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POTENCIAL NEUROPROTETOR DA *CAMELLIA SINENSIS* E DO EXERCICÍO FÍSICO EM UM MODELO DE DOENÇA DE ALZHEIMER

TESE DE DOUTORADO

Helen Lidiane Schimidt

Uruguaiana, RS, Brasil

2018

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Área de concentração: Química e Bioquímica de Produtos Biologicamente Ativos

Orientador: Prof. Dr. Felipe Pivetta Carpes
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2018

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“Estou entre aqueles que acham que a ciência tem uma grande beleza”.

Marie Curie

RESUMO

Tese de Doutorado

Programa de Pós-Graduação em Bioquímica

Universidade Federal do Pampa

POTENCIAL NEUROPROTETOR DA *CAMELLIA SINENSIS* E DO EXERCICÍO FÍSICO EM UM MODELO DE DOENÇA DE ALZHEIMER

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Local e data da defesa: Uruguaiana, 16 de Julho de 2018

A Doença de Alzheimer (DA) causa déficits cognitivos e de memória, entre outros. O mecanismo de desenvolvimento da DA incluí processos inflamatórios e estresse oxidativo em virtude do acúmulo da proteína β-amilóide no cérebro. Por seu potencial antioxidante, chás provenientes da *Camellia sinensis* e o exercício físico são estratégias neuroprotetoras eficazes em diversos modelos de déficits cognitivos. No entanto, não está claro se tanto as variedades dos chás da *Camellia sinensis* quanto os diferentes tipos de exercício possuem o mesmo potencial neuroprotetor na DA. Adicionalmente, poucos estudos investigam estratégias de neuroproteção em modelos de DA associado a fatores de risco da doença, como uma dieta hipercalórica. Considerando esse contexto, determinamos o potencial neuroprotetor de diferentes chás provenientes da *Camellia sinensis* e de diferentes configurações de exercício físico em um modelo de DA associada ou não a dieta hipercalórica. Após a realização de uma série de experimentos bioquímicos e comportamentais, concluímos que (a) o chá verde tem maior potencial neuroprotetor no modelo de DA, (b) uma dieta hipercalórica associada ao modelo de DA não potencializa déficits cognitivos e dano oxidativo no hipocampo, e (c) o exercício de força possui potencial neuroprotetor no modelo de DA estudado e é a única estratégia que também atua no sistema colinérgico.

Palavras chaves: memória; demência; envelhecimento; neuroproteção; chá verde; atividade física; dieta; obesidade.

ABSTRACT

Doctoral Thesis

Graduation Program in Biochemistry

Federal University of Pampa

NEUROPROTECTIVE POTENTIAL OF CAMELLIA SINENSIS AND PHYSICAL EXERCISE IN A MODEL OF ALZHEIMER'S DISEASE

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Place and presentation date: Uruguaiana, July 16th 2018

Alzheimer's disease (AD) causes cognitive and memory deficits, and other. The mechanism of AD onset includes inflammatory processes and oxidative stress due to the accumulation of β-amyloid protein in the brain. Teas from *Camellia sinensis* and physical exercise are effective neuroprotective strategies in several models of cognitive deficits. However, it is unclear whether Camellia sinensis tea varieties have the same neuroprotective potential in AD, as well as whether different exercise configurations have the same neuroprotective potential in AD. Additionally, few studies investigated prevention strategies in AD models associated with additional risk factors, such as a hypercaloric diet. Here we determine the neuroprotective potential of different teas from Camellia sinensis and different exercise configurations in a model of AD associated or not to a hypercaloric diet. After completing a serie of biochemical and behavioral experiments, we conclude (a) that green tea has the greater neuroprotective potential in the AD model, (b) a hypercaloric diet associated with the AD model did not potentiate cognitive deficits and oxidative damage in the hippocampus, and (c) strength exercise has neuroprotective potential in the AD model studied, being the only exercise also influencing the cholinergic system.

Keywords: memory; insanity; aging; neuroprotection; green tea; physical activity; diet; obesity.

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LISTA DE ABREVIATURAS

ALA - Ácido alfa-lipóico

DNA - Ácido desoxirribonucleico

α-secretase - Alfa-secretase

Apoe4 - Apolipoproteína E4

ABRAz - Associação Brasileira de Alzheimer

AVD - Atividades da vida diária

Aβ - Beta amiloide

β-secretase - Beta- secretase

C - Catequina

coq10 - Coenzima Q10

DA - Doença de Alzheimer

EN - Emaranhados neurofibrilares

IDE - Enzima degradadora de insulina

inos - Enzima óxido nítrico sintase induzível

EC - Epicatequina

ECG - Epicatequina galato

EGC - Epigalocatequina

EGCG - Epigalocatequina galato

ERO - Espécies reativas de oxigénio

VEGF - Fator de Crescimento Endotelial Vascular

IGF-I - Fator de crescimento semelhante à insulina tipo 1

TGF-β - Fator de transformação do crescimento-beta

γ-secretase - Gama-secretase

DG - Giro denteado

GSH - Glutationa

GSH-Px - Glutationa peroxidase

HA - Hipertensão arterial

PAI-1 - Inibidor-1 do ativador do plasminogênio

HDL - Lipoproteínas de alta densidade

HPLC - Cromatografia líquida de alta eficiência

LDL - Lipoproteínas de baixa densidade

LC - Locus Coeruleus

MC - Memória de curta duração

ML - Memória de longa duração

MT - Memória de trabalho

MT - Microtúbulos

ICAM - Molécula de adesão intracelular solúvel

PS1 e PS2 - Presenilina 1 e 2

PA - Pressão arterial

PCR - Proteína C reativa

PPA - Proteína precursora amilóide

sppaα - Proteína precursora amilóide alfa solúvel

MCP-1 - Proteína quimiotática para monócitos

SNC - Sistema nervoso central

S - Subículo

SOD - Superóxido dismutase

IL-6 - interleucina-6

TNF-α - Fator de necrose tumoral alfa

PET - Tomografia por emissão de pósitrons

SUMÁRIO

APRESENTAÇÃO

Essa tese está organizada em seis capítulos, conforme detalhamento a seguir. Os capítulos redigidos especificamente para este documento estão no idioma Português. Os artigos que foram publicados ou que estão em fase de submissão para periódicos, estão redigidos no idioma Inglês.

No primeiro capítulo apresentamos uma revisão dissertativa da literatura sobre a Doença de Alzheimer e estratégias de neuroproteção, onde apresentamos conceitos fundamentais e alguns destaques de estudos publicados na temática desta tese. Neste capítulo também apresentamos as justificativas e objetivos da tese.

O segundo capítulo é composto pelo artigo original intitulado "*Green tea supplementation produces better neuroprotective effects than red and black tea in Alzheimer-like rat mode",* que foi publicado na revista *Food Research International*, Qualis B1 nas Ciências Biológicas II; Fator de Impacto 2017: 3.086.

No terceiro capítulo está apresentado o artigo original intitulado "*Effects of cafeteria diet on memory and hippocampal oxidative stress in a rat model of Alzheimer-like disease: neuroprotection of green tea supplementation"*, que se encontra em avaliação pelo pares para publicação na revista *Journal of Functional Foods,* Qualis A2 nas Ciências Biológicas II; Fator de Impacto 2017: 3.144.

O quarto capítulo inclui o artigo de revisão sistemática da literatura intitulado "*The role of regular physical exercise for enhancement of long-term memory in the elderly: a review of recent evidences"*, publicado na *revista Pan American Journal of Aging Research* (PAJAR).

No quinto capítulo apresentamos o manuscrito original intitulado “*Strength and running training elicit different neuroprotection outcomes in Alzheimer like-disease induced by beta-amyloid toxicity”*, que se encontra em avaliação pelo pares para publicação na revista Neuroscience, Qualis A2, nas Ciências Biológicas II; Fator de Impacto 2017: 3.42.

Por fim, o sexto capítulo apresenta uma discussão geral dos resultados desta tese bem como aplicações práticas e perspectivas para investigações futuras.

CAPITULO I

1 INTRODUÇÃO

1.1 Doença de Alzheimer

1.1.1 Definição, história e epidemiologia

A Doença de Alzheimer (DA) tem caráter neurodegenerativo e está associada ao envelhecimento, cujas manifestações cognitivas e neuropsiquiátricas resultam em um déficit progressivo seguido de incapacitação (Cisse e Checler, 2014). A DA foi descrita pela primeira vez por *Alois Alzheimer*, um médico especialista em neuropsiquiatria, no dia 3 de novembro de 1906, em Tübingen, na Alemanha. Na ocasião, *Alois* apresentou um trabalho intitulado "*Eine eigenartige Erkrankung der Hirnrinde"*, que para o português pode ser traduzido como: "Uma doença peculiar dos neurônios do córtex cerebral"; no 37º Encontro de Psiquiatras do Sudeste da Alemanha.

Em seu trabalho, o neuropsiquiatra descreveu o caso clínico de uma paciente do sexo feminino, com 51 anos de idade e que havia sido internada em um sanatório de psiquiatria em Frankfurt. *Alois* relatou que ao exame clínico, a paciente apresentava uma série de sintomas como déficit de memória e raciocínio, afasia, desorientação, distúrbios de comportamento, alucinações auditivas e importante dificuldade para realizar atividades básicas da vida diária e de convívio social, rejeitando qualquer tipo de ajuda. Os primeiros sintomas da paciente em questão ocorreram quase um ano antes da sua internação e pioraram progressivamente, acabando por comprometer as funções mentais ao longo dos cinco anos de sua internação e evoluindo para a morte.

Depois do falecimento da paciente, *Alois* pode realizar a necropsia do seu cérebro, onde foram encontradas placas neuríticas, novelos neurofibrilares e discretas alterações vasculares. Um trabalho com a descrição dos sintomas clínicos e neuropatologia completa desse caso clínico foi publicado no ano seguinte, na revista "*Allgen Z Psychiatr Psych – Gerich Méd*". Esse foi um marco histórico para a DA, e a partir desta publicação casos semelhantes foram relatados. A fisiopatologia descrita por *Alois* até hoje contribui para o avanço em pesquisas sobre a DA. A história da DA está muito bem documentada um artigo intitulado, *"The discovery of Alzheimer's disease"*, publicado por Hippius *et al* em 2003 na revista "Dialogues in Clinical Neuroscience" (Hippius e Neundörfer, 2003).

Atualmente, a DA é o tipo de demência mais comum em todo o mundo (Sachdev *et al.*, 2014), sendo uma ameaça frente ao crescente fenômeno do envelhecimento populacional mundial. Sabe-se que a DA corresponde a 60% do total de casos de demências no mundo, o que representa um número de 3,5 milhões de pessoas com DA. Segundo a organização mundial de saúde (OMS), há uma estimativa de que em 2050 esse número possa chegar a 135,5 milhões de pessoas.

Um dos problemas gerados a partir desse cenário é o alto custo econômico da DA. Um levantamento realizado em 2010 mostrou que a DA leva a um custo mundial anual de US$ 604 bilhões (Qiu *et al.*, 2009). Isso ocorre porque o curso da doença, entre o diagnóstico e o óbito, leva aproximadamente oito anos, e durante esse tempo os pacientes possuem necessidades básicas de atenção em saúde para que tenham uma melhor qualidade de vida. Sabe-se que idosos portadores de DA tem o dobro de internações hospitalares comparados aos idosos sem a doença, além de dependerem de cuidadores, atendimento médico diferenciado e uma extensa lista de medicamentos (Medina *et al.*, 2017).

No Brasil, segundo dados da Associação Brasileira de Alzheimer (ABRAz, 2015), 6% da população com mais de 65 anos tem o diagnóstico da DA. No entanto, pesquisas apontam que mais de 50% dos pacientes não recebem o diagnóstico corretamente, portanto, não recebem tratamento adequado e isso agrava ainda mais o problema. Nos Estados Unidos, segundo o relatório anual da "*Alzheimer's Association*" do ano de 2017, a DA tem índices de mortalidade mais altos do que os de câncer de mama e de próstata juntos. Ainda, segundo o relatório anual de 2017, desde o ano 2000 as mortes por doença cardíaca, por exemplo, diminuíram em 14%, enquanto que as mortes causadas por DA aumentaram 89%. Esses estudos epidemiológicos apontam a problemática envolvendo a DA com o passar dos anos.

1.1.2 Classificação, etiologia, diagnóstico e tratamento

A DA pode ser classificada como de início precoce ou de início tardio. A DA de início precoce está ligada a uma herança autossômica dominante, devido a mutações nos genes da presenilina 1 e 2 (PS1 e PS2). Nesse caso, a doença acomete pacientes com idade entre 40 e 50 anos e representa 5% do total de casos dessa patologia. Assim, o aparecimento da DA de início precoce está mais ligado à herança genética do que a fatores ambientais (Freudenberg-Hua *et al.*, 2018).

A DA de início tardio é a forma mais comum da doença, onde o quadro clínico se inicia comumente após os 65 anos. A idade avançada é sem dúvida o fator de risco mais importante para a DA neste caso. Logo, há outros fatores de risco, como lesão traumática da cabeça, depressão, doença cardiovascular e cerebrovascular, hábitos de vida pouco saudáveis como, por exemplo, tabagismo e sedentarismo (Caruso *et al.*,

2018). A obesidade e doenças metabólicas, como a Diabetes, são fatores de risco muito importantes para o desenvolvimento da DA de início tardio (Frasca *et al.*, 2017).

Embora a etiologia da DA ainda seja desconhecida, o que já é consenso na literatura é que a DA se manifesta a partir de uma combinação de fatores de risco, genéticos e ambientais, que desencadearão os principais mecanismos fisiopatológicos que estão descritos a seguir, como a cascata amiloidogênica e a formação de emaranhados neurofibrilares (Caruso *et al.*, 2018; Kumar e Tsao, 2018).

O diagnóstico da DA é essencialmente clínico, baseado em uma história médica completa, testes e questionários que avaliam a memória e o estado mental, o grau de atenção e concentração, habilidade em resolver problemas e nível de comunicação (Masters *et al.*, 2015). Ainda não se conhece um marcador biológico que confirme de maneira exata a presença da DA, o que dificulta o diagnóstico preciso da doença e torna esse um dos principais focos de pesquisa na área (Mendiola-Precoma *et al.*, 2016).

Outras ferramentas que auxiliam no diagnóstico são os exames de imagem, como por exemplo, a tomografia por emissão de pósitrons (PET), que ajuda a identificar a presença de placas amiloides, e a ressonância magnética nuclear, útil na identificação de atrofia cerebral (Henriques *et al.*, 2018). Contudo, para que exista uma padronização internacional no diagnóstico da doença, há critérios pré-estabelecidos por associações nacionais e internacionais que estudam a DA (Frota *et al.*, 2011). Estes critérios são baseados em estudos científicos epidemiológicos e de coorte, importantes principalmente para o diagnóstico diferencial, excluindo outros tipos de demência e transtornos cognitivos (Kumar e Tsao, 2018).

Embora a DA seja descrita e estudada há muitos anos, ainda não existe cura para doença. Diante disso, existe grande investimento em pesquisas que envolvem o estudo de mecanismos de proteção, prevenção e tratamento da DA. Por ser uma doença

multifatorial e com amplas manifestações clínicas, os métodos de tratamentos envolvem, por exemplo, remoção de placas amiloides, modulação das atividades da enzima secretase, regulação da hiperfosforilação e da agregação da proteína tau, entre outras (Kozlov *et al.*, 2017).

Essas estratégias são baseadas em fármacos e vão resultar em diminuição e restrição da progressão sintomática. Nesse sentido, medicamentos que modulam neurotransmissores, como anticolinesterásicos e moduladores glutamatérgicos tem efeito sobre a evolução da doença, além do uso de antidepressivos (Kivipelto e Solomon, 2008; Freudenberg-Hua *et al.*, 2018).

Com o tratamento, ainda um tanto quanto paliativo, pesquisadores se debruçam sobre a importância do diagnóstico precoce da DA e, sobretudo, na necessidade de identificar intervenções para pacientes nas fases iniciais da doença, quando ainda não são notados sintomas comportamentais e psicológicos significativos nem deterioração funcional grave. Assim, também são foco de interesse de pesquisas as intervenções não farmacológicas, que atuam como protetoras e são entendidas hoje como importantes aliadas para frear a progressão da DA. No contexto de prevenção, um foco especial tem sido dado na suplementação com antioxidantes e atividade física regular (Masters *et al.*, 2015; Kozlov *et al.*, 2017; Ozbeyli *et al.*, 2017; Sun *et al.*, 2018).

1.1.3 Fisiopatologia da Doença de Alzheimer

Os principais achados fisiopatológicos da DA são o aumento da formação de emaranhados neurofibrilares (EN) intracelulares e de placas amiloides no meio extracelular (Masters *et al.*, 2015). Os EN são formados quando a proteína tau sofre hiperfosforilação, por excesso de atividade das quinases ou redução da atividade das

fosfatases, e se desliga dos microtúbulos (MT) (Cheng e Bai, 2018). Os EN provocam neurodegeneração como consequência da perda da função estrutural do citoesqueleto (MT + proteína tau), comprometendo o transporte axonal, gerando disfunção sináptica e morte neuronal. Além da perda de função estrutural, também têm função tóxica, contribuindo para neuroinflamação e estresse oxidativo (Giraldo *et al.*, 2014; Cheng e Bai, 2018).

Já as placas amiloides estão no meio extracelular e são formadas por acúmulo de beta amiloide (Aβ). Aβ são peptídeos com 36-43 aminoácidos oriundos da catálise da proteína precursora amiloide (PPA) (Sun *et al.*, 2018). A função da PPA ainda não está bem definida, embora se saiba que ela está envolvida na regulação e formação das sinapses, na neuroplasticidade e na exportação de ferro (Masters *et al.*, 2015). A deposição de placas amiloides no sistema nervoso central (SNC) ocorre por meio de uma anormalidade na clivagem da PPA (Kozlov *et al.*, 2017). A clivagem da PPA pode acontecer por duas vias principais, a via não amiloidogênica e a via amiloidogênica. A via fisiológica é chamada de via não amiloidogênica, sendo considerada esta a via prevalente, onde a PPA é clivada pela alfa-secretase (α-secretase), seguida por nova clivagem pela enzima gama-secretase (γ-secretase), gerando uma proteína precursora amiloide alfa solúvel (sPPAα) que parece agir como um fator trófico. Já na via amiloidogênica, uma via menos comum, a clivagem da PPA é realizada pela beta secretase (β-secretase), seguida pela γ-secretase, gerando a proteína beta amiloide (Aβ) (Andreeva *et al.*, 2017). A liberação do fragmento Aβ no espaço extracelular é seguido por agregação, formação de depósitos de placas amiloides. Esses fragmentos em excesso no meio extracelular formam agregados com efeitos neurotóxicos e neurodegenerativos (Masters *et al.*, 2015; Jack *et al.*, 2016; Andreeva *et*

al., 2017). A via não amiloidogênica e a via amiloidogênica estão representadas em um desenho esquemático na figura 1.

A formação de placas amiloides é acompanhada pelo aumento em espécies reativas de oxigênio e estresse oxidativo, o que aumenta ainda mais o processo de deposição de ß-amilóide, caracterizando dessa forma um efeito cascata (Giraldo *et al.*, 2014; Da Mesquita *et al.*, 2016). Com a formação das placas amiloides há uma redução do número de sinapses e, consequentemente, uma perda seletiva de neurônios colinérgicos nas vias do lobo frontal e hipocampo (Ghasemi *et al.*, 2014; Sun *et al.*, 2018). Além disso, a neuroinflamação também tem sido descrita na fisiopatologia da DA, pois ao redor dos depósitos da proteína ß-amilóide há um aumento de mediadores inflamatórios, como o fator de necrose tumoral TNF-α e as interleucinas IL-6 e IL-1β (Ferreira *et al.*, 2014; Da Mesquita *et al.*, 2016).

Além dos EN e das placas amiloides, há outros mecanismos citados na fisiopatologia da DA e que, de certa forma, estão interligados e são consequências destes primeiros. Dentre eles, o mecanismo vascular, mitocondrial, oxidativo e inflamatório (Kivipelto e Solomon, 2008; Masters *et al.*, 2015; Jack *et al.*, 2016; Kozlov *et al.*, 2017; Caruso *et al.*, 2018; Kumar e Tsao, 2018). Doenças vasculares cerebrais estão associadas com a DA (Kisler *et al.*, 2017). Adicionalmente, com o envelhecimento ocorre um comprometimento funcional a nível mitocondrial, e isso leva a aumento do estresse oxidativo e, consequentemente, morte neuronal, entrando em um ciclo estresse oxidativo e inflamação (Cheng e Bai, 2018) desencadeando a cascata amiloidogênica e caracterizando assim a progressão da doença (Zussy *et al.*, 2013; Cheng e Bai, 2018).

