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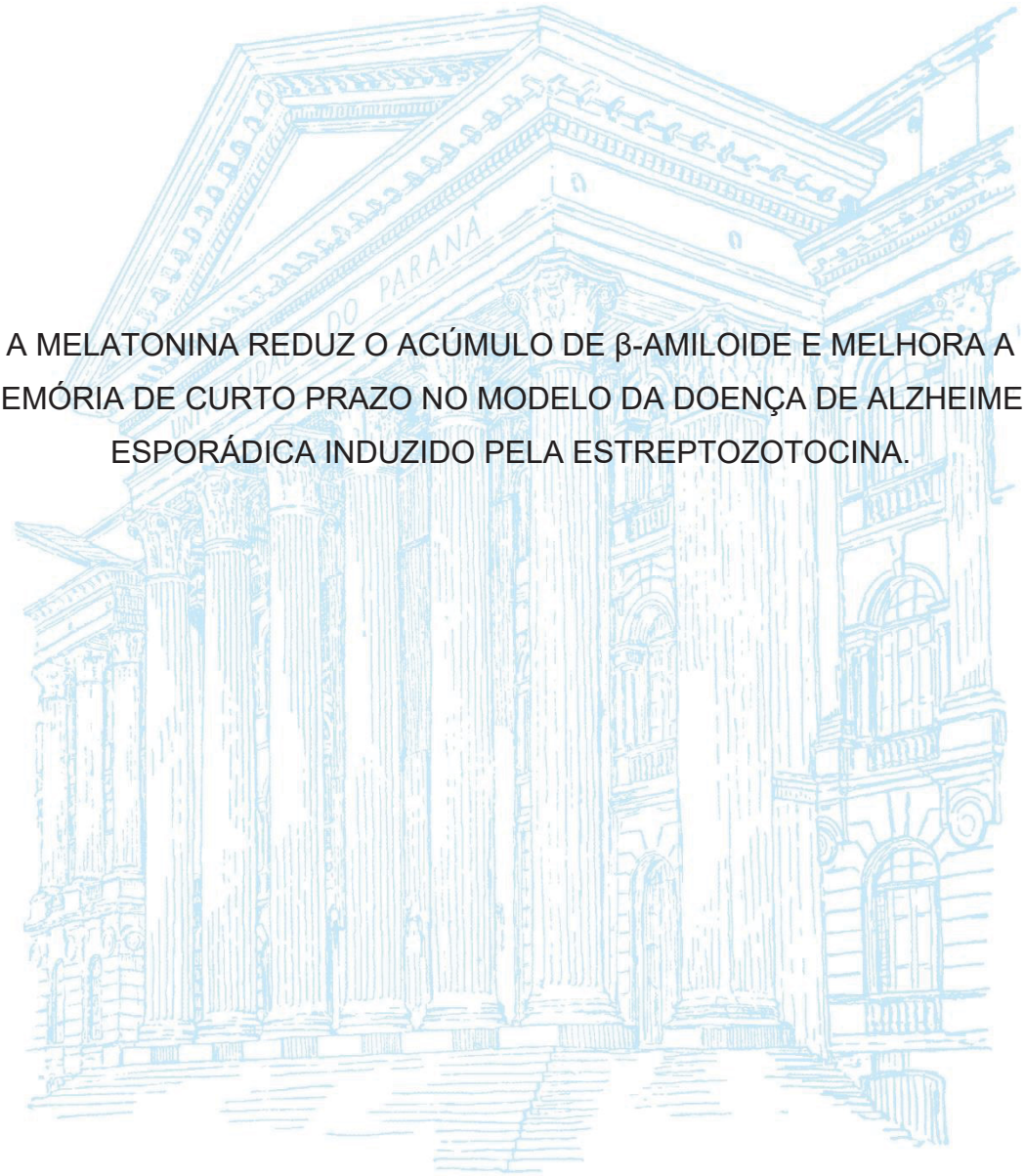
MARCOS KAOANN DE ANDRADE



A MELATONINA REDUZ O ACÚMULO DE β-AMILOIDE E MELHORA A MEMÓRIA DE CURTO PRAZO NO MODELO DA DOENÇA DE ALZHEIMER ESPORÁDICA INDUZIDO PELA ESTREPTOZOTOCINA.

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2022

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Dissertação apresentada como requisito parcial a obtenção do grau de Mestre em Farmacologia, no Curso de Pós-graduação em Farmacologia, Setor de Ciências Biológicas, Universidade federal do Paraná.

Orientadora: Profª. Drª. Maria A. B. F. Vital.

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ATA DE SESSÃO PÚBLICA DE DEFESA DE MESTRADO PARA A OBTENÇÃO DO GRAU DE MESTRE EM FARMACOLOGIA

No dia trinta de março de dois mil e vinte e dois às 14:00 horas, na sala Anfiteatro do Programa de Pós Graduação em Farmacologia., Anexo 1 Setor de Ciências Biológicas, foram instaladas as atividades pertinentes ao rito de defesa de dissertação do mestrando **MARCOS KAOANN DE ANDRADE**, intitulada: **A MELATONINA REDUZ O ACÚMULO DE -AMILOIDE E MELHORA A MEMÓRIA DE CURTO PRAZO NO MODELO DA DOENÇA DE ALZHEIMER ESPORÁDICA INDUZIDO PELA ESTREPTOZOTOCINA**, sob orientação da Profa. Dra. MARIA APARECIDA BARBATO FRAZÃO VITAL. A Banca Examinadora, designada pelo Colegiado do Programa de Pós-Graduação FARMACOLOGIA da Universidade Federal do Paraná, foi constituída pelos seguintes Membros: MARIA APARECIDA BARBATO FRAZÃO VITAL (UNIVERSIDADE FEDERAL DO PARANÁ), TAYSA BERVIAN BASSANI (MAASTRICHT UNIVERSITY), ALEXANDRA ACCO (UNIVERSIDADE FEDERAL DO PARANÁ). A presidência iniciou os ritos definidos pelo Colegiado do Programa e, após exarados os pareceres dos membros do comitê examinador e da respectiva contra argumentação, ocorreu a leitura do parecer final da banca examinadora, que decidiu pela APROVAÇÃO. Este resultado deverá ser homologado pelo Colegiado do programa, mediante o atendimento de todas as indicações e correções solicitadas pela banca dentro dos prazos regimentais definidos pelo programa. A outorga de título de mestre está condicionada ao atendimento de todos os requisitos e prazos determinados no regimento do Programa de Pós-Graduação. Nada mais havendo a tratar a presidência deu por encerrada a sessão, da qual eu, MARIA APARECIDA BARBATO FRAZÃO VITAL, lavrei a presente ata, que vai assinada por mim e pelos demais membros da Comissão Examinadora.

CURITIBA, 30 de Março de 2022.

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TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação FARMACOLOGIA da Universidade Federal do Paraná foram convocados para realizar a arguição da Dissertação de Mestrado de **MARCOS KAOANN DE ANDRADE** intituiada: **A MELATONINA REDUZ O ACÚMULO DE -AMILOIDE E MELHORA A MEMÓRIA DE CURTO PRAZO NO MODELO DA DOENÇA DE ALZHEIMER ESPORÁDICA INDUZIDO PELA ESTREPTOZOTOCINA**, sob orientação da Profa. Dra. MARIA APARECIDA BARBATO FRAZÃO VITAL, que após terem inquirido o aluno e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do titulo de mestre está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

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NOTA EXPLICATIVA

Esta dissertação é apresentada em formato alternativo – artigo para publicação – de acordo com as normas do Programa de Pós-Graduação em Farmacologia da Universidade Federal do Paraná.

RESUMO

A melatonina é um hormônio secretado principalmente pela glândula pineal, podendo estar associada a ritmos circadianos, envelhecimento e neuroproteção. Os níveis de melatonina no líquido cefalorraquidiano estão diminuídos em pacientes com doença de Alzheimer esporádica (sAD), sugerindo uma relação entre o sistema melatonérgico e sAD. Além disso, a melatonina pode reduzir a inflamação, o estresse oxidativo, a hiperfosforilação da proteína TAU e a formação de agregados de proteína β-amilóide (Aβ), bem como reduzir a resistência à insulina no cérebro. Portanto, o objetivo deste trabalho foi investigar o impacto do tratamento com 10 mg/kg de melatonina (i.p) no modelo animal de sAD induzida por infusão intracerebroventricular (ICV) de 3 mg/kg de estreptozotocina (STZ). A ICV-STZ causa alterações no cérebro de ratos semelhantes às encontradas em pacientes com sAD, como declínio progressivo da memória, formação de emaranhados neurofibrilares, placas senis, distúrbios no metabolismo da glicose, resistência à insulina e até mesmo astrogliose reativa caracterizada por upregulation dos níveis da proteína glial fibrilar ácida (GFAP). Os resultados mostram que ICV-STZ causou comprometimento da memória espacial de curto prazo em ratos após 30 dias de infusão de STZ sem comprometimento locomotor que foi avaliado no 27º dia pós-lesão. Além disso, demonstramos que o tratamento prolongado de 30 dias com melatonina pode melhorar o comprometimento cognitivo dos animais no teste do labirinto em Y, mas não no teste de localização de objetos. Finalmente, demonstramos através da quantificação de western blotting que os animais que receberam ICV-STZ apresentam altos níveis de Aβ e GFAP no hipocampo e que o tratamento com melatonina reduz significativamente os níveis de Aβ no hipocampo, mas não reduz os níveis de GFAP, concluindo que a melatonina pode ser útil para controlar a progressão da patologia amiloide na doença de Alzheimer.

