ice-cold acetone for 10 min, under agitation. After removing the acetone, the slides were left to rest until total evaporation, and then washed with distilled water. Afterwards, the slides were placed in a water bath for 20 min at 95-98ºC, in 20 mM sodium citrate buffer (pH 6.0), for antigen recovery. Endogenous peroxidase

blocking was performed with 5% hydrogen peroxide prepared in methanol for 10 min (3 times). Non-specific proteins were blocked with 1% bovine serum albumin (BSA, Sigma-Aldrich®, Reference Number: A9647) prepared in PBS plus 0.1% Triton X-100 for 1 h at room temperature (RT).

Anti-Insulin Receptor Alfa: sections were incubated overnight in a refrigerator at 2-8 °C, with the primary polyclonal anti-insulin receptor alpha antibody (5µm sections): 1:200 (Abcam, Cambridge, UK, England, Reference Number: ab5500). The sections for the insulin receptor were incubated for 30 min at room temperature with HRP-conjugated polymer (Envision+ Dual Link system-HRP kit, Dako Reference Number: K4061). Then, diaminobenzidine (DAB+ Chromogen Substrate Liquid System, Dako Reference Number: K3468) was used to visualize the color of the reactions according to the manufacturer's recommendations [36].

Anti-proliferating cell nuclear antigen (PCNA): sections were incubated overnight in a refrigerator at 2-8°C, with anti-PCNA primary antibody (5µm sections): 1:800 (Millipore®, Burlington, Massachusetts, USA, Reference Number: MAB424). The sections were incubated for 30min at room temperature with HRP-conjugated polymer (Envision+ Dual Link system-HRP kit, Dako Reference Number: K4061). Then, diaminobenzidine (DAB+ Chromogen Substrate Liquid System, Dako Reference Number :K3468) was added to the sections for 5 min and finally the slides were counterstained with Harris Hematoxylin for 20 seconds and mounted using Entellan® (Merck®) [36]. Images were acquired using a Leica DM6-B microscope, Leica DFC 7000-T Camera and Leica Las X Software.

Immunofluorescence

Firstly, the samples, still frozen, were washed with cold acetone for 10 minutes, under agitation. Then, the acetone was allowed to evaporate; the sections were washed in PBS twice (10 min) and blocked/permeabilized with 5% bovine serum albumin (BSA, Sigma-Aldrich®, Reference Number: A9647) prepared in PBS plus 0.1% Triton X-100 for 2 hours at RT. Sections were incubated with primary antibodies diluted in 5% BSA solution: **Anti-NeuN** (sections of 5 µm) 1:6000 Millipore®, Burlington, Massachussetts, USA, Reference Number: MAB377); **Anti-Cleaved Caspase-3** (section of 16 µm) 1:200, Cell Signaling Technology®, Danvers, Massachusetts, EUA, Reference Number: #9661).

After incubation with the primary antibody, samples were washed with PBS-Triton X-100 for 5 minutes (4 times) and incubated in the dark for 2 hours with the anti-rabbit IgG H&L antibody (1:1000, secondary antibody, Alexa Fluor® 555, Abcam® - Cambridge - UK, England, Reference Number: ab150078) for anti-Cleaved Caspase-3. For samples labeled with anti-NeuN, slices were incubated with the anti-mouse IgG H&L secondary antibody (1:500, Alexa Fluor® 488, Abcam®, Cambridge - UK, England, Reference Number: ab150113).

Then, the samples were again washed with PBS-Triton for 5 minutes (4 times) and incubated in the dark with the nuclear marker DAPI (1:10,000, 4',6-diamidino-2-phenylindole, Invitrogen® - Eugene - Oregon - USA; Reference number: D1306) for 10 minutes. The slides were washed 4 times with PBS-triton for 5 minutes each. VectaShield® (Vector, Burlingame - California - USA; Reference Number: H-1000) was added over the sections, which were then overlaid with a coverslip and kept at a temperature of 4ºC, in the dark, until the time of capturing the images. Images were acquired using a Leica DM6-B microscope, Leica DFC 7000-T camera and Leica Las-X Software.

Image Acquisition, Standardization and Selection of Area for Analysis

The analysis of the markers was carried out in the hippocampus in the main regions of CA1, CA3 and DG, in order to evaluate the pattern of immunoreactivity of proteins NeuN and cleaved caspase-3 (CC3). Since the study cells are neurons, images were acquired on the pyramidal cell layer of the CA1 and CA3 (see figure **2A**, region 3) as well as on the granular layer of the DG (see figure **2A**, region 8).

Determining factors for the selection of pyramidal and granular layers were established according to the hippocampal anatomy (see figure **2)**. Briefly, the hippocampus consists of thin layers of neurons, folded one on top of the other. The DG is composed of granulated neurons while the Horn of Ammon (CA, from the Latin "Cornu Ammonis") is composed of pyramidal neurons and consists of four regions (CA1, CA2, CA3 and CA4) that together with the entorhinal cortex and the subicular complex integrate the hippocampal formation [37].

The oriens layer (**2**) is the most superficial layer of the hippocampus, situated just inferior to the alveus fibers (**1**). It is composed mainly of basket-cell interneurons and shares many structural characteristics with the deepest layer of the neocortex. The pyramidal layer (**3**) is the thickest and the most important layer of the hippocampus. It is composed of densely packed pyramidal neurons. The pyramidal layer merges with the internal pyramidal layer of the neocortex (**3**). From this information, the acquisition and quantification of proteins occurred specifically in the pyramidal layer (**3**) of the CA1 and CA3 regions (see figure **2A**). The radiate layer (**4**) is mainly composed of the dendrites of the pyramidal cells and stellate cells while the molecular layer is the deepest layer of the hippocampal arch cortex and this layer is mainly composed of interneurons (**5**) [37].

DG is multilayered and its primary cell is granule cell. Granule cell axons are called mossy fibers. The granular layer (**8**) is composed of granule cells, the main cells of DG (**8**), while the molecular layer (**9**) composed of nerve cell bodies and granule cell dendrites (**9**). Since the granular cells are the most important in the DG, the quantifications were carried out specifically in this region (**8**). Finally, the hilum (**7**) can be observed between the external granular layer (above the hilum) and internal granular layer (below the hilum) (see figure **2A**).

Images were captured using a 20X Objective Lens. From that, a specific area was standardized according to the size of the pyramidal or granular layer and delimited for each image in: **DG** (area of 770,172.7 μm^2), **CA1** (area of 102,664.53 μm^2) and **CA3** (area of 287,693.13 μm^2) (see figure **2B**)

The images obtained for NeuN, and Insulin Receptor-alpha 1 were measured by optical densitometry using ImagePro Plus®6.3 software (Media Cybernetics®). Images obtained for CC3 and PCNA were measured by double counting of positive cells using ImagePro Plus® 6.3 software (Media Cybernetics®).

Statistical Analysis

The normality analysis of the samples was performed by Kolmogorov Smirnov test. Afterwards, parametric data were analyzed using two-way analysis of variance (ANOVA) followed by the Bonferroni test when appropriate. Body weight gain was analyzed by one-way ANOVA followed by post hoc Tukey Test. Results were expressed as the mean ± standard error (SE). P values less than 0.05 ($P<0.05$) were considered as indicative of significance.

Results

Effect of treatment with exendin-4 (EXE-4) in rats subjected to a model of sporadic dementia of the Alzheimer type (SDAT) on body weight gain and blood glucose levels.

Graph **3A** shows that there was no significant difference between treatments in the body weight gain when compared to the vehicle group [$F_{(3,33)}$=2.484; P=0,266) on the start of treatment (Day 1). On the seventh day, there was a significant reduction in body weight gain ($F_{(3,33)}$=9.224; P=0,0001) induced by STZ in relation to the vehicle group [vehicle *vs* STZ, P=0,0024; vehicle *vs* EXE-4+STZ, P<0,0001; EXE-4 *vs* STZ, P=0,0034].

The EXE-4, STZ and EXE-4+STZ groups showed a significant reduction in body weight gain on the fourteenth day compared to the vehicle group ($F_{(3,33)}$=13,73; P<0,0001) [vehicle *vs* EXE-4, P=0,0309; vehicle *vs* STZ, P=0,0001; EXE-4 *vs* STZ, P=0,0005]. The same effect was also observed on day 21, where all treatments reduced body weight gain ($F_{(3,33)}$=15.85; P<0,0001) [vehicle *vs* EXE-4, P=0,0058; vehicle *vs* STZ, P<0,0001; vehicle *vs* EXE-4+STZ, P<0,0001; EXE-4 *vs* EXE-4+STZ, P=0,0006; STZ *vs* EXE-4+STZ, P=0,0159].

Over the 21 days, both STZ and EXE-4 treatment reduced body weight gain. Furthermore, STZ showed a more robust effect in reducing body weight gain when compared to EXE-4. The interaction of EXE-4 *plus* STZ treatments showed a summation effect, further reducing body weight gain.

Graph **3B** shows that STZ reduced blood glucose levels in the STZ and EXE-4+STZ groups compared to the vehicle group ($F_{(1,32)}$=6.668; P=0,0148), excluding a hyperglycemic episode of diabetes. No effects of EXE-4 were observed in relation to vehicle group ($F_{(1,32)}$=0,005; P=0,9812). No significant interaction between treatments EXE-4 or saline *versus* STZ or ACFS ($F_{(1,32)}$=0.024, P=0,877) were observed to blood glucose levels.

Exendin-4 protects spatial memory in the Y-Maze task in rats subjected to a sporadic dementia of the Alzheimer type.

Image 4A shows a schematic execution of the Y-Maze task. Graph **4B** and **4C** show that EXE-4 and STZ do not change the frequency of animals in the familiar arm ($F_{(1,33)}$=1.255; P=0,2707) nor the time spent in the familiar arm ($F_{(1,33)}$=0.123; P=0,7288) during training session.

Graph **4D** shows that there was a significant interaction between treatments, where EXE-4 protects against the reduction in frequency of animals in the novel arm induced by STZ ($F_{(1,33)}$=5,061; P=0,0313) during the test. Furthermore, the same nootropic effect was seen when evaluating the time spent in the novel arm, since EXE-4 protects against the reduction of exploration time in the novel arm induced by STZ ($F_{(1,31)}$=4,909; P=0,0342, graph **4E**) in the test.

Exendin-4 protects discrimination memory in object recognition and object displacement tasks in rats submitted to sporadic dementia of the Alzheimer type.

Image 5A shows a schematic execution of the habituation, training and testing tasks. First, the animals were habituated to the object recognition box. During this session, the number of crossings, rearing and time spent in the center were noted (graph **5B**, **5C** and **5D**). The next day, training for object recognition was

performed, with two identical objects (**5E**). After 4 hours, one of the objects was replaced and the discrimination index (INDEX) was evaluated between the groups (**5F**). Finally, the next day the animals returned to the box and the new object was moved. The INDEX for the displaced object was also evaluated (**5G**).

Graph **5B** shows that the STZ group performed a greater number of crossings compared to the vehicle group ($F_{(1,33)}$=7.056, P=0.0121). No significant interaction was observed between treatments for this parameter ($F_{(1,33)}$=0.289; P=0.8661). There was also no significant interaction for the number of rearing between groups ($F_{(1,33)}$=3.316; P=0,077; graph **5C**).

Graph **5D** shows that EXE-4 and STZ do not change the time spent in the center of the object recognition box [EXE-4: $F_{(1,32)}$=0.5528; P=0,462; STZ: $F_{(1,32)}$=1.627; P=0,65]. In parallel, no significant interaction was found between treatments ($F_{(1,32)}$=0.3662; P=0,5515).

During the training session, none of the treatments changed the object recognition INDEX ($F_{(1,33)}$=0.9702; P=0,3318; graph **5E**). STZ reduced the INDEX in the ORT compared to the vehicle group [$F_{(1,32)}$=4.854; P=0,0351; graph **5F**]. In addition, a significant interaction between treatments was observed (F(1.32)=5.128; P=0.0307; graph **5F**) indicating a protective effect of EXE-4 against memory impairment induced by STZ.

Graph **5G** shows the INDEX for ODT. It was observed that STZ significantly reduced the INDEX when compared to the vehicle group; and EXE-4 protected against this effect [$F_{(1,32)}$=10.45; P=0,0028, graph **5G**]. Furthermore, EXE-4 *per se* increased the INDEX compared to the vehicle group ($F_{(1,32)}$=6.412; P=0,0164).

Exendin-4 protects against the reduction of mature neurons in the hippocampus of rats submitted to a model of sporadic dementia of the Alzheimer type.

Figure **6** shows the immunoreactivity for Neu-N in different regions in the hippocampus of rats with SDAT and treated with EXE-4. Image **6A** shows representative immunoreactions of each group in the DG, CA1 and CA3, respectively. Graph **6B** shows a significance in the interaction between EXE-4 or saline *versus* STZ or ACFS [$F_{(1,26)}$=13.18; P=0,001; graph **6B**] was observed for the granular neurons that make up the DG, suggesting a protective effect of EXE-4 against the deleterious effects of STZ for the population of NeuN-immunoreactive cells.

Finally, a significance in the interaction between EXE-4 or saline *versus* STZ or ACFS [$F_{(1,26)}$=7.180; P=0,0144, graph **6C**], suggesting that EXE-4 protected against the reduction in immunoreactivity to NeuN in the pyramidal layer in the CA1 region induced by STZ. In the CA3 region, a significance was observed in the interaction between EXE-4 or saline *versus* STZ or ACFS [$F_{(1,26)}$=9.951; P=0,0044; graph **6D**], suggesting that EXE-4 also protected against the reduction in immunoreactivity to NeuN in the pyramidal layer in the CA3 region induced by STZ.

Exendin-4 protects against apoptosis of neurons in the hippocampus of rats submitted to a model of sporadic dementia of the Alzheimer type

Figure **7** shows the immunoreactivity for CC3 in the hippocampus of rats with SDAT and treated with EXE-4. Image **7A** shows representative immunoreactions of each group in the DG, CA1 and CA3, respectively.

Graph **7B** shows a significant interaction between EXE-4 or saline *versus* STZ or ACFS [$F_{(1,26)}$=28.00; P<0.0001; graph **7B**] was also observed in DG, suggesting that EXE-4 is effective in reducing $CC3^+$ cells in the granular layer induced by STZ.

Graph 7C shows a significant interaction between EXE-4 or saline *versus* STZ or ACFS [F(1,26)=14.68; P=0.0006; graph 7C], suggesting that EXE-4 reduced the apoptosis induced by STZ in the pyramidal layer of the CA1 region. In the CA3 region, a significance was observed in the interaction between EXE-4 or saline *versus* STZ or ACFS [$F_{(1,26)}$=14.54; P=0.0007; graph **7D**], also suggesting an anti-apoptotic effect of EXE-4 in the pyramidal layer against the deleterious effects of STZ.

Exendin-4 enhances $PCNA^+$ cells in rat hippocampus

Figure **8A** shows the immunoreactivity for PCNA in different regions in the hippocampus of rats with SDAT and treated with EXE-4. Graph **8B** shows that EXE-*4 per se* increased the number of $PCNA^+$ cells in the CA1 region compared to vehicle group [$F_{(1,26)}$=63.98; P<0,0001].

In the CA3 region, EXE-4 *per se* increased $PCNA^+$ cells compared to vehicle group [$F_{(1,26)}$=46.16; P<0,0001, graph **8C**]. A significance in the interaction between EXE-4 or saline *versus* STZ or ACFS [$F_{(1,26)=}$36.36; P<0,0001; graph **8C**] was observed, suggesting that STZ reduces the increase in $PCNA^+$ cells induced by EXE-4.

In DG, a significance in the interaction between EXE-4 or saline *versus* STZ or ACFS [$F_{(1,26)}$=9.577; P=0,0063; graph **8D**] was observed, suggesting that STZ also reduces the increase in the number of $PCNA^+$ cells induced by EXE-4. Furthermore, EXE-4 increased *per se* the number of $PCNA^+$ cells compared to vehicle group [$F_{(1,26)}$=11.06; P=0,0038; graph **8D**]

Exendin-4 enhances insulin receptor immunoreactivity in rat hippocampus

Figure **9** shows the immunoreactivity for insulin receptor in the hippocampus of rats with SDAT and treated with EXE-4. Image **9A** shows the immunoreactions of each group in the CA1, CA3 and DG, respectively. Graph **9B** does not show significance in the interaction between EXE-4 or saline *versus* STZ or ACFS [$F_{(1,26)}$=0.600; P=0,4496] in the CA1. EXE-4 *per se* increases insulin receptor immunoreactivity compared to the vehicle group [$F_{(1,26)}$=5.748; P=0,0291].

Graphs **9C-D** do not show significant effects between treatments for the CA3 [$F_{(1,26)}$=0.9802; P=0,3389; graph **9C**] and DG [$F_{(1,26)}$=1.168; P=0,2381; graph **9D**], respectively.

Discussion

AD is the most common cause of dementia, and its risk is increased by T2DM. It is still unclear whether T2DM and AD are parallel phenomena or synergistic diseases linked by pathological cycles with associated metabolic alterations, but common evidence points to an impairment in the insulin signaling pathway that culminates in a reduction in the cellular metabolism as a key element in this process [38,39]. Based on this hypothesis, the SDAT model was used in this study since the STZ inducing agent has the mechanism of causing a sporadic form of dementia, triggered by through impairments in insulin signaling and in the cascades activated by its receptor, as well as in other cellular events involving neuroinflammation, oxidative stress and hippocampal atrophy, all of which are present in AD [8,40,41] .

GLP-1R agonists, such as EXE-4, exert antidiabetic and anti-obesity effects, including regulation of insulin homeostasis glucose-dependent and regulation of food intake and weight loss [42]. GLP-1R agonist treatments can effectively suppress increased glycemic parameters and body weight [43]. These evidences corroborate data from this study, which showed a reduction in body weight gain in the EXE-4 and EXE-4 *plus* STZ groups. Furthermore, only SDAT animals showed a reduction in blood glucose, confirming that the STZ dose was subdiabetogenic.

In the CNS, GLP-1Rs are observed in several areas of the brain, especially the hypothalamus and hippocampus. [44]. In the hypothalamus, GLP1-R acts on food satiety and stress response [44]; whereas in the hippocampus, they are related to learning and memory function [44]. GLP-1 agonists also promote neuritogenesis and increase long-lasting potentials (LTP) in the hippocampus [45]; and cleavage products of GLP-1 (9-36) increase hippocampal synaptic plasticity in correlation with expression and phosphorylation of Kv4.2 channels (a channel subtype for K^+ ions) [45].

It is known that insulin also plays an important role in cognitive functions. [46] and neuronal activity [47,48]. For example, signaling mediated by this peptide triggers underlying molecular cascades vital for synaptic plasticity, acquisition and consolidation of hippocampal memories [49]. A reduction in this signaling, whether due to insulin deficiency or resistance to this receptor, reduces neural stem cell populations, neuron activity and firing, and compromises hippocampal synaptic plasticity [49]. This evidence highlights the critical role of brain insulin resistance at the "crossroads" between T2DM and AD. From this, finding pharmacological agents capable of reestablishing this signaling assumes an important role as they can contribute to both diabetic neuropathy and dementia caused by AD.

EXE-4 has the ability to elevate peripheral and brain insulin levels by activating GLP1-R [50]. Systemic EXE-4 is able to stimulate the proliferation of pancreatic β-cells [51], increase proinsulin hormone biosynthesis and potentiate insulin responses in a glucose-dependent manner [52]. It was seen in this study that EXE-4 (i.p.) showed a protective effect on short- and long-term memory compromised by SDAT in behavioral tasks for spatial (Y-Maze) and discriminatory (ORT/ODT) memory. It is known that STZ (3mg/kg, icv) induces worsening of learning and memory from the 14th day after injection [10,31]. EXE-4 protected STZ-induced spatial and discriminatory memory. These findings point out that GLP-1R can promote a nootropic effect in neuropathic conditions associated with T2DM and SDAT.

Garabadu and Verma (2019) showed that EXE-4 at a dose of 5 μg/kg i.p also significantly attenuated memory deficits induced by Aβ peptide (bilateral, icv) in rats submitted to Morris Water Maze and Y-maze Tasks. In that same study, a significant decrease in Aβ levels was seen in the hippocampus and prefrontal cortex, as well as an increase in the levels of the neurotransmitter acetylcholine and in the activity of the enzyme choline acetyl-transferase (responsible for producing this neurotransmitter). Garabadu and Verma (2019) also showed that EXE-4 protected against the reduction in the activity of enzymes I, IV and V of the mitochondrial complex, indicating the beneficial capacity of incretin-mimetics on mitochondrial metabolism [53]. Thus, the protective role of systemic administration of EXE-4 on memory corroborates the findings of this study, even using another experimental model for preclinical studies for AD. Interestingly, Wang et al. (2016) showed a similar effect of EXE-4, but drug administration occurred intra-hippocampal [21]. An injection of 0.625 ηmol/1μl/site of EXE-4 protected against memory impairment by Aβ1-42 in the Morris Water Maze. Through electrophysiology studies, there was a recovery of long-term potentiation in the rat hippocampal CA1 region

in vivo [21]. These data show that the neuroprotective effects of GLP1-R agonists may go beyond their ability to stimulate peripheral insulin production and release, which occurs in intraperitoneal administrations.

Grieco et al. (2019) reviewed GLP-1R signaling in cognitive function against neurodegenerative diseases. [54]. To better understand the protective mechanisms coming from GPL-1R agonists, the authors associated the increase in neuronal functions (firing and number of synapses), the control of blood pressure, the stimulation of neurogenesis and the reestablishment of energy homeostasis in the nervous tissue [54]. Interestingly, GLP-1R stimulation also seems to reduce Aβ aggregation, hallmark of AD [55]. To explain the nootropic effect of EXE-4 on memory protection in SDAT, it is not possible to rule out all these biological properties of GLP1-R agonists. However, anti-apoptotic properties were evident and predominate in all hippocampal regions investigated. It was seen that STZ reduced the populations of mature neurons ($NeuN^+$ cells) in the CA1, CA3 and DG regions. In parallel, EXE-4 protected against this reduction, in other words, EXE-4 increased the immunoreactivity of NeuN in the pyramidal layers of CA1 and CA3 as well as in the granular layers of DG. This finding indicates that EXE-4 protects neuronal populations against an injury caused by STZ. From $CC3^+$ cell counts performed in these same layers, an increase in cellular apoptosis in the CA1, CA3 and DG regions was confirmed in SDAT animals. EXE-4 also protected against this effect, attenuating apoptosis in the pyramidal and granular layers that are formed mainly by neurons.