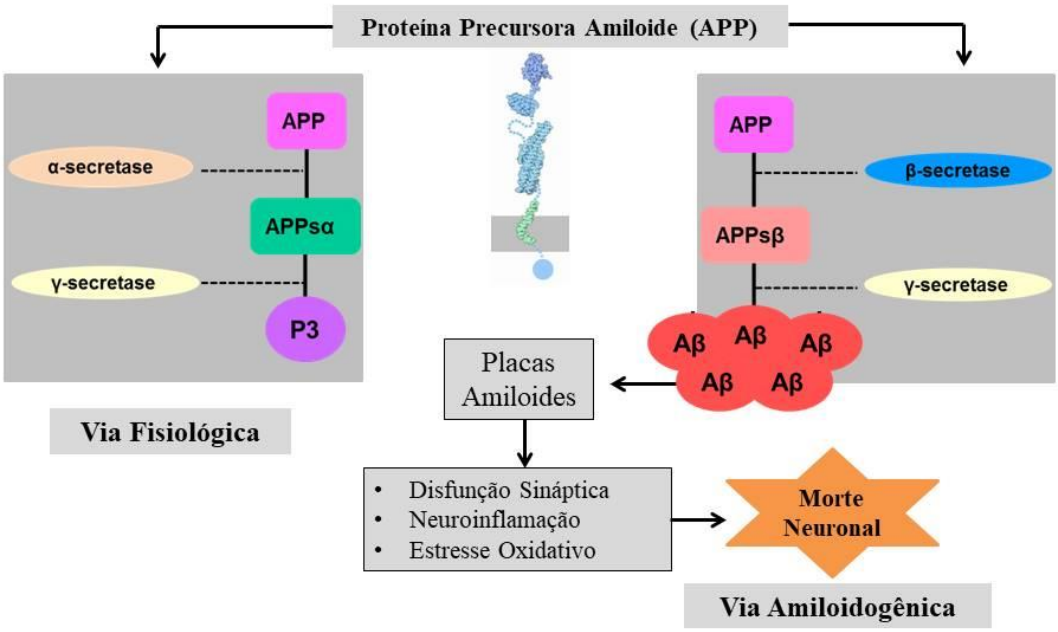

Figura 1. Catálise da proteína precursora amiloide (APP) – Via fisiológica e via Amiloidogênica. Na via fisiológica, a APP é reduzida pelas enzimas α-secretase e γ-secretase a uma proteína precursora solúvel que não trás malefício ao SNC. Já na via amiloidogênica, a APP é reduzida a proteína ß-amilóide (Aβ) que forma agregados de placas amiloides, responsáveis pela disfunção sináptica, neuroinflamação e estresse oxidativo que resulta na morte neuronal.

O mecanismo fisiopatológico da DA resulta como uma enfermidade progressiva. A progressão da doença faz com que seu curso tenha pelo menos três estágios bem definidos por suas características fisiopatológicas e clínicas. No estágio I, as alterações patológicas supracitadas começam no córtex entorrinal, seguidas pelo hipocampo. Nesta fase, os pacientes apresentam perda leve de memória, sem comprometimento funcional significativo em suas atividades diárias. No estágio II, a doença se espalha para as áreas do córtex cerebral responsáveis pela linguagem, raciocínio e processamento sensorial. Há crescente perda de memória e atenção, e surgem problemas comportamentais. Os pacientes na fase II começam a ter dificuldade em reconhecer a própria família e os

amigos. Eles têm apatia, retraimento social e perda de inibição. Fazem declarações repetitivas e têm perda do controle de impulsos. Eles também têm dificuldades com linguagem, leitura e escrita.

Por fim, no estágio III da DA, há uma progressão dos danos para todo o córtex. Nesta fase, os pacientes não reconhecem família e amigos, e são totalmente dependentes de outros para atividades diárias. Juntamente com outras características, eles também se tornam incontinentes da bexiga e do intestino, e podem ter dificuldade em engolir. Nessa fase, os pacientes já estão acamados, quando assumem a posição fetal e seu prognóstico evolui para morte (Masters *et al.*, 2015; Kumar e Tsao, 2018). Na figura 2 apresentamos um esquema da progressão da DA no cérebro, partindo de um cérebro normal sem a DA e logo com os cérebros acometidos com a DA de acordo com cada estágio.

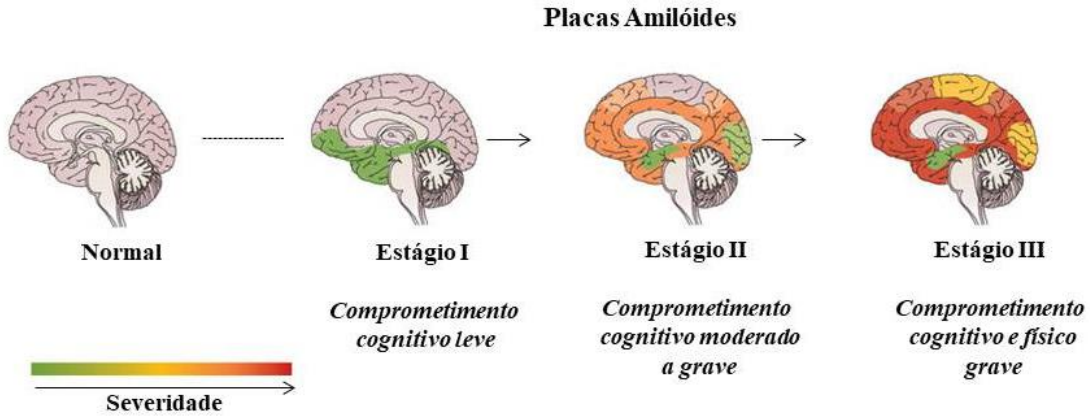

Figura 2. Depósito de placas amiloides de acordo com o estágio da Doença de Alzheimer. Placas amiloides se espalham pelo cérebro humano à medida que a DA

progride, agravando o comprometimento cognitivo (Figura adaptada de Masters *et al*; 2015).

1.1.4 Obesidade, Diabetes e a Doença de Alzheimer

Segundo a Organização Mundial da Saúde (OMS), no Brasil, nas últimas quatro décadas, a prevalência de obesidade aumentou em mais de 2,8% para homens e mais de 8,0% para mulheres. Em idosos, a prevalência de obesidade atingiu 20,2% no ano de 2013, o que significa, em termos absolutos, que mais de três milhões de pessoas com idade acima de 65 anos são obesas. A obesidade na fase do envelhecimento é resultante de uma vida pouco saudável, onde se prioriza uma alimentação rica em carboidratos e gorduras associado ao sedentarismo.

Assim, a obesidade hoje é considerada uma doença crônica e de difícil tratamento, associada com muitas outras doenças, como câncer, Diabetes tipo 2, hipercolesterolemia, hipertensão arterial (HA), acidente vascular cerebral (AVC), osteoartrose, esteatose hepática e doenças neurodegenerativas, aumentando o risco de morbidade e mortalidade (Frasca *et al.*, 2017).

A obesidade está diretamente relacionada a alterações nas funções endócrinas e metabólicas (Mattson, 2009). Em sujeitos obesos, o tecido adiposo aumenta a capacidade de síntese de moléculas com ação pró-inflamatórias, como a proteína C reativa (PCR), o fatore de necrose tumoral alfa (TNF-α), a interleucina-6 (IL-6), a leptina, a enzima óxido nítrico sintase induzível (iNOS), o fator de transformação do crescimento-beta (TGF-β), a proteína quimiotática para monócitos (MCP-1), a molécula de adesão intracelular solúvel (sICAM), o angiotensinogênio e o inibidor-1 do ativador do plasminogênio (PAI-1) (Ríos *et al.*, 2014; Frasca *et al.*, 2017). Todas essas

moléculas em grandes níveis periféricos têm um efeito sistêmico que acabam envolvendo níveis centrais, contribuindo para o desenvolvimento de doenças neurodegenerativas como a DA (Frasca *et al.*, 2017; Pugazhenthi *et al.*, 2017). Além disso, a obesidade e doenças associadas compartilham de mecanismos semelhantes a DA, como a inflamação e o estresse oxidativo, e por isso são consideradas um fator de risco para o desenvolvimento da DA.

Já é sabido que a hipercolesterolemia aumenta o risco de DA e demência. Um estudo mostrou a associação do colesterol ruim, ou LDL acima de 155 mg/dL com o desenvolvimento de placas beta-amiloide em comparação com pessoas cujo LDL era menor do que 106 mg/dL (Matsuzaki *et al.*, 2011). A HA gera um comprometimento circulatório cerebral, que associado ao envelhecimento, reduz a capacidade de autorregulação do fluxo sanguíneo cerebral, especialmente quando ocorrem flutuações súbitas e amplas de pressão arterial (PA). No entanto, a HA controlada com medicamentos e hábitos de vida saldável não oferece risco nenhum ao desenvolvimento da DA (Bonini *et al.*, 2006).

A Diabetes também aumenta em até duas vezes o risco de DA e demência vascular (Vandal *et al.*, 2014). Maiores valores de glicemia, mesmo em não diabéticos, estão associados com déficit cognitivo e atrofia do hipocampo. A hiperglicemia tem um efeito toxico por influxo aumentado de glicose (Matsuzaki *et al.*, 2011). Além disso, em pacientes com DA, o número de receptores de insulina está aumentado e a sinalização está prejudicada, o que pode ser qualificado pela resistência à insulina no SNC (Silzer e Phillips, 2018). A insulina também afeta o metabolismo amiloide, aumentando a produção de Aβ e diminuindo sua depuração, concorrendo pela mesma enzima que promove sua proteólise, a enzima degradadora de insulina (IDE) (Ahmad *et al.*, 2017;

Caruso *et al.*, 2018). A figura 3 mostra um resumo de como as doenças metabólicas e hábitos de vida estão associadas com a DA.

Em modelos animais, como por exemplo, roedores, a Dieta de Cafeteria é um modelo de dieta hipercalórica e palatável, que pode induzir, se oferecida de maneira crônica, a obesidade e ao aumento de glicose (Leffa *et al.*, 2015; Soares, M. B. *et al.*, 2017). Isso acontece porque a dieta é rica em açucares e gorduras, sendo produzida a partir de alimentos consumidos na dieta humana, como chocolates, biscoitos doces e amendoim (Almeida *et al.*, 2015). Um estudo prévio do nosso grupo, realizado com camundongos fêmeas, mostrou que só a dieta de cafeteria, já é capaz de induzir déficits de memória (Soares, M. *et al.*, 2017).

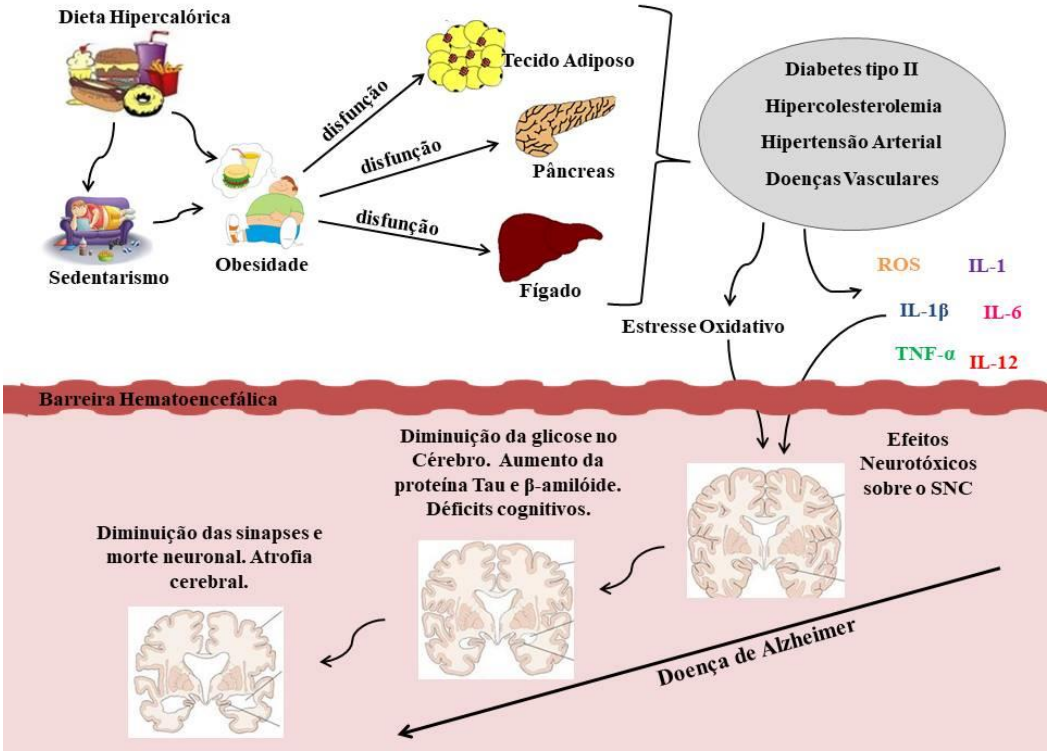

Figura 3. Desenho esquemático da relação entre os hábitos de vida e doenças associadas com o risco de desenvolvimento da DA. Uma dieta hipercalórica associada com inatividade física leva à obesidade crônica e doenças como Diabetes do tipo II, colesterol alto, hipertensão arterial e doenças vasculares. Todas essas doenças têm um mecanismo em comum, o aumento da neuroinflamação e do estresse oxidativo. As citosinas inflamatórias e os radicais livres atingem o SNC causando efeitos neurotóxicos. A frequente exposição a esses efeitos leva a um aumento na deposição das proteínas responsáveis pela formação dos emaranhados neurofibrilares (proteína Tau) e placas amiloide (proteína β-amilóide). Em um efeito mais tardio, há a morte neuronal e a atrofia cerebral. Todo esse processo caracteriza a DA (DE FELICE; FERREIRA, 2014).

1.2 Memória

A perda de memória é a principal característica clínica da DA, e por isso é o tema central desta tese. A memória é a capacidade de adquirir, armazenar e evocar informações, sendo uma habilidade muito importante para o ser humano (Izquierdo, 2011), pois garante a possibilidade de discriminação entre pessoas, objetos e lugares (Bird, 2017). As memórias também são responsáveis pela construção da nossa personalidade e interação com o meio que vivemos (Squire e Larry, 1992). Assim, a memória também pode ser compreendida como a capacidade de um organismo alterar seu comportamento em decorrência de experiências prévias (Izquierdo, 2011). Do ponto de vista fisiológico, essa capacidade é resultado de modificações no circuito neural, mediadas por diferentes sistemas celulares e bioquímicas do sistema nervoso central (SNC), e que funcionam independentemente, mas de forma cooperativa com o ambiente (Izquierdo e Mcgaugh, 2000; Izquierdo *et al.*, 2006).

A memória pode ser dividida nas fases de aquisição, consolidação e evocação de informações (Squire e Larry, 1992). A fase de aquisição é o processo de recebimento da informação. A fase de consolidação é o processo de armazenamento da informação, sendo esse processo o mais complexo, pois envolve mecanismos bioquímicos e eletrofisiológicos distintos para cada tipo de memória. A evocação, também chamada de recordação, diz respeito ao retorno espontâneo ou voluntário das informações consolidadas. A evocação envolve a organização dos traços de memória em uma sequência coerente no tempo (Izquierdo, 2011). A memória consolidada pode passar por outros processos, como por exemplo, o esquecimento natural, quando a informação original não estará mais disponível para a recuperação, e a extinção, quando a memória é inibida por um novo aprendizado e progressivamente substituída por este (Vargas *et*

al., 2014). Além disso, as memórias também podem sofrer reconsolidação, permitindo a incorporação de novas informações à memória original que está sendo evocada (Izquierdo, 2011; Vargas *et al.*, 2017).

As memórias podem ser classificadas em relação à duração, sendo elas memórias de trabalho (MT), memórias de curta duração (MCD) e memórias de longa duração (MLD). As memórias também podem ser classificadas quanto ao conteúdo, como memórias declarativas e memórias não declarativas. A MT é sustentada pela atividade elétrica de neurônios do córtex pré-frontal, em rede via córtex entorrinal com o hipocampo e a amígdala, durante a percepção, a aquisição ou a evocação e dura apenas segundos, não deixando traços. A MC dura de 30 minutos a seis horas e utiliza processos bioquímicos breves no hipocampo e córtex entorrinal. A ML perdura muitas horas, dias ou anos. Quando duram anos, denomina-se remota. Sua formação requer uma sequência de passos moleculares que duram pelo menos de 3 a 6 horas durante as quais é suscetível a numerosas influências, em processos que ocorrem no hipocampo, nos núcleos amigdalinos, e em outras áreas (Squire e Kandel, 2003; Mello, P. *et al.*, 2008; Izquierdo, 2011).

As memórias não declarativas estão relacionadas com capacidades e/ou habilidades motoras e/ou sensoriais, como por exemplo, a memória de procedimento, sistema de representação perceptivo, condicionamento clássico e aprendizado não associativo (habituação/sensibilização). Já as memórias declarativas registram fatos, eventos e conhecimentos. Elas podem ser divididas em semânticas, que estão relacionadas com conhecimentos gerais, e episódicas, que se relacionam com eventos ocorridos (Izquierdo *et al.*, 2002; Riedel e Blokland, 2015).

1.2.1 Memória de reconhecimento

A memória de reconhecimento é um tipo de memória discriminativa, ou seja, uma memória relacionada a fatos e eventos que podem ser conscientemente recordados (Hart *et al.*, 1985; Riedel e Blokland, 2015). Um exemplo seria aquela memória que frequentemente falamos: “Não lembro, mas quando eu ver vou lembrar”. Em outras palavras, é a capacidade de reconhecer pessoas, objetos e lugares os quais já fomos apresentados antes, sendo essa uma função cognitiva vital para o convívio social e ambiental (Bird, 2017). A memória de reconhecimento não é um processo unitário, pois distintos tipos de informações são usados para sua formação, como a familiaridade relativa com um objeto ou local, ou quando e onde o objeto foi encontrado anteriormente (Barker e Warburton, 2011).

A memória de reconhecimento, por ser um tipo de memória muito importante, é amplamente testada em estudos científicos que envolvem déficits cognitivos, podendo ser testada inclusive em estudos com modelos animais (Hart *et al.*, 1985; Mello, P. B. *et al.*, 2008; Da Silveira *et al.*, 2013; Vargas *et al.*, 2014). Dentre os testes realizados em roedores, está o teste de reconhecimento de objetos e suas variações (Riedel e Blokland, 2015). Nesse tipo de teste é avaliada a capacidade do animal de diferenciar um objeto familiar, que já foi apresentado anteriormente, de um objeto novo. Já o teste de reconhecimento social, avalia se o animal consegue reconhecer outro animal que foi apresentado a ele anteriormente, em comparação com um animal não familiar. Os animais que vivem em sociedades utilizam a memória social para exibir os comportamentos sociais apropriados, como agressão, aversão, comportamento cooperativo, e até comportamento de acasalamento (Riedel e Blokland, 2015; Okuyama, 2018). Ao longo da hierarquia de formação da memória, a memória social é também a

mais complexa, uma vez que sua formação integra outros tipos de memória, como a memória olfativa, a memória espacial (onde) e à memória temporal (quando) (Okuyama, 2018), enquanto um objeto dificilmente possui odor, e para reconhecê-lo temos que associá-lo ao lugar e tempo.

As principais estruturas cerebrais envolvidas no processo de memória são a amígdala, a formação hipocampal, giro denteado, região CA do hipocampo (CA1, CA2 e CA3), subículo (S), e os córtices entorrinal, perirrinal e para-hipocampal (Ramón y Cajal, 1893). Essas estruturas formam um circuito (Figura 4) com projeções aferentes e eferentes (Strange *et al.*, 2014). Existem divisões funcionais para cada estrutura envolvida no processamento da memória. O hipocampo processa principalmente informações espaciais e contextuais, a amígdala informações de origem emocionais e aversivas, e o córtex perirrinal principalmente a memória discriminativa. O córtex entorrinal processa vários tipos de memória e possivelmente integra as informações processadas pela amígdala e hipocampo (Kesner e Dimattia, 1987; Squire *et al.*, 2004).

Na figura 2 já mostramos a progressão da DA de acordo com a deposição das placas amiloides. As primeiras estruturas a serem comprometidas são o hipocampo e as regiões adjacentes, o que explica os sintomas do início da doença e sua progressão (Masters *et al.*, 2015). No início da DA, o paciente já apresenta déficit na MT e na MC, enquanto que a ML permanece intacta até os pacientes apresentarem um quadro mais avançado da doença. No quadro mais avançado, os pacientes apresentam perda da organização da memória semântica, e por isso há também uma dificuldade na fluência verbal (Balouch e Rusted, 2014; Sun e Alkon, 2014). A memória implícita, utilizada para realizar tarefas com bases em experiências anteriores, que não requer a evocação intencional e é subconsciente, como por exemplo, o ato de dirigir ou levar uma colher a boca, é o tipo de memória que mais demora a sofrer consequências da DA. Nesse

sentido, ela é utilizada como estratégia compensatória nas atividades da vida diária (AVD) de modo a neutralizar os déficits da memória explícita (MT, MC e ML) (Machado *et al.*, 2009).

1.3 Neuroproteção

Neuroproteção é definida como qualquer intervenção capaz de prevenir morte neuronal, podendo ser farmacológica ou não farmacológica (Whitcup, 2008). No entanto, quando se trata de doenças neurodegenerativas que ainda não tem cura, como a DA, as intervenções neuroprotetivas não farmacológicas são abundantemente investigadas (Lalkovičová e Danielisová, 2016). Estratégias como suplementação com produtos naturais (Heber *et al.*, 2014; Tewari *et al.*, 2014; Martins *et al.*, 2017) e exercício físico são instrumentos utilizados como neuroprotetores em estudos com o objetivo de prevenir déficits de doenças crônicas do SNC (Rolland *et al.*, 2010; Marques-Aleixo. *et al.*, 2012; Schimidt, H. *et al.*, 2014). O foco principal dessas estratégias são proporcionar um maior aporte antioxidante exógeno e endógeno, prevenindo, principalmente, o aumento de espécies reativas de oxigénio (EROs) e, consequentemente, o estresse oxidativo e inflamação, mecanismos importantes envolvidos no desenvolvimento e progressão de doenças crônicas.