Palavras-chave: Doença de Alzheimer; Melatonina; β-amiloide; GFAP; Labirinto em Y; Memoria de curto prazo.

ABSTRACT

Melatonin is a hormone secreted mainly by the pineal gland, it can be associated with circadian rhythms, aging and neuroprotection. Melatonin levels in cerebrospinal fluid are decreased in sporadic Alzheimer's disease (sAD) patients suggesting a relationship between the melatonergic system and sAD. In addition, melatonin may reduce inflammation, oxidative stress, TAU protein hyperphosphorylation, and the formation of β-amyloid (Aβ) protein aggregates, as well as reduce brain insulin resistance. Therefore, the objective of this work was to investigate the impact of treatment with 10 mg/kg of melatonin (i.p) in the animal model of *sAD* induced by intracerebroventricular (ICV) infusion of 3 mg/kg of streptozotocin (STZ). ICV-STZ causes changes in the brain of rats similar those found in patients with sAD, such as progressive memory decline, formation of neurofibrillary tangles, senile plaques, disturbances in glucose metabolism, insulin resistance and even reactive astrogliosis characterized by upregulation of glucose levels and glial fibrillary acidic protein (GFAP). The results show that ICV-STZ caused short-term spatial memory impairment in rats after 30 days of STZ infusion without locomotor impairment which was evaluated on the 27th post-injury day. Furthermore, we demonstrated that prolonged 30-day treatment with melatonin can improve the cognitive impairment of animals in the Y-maze test, but not in the object location test. Finally, we demonstrated through western blotting quantification that animals receiving ICV-STZ have high levels of Aβ and GFAP in the hippocampus and that treatment with melatonin significantly reduces Aβ levels in the hippocampus but does not reduce GFAP levels, concluding that melatonin may be useful to control the progression of amyloid pathology in the brain.

Keywords: Alzheimer's Disease; Melatonin; β-amyloid; GFAP; Y maze; Short-term memory.

LISTA DE ABREVIATURAS

Aβ – Beta amiloide

AICD – Dominio intracelular da APP

APOE – Apolipoproteina E

APP – Proteina precursora amiloide

APPs a – Fragmento de ectodomínio da APP gerado pela α-secretase

APPs b – Fragmento de ectodomínio da APP gerado pela β-secretase

ATP – Adenosina trifosfato

BACE 1 – Beta secretase

C83 – Fragmento C terminal α

C99 – Fragmento C terminal β

CFT-a – Fragmento C terminal α

CFT-b – Fragmento C terminal β

DM2 – Diabetes mellitus 2

GABA – Ácido gama-aminobutírico

GFAP – Proteina fibrilar glial ácida

ICV – Intracerebroventricular

IDE – Enzima degradadora de insulina

IL-1β – Interleucina-1β

IL-6 – Interleucina-6

IRS-1 – Substrato receptor de insulina-1

LTP – Potenciação de longo prazo

MT1 – Receptor de melatonina 1

MT2 – Receptor de melatonina 2

Nf-Kβ – Fator nuclear de kappa beta

NFTs – Emaranhado neurofibrilares

NO – Oxido nitrico

P3 – Peptideo P3

sAD – Doença de Alzheimer esporádica

STZ – Estreptozotocina

TNF-α – Fator de necrose tumoral-a

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1.INTRODUÇÃO

A doença de Alzheimer esporádica (sAD) é uma desordem neurodegenerativa, caracterizada pelo declínio progressivo das funções cognitivas, principalmente a memória. Devido ao acometimento múltiplas regiões do cérebro, podem ocorrer problemas na linguagem, distúrbios do humor, distúrbios comportamentais e do sono impactando amplamente a qualidade de vida do portador (NIEDOWICZ; NELSON; MURPHY, 2011; SANTIAGO; POTASHKIN, 2021).

A doença de Alzheimer é responsável por até 70% dos casos de demência, sendo considerada a principal causa de demência no mundo. Atualmente a demência acomete cerca de 50 milhões de pessoas em todo o mundo, e estimativas sugerem que esse número poderá triplicar até 2050 (MAYEUX; STERN, 2012; ZHANG *et al.*, 2021). O aumento da incidência da doença de Alzheimer, parece estar vinculada com o aumento da expectativa de vida, já que o envelhecimento e idade a partir dos 65 anos é considerado um dos principais fatores de risco para o desenvolvimento da doença de Alzheimer esporádica (sAD) que é a forma mais frequente da doença correspondendo mais de 95% dos casos (CHAKRABARTI *et al.*, 2015; ALZHEIMER'S ASSOCIATION, 2016).

Os fatores de risco são diversos, e podem ser separados em fatores não modificáveis e ambientais, em razão da patogenia multifatorial e complexa. Os fatores ambientais direcionam ao estilo de vida dado que, hábitos como tabagismo e consumo de álcool aumentam a deposição da β amiloide (Aβ) no cérebro e induzem a perda de neurônios colinérgicos (VETRENO; CREWS, 2018). A nicotina presente no tabaco aumenta significativamente tanto hiperfosforilação quanto a agregação da proteína TAU, e parece não reduzir os níveis de Aβ como estudos anteriores demonstram compondo mais um fator de risco (Abner, E. L et al.,2019; ODDO et al., 2005).

Dentre os fatores genéticos, o gene *APOE é importante no desenvolvimento do Alzheimer esporádico, esse gene é* responsável pela formação de três isoformas da Apolipoproteína E (ApoE ε2, ε3 e ε4), responsáveis por conduzir o colesterol para os neurônios. Contudo, a isoforma ε4 é considerada fator de risco para a doença de Alzheimer, visto que, o alelo ε4 é capaz de aumentar até quatro vezes o risco do desenvolvimento da doença e aproximadamente 40% dos portadores do Alzheimer a ApoE ε4 é detectada (SPINNEY, 2014, HICKMAN; A FAUSTIN; WISNIEWSKI, 2017). A ApoE ε4 interfere no processamento da proteína precursora amiloide (APP),

elevando a produção, deposição e prejudicando a depuração de peptídeos Aβ no cérebro, além disso, a ApoE ε4 parece reduzir a plasticidade sináptica. Esses eventos desempenham papeis centrais na patogênese da doença de Alzheimer (ZHAO et al., 2018, BU, 2009).

O tratamento da doença de Alzheimer esporádica é sintomático e consiste na utilização dos inibidores da acetilcolinesterase como donepezila, rivastigmina e galantamina, os quais podem ser utilizados em qualquer estágio. A memantina um antagonista do receptor de N-metil-D-aspartato é utilizada por pacientes em estágio moderado a grave concomitantemente aos inibidores da acetilcolinesterase (WELLER; BUDSON, 2018, LOCKHART; ORME; MITCHELL, 2011). Atualmente pesquisas estão direcionadas a busca de drogas que alterem o curso da doença, dentre elas o anticorpo monoclonal (Aducanumab) contra Aβ que reduz de forma significativa e dose dependente os agregados insolúveis de Aβ no cérebro, porém a desaceleração do declínio cognitivo não foi tão evidente (SELKOE, 2019; SEVIGNY *et al.*, 2016).

A patogenia da doença de Alzheimer é multifatorial e pode ser descrita principalmente pela hipótese da hiperfosforilação da TAU e pela hipótese da cascata amiloide. Além disso, fenômenos como neuroinflamação, estresse oxidativo e resistência à insulina, desempenham papeis cruciais na patogênese da doença de Alzheimer (KELLAR; CRAFT, 2020). A hipótese da resistência insulínica cerebral tem sido sugerida por contribuir na patogênese da doença de Alzheimer (BOMFIM et al., 2012). A resistência à insulina no cérebro pode ser vista por alterações nas vias de sinalização da insulina, como a fosforilação aberrante do substrato receptor de insulina 1 (IRS-1) em resíduos serina e treonina resultando na sensibilidade reduzida à insulina (MULLINS et al., 2017). Além do papel clássico de regulação do metabolismo da glicose, a insulina pode modular a plasticidade sináptica, sinaptogênese e neurogênese no hipocampo, esses fenômenos estão intimamente vinculados com processos cognitivos de memória, portanto relacionados com a doença de Alzheimer, já que déficits de memória é um dos principais sintomas da doença (SPINELLI; FUSCO; GRASSI, 2019).

A hipótese da hiperfosforilação da proteina TAU postula que essa proteína é hiperfosforilada em condições patológicas e se agregada em filamentos formando os emaranhados neurofibrilares intraneuronais (NFTs) favorecendo a neuroinflamação, estresse oxidativo, resistência à insulina cerebral que resulta em disfunção neuronal

(GONÇALVES et al., 2019; LIU et al., 2015; LAURENT; BUÉE; BLUM, 2018; FAN et al., 2020).

Outra hipótese que complementa a explicação da patogênese da doença de alzheimer é a hipótese da cascata amiloide que descreve o processamento anormal da proteína precursora amiloide (APP) e resulta na elevação dos níveis da Aβ em várias regiões do cérebro. A APP é uma proteína transmembranar que desempenha inúmeros papeis no sistema nervoso central, incluindo desenvolvimento/sobrevivência neuronal, neurogênese, estrutura e funções sinápticas no cérebro (MÜLLER; DELLER; KORTE, 2017).