Qiu and colleagues (2016) investigated the anti-apoptotic mechanism of EXE-4 in neurons exposed in vitro to Aβ 1-42 oligomers [56]. EXE-4 concentrations in a range of 100 to 300 ηM increase the viability of neurons (PC12 cells), reduce the active expression of pro-caspase-3 and Bcl-2. Finally, EXE-4 re-established the AKT pathway and phosphorylation of cAMP-response binding protein (CREB) element, which is described to regulate neuronal survival and stimulate neurotrophin production [56]. Xie et al. (2018) found a similar result in vivo using an experimental model of injury after subarachnoid hemorrhage in rats. EXE-4 (i.p.) also attenuated neuronal death via the GLP1-R/PI3K/AKT pathway and reduced brain Bcl-2/Bax levels [57]. These findings are in agreement with those reported in this study since systemic EXE-4 reduced apoptosis in the layers formed by neurons in the hippocampus of SDAT rats.

PCNA, or proliferating cell nuclear antigen, plays an essential role in nucleic acid metabolism, replication and repair. This protein surrounds DNA and confers processability to DNA polymerase enzymes, interacts with proteins involved in cell cycle progression, and has a direct effect on DNA synthesis [58]. Since then, PCNA has been used in studies to investigate cell proliferation and nucleic acid metabolism [59]. EXE-4 has been identified as a pharmacological agent capable of stimulating cell proliferation. EXE-4 increased cell proliferation and neuroblastic differentiation in adult rat hippocampus [60], with emphasis on the sub-granular area of the DG [15], and increased neurogenesis in the sub-ventricular zone [14]. Here, the results showed that EXE-4 increased immunoreactivity for PCNA in cells that make up the pyramidal layer of the CA1 and CA3 regions as well as the granular layer of DG, suggesting an increase in nucleic acid metabolism and proliferation in the cells that make up these regions, that is, pyramidal and granular neurons. The association between STZ *plus* EXE-4 blocked the effect of EXE-4 on the increase of PCNA in the CA3 and DG regions. This effect can be explained since this SDAT model is able to reduce neurogenesis and cell proliferation in the hippocampus of rats [61] and mice [62]. Although no effect per se was observed in the SDAT group on PCNA immunoreactivity, STZ was able to reduce the properties of EXE-4 on cell proliferation and nucleic acid metabolism. It is important to highlight that cell cycle mechanisms can be active in cell proliferation processes,

but also be aberrantly activated in an injured or aged brain, and this effect is seen in AD [63,64]. *Post-mortem* analysis revealed that the hippocampi of elderly people affected by this pathology show an increase in the expression of PCNA, Mcm2 and Ki67 (markers of cell proliferation), and that there is a greater increase in the expression of these markers in association with more advanced cases of AD. [63,64]. However, this effect was not observed in SDAT animals.

Recently, it was reported that EXE-4 was able to increase insulin levels in the cerebrospinal fluid of diabetic mice. [22]. Liraglutide, another GLP1-R analogue, prevented the loss of insulin receptors and synapses in the brain of mice subjected to the Aβ injection model. The infusion of Aβ into the lateral cerebral ventricle of non-human primates (NHPs) led to a marked loss of insulin receptors and synapses in memory-related brain regions, an effect attenuated by liraglutide [65]. Batista et al. (2018) showed, in this study with NHPs, that the damage and elimination of insulin synapses are among the first known pathological changes and the best correlates of memory impairment in AD and that evidence of this level shows the importance of understanding the neuroprotective mechanisms of incretin-mimetics by activating GLP-1R to protect brain insulin receptors and synapses in AD [65]. Here, the SDAT model showed no change in immunoreactivity for the insulin receptor in the hippocampus, but EXE-4 increased the immunoreactivity of insulin receptors in the CA1 region.

In conclusion, agonists for GLP-1R have been reported as neuroprotective agents in preclinical models of AD. The anti-apoptotic properties of EXE-4 on hippocampal neuronal populations can compose one of the main mechanisms in the nootropic action of EXE-4 for protecting learning and memory in SDAT. Furthermore, EXE-4 has shown the ability to stimulate nuclear metabolism and hippocampal cell proliferation as well as the density of insulin receptors in the CA1 region. These results demonstrate the potential properties of GLP-1R agonists for the prevention and treatment of dementia associated with T2DM and AD. From this, more preclinical studies should be performed in order to better elucidate the mechanisms of action of these receptors as well as their nootropic properties for neurodegenerative diseases, including AD.

References

1. Haines JL (2018) Alzheimer Disease: Perspectives from Epidemiology and Genetics. The Journal of law, medicine & ethics : a journal of the American Society of Law, Medicine & Ethics 46 (3):694-698. doi:10.1177/1073110518804230

2. Reitz C, Brayne C, Mayeux R (2011) Epidemiology of Alzheimer disease. Nature reviews Neurology 7 (3):137-152. doi:10.1038/nrneurol.2011.2

3. Knopman DS, Amieva H, Petersen RC, Chetelat G, Holtzman DM, Hyman BT, Nixon RA, Jones DT (2021) Alzheimer disease. Nature reviews Disease primers 7 (1):33. doi:10.1038/s41572-021-00269-y

4. Duran-Aniotz C, Hetz C (2016) Glucose Metabolism: A Sweet Relief of Alzheimer's Disease. urrent biology : CB 26 (17):R806-809. doi:10.1016/j.cub.2016.07.060

5. Calsolaro V, Edison P (2016) Alterations in Glucose Metabolism in Alzheimer's Disease. Recent patents on endocrine, metabolic & immune drug discovery 10 (1):31-39. doi:10.2174/1872214810666160615102809

6. Chen Z, Zhong C (2013) Decoding Alzheimer's disease from perturbed cerebral glucose metabolism: implications for diagnostic and therapeutic strategies. Progress in neurobiology 108:21-43. doi:10.1016/j.pneurobio.2013.06.004

7. Mosconi L, Andrews RD, Matthews DC (2013) Comparing brain amyloid deposition, glucose metabolism, and atrophy in mild cognitive impairment with and without a family history of dementia. Journal of Alzheimer's disease : JAD 35 (3):509-524. doi:10.3233/JAD-121867

8. Mehla J, Pahuja M, Gupta YK (2013) Streptozotocin-induced sporadic Alzheimer's disease: selection of appropriate dose. Journal of Alzheimer's disease : JAD 33 (1):17-21. doi:10.3233/JAD-2012-120958

9. Gerzson MFB, Bona NP, Soares MSP, Teixeira FC, Rahmeier FL, Carvalho FB, da Cruz Fernandes M, Onzi G, Lenz G, Goncales RA, Spanevello RM, Stefanello FM (2020) Tannic Acid Ameliorates STZ-Induced Alzheimer's Disease-Like Impairment of Memory, Neuroinflammation, Neuronal Death and Modulates Akt Expression. Neurotox Res 37 (4):1009-1017. doi:10.1007/s12640-020-00167-3

10. Pacheco SM, Soares MSP, Gutierres JM, Gerzson MFB, Carvalho FB, Azambuja JH, Schetinger MRC, Stefanello FM, Spanevello RM (2018) Anthocyanins as a potential pharmacological agent to manage memory deficit, oxidative stress and alterations in ion pump activity induced by experimental sporadic dementia of Alzheimer's type. The Journal of nutritional biochemistry 56:193-204. doi:10.1016/j.jnutbio.2018.02.014

11. Rodrigues MV, Gutierres JM, Carvalho F, Lopes TF, Antunes V, da Costa P, Pereira ME, Schetinger MRC, Morsch VM, de Andrade CM (2019) Protection of cholinergic and antioxidant system contributes to the effect of Vitamin D3 ameliorating memory dysfunction in sporadic dementia of Alzheimer's type. Redox Rep 24 (1):34-40. doi:10.1080/13510002.2019.1617514

12. Javadpour P, Askari S, Rashidi FS, Dargahi L, Ahmadiani A, Ghasemi R (2021) Imipramine alleviates memory impairment and hippocampal apoptosis in STZ-induced sporadic Alzheimer's rat model: Possible contribution of MAPKs and insulin signaling. Behav Brain Res 408:113260. doi:10.1016/j.bbr.2021.113260

13. Yap MKK, Misuan N (2019) Exendin-4 from Heloderma suspectum venom: From discovery to its latest application as type II diabetes combatant. Basic Clin Pharmacol Toxicol 124 (5):513-527. doi:10.1111/bcpt.13169

14. Bertilsson G, Patrone C, Zachrisson O, Andersson A, Dannaeus K, Heidrich J, Kortesmaa J, Mercer A, Nielsen E, Ronnholm H, Wikstrom L (2008) Peptide hormone exendin-4 stimulates subventricular zone neurogenesis in the adult rodent brain and induces recovery in an animal model of Parkinson's disease. Journal of neuroscience research 86 (2):326-338. doi:10.1002/jnr.21483

15. Li H, Lee CH, Yoo KY, Choi JH, Park OK, Yan BC, Byun K, Lee B, Hwang IK, Won MH (2010) Chronic treatment of exendin-4 affects cell proliferation and neuroblast differentiation in the adult mouse hippocampal dentate gyrus. Neuroscience letters 486 (1):38-42. doi:10.1016/j.neulet.2010.09.040

16. Holscher C (2012) Potential role of glucagon-like peptide-1 (GLP-1) in neuroprotection. CNS drugs 26 (10):871-882. doi:10.2165/11635890-000000000-00000

17. Wang M, Yoon G, Song J, Jo J (2021) Exendin-4 improves long-term potentiation and neuronal dendritic growth in vivo and in vitro obesity condition. Sci Rep 11 (1):8326. doi:10.1038/s41598-021-87809-4

18. Bu LL, Liu YQ, Shen Y, Fan Y, Yu WB, Jiang DL, Tang YL, Yang YJ, Wu P, Zuo CT, Koprich JB, Liu FT, Wu JJ, Wang J (2021) Neuroprotection of Exendin-4 by Enhanced Autophagy in a Parkinsonian Rat Model of alpha-Synucleinopathy. Neurotherapeutics 18 (2):962-978. doi:10.1007/s13311-021-01018-5

19. Ahn YJ, Shin HJ, Jeong EA, An HS, Lee JY, Jang HM, Kim KE, Lee J, Shin MC, Roh GS (2021) Exendin-4 Pretreatment Attenuates Kainic Acid-Induced Hippocampal Neuronal Death. Cells 10 (10). doi:10.3390/cells10102527

20. Teramoto S, Miyamoto N, Yatomi K, Tanaka Y, Oishi H, Arai H, Hattori N, Urabe T (2011) Exendin-4, a glucagon-like peptide-1 receptor agonist, provides neuroprotection in mice transient focal cerebral ischemia. J Cereb Blood Flow Metab 31 (8):1696-1705. doi:10.1038/jcbfm.2011.51

21. Wang X, Wang L, Jiang R, Xu Y, Zhao X, Li Y (2016) Exendin-4 antagonizes Abeta1-42-induced attenuation of spatial learning and memory ability. Experimental and therapeutic medicine 12 (5):2885-2892. doi:10.3892/etm.2016.3742

22. Peng X, Shi X, Huang J, Zhang S, Yan Y, Ma D, Xu W, Xu W, Dong K, Tao J, Li M, Yang Y (2021) Exendin-4 Improves Cognitive Function of Diabetic Mice via Increasing Brain Insulin Synthesis. Current Alzheimer research 18 (7):546-557. doi:10.2174/1567205018666210929150004

23. Jo D, Yoon G, Song J (2021) Role of Exendin-4 in Brain Insulin Resistance, Mitochondrial Function, and Neurite Outgrowth in Neurons under Palmitic Acid-Induced Oxidative Stress. Antioxidants (Basel) 10 (1). doi:10.3390/antiox10010078

24. Diehl T, Mullins R, Kapogiannis D (2017) Insulin resistance in Alzheimer's disease. Transl Res 183:26-40. doi:10.1016/j.trsl.2016.12.005

25. Zhang J, Chen C, Hua S, Liao H, Wang M, Xiong Y, Cao F (2017) An updated meta-analysis of cohort studies: Diabetes and risk of Alzheimer's disease. Diabetes Res Clin Pract 124:41-47. doi:10.1016/j.diabres.2016.10.024

26. Biessels GJ, Staekenborg S, Brunner E, Brayne C, Scheltens P (2006) Risk of dementia in diabetes mellitus: a systematic review. Lancet Neurol 5 (1):64-74. doi:10.1016/S1474-4422(05)70284-2

27. Cukierman T, Gerstein HC, Williamson JD (2005) Cognitive decline and dementia in diabetes--systematic overview of prospective observational studies. Diabetologia 48 (12):2460-2469. doi:10.1007/s00125-005-0023-4

28. Strachan MW, Deary IJ, Ewing FM, Frier BM (1997) Is type II diabetes associated with an increased risk of cognitive dysfunction? A critical review of published studies. Diabetes Care 20 (3):438-445. doi:10.2337/diacare.20.3.438

29. Zanotto C, Hansen F, Galland F, Batassini C, Federhen BC, da Silva VF, Leite MC, Nardin P, Goncalves CA (2019) Glutamatergic Alterations in STZ-Induced Diabetic Rats Are Reversed by Exendin-4. Mol Neurobiol 56 (5):3538-3551. doi:10.1007/s12035-018-1320-5

30. Zanotto C, Simao F, Gasparin MS, Biasibetti R, Tortorelli LS, Nardin P, Goncalves CA (2017) Exendin-4 Reverses Biochemical and Functional Alterations in the Blood-Brain and Blood-CSF Barriers in Diabetic Rats. Mol Neurobiol 54 (3):2154-2166. doi:10.1007/s12035-016-9798-1

31. Gutierres JM, Carvalho FB, Schetinger MR, Marisco P, Agostinho P, Rodrigues M, Rubin MA, Schmatz R, da Silva CR, de PCG, Farias JG, Signor C, Morsch VM, Mazzanti CM, Bogo M, Bonan CD, Spanevello R (2014) Anthocyanins restore behavioral and biochemical changes caused by streptozotocin-induced sporadic dementia of Alzheimer's type. Life sciences 96 (1-2):7-17. doi:10.1016/j.lfs.2013.11.014

32. Paxinos G, Watson C (2006) The Rat Brain in Stereotaxic Coordinates. Hard Cover Edition: 6th Edition

33. Gotz J, Ittner LM (2008) Animal models of Alzheimer's disease and frontotemporal dementia. Nat Rev Neurosci 9 (7):532-544. doi:10.1038/nrn2420

34. Carvalho FB, Gutierres JM, Bueno A, Agostinho P, Zago AM, Vieira J, Fruhauf P, Cechella JL, Nogueira CW, Oliveira SM, Rizzi C, Spanevello RM, Duarte MMF, Duarte T, Dellagostin OA, Andrade CM (2017) Anthocyanins control neuroinflammation and consequent memory dysfunction in mice exposed to lipopolysaccharide. Molecular neurobiology 54 (5):3350-3367. doi:10.1007/s12035-016-9900-8

35. Silva SP, Zago AM, Carvalho FB, Germann L, Colombo GM, Rahmeier FL, Gutierres JM, Reschke CR, Bagatini MD, Assmann CE, Fernandes MDC (2021) Neuroprotective Effect of Taurine against Cell Death, Glial Changes, and Neuronal Loss in the Cerebellum of Rats Exposed to Chronic-Recurrent Neuroinflammation Induced by LPS. J Immunol Res 2021:7497185. doi:10.1155/2021/7497185

36. Rahmeier FL, Zavalhia LS, Tortorelli LS, Huf F, Gea LP, Meurer RT, Machado AC, Gomez R, Fernandes MDC (2016) The effect of taurine and enriched environment on behaviour, memory and hippocampus of diabetic rats. Neurosci Lett 630:84-92. doi:10.1016/j.neulet.2016.07.032

37. Fogwe LA, Reddy V, Mesfin FB (2022) Neuroanatomy, Hippocampus. In: StatPearls. Treasure Island (FL),

38. Su M, Naderi K, Samson N, Youssef I, Fulop L, Bozso Z, Laroche S, Delatour B, Davis S (2019) Mechanisms Associated with Type 2 Diabetes as a Risk Factor for Alzheimer-Related Pathology. Molecular neurobiology 56 (8):5815-5834. doi:10.1007/s12035-019-1475-8

39. Lester-Coll N, Rivera EJ, Soscia SJ, Doiron K, Wands JR, de la Monte SM (2006) Intracerebral streptozotocin model of type 3 diabetes: relevance to sporadic Alzheimer's disease. Journal of Alzheimer's disease : JAD 9 (1):13-33. doi:10.3233/jad-2006-9102

40. Grieb P (2016) Intracerebroventricular Streptozotocin Injections as a Model of Alzheimer's Disease: in Search of a Relevant Mechanism. Molecular neurobiology 53 (3):1741-1752. doi:10.1007/s12035-015-9132-3

41. Knezovic A, Loncar A, Homolak J, Smailovic U, Osmanovic Barilar J, Ganoci L, Bozina N, Riederer P, Salkovic-Petrisic M (2017) Rat brain glucose transporter-2, insulin receptor and glial expression are acute targets of intracerebroventricular streptozotocin: risk factors for sporadic Alzheimer's disease? Journal of neural transmission 124 (6):695-708. doi:10.1007/s00702-017-1727-6

42. Chatterjee S, Ghosal S, Chatterjee S (2016) Glucagon-like peptide-1 receptor agonists favorably address all components of metabolic syndrome. World journal of diabetes 7 (18):441-448. doi:10.4239/wjd.v7.i18.441

43. Kodera R, Shikata K, Kataoka HU, Takatsuka T, Miyamoto S, Sasaki M, Kajitani N, Nishishita S, Sarai K, Hirota D, Sato C, Ogawa D, Makino H (2011) Glucagon-like peptide-1 receptor agonist ameliorates renal injury through its anti-inflammatory action without lowering blood glucose level in a rat model of type 1 diabetes. Diabetologia 54 (4):965-978. doi:10.1007/s00125-010-2028-x

44. Merchenthaler I, Lane M, Shughrue P (1999) Distribution of pre-pro-glucagon and glucagon-like peptide-1 receptor messenger RNAs in the rat central nervous system. The Journal of comparative neurology 403 (2):261-280. doi:10.1002/(sici)1096-9861(19990111)403:2<261::aid-cne8>3.0.co;2-5

45. Day SM, Yang W, Ewin S, Zhou X, Ma T (2017) Glucagon-like peptide-1 cleavage product GLP-1 (9-36) amide enhances hippocampal long-term synaptic plasticity in correlation with suppression of Kv4.2 expression and eEF2 phosphorylation. Hippocampus 27 (12):1264-1274. doi:10.1002/hipo.22795

46. Bloemer J, Bhattacharya S, Amin R, Suppiramaniam V (2014) Impaired insulin signaling and mechanisms of memory loss. Progress in molecular biology and translational science 121:413-449. doi:10.1016/B978-0-12-800101-1.00013-2

47. Dodd GT, Michael NJ, Lee-Young RS, Mangiafico SP, Pryor JT, Munder AC, Simonds SE, Bruning JC, Zhang ZY, Cowley MA, Andrikopoulos S, Horvath TL, Spanswick D, Tiganis T (2018) Insulin regulates POMC neuronal plasticity to control glucose metabolism. eLife 7. doi:10.7554/eLife.38704

48. Garcia SM, Hirschberg PR, Sarkar P, Siegel DM, Teegala SB, Vail GM, Routh VH (2021) Insulin actions on hypothalamic glucose-sensing neurones. Journal of neuroendocrinology 33 (4):e12937. doi:10.1111/jne.12937

49. Spinelli M, Fusco S, Grassi C (2020) Brain insulin resistance impairs hippocampal plasticity. Vitamins and hormones 114:281-306. doi:10.1016/bs.vh.2020.04.005

50. Egido EM, Silvestre RA, Hernandez R, Marco J (2004) Exendin-4 dose-dependently stimulates somatostatin and insulin secretion in perfused rat pancreas. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme 36 (9):595-600. doi:10.1055/s-2004-825921

51. Fusco J, Xiao X, Prasadan K, Sheng Q, Chen C, Ming YC, Gittes G (2017) GLP-1/Exendin-4 induces beta-cell proliferation via the epidermal growth factor receptor. Scientific reports 7 (1):9100. doi:10.1038/s41598-017-09898-4

52. Alarcon C, Wicksteed B, Rhodes CJ (2006) Exendin 4 controls insulin production in rat islet beta cells predominantly by potentiation of glucose-stimulated proinsulin biosynthesis at the translational level. Diabetologia 49 (12):2920-2929. doi:10.1007/s00125-006-0433-y