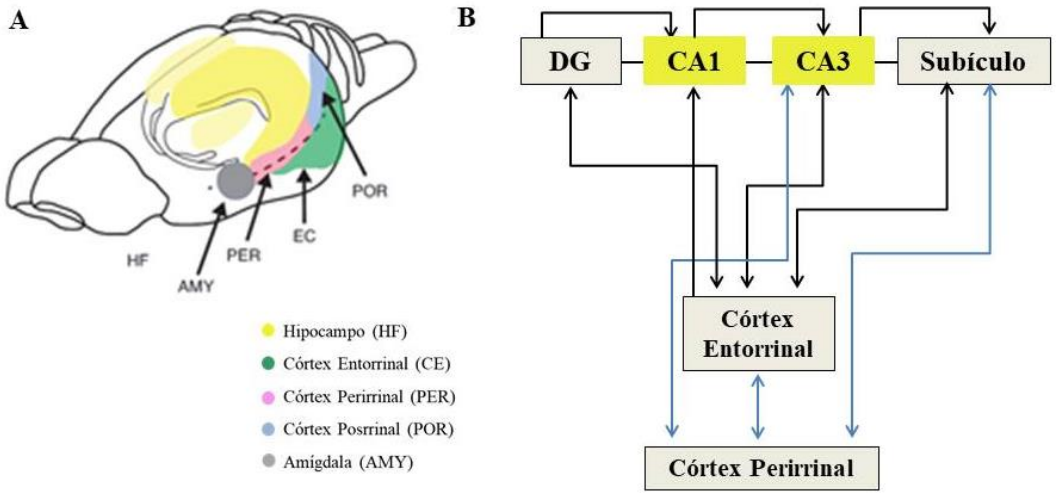

Figura 4. A. Estruturas neurais envolvidas nos processos de memória. B. Circuitos neurais de aferências e eferências relacionados aos processos de memória. Em preto, as comunicações referentes a processos de memória em geral. Em azul, as comunicações referentes a memória de reconhecimento (Kesner e Dimattia, 1987; Squire *et al.*, 2004; Strange *et al.*, 2014).

As espécies reativas de oxigénio (EROs) são responsáveis por um conjunto de processos oxidativos, como a peroxidação lipídica, oxidação proteica e dano no ácido desoxirribonucleico celular (DNA) (Ahmad *et al.*, 2017). As defesas antioxidantes para proteção das membranas e de outras estruturas celulares, atuam na remoção e decomposição das EROs. Na DA há um desiquilíbrio entre as defesas antioxidantes e a produção de EROs, um processo de estresse oxidativo (Giraldo *et al.*, 2014). O SNC é particularmente vulnerável ao estresse oxidativo, uma vez que, para além de elevadas taxas de consumo de oxigênio e abundante conteúdo lipídico, parece apresentar sistemas antioxidantes menos eficientes comparativamente a outros tecidos (Zlatkovic *et al.*, 2014).

As defesas antioxidantes podem ser endógenas que são produzidas pelo nosso próprio organismo, como por exemplo temos a superóxido dismutase (SOD), catalase,

coenzima Q10 (CoQ10), o ácido alfa-lipóico (ALA), glutationa peroxidase (GSH-Px) e a glutationa (GSH), sendo esta última considerada o principal antioxidante endógeno. A SOD, a catalase e a GSH-Px atuam em conjunto na conversão dos radicais livres em elementos neutros, como água e oxigénio. A GSH consegue ainda reparar danos celulares, e em conjunto com o ALA e CoQ10 regenera e reutiliza antioxidantes endógenos(Lalkovičová e Danielisová, 2016). Já os antioxidantes exógenos são aqueles que podem ser adquiridos por meio da alimentação. Também ajudam a minimizar e bloquear os danos causados pelos radicais livres (Lalkovičová e Danielisová, 2016; Ahmad *et al.*, 2017).

A medida que envelhecemos a capacidade de o nosso organismo de produzir antioxidantes endógenos diminui, por isso muitas pesquisas tem focado em buscar potenciais antioxidantes exógenos que atuem na proteção e prevenção da DA (Liguori *et al.*, 2018). A suplementação com vitaminas C e/ou E, e mais recentemente com os polifenóis encontrados no vinho tinto e alguns chás são os compostos mais investigados como possíveis neuroprotetores da DA (Lloret *et al.*, 2009; Albarracin *et al.*, 2012; Granzotto e Zatta, 2014; Monacelli *et al.*, 2017).

1.3.1 *Camellia Sinensis*

A *Camellia sinensis* (família *Theaceae*) é uma planta originária dos países do leste asiático (Banerjee e Chatterjee, 2015). Os chás ou infusões preparadas a partir das folhas vaporizadas e secas da *Camellia sinensis*, são amplamente utilizados em todo o mundo em suas variedades como chá verde, chá preto e chá vermelho (Heber *et al.*, 2014; Banerjee e Chatterjee, 2015). Estas variedades de chás se dão devido às diferentes formas de cultivo da planta (clima, tipo de solo e outras condições de crescimento),

tempo de colheita, e processamento das folhas. Tanto o chá preto quanto o vermelho passam por um processo de fermentação, o que não acontece com o chá verde. A figura 5 mostra um desenho esquemático do processo de fabricação do chá verde, chá vermelho e chá preto.

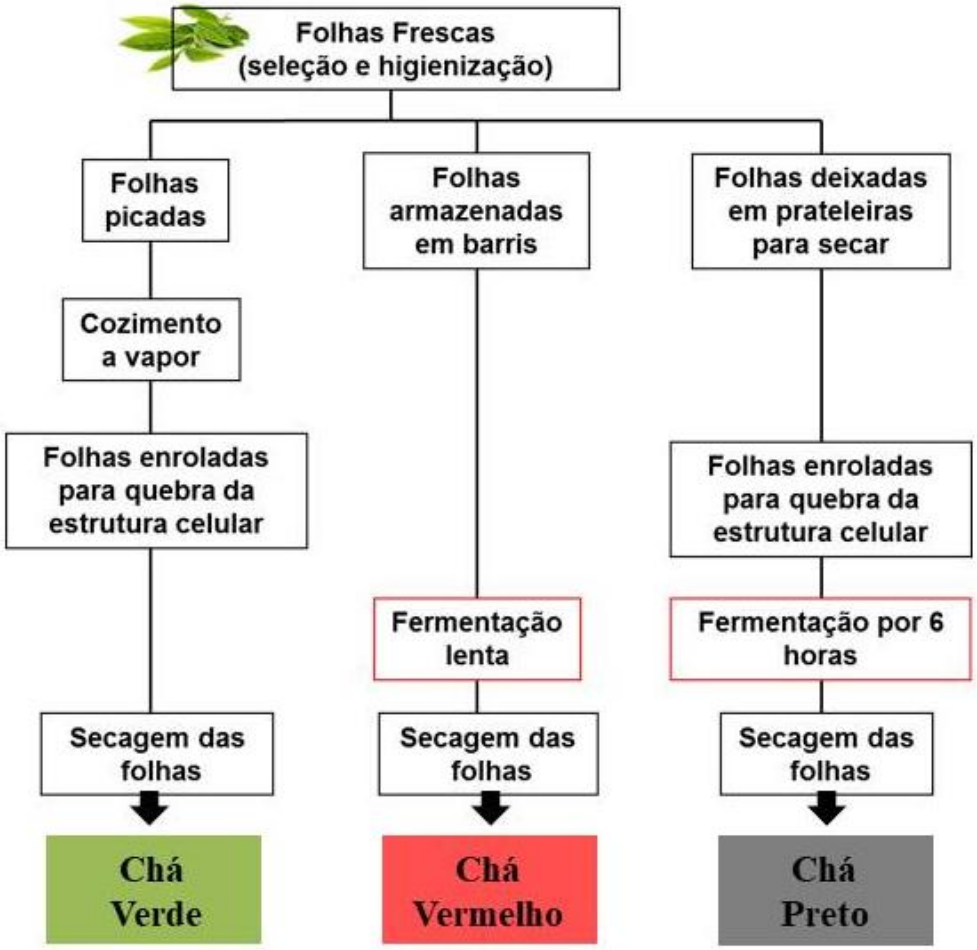

Figura 5. Desenho esquemático do processo de produção dos três principais chás provenientes da *Camellia sinensis,* o chá verde, chá vermelho e chá preto.

A composição dos chás oriundos da *Camellia sinensis* é feita por carboidratos celulósicos, proteínas e lipídios. Uma vez que são insolúveis, eles não se tornam parte da bebida de chá a partir da infusão, apenas componentes de baixo peso molecular, tais como polifenóis, cafeína, teobromina, vitamina C, metais e íons fluoreto passam à infusão.

Os benefícios atribuídos a *Camellia sinensis* estão relacionados com a presença de catequinas. As catequinas são polifenóis que atuam como antioxidantes, neutralizando radicais livres. As principais catequinas encontradas na infusão da planta são epigalocatequina galato (EGCG), epigalocatequina (EGC), epicatequina galato (ECG), epicatequina (EC) e catequina (C) (Soares *et al.*, 2013). Essas catequinas são consideradas agentes preventivos e terapêuticos com capacidade de alterar o envelhecimento cerebral e a progressão de doenças, sendo também capazes de diminuir a toxicidade e os sinais pró-apoptóticos (Assuncao *et al.*, 2011).

Na DA, o extrato da *Camellia sinensis* tem ação antioxidante e impede a toxicidade da proteína β-amilóide atuando como neuroprotetor (Weinreb *et al.*, 2004). No entanto, a maioria dos estudos utiliza apenas o chá verde, ou ainda, as catequinas isoladas da *Camellia sinensis*. Com isso, desconsidera-se o fato de que há uma grande variedade de chás obtidos a partir das folhas novas da planta *Camellia sinensis*, com variações no processamento e na composição, o que pode repercutir em diferentes efeitos neuroprotetores (Rezai-Zadeh *et al.*, 2008; Park *et al.*, 2009; Biasibetti *et al.*, 2013).

1.3.2 Exercício físico

Da mesma forma que a suplementação com produtos naturais, o exercício físico tem sido bastante citado em trabalhos que buscam neuroproteção (Cancela *et al.*, 2016; Morris *et al.*, 2017; Ozbeyli *et al.*, 2017; Sun *et al.*, 2018). A neuroproteção pelo exercício está relacionada com o fato de que o exercício físico estimula a neurogênese e melhora níveis de antioxidantes no cérebro, corroborando um controle mais efetivo da produção de radicais livres (Schimidt, H. *et al.*, 2014; Nokia *et al.*, 2016; Sun *et al.*, 2018). Além disso, o exercício físico é capaz de reduzir a resposta inflamatória, promover a função capilar, inibir a supraexpressão de glutamato, e combater a apoptose neural em doenças neurodegenerativas (Yang *et al.*, 2012; Nokia *et al.*, 2016).

No entanto, a maioria dos estudos sobre o papel neuroprotetor do exercício físico investiga o exercício aeróbico, sendo o papel do exercício de força ainda pouco explorado (Ozbeyli *et al.*, 2017). Por exemplo, em estudos com roedores, os dois principais modelos experimentais de atividade física são atividades aeróbicas voluntárias, como atividades em rodas de correr e ambientes enriquecidos e atividades aeróbicas não voluntárias, como a natação e a corrida em esteira (Aguiar Jr. e Pinho, 2007).

Dessa forma, o exercício físico aeróbico é apontado como protetor de doenças relacionadas ao SNC (Pietrelli *et al.*, 2012; Alves *et al.*, 2014; Cancela *et al.*, 2016; Morris *et al.*, 2017). Há estudos que indicam benefícios, inclusive agudos, de uma única sessão de exercício aeróbico. Com uma única sessão de exercício físico de intensidade moderada, onde o VO_2 atinge no máximo 70%, há um aumento do fluxo de sangue no cérebro, o que representa maior oferta de oxigênio e, por conseguinte, maior aporte energético, favorecendo o desempenho cognitivo (Alves *et al.*, 2014). Também, uma

única sessão de exercício físico aeróbico é capaz de elevar a síntese de neurotransmissores sinápticos tais como noradrenalina, β-endorfina e a própria dopamina (Alves *et al.*, 2014; Vargas *et al.*, 2017).

De forma crônica, o exercício aeróbico de intensidade moderada contribui para neuroplasticidade, com proliferação de novos capilares cerebrais, neurogênese e surgimento de novas conexões sinápticas em regiões cerebrais responsáveis pela memória e cognição (Nokia *et al.*, 2016; Morris *et al.*, 2017). Participam destas alterações importantes hormônios, como fator de crescimento semelhante à insulina tipo 1 (IGF-I) e fator de crescimento endotelial vascular (VEGF), que tem a função de estimular o crescimento neuronal (Merege Filho *et al.*, 2014).

Quando se fala em capacidade de neuroproteção do exercício físico, é muito importante considerar a intensidade do esforço. No exercício aeróbico, que deve ser de longa duração, a alta intensidade pode provocar um quadro de fadiga sistêmica, resultando tanto na queda do desempenho físico quanto cognitivo, uma vez que a fadiga está associada à redução do aporte sanguíneo e energético cerebral, bem como supressão da síntese de neurotransmissores (Tomporowski, 2003). Já no exercício anaeróbico e de força, que pode ser realizado com intervalos de descanso, a alta intensidade não prejudica a memória de curto prazo, e pode até mesmo melhorar a velocidade de processamento, uma vez que as pausas ativas realizadas entre os esforços minimizam o quadro de fadiga, evitando os prejuízos na cognição (Alves *et al.*, 2014).

Além do mencionado até aqui, é importante destacar a ação do exercício físico sobre o estresse oxidativo (Cechetti *et al.*, 2008). O exercício físico, tanto aeróbico quanto de força, promove de forma aguda um aumento no estresse oxidativo, que de forma geral é controlado pelo sistema antioxidante do organismo. Como resposta crônica à elevação da concentração de espécies reativas de oxigênio, ocorre uma

melhora na atuação do sistema antioxidante protegendo o sujeito de doenças que estão relacionadas ao estresse oxidativo (Teixeira-Lemos *et al.*, 2011).

Na DA o exercício físico atua como neuroprotetor, retardando o declínio cognitivo e o progresso da doença. Ainda não há um consenso na literatura sobre qual a melhor modalidade de exercício físico para a prevenção de doenças relacionadas ao envelhecimento. Enquanto que o exercício aeróbico é capaz de melhorar a função cognitiva e prevenir a perda de memória em pacientes com DA. Adicionalmente, sabemos que pacientes com DA têm perda muscular associada ao envelhecimento e que se acentua com a inatividade física correspondente a doença, o que pode ter consequências como o déficits de equilíbrio, coordenação e maior risco de quedas. Assim, o exercício com o objetivo de ganho de força e manutenção ou aumento de massa muscular, é recomendado para essa população. Dessa forma, buscar benefícios do exercício de força também sobre o sistema nervoso é importante (Pizzigalli *et al.*, 2011; Menant *et al.*, 2016; Lopez *et al.*, 2017).

2 JUSTIFICATIVA

Assim, sendo a DA uma patologia de difícil tratamento e de ordem progressiva, buscar estratégias de proteção da doença é muito importante, e utilizar modelos animais da doença associada a fatores de risco aproxima ainda mais da realidade em humanos. No contexto da prevenção, é importante considerar estratégias que são comuns e acessíveis a toda população, tais como o exercício físico e a utilização de chás. Considerando a combinação de intervenções, o exercício físico tem sido associado à ingestão de chá verde, não de outros chás provenientes da *Camellia sinensis*, para diminuir o dano muscular induzido pelo exercício (Da Silva *et al.*, 2018). Entretanto, no que diz respeito à cognição, os efeitos bioquímicos e comportamentais da combinação do exercício e chá foram investigados pelo nosso grupo em um modelo de envelhecimento e de acidente vascular cerebral, mas não em modelos de DA (Flores *et al.*, 2014; Schimidt, H. *et al.*, 2014). Já foi previamente sugerido que os efeitos do exercício físico sobre o SNC podem ser potencializados pelo consumo de produtos naturais, tais como os polifenóis, uma vez que estas intervenções envolvem efeitos em vias celulares similares que são importantes para neurogênese, sobrevivência celular, plasticidade sináptica e função vascular (Haramizu *et al.*, 2013; Walker *et al.*, 2015).

3 OBJETIVOS

3.1 Objetivo Geral

Determinar os efeitos neuroprotetores da *Camellia sinensis* e do exercício físico aeróbico e de força sobre déficits cognitivos e funcionais em modelos animais da DA.

3.2 Objetivos Específicos

- Verificar os efeitos dos chás provenientes da *Camellia Sinensis* sobre o dano cognitivo e oxidativo induzido pela injeção de ß-amilóide;
- Investigar qual dos chás provenientes da *Camellia Sinensis* tem maior efeito neuroprotetor para déficits resultantes de um modelo de DA;
- Investigar o efeito da dieta de cafeteria sobre o dano cognitivo e oxidativo induzido pela injeção de ß-amilóide;
- Investigar o efeito da dieta de cafeteria associado ao chá com maior potencial neuroprotetor sobre os déficits resultantes de um modelo de DA;
- Verificar o efeito do exercício físico aeróbico e de força sobre o dano cognitivo e oxidativo induzido pela injeção de ß-amilóide;
- Comparar os efeitos do exercício físico aeróbico e de força sobre os déficits resultantes de um modelo de DA;

CAPÍTULO II

ARTIGO ORIGINAL

GREEN TEA SUPPLEMENTATION PRODUCES BETTER NEUROPROTECTIVE EFFECTS THAN RED AND BLACK TEA IN ALZHEIMER-LIKE RAT MODEL

Short title: Neuroprotection in Alzheimer-like disease

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Highlights

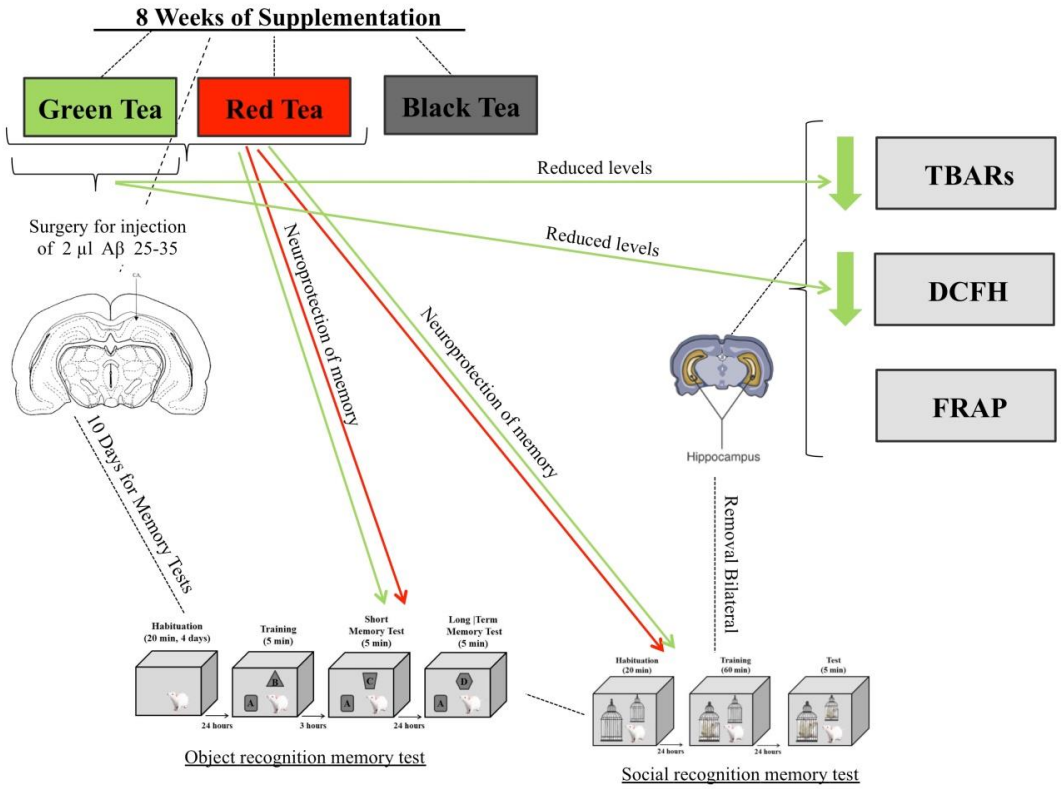
- Teas from *Camellia Sinensis* present different neuroprotective potentials.
- Green and red teas avoid deficits in the short and long-term memories in AD rats.
- Green tea avoids oxidative damage in the hippocampus.
- Green tea presents the highest content of EGCG.

Abstract

Green tea from Camellia sinensis plays a neuroprotective role in different neurodegenerative conditions, such as memory deficits in Alzheimer disease (AD). However, whether other teas from Camellia sinensis present similar neuroprotective effect still is not clear. Here we investigate effects of green, red and black tea supplementation on memory and hippocampus oxidative status in a rat model of Alzheimer-like disease (AD-like). Method: Wistar male rats were supplemented with green, red or black tea during 8 weeks before Aβ intra-hippocampal injection (2µL of Aβ-25-35, CA1 region). AD and sham rats were submitted to memory tests. After euthanasia, oxidative status in the bilateral hippocampus was quantified. Green and red teas avoid memory deficits in AD rats, but only green tea also avoids oxidative stress and damage in the hippocampus. Green tea was more effective for neuroprotection than red and black teas from the Camellia sinensis in the AD rat model.

Keywords: epigallocatechin gallate; Camellia sinensis; oxidative stress; brain; memory; diet.