A APP pode ser clivada por várias vias proteolíticas distintas (GARCÍA-GONZÁLEZ et al., 2019), dentre elas, destacam-se a via não amiloidogênica e a amiloidogênica que está intimamente relacionada com a fisiopatologia da doença de Alzheimer. A chamada via não amiloidogênica, forma inicialmente os fragmentos APPsα (fragmento de ectodomínio gerado pela α-secretase) e C83/CFT-α (fragmento C terminal α) pela clivagem da α-secretase, sequencialmente a protease γ-secretase cliva o fragmento C83 formando os peptídeos P3 e AICD (domínio intracelular da APP) (Cho et al. 2022). No entanto, quando a APP é clivada de forma sequencial pela β- e γ-secretase respectivamente, ocorre formação do peptídeo neurotóxico Aβ caracterizando a via amiloidogênica. A clivagem inicial da APP com a protease β-secretase (também chamada de BACE – β secretase) gera os subprodutos APPsβ e C99/CFT-β. Posteriormente o fragmento C99 ainda ligado a membrana, é clivado, e ocorre a formação e liberação para o meio extracelular de monômeros de Aβ contendo de 37 a 49 aminoácidos (HAMPEL et al., 2021). Os monômeros Aβ produzidos mais abundantemente contém 40 aminoácidos ($A\beta_{1-40}$) e são menos suscetíveis a formar agregados quando comparados com os monômeros de 42 aminoácidos ($A\beta_{1-42}$) que são menos solúveis e mais propensos a formar oligômeros e apresentam maior neurotoxicidade (FINDER et al., 2010; HUBIN et al., 2014).

A dinâmica de formação das placas senis (placas amiloides) ocorre pelo processo denominado de nucleação (XUE, 2015). A nucleação consiste na transição de fase bidirecional das formas monoméricas de Aβ, para oligômeros, protofibrilas e por fim, para a forma fibrilar que agrega formando as placas senis insolúveis, considerada marca neuropatológica da doença de Alzheimer (HAMPEL et al., 2021).

As diferentes espécies de Aβ produzidas pela nucleação podem diferir na toxicidade, onde as formas oligoméricas parecem ser mais neurotóxicas e causam

maior comprometimento cognitivo quando infundidas no hipocampo de ratos. Além disso, os oligômeros podem elevar mais significativamente os níveis de marcadores inflamatórios como o TNF- α e NF-kB sugerindo diferença na resposta inflamatória produzidas pelos oligômeros ou fibrilas Aβ (HE et al., 2012). Mais ainda, as distintas formas de Aβ como oligômeros, protofibrilas e fibrilas são capazes de reduzir a potenciação de longo prazo (LTP) no hipocampo, induzir a neuroinflamação, resistência insulínica central e deterioração sináptica em neurônios hipocampais (ONUALLAIN et al., 2010; LI et al., 2011; PUZZO et al., 2017). Por fim, o Aβ oligomérico pode ser fagocitado por astrócitos ativados (MORIZAWA et al., 2017) e induzir alterações críticas na sobrevivência neuronal resultando em morte neuronal e astrocítica (LASAGNA-REEVES; KAYED, 2011; BATARSEH et al., 2016).

Os astrócitos são células gliais de suporte que diferem em funções e morfologicamente de outras células do sistema nervoso central (ROSSI, 2015). Em condições fisiológicas, por sua morfologia altamente ramificada, os astrócitos fazem contato físico com vasos sanguíneos por meio das suas projeções, por isso, são capazes de captar nutrientes como glicose dos vasos sanguíneos e fornecer aos neurônios conectados (SOFRONIEW; VINTERS, 2009; SIRACUSA; FUSCO; CUZZOCREA, 2019). Além disso, os astrócitos são capazes de regular o fluxo sanguíneo local, controlar os níveis de íons extracelulares, pH e participam ativamente na liberação de neurotransmissores como glutamato, GABA, ATP e do aminoácido D-serina em um processo modulado pelo Ca^{+2} e denominado de gliotransmissão, tudo isso pode contribuir para plasticidade sináptica em diferentes estruturas como córtex e hipocampo (GONZÁLEZ-REYES et al., 2017; KIM; PARK; CHOI, 2019).

Alterações nos padrões de expressão de proteínas e mudanças morfológicas dos astrócitos também são observadas no cérebro de pacientes com doença de alzheimer, essa mudança dos astrócitos é denominada astrogliose reativa (COLANGELO; ALBERGHINA; PAPA, 2014; FAKHOURY, 2018). Na doença de Alzheimer a astrogliose reativa é observada ao redor das placas senis e é evidenciada pela elevação na expressão da proteína glial fibrilar acida (GFAP) em resposta aos oligômeros e fibrilas Aβ (OSBORN et al., 2016). O acúmulo de espécies de Aβ estimula os astrócitos a produzir citocinas pró-inflamatórias como IL-1β, IL-6, NO e TNFα que caracteriza a astrogliose como um estado inflamatório dos astrócitos, além disso, as citocinas pró-inflamatórias também parecem regular a produção de espécies

de Aβ que contribui para fisiopatologia da doença de Alzheimer (GONZÁLEZ-REYES et al., 2017; GUTTENPLAN et al., 2021).

Modelos animais são frequentemente utilizados para mimetizar alterações fisiopatológicas de diversas doenças (ERICSSON; CRIM; FRANKLIN, 2013). Com isso, através de uma injeção intracerebroventricular (ICV) de estreptozotocina (STZ) em doses subdiabetogênicas em ratos é possível observar alterações neuroquímicas, comportamentais e histológicas similares à doença de Alzheimer (GRIEB, 2015). A injeção ICV-STZ ocasiona distúrbios relacionados ao metabolismo da glicose, como a resistência à insulina e baixo consumo de glicose. É observado também deficiência insulínica no cérebro que parece estar vinculada com a elevação da expressão da IDE (enzima degradadora de insulina) em certas regiões do cérebro (DELIKKAYA et al., 2019). A injeção ICV-STZ provoca neuroinflamação intensa que se cronifica e ocorre a elevação da produção de citocinas pró-inflamatórias e neurotóxicas como o TNF-α, juntamente com alterações na atividade microglial de captar e depurar os oligômeros da β-amiloide de maneira eficiente caracterizando o modelo de STZ (MAWUENYEGA et al., 2010).

A injeção ICV-STZ eleva a expressão de proteínas vinculadas à via amiloidogênica como a proteína precursora amiloide (APP) e a enzima de clivagem 1 (BACE 1) tanto no hipocampo como no córtex cerebral. Em ambas as estruturas anatômicas a fosforilação da TAU está elevada. Tais elevações, estão relacionadas com o aumento da formação de placas amiloides e emaranhados neurofibrilares que caracterizam a neuropatologia da doença de Alzheimer esporádica (MISHRA et al., 2018). No aspecto comportamental a STZ causou significativos déficits de memória espacial e reconhecimento de curto prazo. Bem como, neuroinflamação intensa e diminuição da neurogênese (RAVELLI et al., 2016; BASSANI et al., 2017).

Sabe-se que várias regiões e sistemas podem estar alterados na doença de Alzheimer esporádica como os sistemas serotonérgico, glutamatérgico e colinérgico. Diversos estudos mostram perturbações no sistema melatoninérgico (SANGUBOTLA; KIM, 2018; REDDY, 2017; NOUS; ENGELBORGHS; SMOLDERS, 2021). Tais alterações consistem na diminuição nos níveis de melatonina (N-aceil-5-metoxitriptamina) um hormônio sintetizado principalmente pela glândula pineal, sendo responsável pela modulação de ritmos circadianos, diminuição de radicais livres, melhora na imunidade e inflamação (SONG, 2019). Além da diminuição nos níveis da melatonina, também é observada redução da expressão da receptores MT2 no

hipocampo de idosos com Alzheimer (SAVASKAN et al., 2005). Em adição, os receptores MT1 e MT2 também estão diminuídos na glândula pineal e córtex occipital de pacientes com a doença de Alzheimer (Brunner et al. 2006).

Os níveis normais de melatonina estão vinculados com o a regulação no metabolismo da proteína percursora amiloide e diminuição da geração e deposição da Aβ (MATSUBARA et al., 2003). A melatonina é capaz de atenuar a hiperfosforilação da TAU, além disso, é responsável por modular e proteger o sistema colinérgico (Feng et al., 2004, Wang JZ et al 2006; VINCENT, 2018).

A melatonina participa ainda na regulação do sistema imunológico e em várias fases da inflamação, como na redução da expressão de moléculas de adesão limitando a migração celular para o local inflamado. Além disso, a melatonina induziu a polarização microglial para um fenótipo anti-inflamatório (fenótipo micróglial M2), sendo que esse fenótipo induz a resolução da neuroinflamação. Em adição, a melatonina reduziu a disfunção cognitiva e reduziu depósitos de Aβ e emaranhados neurofibrilares (CHITRE; GHUMATKAR; SATHAYE, 2016; PIRES-LAPA et al., 2013; ZHU et al., 2016; YAO; ZU, 2019). Estudos sugerem que a melatonina é capaz de atenuar a redução da neurogênese e sinaptogênese no hipocampo em um modelo de animal de DM2 induzido por STZ (WONGCHITRAT et al., 2016). O mesmo grupo de pesquisadores, demonstrou que a melatonina foi capaz de reduzir o comprometimento da memória, a hiperfosforilação da TAU e reduzir o acúmulo da deposição da β-amiloide em ratos (KAMSRIJAI et al., 2020).