687 53. Garabadu D, Verma J (2019) Exendin-4 attenuates brain mitochondrial toxicity through PI3K/Akt-
688 dependent pathway in amyloid beta (1-42)-induced cognitive deficit rats. Neurochemistry international
689 128:39-49. doi:10.1016/j.neuint.2019.04.006
690 54. Grieco M, Giorgi A, Gentile MC, d'Erme M, Morano S, Maras B, Filardi T (2019) Glucagon-Like
691 Peptide-1: A Focus on Neurodegenerative Diseases. Frontiers in neuroscience 13:1112.
692 doi:10.3389/fnins.2019.01112
693 55. Li Y, Duffy KB, Ottinger MA, Ray B, Bailey JA, Holloway HW, Tweedie D, Perry T, Mattson
694 MP, Kapogiannis D, Sambamurti K, Lahiri DK, Greig NH (2010) GLP-1 receptor stimulation reduces
695 amyloid-beta peptide accumulation and cytotoxicity in cellular and animal models of Alzheimer's disease.
696 Journal of Alzheimer's disease : JAD 19 (4):1205-1219. doi:10.3233/JAD-2010-1314
697 56. Qiu C, Wang YP, Pan XD, Liu XY, Chen Z, Liu LB (2016) Exendin-4 protects Abeta(1-42)
698 oligomer-induced PC12 cell apoptosis. American journal of translational research 8 (8):3540-3548
699 57. Xie Z, Enkhjargal B, Wu L, Zhou K, Sun C, Hu X, Gospodarev V, Tang J, You C, Zhang JH
700 (2018) Exendin-4 attenuates neuronal death via GLP-1R/PI3K/Akt pathway in early brain injury after
701 subarachnoid hemorrhage in rats. Neuropharmacology 128:142-151. doi:10.1016/j.neuropharm.2017.09.040
702 58. Kelman Z (1997) PCNA: structure, functions and interactions. Oncogene 14 (6):629-640.
703 doi:10.1038/sj.onc.1200886
704 59. Raucci F, Di Fiore MM, Pinelli C, D'Aniello B, Luongo L, Polese G, Rastogi RK (2006)
705 Proliferative activity in the frog brain: a PCNA-immunohistochemistry analysis. Journal of chemical
706 neuroanatomy 32 (2-4):127-142. doi:10.1016/j.jchemneu.2006.08.001
707 60. Solmaz V, Cinar BP, Yigitturk G, Cavusoglu T, Taskiran D, Erbas O (2015) Exenatide reduces
708 TNF-alpha expression and improves hippocampal neuron numbers and memory in streptozotocin treated rats.
709 European journal of pharmacology 765:482-487. doi:10.1016/j.ejphar.2015.09.024
710 61. Fronza MG, Baldinotti R, Sacramento M, Gutierres J, Carvalho FB, Fernandes MDC, Sousa FSS,
711 Seixas FK, Collares T, Alves D, Pratico D, Savegnago L (2021) Effect of QTC-4-MeOBnE Treatment on
712 Memory, Neurodegeneration, and Neurogenesis in a Streptozotocin-Induced Mouse Model of Alzheimer's
713 Disease. ACS chemical neuroscience 12 (1):109-122. doi:10.1021/acschemneuro.0c00615
714 62. Martini F, Rosa SG, Klann IP, Fulco BCW, Carvalho FB, Rahmeier FL, Fernandes MC, Nogueira
715 CW (2019) A multifunctional compound ebselen reverses memory impairment, apoptosis and oxidative
716 stress in a mouse model of sporadic Alzheimer's disease. Journal of psychiatric research 109:107-117.
717 doi:10.1016/j.jpsychires.2018.11.021
718 63. Wharton SB, Williams GH, Stoeber K, Gelsthorpe CH, Baxter L, Johnson AL, Ince PG, Mrc C
719 (2005) Expression of Ki67, PCNA and the chromosome replication licensing protein Mcm2 in glial cells of
720 the ageing human hippocampus increases with the burden of Alzheimer-type pathology. Neuroscience letters
721 383 (1-2):33-38. doi:10.1016/j.neulet.2005.04.019
722 64. Toschi L, Bravo R (1988) Changes in cyclin/proliferating cell nuclear antigen distribution during
723 DNA repair synthesis. The Journal of cell biology 107 (5):1623-1628. doi:10.1083/jcb.107.5.1623
724 65. Batista AF, Forny-Germano L, Clarke JR, Lyra ESNM, Brito-Moreira J, Boehnke SE, Winterborn
725 A, Coe BC, Lablans A, Vital JF, Marques SA, Martinez AM, Gralle M, Holscher C, Klein WL, Houzel JC,
726 Ferreira ST, Munoz DP, De Felice FG (2018) The diabetes drug liraglutide reverses cognitive impairment in

mice and attenuates insulin receptor and synaptic pathology in a non-human primate model of Alzheimer's disease. The Journal of pathology 245 (1):85-100. doi:10.1002/path.5056

Statements & Declarations

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Competing Interests

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

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Adriana M. Zago], [Fabiano B. Carvalho], [Francine L. Rahmeier], [Marta Santin], [Giuliano R. Guimarães], [Jessié M. Gutierres] and [Marilda da C. Fernandes]. The first draft of the manuscript was written by [Adriana M Zago] and [Fabiano B Carvalho] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Committee of Ethics and Animal Experimentation (CEUA) of the Federal University of Health Sciences of Porto Alegre (UFCPSPA, Brazil), under Protocol Number 191/16, Identification Code: 488/16, Date: 11/01/2017.

List of legends

Figure 1. (A). Experimental design: Execution of the treatment with exendin-4 (EXE-4) and stereotaxic procedure for the administration of streptozotocin (STZ) as an induction model for sporadic dementia of the Alzheimer's type (SDAT). Rats were acclimatized for ten days. On day 0, stereotaxic surgery was performed for injection of STZ or the vehicle (Artificial Cerebrum-Spinal Fluid, ACFS, pH 7.4). STZ was injected intracerebroventricular in a total volume of 10 µl (5 µl, bilaterally; 1 µl/minute). After this procedure, the rats were allowed to recover for two days. On day 2, treatment started with EXE-4 at a dose of 10 µg/kg, administered intraperitoneally (volume of 1 ml/kg). EXE-4 was prepared in sterile saline (vehicle). The end of treatment with EXE-4 occurred on day 21 (2 hours before the start of behavioral tasks). On day 21, the animals were trained on the Y-Maze task. After 4 hours, the animals were tested for the Y-Maze task. On day 22, the animals were placed in the open field for 10 minutes in order to acclimatize the box. Here, the number of crossings, rearings and time spent in center (s) were noted. On day 23, the animals returned to the box for object recognition task (ORT) training. After 4 hours, the animals returned to perform the object recognition test (short-term memory). On day 24, the animals returned to the box to perform the object displacement task (ODT, long-term memory). After behavioral tests, the animals were immediately anesthetized and euthanized to obtain the brain. **(B)**. Experimental groups: Vehicle Group (Saline *plus* ACSF, n=9), STZ Group (Saline *plus* STZ, n=10), EXE-4 Group (EXE-4 *plus* ACSF, n=9) and EXE-4+STZ Group (EXE-4 *plus* STZ, n=10).

Figure 2. (A). Hippocampus anatomy and distribution of the layers that constitute the Ammon's Horn and dentate gyrus regions. **(B)**. Area selected for image acquisition [**DG** (area of 770,172.7 μm^2), **CA1** (area of 102,664.53 μm^2) and **CA3** (area of 287,693.13 μm^2)].

Figure 3. Effect of exendin-4 (EXE-4) treatment in rats submitted to a model of sporadic dementia of the Alzheimer type (SDAT, STZ) on body weight gain (BW) and blood glucose levels. (**A**). The body weight gain of the different groups was assessed on days 1, 7, 14 and 21. ● Denotes vehicle, ● denotes STZ, □ denotes EXE-4, ■ denotes STZ+EXE-4. Statistical analysis was performed by one-way analysis of variance followed by Tukey's post hoc. Letters a, b and c denote the significant difference between groups on the respective day. $P<0.05$ was considered significant. Data expressed as mean ± standard error of mean for n=9-10. (**B**) Blood glucose levels of the different experimental groups. After euthanasia, the animals' blood was collected for measurement of blood glucose levels (day 24). *Means significant difference compared to vehicle group. Two-way analysis of variance. $P<0.05$ was considered significant. Data expressed as mean ± standard error of mean for n=9-10.

Figure 4. Effect of treatment with exendin-4 (EXE-4, 10µg/kg, ip) in rats submitted to a model of sporadic dementia of the Alzheimer type (STZ, 3mg/kg, icv) on performance in the Y-Maze Task. (**A**) Schematic drawing of the execution in the Y-maze Task. The test was performed 4 hours after training to check short-term memory (STM). Frequency (**B**) and time (**C**) spent on the familiar arm during the training session. Frequency (**D**) and time (**E**) spent on the novel arm during the test session. * Denotes significant difference in relation to the vehicle group. #Denotes significant interaction between treatments (*versus* STZ group). Two-

way analysis of variance. P<0.05 was considered significant. Data expressed as mean ± standard error of mean for n=9-10.

Figure 5. Effect of treatment with exendin-4 (EXE-4, 10µg/kg, ip) in rats submitted to a model of sporadic dementia of the Alzheimer type (STZ, 3mg/kg, icv) on performance in the Object Recognition (ORT) and Object Displacement (ODT) Tasks. (**A**) Scheme of the execution of the Habituation, Training and Test. First, the animals were habituated to the Object Recognition box. During this session, the number of crossings, rearing and time spent in the center were noted (**B**, **C** and **D**, respectively). The next day, object recognition training was performed with two identical objects (**E**). After 4 hours, one of the objects was replaced, and the discrimination index (INDEX) was evaluated between groups (**F**). Finally, the next day the animals returned to the box and the new object was moved. The INDEX for the displaced object was also evaluated (**G**). Crossing (**B**), rearing (**C**), time spent in center (**D**). (**E**) INDEX for training in the ORT. (**F**) INDEX for the test in the ORT (4 hours post-training, STM). (**G**) INDEX for test in the ODT (24 hours, LTM). *Denotes significant difference *versus* vehicle group. #Denotes significant interaction between treatments (*versus* STZ group). Two-way analysis of variance. P<0.05 was considered significant. Data expressed as mean ± standard error of mean for n=9-10.

Figure 6. Effect of treatment with exendin-4 (EXE-4, 10µg/kg, ip) in rats submitted to a sporadic dementia of the Alzheimer type (STZ, 3mg/kg, icv) on the NeuN immunoreactivity on the Granular Layer of the DG, Pyramidal Layer of the CA1 and CA3 regions. (**A)** Representative immunoreactions of each group (DAPI in blue, NeuN in green and Merge). Graph with the results of the statistical analysis of optical densities for NeuN in the CA1 (**B**), CA3 (**C**) and DG (**D**) regions. *Denotes significant difference *versus* vehicle group. #Denotes significant interaction between treatments (versus STZ group). Two-way analysis of variance. P<0.05 was considered significant. Data expressed as mean ± standard error of mean for n = 5-6.

Figure 7. Effect of treatment with exendin-4 (EXE-4, 10µg/kg, ip) in rats submitted to a sporadic dementia of the Alzheimer type (STZ, 3mg/kg, icv) on the Cleaved Caspase-3 (CC3) immunoreactivity immunoreactivity on the Granular Layer of the DG, Pyramidal Layer of the CA1 and CA3 regions. (**A)** Representative immunoreactions of each group (DAPI in blue, CC3 in red and Merge). Graph with the results of the statistical analysis of number of $CC3^+$ cells in the CA1 (**B**), CA3 (**C**) and DG (**D**) regions. *Denotes significant difference versus vehicle group. #Denotes significant interaction between treatments (*versus* STZ group). Two-way analysis of variance. P<0.05 was considered significant. Data expressed as mean ± standard error of mean for n = 5-6.

Figure 8. Effect of treatment with exendin-4 (EXE-4, 10µg/kg, ip) in rats submitted to a sporadic dementia of the Alzheimer type (STZ, 3mg/kg, icv) on the Proliferating Cell Nuclear Antigen (PCNA) immunoreactivity in the Pyramidal Layer of the CA1 and CA3 regions; and on the Granular Layer of the DG. (**A)** Representative immunoreactions of each group. Graph with the results of the statistical analysis of number of $PCNA^+$ cells in the CA1 (**B**), CA3 (**C**) and DG (**D**) regions. *Denotes significant difference *versus* vehicle group. #Denotes

834 significant interaction between treatments (*versus* EXE-4 group). Two-way analysis of variance. P<0.05 was
835 considered significant. Data expressed as mean ± standard error of mean for n = 5-6.
836
837 **Figure 9**. Effect of treatment with exendin-4 (EXE-4, 10μg/kg, ip) in rats submitted to a sporadic dementia of
838 the Alzheimer type (STZ, 3mg/kg, icv) on the Insulin alfa-1 Receptor (IR) immunoreactivity in the Pyramidal
839 Layer of the CA1 and CA3 regions; and on the Granular Layer of the DG. (**A)** Representative immunoreactions
840 of each group. Graph with the results of the statistical analysis of optical density to IR in the CA1 (**B**), CA3
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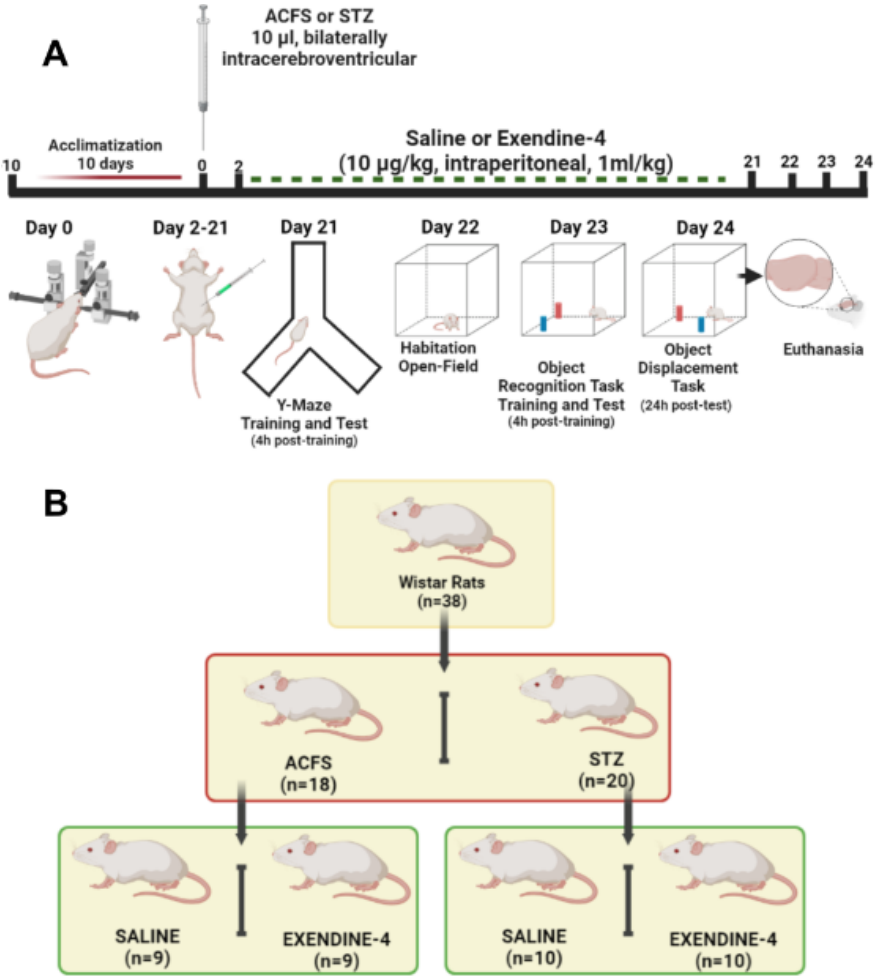

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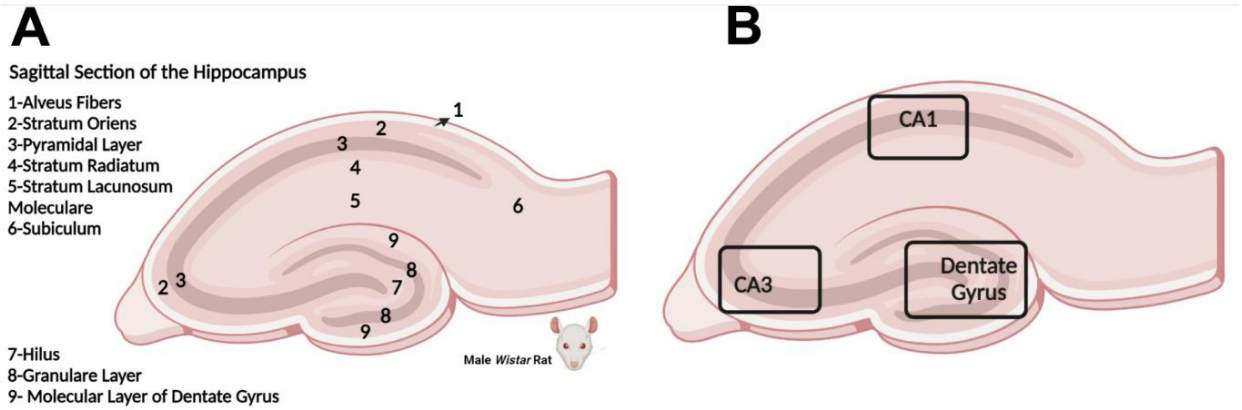

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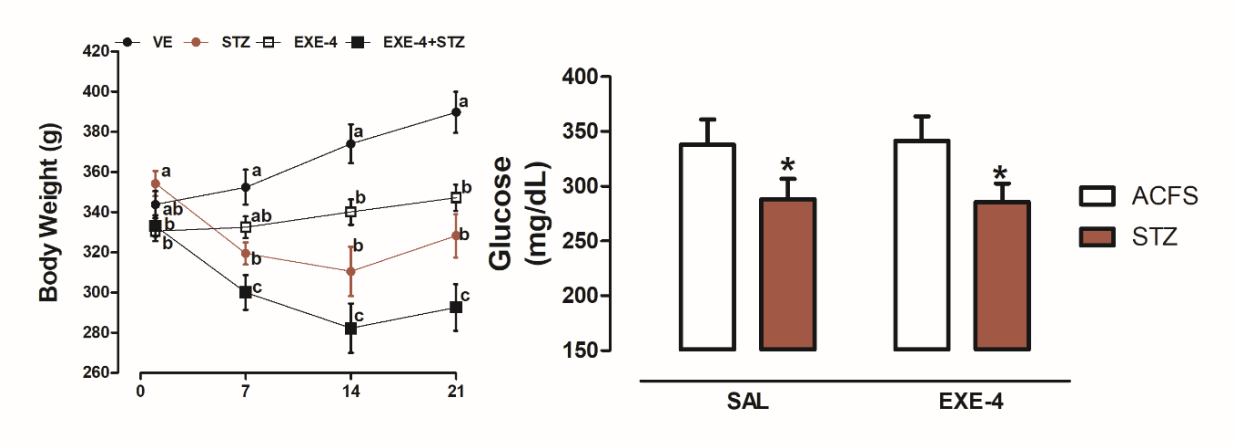

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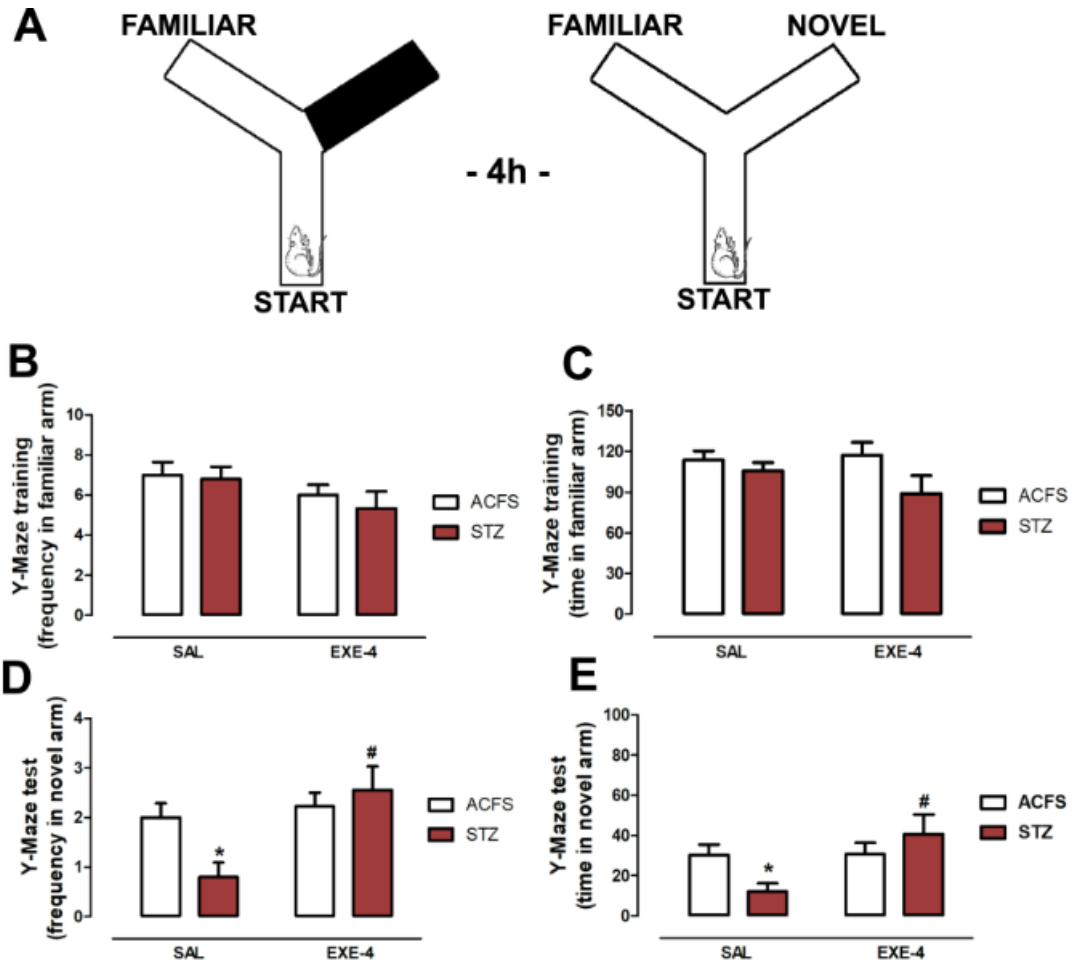