Graphical abstract



1 Introduction

The Alzheimer disease (AD) is a progressive neurodegenerative disorder characterized by declines in memory and cognition (Jack et al., 2016). AD is a major disease and a socio-economic challenge, with estimation of 100 million cases worldwide by the year 2050 (Z. Liu et al., 2015). There is no current cure for AD and its origin still not elucidated, although some contributing factors have been described. One of them is the accumulation of Amyloid β (Aβ) plaques in the brain (Jack et al., 2016; Zuo, Hemmelgarn, Chuang, & Best, 2015) leading to synaptic depression, abnormal network activity (Jack et al., 2016) and excessive production of reactive oxygen species

(ROS) (Zuo et al., 2015). The high oxidative metabolic activity in the brain and intense production of ROS leads to the generation of free radicals that are involved in chronic neurodegenerative diseases (Ayyappan, Palayyan, & Kozhiparambil Gopalan, 2016). This oxidative stress condition promotes progressive neuronal loss, predominantly by apoptosis (Z. Liu et al., 2015) and contributes to the onset of memory deficits (T. Liu, Liu, Xiao, & Shi, 2016; Yi, Park, Kim, Kim, & Ryu, 2016). Recognition memory, which is one type of explicit memory (T. Liu et al., 2016), is important to perform daily activities and depends on the integrity of the hippocampus (Yi et al., 2016). The hippocampus is vulnerable to oxidative stress (Zuo et al., 2015); thus, improving antioxidant defenses may prevent the memory deficits resulting from AD (T. Liu et al., 2016; Yi et al., 2016).

The Camellia sinensis (family Theaceae) has strong antioxidant properties (Soung, Wang, Tseng, Fang, & Chang, 2015; Yasmeen & Hasnain, 2015). The antioxidant mechanism is related to the ability of their catechins to sequester free radicals (Yasmeen & Hasnain, 2015). The green tea obtained from Camellia sinensis has a neuroprotective role in memory deficits resulting from aging (Flores et al., 2014), ischemia-reperfusion (Flores et al., 2014) and AD in transgenic mice (Rezai-Zadeh et al., 2008). Green tea has the potential to work as free radical species scavenger (Okello, McDougall, Kumar, & Seal, 2011; Zuo et al., 2015), most likely due to the presence of polyphenols (Banerjee & Chatterjee, 2015). Polyphenols neutralize the excess of free iron by acting as chelating and leading to suppression of amyloid precursor protein (APP), which plays an important role in AD. The neuroprotective role of Camellia sinensis was investigated in other teas processed from the same plant but showing different results. These differences were argued to be a consequence of different

amounts of polyphenols, especially catechins (Banerjee & Chatterjee, 2015), between teas (Heber et al., 2014; Soares et al., 2013).

Green tea extract was shown to protects human red blood cells against oxidative stress, but black or white tea extracts did not show the same effect (Gawlik & Czajka, 2007). Both green and black tea increase antioxidant capacity, but green tea is six-fold more potent than black tea (Serafini, Ghiselli, & Ferro-Luzzi, 1996). The presence of epigallocatechin gallate (ECGC) might be the key factor in determining the neuroprotective role of green tea in brain diseases and cognitive performance (Xicota, Rodriguez-Morato, Dierssen, & de la Torre, 2015). The processing of Camellia sinensis by fermentation results in different teas (Banerjee & Chatterjee, 2015). Green tea is not fermented, whereas red tea is partially fermented and black tea is completely fermented (Okello et al., 2011; Soares et al., 2013). Fermentation influence catechins contents (Banerjee & Chatterjee, 2015) and, therefore, the antioxidant outcomes may differ among different teas. To be best of our knowledge, the neuroprotective potential of different teas obtained from Camellia sinensis has not been tested. Here we investigated the neuroprotective potential of green, red and black teas obtained from Camellia sinensis in rats that had an AD-like disease. Our results showed that green tea has a better neuroprotective effect on memory deficits and hippocampal oxidative status than black and red teas.

2 Material and methods

2.1 Animals and experimental design

Two months old male Wistar rats bought from the Federal University of Santa Maria/RS/Brazil Central Vivarium were housed three per cage under controlled light

and environmental conditions (12 h light/dark cycle at 23 ± 2°C and humidity 50 ± 10%) with food and water or tea ad libitum. Experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH, 1996) and the Local Institution Animal Care and Use Committee (IRB #012015). The rats were randomly assigned to 4 groups: (a) control: not supplemented; (b) supplemented with green tea; (c) supplemented with red tea, and (d) supplemented with black tea. After 8 weeks, sham or AD-like surgeries (see details below) were performed and groups were reorganized (n = 8-12/group), as follow:

- group 1 – Sham: submitted to the sham surgery receiving saline injection;
- group 2 – Green tea and sham: supplemented with green tea before sham surgery;
- group 3 – Red tea and sham: supplemented with red tea before sham surgery;
- group 4 – Black tea and sham: supplemented with black tea before sham surgery;
- group 5 – AD-like: submitted to surgery with hippocampal Amyloid β injection;
- group 6 – Green tea and AD-like: supplemented with green tea before surgery with hippocampal Amyloid β injection;
- group 7 – Red tea and AD-like: supplemented with red tea before surgery with hippocampal Amyloid β injection;
- group 8 – Black tea and AD-like: supplemented with black tea before surgery with hippocampal Amyloid β injection.

Ten days after surgery, the time necessary for beta-amyloid aggregation and plaque formation (Maurice, 2016), rats were submitted to behavioral tests and

euthanized. Biochemical analyses were performed in the bilateral hippocampus to determine levels of reactive oxygen species (ROS), lipid peroxidation by thiobarbituric acid reactive substances (TBARS), and the total antioxidant capacity by ferric reducing/antioxidant power (FRAP). Figure 1 depicts the experimental design.

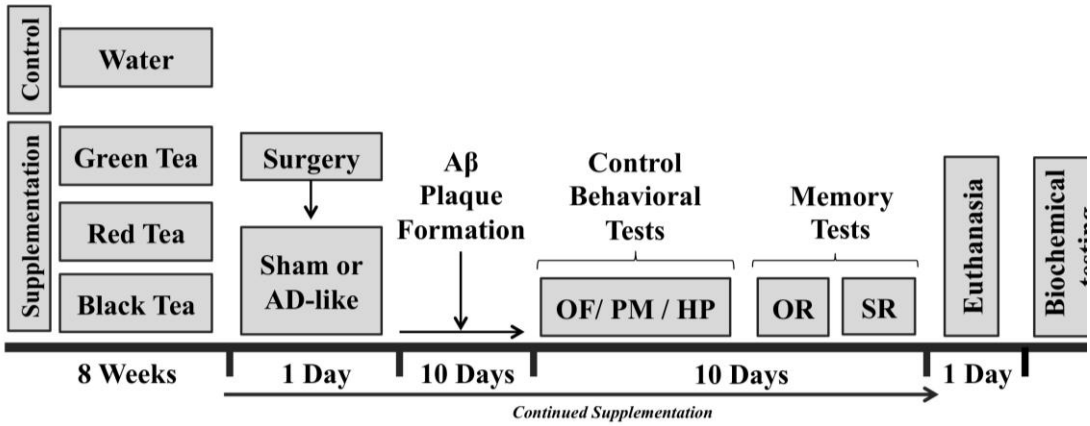


Figure 1. Experimental design. Rats were supplemented with green, red or black tea during 8 weeks. Behavioral testing started 10 days after surgery. Euthanasia occurred 20 days after surgery; biochemical testing was the last step of the study. OF – open field; PM – plus maze; HP – hot plate; OR – object recognition memory test; SR – social recognition memory test.

2.2 Tea Supplementation and Chromatography Analysis

Rats received green, red or black tea. The teas were acquired all in the same season and the same production batch from the local market (Madrugada Alimentos LTDA/ Venâncio Aires/RS/Brazil) as used in previous researches (Martins et al., 2017; Schimidt et al., 2014; Sosa et al., 2015). Teas were prepared daily by mixing 13.33g of dry tea extract in one liter of filtered water at 90°C in the concentration of 1,333 mg/mL (Martins et al., 2017; Schimidt et al., 2014; Sosa et al., 2015). Teas were administered at ambient temperature (23 ± 2°C) as a substitute for drinking water, for free consumption.

Tea intake volume per box home was monitored daily. To calculate the average daily intake per rat, the total consumption in each box home was divided by the number of rats in each box home. Supplementation continued until the day of euthanasia.

The presences of Epigallocatechin (EGC), Epicatechin (EC), Epigallocatechin gallate (EGCG) and Epicatechin gallate (ECG) were determined by high-performance liquid chromatography (HPLC) (see Table 1 for details). HPLC was performed with a Shimadzu Prominence Auto Sampler (YL9100) HPLC system (Shimadzu, Kyoto, Japan), equipped with Shimadzu YL9110 reciprocating pumps connected to an YL9101 degasser with an YL9150 integrator, and YL9160 diode array detector. To determine compounds profile the extracts were analyzed using a reversed phase carried out under gradient conditions using Synergi Fusion-RP 80A column (4.6 × 250 mm). The mobile phase was composed of water (pH = 3): acetonitrile (5:95, v/v) in a gradient mode, until 35 min, in which the mobile phase was 100 % acetonitrile. At 38 min water (pH = 3): acetonitrile (5:95, v/v) was used again, in isocratic mode, as a mobile phase, until 50 min. A flow rate of 0.8 mL/min was used and 20 μL of sample were injected. Phenolic compounds were identified and quantified by comparing the retention time and UV–Visible spectral data to known previously injected standards. The chromatography peaks were confirmed by comparing the retention time with those of reference standards and by DAD spectra. Calibration curves were determined for EGC (y = 101,79x - 10,283); EC (y = 91,872x + 7,657); EGCG (y = 103,5x - 93,211); ECG (y = 112,17x - 81,22). All chromatography operations were carried out at ambient temperature and in triplicate.

2.3 Preparation of Amyloid β 25-35

β-Amyloid peptide (25-35) (Sigma Aldrich; product number: A4559) was dissolved in saline (vehicle) at a concentration of 100μM. Before intrahippocampal injection, the Aβ was incubated at 37°C during 4 days (in vitro) to induce Aβ 25-35 aggregation (Ghasemi, Zarifkar, Rastegar, Maghsoudi, & Moosavi, 2014).

2.4 Surgery

The stereotaxic surgeries for intrahippocampal injection of 2 μL Aβ 25-35 (groups 5-8) or vehicle (groups 1-4) were performed after supplementation. Rats were anesthetized with ketamine and xylazine (i.p. 75 mg/kg and 10 mg/kg, respectively). When the anesthetic plan was confirmed, rats were mounted into a stereotaxic frame and the amon horn 1 (CA1, from Cornu Ammonis) region of the dorsal hippocampus was located based on the Paxinos brain atlas (AP - 4.2, LL ± 3.0, VM - 2.0 mm) (Paxinos, Watson, & Emson, 1980). Bilateral infusions were performed using a Hamilton syringe and an infusion bomb (Limon et al., 2012). After surgery, rats were returned to their cages and monitored during recovery.

2.5 Behavioral control tests

Exploratory and locomotor activities were assessed to ensure injection did not impair such behaviors, 10 days after surgery rats were placed in the left quadrant of a 50 × 50 × 39 cm open field (OF) made with wooden painted white, with a frontal glass wall. Black lines were drawn on the floor to divide the arena into 12 equal quadrants.

Crossing and rearing, as measures of locomotor and exploratory activities, respectively, were measured over 5 min (Bonini et al., 2006). Anxiety state was analyzed using an elevated plus maze. Time spent and the total number of entries into the open arms were recorded over 5 min (Pellow, Chopin, File, & Briley, 1985). Nociception, as a measure of peripheral sensibility, was assessed using a hot plate at a constant temperature of 55 ± 0.5 °C and the latency for paw withdrawal was determined (Y. Zhang, Sun, Meng, Guo, & Chen, 2016).

2.6 Object recognition memory

Object recognition task (OR) training and testing were performed in an open-field arena (50 × 50 × 50 cm) built with polyvinyl chloride plastic, plywood and transparent acrylic (Ennaceur & Delacour, 1988). Rats were habituated to the apparatus during 20 min of free exploration in 4 consecutive days. For training, two different objects (A and B) were placed in the apparatus and rats were allowed to freely explore them during 5 min. Exploration was defined as sniffing or touching the objects with the nose and/or forepaws. Short-term memory test (STM) was performed 3 h after training. During the STM test, one of the objects was randomly changed for a novel one (C) and the rats were reintroduced into the apparatus to freely explore the objects (known and novel) during 5 min. The long-term memory test (LTM) was performed 24h after the STM. Object C was changed for a novel object (D) and the rats were reintroduced into the apparatus to freely explore (known and novel) during 5 min. To avoid confounds by lingering olfactory stimuli and preferences, the objects and the arena were cleaned with 70% ethanol after each test. The discrimination index (Martins et al., 2017) was determined by the difference of time spent exploring the new (Tnovel) and the familiar

(Tfamiliar) objects: [(Tnovel - Tfamiliar)/(Tnovel + Tfamiliar) x 100 (%)], and used as a memory parameter.

2.7 Social recognition memory test

The social recognition memory test was completed in three days. In the first day, the rats were placed in an arena with two small cages during 20 min for free exploration (habituation). In the second day, training was performed with the inclusion of an unfamiliar rat in the cages for 1 hour of free exploration. After 24 hours, testing was performed with the same rat from training (familiar rat) and a new rat was placed for exploration during 5 minutes. The time spent exploring the new and familiar rats was recorded. Exploration was defined as sniffing or touching the small cages with the nose and/or forepaws. The discrimination index (Martins et al., 2017) was determined by the difference of time spent exploring the new (Tnovel) and the familiar (Tfamiliar) rats: [(Tnovel - Tfamiliar)/(Tnovel + Tfamiliar) x 100 (%)], and used as a memory parameter.

2.8 Biochemical testing

2.8.1 Tissue preparation

Rats were euthanized 24 h after the behavioral experiments. The brain was removed and bilateral hippocampi were quickly dissected and homogenized in 50 mM Tris-HCl, pH 7.4, (1/5 or 1/10, w/v). Afterward, samples were centrifuged at 2400 g for 20 min and supernatants (S1) were used for the assay.

2.8.2 Reactive oxygen species (ROS)

Reactive oxygen species (ROS) content was assessed by a spectrofluorimetric method using 2′,7′-dichlorofluorescein diacetate (DCFH-DA) as a probe (Loetchutinat et al., 2005). The sample (S1) was incubated in darkness with 5 μL DCFH-DA (1 mM). The oxidation of DCHF-DA to fluorescent dichlorofluorescein (DCF) was measured for the detection of intracellular ROS. The formation of the oxidized fluorescent derivative (DCF), measured by DCF fluorescence intensity, was recorded at 520 nm (480 nm excitation), 30 min after the addition of DCFH-DA to the medium. Results were expressed as AU (arbitrary units).

2.8.3 Detection of TBARS level

Lipoperoxidation was evaluated by the thiobarbituric acid reactive substance (TBARS) test (Ohkawa, Ohishi, & Yagi, 1979). One aliquot of S1 was incubated with a 0.8% thiobarbituric acid solution, acetic acid buffer (pH 3.2) and sodium dodecyl sulfate solution (8%) at 95° C for 2 h, and the color reaction was measured at 532 nm. Results were expressed as nmol of malondialdehyde (MDA) per mg protein.

2.8.4 Ferric reducing/antioxidant power (FRAP) assay

The working FRAP reagent was prepared by mixing 25 ml acetate buffer, 2.5 ml 2,4,6-Tris(2-pyridyl)-s-triazine(TPTZ) solution, and 2.5 ml FeCl3.6H2O solution. A volume of 10 μL of homogenate was added in the 300 μL working FRAP reagent in microplate (Benzie & Strain, 1996). Additionally, a standard curve with 10 μL Trolox

concentrations of 15, 30, 60, 120 e 240 mM more 300 μL working FRAP reagent was used. The microplate was incubated at 37° C for 15 min before reading in SpectraMax M5 Microplate Reader at 593 nm.

2.9 Statistical analysis

Data are reported as mean and standard deviation. Normality of data distribution was confirmed using the Shapiro-Wilk test. The daily intakes of water and tea were compared between the groups using ANOVA one-way. The weight of each animal was measured at the start and end of supplementation and compared within groups using paired t-tests. Discrimination index for object and social recognition tests were compared using an ANOVA two-way (2 groups – sham and Alzheimer × 4 interventions – water, green, red and black teas) with Bonferroni correction for multiple comparisons. Where significant main effects or interactions were observed, t-tests or ANOVA one-way with Duncan post hoc were conducted to compare groups and interventions, respectively. OF, PM and HP tests results of all groups were compared using an ANOVA one-way. Biochemical results of all groups were compared using an ANOVA two-way (2 groups – sham and Alzheimer × 4 interventions – water, green, red and black teas) with Bonferroni correction for multiple comparisons. Where significant main effects or interactions were observed, t-tests or ANOVA one-way with Duncan post hoc were conducted to compare groups and interventions, respectively. The significance level was set at 0.05.

3 Results

Concentration (μg/mL) of catechins found in infusions of green, red and black teas were determined and results are shown in Table 1.

3.1 Fluid intake and body weight

Daily intake of water was 49.66 ± 3.02 ml, green tea 59.30 ± 4.87 ml, red tea 59.75 ± 0.59 ml, and black tea 68.41 ± 4.79 ml. Daily intake of water was significantly lower than the intake of any tea, and black tea intake was higher than red and green tea ($P < 0.01$; ANOVA one-way). Body weight did not differ between groups, before and after supplementation and control period ($P = 0.372$).

Table 1 - Concentration (µg/mL) of catechins found in infusions of green, red and black teas. Data of concentrations were obtained from HPLC analyses in comparison to a standard references solution.

	Green tea (13.33g/L)	Red tea (13.33g/L)	Black tea (13.33g/L)
(-)-Epigallocatechin (µg/mL)	213.68	ND	ND
(-)-Epicatechin (µg/mL)	191.15	181.36	570.98
(-)-Epigallocatechin gallate (µg/mL)	313.43	ND	ND
(-)-Epicatechin gallate (µg/mL)	86.95	ND	49.20

ND = not detected.

3.2 Object recognition memory test

A significant interaction was observed in object discrimination index for short-term recognition memory ($F(7,59) = 6.46$; $P = 0.0007$; Figure 2A). AD-like group showed a lower discrimination index for novel object compared to sham ($t(12) = 2.87$; $P = 0.01$; Figure 2A). Importantly, green and red teas avoided short-term memory deficits ($t(14) = 2.99$; $P = 0.009$ for green tea; $t(12) = 2.54$; $P = 0.02$ for red tea; Figure 2A), but black tea not ($t(16) = 0.31$; $P = 0.75$; Figure 2A).

In long-term object discrimination index a main effect for condition ($F(1,63) = 4.96$; $P = 0.002$; Figure 2B) and for treatment was observed ($F(3,63) = 3.37$; $P = 0.002$; Figure 2B). AD-like group showed a lower discrimination index for novel object compared to sham ($t(15) = 3.63$; $P = 0.002$; Figure 2B). Importantly, all teas avoided long-term memory deficits ($t(16) = 4.97$; $P = 0.0001$ for green tea; $t(12) = 4.23$; $P = 0.001$ for red tea; $t(17) = 2.12$; $P = 0.04$ for black tea; Figure 2B).

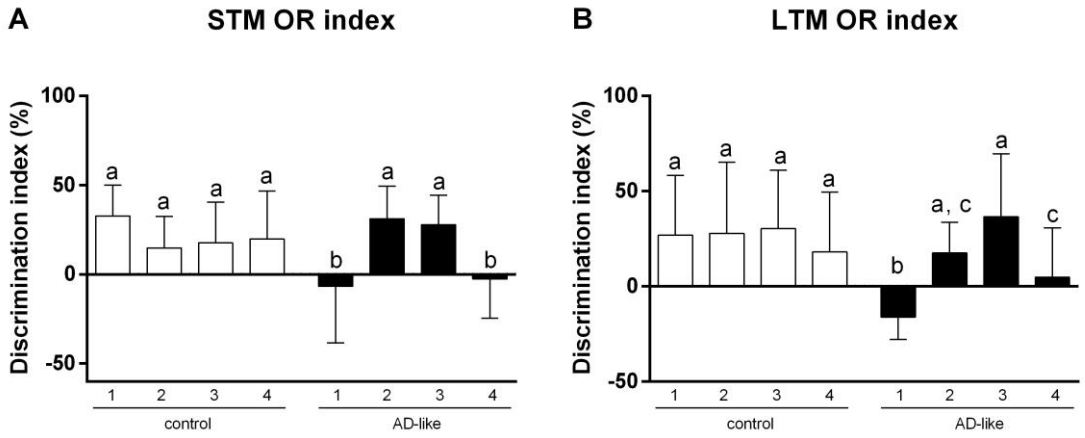


Figure 2. Supplementation with green and red tea protected object recognition memory, avoiding memory deficits in an AD-like rat model. A: short-term memory discrimination index (STM OR test); B: long-term memory discrimination index (LTM OR test). Results (mean ± standard deviation of the discrimination index) from the different experimental groups (1: sham; 2: green tea and sham; 3: red tea and sham; 4: black tea and sham; 5: AD-like; 6: green tea and AD-like: 7: red tea and AD-like; 8: black tea and AD-like). Different letters indicate significant difference considering $P < 0.05$; n = 8–10 per group.

3.3 Social recognition memory test

In social discrimination index a main effect for condition ($F(1,63) = 9.23$; $P = 0.003$; Figure 3) and for treatment was observed ($F(3,63) = 4.66$; $P = 0.005$; Figure 3). Additionally, a significant interaction was observed ($F(3,63) = 3.32$; $P = 0.02$; Figure 3). AD-like group showed a decrease in discrimination index for social recognition compared to sham group ($t(14) = 4.76$; $P = 0.0003$, Figure 2). Importantly, green and red tea avoided social recognition memory deficits ($t(14) = 4.14$; $P = 0.001$ for green tea; $t(12) = 4.42$; $P = 0.0008$ for red tea; Figure 2A), but black tea not ($t(16) = 1.94$; $P = 0.06$; Figure 2A).