Diante do exposto, o objetivo do trabalho foi verificar o efeito do tratamento com melatonina no modelo animal de STZ. E se este neuro-hormônio poderia reduzir a formação de agregados de peptídeos β-amiloides e prevenir o declínio cognitivo.

2.OBJETIVOS

2.1OBJETIVO GERAL

Avaliar os efeitos do tratamento prolongado com a melatonina do modelo da doença de Alzheimer esporádica induzida por injeção ICV de STZ em ratos wistar.

2.2 OBJETIVOS ESPECÍFICOS

- ✓ Verificar os efeitos do tratamento com a melatonina no declínio cognitivo induzido pela injeção ICV de STZ por meio dos testes de localização de objetos e labirinto em Y.
- ✓ Avaliar o impacto do tratamento com a melatonina na locomoção dos animais.
- ✓ Verificar os efeitos do tratamento com a melatonina nos níveis de β-amiloide no hipocampo dos animais.
- ✓ Verificar os efeitos do tratamento com a melatonina no marcador de astrogliose (GFAP) no hipocampo.

MELATONIN REDUCES β-AMYLOID ACCUMULATION AND IMPROVES SHORT-TERM MEMORY IN STREPTOZOTOCIN-INDUCED SPORADIC ALZHEIMER'S DISEASE MODEL

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ABSTRACT

Melatonin is a hormone secreted mainly by the pineal gland, it can be associated with circadian rhythms, aging and neuroprotection. Melatonin levels in cerebrospinal fluid are decreased in sporadic Alzheimer's disease (sAD) patients suggesting a relationship between the melatonergic system and sAD. In addition, melatonin may reduce inflammation, oxidative stress, TAU protein hyperphosphorylation, and the formation of β-amyloid (Aβ) protein aggregates, as well as reduce brain insulin resistance. Therefore, the objective of this work was to investigate the impact of treatment with 10 mg/kg of melatonin (i.p) in the animal model of *sAD* induced by intracerebroventricular (ICV) infusion of 3 mg/kg of streptozotocin (STZ). ICV-STZ causes changes in the brain of rats similar those found in patients with sAD, such as progressive memory decline, formation of neurofibrillary tangles, senile plaques, disturbances in glucose metabolism, insulin resistance and even reactive astrogliosis characterized by upregulation of glucose levels and glial fibrillary acidic protein (GFAP). The results show that ICV-STZ caused short-term spatial memory impairment in rats after 30 days of STZ infusion without locomotor impairment which was evaluated on the 27th post-injury day. Furthermore, we demonstrated that prolonged 30-day treatment with melatonin can improve the cognitive impairment of animals in the Y-maze test, but not in the object location test. Finally, we demonstrated through western blotting quantification that animals receiving ICV-STZ have high levels of Aβ and GFAP in the hippocampus and that treatment with melatonin significantly reduces Aβ levels in the hippocampus but does not reduce GFAP levels, concluding that melatonin may be useful to control the progression of amyloid pathology in the brain.

Keywords: Alzheimer's Disease; Melatonin; β-amyloid; GFAP; Y maze; Short-term memory.

1. INTRODUCTION

Sporadic Alzheimer's disease (sAD) is the most common form of dementia in the world, data from 2015 indicate that about 40 million people have some form of dementia and approximately 70% are attributed to sAD (REITZ; BRAYNE; MAYEUX, 2011). Recent estimates predict that dementia could affect 131 million people in the year 2050. Such an increase in the number of cases seems to be linked to the increase in life expectancy, as aging and age over 65 is considered one of the main risk factors for the development of Alzheimer's (MAYEUX; STERN, 2012; ZHANG et al., 2021).

sAD is a neurodegenerative disorder characterized by the progressive decline of cognitive functions, especially short-term memory. Pathological changes in sAD include the formation of extracellular senile plaques, intracellular neurofibrillary tangles, inflammation, reduced glucose metabolism, cerebral insulin resistance, neuronal and synaptic loss in different regions of the brain (NIEDOWICZ; NELSON; MURPHY, 2011). The formation of senile plaques occurs by shifting the metabolism of the amyloid precursor protein (APP), where enzymes such as β- and γ-secretases increase the formation of amyloid peptides more likely to form insoluble aggregates and accumulate in the brain with sAD (MARR; HAFEZ, 2014). In addition, the accumulation of Aβ is capable of triggering activation of astrocytes located around the senile plaques and giving rise to the process called reactive astrogliosis characterized by the upregulation of glial fibrillary acidic protein (GFAP), which may present in prodromal stages of Alzheimer's disease (OSBORN et al., 2016; CHATTERJEE et al., 2021). Thus, reactive astrocytes in Alzheimer's disease play active roles in the inflammatory process as they can secrete pro-inflammatory cytokines such as TNF-α and interleukin-1β, generate excitotoxicity and lead to neuronal death (HENEKA et al., 2015).

It’s known that some systems are altered in people with sAD, among them the melatonin system seems to have a strong relation with the disease, since reduced levels of melatonin in fluids and reduced expression of MT1 and MT2 receptors are observed in brains with Alzheimer's disease (WU et al., 2007, SAVASKAN et al., 2005). Melatonin (n-acetyl-5-methoxytryptamine) is a hormone mainly synthesized by the pineal gland, being responsible for

modulating circadian rhythms, oxidative stress reduction, improving immunity and inflammation (SONG, 2019).

Normal melatonin levels are linked with important features of the disease, as well as regulation of amyloid precursor protein metabolism and decreased β-amyloid generation and deposition (MATSUBARA et al., 2003). Melatonin is also capable of attenuating tau hyperphosphorylation, in addition, it is responsible for modulating and protecting the cholinergic system (CHENG et al., 2006; WANG et al., 2005).

Melatonin exhibits a regulatory property of the immune system and involves several stages of inflammation, such as the reduction in the expression of adhesion molecules, limiting cell migration to the inflamed site. Furthermore, melatonin can increase Aβ clearance in rats improving cognitive dysfunction (ZHU et al., 2016; YAO; ZU, 2019). Several studies suggest that melatonin is able to increase neurogenesis and synaptogenesis in the hippocampus in animal models (WONGCHITRAT et al., 2016). Moreover, melatonin can reduce the impairment of memory and Tau hyperphosphorylation (KAMSRIJAI et al., 2020).

It's difficult to establish an experimental animal model that would faithfully mimic the developmental pathology of the prevailing sporadic form of Alzheimer disease in humans (SALKOVIC-PETRISIC et al., 2013). ICV-STZ animal model has been considered as an appropriate model of sporadic dementia of alzheimer’s disease (sAD). Thus, through an intracerebroventricular (ICV) injection of streptozotocin (STZ) in subdiabetogenic doses in rats, it is possible to observe neurochemical, behavioral and histological alterations like Alzheimer's disease (GRIEB, 2015). ICV-STZ injection causes disorders related to glucose metabolism, such as insulin resistance and glucose uptake by neurons (DELIKKAYA et al., 2019). ICV-STZ injection causes intense neuroinflammation that becomes chronic, increasing the production of pro-inflammatory and neurotoxic cytokines leading to neuronal death. Furthermore, STZ increase microglial activity of clearance β-amyloid oligomers in an efficient manner characterizing the STZ model (MAWUENYEGA et al., 2010).

ICV-STZ injection elevates the expression of proteins linked to the amyloidogenic pathway, such as β- and γ-secretases enzymes in both the hippocampus and the cerebral cortex besides tau hyperphosphorylation**.** Such elevations are related to the increased formation of amyloid plaques and

neurofibrillary tangles that characterize the neuropathology of sAD (MISHRA et al., 2018). In the behavioral aspect, STZ causes significant deficits in spatial memory and short-term recognition. As well as intense neuroinflammation and decreased neurogenesis (RAVELLI et al., 2016; BASSANI et al., 2017). Thus, the objective of this work was to investigate the effect of treatment with melatonin in the animal model of STZ, the formation of β-amyloid peptides aggregates and upregulation of GFAP, verifying whether this hormone can reverse or prevent the cognitive decline.

2 MATERIAL AND METHODS

2.1 ANIMALS

For this study, male *Rattus norvegicus* Wistar rats, 3 to 4 months old, with a mean weight ±350g at the beginning of the experiments, were used. All rats used in the experiments came from the central animal house of the Federal University of Paraná (UFPR). The animals were housed in a room with controlled temperature (22 ± 2ºC) and humidity, with free access to food and water and respecting the light-dark phase, where the light phase started at 7 am and the dark phase at 7 pm. All protocols used for this study were approved by the Ethics Committee for Animal Use of the Biological Sciences Sector of the Federal University of Paraná (CEUA/BIO - UFPR, authorization number 1367).

2.2 DRUGS

- Streptozotocin (STZ; Santa Cruz Biotechnology Inc,. Califórnia, EUA).
- Melatonin (MEL; Sigma-Aldrich, St. Louis, MO, USA).

The streptozotocin was infused in lateral ventricles of the animals at a dose of 3 mg/kg whose vehicle used was sterile saline. The dose of melatonin used was 10 mg/kg (same dose used by other authors Omeiza et al., 2021; LUENGO et al., 2019) in which it was dissolved in propylene glycol and diluted in saline, resulting in a final ratio of 5:95 propylene glycol/saline, the final concentration of suspended melatonin was 10 mg/ml, which was infused intraperitoneally (i.p). The control groups received only 1 ml/kg of propylene glycol/5% saline solution.