Figure 4

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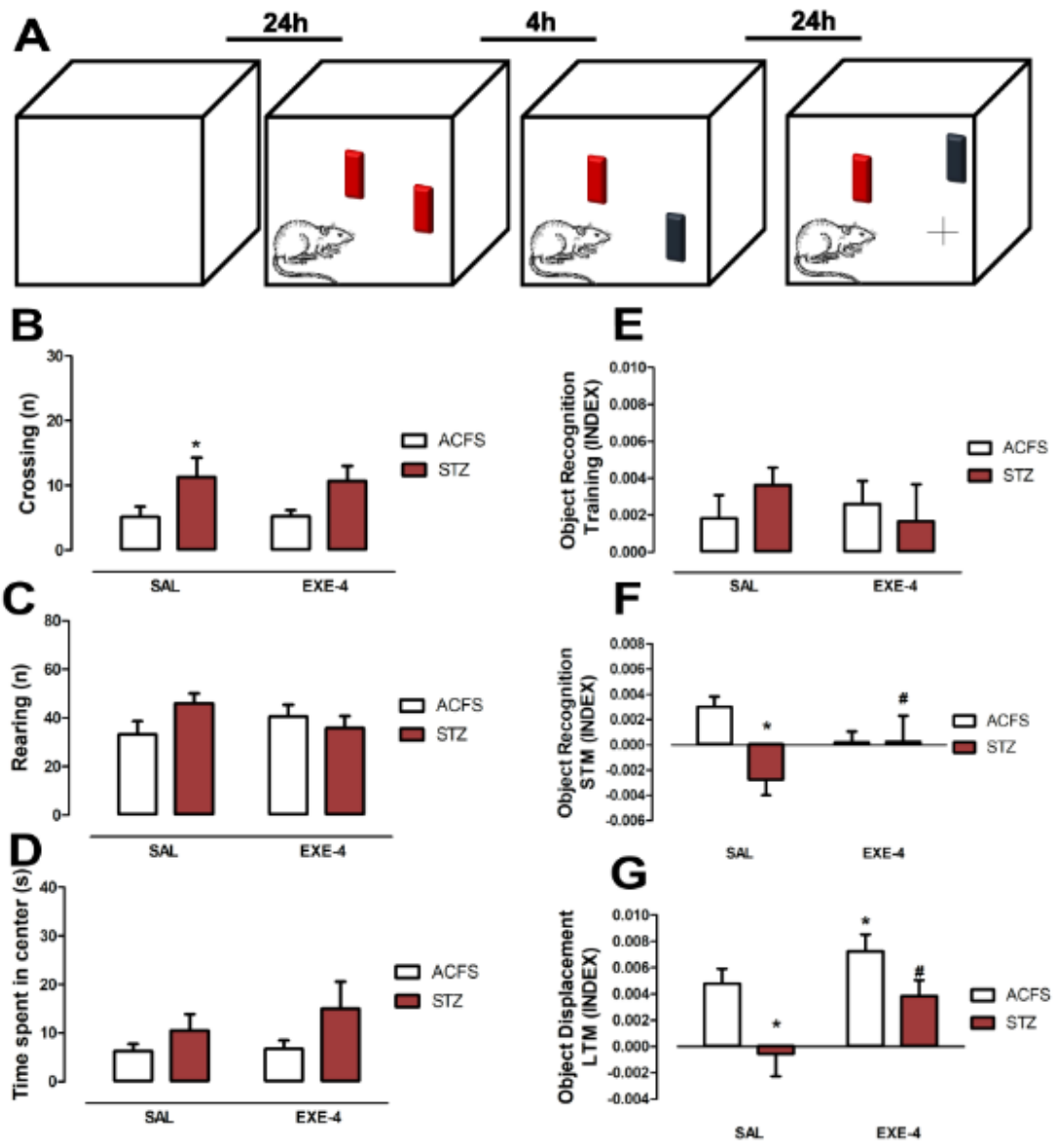

2 Figure 5

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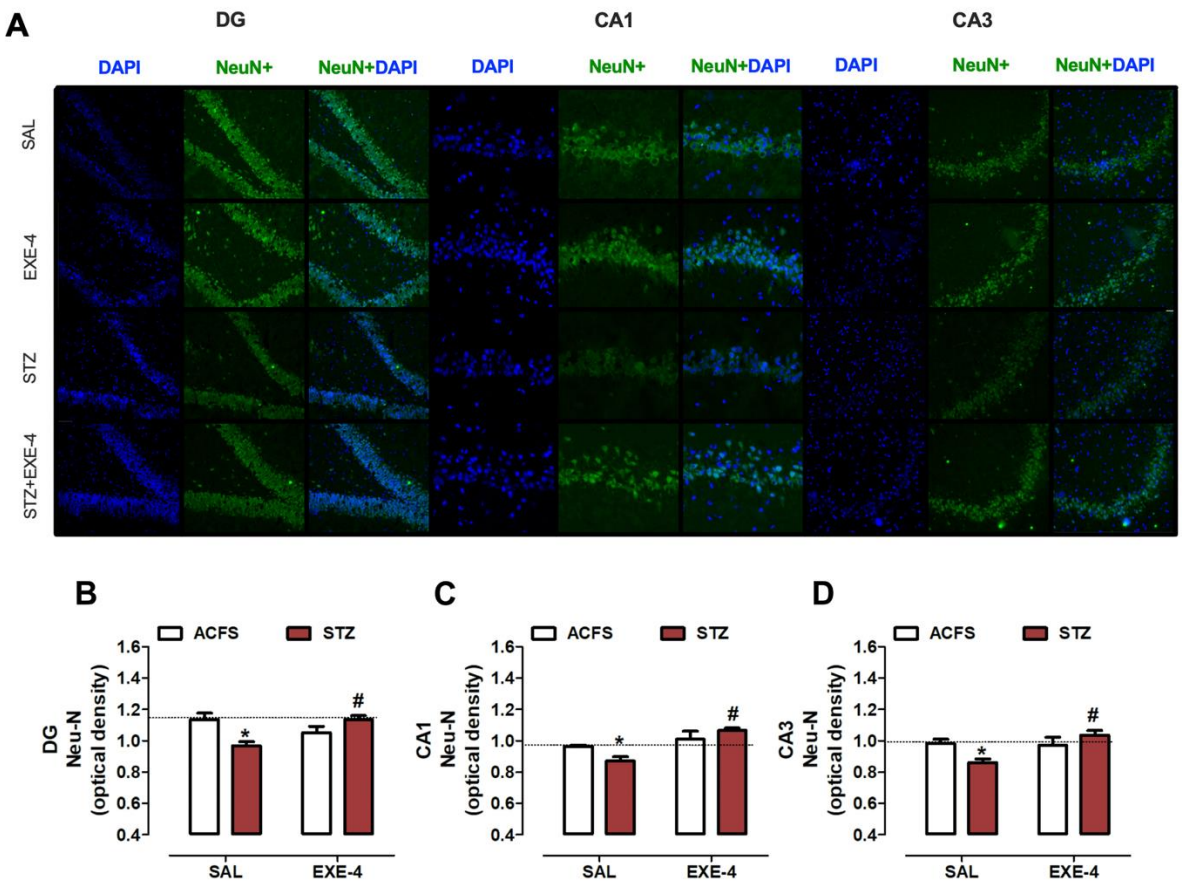
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CA1
CA3
DAPI
NeuN+
NeuN+DAPI
SAL
EXE-4
STZ
STZ+EXE-4
B
C
D
ACFS
STZ
DG Neu-N (optical density)
CA1 Neu-N (optical density)
CA3 Neu-N (optical density)
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1.2
1.0
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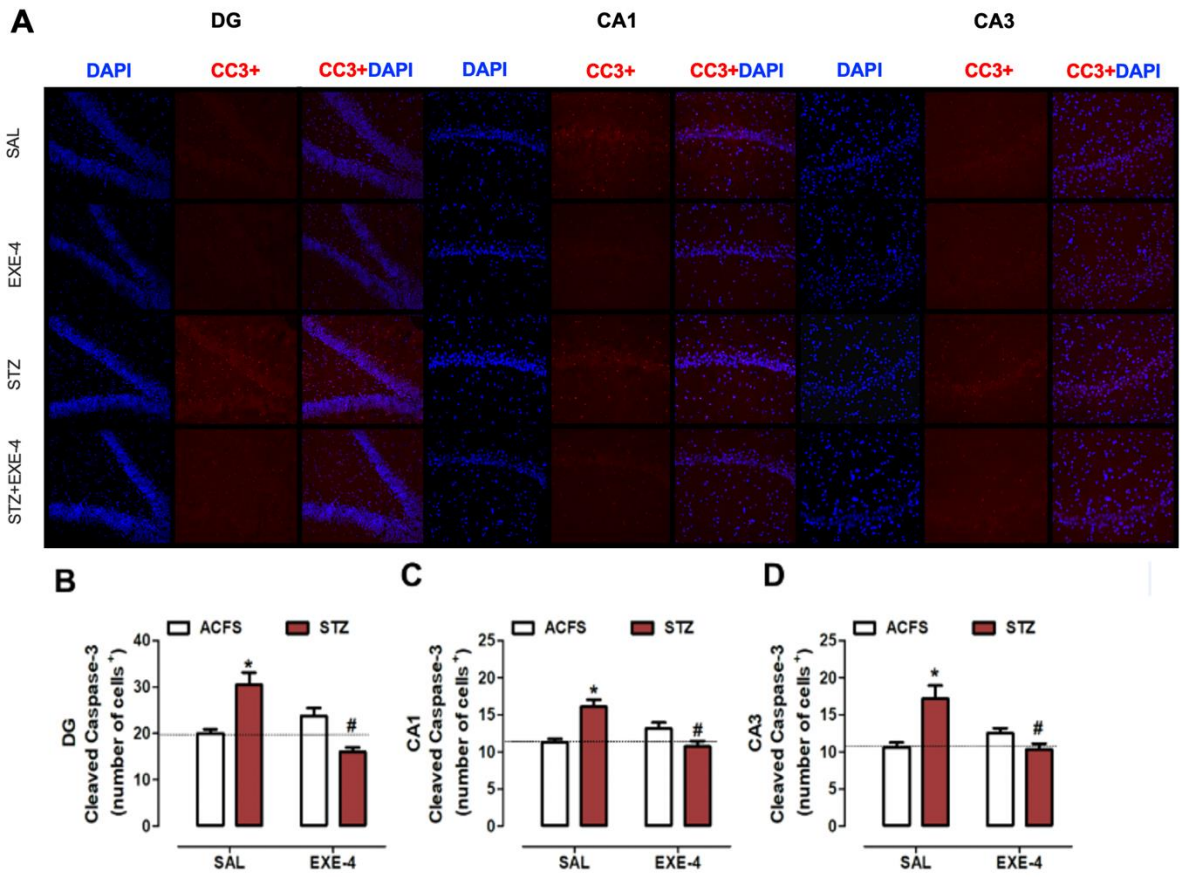
#
SAL
EXE-4

Figure 6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STZ+EXE-4
B
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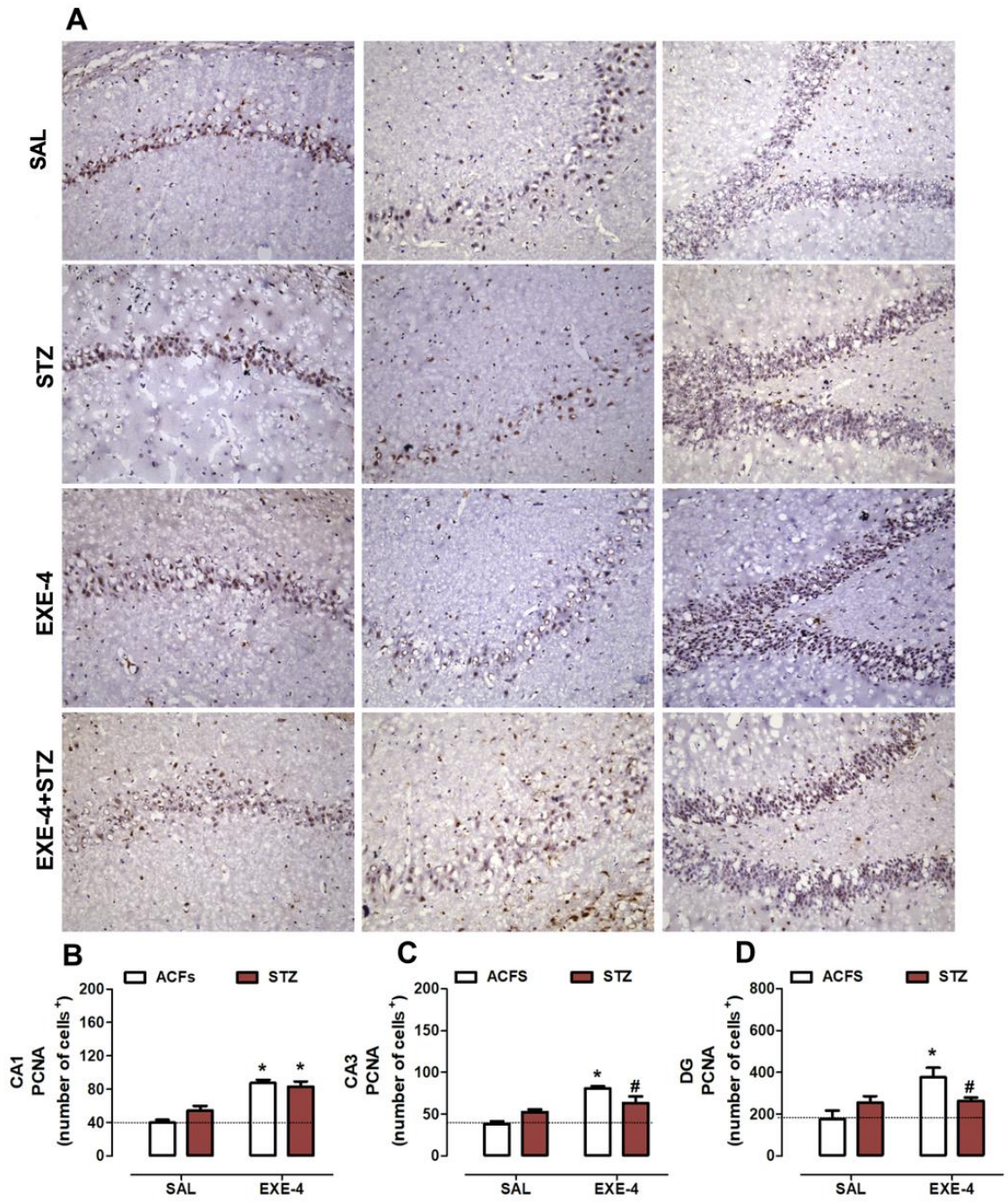
ACFS
STZ
DG Cleaved Caspase-3 (number of cells+)
CA1 Cleaved Caspase-3 (number of cells+)
CA3 Cleaved Caspase-3 (number of cells+)
SAL
EXE-4

Figure 7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CA1 PCNA (number of cells+)
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80
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SAL
EXE-4
C
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CA3 PCNA (number of cells+)
200
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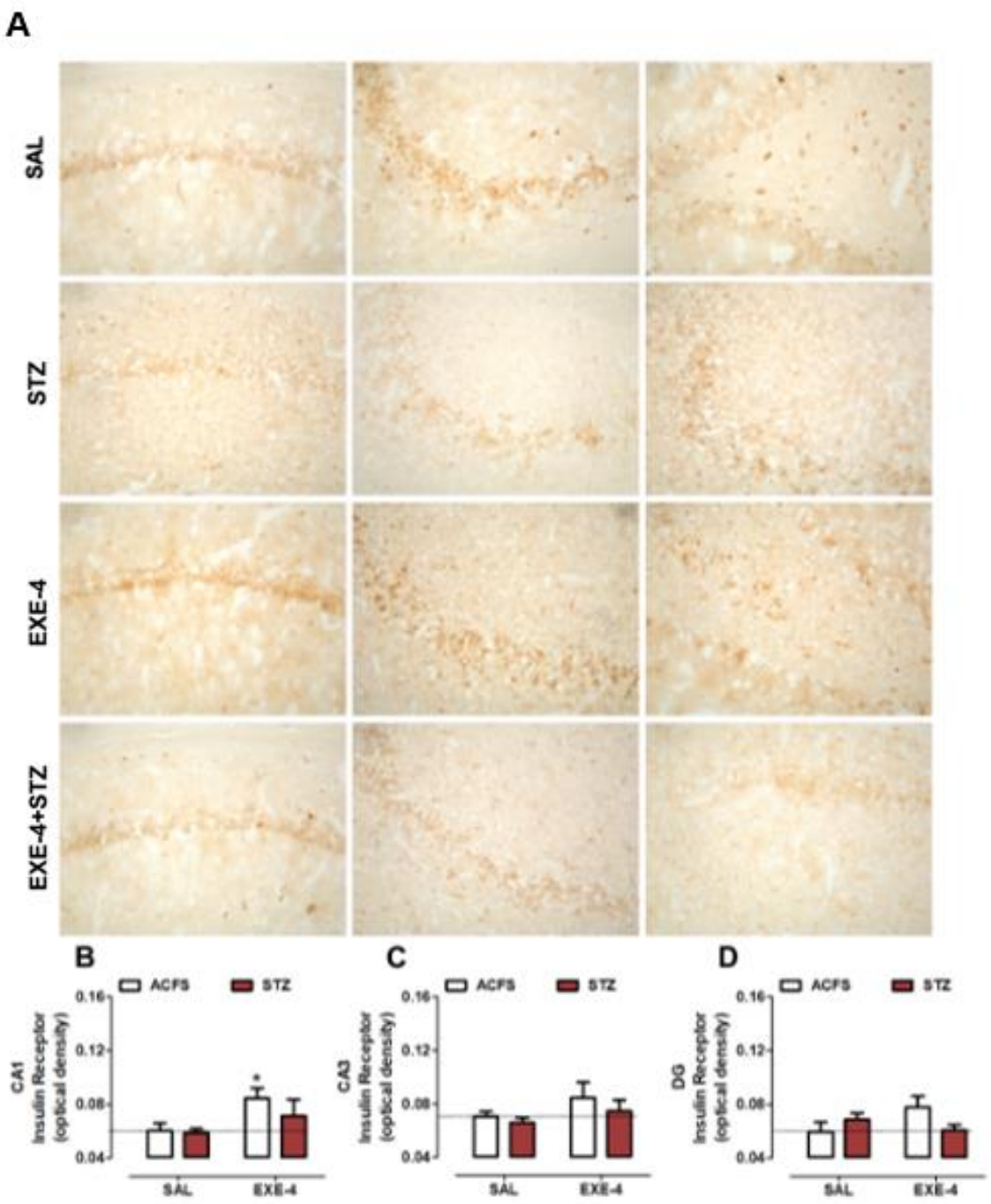
EXE-4

Figure 8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EXE-4
EXE-4+STZ
B
C
D
ACFS
STZ
CA1 Insulin Receptor (optical density)
CA3 Insulin Receptor (optical density)
DG Insulin Receptor (optical density)
0.16
0.12
0.08
0.04
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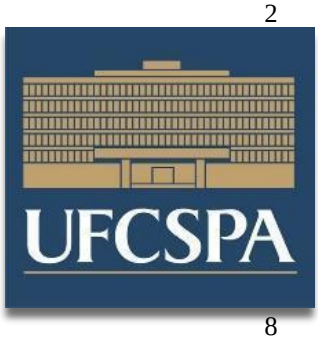
Figure 9

4.2 MANUSCRITO 2



FEDERAL UNIVERSITY OF HEALTH SCIENCES OF PORTO ALEGRE
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To: Editors-in-chief of **Glia**,

Bruce R. Ransom and Helmut Kettenmann

December 2022

Dear Editor,

I would like to submit the research article for publication in the **Glia** entitled "*Exendin-4 reduces astrogliosis, microgliosis and hippocampal neuroinflammation in Sporadic Dementia of the Alzheimer's Type*" (Total Words: ± 4.834, Figures: 7, References: 79).

The manuscript was co-authored by Adriana M. Zago (first author), Fabiano B. Carvalho, Francine L. Rahmeier, Marta Santin, Fernanda Huf, Giuliano R. Guimarães, Jessié M. Gutierres and Marilda da C. Fernandes (corresponding author).

The main contribution of our work is that it describes the beneficial effects of treatment with exendin-4 (EXE-4), a glucagon-like peptide 1 receptor (GLP1-R) agonist and drug for the treatment of type 2 diabetes mellitus, in a model of sporadic dementia of the Alzheimer type (SDAT). Through morphology studies, we verified a protection in the glial alterations present in the SDAT. Furthermore, EXE-4 was shown to be effective in reducing glial apoptosis in the CA1, CA3 and DG regions.

This study is of interest to neuroscience, nutrition, and metabolism professionals as these results may contribute to new insights into the role of incretin-mimetics against neuropathological conditions found in dementias such as Alzheimer's disease. In parallel, this study seeks to find new supporting tools in the protection of populations of hippocampal neurons against the neurotoxic effects present in SDAT.

We read and understand your journal's policies on copyright, ethics, etc., and we believe that neither the manuscript nor the study violates any of them.

Thanks for your consideration. We hope that our manuscript will be suitable for publication in your journal.