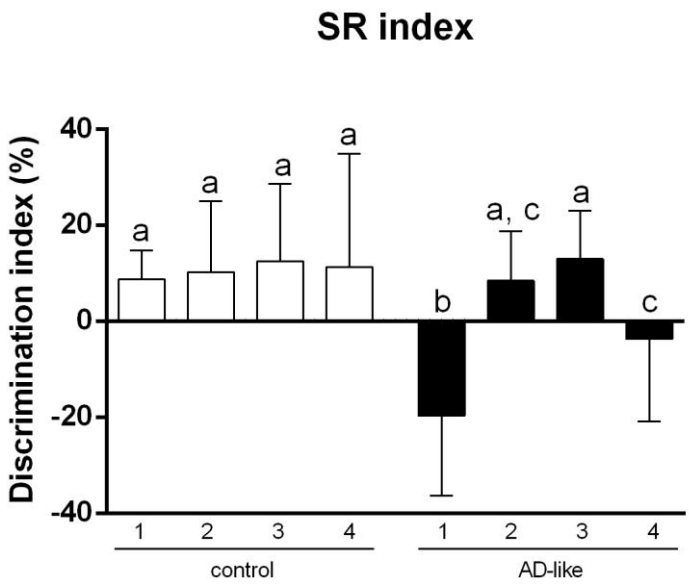


Figure 3. Supplementation with green tea and red tea showed a neuroprotective effect on social recognition memory deficit induced by AD-like rat model. Results expressed as mean ± standard deviation of the discrimination index from the different groups (1: sham; 2: green tea and sham; 3: red tea and sham; 4: black tea and sham; 5: AD-like; 6: green tea and AD-like; 7: red tea and AD-like; 8: black tea and AD-like). Different letters indicate significant difference considering $P < 0.05$; $n = 8–10$ per group.

3.4 Control behavioral tasks

The locomotor, exploratory activities, anxiety and pain threshold were not influenced by the AD-like model or tea supplementation (see Table 2 for numeric results).

3.5 Reactive oxygen species (ROS)

A main effect for condition (F(1,48) = 11.27; P = 0.001; Figure 4A) and for treatment was observed (F(3,48) = 4.70; P = 0.005; Figure 4A) on ROS measurement. AD-like rats showed increased ROS levels in the hippocampus (t(10) = 3.11; P = 0.01 Fig. 4). Green tea avoided ROS increase (t(10) = 3.31; P = 0.007 Fig. 4A), but red and black tea no (t(10) = 0.91; P = 0.38 for red tea; t(10) = 1.56; P = 0.14 for black tea; Fig. 4A).

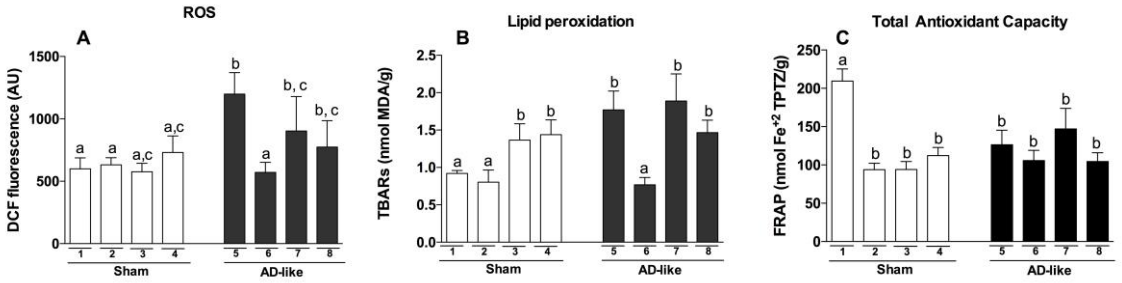


Figure 4. Supplementation with green tea showed a neuroprotective effect on hippocampal oxidative status induced by the AD-like model in rat. A: reactive oxygen species (ROS); B: lipid peroxidation; c: total antioxidant activity. Experimental groups: 1: sham; 2: sham and green tea; 3: sham and red tea; 4: sham and black tea; 5: AD-like; 6: AD-like and green tea: 7: AD-like and red tea; 8: AD-like and black tea. Different letters indicate significant difference considering P < 0.05; n = 8–10 per group.

3.6 Thiobarbituric acid reactive substances (TBARS)

A main effect for condition (F(1,68) = 7.22; P = 0.009; Figure 4B) and for treatment was observed (F(3,68) = 8.20; P < 0.0001; Figure 4B) on ROS measurement. Additionally, a significant interaction was observed (F(3,68) = 2.76; P = 0.04; Figure

4B). AD-like rats showed increased lipid peroxidation in the hippocampus ($t(17) = 3.53$; $P = 0.002$ Fig. 4B). Green tea avoided the lipid peroxidation increase ($t(22) = 4.36$; $P = 0.0002$ Fig. 4B), but red and black tea no ($t(13) = 0.27$; $P = 0.78$ for red tea; $t(13) = 0.90$; $P = 0.38$ for black tea; Fig. 4B). Surprisingly, red and black tea promoted the increase of lipid peroxidation per se ($t(14) = 2.55$; $P = 0.02$ for red tea comparing to sham; $t(14) = 3.28$; $P = 0.005$ for black tea comparing to sham; Fig. 4B).

3.7 Ferric Reducing/Antioxidant Power (FRAP)

A main effect for treatment was observed ($F(3,48) = 8.94$; $P < 0.0001$; Figure 4C) on total antioxidant capacity measurement. Additionally, a significant interaction was observed ($F(3,48) = 7.86$; $P = 0.0002$; Figure 4C). AD-like rats showed decreased antioxidant capacity ($t(10) = 3.36$; $P = 0.007$ Fig. 4C). Green, red and black tea were not able to avoid the antioxidant capacity decrease ($t(10) = 0.89$; $P = 0.38$ for green tea; $t(10) = 0.63$; $P = 0.54$ for red tea; $t(10) = 0.99$; $P = 0.34$ for black tea; Fig. 4C). Surprisingly, green, red and black tea promoted the decrease of total antioxidant capacity per se ($t(10) = 6.40$; P , 0.0001 for green tea comparing to sham; $t(10) = 6.04$; $P = 0.0001$ for red tea comparing to sham; $t(10) = 5.06$; $P = 0.0005$ for black tea comparing to sham; Fig. 4C).

Table 2. The AD-like model and the supplementation with different teas did not affect results of control behavioral tasks. Eight-week supplementation with different *Camilia Sinensis* teas and the AD-like rat model had no effect on locomotor and exploratory activities, anxiety and pain thresholds. Test results [mean (standard deviation)] from total exploration time in object recognition training (OR), short-term memory (STM), long-term memory (LTM), total exploration time in social recognition task (SR), number of crossing and rearing (open field), number of entries and time spent in open arms (plus maze), and the time latency on the hot plate (hot plate). There were no differences between the groups for all measures ($P > 0.05$, ANOVA one-way). Experimental groups: 1: sham; 2: green tea and sham: 3: red tea and sham; 4: black tea and sham; 5: AD-like; 6: AD-like and green tea: 7: AD-like and red tea; 8: AD-like and black tea (n = 8-10 per group).

Behavioral tasks		**Sham**				**AD-like**				**F and p-values**
		1	2	3	4	5	6	7	8	
Exploration time in OR	Total exploration time in training (s)	43.73 (8.30)	42.16 (17.09)	38.91 (15.67)	32.36 (16.81)	41.33 (22.86)	41.21 (16.62)	36.05 (12.58)	29.71 (15.79)	F = 1.50 P = 0.18
	Total exploration time in STM test (s)	42.16 (17.09)	40.27 (25.46)	35.64 (8.05)	32.99 (12.85)	42.09 (13.68)	41.49 (14.02)	42.81 (23.84)	33.63 (11.70)	F = 1.38 P = 0.22
	Total exploration time in LTM test (s)	38.91 (15.67)	42.84 (15.61)	36.53 (14.24)	34.34 (11.99)	39.62 (10.55)	36.81 (12.31)	35.36 (8.82)	25.73 (5.78)	F = 1.32 P = 0.25
Exploration time in SR	Total exploration time in SR test (s)	96.21 (22.21)	81.90 (16.53)	93.73 (25.06)	86.94 (26.41)	83.10 (34.64)	84.86 (31.90)	85.03 (11.15)	98.93 (25.80)	F = 1.59 P = 0.15
Open field	Crossings (n)	50.50 (9.69)	54.62 (13.09)	55.50 (10.35)	53.37 (7.36)	55.50 (17.81)	56.50 (17.67)	51.62 (10.02)	52.87 (14.19)	F = 1.77 P = 0.11
	Rearing (n)	15.25 (4.55)	15.88 (4.34)	14.87 (6.83)	15.18 (9.35)	12.00 (7.92)	13.37 (4.34)	14.00 (6.61)	15.62 (9.88)	F = 0.77 P = 0.60
Plus maze	Total entries (n)	25.73 (9.10)	24.41 (10.38)	23.70 (9.12)	23.04 (8.42)	23.13 (8.85)	25.52 (7.79)	24.17 (8.22)	26.04 (8.42)	F = 0.77 P = 0.99
	Time in open arms (s)	31.51 (15.36)	32.88 (29.48)	29.73 (17.29)	31.73 (25.22)	30.31 (33.55)	35.81 (16.48)	26.68 (16.97)	34.25 (14.53)	F = 0.98 P = 0.45
Hot Plate	Time (s)	9.55 (2.69)	9.10 (2.80)	12.33 (6.31)	8.81 (4.99)	8.50 (2.56)	11.81 (6.04)	9.70 (5.73)	12.85 (5.08)	F = 1.06 P = 0.39

4 Discussion

The goal of this study was to investigate whether different teas obtained from *Camellia sinensis* could prevent or minimize memory impairments and hippocampus oxidative status in an AD-like rat model. Our main results suggest that supplementation with green and red tea can prevent impairments in object and social recognition memories but only green tea prevents oxidative stress and damage in the hippocampus of AD-like rats.

The effects of green tea supplementation on both behavioral and oxidative stress of AD-like rats suggest a greater potential to prevent or minimize memory impairments and hippocampus oxidative stress and damage in comparison to the other teas from Camellia sinensis. A possible explanation for this result is the presence of EGCG, which was detected only in the green tea samples. The neuroprotective effect of green tea on both behavioral (memory performance) and neurochemical outcomes (oxidative stress and antioxidant defenses) has been explored in different models of brain diseases (Banerjee & Chatterjee, 2015; Flores et al., 2014). The focus of previous studies on green tea does not mean that other teas from the same plant have no potential benefits. As shown here, green tea showed the best neuroprotective results (i.e. induced behavioral gains and reduced brain oxidative stress); however, red tea also presented a significant role in reducing recognition memory losses.

Considering that increased ROS and lipid peroxidation induced by deposition of the amyloid plaques are mechanisms involved in AD memory deficits (Zuo et al., 2015), strategies to prevent oxidative stress could be a neuroprotective tool against impairments of recognition memory resulting from AD (T. Liu et al., 2016). We demonstrated the neuroprotective role of green and red tea supplementation in the

recognition memory deficits. These results are in agreement with previous reports of green tea promoting neuroprotection in different brain regions of the rat (Flores et al., 2014).

The different catechins in the Camellia sinensis work to decrease ROS and lipid peroxidation. Green tea has a higher concentration of total catechins, especially the EGCG, the catechin with the highest neuroprotective capacity in the hippocampus (Choi et al., 2014; Soung et al., 2015) and potential to inhibit Aβ-induced neuronal death (Choi et al., 2014). In our experiments, red and black tea did not present detectable concentrations of EGCG (see Table 1). Red tea did not present the same effects of green tea in oxidative stress measures but prevented recognition memory deficits. Therefore, effects of red tea on memory could be related to other mechanisms than the ones previously described, requiring further investigations. In previous studies, the antioxidant potential of green tea was more potent than from black (Serafini et al., 1996) or black and white teas (Gawlik & Czajka, 2007).

Our HPLC analyses showed higher amounts of EGCG, EGC and ECG in the green tea samples. EGCG supplementation attenuates cognitive deficits (Walker et al., 2015) and oxidative damage (Zuo et al., 2015) related to brain insults or neurodegenerative diseases. Taken together, these evidences suggest that the neuroprotective effects of green tea on oxidative damage in the hippocampus may rely on the presence of EGCG. Our results showed that red tea and black tea raised TBARs compared to water in the sham rats. Additionally, although beta-amyloid injection did not alter the total antioxidant capacity, all teas promoted its decrease. This decrease was not expected, but in a recent study no effect of green tea on FRAP was observed as well (Maurice, 2016), suggesting that Camellia sinensis tea antioxidant effects are more related to specific antioxidant systems (GSH, GPx, catalase, and others), as

demonstrated by previously researches (Flores et al., 2014; Schimidt et al., 2014), than to chelating activity, since in FRAP we measured the tea capacity of promote the reduction of ferric tripyridyltriazine complex to the ferrous tripyridyltriazine (Benzie & Strain, 1996).

Furthermore, raised TBARs in black tea group may be a result of the larger tea intake observed. The sham black tea group consumed more tea than others groups. Additionally, the catechins presence and concentration in red and black tea was different from green tea. The main component (according to the HPLC testing) of black tea was Epicatechin, which is the catechin that in great amounts accentuates a hormetic effect (Z. Zhang, Wu, & Huang, 2016). Besides the catechins, which were measured here, there could be significant differences in the content of amino acids, flavonol, flavone glycosides, and other compounds in the Camellia, teas, although they were manufactured from the same fresh tea leaves (Dai et al., 2017; Khanum, Faiza, Sulochanamma, & Borse, 2017), which may help to explain our results. It is important consider that teas also presented purine alkaloids, as caffeine and theophylline, and these compounds have biological effects, acting like vasodilator and relaxant (Bi et al., 2016). Caffeine, for example, has reported neuroprotective effects on different CNS injuries (Endesfelder et al., 2017), although caffeine cravings seems to impair cognitive functions (Palmer, Sauer, Ling, & Riza, 2017).

These controversy findings on oxidative status will be more investigated in our next studies by measuring endogenous enzymes and glutathione metabolism in the hippocampus. Additionally, it could be of interest to investigate the DPPH radical scavenging activity of green, red and black tea decoction and EGCG to further detail their antioxidant activity as discussed elsewhere (Kerio, Wachira, Wanyoko, & Rotich,

2013; Prathapan et al., 2011). The amount of alkaloid theophylline and its potential effects should not be discarded and will be considered in further studies as well.

A limitation of our study was that the tea concentration was determined based on previous studies showing positive effects of green tea supplementation. We cannot affirm that a larger concentration of other teas could have resulted in different outcomes. However, testing different concentrations for different teas would be not possible in our experiment. Furthermore, tea characteristics may differ considering samples from different regions of the world, which is also a limitation of other studies with similar scope. Oxidative stress is reported to be one of the earliest events in AD and can induce tau hyperphosphorylation (Z. Liu et al., 2015), which was not addressed here. We cannot exclude that other complex action mechanisms of EGCG, in addition to its antioxidant activity (Soung et al., 2015; Xicota et al., 2015), could have accounted for the results observed in our experiments. However, this limitation is common to other studies with similar scope.

5 Conclusion

Green tea presents the highest content of EGCG and the stronger neuroprotective capacity in the Alzheimer-like rat model, avoiding memory deficits and increases in ROS and TBARS levels in the hippocampus. These data suggest that green tea supplementation could be an effective prophylactic strategy to reduce AD effects, probably due to its higher EGCG concentration.

References

Ayyappan, P., Palayyan, S. R., & Kozhiparambil Gopalan, R. (2016). Attenuation of Oxidative Damage by Boerhaavia diffusa L. Against Different Neurotoxic Agents in Rat Brain Homogenate. *Journal of Dietary Supplements, 13*(3), 300-312. doi: 10.3109/19390211.2015.1036186

Banerjee, S., & Chatterjee, J. (2015). Efficient extraction strategies of tea (Camellia sinensis) biomolecules. [Review]. *Journal of Food Science and Technology, 52*(6), 3158-3168. doi: 10.1007/s13197-014-1487-3

Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. [Research Support, Non-U.S. Gov't]. *Analls of Biochemistry, 239*(1), 70-76. doi: 10.1006/abio.1996.0292

Bi, W., He, C., Ma, Y., Shen, J., Zhang, L. H., Peng, Y., & Xiao, P. (2016). Investigation of free amino acid, total phenolics, antioxidant activity and purine alkaloids to assess the health properties of non-Camellia tea. *Acta Pharm Sin B, 6*(2), 170-181. doi: 10.1016/j.apsb.2015.11.003

Bonini, J. S., Bevilaqua, L. R., Zinn, C. G., Kerr, D. S., Medina, J. H., Izquierdo, I., & Cammarota, M. (2006). Angiotensin II disrupts inhibitory avoidance memory retrieval. *Hormones and Behavior, 50*(2), 308-313. doi: S0018-506X(06)00089-4 [pii]10.1016/j.yhbeh.2006.03.016

Choi, S. M., Kim, B. C., Cho, Y. H., Choi, K. H., Chang, J., Park, M. S., . . . Kim, J. K. (2014). Effects of Flavonoid Compounds on beta-amyloid-peptide-induced Neuronal Death in Cultured Mouse Cortical Neurons. *Chonnam Medical Journal, 50*(2), 45-51. doi: 10.4068/cmj.2014.50.2.45

Dai, W., Xie, D., Lu, M., Li, P., Lv, H., Yang, C., . . . Lin, Z. (2017). Characterization of white tea metabolome: Comparison against green and black tea by a nontargeted metabolomics approach. *Food Res Int, 96*, 40-45. doi: 10.1016/j.foodres.2017.03.028

Endesfelder, S., Weichelt, U., Strauss, E., Schlor, A., Sifringer, M., Scheuer, T., Schmitz, T. (2017). Neuroprotection by Caffeine in Hyperoxia-Induced Neonatal Brain Injury. *Int J Mol Sci, 18*(1). doi: 10.3390/ijms18010187

Ennaceur, A., & Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. [Research Support, Non-U.S. Gov't]. *Behavioral Brain Research, 31*(1), 47-59.

Flores, M. F., Martins, A., Schimidt, H. L., Santos, F. W., Izquierdo, I., Mello-Carpes, P. B., & Carpes, F. P. (2014). Effects of green tea and physical exercise on memory impairments associated with aging. [Research Support, Non-U.S. Gov't]. *Neurochemistry International, 78*, 53-60. doi: 10.1016/j.neuint.2014.08.008

Gawlik, M., & Czajka, A. (2007). The effect of green, black and white tea on the level of alpha and gamma tocopherols in free radical-induced oxidative damage of human red blood cells. *Acta Poloniae Pharmaceutica, 64*(2), 159-164.

Ghasemi, R., Zarifkar, A., Rastegar, K., Maghsoudi, N., & Moosavi, M. (2014). Repeated intra-hippocampal injection of beta-amyloid 25-35 induces a reproducible impairment of learning and memory: considering caspase-3 and MAPKs activity. [Research Support, Non-U.S. Gov't]. *European Journal of Pharmacology, 726*, 33-40. doi: 10.1016/j.ejphar.2013.11.034

Heber, D., Zhang, Y., Yang, J., Ma, J. E., Henning, S. M., & Li, Z. (2014). Green tea, black tea, and oolong tea polyphenols reduce visceral fat and inflammation in

mice fed high-fat, high-sucrose obesogenic diets. [Research Support, Non-U.S. Gov't]. *Journal of Nutrition, 144*(9), 1385-1393. doi: 10.3945/jn.114.191007

Jack, C. R., Jr., Knopman, D. S., Chetelat, G., Dickson, D., Fagan, A. M., Frisoni, G. B., . . . Vos, S. J. (2016). Suspected non-Alzheimer disease pathophysiology - concept and controversy. [Review]. *Nature Reviews Neurology*. doi: 10.1038/nrneurol.2015.251

Kerio, L. C., Wachira, F. N., Wanyoko, J. K., & Rotich, M. K. (2013). Total polyphenols, catechin profiles and antioxidant activity of tea products from purple leaf coloured tea cultivars. *Food Chemistry, 136*(3-4), 1405-1413. doi: 10.1016/j.foodchem.2012.09.066

Khanum, H., Faiza, S., Sulochanamma, G., & Borse, B. B. (2017). Quality, antioxidant activity and composition of Indian black teas. *J Food Sci Technol, 54*(5), 1266-1272. doi: 10.1007/s13197-017-2506-y

Limon, D., Diaz, A., Hernandez, M., Fernandez, G. J., Torres-Martinez, A. C., Perez-Severiano, F., . . . Guevara, J. (2012). Neuroprotective effect of the aminoestrogen prolame against impairment of learning and memory skills in rats injected with amyloid-beta-25-35 into the hippocampus. [Research Support, Non-U.S. Gov't]. *European Journal of Pharmacology, 685*(1-3), 74-80. doi: 10.1016/j.ejphar.2012.04.020

Liu, T., Liu, X., Xiao, T., & Shi, J. (2016). Human recognition memory and conflict control: An event-related potential study. *Neuroscience, 313*, 83-91. doi: 10.1016/j.neuroscience.2015.11.047

Liu, Z., Li, T., Li, P., Wei, N., Zhao, Z., Liang, H., . . . Wei, J. (2015). The Ambiguous Relationship of Oxidative Stress, Tau Hyperphosphorylation, and Autophagy Dysfunction in Alzheimer's Disease. [Research Support, Non-U.S. Gov't

Review]. *Oxidative Medicine and Cellular Longevity, 2015*, 352723. doi: 10.1155/2015/352723

Loetchutinat, C., Kothan, S., Dechsupa, S., Meesungnoen, J., Jay-Gerin, J., & Mankhetkorn, S. (2005). Spectrofluorometric determination of intracellular levels of reactive oxygen species in drug-sensitive and drug-resistant cancer cells using the 2′,7′-dichlorofluorescein diacetate assay. *Radiation Physics and Chemistry, 72*(2-3), 323-331. doi: doi:10.1016/j.radphyschem.2004.06.011

Martins, A., Schimidt, H. L., Garcia, A., Colletta Altermann, C. D., Santos, F. W., Carpes, F. P., . . . Mello-Carpes, P. B. (2017). Supplementation with different teas from Camellia sinensis prevents memory deficits and hippocampus oxidative stress in ischemia-reperfusion. *Neurochem Int*. doi: 10.1016/j.neuint.2017.04.019

Maurice, T. (2016). Protection by sigma-1 receptor agonists is synergic with donepezil, but not with memantine, in a mouse model of amyloid-induced memory impairments. *Behav Brain Res, 296*, 270-278. doi: 10.1016/j.bbr.2015.09.020

Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analls of Biochemistry, 95*(2), 351-358.