2.3 DESIGN EXPERIMENTAL

Thirty-two Wistar rats underwent stereotaxic surgery to bilaterally infuse vehicle or streptozotocin into the animals lateral ventricles, such animals are called SHAM (n=16) and STZ injured (n=16) respectively. These two large groups were randomized to intervention treatment with melatonin or vehicle (VEH) which is injected daily at the end of the light phase between 5:30 and 6:30 pm for 30 days, these groups were called SHAM+VEH, SHAM+MEL, STZ+VEH and STZ+MEL. After 27 days of surgery, the animals were submitted to an open field test to assess locomotor activity and make the first habituation on the apparatus at the same time. On the 28th day after surgery, the animals were again submitted to a second habituation to the apparatus, followed by the object location test to assess short-term spatial memory. And on the 30th day, the animals were evaluated in the Y-maze for short-term spatial memory. After the last cognitive test, the animals were deeply anesthetized with chloral hydrate and dissected to collect the prefrontal cortex and hippocampus for future analysis. (Fig. 1).

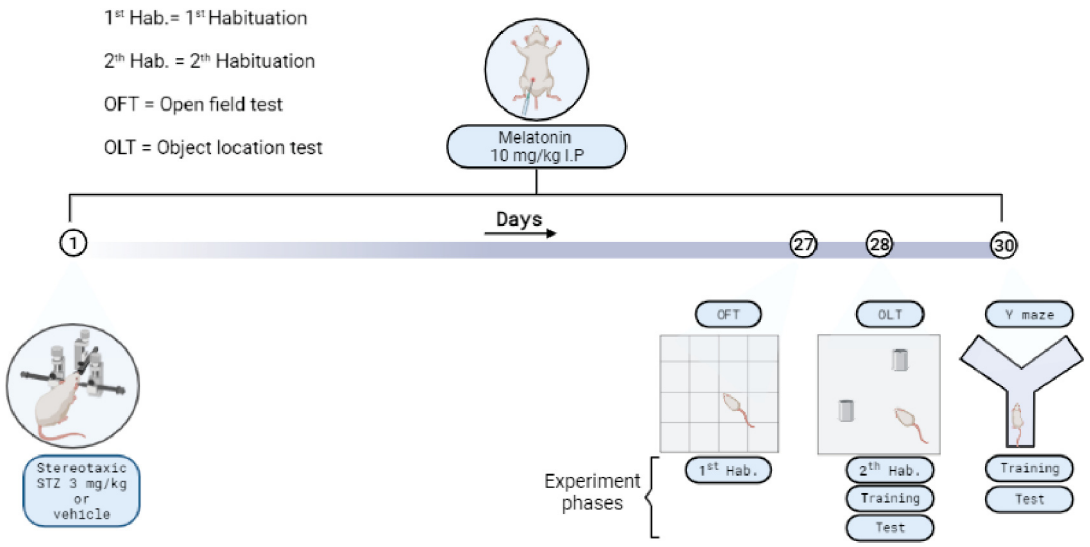

Fig.1 – Experimental design. Diagram showing each procedure performed with its respective days

2.4 STEREOTAXIC SURGERY AND STREPTOZOTOCIN INFUSION

Initially, by intraperitoneal (ip) injection, rats received a dose of the anesthetic Equitesin 3 ml/kg (1% sodium thiopental, 4.25% chloral hydrate,

2.13% magnesium sulfate, 42.8% propylene glycol, and 3.7% ethanol in water) and a dose of atropine sulfate (0.4 mg/kg) to reduce the production of secretions. After anesthetized, the rats were submitted to trichotomy of the head region, and then fixed in the stereotaxic apparatus (David Kopf, model 957L). With a scalpel the animal's skull was exposed and the stereotaxic coordinates were adjusted for infusion of the drug or vehicle into the lateral ventricles: anteroposterior -0.8 mm, lateral 1.5 mm and dorsoventral 4.0 mm relative to the bregma and ventral from the dura with the bar set to 0 mm. Trepanation was performed with a dental surgical drill at points relative to the ventricles, then a 30-gauge needle was inserted, connected to a polyethylene cannula attached to a 10 µL microsyringe (Hamilton, USA) that was attached to a pump of infusion (Harvard Apparatus, USA).

The dose of STZ used was 3 mg/kg which was dissolved in 0.9% sterile saline (vehicle) which has been prepared before infusion to avoid degradation by light and temperature. Rats received vehicle (SHAM group) or STZ (injured group) according to the belonging group, the volume infused into each ventricle is 4.5 µL at an infusion rate of 1 µL/min. After infusion, the needle remains in place for another 2 minutes to prevent reflux of the infused solution. After surgery, all the rats received Pentabiotic (0.1 ml, intramuscular) to prevent infection and were allowed to recover from anesthesia for 2–4 h in a heated and well-ventilated room. Food and water were placed inside the cage for 10–15 days so that the animals could easily access it without physical trauma caused by head surgery.

2.5 BEHAVIORAL TESTS

The tests were conducted in a square box with dimensions of 100-cm length, 100-cm width and 40-height (for OFT and OLT tests), or in a Y-shaped apparatus with 50-cm length, 27-cm height, 12-cm width (for Y-Maze) Both devices were composed black painted wood. The tests were always carried out in the same room, with light (20 lx) and controlled temperature (22 ±2ºC). A camera was positioned above the apparatus to record the tests and between each animal the box was cleaned with 20% alcohol to eliminate olfactory bias.

2.5.1 Open field test (OFT)

OFT was used to analyze the spontaneous locomotor behavior of rats in the 27th after stereotaxic surgery. Each rat was placed in the center of a black box, square (dimensions 100x100x40cm) and made of wood. A camera was positioned above the box to record the animal's behavior for 5 minutes. The total distance covered (m) was analyzed using the Anymaze 4.98 path-tracking software (Stoelting, Wood Dale, IL, USA).

2.5.2 Object location test (OLT).

OLT was used to assess the spatial short-term memory of rats. The OLT was performed in 4 sessions, 1st habituation, 2nd habituation, training and testing, which starts on the 27th day and ends on the 28th day after stereotaxic surgery. The 1st habituation lasts 5 minutes, was carried out on the 27th in order to familiarize the animal with the environment. The recording of the 1st habituation was used to perform OFT as suggested by *Lueptow et al. (2017)* since the 1st habituation consists of the free exploration of the arena without the presence of objects. After 24 hours of the 1st habituation, on the 28th day the 2nd habituation were performed, lasting 5 minutes. After 1 hour of the 2nd habituation, the OLT training session begins, where the animal can freely explore two identical objects (cylindrical and made of acrylic) for 5 minutes. In OLT, objects were positioned parallel to each other, and are at a distance of 10 cm from the walls of the box. After an interval of one hour the rats were submitted to the test session. In the OLT test session, one of the objects was moved from its original position (novel location), and the time that the animal explores the object with familiar or new location was measured and analyzed by the method described by Bassani et.al (2017) (BASSANI et al., 2017). The time of test session was 3 minutes and was recorded for further analysis by a trained observer (**Fig.2**).

2.5.3 Y Maze (spatial version)

The spatial version Y-maze test was used to assess the animals' spatial short-term memory. This version of the Y-maze is composed of two sessions, one training session and one testing session, as described by Bassani et al. (2017) (BASSANI et al., 2017). In the training session one of the 3 arms

(randomly chosen) of the apparatus was inaccessible (blocked by a wooden door), so the animals were placed in the apparatus to freely explore the rest of the apparatus for 5 minutes. After an interval of one hour, the animals were submitted to a test session, where the wooden door was removed, and the animal can freely explore all the arms of the apparatus for 3 minutes. For further analysis, the apparatus was divided into a central region that interconnects the 3 arms, the previously blocked arm and the animal's released arm, which is where the animal was placed at the beginning of the test (**Fig.2 B**) (BASSANI et al., 2017; KRAEUTER et al., 2018). The assessment of short-term memory was calculated by the time spent by the animals in the novel arm, which must be greater than 33% of the total time, being corrected by the exit latency of the starting arm and time spent in the central region.