Sincerely,

Marilda da Cruz Fernandes
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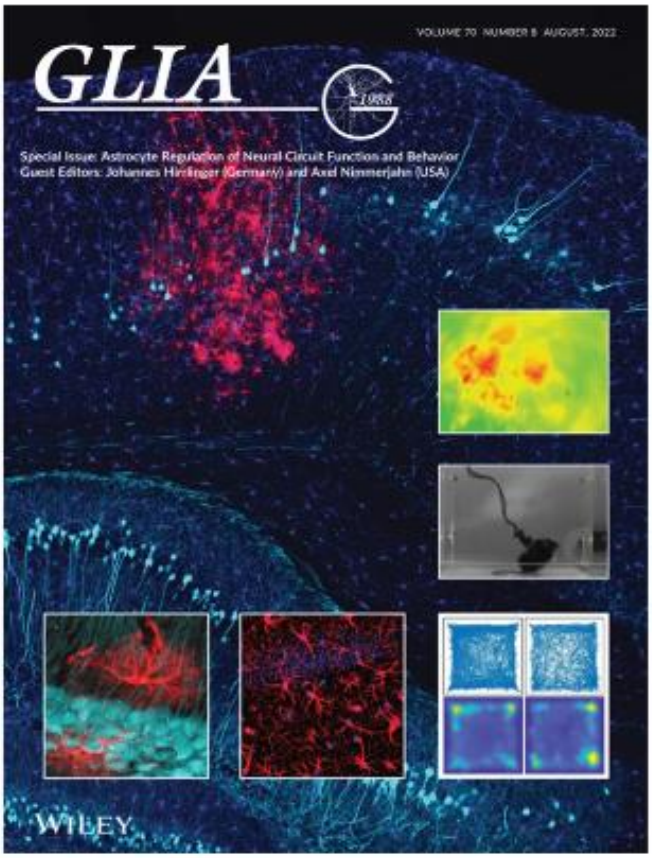

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ISSN	Título	Área de Avaliação	Classificação
0894-1491	GLIA (NEW YORK, N.Y. : PRINT)	MEDICINA II	A1

EXENDIN-4 REDUCES ASTROGLIOSIS, MICROGLIOSIS AND HIPPOCAMPAL NEUROINFLAMMATION IN SPORADIC DEMENTIA OF THE ALZHEIMER'S TYPE

Adriana M. Zago[1], Fabiano B. Carvalho,[1] Francine L. Rahmeier[1], Marta Santin[1], Fernanda Huf[1], Giuliano R. Guimarães[1], Jessié M. Gutierres[1] and Marilda da C. Fernandes[1]

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Abstract

Alzheimer's disease (AD) is a pathology characterized by progressive and irreversible deterioration of cognitive functions. Astrocytes and microglia are immune cells that when activated acquire a reactive phenotype acting rapidly in response to tissue injury, this phenomenon is known by astrogliosis and microgliosis. This study evaluated the effects of exendin-4 (EXE-4), a glucagon-like peptide-1 receptor (GLP-1R) agonist, on the hippocampal astrocytic and microglial population in rats submited to sporadic dementia of the Alzheimer's type (SDAT). For that, male *Wistar* rats received STZ (icv, 10 µl, 3 mg/kg, artificial cerebrospinal fluid as vehicle) and treated with EXE-4 (10 µg/kg, i.p., saline as vehicle). Four groups were formed: Vehicle, EXE-4, STZ and STZ *plus* EXE-4. Then, brains were used for immunofluorescence studies with antibodies to GFAP, vimentin, Iba-1, CD11b and cleaved caspase-3 (CC3). STZ increased immunoreactivity for GFAP, CD11b and Iba-1 in CA1, CA3 and DG compared to the vehicle group. EXE-4 protected against the increase in immunoreactivity for GFAP, Iba-1 and CD11b induced by STZ. STZ increased immunoreactivity for vimentin only in DG, and EXE-4 attenuated this effect. STZ increased $CC3^+$ cells in all regions, and EXE-4 reduced this apoptosis induced by STZ. In conclusion, EXE-4 showed an anti-apoptotic effect on glial cells as well, the ability to reduce astrogliosis and microgliosis in SDAT. These findings show a potential neuroprotective role of this agonist to GLP-1R in glial injury in a preclinical model that mimics the sporadic form of AD.

Keywords: Alzheimer's disease; exendin-4; hippocampus; astrocytes; microglia; apoptosis.

1. Introduction

Alzheimer's Disease (AD) constitutes 60-70% of cases of dementia and it is estimated that there are 35.6 million people affected in the world (Association, 2022). The sporadic form represents 90-95% of all cases and, although its etiology is multifactorial, age is the main risk factor (Meraz-Rios, Toral-Rios, Franco-Bocanegra, Villeda-Hernandez, & Campos-Pena, 2013). Clinically, AD is defined by the progressive decline in memory, characterized by the presence of extracellular aggregates of beta-amyloid peptide (Aβ) arising from the abnormal proteolytic cleavage of the amyloid precursor protein (APP) by beta and gamma-secretase enzymes (Cho, Bae, Okun, Arumugam, & Jo, 2022; Hur, 2022; Y. W. Zhang, Thompson, Zhang, & Xu, 2011). In addition, intraneural neurofibrillary tangles (NFTs) are formed, which are composed of hyperphosphorylated tau protein (Avila-Munoz & Arias, 2014). Amyloid plaques accumulate around neurons and cause abnormalities in synaptic transmission and disrupt mitochondrial functioning (Bronzuoli, Iacomino, Steardo, & Scuderi, 2016). The deposition of Aβ and NFTs leads to neuronal cell death, microtubule destabilization and cell membrane degeneration (Calsolaro & Edison, 2016). In parallel, glial cells are also the target of injury caused by Aβ deposition. This peptide disrupts gliotransmission, impairs Ca^{2+}-mediated signaling, increases cytotoxicity and cellular apoptosis. These events also contribute to the reduction of synaptic plasticity and cognitive impairment in the AD (Sajja, Hlavac, & VandeVord, 2016).

Astrocytes and microglia are the main immune cells of the central nervous system (CNS) and, therefore, are responsible for initiating inflammatory responses to insults in the brain, as well as mediating tissue recovery from stress and injury (Farfara, Lifshitz, & Frenkel, 2008; Molofsky & Deneen, 2015; Ransohoff & Brown, 2012). Increased astrocyte immunoreactivity can trigger beneficial or harmful responses, such as promoting tissue repair and maintaining homeostasis; or in the exacerbation of inflammatory reactions, cell damage and apoptosis (Colombo & Farina, 2016). Reactive astrocytosis has become an important tool for investigating glia, as it is a pro-inflammatory phenomenon with alterations in the astrocytic spectrum in the face of tissue injury and is found in several pathologies that affect the CNS, especially AD (Sajja et al., 2016).

Microglia is in a quiescent state and monitor the presence of pathogens in nervous tissue (Hristovska & Pascual, 2015). Hyper-reactive microglia are reported with the progression of neurodegeneration. There is a complexity in the factors that regulate the balance between microglial beneficial and harmful effects (Di Benedetto et al., 2022) since in the early stages of the disease, microglia activation has been associated with neuroprotective effects, removing

harmful stimuli represented by Aβ hyperproduction, cleaning up cellular debris, phagocytosing dead cells and releasing neurotrophic factors (Simon, Obst, & Gomez-Nicola, 2019). However, the persistence of harmful stimuli throughout the disease subjects these cells to a state of chronic activation and release of pro-inflammatory cytokines, which lead to neurotoxicity and neurodegeneration (Ransohoff & Brown, 2012; Ransohoff & El Khoury, 2015). Activated microglia produce and secrete several pro-inflammatory mediators, including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and nitric oxide (NO), which confer the onset and progression of AD (Ahmad, Fatima, & Mondal, 2019).

The activation of microglia and astrocytes stimulated by the deposition of Aβ induces reactive gliosis and triggers a inflammatory signaling cascade. While prolonged activation of pro-inflammatory cytokines affects APP processing through beta-secretase, Aβ production is accelerated. This results in reduction from Aβ debugging and phagocytosis by activated glial cells that lead to elevation Aβ content (Sastre et al., 2004; Sastre et al., 2003; Sastre et al., 2001). Astrogliosis and microgliosis can promote the removal of the harmful stimulus providing protection to the brain (Sofroniew, 2000, 2011, 2015, 2020). However, glial activation diverse and continuous can exercise an adverse effect by promoting neurodegeneration (Linnerbauer, Wheeler, & Quintana, 2020; Madore, Yin, Leibowitz, & Butovsky, 2020).

Finding a suitable molecular target and a successful multifaceted pharmacological approach to AD is a major challenge for drug development. In addition, a therapy that aims to treat disorders linked to insulin signaling, either through resistance or interruption in signaling, which interfere with glucose metabolism and neuronal activity, may be beneficial. Insulin resistance, hyperglycemia and hyperinsulinemia are metabolic characteristics that have been associated with AD and progress to mitochondrial dysfunction, oxidative stress, bioenergetic failure, negatively impacting neurons and glial cells (Ghasemi et al., 2013; Talbot et al., 2012; Yin, Yao, Brinton, & Cadenas, 2017).

Exendin-4 (EXE-4) is a 39-amino acid polypeptide that acts as glucagon-like peptide-1 receptor (GLP-1R) agonist and is a well-established drug for the treatment of type 2 diabetes mellitus (TDM2). EXE-4, when administered peripherally, crosses the blood-brain barrier (BBB) (Luciani et al., 2010). The beneficial effects of EXE-4 on the CNS include improvement in neurogenesis, cell repair and suppression of the inflammatory response, as well as cognitive improvement (Holscher, 2014; Huang et al., 2012). Given this evidence, the aim of this study was to investigate the immunoreactivity for markers of astrogliosis, microgliosis and apoptosis in the hippocampus of rats submitted to sporadic dementia of the Alzheimer's type (SDAT).

Through this study, it is expected to find evidence on the role of the GLP-1 receptor agonist in modulating microglial and astrocytic activity, as well as reducing cell death present in SDAT.

2. Materials and methods

2.1 Chemicals

Streptozotocin (STZ, Sigma-Aldrich® - Sant Louis, Missouri, USA; product number: S0130) and EXE-4 ≥ 97%, Reference code: E7144 - Sigma Aldrich Chemical Co. (St. Louis, MO, USA) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other reagents used in the experiments were of analytical grade and of highest purity.

2.2 Animals

Adult male *Wistar* rats (n= 28) weighing 300g on average, from the local breeding colony of Federal University of Health Sciences of Porto Alegre (UFCSPA, Brazil). The animals were maintained in the Central Animal House of the UFCSPA in colony cages at an ambient temperature of 23 ± 2°C and relative humidity of 45–55% with 12 h light/dark cycles and with free access to a standard rodent pellet diet and water *ad libitum*.

The Committee of Ethics and Animal Experimentation of the UFCSPA, under protocol number 191/16, identification code: 488/16, approved all procedures. The animals were handled in accordance with international and national laws for the ethical care and handling of laboraty animals (Guidelines of the Council of the European Communities of 22 September 2010, 2010/63/EU and Law 11.794/08).

2.3 Treatments

The rats received EXE-4 at a dose of 10 µg/kg body weight, previously dissolved in saline via intraperitoneal (ip) (1ml/kg) for 21 days (a total of 21 administrations were performed). The dose was chose based on a previous study as describe (Tortorelli et al., 2015; Zanotto et al., 2017). The control groups received only the vehicle (1ml/kg of salina, ip). Rats were randomly distributed into four groups: vehicle, EXE-4 10µg/kg (EXE-4), Streptozotocin (STZ), and STZ *plus* EXE-4 10µg/kg. Further information can be viewed in the experimental design (see figure 1).

2.4 Induction of Sporadic Dementia of the Alzheimer's Type

The animals were anesthetized with ketamine (Dopalen® 10%, Ceva® - São Paulo - Brazil, 80-100 mg/kg ip) and xylazine (Anasedan® 2%, Ceva® - São Paulo - Brazil, 5-10 mg/kg i. p.) and then the animal's head hair was shaved before being placed in the stereotaxis.

Asepsis was performed with 3% hydrogen peroxide (10% hydrogen peroxide - Lifar brand) for subsequent sagittal incision on the midline to expose the cranial scalp. Two bilateral holes were drilled with a drill, following the stereotaxic coordinates of the lateral ventricles provided by Paxinos and Watson's atlas of stereotaxic coordinates (0.8 mm in the anteroposterior axis, 1.5 mm in the medial-lateral axis, and 4 mm in the dorsoventral axis) (Paxinos & Watson, 2013). Through these orifices, with a microliter microsyringe (Hamilton®, 10µl, Product code, 80300), STZ (Sigma-Aldrich® - Sant Louis, Missouri, USA; product number: S0130) dissolved in sterile saline (5 µL/ventricle) was injected at a dose of 3 mg/kg. This dose/volume has been pre-established in previous work by the project's collaborating group as well as described by other research groups and is shown to be effective in the development of dementia-related cognitive deficit (Gutierres et al., 2014; L. Rodrigues et al., 2009; M. V. Rodrigues et al., 2019). Animals not induced SDAT received saline only. After surgery the animals were kept warm by eletric heater and hot water bags, and after regaining consciousness they were put back into the housing box with water and food ad libitum. Two days after surgery, the animals received a bottle with a watery solution of nest milk (70g/3000ml) renewed each day for five consecutive days to avoid body weight loss. The degree of invasiveness for this stereotactic procedure is considered moderate (see figure 2).

2.5 Immunofluorescence morphological studies

On day 24, the animals were deeply anesthetized by injection with ketamine (Dopalen® 10%, Ceva® - São Paulo - Brazil, 80-100 mg/kg ip) and xylazine (Anasedan® 2%, Ceva® - São Paulo - Brazil, 5-10 mg/kg ip), and were euthanized by intracardic transfusion, heparinized with 0.1 ml of heparin (Heparin 5000UI®, Cristália® - São Paulo, Brazil) and perfused with 0.9% saline for 10 minutes, followed by paraformaldehyde (Sigma-Aldrich® - Sant Louis, Missouri, USA; product number: 158127) 4% diluted in 0.1 M phosphate buffer (PBS at pH 7.4) for 30 minutes (Rahmeier et al., 2016). The brains were post fixed in 4% paraformaldehyde (24 hours), transferred to a solution of 30% sucrose in PBS-1% until total submersion. Then, the frozen fixed brains were sectioned (5 and 16 µm) as per the stereotaxic coordinate atlas (Paxinos & Watson, 2013), using cryostat Leica CM3050S (Leica Microsystem, German) at a temperature of -30ºC. Next, the sections were mounted on slides coated with 2% gelatin *plus* 0.08% chromalin (chromium and potassium sulfate, from Sigma-Aldrich, Brazil) and finally

allowed to dry at room temperature for 24 hours. At last, all sections were stored at -20ºC until use (Rahmeier et al., 2016).

For the immunofluorescence (IF) techniques, five markers were selected: GFAP (marker of reactive astrocytes), CD11b and Iba-1 (markers of microglia), vimentin (marker of astrocytosis and reactive gliosis in responses to tissue injuries) cleaved caspase-3 (CC3) (marker of cellular apoptosis). The slides with the frozen samples were washed in ice-cold acetone for 10 min, under agitation. After removing the acetone, the slides were left to rest until their total evaporation and then washed for 5 min (three times) with phosphate buffer containing Triton® X-100 (PBS-tx). Blocking of non-specific proteins was performed with 5% bovine serum albumin (BSA - Sigma-Aldrich® - Sant Louis, Missouri, USA; product number: A9647) diluted in PBS-tx for 2 hours at room temperature (RT). The sections were then incubated with the primary antibody diluted in 5% BSA for 1 hour at room temperature and overnight at 4°C.

The antibodies used were Anti-GFAP (16µm sections) 1:800, Sigma- Aldrich®, Sant Louis, Missouri, USA (product number: G9269-2ML); Anti-Iba1 (16 µm sections) 1:800, Genetex®, Irvine, California, USA (product number: GTX100042); Anti-CD11b (16 µm sections) 1:800, Abcam®, Cambridge, UK, England (product number: ab1211); Anti-vimentin (16 µm sections): 1:800 DakoCytomation®, Santa Clara, California, USA (product number: M0725) and anti-CC3 (16 µm sections) 1:200, Cell Signaling Technology®, Danvers, Massachusetts, USA (product number: #9661).

After incubation with the primary antibody, the samples were washed with PBS-tx for 5 minutes (4 times) and incubated in the dark for 2 hours with the goat anti-rabbit IgG H&L antibody: 1:1000 secondary antibody (Alexa Fluor® 555, Abcam® - Cambridge - UK, England, product number: ab150078) for samples with Iba-1, GFAP and CC3. CD11b and vimentin were incubated with the secondary antibody goat anti-mouse IgG H&L: 1:500 (Alexa Fluor® 488, Abcam® - Cambridge - UK, England, product number: ab150113).

Afterwards, the samples were washed again with PBS-tx for 5 minutes (4 times) and incubated in the dark with the nuclear marker DAPI: 1:10,000 (4',6-diamidino-2-phenylindole, Invitrogen® - Eugene - Oregon - USA; product number: D1306) for 10 minutes. Then the slides were washed 4 times with PBS-tx for 5 minutes each. VectaShield® (Vector, Burlingame - California - USA; product number: H-1000) was added over the sections which were then overlaid with a coverslip and kept at a temperature of 4ºC, in the dark, until the moment of capturing the images. Images were acquired using a Leica DM6-B microscope, Leica DFC 7000-T Camera and Leica Las X Software. For each section, images were acquired in the hippocampus regions (CA1, CA3 and Dentate Gyrus, DG). The slides were evaluated in a wide-

field optical microscope for fluorescence observations (Leica DM6-B) and the images obtained were captured by a camera (Leica DFC7000 T) coupled to the microscope. GFAP, Iba-1, CD11b and vimentin were measured by optical densitometry using ImagePro Plus®6.3 software (Media Cybernetics®). $CC3^+$ was measured by double counting of positive cells ($CC3^+$ cells).

In CA1 and CA3, Stratum Oriens, Stratum Radiatum and Stratum Moleculare layers were analyzed; except the pyramidal layer. In the DG, the Moleculare layer and the Hilus region were analyzed, except for the granulare layer.

2.6 Statistical Analysis

The normality analysis of the samples was performed by Kolmogorov-Smirnov test. Afterwards, parametric data were analyzed using two-way analysis of variance (ANOVA) followed by the Bonferroni test when appropriate. Results were expressed as the mean ± standard error (SE). P values less than 0.05 ($P<0.05$) were considered as indicative of significance.

3. Results

3.1. Exendin-4 protects against increased immunoreactivity to GFAP induced by sporadic dementia of the Alzheimer's type

Figure 3 shows GFAP immunoreactivity in the hippocampus of rats treated with EXE-4 and submitted to SDAT. Figure 3A shows representative immunoreactions of each group in the CA1, CA3 and DG respectively. Graph 3B shows the significance in the interaction between vehicle or EXE-4 *versus* vehicle or STZ [$F_{(1,24)}=22.24$; $P<0.0001$; graph 3B], suggesting that STZ increased immunoreactivity for GFAP and that EXE-4 protected against this effect induced by the SDAT in the CA1.

In the CA3, significant interaction was observed between vehicle or EXE-4 *versus* vehicle or STZ [$F_{(1,24)}=14.28$; $P=0.0009$; graph 3C], suggesting that EXE-4 protected against the increase in GFAP induced by SDAT.

In DG, significance in the interaction between vehicle or EXE-4 *versus* vehicle or STZ [$F_{(1,24)}=17.10$; $P=0.0004$; 3D graph] was observed, suggesting a neuroprotective effect of EXE-4 against the increase in the GFAP induced by STZ.

3.2 Exendin-4 protects against increased immunoreactivity to vimentin in the DG induced by sporadic dementia of the Alzheimer's type

Figure 4 shows the immunoreactivity for vimentin in the hippocampus of rats with SDAT and treated with EXE-4. Figure 4A shows representative immunoreactions of each group in the CA1, CA3 and DG, respectively. Graph 4B shows no significant effect of both treatments for the CA1 [$F_{(1,24)}$=2,682; P=0.1145; graph 4B] and CA3 [$F_{(1,24)}$=0.7708; P=0.3877; graph 4C]. A significance in the interaction between vehicle or EXE-4 *versus* vehicle or STZ [$F_{(1,24)}$=7,828; P=0.0105; graph 4D] was observed only in DG, suggesting that STZ increase the immunoreactivity to vimentin and that EXE-4 protects against this effect.

3.3 Exendin-4 protects against increased immunoreactivity to Iba-1 induced by sporadic dementia of the Alzheimer's type

Figure 5 shows Iba-1 immunoreactivity in the hippocampus of rats with SDAT and treated with EXE-4. Figure 5A shows representative immunoreactions of each group in the CA1, CA3 and DG, respectively. Graph 5B shows a significance in the interaction between vehicle or EXE-4 *versus* vehicle or STZ [$F_{(1,24)}$=5.241; P=0.0301; graph 5B], suggesting that STZ increases Iba-1 immunoreactivity and EXE-4 protects against this effect.

In parallel, EXE-4 showed an effect *per se* in reducing Iba-1 immunoreactivity compared to the vehicle group [$F_{(1,24)}$=188.9; P<0.0001; graph 5B]. In the CA3 region, a significance was observed in the interaction between vehicle or EXE-4 *versus* vehicle or STZ [$F_{(1,24)}$= 6,981; P=0.0133; graph 5C], also suggesting that EXE-4 reduces the increase in immunoreactivity for Iba-1 induced by STZ. EXE-4 also reduced immunoreactivity for Iba-1 *per se*, when compared to the vehicle group [F(1,24)=222.7; P<0.0001; graph 5C].

Finally, a significance in the interaction between vehicle or EXE-4 *versus* vehicle or STZ [$F_{(1,24)}$=7,844; P=0.0095; graph 5D] was also observed in DG. Furthermore, the same effect *per se* of EXE-4 was also seen for this region [$F_{(1,24)}$=221.1; P<0.0001; graph 5D] suppressing microglial immunoreactivity.

3.4 Exendin-4 protects against increased immunoreactivity to CD11b induced by sporadic dementia of the Alzheimer type

Figure 6 shows the immunoreactivity for CD11b in the hippocampus of rats with SDAT and treated with EXE-4. Figure 6A shows representative immunoreactions from each group in the CA1, CA3 and DG, respectively. Graph 6B shows a significance in the interaction between vehicle or EXE-4 *versus* vehicle or STZ [$F_{(1,24)}$=5.075; P=0.0320; graph 6B], suggesting that

EXE-4 treatment was effective in reducing the immunoreactivity to CD11b in the CA1 induced by STZ.

In the CA3, a significance was observed in the interaction between vehicle or EXE-4 *versus* vehicle or STZ [$F_{(1,24)}$=5,391; P=0.0270; graph 6C], suggesting that EXE-4 treatment protected against the increase in the CD11b immunoreactivity induced by STZ.

Finally, a significance in the interaction between vehicle or EXE-4 *versus* vehicle or STZ [$F_{(1,24)}$=6,909; P<0.0136; graph 6D] was observed at the DG, suggesting that EXE-4 is effective in protecting against the increase in the CD11b immunoreactivity induced by STZ.