Okello, E. J., McDougall, G. J., Kumar, S., & Seal, C. J. (2011). In vitro protective effects of colon-available extract of Camellia sinensis (tea) against hydrogen peroxide and beta-amyloid (Abeta((1-42))) induced cytotoxicity in differentiated PC12 cells. [Research Support, Non-U.S. Gov't]. *Phytomedicine, 18*(8-9), 691-696. doi: 10.1016/j.phymed.2010.11.004

Palmer, M. A., Sauer, J. D., Ling, A., & Riza, J. (2017). Caffeine cravings impair memory and metacognition. *Memory*, 1-10. doi: 10.1080/09658211.2017.1282968

Paxinos, G., Watson, C. R., & Emson, P. C. (1980). AChE-stained horizontal sections of the rat brain in stereotaxic coordinates. [Research Support, Non-U.S. Gov't]. *Journal of Neuroscience Methods, 3*(2), 129-149.

Pellow, S., Chopin, P., File, S. E., & Briley, M. (1985). Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. [Comparative Study]. *Journal of Neuroscience Methods, 14*(3), 149-167.

Prathapan, A., Singh, M. K., Anusree, S. S., Kumar, D. R. S., Sundaresan, A., & Raghu, K. G. (2011). Antiperoxidative, free radical scaveging and metal chelating activities of Boerhaavia Diffuda L. *Journal of Food Biochemistry, 35*, 1548–1554.

Rezai-Zadeh, K., Arendash, G. W., Hou, H., Fernandez, F., Jensen, M., Runfeldt, M., . . . Tan, J. (2008). Green tea epigallocatechin-3-gallate (EGCG) reduces beta-amyloid mediated cognitive impairment and modulates tau pathology in Alzheimer transgenic mice. [Research Support, N.I.H., Extramural]. *Brain Research, 1214*, 177-187. doi: 10.1016/j.brainres.2008.02.107

Schimidt, H. L., Vieira, A., Altermann, C., Martins, A., Sosa, P., Santos, F. W., . . . Carpes, F. P. (2014). Memory deficits and oxidative stress in cerebral ischemia-reperfusion: neuroprotective role of physical exercise and green tea supplementation. *Neurobiol Learn Mem, 114*, 242-250. doi: 10.1016/j.nlm.2014.07.005

Serafini, M., Ghiselli, A., & Ferro-Luzzi, A. (1996). In vivo antioxidant effect of green and black tea in man. [Clinical Trial Comparative Study Randomized Controlled Trial Research Support, Non-U.S. Gov't]. *European Journal of Clinical Nutrition, 50*(1), 28-32.

Soares, M. B., Izaguirry, A. P., Vargas, L. M., Mendez, A. S., Spiazzi, C. C., & Santos, F. W. (2013). Catechins are not major components responsible for the beneficial effect of Camellia sinensis on the ovarian delta-ALA-D activity inhibited by cadmium. [Research Support, Non-U.S. Gov't]. *Food and Chemical Toxicology, 55*, 463-469. doi: 10.1016/j.fct.2013.01.032

Sosa, P. M., Schimidt, H. L., Altermann, C., Vieira, A. S., Cibin, F. W., Carpes, F. P., & Mello-Carpes, P. B. (2015). Physical exercise prevents motor disorders and striatal oxidative imbalance after cerebral ischemia-reperfusion. *Braz J Med Biol Res, 48*(9), 798-804. doi: 10.1590/1414-431X20154429

Soung, H. S., Wang, M. H., Tseng, H. C., Fang, H. W., & Chang, K. C. (2015). (-)Epigallocatechin-3-gallate decreases the stress-induced impairment of learning and memory in rats. *Neuroscience Letters, 602*, 27-32. doi: 10.1016/j.neulet.2015.06.035S0304-3940(15)00478-4 [pii]

Walker, J. M., Klakotskaia, D., Ajit, D., Weisman, G. A., Wood, W. G., Sun, G. Y., . . . Schachtman, T. R. (2015). Beneficial effects of dietary EGCG and voluntary exercise on behavior in an Alzheimer's disease mouse model. [Research Support, N.I.H., Extramural]. *Journal of Alzheimers Disease, 44*(2), 561-572. doi: 10.3233/JAD-140981

Xicota, L., Rodriguez-Morato, J., Dierssen, M., & de la Torre, R. (2015). Potential Role of (-)-epigallocatechin-3-gallate (EGCG) in the Secondary Prevention of Alzheimer Disease. *Current Drug Targets*.

Yasmeen, H., & Hasnain, S. (2015). In vitro antioxidant effect of Camellia sinensis on human cell cultures. *Pakistan Journal of Pharmaceutical Sciences, 28*(5), 1573-1581.

Yi, J. H., Park, H. J., Kim, B. C., Kim, D. H., & Ryu, J. H. (2016). Evidences of the role of the rodent hippocampus in the non-spatial recognition memory. *Behavioral Brain Research, 297*, 141-149. doi: 10.1016/j.bbr.2015.10.018

Zhang, Y., Sun, D., Meng, Q., Guo, W., & Chen, Q. (2016). Calcium Channels Contribute to Albiflorin-mediated Antinociceptive Effects in Mouse Model. *Neurosci Letters, 628*, 105-109. doi: 10.1016/j.neulet.2016.03.054

Zhang, Z., Wu, H., & Huang, H. (2016). Epicatechin Plus Treadmill Exercise are Neuroprotective Against Moderate-stage Amyloid Precursor Protein/Presenilin 1 Mice. *Pharmacognosy Magazine, 12*(Suppl 2), S139-146. doi: 10.4103/0973-1296.182174

Zuo, L., Hemmelgarn, B. T., Chuang, C. C., & Best, T. M. (2015). The Role of Oxidative Stress-Induced Epigenetic Alterations in Amyloid-beta Production in Alzheimer's Disease. [Review]. *Oxidative Medicine and Cellular Longevity, 2015*, 604658. doi: 10.1155/2015/604658

CAPITULO III

MANUSCRITO SUBMETIDO

EFFECTS OF CAFETERIA DIET AND GREEN TEA SUPPLEMENTATION ON MEMORY DEFICITS AND HIPPOCAMPAL OXIDATIVE STRESS IN A RAT MODEL OF ALZHEIMER-LIKE DISEASE

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Highlights

- CAF diet did not influence long-term memory after Aβ injection in the hippocampus.

- CAF diet reduces oxidative stress and improves antioxidant status in the hippocampus.

- Green tea preserves short and long-term memories in a rat model of AD-like disease.

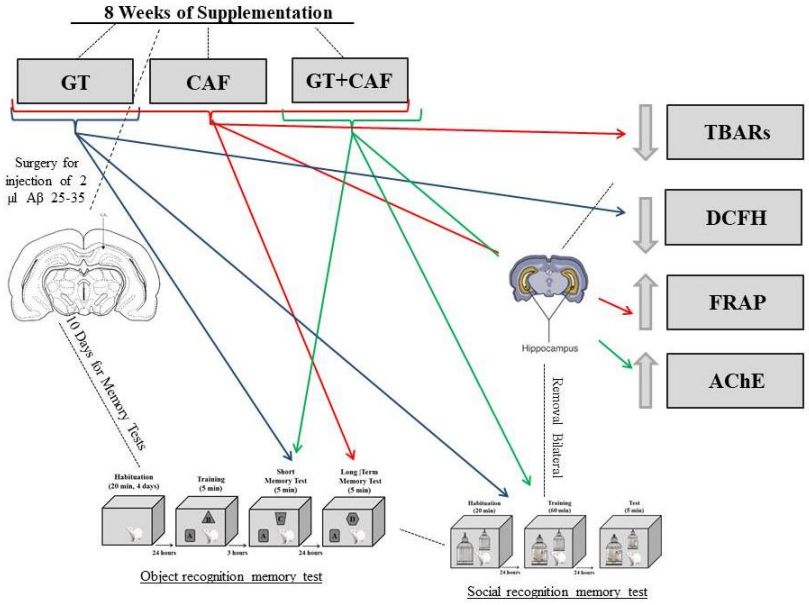
- CAF diet combined with green tea has better effects on memory than only green tea.

Abstract

Objectives: Metabolic diseases developed throughout life increase risk factors for brain diseases such as Alzheimer disease (AD). Antioxidant dietary strategies like green tea intake play a neuroprotective role in models of brain diseases, including AD-like models. Here we investigate the neuroprotective role of green tea associated with a cafeteria diet in a model of β-Amyloid injection that induces impairments related to AD. **Methods**: Wistar male rats were supplemented with green tea, cafeteria diet (CAF) or green tea plus CAF during 8 weeks before intra-hippocampal injection of β-amyloid peptide (2μL of Aβ-25–35, CA1 region). AD-like and sham rats were submitted to memory tests. After euthanasia, oxidative status was quantified in the bilateral hippocampus, and plasmatic triglycerides, total cholesterol, glucose, and AChE activity were determined. **Results**: Green tea preserves its neuroprotective role even regardless of the CAF and its combination with AD-like. CAF per se does not impair object and social recognition memories. Green tea supplementation and CAF, either administrated in separate or in combination, avoid oxidative stress and damage in the hippocampus of AD-like rats. **Conclusion**: In addition to the neuroprotective benefits of green tea, we conclude that CAF did not influence oxidative damage and memory deficits resultant of β-Amyloid injection when green tea is simultaneously ingested.

Keywords: metabolic diseases; dementia; catechins; neuroprotection; Wistar.

Graphic Abstract



1 Introduction

Processed foods rich in fat and sugar are popular in the modern society (De Macedo *et al.*, 2016), and the high intake of fat and sugar leads to a hypercaloric condition that increase risk of metabolic diseases (Sack *et al.*, 2016b; Kang *et al.*, 2017). A hypercaloric diet may cause peripheral insulin resistance, increase brain oxidative stress, hippocampal synaptic dysfunction, brain mitochondrial dysfunction, and brain insulin resistance (Vandal *et al.*, 2014; Husain *et al.*, 2017; Kang *et al.*, 2017). Taken together, these adaptations increase risk for onset of dementias, especially the Alzheimer's disease (AD), given that this diet also increases deposition of Aβ plaques in the brain (Leffa *et al.*, 2015; Pugazhenthi *et al.*, 2017).

AD is characterized by declines in memory and cognition (Mendiola-Precoma *et al.*, 2016) being one of its precursor mechanism a condition of neurotoxicity resultant of β-Amyloid plaques accumulation in the brain. β-Amyloid plaques increase the production of reactive oxygen species and promote oxidative stress, further increasing the β-Amyloid deposition in a cascade effect (Mendiola-Precoma *et al.*, 2016), neuronal losses, predominantly by apoptosis (Mendiola-Precoma *et al.*, 2016), and cognitive deficits (Grizzanti *et al.*, 2016).

Green tea (*Camellia sinensis, Theaceae*) has been shown as a potential nutritional intervention in conditions of brain diseases and injuries. Catechins present in the green tea promoted neuroprotection against oxidative and inflammatory stressors in obese rats supplemented with a cafeteria diet (Macedo *et al.*, 2017a). The antioxidant properties from green tea were also useful in protecting memory against deficits resultant of aging (Flores *et al.*, 2014) and brain insults like ischemia reperfusion (Martins *et al.*, 2017; Schimidt *et al.*, 2017). Recently, its neuroprotective potential was also reported in a model of AD-like disease (Schimidt *et al.*, 2017).

Whether a hypercaloric diet combined or not with green tea ingest has effects on brain functions and oxidative damage in models of neurodegenerative disease still

unknown. It would be valuable to known if an intervention based in green tea intake may have potential to influence the effects of an hypercaloric diet on memory and brain oxidative status. Therefore, the purpose of our study was to determine the neuroprotective potential of the green tea from *Camellia Sinensis* in a rat model of Alzheimer-like disease associated with a cafeteria diet with high content of fat and sugar. We hypothesized that cafeteria diet could impair redox status in the hippocampus and therefore negatively impact on memory, whereas the green tea would confirm its role as a neuroprotetor in the AD-like model as previously reported (Schimidt *et al.*, 2017).

2 Material and methods

2.1 Animals and experimental design

Animals and experimental design

Male Wistar rats aged two months were bought from the Central Vivarium of Federal University of Santa Maria (RS/Brazil). They were housed three per cage under controlled light and environmental conditions (12 h light/12 h dark cycle at temperature of 23 ± 2°C and 50 ± 10% air humidity). Food and water, or tea, were available *ad libitum*. Experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and the Local Institution Animal Care and Use Committee (IRB #012015). Upon arrival, rats were randomly assigned into experimental groups: (a) control: rats treated with standard diet (STAN diet) and not supplemented; (b) rats treated with cafeteria diet (CAF diet); (c) rats treat with standard diet (STAN diet) and supplemented with green tea; and (d) rats treat with green tea plus CAF diet.

After 8 weeks, sham (saline injection/group 1) or A-beta neurotoxicity induced surgery (β-Amyloid injection) were performed and groups were reorganized (n = 12/group), as follow (see Figure 1 and the details below):

- group 1 – Control: STAN diet; sham surgery without green tea supplementation;
- group 2 – β-Amyloid hippocampal infusion (AD-Like): STAN diet; β-Amyloid hippocampal infusion; not supplemented with green tea;
- group 3 – AD-Like + Green Tea: STAN diet; β-Amyloid hippocampal infusion; supplemented with green tea;
- group 4 – AD-Like + CAF diet: CAF diet; β-Amyloid hippocampal infusion; not supplemented with green tea;
- group 5 – AD-Like + Green Tea + CAF diet: supplemented with green tea and CAF diet; β-Amyloid injection hippocampal infusion.

Ten days after surgery, rats were submitted to behavioral tests and euthanized (Figure 1). Blood samples served to determine glucose, triglycerides and cholesterol levels. Abdominal fat was removed and quantified. Bilateral hippocampus was removed and biochemical analyses determined the levels of reactive oxygen species (ROS), thiobarbituric acid reactive substances (TBARS), ferric reducing/antioxidant power (FRAP), and acetylcholinesterase (AChE) activity.

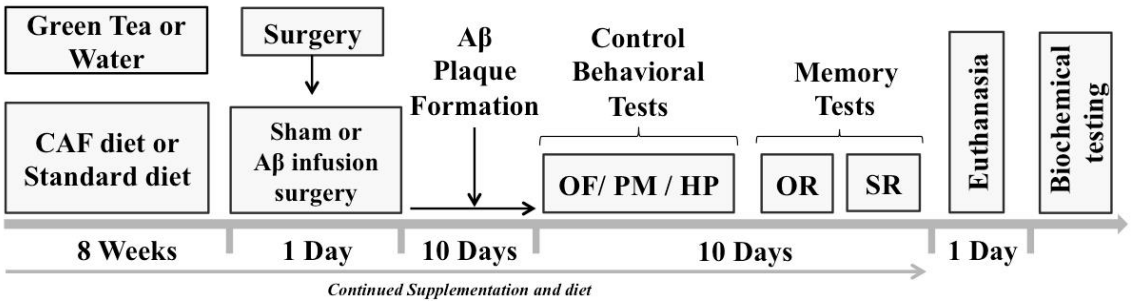


Figure 1. Experimental design. Rats were supplemented with green tea and/or CAF diet during 8 weeks before surgeries. Behavioral testing started 10 days after surgery. Euthanasia occurred 20 days after surgery; biochemical testing was the last step of the study. OF – open field; PM – plus maze; HP – hot plate; OR – object recognition memory test; SR – social recognition memory test.

2.2 Cafeteria diet

Rats from groups 1, 2 and 3 received STAN diet and rats from groups 4 and 5 received CAF diet to resemble the modern patterns of human diet (Estadella et al., 2004; Soares et al., 2017). Using the manufacturers' nutritional information was performed a detailed evaluation of diet composition for late to analyze the feeding behavior. The STAN diet consisted of commercial rat chow (J A Teixeira Veterinária Ltda, Santo Augusto/RS - Brazil) achieves in average 22% protein, 8% fat, 41% carbohydrate and 3% fiber in 100 g. The CAF diet consisted of commercial rat chow, peanuts, milk chocolate, and sweet biscuit in the proportion of 3:2:2:1, with all components powdered, mixed and pellete. The cafeteria diet achieves in average 16% protein, 35% fat, 40% carbohydrate, and 8% fiber in 100 g. This stands that the peanut composed by 6% protein, 56.6% fat, 21.5% carbohydrate and 8% fiber in 100 g; the chocolate composed by 23.7% protein, 62.8% fat,48% carbohydrate and 10.8% fiber in 100 g and sweet biscuit were composed by 3.2% protein, 11% fat, 79% carbohydrate and 2.4% fiber in 100 g. Based on these values, 100g of the STAN diet offers 1429 KJ/g and the CAF diet 4587 KJ/g. The average daily intake per rat was determined by the total consumption from each home cage divided by the number of rats in each home cage. To compare the groups we considered the caloric densities in kJ/g. The diets were maintained until the day of euthanasia.

2.3 Green tea supplementation

Rats from groups 3 and 5 received green tea mixed with drinking water (13.33 g/L), as described elsewhere (Flores et al., 2014; Schimidt et al., 2014; Schimidt et al., 2017). The tea was purchased from a local supplier (Madrugada Alimentos LTDA, RS, Brazil) and daily prepared with water boiled to 90°C, brewed for 3 min, filtered, cooled down and protected from light with aluminum foil, being administered at ambient temperature (Schimidt et al., 2014). The average daily tea intake per rat was determined by the total consumption from each home cage divided by the number of rats in each home cage. Tea supplementation continued until the day of euthanasia.

The tea was analyzed concerning the presence of Epigallocatechin (EGC; 213.68 μg/mL), Epicatechin (EC; 191.15 μg/mL), Epigallocatechin gallate (EGCG; 313.43 μg/mL) and Epicatechin gallate (ECG; 86.95 μg/mL) by high-performance liquid chromatography (HPLC system YL9100, Young Lin, with diode array detector). HPLC was performed with a Shimadzu Prominence Auto Sampler (YL9100) HPLC system (Shimadzu, Kyoto, Japan), equipped with Shimadzu YL9110 reciprocating pumps connected to an YL9101 degasser with an YL9150 integrator, and YL9160 diode array detector. To determine compounds profile the extracts were analyzed using a reversed phase carried out under gradient conditions using Synergi Fusion-RP 80A column (4.6 × 250 mm). The mobile phase was composed of water (pH = 3): acetonitrile (5:95, v/v) in a gradient mode, until 35 min, in which the mobile phase was 100% acetonitrile. At 38 min water (pH = 3): acetonitrile (5:95, v/v) was used again, in isocratic mode, as a mobile phase, until 50 min. A flow rate of 0.8mL/min was used and 20μL of sample were injected. Phenolic compounds were identified and quantified by comparing the retention time and UV–Visible spectral data to known previously injected standards. The chromatography peaks were confirmed by comparing the retention time with those of reference standards and by DAD spectra. Calibration curves were determined for EGC (y = 101,79x − 10,283); EC (y =91,872x + 7657); EGCG (y =103,5x − 93,211); ECG (y =112,17x − 81,22). All chromatography operations were performed at ambient temperature and in triplicate.

2.4 Preparation of Aβ 25-35

Aβ peptide (25-35) (Sigma Aldrich, São Paulo, Brazil; Product Number: A4559) was dissolved in saline at a concentration of 100μM. Before intrahippocampal injection,

the Aβ was incubated at 37°C during 4 days (*in vitro*) to induce Aβ 25-35 aggregation (Soultanov et al., 2017)

2.5 Surgeries

The stereotaxic surgeries for intrahippocampal injection of 2 µl Aβ 25-35 or vehicle were performed after 8 weeks of diet/supplementation. Rats were anesthetized with ketamine and xylazine (i.p. 75 mg/kg and 10 mg/kg, respectively). After the confirmation of the anesthesia, rats were mounted into a stereotaxic frame. The CA1 region of the dorsal hippocampus was located according to Paxinos' brain (AP -4.2, LL ± 3.0, VM - 2.0 mm) (Paxinos, Watson, & Emson, 1980). Bilateral infusions were performed using a Hamilton syringe and an infusion bomb. Rats were returned to their cages and monitored during surgery recovery and in the following 10 days during the formation of amyloids plates (Deng et al., 2010; Raha et al., 2016; Soultanov, et al., 2017).