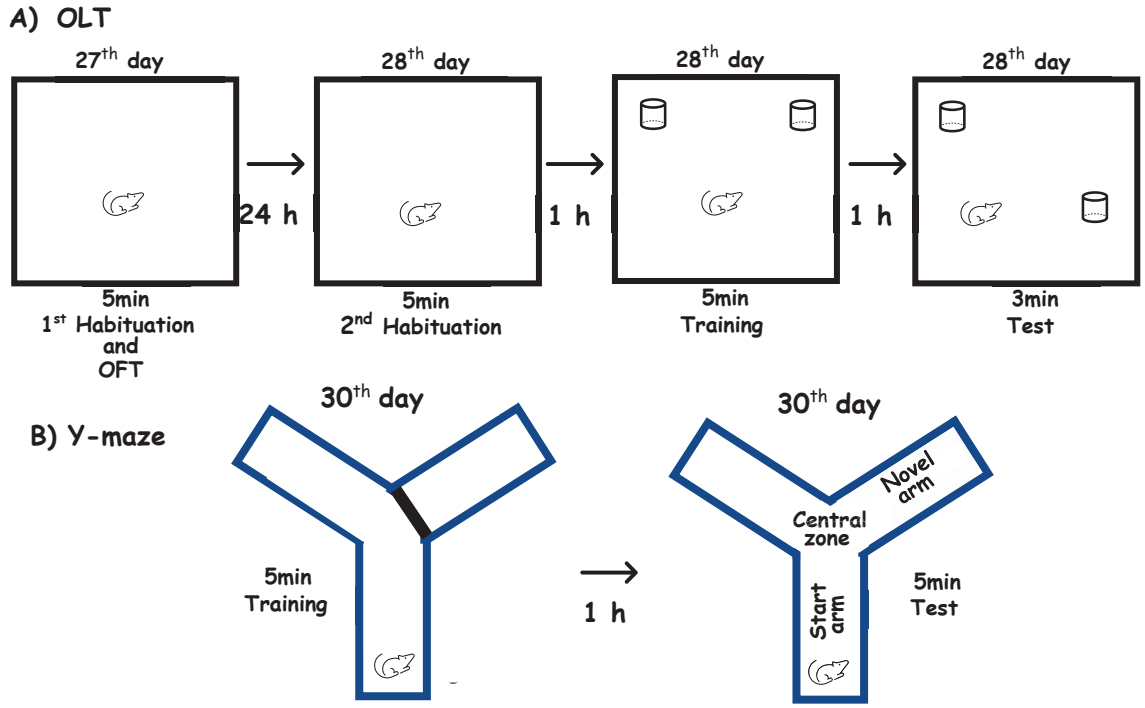

Fig. 2 - Schematic of the experimental configuration of the Object location test (**A**) and Y maze (spatial version) (**B**). OLT:Object location test; OFT:Open field test

2.6 Western blot

On the 30th day after the last behavioral test, the rats were anesthetized with chloral hydrate and decapitated for immediate dissection of the

hippocampus, then the tissues were frozen with liquid nitrogen and stored at -80°C until the day of processing (BAKHACH et al., 2009). The structures were then homogenized (with an ultrasonic processor) with lysis buffer (150 mM NaCl, 1.0% NP-40, 50 mM Tris-HCl, pH 8.0 and protease inhibitors), then the samples were centrifuged at 4ºC at 20000 rpm for 20 min. Protein quantification was performed by the Bradford method with the sample supernatant. The supernatants are then boiled with Laemmli's buffer (4% SDS, 10% 2-mercaptoethanol, 20% glycerol, 0.004% bromophenol blue, 0.125 M Tris-HC with final pH adjustment of buffer to 6.3) for 10 min at 95°C. After reduction, the samples were subjected to electrophoresis and later transferred to a nitrocellulose membrane (Bio-rad, 0.45 µm). The membranes are blocked with powdered milk (5% non-fat Molico®) diluted in a TBS-Tween 20 solution. The membranes were incubated overnight at 4ºC with the primary Anti-β-amyloid antibodies (sc-28365) at a 1:750 dilution, anti-β-actin (sc-130657) at 1:500 dilution, anti-GFAP (sc-51908) at a 1:1000 dilution, anti-GAPDH (SC-32233) at a 1:1000 dilution and the secondary antibody was Goat anti-rabbit (SC-2004) at 1:5000 dilution for 1 hour. The chemiluminescence reaction was produced by the Pierce ECL kit (Thermo scientific) and exposed to the Amersham ™ Imager 600 (GE Healthcare) device for image capture and storage. Subsequently, the images were analyzed by densitometry using the image J software (USA).

4.7 Statistical analysis

All data were analyzed with Graph Pad Prism Software 8.0. Data were expressed as mean ± standard error of the mean (SEM). The normality of the data, the Kolmogorov-Smirnov and D'Agostino-Pearson tests were used. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used for multiple comparisons. Values of $p < 0.05$ were considered statistically significant.

3 RESULTS

3.1 Effects of melatonin in open field test

The spontaneous locomotor activity of the animals was evaluated through the number of crossings in the open field test (**Fig.3**). The post hoc test showed

an increase in locomotor activity only in the SHAM+MEL group when compared to the STZ+MEL group (p= 0.0268). Furthermore, the ICV-STZ groups did not show significant differences when compared to the SHAM+VEH group.

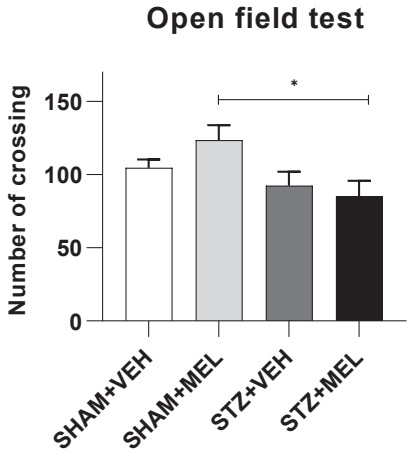

Fig. 3 - Effect of 30-days treatment with 10 mg/kg melatonin (i.p) or vehicle on spontaneous locomotor activity in ICV-STZ or SHAM animals in open field test. The data are expressed as mean ± SEM and were analyzed by one-way (ANOVA) with Tukey's multiple comparison test (*p<0.05). SHAM+VEH (n=8), SHAM+MEL (n=8), STZ+VEH (n=6), STZ+MEL (n=9).

3.2 Melatonin reverses cognitive impairment in the Y-maze (spatial version) but not in the OLT.

Short-term spatial memory was assessed by the OLT and Y-maze tests (spatial version). As shown in **Fig. 4 A** short-term spatial memory was impaired in OLT and treatment with melatonin was not able to reverse the changes caused by the toxin, as there was a significant reduction in the discrimination index of the STZ+VEH groups (p = 0,0021) and STZ+MEL (p=0.0181) when compared to the SHAM+VEH groups, while the SHAM+MEL group differed only from the STZ+VEH group (p=0.0152).

In the Y-maze (spatial version) it is possible to observe a significant reduction in the percentage of time exploring the novel arm only in the STZ+VEH group when compared to the SHAM+VEH (p=0.0093) and SHAM+MEL (p=0.0282) suggesting memory impairment (**Fig.4 B**). In contrast, treatment with melatonin reversed the memory impairment induced by ICV-STZ, observed by the significant increase in time spent in the new arm of the STZ+MEL group compared to STZ+VEH (p=0.0065).

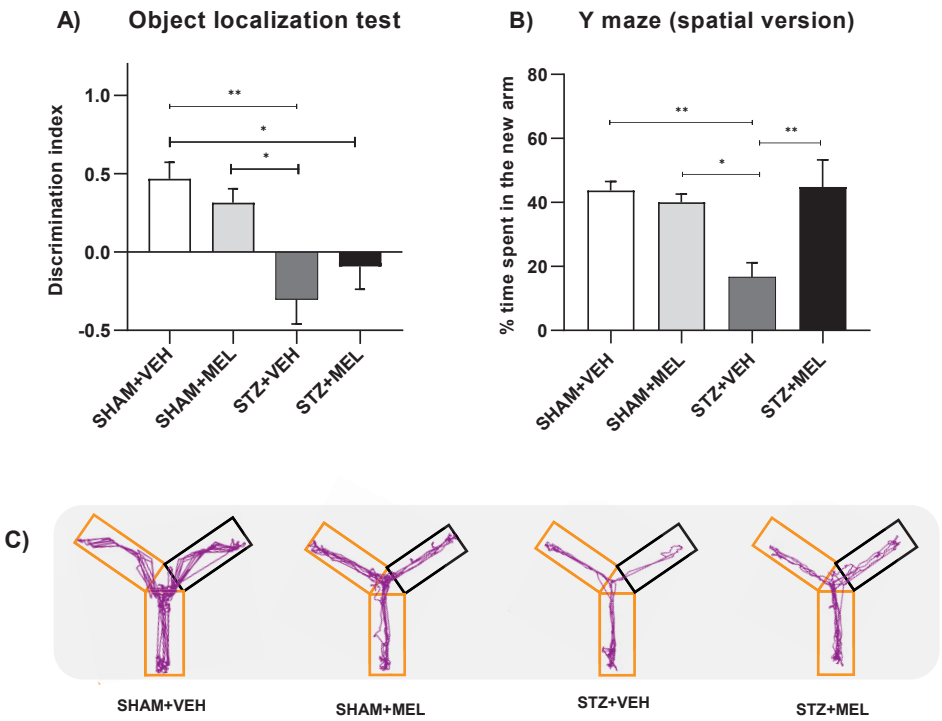

FIG. 4 - Effects of prolonged melatonin treatment in the memory test in STZ-lesioned rats. Evaluation short-term spatial memory expressed by object discrimination index in object location test (**A**) and by the time spent in the new arm of the Y-maze (**B**). (**C**) Representative image of the route traveled by an animal from each treatment group in the Y-maze. The arm skirted with the color black indicates the novel arm of the apparatus. The image illustrates the exploration of the apparatus by the rats of these groups, and the STZ+VEH group explores less of the novel arm. The data are expressed as mean ± SEM and were analyzed by one-way (ANOVA) with Tukey's multiple comparison test (**p*<0.05, ***p*<0.01). SHAM+VEH (n=8), SHAM+MEL (n=8), STZ+VEH (n=6), STZ+MEL (n=8).

3.3 ICV-STZ did not modify APP levels in the rat hippocampus

The APP levels was quantified in hippocampus of rats by western blotting. The results showed that APP was not changed in the hippocampus of STZ-lesioned rats ($F_{3,12} = 0{,}878$, $p>0{,}05$).