3.5 Exendin-4 protects against apoptosis in the hippocampus of rats induced by sporadic dementia of the Alzheimer's type

Figure 7 shows the number of $CC3^+$ cells in the hippocampus of rats with SDAT and treated with EXE-4. Figure 7A shows representative immunoreactions of each group in the CA1, CA3 and DG, respectively. Graph 7B shows a significance in the interaction between vehicle or EXE-4 *versus* vehicle or STZ [$F_{(1,24)}$=16.09; P=0.0004; graph 7B], suggesting that EXE-4 treatment reduced apoptosis in the CA1 region induced by STZ.

In the CA3 region, a significance was observed in the interaction between vehicle or EXE-4 *versus* vehicle or STZ [$F_{(1,24)}$=19.06; P=0.0002; graph 7C], also suggesting an anti-apoptotic effect of EXE-4 against the deleterious effects of STZ. In the DG, a significance was also observed in the interaction between vehicle or EXE-4 *versus* vehicle or STZ [$F_{(1,24)}$=21.87; P<0.0001; graph 7D] suggesting that EXE-4 is effective in reducing $CC3^+$ cells increased in SDAT.

4. Discussion

The administration of STZ-icv mimics a model of SDAT, characterized by glucose hypometabolism and insulin resistance in the brain (de la Monte & Tong, 2014; Hoyer & Lannert, 2007). This model reproduces AD-like symptoms such as memory deficits, mitochondrial dysfunction, increased oxidative stress, astrocyte and microglia activation, cholinergic dysfunction, neuroinflammation, and neurodegeneration (Mishra, Singh, Shukla, & Shukla, 2018; Salkovic-Petrisic & Hoyer, 2007; Yuliani, Lobentanzer, & Klein, 2021). Due to the important role of glia in nervous system homeostasis, the present study investigated whether EXE-4 has the ability to protect astrocytes and microglia from tissue injury caused by SDAT. Thus, markers of astrogliosis (GFAP and vimentin), microgliosis (Iba-1 and CD11b) and apoptosis ($CC3^+$) were investigated in the Stratum Oriens, Stratum Radiatum and Stratum

Moleculare in the CA1 e CA3; and Moleculare Layer and *Hilus* region of the DG (layers formed mostly by glial cells).

Our findings show that SDAT increases the GFAP immunoreactivity in the CA1, CA3 and DG. These results corroborate with previous studies that also showed reactive astrogliosis for SDAT. Teixeira et al. (2021) showed increase in GFAP in the hippocampus of rats with SDAT 25 days after administration of STZ (3 mg/kg) (Teixeira et al., 2022). Yuliani et al (2020) observed an increase in GFAP immunoreactivity in the hippocampus of rats 21 days after STZ administration (Yuliani et al., 2021).

GFAP is a protein present in the intermediate filaments of the cytoskeleton being considered an important marker in pathological conditions once astrocytes become more reactive against an imbalance of cellular homeostasis and loss of cellular functions (Agulhon et al., 2012; Santos et al., 2013). Reactive astrogliosis is seen in neurological disorders such as neurotrauma, ischemic stroke, and neurodegenerative diseases, including AD (Kraft et al., 2013; Macauley, Pekny, & Sands, 2011).

In this study, EXE-4 was seen to reduce astrogliosis and microgliosis in animals with 21 days of SDAT. MiaoJun et al., (2021) showed that different GLP-1R agonists, including EXE-4 (25nmol/kg ip), were also able to inhibit astrogliosis using a model of Parkinson's disease in mice (MPTP model).In this study, the ability of incretinomimetics reduced the content of GFAP and Iba-1 induced by MPTP (Lv et al., 2021). In addition, GLP1-R agonists inhibited the NF-Kβ inflammatory pathway and reestablished the levels of neurotrophins BNDF and GDNF, important for cell survival and tissue repair (MiaoJung et al., 2021). Shi et al., (2017) showed that a new agonist for GIP/GLP-1 receptors (10nm/kg, ip) was able to reduce astrogliosis (GFAP positive astrocytes) and microgliosis (Iba-1 positive microglia) by modulation of the AKT enzyme and phosphorylation of insulin receptors 1 in an SDAT model (14 days after STZ injection, in rats) (Shi, Zhang, Li, & Holscher, 2017). Although the mechanism by which EXE-4 regulates astrocytic reactivity has not been fully elucidated, the authors suggest a reduction in the ratio of the content of pro-apoptotic factors Bax *versus* anti-apoptotic Bcl-2 factors is a key factor in this process (Shi et al., 2017).

In contrast to these findings, liraglutide (another GLP-1R agonist) has been shown to increase GFAP immunoreactivity in the face of ischemic injury. Using the cerebral ischemia model (MCAO), Dong et al (2017) found that liraglutide (50-200 ug/kg, ip) only protected neuronal populations from ischemic damage. Furthermore, liraglutide further increased astrogliosis caused by MCAO, which would be justified by the remodeling of the

vascular/astrocyte system, a recurrent process after a tissue ischemic process (Dong et al., 2017).

VIM is the major structural component of intermediate filaments in cells of mesenchymal origin. VIM expression demonstrates functions beyond the maintenance of cell cytoarchitecture (Eckes et al., 1998). During development of rodents, for example, VIM is associated with the mobility of neural crest cells (Cochard & Paulin, 1984) and mesenchymal cells (Franke et al., 1982). VIM also plays a role in the structuring and phenotypic rearrangement of immature astrocytes and its expression increases for cell division and migration (de Pablo et al., 2019).

SDAT increases immunoreactivity for VIM, and this effect was unique to DG. EXE-4 reduces VIM immunoreactivity in SDAT animals. As discussed earlier, cells of the astrocytic lineage undergo changes in the intermediate filaments (Fedoroff, McAuley, Houle, & Devon, 1984). In the development of radial glia, the VIM appears as the only important and predominant intermediate filament (Bignami, Raju, & Dahl, 1982). The subgranular zone (SGZ) of the DG contains many radial-type astrocytes that arise from the radial glia (Eckenhoff & Rakic, 1984; Rickmann, Amaral, & Cowan, 1987). These astrocytes express VIM mostly, but other filaments such as nestin and GFAP are found in these cells (Eckenhoff & Rakic, 1984; Lendahl, Zimmerman, & McKay, 1990; Rickmann et al., 1987; Seri, Garcia-Verdugo, Collado-Morente, McEwen, & Alvarez-Buylla, 2004). From this, this fact may explain the change in VIM immunoreactivity occurring only in the DG of SDAT animals.

It is important to note that the granular layer of the DG was excluded for the measurement of immunoreactivity, with the aim of eliminating a possible quantification of immature neurons that can be formed in the process of adult neurogenesis. It is known that new neurons do form in the adult mammalian brain from neural stem cells, especially in the border of the lateral ventricles of the brain (subventricular zone) and (b) the subgranular zone (SGZ) of the DG of the hippocampus (Abbott & Nigussie, 2020). It has been described that astrocyte reactive in AD concomitantly overexpressed GFAP and VIM (Wilhelmsson et al., 2006). This effect is also present in the hippocampus of SDAT animals, but more focused on the DG. Stanojevic et al. (2022) recently reported a concomitant increase in GFAP and VIM content in the hippocampal and periventricular areas in SDAT rats (Stanojevic et al., 2022).

Microglia can change their phenotype and transition from a neuroprotective to a pro-inflammatory process, especially in pathologies characterized due to signaling dysfunction insulin receptor mediated or in insulin resistance events (Kwon & Koh, 2020). Under this situation, activated microglia produce and secretes several pro-inflammatory mediators (Z. Cai,

Hussain, & Yan, 2014; Dheen, Kaur, & Ling, 2007), including tumor necrosis factor-α (TNF-α), interleukin-1beta (IL-1β), IL-6 and nitric oxide (NO); molecular products that trigger the onset and contribute to the progression of AD (Akiyama et al., 2000; H. Cai, Liang, & Ge, 2016; Sajja et al., 2016).

CD11b is a frequently used marker for microglial study. In the healthy brain, microglia are the only cells that express CD11b. However, when the blood-brain barrier is broken, neutrophils and monocytes, which express CD11b, infiltrate the brain. Therefore, anti-CD11b antibodies cannot distinguish microglia from neutrophils and monocytes, but can provide pertinent information about the presence of inflammatory cells and microglial activity (Jeong, Ji, Min, & Joe, 2013). In contrast, ionized calcium-binding adapter protein 1 (Iba-1) is specifically expressed in microglia-like macrophages and increases in Iba-1 expression have been reported after tissue damage in ischemic processes and neurodegenerative diseases (Jeong et al., 2013).

SDAT increases immunoreactivity for Iba-1 and CD11-b in all hippocampal regions. These data are in agreement with Chen et al., (2014) in which the SDAT increased Iba-1 levels in the hippocampus of 3xTg-AD mice. Iba-1 and CD11b levels are also increased in other experimental models for AD and in patients affected by this pathology (Bronzuoli, Facchinetti, Steardo, & Scuderi, 2017; Bronzuoli et al., 2016). On the immunomodulatory effect of microglia by GLP-1R and GIP-R agonists, DA5-CH (GLP-1/GIP dual-receptor agonist) and NLY01 (GLP-1 single-receptor agonist) were reported to be able to reduce microgliosis in Parkinson disease (Lv et al., 2021) and SDAT models (Shi et al., 2017). In order to better understand the anti-inflammatory effect of EXE-4, Huang et al., (2012) verified the capacity of this peptide (10μg/kg ip) against the inflammatory responses triggered by hyperglycemia and by hyperglycemia associated with lipopolysaccharide (an inducing agent of neuroinflammation). There was a suppression of the NF-kβ pathway, reduction of COX1/2 levels, reduction of astrocytosis and microgliosis caused by EXE-4 in hyperglycemic and pro-inflammatory injuries (Huang et al., 2012). These findings lead to believe that EXE-4 has the potential anti-inflammatory ability to down-regulate hyper reactive microglia, and may favor against deleterious processes associated with TDM2 and AD.

To assess the impact of astrogliosis and microgliosis on cell viability, the number of $CC3^{+}$ cells was counted in SDAT and EXE-4 treated animals. SDAT increased apoptosis in all hippocampal regions. Furthermore, EXE-4 was able to reduce this cell death caused by SDAT. Increased cell death in SDAT models was reported by Martini, et al., (2019) and Du et al., (2015), evidencing an increase in apoptotic cells in mice (Martini et al., 2019) and SDAT rats

(Du et al., 2015), respectively. Other evidence suggests that STZ-icv is capable of inducing hippocampal atrophy and increasing ventricular areas (Roy et al., 2022). On the neuroprotective mechanism of EXE-4, it is known that this molecule has been described as anti-apoptotic by Huang et al (2012), where they showed that EXE-4 (10µg/kg, ip) reduced apoptosis caused by LPS, as well as apoptosis induced by the association between hyperglycemia plus LPS in the CA1 region (Huang et al., 2012). Similar effects were also noted by induced apoptosis by cerebral ischemia/reperfusion in diabetic rats (Chung, Chung, Cheng, Yang, & Chien, 2022) by glucose/oxygen deprivation in rat primary cortical neuron (EXE-4, concentrations between 0.2-0.8 µg/ml) (Wang et al., 2012) and in a preclinical model for Parkinson's disease (6-hydroxydopamine) in rats (L. Y. Zhang, Jin, Holscher, & Li, 2021).

It has been seen that a chronic treatment (21 days) with EXE-4 is able to stimulate cell proliferation and neuroblastic differentiation in the DG of adult mice (Li et al., 2010). In this study, EXE-4 treatment increases the number of $Ki67^{+}$, $doublecortin^{+}$ and $BrdU^{+}$ cells (Li et al., 2010). (Bertilsson et al., 2008) found that EXE-4 stimulates neurogenesis in the subventricular zone of rats submitted to a model for Parkinson's disease. (Hamilton, Patterson, Porter, Gault, & Holscher, 2011) showed that agonists for GLP-1R, highlighting EXE-4, as agents promoting the proliferation of brain progenitor cells. Although the mechanism of EXE-4 and other GLP-1R agonists on cell proliferation has not been elucidated, mimetics are identified as neuroprotective agents for contributing to the tissue repair process, especially in neurodegeneration conditions (Holscher, 2014). As reviewed by Christian Hölscher (2014), stimulation of GIP/GLP-1 receptors increase the production of second messengers and activate vital signaling pathways for the control of energy metabolism, mitogenesis, mitophagy, autophagy, inhibition of apoptosis, cell and synapse growth, reduction of inflammation and regulation of translation and transcription of genes involved in cellular stress (Holscher, 2014).

Through this study, an increase was observed of astrocytosis/microgliosis accompanied by cell death in SDAT animals. EXE-4 has been shown to be effective to mitigate these effects indicating that GLP-1R agonists may contribute to the protection of glial cells against injuries found in TDM2 and AD. These findings point out that EXE-4 can reduce neuroinflammation by negative modulation on astrocytic and microglial cells as well as, reduce the death of these cells in a condition of sporadic dementia of the Alzheimer type. Through this, this work points to a potential neuroprotective effect of EXE-4 for neurodegenerative diseases, especially the DA. Although neurons have been the protagonists in most studies related to neurodegenerative diseases, it is important to understand the role of astrogliosis and microgliosis in pathological conditions, especially AD, and to find molecules capable of protecting against this disease.

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Compliance with Ethical Standards

Ethics Approval

The study was approved by the Ethics Committee of UFCSPA (Protocol number 488/16).

Competing Interests

The authors declare that they have no competing interests

References

Abbott, L. C., & Nigussie, F. (2020). Adult neurogenesis in the mammalian dentate gyrus. *Anat Histol Embryol, 49*(1), 3-16. doi:10.1111/ahe.12496

Agulhon, C., Sun, M. Y., Murphy, T., Myers, T., Lauderdale, K., & Fiacco, T. A. (2012). Calcium Signaling and Gliotransmission in Normal vs. Reactive Astrocytes. *Front Pharmacol, 3*, 139. doi:10.3389/fphar.2012.00139

Ahmad, M. H., Fatima, M., & Mondal, A. C. (2019). Influence of microglia and astrocyte activation in the neuroinflammatory pathogenesis of Alzheimer's disease: Rational insights for the therapeutic approaches. *J Clin Neurosci, 59*, 6-11. doi:10.1016/j.jocn.2018.10.034

Akiyama, H., Barger, S., Barnum, S., Bradt, B., Bauer, J., Cole, G. M., . . . Wyss-Coray, T. (2000). Inflammation and Alzheimer's disease. *Neurobiol Aging, 21*(3), 383-421. doi:10.1016/s0197-4580(00)00124-x

Association, A. s. (2022). Alzheimer’s Disease: Facts and Figures. *Alzheimers Dement*(18), 20.

Avila-Munoz, E., & Arias, C. (2014). When astrocytes become harmful: functional and inflammatory responses that contribute to Alzheimer's disease. *Ageing Res Rev, 18*, 29-40. doi:10.1016/j.arr.2014.07.004

Bertilsson, G., Patrone, C., Zachrisson, O., Andersson, A., Dannaeus, K., Heidrich, J., . . . Wikstrom, L. (2008). Peptide hormone exendin-4 stimulates subventricular zone neurogenesis in the adult rodent brain and induces recovery in an animal model of Parkinson's disease. *J Neurosci Res, 86*(2), 326-338. doi:10.1002/jnr.21483

Bignami, A., Raju, T., & Dahl, D. (1982). Localization of vimentin, the nonspecific intermediate filament protein, in embryonal glia and in early differentiating neurons. In vivo and in vitro immunofluorescence study of the rat embryo with vimentin and neurofilament antisera. *Dev Biol, 91*(2), 286-295. doi:10.1016/0012-1606(82)90035-5

Bronzuoli, M. R., Facchinetti, R., Steardo, L., & Scuderi, C. (2017). Astrocyte: An Innovative Approach for Alzheimer's Disease Therapy. *Curr Pharm Des, 23*(33), 4979-4989. doi:10.2174/1381612823666170710163411

Bronzuoli, M. R., Iacomino, A., Steardo, L., & Scuderi, C. (2016). Targeting neuroinflammation in Alzheimer's disease. *J Inflamm Res, 9*, 199-208. doi:10.2147/JIR.S86958

Cai, H., Liang, Q., & Ge, G. (2016). Gypenoside Attenuates beta Amyloid-Induced Inflammation in N9 Microglial Cells via SOCS1 Signaling. *Neural Plast, 2016*, 6362707. doi:10.1155/2016/6362707

Cai, Z., Hussain, M. D., & Yan, L. J. (2014). Microglia, neuroinflammation, and beta-amyloid protein in Alzheimer's disease. *Int J Neurosci, 124*(5), 307-321. doi:10.3109/00207454.2013.833510

Calsolaro, V., & Edison, P. (2016). Neuroinflammation in Alzheimer's disease: Current evidence and future directions. *Alzheimers Dement, 12*(6), 719-732. doi:10.1016/j.jalz.2016.02.010

Cho, Y., Bae, H. G., Okun, E., Arumugam, T. V., & Jo, D. G. (2022). Physiology and pharmacology of amyloid precursor protein. *Pharmacol Ther, 235*, 108122. doi:10.1016/j.pharmthera.2022.108122

Chung, C. H., Chung, S. D., Cheng, Y. H., Yang, C. P., & Chien, C. T. (2022). Long-Lasting Exendin-4-Loaded PLGA Nanoparticles Ameliorate Cerebral Ischemia/Reperfusion Damage in Diabetic Rats. *J Pers Med, 12*(3). doi:10.3390/jpm12030390

Cochard, P., & Paulin, D. (1984). Initial expression of neurofilaments and vimentin in the central and peripheral nervous system of the mouse embryo in vivo. *J Neurosci, 4*(8), 2080-2094. doi:10.1523/JNEUROSCI.04-08-02080.1984

Colombo, E., & Farina, C. (2016). Astrocytes: Key Regulators of Neuroinflammation. *Trends Immunol, 37*(9), 608-620. doi:10.1016/j.it.2016.06.006

de la Monte, S. M., & Tong, M. (2014). Brain metabolic dysfunction at the core of lzheimer's disease. *Biochem Pharmacol, 88*(4), 548-559. doi:10.1016/j.bcp.2013.12.012

de Pablo, Y., Marasek, P., Pozo-Rodrigalvarez, A., Wilhelmsson, U., Inagaki, M., Pekna, M., & Pekny, M. (2019). Vimentin Phosphorylation Is Required for Normal Cell Division of mmature Astrocytes. *Cells, 8*(9). doi:10.3390/cells8091016

Dheen, S. T., Kaur, C., & Ling, E. A. (2007). Microglial activation and its implications in the brain diseases. *Curr Med Chem, 14*(11), 1189-1197. doi:10.2174/092986707780597961

Di Benedetto, G., Burgaletto, C., Bellanca, C. M., Munafo, A., Bernardini, R., & Cantarella, G. (2022). Role of Microglia and Astrocytes in Alzheimer's Disease: From Neuroinflammation to Ca(2+) Homeostasis Dysregulation. *Cells, 11*(17). doi:10.3390/cells11172728

Du, L. L., Chai, D. M., Zhao, L. N., Li, X. H., Zhang, F. C., Zhang, H. B., . . . Zhou, X. W. (2015). AMPK activation ameliorates Alzheimer's disease-like pathology and spatial memory impairment in a streptozotocin-induced Alzheimer's disease model in rats. *J Alzheimers Dis, 43*(3), 775-784. doi:10.3233/JAD-140564

Eckenhoff, M. F., & Rakic, P. (1984). Radial organization of the hippocampal dentate gyrus: a Golgi, ultrastructural, and immunocytochemical analysis in the developing rhesus monkey. *J Comp Neurol, 223*(1), 1-21. doi:10.1002/cne.902230102

Eckes, B., Dogic, D., Colucci-Guyon, E., Wang, N., Maniotis, A., Ingber, D., . . . Krieg, T. (1998). Impaired mechanical stability, migration and contractile capacity in vimentin-deficient fibroblasts. *J Cell Sci, 111 (Pt 13)*, 1897-1907. doi:10.1242/jcs.111.13.1897

Farfara, D., Lifshitz, V., & Frenkel, D. (2008). Neuroprotective and neurotoxic properties of glial cells in the pathogenesis of Alzheimer's disease. *J Cell Mol Med, 12*(3), 762-780. doi:10.1111/j.1582-4934.2008.00314.x

Fedoroff, S., McAuley, W. A., Houle, J. D., & Devon, R. M. (1984). Astrocyte cell lineage. V. Similarity of astrocytes that form in the presence of dBcAMP in cultures to reactive astrocytes in vivo. *J Neurosci Res, 12*(1), 14-27. doi:10.1002/jnr.490120103

Ghasemi, R., Dargahi, L., Haeri, A., Moosavi, M., Mohamed, Z., & Ahmadiani, A. (2013). Brain insulin dysregulation: implication for neurological and neuropsychiatric disorders. *Mol Neurobiol, 47*(3), 1045-1065. doi:10.1007/s12035-013-8404-z

Gutierres, J. M., Carvalho, F. B., Schetinger, M. R., Marisco, P., Agostinho, P., Rodrigues, M., . . . Spanevello, R. (2014). Anthocyanins restore behavioral and biochemical changes caused by streptozotocin-induced sporadic dementia of Alzheimer's type. *Life Sci, 96*(1-2), 7-17. doi:10.1016/j.lfs.2013.11.014

Hamilton, A., Patterson, S., Porter, D., Gault, V. A., & Holscher, C. (2011). Novel GLP-1 mimetics developed to treat type 2 diabetes promote progenitor cell proliferation in the brain. *J Neurosci Res, 89*(4), 481-489. doi:10.1002/jnr.22565

Holscher, C. (2014). Central effects of GLP-1: new opportunities for treatments of neurodegenerative diseases. *J Endocrinol, 221*(1), T31-41. doi:10.1530/JOE-13-0221