2.6 Behavioral control tests

Exploratory and locomotor activities were analyzed to ensure that saline or Aβ infusion did not impair such behaviors. Ten days after surgery rats were individually placed on the left quadrant of a 50 × 50 × 39 cm open field (OF) made with all painted in white except for one transparent glass wall. Black lines were drawn on the floor to divide the arena into 12 equal rectangular quadrants. Crossing and rearing, as measures of locomotor and exploratory activities, respectively, were recorded over 5 min. Anxiety

state was analyzed using an elevated plus maze to record the time spent and the total number of entries into the open arms over 5 min (Pellow, Chopin, File, & Briley, 1985). Nociception was assessed as a measure of peripheral sensibility, by placing rats on a hot plate at 55 ± 0.5°C and determining the latency for paw withdrawal (Zhang, Sun, Meng, Guo, & Chen, 2016).

2.6.1 Object recognition memory

Training and test in object recognition task (OR) were performed in an open-field arena (50 × 50 × 50 cm) built with polyvinyl chloride plastic, plywood and transparent acrylic (Ennaceur & Delacour, 1988). Rats were habituated to the apparatus during 20 min of free exploration in 4 consecutive days. For training, two different objects (A and B) were placed in the apparatus and rats were allowed to freely explore them during 5 min. Exploration was defined as sniffing or touching the objects with the nose and/or forepaws. The behavior was recorded using a video tracking system. Short-term memory (STM) was tested 3 h after training when one of the objects was randomly changed for a novel one (C) and the rats were reintroduced into the apparatus to freely explore the objects (familiar and new one) during 5 min. Long-term memory (LTM) was tested 24 h after training when the object C was changed for a new object (D) and the rats were reintroduced into the apparatus to freely explore the objects (familiar and new one) during 5 min. To avoid confounds by lingering olfactory stimuli and preferences, the objects and the arena were cleaned with 70% ethanol after each test. The discrimination index was determined by the difference of time spent exploring the

new (Tnovel) and the familiar (Tfamiliar) objects: [(Tnovel - Tfamiliar)/(Tnovel + Tfamiliar) x 100 (%)], and used as a memory parameter (Martins, et al., 2017).

2.6.2 Social recognition memory test

This task is an adaptation of the social recognition memory test (Kaidanovich-Beilin, Lipina, Vukobradovic, Roder, & Woodgett, 2011). The task was completed in three days in a row. On the first day, the rats were placed in an arena with two small cages during 20 min for free exploration (habituation). On the second day, training was performed with inclusion of one unfamiliar rat in one of the small cages that allow physical/sensory contact, and the tested rat had 1 hour to freely explore. After 24 hours, testing was performed, when the same rat of training (familiar rat) and a new rat were placed in the small cages for exploration during 5 minutes. The time spent exploring the new and the familiar rat were recorded. Exploration was defined as sniffing or touching the small cages with the nose and/or forepaws. The discrimination index was determined by the difference of time spent exploring the new (Tnovel) and the familiar (Tfamiliar) rats: [(Tnovel - Tfamiliar)/(Tnovel + Tfamiliar) x 100 (%)], and used as a memory parameter (Martins, et al., 2017).

2.7 Biochemical testing

2.7.1 Tissue preparation

Rats were euthanized 24 h after the behavioral experiments. The brain was removed and bilateral hippocampus were quickly dissected out and homogenized in 50

mM Tris HCl, pH 7.4, (1/5 or 1/10, w/v). Afterwards, samples were centrifuged at 2400 g for 20 min, and supernatants (S1) were used for biochemical assay. Blood samples were collected through cardiac puncture in tubes with anticoagulant. Samples were centrifuged and had the plasma separate for the biochemical analyses. The retroperitoneal and gonadal fat tissues were dissected and weighted by the same trained experimenter, and were considered as abdominal fat.

2.7.2 Plasmatic triglycerides, total cholesterol, and glucose

Dosages (mg/dl) of plasmatic triglycerides, total cholesterol, and glucose were performed using commercial kits (Labtest Diagnostica, MG, Brazil).

2.7.3 Hippocampal reactive oxygen species (ROS)

Reactive oxygen species (ROS) content was assessed by a spectrofluorimetric method using 2′,7′-dichlorofluorescein diacetate (DCFH-DA) as a probe (Loetchutinat et al., 2005). The hippocampus homogenate was incubated in darkness with 5 μL DCFH-DA (1 mM). The oxidation of DCHF-DA to fluorescent dichlorofluorescein (DCF) was measured for the detection of intracellular ROS. The formation of the oxidized fluorescent derivative (DCF), measured by DCF fluorescence intensity, was recorded at 520 nm (480 nm excitation), 30 min after the addition of DCFH-DA to the medium. Results were expressed as AU (arbitrary units).

2.7.4 Detection of hippocampal lipoperoxidation

Lipoperoxidation was evaluated though the thiobarbituric acid reactive substance (TBARS) test (Ohkawa, Ohishi, & Yagi, 1979). One aliquot of hippocampus homogenate was incubated with a 0.8% thiobarbituric acid solution, acetic acid buffer (pH = 3.2) and sodium dodecyl sulfate solution (8%) at 95° C for 2 h, and the color reaction was measured at 532 nm. Results were expressed as nmol of malondialdehyde (MDA) per mg of protein.

2.7.5 Hippocampal ferric reducing/antioxidant power (FRAP) assay

The working ferric reducing/antioxidant power (FRAP) reagent was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution, and 2.5 ml FeCl3.6H2O solution. 10 μL of homogenate was added in 300 μL working FRAP reagent in microplate (Benzie & Strain, 1996). Additionally, we used a standard curve with 10 μL Trolox concentrations of 15, 30, 60, 120 e 240 mM more 300 μL working FRAP reagent. The microplate was incubated at 37° C for 15 min before reading in SpectraMax M5 Microplate Reader at 593 nm.

2.7.6 Estimation of hippocampal acetylcholinesterase (AChE) activity

The activity of acetylcholinesterase (AChE) was determined using acetylthiocholine as substrate (Ellman, Courtney, Andres, & Feather-Stone, 1961). The

activity of AChE was determined by spectrophotometry at 412 nm. The activity of AChE was expressed as nmol AcSCh/hour/mg protein.

2.8 Statistical analyses

Data are presented as mean and standard deviation. Normality of data distribution was checked using Shapiro-Wilk test. For all variables, one-way ANOVA (memory results) or Kruskall-Wallis (biochemical data) was applied to compare the groups. When main effects were found, Tukey or Dunn's test served as *post hoc*. Significance level was set at 0.05 for all comparisons.

3 Results

3.1 Food and fluid intake and abdominal fat

All groups gained weight throughout the 8 weeks, however we did not find significant difference between the groups (table 1). The groups AND-like with CAF diet and AD-like with CAF diet plus green tea ingested less amount of food when compared to the groups SHAM, AD-like with STAN diet and AD-like with STAN diet plus green tea (P = 0.0031; ANOVA one way; SHAM: 38.75 ±0.73; STAN: 38.,23g ±0.49; STAN plus green tea: 39.26g ±0.5625; CAF: 32.82g ±3.03; CAF plus green tea: 35.35g ±0.01).But for daily caloric intake the groups the groups SHAM, AD-like with STAN diet and AD-like with STAN diet plus green tea presented higher caloric intake (P <

0,0001; ANOVA one way; SHAM: 554.00 KJ ±10.52; STAN: 546.60 KJ ±7.04; STAN plus green tea: 561.30 KJ ±8.042; CAF: 1505.00 KJ ±139.10; CAF plus green tea: 1622.00 KJ ± 0.76) (Table 1). Green tea intake (59.30 ± 4.87 ml) was higher than water intake (49.66 ± 3.02 ml) regardless of the diet (P = 0.028). CAF increased abdominal fat (Table 1, P < 0.001; 8.37 g ± 0.44 for STAN vs. 18.16 ± 1.39 g for CAF).

3.2 Cholesterol, triglycerides, and glucose

Cholesterol (F = 2.19; P = 0.092; ANOVA one way) and glucose levels did not differ between the groups (Table 2, F = 0.29; P = 0.882; ANOVA one way). Higher levels of triglycerides were found in the AD-like compared to the sham (Table 2, F = 5.55; P = 0.001; ANOVA one way). AD-like group also showed higher levels of triglycerides than AD-like supplemented with green tea (P = 0.015) and CAF (P < 0.001).

3.3 Behavioral tests

Locomotor and exploratory behavior, anxiety, and pain sensitivity did not differ between the groups (Table 2).

Table 1 - Mean (standard deviation) results of plasma cholesterol, triglycerides, glucose and amount of abdominal fat in the different groups. The levels of triglycerides were higher in AD-Like that, SHAM, AD-Like + GT and AD-Like + CAF diet. * $P < 0.05$ difference from AD-Like. For abdominal fat the groups AD-Like + CAF diet and AD-Like + GT+ CAF diet has more accumulation. # $P < 0.05$ difference from your controls with STAN diet.

	SHAM	**AD-Like**	**AD-Like + GT**	**AD-Like + CAF diet**	**AD-Like + GT + CAF diet**
Cholesterol (mg/dl)	81.05 (19.90)	91.37 (16.73)	96.32 (9.32)	83.04 (20.94)	102.09 (12.91)
Triglycerides (mg/dl)	113.73* (54.91)	227.80 (78.15)	144.40* (27.12)	98.72* (37.60)	168.55 (70.40)
Glucose (mg/dl)	150.10 (56.98)	147.25 (34.91)	148.68 (35.12)	125.40 (35.12)	153.53 (31.23)
Abdominal fat (g)	8.3 (1.66)	8.95 (1.19)	7.36 (2.15)	17.08# (6.08)	17.43# (4.54)

Table 2 - Results of total exploration time on memory tasks and control behavioral tasks did not differ between the groups. Test results [mean (standard deviation)] from total exploration time in object recognition training (OR), short-term memory (STM), long-term memory (LTM), total exploration time in social recognition task (SR), number of crossing and rearing (open field), number of entries and time spent in open arms (plus maze), and the time latency on the hot plate (HP). GT= green tea; CAF = cafeteria diet.

		SHAM	AD-like	AD-like +GT	AD-like + CAF	AD-like + GT + CAF
Exploration time in OR	Total exploration time in training (s)	51.56 (12.83)	45.63 (15.50)	44.75 (8.72)	44.83 (10.50)	52.15 (19.03)
	Total exploration time in STM test (s)	37.24 (4.97)	42.20 (16.61)	44.72 (23.52)	42.29 (16.07)	41.93 (6.95)
	Total exploration time in LTM test (s)	37.10 (21.91)	39.58 (15.35)	42.17 (24.86)	54.01 (10.69)	49.44 (15.99)
Exploration time in SR	Total exploration time in SR test (s)	119.67 (35.55)	107.16 (45.02)	120.86 (30.58)	121.57 (21.62)	110.13 (34.07)
Open field	Crossings (n)	51.00 (10.79)	43.83 (19.94)	50.62 (27.60)	50.90 (15.37)	55.70 (36.69)
	Rearing (n)	13.90 (5.17)	16.10 (8.63)	15.75 (11.67)	13.80 (9.00)	14.75 (11.46)
Plus maze	Total entries (n)	46.40 (53.45)	99.16 (64.58)	102.50 (74.84)	114.20 (67.89)	138.66 (55.68)
	Time in open arms (s)	20.59 (5.83)	29.44 (17.94)	21.51 (14.43)	14.82 (8.07)	16.75 (17.86)
Hot plate	Latency (s)	12.40 (3.57)	9.90 (4.27)	10.25 (2.43)	8.70 (3.68)	11.77 (5.69)

3.4 Object recognition memory

Object discrimination index for STM differed between the groups (Fig. 2A; F= 2.82; p = 0.036; one-way ANOVA). AD-like group showed impaired discrimination index compared to the SHAM (Fig. 2A; F = 2.51; p= 0.033, one-way ANOVA). AD-like+GT, AD-like + CAF diet and AD-like + GT + CAF diet groups did not show deficits in STM (not different of sham; p = 0.344). However, AD-like + GT and AD-like + GT + CAF diet presented higher discrimination index than AD-like group (p= 0.035 and p = 0.035, respectively).

LTM object recognition memory differed between the groups (Fig. 2B; F = 6.64; p < 0.001, one-way ANOVA). AD-like group showed impaired LTM discrimination index (Fig. 2B; p = 0.002), and the groups AD-like + GT, AD-like + CAF diet, AD-like + GT + CAF diet, avoided deficits in LTM discrimination index (Fig. 2B; p = 0.001 for AD-like +GT; p = 0.017 for AD-like + CAF; p < 0.001 for AD-like + GT + CAF diet).

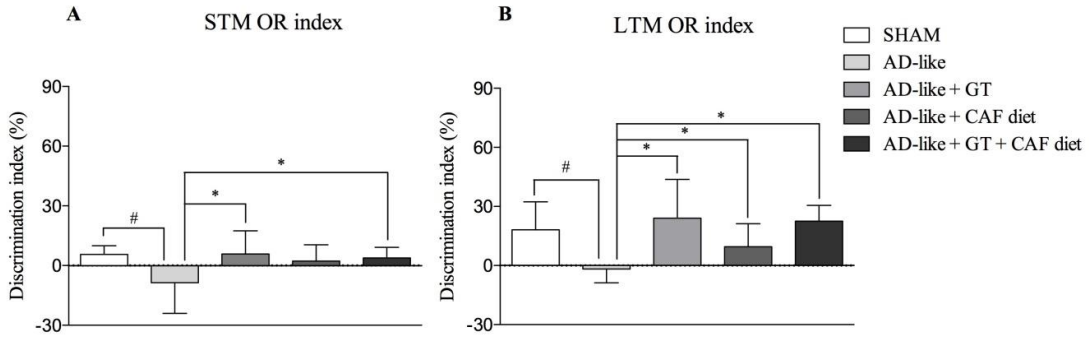


Figure 2. Supplementation with green tea, CAF diet and green tea plus CAF diet avoided short-term (A) and long-term (B) memory deficits in an AD-like rat model. Results (mean and standard deviation of the percent of the total exploration time) from the different experimental groups (GT= green tea; CAF diet = cafeteria diet). # P < 0.05 SHAM vs AD-like; * difference between groups; ANOVA followed by Dunn's multiple comparison; n = 8–10 per group.

3.5 Social recognition memory

Social discrimination index differed between the groups (Fig. 3; F = 5.82; p < 0.001, one-way ANOVA). AD-like showed impaired social recognition index memory compared to SHAM (Fig. 3; p = 0.001). AD-like + GT, AD-like + CAF diet, AD-like + GT + CAF diet groups did not show deficits in social memory (not different of SHAM; p= 0.267, p = 0.068, p= 0.498, respectively). AD-like + GT and AD-like + GT + CAF diet showed higher social discrimination index than AD-like group (Fig. 3; p = 0.001 for green tea; p = 0.001 for CAF + green tea).

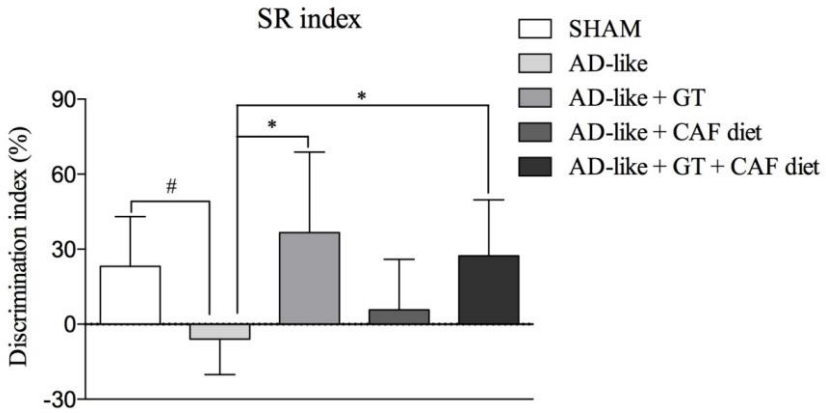


Figure 3. Supplementation with green tea and green tea plus CAF diet showed a neuroprotective effect on SR memory deficit in the AD-like rat model. Results (mean and standard deviation of the percent of the total exploration time) from the different groups (GT= green tea; CAF diet = cafeteria diet). # P < 0.05 SHAM vs AD-like; * difference between groups; ANOVA followed by Dunn's multiple comparison; n = 8–10 per group.

3.6 Hippocampal oxidative stress

ROS levels differed between the groups (Fig. 4A; F=4.43, p = 0.005, one-way ANOVA). AD-like showed higher ROS compared to SHAM (Fig. 4A; p = 0.001). ROS levels did not differ of sham in the AD-like + CAF diet group (Fig.4A; p = 0.083) and the AD-like + GT + CAF diet (Fig.4A; p = 0.055). AD-like rats showed higher lipid peroxidation than SHAM (Fig. 4B; F = 15.58, p < 0.001, one-way ANOVA). AD-like + GT (p = 0.004), AD-like + CAF diet (p < 0.001) and AD-like + GT + CAF (p < 0.001) avoided increases in lipid peroxidation levels compared to SHAM (Fig. 4B). AD-like showed lower FRAP compared to SHAM< (Fig. 5A; F= 4.32, p= 0.008, one-way ANOVA). AD-like +GT (p < 0.001), AD-like+ CAF diet (p = 0.018) and AD-like + GT + CAF diet (p < 0.001) avoided decreases in FRAP (Fig. 5A).

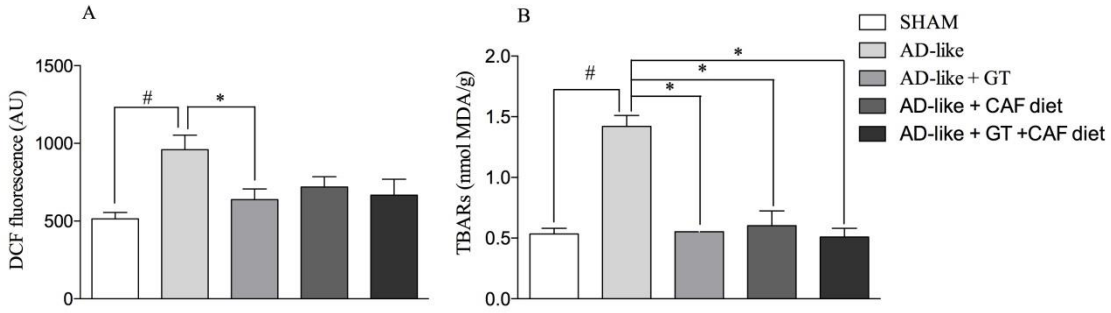


Figure 4. Supplementation with green tea, CAF diet and green tea plus CAF diet showed a neuroprotective effect on hippocampal oxidative stress induced by the AD-like model in rat. A: ROS; B: lipid peroxidation. GT= green tea; CAF diet = cafeteria diet. # P < 0.05, difference between sham and AD-like group, ANOVA followed by Dunn's multiple comparison; * P < 0.05, different from AD-like group, ANOVA followed by Dunn's multiple comparison; n = 8–10 per group.

3.7 Activity of AChE

Lower AChE activity was found in the AD-like group compared to SHAM (Fig. 5B; F = 3.17; *p*= 0.011, one-way ANOVA). AD-like + GT + CAF diet prevented the decrease of AChE activity (Fig. 5B; $p < 0.001$).

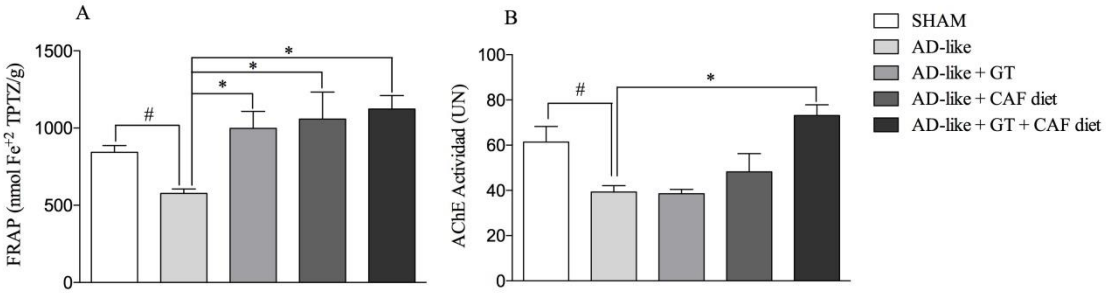


Figure 5. Supplementation with green tea, CAF diet and green tea more CAF diet preserve the total antioxidant capacity; supplementation with green tea plus CAF diet prevents the decreased of AchE activity. A: FRAP; B: AChE. (GT= green tea; CAF diet = cafeteria diet). # P < 0.05, difference between sham and AD-like group, ANOVA followed by Dunn's multiple comparison; * P < 0.05, different from AD-like group, ANOVA followed by Dunn's multiple comparison; n = 8–10 per group.

4 Discussion

Discussion

Green tea has effective antioxidant properties against brain insults and also inflammatory stressors. Cafeteria diet is a potential risk factor for onsets of metabolic diseases that are also related to cognitive deficits observed in the Alzheimer disease (AD). However, literature is not conclusive regarding deleterious effects of high fat diet on memory. A high-fat diet did not influence memory in aged mice (Roberts et al., 2017). The same outcome was observed in studies considering a low-carbohydrate (Dehghan et al., 2017), and palatable diets (Macedo et al., 2017a; Pini et al., 2017). This

motivated us to investigate the influence of the cafeteria diet in the context of a neurological diseases, and considering a hypothetic negative effect of the diet on memory, we tested the effectiveness of a nutritional strategy for neuroprotection based in green tea intake.

We hypothesized that cafeteria diet could impair redox status in the hippocampus and therefore negatively impact on memory, whereas the green tea would confirm his role as a neuroprotetor and reverse deficits. Our main findings show that CAF diet increases the abdominal fat as expected, but does not increase oxidative stress in the hippocampus along with lack of impairments in recognition memory. We confirmed the neuroprotetor potential of green tea.