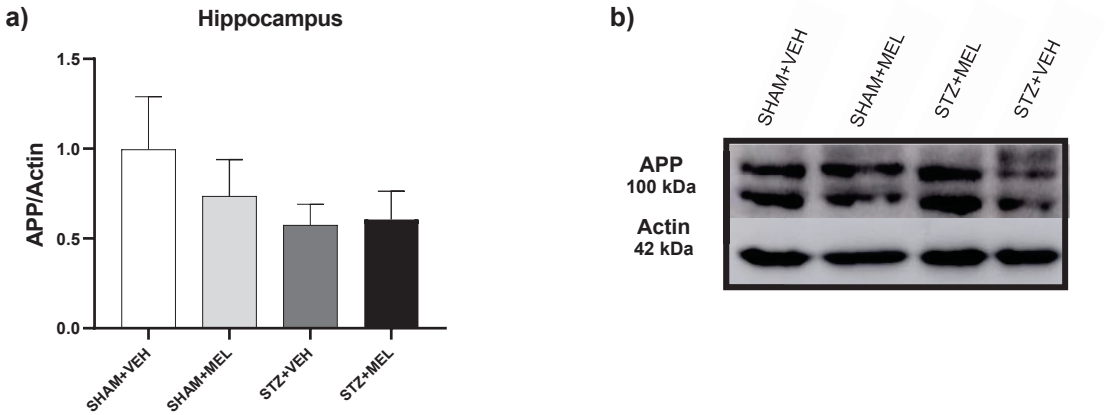

Fig. 5 – ICV-STZ at a dose 3 mg/kg did not cause changes in APP levels. (**A**) Quantification of APP levels. (**B**) Representative western blot image for APP. The data are expressed as mean ± SEM (n =4 per group) and were analyzed by one-way (ANOVA) with Tukey's multiple comparison test.

3.4 Melatonin reduced the increase in β-amyloid induced by ICV-STZ

The **Fig.6** showed that β-amyloid expression in the hippocampus of STZ+VEH group of rats had an increased in this protein when compared to SHAM groups ($p<0.0001$ vs SHAM groups). On the other hand, melatonin was able to reduce β-amyloid levels in STZ-lesioned rats when they were compared to animals of STZ+VEH group ($p < 0.0001$).

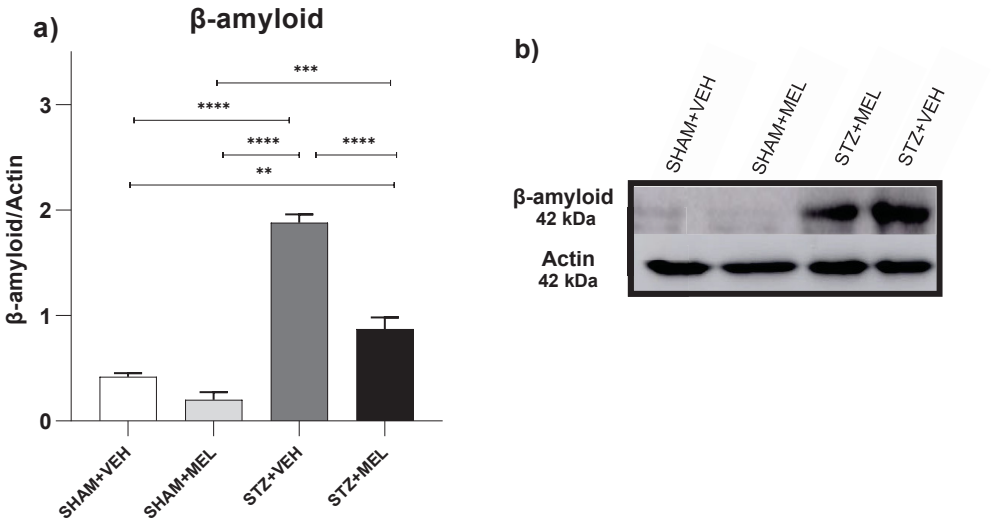

Fig. 6 – Effects of 30-days treatment with 10mg/kg melatonin reduced increase of β-amyloid in the hippocampus of rats induced by STZ. (**A**) Quantification of β-amyloid protein levels. (**B**) Representative western blot image for β-amyloid protein. The data are expressed as mean ± SEM (n =4 per group) and were analyzed by one-way (ANOVA) with Tukey's multiple comparison test (**p<0.01, ***p<0.001, ****p<0.0001).

3.5 Melatonin did not decrease GFAP levels in the hippocampus

One-way ANOVA showed a significant increase in hippocampal GFAP expression ($F_{3,12} = 8.904$, $p<0.01$) in STZ-lesioned rats in comparison to SHAM groups. The prolonged melatonin treatment was not able to reduce this increase (**Fig. 7 A and B**).

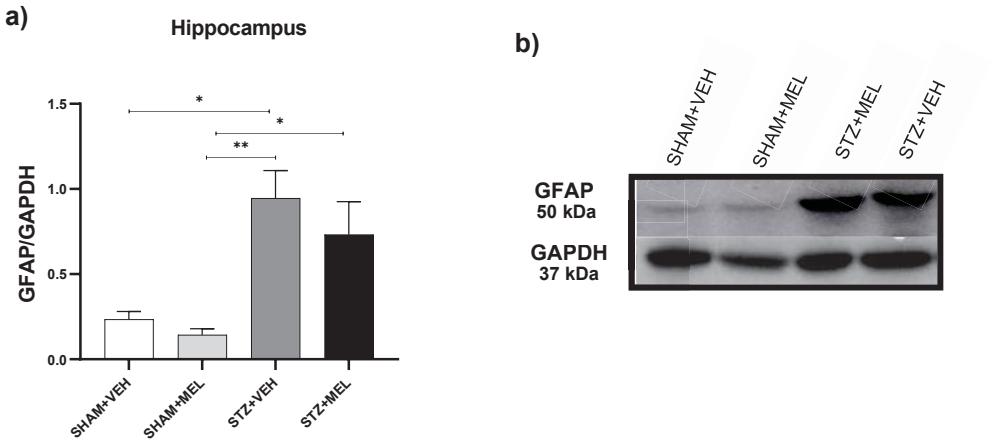

Fig. 7 – 30-days treatment with 10mg/kg melatonin not reduced increase of GFAP in the hippocampus of rats induced by STZ. (**A**) Quantification of GFAP levels. (**B**) Representative western blot image for GFAP. The data are expressed as mean ± SEM (n =4 per group) and were analyzed by one-way (ANOVA) with Tukey's multiple comparison test (**p*<0.05, ***p*<0.01, ****p*<0.001, *****p*<0.0001).

3.6 Correlations

Pearson's correlation coefficients revealed a moderate negative correlation (r = -0.59; p <0.05) between % time spent in the new arm and hippocampal Aβ levels (**Fig. 8 A**). Furthermore, Y-maze performance and hippocampal GFAP levels (r = -0.33; p >0.05) did not reach statistical significance (**Fig. 8 B**). Finally, we found a positive and significant correlation (r = 0.73; p <0.01) between GFAP levels and Aβ levels in the hippocampus of rats (**Fig. 8 C**).

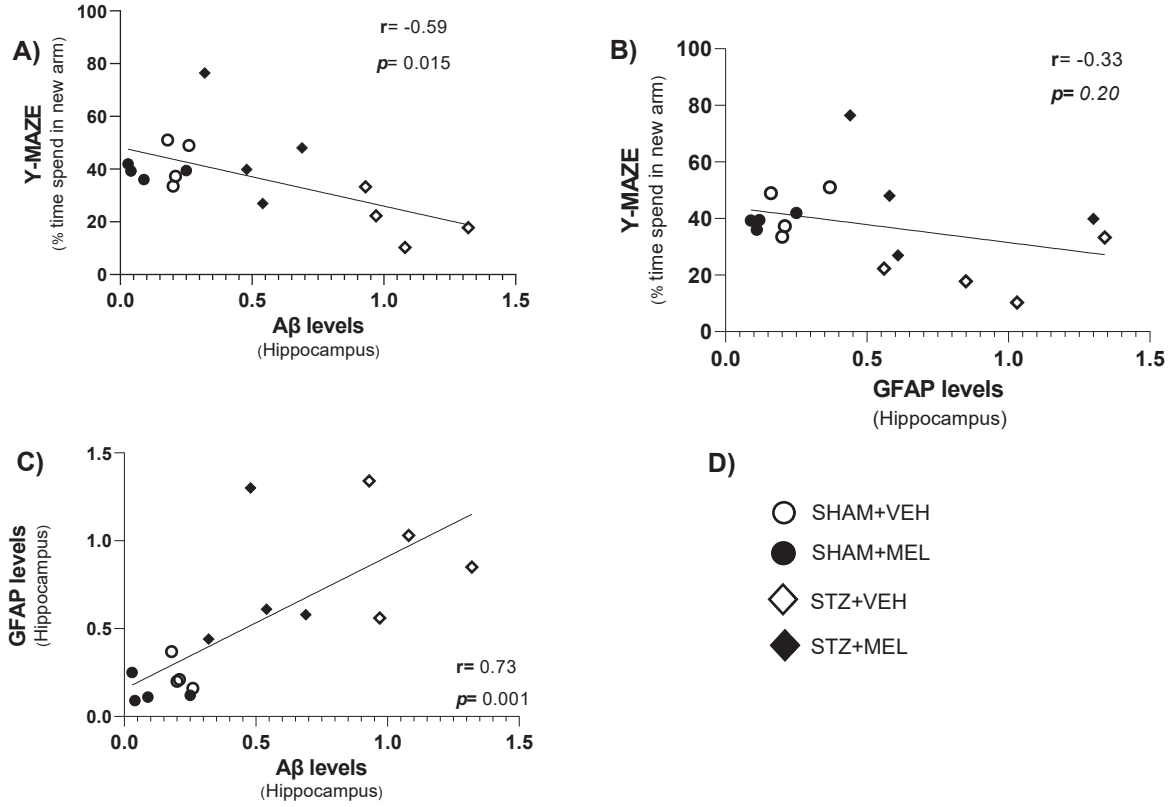

Fig. 8 – Correlations between % time spent in the new arm (Y-maze), Aβ and hippocampal GFAP in the animal model of sporadic Alzheimer's disease induced by ICV-STZ. (**A**) % time spent on the new arm and Aβ levels. (**B**) % time spent on the new arm and GFAP levels. (**C**) GFAP levels and Aβ levels. (**D**) Legend representing the symbols and experimental groups respectively. Pearson correlation coefficient, (**p*<0.05, ***p*<0.01).