Hoyer, S., & Lannert, H. (2007). Long-term abnormalities in brain glucose/energy metabolism after inhibition of the neuronal insulin receptor: implication of tau-protein. *J Neural Transm Suppl*(72), 195-202. doi:10.1007/978-3-211-73574-9_25

Hristovska, I., & Pascual, O. (2015). Deciphering Resting Microglial Morphology and Process Motility from a Synaptic Prospect. *Front Integr Neurosci, 9*, 73. doi:10.3389/fnint.2015.00073

Huang, H. J., Chen, Y. H., Liang, K. C., Jheng, Y. S., Jhao, J. J., Su, M. T., . . . Hsieh-Li, H. M. (2012). Exendin-4 protected against cognitive dysfunction in hyperglycemic mice

receiving an intrahippocampal lipopolysaccharide injection. *PLoS One, 7*(7), e39656. doi:10.1371/journal.pone.0039656

Hur, J. Y. (2022). gamma-Secretase in Alzheimer's disease. *Exp Mol Med, 54*(4), 433-446. doi:10.1038/s12276-022-00754-8

Jeong, H. K., Ji, K., Min, K., & Joe, E. H. (2013). Brain inflammation and microglia: facts and misconceptions. *Exp Neurobiol, 22*(2), 59-67. doi:10.5607/en.2013.22.2.59

Kraft, A. W., Hu, X., Yoon, H., Yan, P., Xiao, Q., Wang, Y., . . . Lee, J. M. (2013). Attenuating astrocyte activation accelerates plaque pathogenesis in APP/PS1 mice. *FASEB J, 27*(1), 187-198. doi:10.1096/fj.12-208660

Kwon, H. S., & Koh, S. H. (2020). Neuroinflammation in neurodegenerative disorders: the roles of microglia and astrocytes. *Transl Neurodegener, 9*(1), 42. doi:10.1186/s40035-020-00221-2

Lendahl, U., Zimmerman, L. B., & McKay, R. D. (1990). CNS stem cells express a new class of intermediate filament protein. *Cell, 60*(4), 585-595. doi:10.1016/0092-8674(90)90662-x

Li, H., Lee, C. H., Yoo, K. Y., Choi, J. H., Park, O. K., Yan, B. C., . . . Won, M. H. (2010). Chronic treatment of exendin-4 affects cell proliferation and neuroblast differentiation in the adult mouse hippocampal dentate gyrus. *Neurosci Lett, 486*(1), 38-42. doi:10.1016/j.neulet.2010.09.040

Linnerbauer, M., Wheeler, M. A., & Quintana, F. J. (2020). Astrocyte Crosstalk in CNS Inflammation. *Neuron, 108*(4), 608-622. doi:10.1016/j.neuron.2020.08.012

Luciani, P., Deledda, C., Benvenuti, S., Cellai, I., Squecco, R., Monici, M., . . . Peri A. (2010). Differentiating effects of the glucagon-like peptide-1 analogue exendin-4 in a human neuronal cell model. *Cell Mol Life Sci, 67*(21), 3711-3723. doi:10.1007/s00018-010-0398-3

Lv, M., Xue, G., Cheng, H., Meng, P., Lian, X., Holscher, C., & Li, D. (2021). The GLP-1/GIP dual-receptor agonist DA5-CH inhibits the NF-kappaB inflammatory pathway in the MPTP mouse model of Parkinson's disease more effectively than the GLP-1 single-receptor agonist NLY01. *Brain Behav, 11*(8), e2231. doi:10.1002/brb3.2231

Macauley, S. L., Pekny, M., & Sands, M. S. (2011). The role of attenuated astrocyte activation in infantile neuronal ceroid lipofuscinosis. *J Neurosci, 31*(43), 15575-15585. doi:10.1523/JNEUROSCI.3579-11.2011

Madore, C., Yin, Z., Leibowitz, J., & Butovsky, O. (2020). Microglia, Lifestyle Stress, and Neurodegeneration. *Immunity, 52*(2), 222-240. doi:10.1016/j.immuni.2019.12.003

Martini, F., Rosa, S. G., Klann, I. P., Fulco, B. C. W., Carvalho, F. B., Rahmeier, F. L., . . . Nogueira, C. W. (2019). A multifunctional compound ebselen reverses memory impairment, apoptosis and oxidative stress in a mouse model of sporadic Alzheimer's disease. *J Psychiatr Res, 109*, 107-117. doi:10.1016/j.jpsychires.2018.11.021

Meraz-Rios, M. A., Toral-Rios, D., Franco-Bocanegra, D., Villeda-Hernandez, J., & Campos-Pena, V. (2013). Inflammatory process in Alzheimer's Disease. *Front Integr Neurosci, 7*, 59. doi:10.3389/fnint.2013.00059

Mishra, S. K., Singh, S., Shukla, S., & Shukla, R. (2018). Intracerebroventricular streptozotocin impairs adult neurogenesis and cognitive functions via regulating neuroinflammation and insulin signaling in adult rats. *Neurochem Int, 113*, 56-68. doi:10.1016/j.neuint.2017.11.012

Molofsky, A. V., & Deneen, B. (2015). Astrocyte development: A Guide for the Perplexed. *Glia, 63*(8), 1320-1329. doi:10.1002/glia.22836

Paxinos, G., & Watson, C. (2013). The Rat Brain in Stereotaxic Coordinates. *Hard Cover Edition, 7th*.

Rahmeier, F. L., Zavalhia, L. S., Tortorelli, L. S., Huf, F., Gea, L. P., Meurer, R. T., . . . Fernandes, M. D. C. (2016). The effect of taurine and enriched environment on behaviour, memory and hippocampus of diabetic rats. *Neurosci Lett, 630*, 84-92. doi:10.1016/j.neulet.2016.07.032

Ransohoff, R. M., & Brown, M. A. (2012). Innate immunity in the central nervous system. *J Clin Invest, 122*(4), 1164-1171. doi:10.1172/JCI58644

Ransohoff, R. M., & El Khoury, J. (2015). Microglia in Health and Disease. *Cold Spring Harb Perspect Biol, 8*(1), a020560. doi:10.1101/cshperspect.a020560

Rickmann, M., Amaral, D. G., & Cowan, W. M. (1987). Organization of radial glial cells during the development of the rat dentate gyrus. *J Comp Neurol, 264*(4), 449-479. doi:10.1002/cne.902640403

Rodrigues, L., Biasibetti, R., Swarowsky, A., Leite, M. C., Quincozes-Santos, A., Quilfeldt, J. A., . . . Goncalves, C. A. (2009). Hippocampal alterations in rats submitted to streptozotocin-induced dementia model are prevented by aminoguanidine. *J Alzheimers Dis, 17*(1), 193-202. doi:10.3233/JAD-2009-1034

Rodrigues, M. V., Gutierres, J. M., Carvalho, F., Lopes, T. F., Antunes, V., da Costa, P., . . . de Andrade, C. M. (2019). Protection of cholinergic and antioxidant system contributes to the effect of Vitamin D(3) ameliorating memory dysfunction in sporadic dementia of Alzheimer's type. *Redox Rep, 24*(1), 34-40. doi:10.1080/13510002.2019.1617514

Roy, A., Sharma, S., Nag, T. C., Katyal, J., Gupta, Y. K., & Jain, S. (2022). Cognitive Dysfunction and Anxiety Resulting from Synaptic Downscaling, Hippocampal Atrophy, and Ventricular Enlargement with Intracerebroventricular Streptozotocin Injection in Male Wistar Rats. *Neurotox Res*. doi:10.1007/s12640-022-00563-x

Sajja, V. S., Hlavac, N., & VandeVord, P. J. (2016). Role of Glia in Memory Deficits Following Traumatic Brain Injury: Biomarkers of Glia Dysfunction. *Front Integr Neurosci, 10*, 7. doi:10.3389/fnint.2016.00007

Salkovic-Petrisic, M., & Hoyer, S. (2007). Central insulin resistance as a trigger for sporadic Alzheimer-like pathology: an experimental approach. *J Neural Transm Suppl*(72), 217-233. doi:10.1007/978-3-211-73574-9_28

Santos, L. E., da Silveira, G. A., Costa, V. D., Batista, A. G., Madureira, A. P., Rodrigues, A. M., . . . de Almeida, A. C. (2013). Alcohol abuse promotes changes in non-synaptic epileptiform activity with concomitant expression changes in cotransporters and glial cells. *PLoS One, 8*(11), e78854. doi:10.1371/journal.pone.0078854

Sastre, M., Calero, M., Pawlik, M., Mathews, P. M., Kumar, A., Danilov, V., . . . Levy, E. (2004). Binding of cystatin C to Alzheimer's amyloid beta inhibits in vitro amyloid fibril formation. *Neurobiol Aging, 25*(8), 1033-1043. doi:10.1016/j.neurobiolaging.2003.11.006

Sastre, M., Dewachter, I., Landreth, G. E., Willson, T. M., Klockgether, T., van Leuven, F., & Heneka, M. T. (2003). Nonsteroidal anti-inflammatory drugs and peroxisome proliferator-activated receptor-gamma agonists modulate immunostimulated processing of amyloid precursor protein through regulation of beta-secretase. *J Neurosci, 23*(30), 9796-9804. doi:10.1523/JNEUROSCI.23-30-09796.2003

Sastre, M., Steiner, H., Fuchs, K., Capell, A., Multhaup, G., Condron, M. M., . . . Haass, C. (2001). Presenilin-dependent gamma-secretase processing of beta-amyloid precursor protein at a site corresponding to the S3 cleavage of Notch. *EMBO Rep, 2*(9), 835-841. doi:10.1093/embo-reports/kve180

Seri, B., Garcia-Verdugo, J. M., Collado-Morente, L., McEwen, B. S., & Alvarez-Buylla, A. (2004). Cell types, lineage, and architecture of the germinal zone in the adult dentate gyrus. *J Comp Neurol, 478*(4), 359-378. doi:10.1002/cne.20288

Shi, L., Zhang, Z., Li, L., & Holscher, C. (2017). A novel dual GLP-1/GIP receptor agonist alleviates cognitive decline by re-sensitizing insulin signaling in the Alzheimer icv. STZ rat model. *Behav Brain Res, 327*, 65-74. doi:10.1016/j.bbr.2017.03.032

Simon, E., Obst, J., & Gomez-Nicola, D. (2019). The Evolving Dialogue of Microglia and Neurons in Alzheimer's Disease: Microglia as Necessary Transducers of Pathology. *Neuroscience, 405*, 24-34. doi:10.1016/j.neuroscience.2018.01.059

Sofroniew, M. V. (2000). Astrocyte failure as a cause of CNS dysfunction. *Mol Psychiatry, 5*(3), 230-232. doi:10.1038/sj.mp.4000753

Sofroniew, M. V. (2011). Neuromyelitis optica: circulating autoantibodies provoke astrocyte-mediated neural dysfunction. *Neurology, 76*(14), 1202-1203. doi:10.1212/WNL.0b013e3182143355

Sofroniew, M. V. (2015). Astrocyte barriers to neurotoxic inflammation. *Nat Rev Neurosci, 16*(5), 249-263. doi:10.1038/nrn3898

Sofroniew, M. V. (2020). Astrocyte Reactivity: Subtypes, States, and Functions in CNS Innate Immunity. *Trends Immunol, 41*(9), 758-770. doi:10.1016/j.it.2020.07.004

Stanojevic, J., Dragic, M., Stevanovic, I., Ilic, T., Stojanovic, I., Zeljkovic, M., & Ninkovic, M. (2022). Intermittent theta burst stimulation ameliorates cognitive impairment and hippocampal gliosis in the Streptozotocin-induced model of Alzheimer's disease. *Behav Brain Res, 433*, 113984. doi:10.1016/j.bbr.2022.113984

Talbot, K., Wang, H. Y., Kazi, H., Han, L. Y., Bakshi, K. P., Stucky, A., . . . Arnold, S. E. (2012). Demonstrated brain insulin resistance in Alzheimer's disease patients is associated with IGF-1 resistance, IRS-1 dysregulation, and cognitive decline. *J Clin Invest, 122*(4), 1316-1338. doi:10.1172/JCI59903

Teixeira, F. C., Soares, M. S. P., Blodorn, E. B., Domingues, W. B., Reichert, K. P., Zago, A. M., . . . Spanevello, R. M. (2022). Investigating the Effect of Inosine on Brain Purinergic Receptors and Neurotrophic and Neuroinflammatory Parameters in an Experimental Model of Alzheimer's Disease. *Mol Neurobiol, 59*(2), 841-855. doi:10.1007/s12035-021-02627-z

Tortorelli, L. S., Engelke, D. S., Lunardi, P., Mello, E. S. T., Santos-Junior, J. G., & Goncalves, C. A. (2015). Cocaine counteracts LPS-induced hypolocomotion and triggers locomotor sensitization expression. *Behav Brain Res, 287*, 226-229. doi:10.1016/j.bbr.2015.03.054

Wang, M. D., Huang, Y., Zhang, G. P., Mao, L., Xia, Y. P., Mei, Y. W., & Hu, B. (2012). Exendin-4 improved rat cortical neuron survival under oxygen/glucose deprivation through PKA pathway. *Neuroscience, 226*, 388-396. doi:10.1016/j.neuroscience.2012.09.025

Wilhelmsson, U., Bushong, E. A., Price, D. L., Smarr, B. L., Phung, V., Terada, M., . . . Pekny, M. (2006). Redefining the concept of reactive astrocytes as cells that remain within their unique domains upon reaction to injury. *Proc Natl Acad Sci U S A, 103*(46), 17513-17518. doi:10.1073/pnas.0602841103

Yin, F., Yao, J., Brinton, R. D., & Cadenas, E. (2017). Editorial: The Metabolic-Inflammatory Axis in Brain Aging and Neurodegeneration. *Front Aging Neurosci, 9*, 209. doi:10.3389/fnagi.2017.00209

Yuliani, T., Lobentanzer, S., & Klein, J. (2021). Central cholinergic function and metabolic changes in streptozotocin-induced rat brain injury. *J Neurochem, 158*(6), 1307-1319. doi:10.1111/jnc.15155

Zanotto, C., Simao, F., Gasparin, M. S., Biasibetti, R., Tortorelli, L. S., Nardin, P., & Goncalves, C. A. (2017). Exendin-4 Reverses Biochemical and Functional Alterations in the Blood-Brain and Blood-CSF Barriers in Diabetic Rats. *Mol Neurobiol, 54*(3), 2154-2166. doi:10.1007/s12035-016-9798-1

Zhang, L. Y., Jin, Q. Q., Holscher, C., & Li, L. (2021). Glucagon-like peptide-1/glucose-dependent insulinotropic polypeptide dual receptor agonist DA-CH5 is superior to exendin-4 in protecting neurons in the 6-hydroxydopamine rat Parkinson model. *Neural Regen Res, 16*(8), 1660-1670. doi:10.4103/1673-5374.303045

Zhang, Y. W., Thompson, R., Zhang, H., & Xu, H. (2011). APP processing in Alzheimer's disease. *Mol Brain, 4*, 3. doi:10.1186/1756-6606-4-3

List of Legends

Figure 1

Figure 1. Division of Experimental Groups. Vehicle (saline ip *plus* ACFS icv), Exendin-4 (EXE-4 ip *plus* ACFS icv), STZ (saline ip *plus* STZ icv), and STZ+EXE-4 (EXE-4 ip *plus* STZ icv).

Figure 2

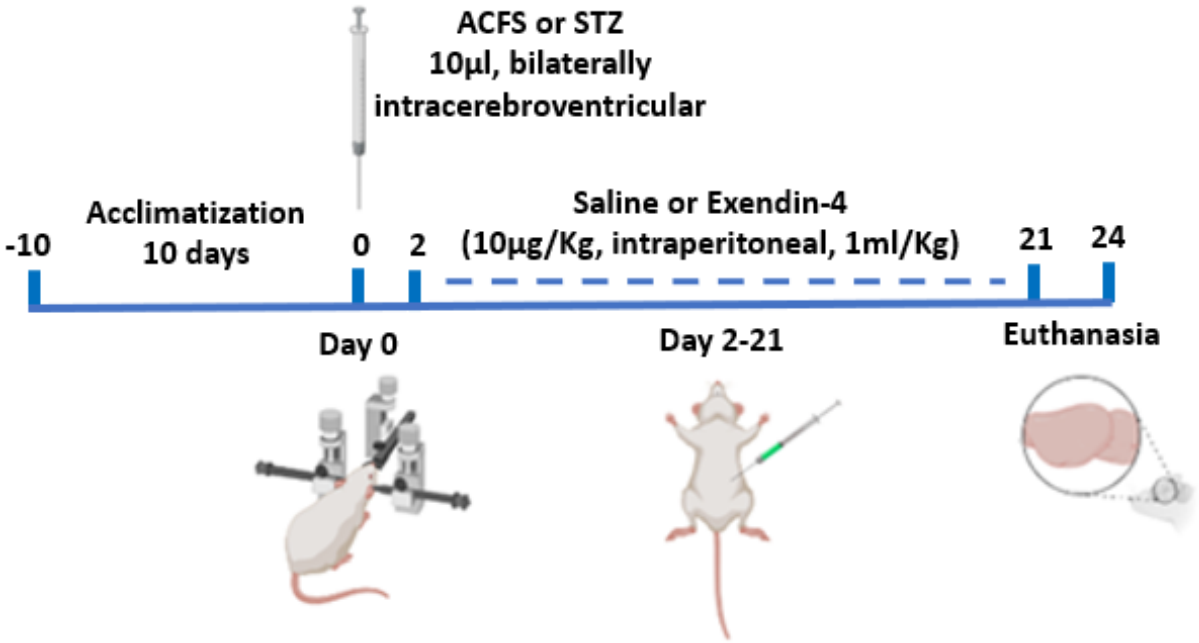

Figure 2. Experimental design of the treatment with EXE-4 and the stereotaxic procedure for the Sporadic Dementia Alzheimer's Type (SDAT). Day **0**: stereotaxic surgery was performed for STZ or ACFS injection (icv, in a total volume of 10 µl - 5 µl, bilaterally; 1 µl/minute). After this procedure, the rats were allowed to recovery for two days. Day **2**: treatment with EXE-4 or saline was started, until day **21**, at a dose of 10 µg/kg, administered intraperitoneal (volume of 1 ml/kg). EXE-4 was prepared in sterile saline. On day **24**, the animals were anesthetized and euthanized to obtain the brain.

Figure 3

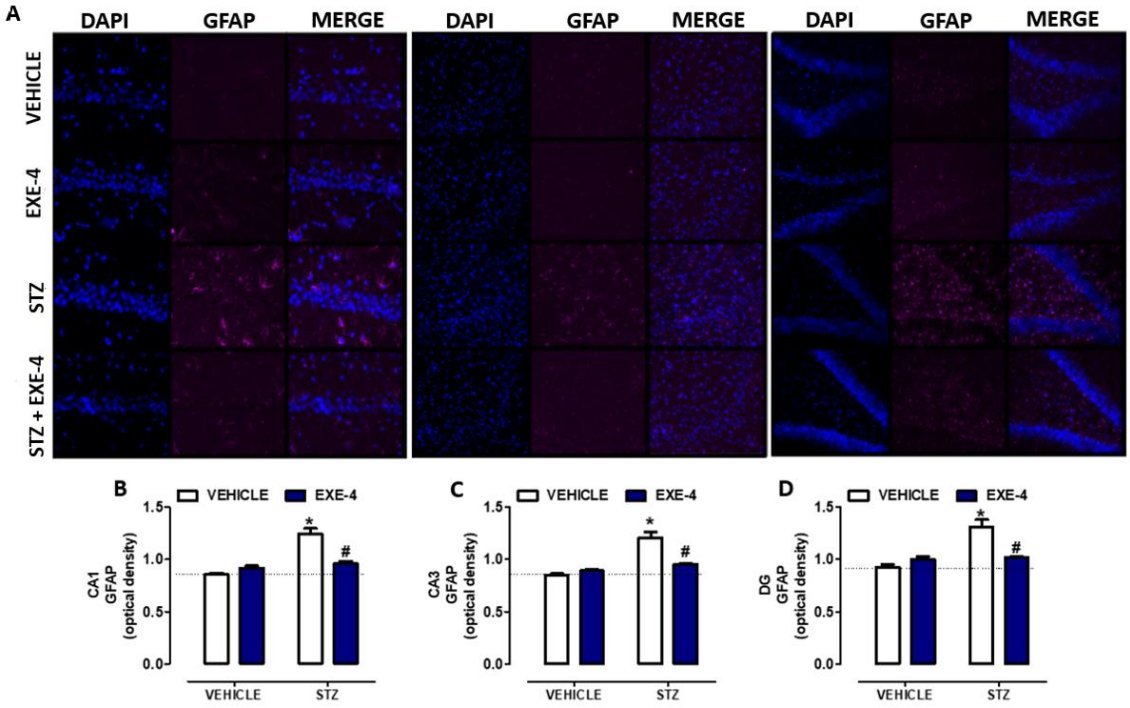

Figure 3. Effect of treatment with exendin-4 (EXE-4, 10µg/kg, ip) in rats submitted to a sporadic dementia of the Alzheimer type (SDAT, STZ 3mg/kg, icv) on the GFAP immunoreactivity of the CA1, CA3 and DG. (**A)** Representative immunoreactions of each group (DAPI in blue, GFAP in pink and Merge, 20X). Graph with optical densities for GFAP in the CA1 (**B**), CA3 (**C**) and DG (**D**). *Denotes significant difference *versus* vehicle group (saline *plus* ACFS). #Denotes significant interaction between treatments (difference *versus* STZ group). Two-way analysis of variance. * or # $P<0.05$ was considered significant. Data expressed as mean ± standard error of mean for n = 7.

Figure 4

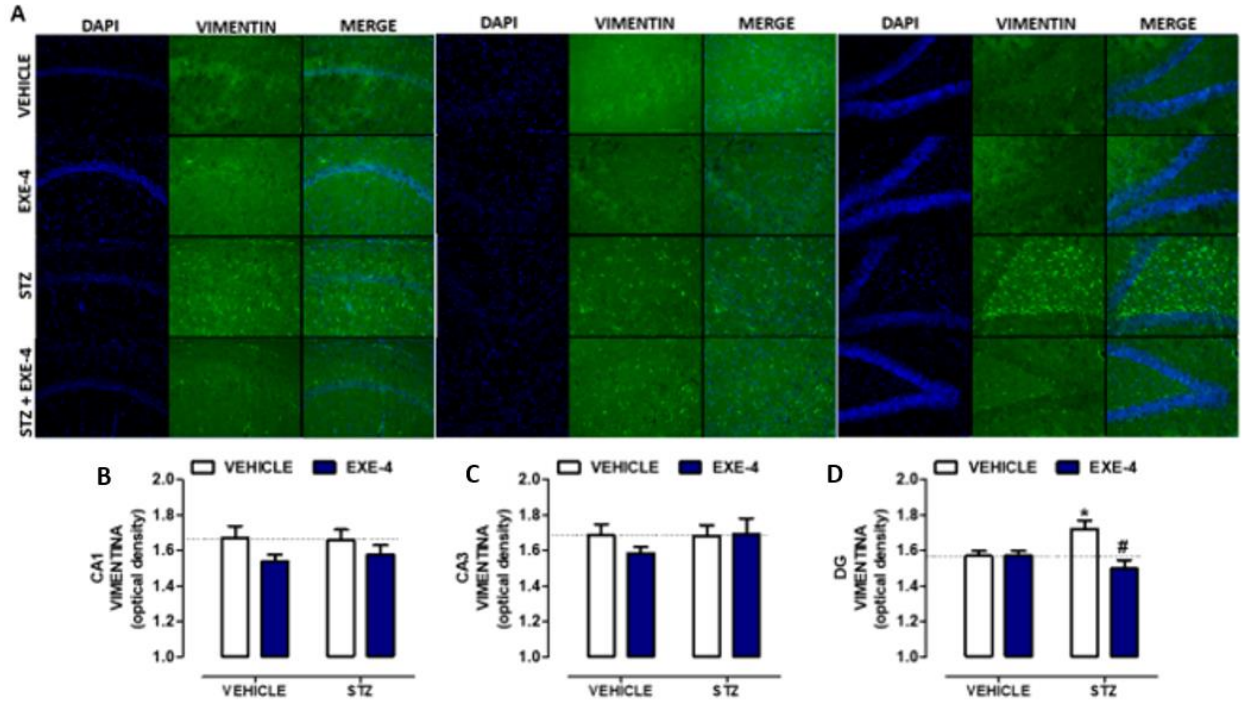

Figure 4. Effect of treatment with exendin-4 (EXE-4, 10μg/kg, ip) in rats submitted to a sporadic dementia of the Alzheimer type (SDAT, STZ 3mg/kg, icv) on the vimentin immunoreactivity of the CA1, CA3 and DG. (**A)** Representative immunoreactions of each group (DAPI in blue, vimentin in green and Merge, 20X). Graph with optical densities for vimentin in the CA1 (**B**), CA3 (**C**) and DG (**D).** *Denotes significant difference *versus* vehicle group (saline *plus* ACFS). #Denotes significant interaction between treatments (difference *versus* STZ group). Two-way analysis of variance. * or # $P<0.05$ was considered significant. Data expressed as mean ± standard error of mean for n = 7.

Figure 5

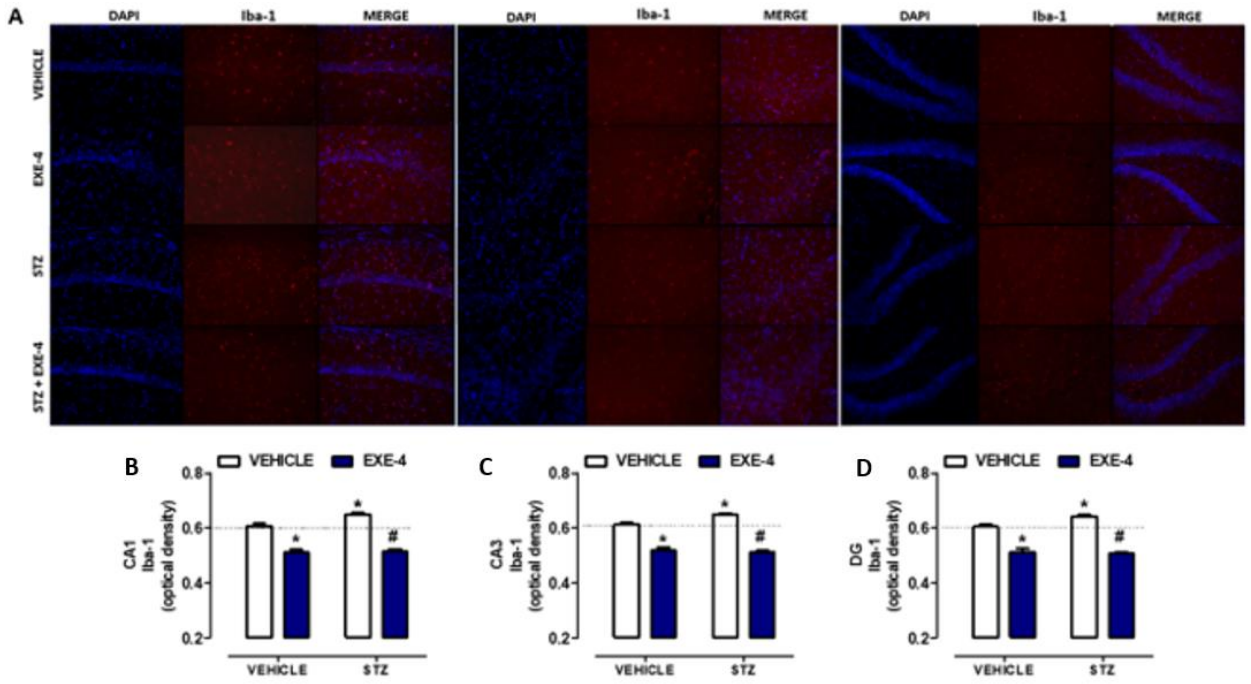

Figure 5. Effect of treatment with exendin-4 (EXE-4, 10μg/kg, ip, saline vehicle) in rats submitted to a sporadic dementia of the Alzheimer type (SDAT, STZ, 3mg/kg, icv, ACFS as vehicle) on the Iba-1 immunoreactivity of the CA1, CA3 and DG. (**A)** Representative immunoreactions of each group (DAPI in blue, Iba-1 in red and Merge, 20X). Graph with the optical densities for Iba-1 in the CA1 (**B**), CA3 (**C**) and DG (**D**). *Denotes significant difference *versus* vehicle group (saline *plus* ACFS). # Denotes significant interaction between treatments (difference *versus* STZ group). Two-way analysis of variance. * or # $P<0.05$ was considered significant. Data expressed as mean ± standard error of mean for n =7.

Figure 6

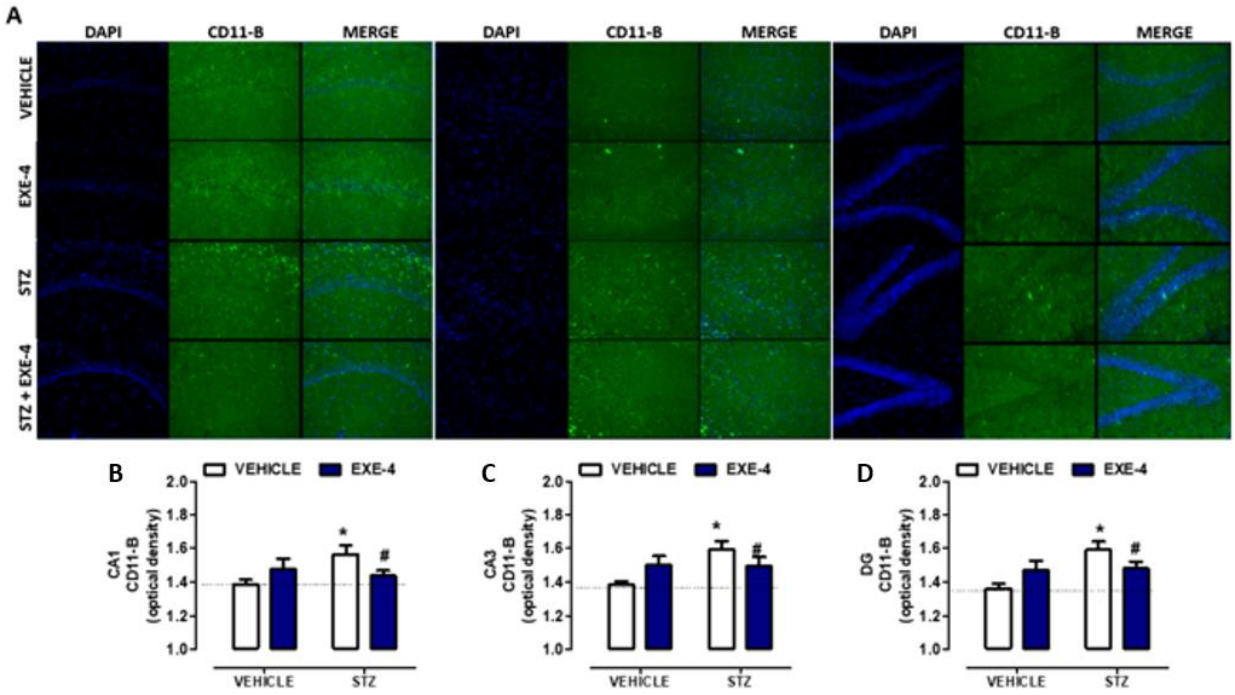

Figure 6. Effect of treatment with exendin-4 (EXE-4, 10μg/kg, ip, vehicle as saline) in rats submitted to a sporadic dementia of the Alzheimer type (SDAT, STZ, 3mg/kg, icv, ACFS as vehicle) on the CD11b immunoreactivity of the CA1, CA3 and DG regions. (**A)** Representative immunoreactions of each group (DAPI in blue, CD11-B in green and Merge, 20X). Graph with optical densities for CD11b in the CA1 (**B**), CA3 (**C**) and DG (**D**). *Denotes significant difference versus vehicle group (saline *plus* ACFS). #Denotes significant interaction between treatments (difference *versus* STZ group). Two-way analysis of variance. * or # $P<0.05$ was considered significant. Data expressed as mean ± standard error of mean for n = 7.

Figure 7

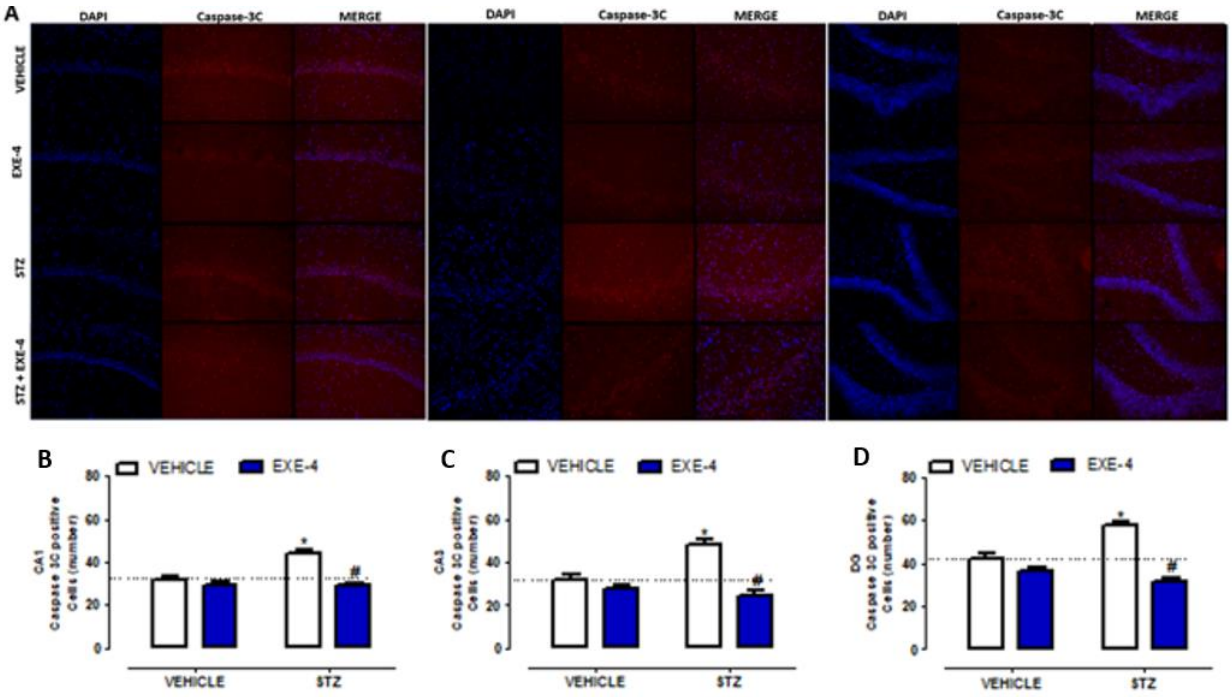

Figure 7. Effect of treatment with exendin-4 (EXE-4, 10μg/kg, ip, saline as vehicle) in rats submitted to a sporadic dementia of the Alzheimer type (SDAT, STZ, 3mg/kg, icv. ACFS as vehicle) on the Cleaved Caspase-3^{+} (CC3) cells in the CA1, CA3 and DG. (**A**) Representative immunoreactions of each group (DAPI in blue, CC3 in red and Merge, 20X). Graph with the number of CC^{3+} cells in the CA1 (**B**), CA3 (**C**) and DG (**D**). *Denotes significant difference versus vehicle group (saline *plus* ACFS). #Denotes significant interaction between treatments (difference *versus* STZ group). Two-way analysis of variance. * or # $P<0.05$ was considered significant. Data expressed as mean ± standard error of mean for n = 7.

5 CONCLUSÕES

✓ Nossos resultados e outros estudos já citados na literatura mostraram que o modelo de demência esporádica do tipo Alzheimer induzido por estreptozotocina (DETA) foi capaz de reproduzir sintomas semelhantes aos da DA, como déficits de memória, ativação de astrócitos e micróglia, apoptose celular, neuroinflamação e neurodegeneração.

✓ A administração de EXE-4 foi capaz de proteger a memória de curta e longa duração dos animais no modelo de demência esporádica do tipo Alzheimer.

✓ A EXE-4 apresentou um efeito benéfico nos neurônios piramidais e granulares no hipocampo dos animais DETA, mostrando propriedades anti-apoptóticas e promovendo a proliferação celular.

✓ A EXE-4 aumentou o conteúdo de receptores para a insulina no hipocampo, o que pode melhorar as respostas biológicas mediadas pela insulina nessa estrutura.

✓ No hipocampo dos animais DETA, foi observado um aumento de astrocitose/microgliose acompanhada de morte celular. EXE-4 foi capaz de abrandar esses efeitos, indicando que os agonistas de GLP-1R podem contribuir para a proteção das células gliais contra lesões encontradas no DM2 e DA.

✓ Este trabalho aponta para um potencial efeito neuroprotetor da EXE-4 para doenças neurodegenerativas, principalmente a DA.

✓ Embora os neurônios tenham sido protagonistas na maioria dos estudos relacionados às doenças neurodegenerativas, é importante entender o papel da astrogliose e da microgliose nas condições patológicas, principalmente na DA, e encontrar moléculas capazes de proteger contra essa doença.

6 CONSIDERAÇÕES FINAIS

O presente estudo faz parte de um projeto maior intitulado “Caracterizações comportamentais, histopatológicas e inflamatórias no modelo da doença de Alzheimer induzida por estreptozotocina”: possíveis mecanismos de toxicidade e proteção**”,** aprovado pelo Comitê de Ética em Pesquisa (CEP) da UFCSPA
(Protocolo N° 191/16; Parecer Nº 488/16). Deste projeto maior além da minha tese de doutorado foram desenvolvidos outros trabalhos, incluindo uma dissertação de mestrado.

A ideia da minha tese de doutorado foi pesquisar o efeito neuroprotetor da EXE-4, um análogo do receptor do peptídeo semelhante ao glucagon-1 (GLP-1), um medicamento bem estabelecido para o tratamento de diabetes mellitus tipo 2 (DM2) em ratos submetidos a um modelo de demência esporádica do tipo Alzheimer induzido por estreptozotocina.

Na pesquisa, verificamos que a EXE-4 apresentou efeitos benéficos sobre os neurônios piramidais e granulares que constituem o hipocampo, sobre o processo de apoptose, protegeu a memória de curta e longa duração, melhorou a proliferação celular e foi capaz de abrandar a astrocitose/microgliose. Com nossos resultados e outros estudos realizados na literatura, a EXE-4 pode ser um adjuvante para o tratamento da doença de Alzheimer, melhorando a qualidade de vida das pessoas acometidas por essa patologia.

ANEXOS - PARECER DO COMITÊ DE ÉTICA DA UFCSPA

REPÚBLICA FEDERATIVA DO BRASIL
MINISTÉRIO DA EDUCAÇÃO

UFCSPA
UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE

CEUA –COMISSÃO DE ÉTICA NO USO DE ANIMAIS

PARECER CONSUBSTANCIADO DE PROJETO DE PESQUISA E ENSINO

1) PROTOCOLO Nº: 191/16 **Parecer 488/16**

2) DATA DO PARECER: 11/01/2017

3) TÍTULO DO PROJETO:

Caracterizações comportamentais.histopatológicas e inflamatórias no modelo da doença de Alzheimer induzida por estreptozotocina: possíveis mecanismos de toxicidade e proteção

4) PESQUISADOR RESPONSÁVEL:

Profa. Marilda da Cruz Fernandes

5) RESUMO DO PROJETO:

Trata-se de projeto experimental em ratos com 3 experimentos onde se busca estudar os efeitos da injeção intracerebroventricular de estreptozotocina (com e sem LPS e EX4) e avaliaão da ocorrência de alterações de memória (no labirinto aquático) cerebrais a serem testadas quanto à presença de placas senis, emaranhados neurofibrilares, 'corpos amiláceos', marcados inflamatórios (S100B e TLR4 e deposição de LPS) no tecido nervoso e no líquido cefalorraquidiano (neste caso, S100B, proteína C reativa, componentes C3 e C4 do sistema complemento).

6) OBJETIVOS DO PROJETO:

Acima indicados

7) FINALIDADE DO PROJETO: ☐ Ensino ☒ Pesquisa

8) ITENS METODOLÓGICOS E ÉTICOS DO PROJETO:

Título	☒ Adequado	☐ Comentários
Introdução	☒ Adequada	☐ Comentários
Objetivos	☒ Adequados	☐ Comentários

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Relevância e Justificativa	[ ] Adequados	[x] Comentários
Materiais e Métodos	[ ] Adequados	[x] Comentários
Cronograma para execução da pesquisa	[x] Adequado	[ ] Comentários
Orçamento e fonte financiadora (não consta fonte financiadora)	[ ] Adequados	[x] Comentários
Referências Bibliográficas	[ ] Adequadas	[x] Comentários

9) O PROJETO ESTÁ ADEQUADO À LEGISLAÇÃO VIGENTE:
(vide comentários referentes à metodologia a serem respondidos)
[ ] Sim [x] Não

10) INFORMAÇÕES RELATIVAS AOS ANIMAIS:

Grau de dor/estresse: **B** [ ] **C** [ ] **D** [X] **E** [ ]
Justifique:

Procedimentos invasivos com necessidade de anestesia e analgesia e, por fim, eutanásia.

Espécie: Rato **Número Amostral:** 100

Redução Amostral: [ ] Sim [x] Não
Justifique:

Substituição de Metodologia: [x] Sim [ ] Não
Se achar necessário, justifique e sugira uma nova metodologia:

Vide comentários abaixo

Aprimoramento da Metodologia: [x] Sim [ ] Não
Se achar necessário, justifique e sugira aprimoramentos da metodologia:

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Vide comentários abaixo

Acomodação e manutenção dos animais: [x] Adequada [ ] Inadequada
Se achar inadequada cite abaixo as melhorias necessárias:

Manipulação dos animais: [x] Adequada [ ] Inadequada
Se achar inadequada cite abaixo as melhorias necessárias:

Analgesia dos animais (se aplicável): [ ] Adequada [ ] Inadequada
Se achar inadequada cite abaixo as melhorias necessárias com analgésico substituto:

Vide comentários abaixo

Anestesia dos animais (se aplicável): [x] Adequada [ ] Inadequada
Se achar inadequada cite abaixo as melhorias necessárias com anestésico substituto:

Eutanásia dos animais (se aplicável): [ ] Adequada [ ] Inadequada
Se achar inadequada cite abaixo as melhorias necessárias com metodologia substituta:

Vide comentários abaixo

Local de Realização (Biotério/Labotarório): Laboratório de Pesquisa em Patologia/UFCSPA

Outra instituição. Qual? Laboratório de "Proteínas Ligantes de Cálcio"/UFRGS e Laboratório de Bioquímica do Hospital de Clínicas de Porto Alegre

11) CRONOGRAMA DE UTILIZAÇÃO DE ANIMAIS

Data	Espécie	Sexo	Quantidade
2017/1	Rato	Macho	20
2018/1	Rato	Macho	40
2019/1	Rato	Macho	40

12) RECOMENDAÇÃO:

[x] Aprovado

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UFCSPA

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☐ Com Pendência

☐ Não aprovado

Data de início 01/2017

Data de Término 07/2021

Comentários gerais sobre o projeto:

Os comentários são apresentados de acordo com a ordem dos documentos apresentados, sugerindo-se, respeitosamente, aos autores os seguintes pontos a considerar:

Sugestões:
Utilizar anestésico geral com isoflurano ou tiopental, antes da perfusão, levando em consideração o possível estresse de ser causado no animal. O uso de anestésico com está proposto, não seria o mais indicado pois não possui efeito anestésico profundo.

Da mesma forma sugerimos aumentar a concentração do fármaco a ser injetado pela via intracérebro ventricular. Adequar para o volume máximo de 3ml conforme literatura (8).