4 DISCUSSION

The animal model of sAD induced by ICV-STZ causes brain changes similar those found in patients with sAD, such as oxidative stress, intense neuroinflammation, excitotoxicity, deficits in the cholinergic system, formation of senile plaques and neurofibrillary tangles, deterioration of cognitive functions and glicose metabolism impairment (MISHRA et al., 2018; WILLETTE et al., 2015; REETA; SINGH; GUPTA, 2017; OBULESU; JHANSILAKSHMI, 2013; JOHANSSON et al., 2021). Such alterations are believed to be produced by the state of insulin resistance and intense oxidative stress produced by the STZ lesion (AGRAWAL et al., 2011; KAMAT et al., 2015; KRASKA et al., 2012). Our results from the present study confirm some of these findings, since the STZ lesion was able to produce memory deficits in the animals (object location test and Y maze), increase in the levels of β-amyloid and GFAP in the hippocampus of rats. Furthermore, we showed that bilateral infusion of 3 mg/kg STZ are not sufficient to cause significant changes in APP levels in the hippocampus of rats (*Rattus norvergicus* - Wistar). However, other authors such as Mishra, SK et al. (2018) and Wang et al. (2017) found significant differences in APP levels in Sprague Dawley rats species.

The OFT results suggest that ICV-STZ lesion did not affect locomotor activity of rats. These results are confirmed by other groups (MARTINI et al., 2019; THOMÉ et al., 2018; JIANG et al., 2019) and may indicate that the results obtained in the spatial memory assessment tests are not negatively influenced by a low or high locomotor activity of the animals.

Impaired spatial memory is strongly related to sAD, being considered one of the initial manifestations of sAD in humans (CHERRIER et al., 2005; VLCEK; LACZO, 2014). Several studies demonstrate that spatial memory in humans is dependent on structures such as the medial temporal lobe, entorhinal cortex and right hippocampus (MILLER et al., 2018; PARSLOW et al., 2004). These structures are affected in early stages of sAD, justifying the degeneration of these functions (KESSELS et al., 2009), however, other author such as Pennanem et al. 2014 (PENNANEN et al., 2004) demonstrate that the deficit in spatial memory may depend on other structures such as the anterior cingulate cortex.

Present results showed that the STZ-induced lesion was able to cause short-term memory impairment in the rats, which can be observed through

discrimination index result in the OLT and the short time spent in the new arm of the Y maze test. These results corroborate several studies (BASSANI et al., 2017; MARTINI et al., 2019; SILVA et al., 2020; LI et al., 2020) and indicate that the sAD model induced by ICV-STZ infusion is useful to study possible treatments to improve the cognitive impairments of sAD. In addition, melatonin treatment prevented the cognitive impairment caused by STZ in the Y maze test, but, not in the OLT (**Fig 4 A and B**). One possible explanation for this result, is that the Y-maze and OLT are dependent on different processes in the brain or even different brain substrates (VORHEES; WILLIAMS, 2014; AGGLETON; KYD; BILKEY, 2004). Furthermore, although the role of the hippocampus in spatial memory in mice is well described, some studies suggest (ENNACEUR; NEAVE; AGGLETON, 1997) that lesions in structures such as the cingulate cortex and cerebral fornix more significantly affect spatial memory in the OLT while in tests such as the alternation test in the T-maze show that permanent lesions in the posterior cingulate cortex of mice appear to be less impactful (NELSON et al., 2015). Another important point is that immunoreactivity studies show that the cingulate cortex has high expression of MT1 receptors, whereas the MT2 receptor seems to have lower expression in this area and such receptor has been associated with inhibition of synaptic plasticity (WANG et al., 2005). Some studies show improvement in the performance of melatonin-treated rats in the OLT test, but these studies use different animal models and variations of OLT in which the time between the training phase and the test phase is shorter (LABBAN et al., 2021; ARANAROCHANA et al., 2019). In other studies, in which the time between training and test is longer, melatonin improved the discrimination index in OLT only at doses above 40 mg/kg. This last result corroborates present data despite in our protocol melatonin dose was 10 mg/kg and was similar to the result demonstrated by MADHU et al. (2021).

It is known that melatonin is involved in multiple physiological processes and that disturbances in the melatoninergic system are related to several diseases, including sAD (SAVASKAN et al., 2005; FAREZ et al., 2015; JACOB et al., 2002; GUNATA; PARLAKPINAR; ACET, 2020). Patients with Alzheimer's disease have decreased melatonin levels in various fluids (NOUS; ENGELBORGHS; SMOLDERS, 2021). Furthermore, many preclinical studies

demonstrate that melatonin, can reverse important pathophysiological features in several models of Alzheimer's disease (SRINIVASAN et al., 2006).

Regarding the β-amyloid and GFAP levels in the hippocampus of animals, melatonin significantly reduced β-amyloid levels in the hippocampus of STZ-injured rats (**Fig.6 A and B**). This result may explain the improve of performance of the animals in Y-maze. Whereas elevated levels of β-amyloid in the hippocampus are strongly linked to increased neuroinflammation, oxidative stress, and poor cognitive performance in humans and animal models (PETRY et al., 2021; HARRINGTON et al., 2017). The reduction in β-amyloid levels can be explained at several levels (LI et al., 2020). Acted through MT1 and MT2 receptors, melatonin can reduce amyloidogenic processing by reducing β- and γ-secretase expression and increasing α-secretase expression in the hippocampus. Furthermore, in vitro melatonin has demonstrated β- and γ-secretase inhibitory activity (CHEN et al., 2021) and recent articles show that melatonin binds strongly to β-amyloid fibers and can promote depolymerization of amyloid plaques (DAI et al., 2021).

In the present study, we observed that the infusion of 3 mg/kg of STZ in the animal ventricles was able to increase the levels of GFAP (**Fig. 7 A**) in the rat hippocampus, indicating reactive astrogliosis, as shown by several authors (BASSANI et al., 2017; VILLAR et al., 2018; RAI et al., 2014; KNEZOVIC et al., 2017) and this may be associated with the death of neurons in the hippocampus (RAI et al., 2014). In addition, melatonin was not able to reduce the increase of GFAP induced by ICV-STZ (**Fig. 7 A**). Furthermore, this elevation of GFAP appears to be uncorrelated with the cognitive performance of rats on the Y maze ($r = -0.33$; $p > 0.05$; **Fig. 8 A**). Finally, we show that the increase in Aβ levels has a strong positive correlation ($r = 0.73$; $p < 0.01$) with elevated GFAP levels in the hippocampus, suggesting a relationship between the variables since Aβ can induce reactive astrogliosis, as well as neuroinflammation and oxidative stress in the rat brain (PEREIRA et al., 2021; OLSEN et al., 2018).

5 CONCLUSION

In summary, the present study corroborrate that the STZ model of sAD is useful to assess cognitive impairment in AD and the effect of pharmacological

interventions. Furthermore, we showed that prolonged melatonin treatment improved short-term spatial memory in the Y-maze test and no changes in the discrimination index in the OLT. We also show that the increase in β-amyloid levels in the hippocampus by STZ may be associated with cognitive deficits in the model and the increase in GFAP levels since astrogliosis is also found in response to Aβ. Finally, we showed that melatonin treatment can significantly reduce β-amyloid levels in the hippocampus.

The authors have no conflicts of interest to declare.

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4.CONCLUSÃO

Em conclusão, nosso estudo reforça que o modelo induzido pela ICV-STZ da sAD é útil para avaliar o comprometimento cognitivo similar a DA bem como, efeito de intervenções farmacológicas. Além disso, mostramos que o tratamento de longo prazo com melatonina pode melhorar a memória espacial de curto prazo no teste do labirinto em Y, porém o mesmo tratamento parece não impactar significativamente o índice de discriminação no OLT. Mostramos também que o aumento dos níveis de β-amilóide no hipocampo pela STZ pode estar associado aos déficits cognitivos no modelo e o aumento dos níveis de GFAP já que a astrogliose também é encontrada em resposta a Aβ. Por fim, mostramos que o tratamento com melatonina pode reduzir significativamente os níveis de β-amilóide no hipocampo.

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