β-Amyloid injection increased ROS and lipid peroxidation in the hippocampus, which was accompanied by deficit in recognition memory. As expected, green tea avoided oxidative stress and preserved antioxidant capacity in the hippocampus (Flores et al., 2014; Schimidt et al., 2014; Martins et al., 2017; Schimidt et al., 2017). These effects of green tea can be attributed to its high content of catechins that can neutralize ROS (Macedo et al., 2017b). Besides the catechins, green tea has other compounds with neuroactive effects, like caffeine and theaflavin (Anandhan et al., 2013; Kolahdouzan e Hamadeh, 2017) that may also account to the results observed here and in previous studies.

Considering that CAF did not impair LTM index, lipid peroxidation and total antioxidant capacity, we could expect that CAF and green tea in combination might lead to additional gains in the parameters evaluated. However, the combination of the two interventions did not lead to additional benefits, which is similar to the observed in previous reports that combined green tea with other antioxidant strategies (Flores et al., 2014; Schimidt et al., 2014).

Different of what we initially hypothesized, impairments due to β-Amyloid injection were not aggravated by CAF. AD-like rats in the CAF group showed preserved long-term object recognition memory. CAF group did not show increases in lipid peroxidation and decreases of antioxidant capacity that would suggest oxidative damage. Obesity and metabolic diseases are cited as risk factors for AD (Pugazhenthi et al., 2017). Many studies tried to explain the mechanisms involved in this relationship and showed that increases in peripheral cytokine levels, insulin resistance and excessive adiposity generate immune responses that lead to cognitive impairment (De Macedo et al., 2016; Sack et al., 2016b; Pini et al., 2017). One could argue that our results are unexpected. First we have to consider that there are studies not supporting the association between the CAF (Leffa et al., 2015; Sack et al., 2016a; Pini et al., 2017) or diets rich in fat (Newman et al., 2017; Roberts et al., 2017), and cognitive impairments. CAF increased gray matter increase and only mild deficits were found on long-term memory assessed by the puzzle-box paradigm, while executive functions and short-term memory were not affected (Sack et al., 2016a). Despite of this, it is important to note is that our study has some inherent limitations. We administrated the CAF during 8 weeks of supplementation. Evidences suggest that rats may have the ability to balance the nutrient intake among available food based in the amount needed for their survival (Macedo et al., 2017b). As a result, we cannot exclude the possibility that caloric intake may have been similar in the different groups. Furthermore, our CAF included peanuts. Peanut has antioxidant properties that can improve memory via modulation of anti-oxidative stress and activation of BDNF/ERK/CREB signaling pathways (Xiang et al., 2016; Barbour et al., 2017).

Another possible source of confusion concerning CAF and its effects on cognition is the presence of casein in the CAF. Casein is rich in tryptophan, the direct

precursor of serotonin (Stancampiano et al., 1999; Sack et al., 2016b). The hippocampus is rich in serotonin receptors that are important for memory consolidation (Matsukawa et al., 1997; Stancampiano et al., 1999). It is possible that CAF increases availability of serotonin. Further investigations of mechanisms of protection involving the serotonergic system may help to explain the effects of CAF. This hypothesis concerning the serotonergic system might found support in our AChE results, since acetylcholine and serotonin play important roles on memory preservation (Matsukawa et al., 1997; Stancampiano et al., 1999; Wang et al., 2017). Green tea has L-theanine (N-ethyl-L-glutamine) or theanine, an amino acid that also increases synthesis of serotonin (Nathan et al., 2006).

5 Conclusion

We found a neuroprotective potential of green tea in the Alzheimer-like disease model tested. The cafeteria diet does not impair recognition memory, does not alter the redox state in the hippocampus, and in some cases avoids the memory deficits due to the AD-like model.

References

Anandhan, A., Essa, M. M., & Manivasagam, T. (2013). Therapeutic attenuation of neuroinflammation and apoptosis by black tea theaflavin in chronic

MPTP/probenecid model of Parkinson's disease. *Neurotox Res, 23*(2), 166-173. doi: 10.1007/s12640-012-9332-9

Barbour, J. A., Howe, P. R. C., Buckley, J. D., Bryan, J., & Coates, A. M. (2017). Cerebrovascular and cognitive benefits of high-oleic peanut consumption in healthy overweight middle-aged adults. *Nutr Neurosci, 20*(10), 555-562. doi: 10.1080/1028415X.2016.1204744

Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal Biochem, 239*(1), 70-76. doi: S0003-2697(96)90292-4 [pii]10.1006/abio.1996.0292

de Macedo, I. C., de Freitas, J. S., & da Silva Torres, I. L. (2016). The Influence of Palatable Diets in Reward System Activation: A Mini Review. *Adv Pharmacol Sci, 2016*, 7238679. doi: 10.1155/2016/7238679

Dehghan, M., Mente, A., Zhang, X., Swaminathan, S., Li, W., Mohan, V., . . . Yusuf, S. (2017). Associations of fats and carbohydrate intake with cardiovascular disease and mortality in 18 countries from five continents (PURE): a prospective cohort study. *Lancet*. doi: 10.1016/S0140-6736(17)32252-3

Deng, J., Shen, C., Wang, Y. J., Zhang, M., Li, J., Xu, Z. Q., . . . Zhou, H. D. (2010). Nicotine exacerbates tau phosphorylation and cognitive impairment induced by amyloid-beta 25-35 in rats. [Comparative Study]. *Eur J Pharmacol, 637*(1-3), 83-88. doi: 10.1016/j.ejphar.2010.03.029

Ellman, G. L., Courtney, K. D., Andres, V., Jr., & Feather-Stone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol, 7*, 88-95.

Ennaceur, A., & Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. [Research Support, Non-U.S. Gov't]. *Behav Brain Res, 31*(1), 47-59.

Flores, M. F., Martins, A., Schimidt, H. L., Santos, F. W., Izquierdo, I., Mello-Carpes, P. B., & Carpes, F. P. (2014). Effects of green tea and physical exercise on memory impairments associated with aging. [Research Support, Non-U.S. Gov't]. *Neurochem Int, 78*, 53-60. doi: 10.1016/j.neuint.2014.08.008

Grizzanti, J., Lee, H. G., Camins, A., Pallas, M., & Casadesus, G. (2016). The therapeutic potential of metabolic hormones in the treatment of age-related cognitive decline and Alzheimer's disease. [Review]. *Nutr Res, 36*(12), 1305-1315. doi: 10.1016/j.nutres.2016.11.002

Husain, I., Akhtar, M., Abdin, M. Z., Islamuddin, M., Shaharyar, M., & Najmi, A. K. (2017). Rosuvastatin ameliorates cognitive impairment in rats fed with high-salt and cholesterol diet via inhibiting acetylcholinesterase activity and amyloid beta peptide aggregation. *Hum Exp Toxicol*, 960327117705431. doi: 10.1177/0960327117705431

Kaidanovich-Beilin, O., Lipina, T., Vukobradovic, I., Roder, J., & Woodgett, J. R. (2011). Assessment of social interaction behaviors. [Research Support, Non-U.S. Gov'tVideo-Audio Media]. *J Vis Exp*(48). doi: 10.3791/2473

Kang, S., Lee, Y. H., & Lee, J. E. (2017). Metabolism-Centric Overview of the Pathogenesis of Alzheimer's Disease. [Review]. *Yonsei Med J, 58*(3), 479-488. doi: 10.3349/ymj.2017.58.3.479

Kolahdouzan, M., & Hamadeh, M. J. (2017). The neuroprotective effects of caffeine in neurodegenerative diseases. *CNS Neurosci Ther, 23*(4), 272-290. doi: 10.1111/cns.12684

Leffa, D. D., Valvassori, S. S., Varela, R. B., Lopes-Borges, J., Daumann, F., Longaretti, L. M., . . . Andrade, V. M. (2015). Effects of palatable cafeteria diet on cognitive and noncognitive behaviors and brain neurotrophins' levels in mice. *Metab Brain Dis, 30*(4), 1073-1082. doi: 10.1007/s11011-015-9682-0

Loetchutinat, C., Kothan, S., Dechsupa, S., Meesungnoen, J., Jay-Gerin, J., & Mankhetkorn, S. (2005). Spectrofluorometric determination of intracellular levels of reactive oxygen species in drug-sensitive and drug-resistant cancer cells using the 2′,7′-dichlorofluorescein diacetate assay. *Radiat Phys Chem, 72*(2-3), 323-331. doi: doi:10.1016/j.radphyschem.2004.06.011

Macedo, R. C., Bondan, E. F., & Otton, R. (2017a). Redox status on different regions of the central nervous system of obese and lean rats treated with green tea extract. *Nutr Neurosci*, 1-13. doi: 10.1080/1028415X.2017.1356030

Macedo, R. C., Bondan, E. F., & Otton, R. (2017b). Redox status on different regions of the central nervous system of obese and lean rats treated with green tea extract. *Nutr Neurosci, 31*, 1-13. doi: 10.1080/1028415X.2017.1356030

Martins, A., Schimidt, H. L., Garcia, A., Colletta Altermann, C. D., Santos, F. W., Carpes, F. P., . . . Mello-Carpes, P. B. (2017). Supplementation with different teas from Camellia sinensis prevents memory deficits and hippocampus oxidative stress in ischemia-reperfusion. *Neurochem Int, 108*, 287-295. doi: 10.1016/j.neuint.2017.04.019

Matsukawa, M., Ogawa, M., Nakadate, K., Maeshima, T., Ichitani, Y., Kawai, N., & Okado, N. (1997). Serotonin and acetylcholine are crucial to maintain hippocampal synapses and memory acquisition in rats. *Neurosci Lett, 230*(1), 13-16. doi: S0304-3940(97)00460-6 [pii]

Mendiola-Precoma, J., Berumen, L. C., Padilla, K., & Garcia-Alcocer, G. (2016). Therapies for Prevention and Treatment of Alzheimer's Disease. [Review]. *Biomed Res Int, 2016*, 2589276. doi: 10.1155/2016/2589276

Nathan, P. J., Lu, K., Gray, M., & Oliver, C. (2006). The neuropharmacology of L-theanine(N-ethyl-L-glutamine): a possible neuroprotective and cognitive enhancing agent. [Review]. *J Herb Pharmacother*, *6*(2), 21-30.

Newman, J. C., Covarrubias, A. J., Zhao, M., Yu, X., Gut, P., Ng, C. P., . . . Verdin, E. (2017). Ketogenic Diet Reduces Midlife Mortality and Improves Memory in Aging Mice. *Cell Metab, 26*(3), 547-557 e548. doi: 10.1016/j.cmet.2017.08.004

Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem, 95*(2), 351-358.

Paxinos, G., Watson, C. R., & Emson, P. C. (1980). AChE-stained horizontal sections of the rat brain in stereotaxic coordinates. [Research Support, Non-U.S. Gov't]. *J Neurosci Methods, 3*(2), 129-149.

Pellow, S., Chopin, P., File, S. E., & Briley, M. (1985). Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. [Comparative Study]. *J Neurosci Methods, 14*(3), 149-167.

Pini, R. T. B., Ferreira do Vales, L. D. M., Braga Costa, T. M., & Almeida, S. S. (2017). Effects of cafeteria diet and high fat diet intake on anxiety, learning and memory in adult male rats. *Nutr Neurosci, 20*(7), 396-408. doi: 10.1080/1028415X.2016.1149294

Pugazhenthi, S., Qin, L., & Reddy, P. H. (2017). Common neurodegenerative pathways in obesity, diabetes, and Alzheimer's disease. *Biochim Biophys Acta, 1863*(5), 1037-1045. doi: 10.1016/j.bbadis.2016.04.017

Raha, S., Lee, H. J., Yumnam, S., Hong, G. E., Venkatarame Gowda Saralamma, V., Ha, Y. L., . . . Kim, G. S. (2016). Vitamin D2 suppresses amyloid-beta 25-35 induced microglial activation in BV2 cells by blocking the NF-kappaB inflammatory signaling pathway. *Life Sci, 161*, 37-44. doi: 10.1016/j.lfs.2016.07.017

Roberts, M. N., Wallace, M. A., Tomilov, A. A., Zhou, Z., Marcotte, G. R., Tran, D., . . . Lopez-Dominguez, J. A. (2017). A Ketogenic Diet Extends Longevity and Healthspan in Adult Mice. *Cell Metab, 26*(3), 539-546 e535. doi: 10.1016/j.cmet.2017.08.005

Sack, M., Lenz, J. N., Jakovcevski, M., Biedermann, S. V., Falfan-Melgoza, C., Deussing, J., . . . Auer, M. K. (2016a). Early effects of a high-caloric diet and physical exercise on brain volumetry and behavior: a combined MRI and histology study in mice. *Brain Imaging Behav*. doi: 10.1007/s11682-016-9638-y10.1007/s11682-016-9638-y [pii]

Sack, M., Lenz, J. N., Jakovcevski, M., Biedermann, S. V., Falfan-Melgoza, C., Deussing, J., . . . Auer, M. K. (2016b). Early effects of a high-caloric diet and physical exercise on brain volumetry and behavior: a combined MRI and histology study in mice. *Brain Imaging Behav, 11*(5), 1385-1396. doi: 10.1007/s11682-016-9638-y10.1007/s11682-016-9638-y [pii]

Schimidt, H. L., Garcia, A., Martins, A., Mello-Carpes, P. B., & Carpes, F. P. (2017). Green tea supplementation produces better neuroprotective effects than red and black tea in Alzheimer-like rat model. *Food Res Int, 100*(Pt 1), 442-448. doi: 10.1016/j.foodres.2017.07.026

Schimidt, H. L., Vieira, A., Altermann, C., Martins, A., Sosa, P., Santos, F. W., . . . Carpes, F. P. (2014). Memory deficits and oxidative stress in cerebral ischemia-

reperfusion: neuroprotective role of physical exercise and green tea supplementation. *Neurobiol Learn Mem, 114*, 242-250. doi: 10.1016/j.nlm.2014.07.005

Soares, M. B., Ramalho, J. B., Izaguirry, A. P., Pavin, N. F., Spiazzi, C. C., Schimidt, H. L., . . . Santos, F. W. (2017). Comparative effect of Camellia sinensis teas on object recognition test deficit and metabolic changes induced by cafeteria diet. *Nutr Neurosci*, 1-10. doi: 10.1080/1028415x.2017.1418726

Soultanov, V., Fedotova, J., Nikitina, T., Roschin, V., Ordyan, N., & Hritcu, L. (2017). Antidepressant-Like Effect of Ropren(R) in beta-Amyloid-(25-35) Rat Model of Alzheimer's Disease with Altered Levels of Androgens. *Mol Neurobiol, 54*(4), 2611-2621. doi: 10.1007/s12035-016-9848-8

Stancampiano, R., Cocco, S., Cugusi, C., Sarais, L., & Fadda, F. (1999). Serotonin and acetylcholine release response in the rat hippocampus during a spatial memory task. [Research Support, Non-U.S. Gov't]. *Neuroscience, 89*(4), 1135-1143.

Vandal, M., White, P. J., Tremblay, C., St-Amour, I., Chevrier, G., Emond, V., . . . Calon, F. (2014). Insulin Reverses the High-Fat Diet-Induced Increase in Brain Abeta and Improves Memory in an Animal Model of Alzheimer Disease. *Diabetes*. doi: 10.2337/db14-0375

Wang, A. L., Fazari, B., Chao, O. Y., Nikolaus, S., Trossbach, S. V., Korth, C., . . . de Souza Silva, M. A. (2017). Intra-nasal dopamine alleviates cognitive deficits in tgDISC1 rats which overexpress the human DISC1 gene. *Neurobiol Learn Mem, 146*, 12-20. doi: S1074-7427(17)30166-1 [pii]10.1016/j.nlm.2017.10.015

Xiang, L., Cao, X. L., Xing, T. Y., Mori, D., Tang, R. Q., Li, J., . . . Qi, J. H. (2016). Mixture of Peanut Skin Extract and Fish Oil Improves Memory in Mice via Modulation of Anti-Oxidative Stress and Regulation of BDNF/ERK/CREB

Signaling Pathways. *Nutrients, 8*(5). doi: 10.3390/nu8050256E256 [pii]nu8050256 [pii]

Zhang, Y., Sun, D., Meng, Q., Guo, W., & Chen, Q. (2016). Calcium Channels Contribute to Albiflorin-mediated Antinociceptive Effects in Mouse Model. *Neurosci Lett, 628*, 105-109. doi: 10.1016/j.neulet.2016.03.054

CAPITULO IV

ARTIGO ORIGINAL

THE ROLE OF REGULAR PHYSICAL EXERCISE FOR ENHANCEMENT OF LONG-TERM MEMORY IN THE ELDERLY: A REVIEW OF RECENT EVIDENCES

O papel do exercício físico regular na melhora da memória de longo prazo em idosos: uma revisão de evidências recentes

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Abstract

We performed a systematic review of recent literature to discuss the role of physical exercise on cognition of the elderly, especially long-term memory (LTM). Eight articles were included. Exercise programs improving LTM in the elderly involved aerobic activities performed 2-3 times per week. LTM improvements were correlated with brain-specific regions changing volume and depend on intensity at least moderate performed at least during 2.5 to 3 months. Exercise programs to improve LTM require strict control of exercise intensity while different training duration and weekly sessions seem effective. Training focusing only in walking sessions requires further investigations to ensure its potential in improving LTM in the elderly.

Keywords: physical training, aging, learning, cognition, memory.

1 Introduction

Physical exercise is a source for health and quality of life in the elderly. It contributes to strength gains (Cadore, Pinto, Bottaro, & Izquierdo, 2014), balance improvement (Weerdesteyn et al., 2006), independent locomotion and better general health status in the elderly (M. Izquierdo & Cadore, 2014). Psychological benefits are also observed in the elderly engaged in programs of regular physical exercise (Ko, Jung, & Jeong, 2014). Besides these benefits enhancing performance of daily life activities, the impact of physical exercise on cognitive function has been extensively investigated in different contexts, and often suggested as positively influencing cognitive function (Angevaren, Aufdemkampe, Verhaar, Aleman, & Vanhees, 2008; Roig, Nordbrandt, Geertsen, & Nielsen, 2013). One important cognitive function that continuously brings attention from scientists is the long-term memory.

The long-term memory (LTM) plays important roles for the cognitive integrity in the elderly. The LTM can last days, months and even years. They require time for its establishment and consolidation processes, which may take several hours (I. Izquierdo et al., 1998). Along this process, increase in brain gene expression and protein synthesis is observed. While the LTM is in formation, the short-term memory, which lasts until 6 hours, still is available (I. Izquierdo et al., 1998). The normal aging process during the life span can impairs LTM formation/consolidation (Snigdha, de Rivera, Milgram, & Cotman, 2014). Such impairments will negatively impact the health status and quality of life in the elderly, limiting independence and security in the performance of daily life activities, such as cooking (McDaniel et al., 2014). A number of studies addressed the effects of exercise on memory in the elderly, and at least two recent reviews have extensively described the relationships between exercise and LTM (Angevaren et al.,

2008; Roig et al., 2013). In fact, most of the studies on this topic are considering animal studies (Barnes, 2015).

Additionally, an expressive number of articles consider the acute effects of exercise on the memory; for examples, see additional references (Hogan, Mata, & Carstensen, 2013; Nielson, Wulff, & Arentsen, 2014) and a recent review (Roig et al., 2013). It is known that among the acute effects of exercise are the releases of catecholamine (Krzeminski, Cybulski, Ziemba, & Nazar, 2012) and arousal manipulation, which may contribute to speed up or up regulate specific brain mechanics related to cognitive processes (Nielson & Powless, 2007; Nielson, Radtke, & Jensen, 1996). However, such effects can be transient, sometimes not related to permanent gains, and not directly related to physical exercise effects, but to effects of any intervention that manipulate the arousal (Nielson & Meltzer, 2009). The evidences seems to support that acute effect of exercise on acquisition of new memories can be very positive in the elderly, and long-term exercise would contribute to responses of the molecular machinery responsible for memory processing (Roig et al., 2013).

Although we are able to find some reviews addressing memory processes in the elderly; for examples, see additional references (Barnes, 2015; Hernandez et al., 2014; Yau, Gil-Mohapel, Christie, & So, 2014), most of them present outcomes limited to particular groups, for example, Alzheimer patients. Discussing a particular group affected by a disease is important, but presents a significant limitation when trying to extrapolate these results to the independent elderly. Although the incidence of cognitive impairments is high in the late age, we cannot neglect the number of elderly carrying on daily activities without such major cognitive deficits.

We found recent reviews addressing the role of physical exercise for enhancing memory in the elderly without cognitive impairment (Angevaren et al., 2008; Roig et

al., 2013). Most of the studies included (considering a larger time window than we considered in the present review, the number of included articles was relatively low, i.e., 22 and 19 articles for acute and long-term effects of exercise, respectively (Roig et al., 2013), and 11 articles addressing the relationships between cardiorespiratory fitness and long-term memory (Angevaren et al., 2008). If the recent investigations have considered this previous evidences and suggestions when designing interventions in the elderly, still unknown. Furthermore, characteristics of recent interventions to improve LTM in the elderly by means of physical training have not been reviewed. Therefore, here we conducted a systematic review of studies addressing the effects of physical exercise on LTM in the elderly. Through this systematic review we tried to gather the literature of current exercises programs that aimed to improve long-term memory in the elderly and to present their main results and clinical application, more specifically, discussing the exercise configurations and its specific effects on LTM in independent elderly.

From a clinical perspective, human research still raises questions concerning how exercise can be used to improve LTM or to minimize losses related to the expected aging process. Based in the controversial results in the literature, our systematic review discusses this topic in light of studies that trained the elderly and evaluated their cognitive function in well-controlled conditions. The findings of our systematic review provide important information that can help in the clinical decision of exercise administration in the elderly. More than describing exercise interventions and its main results, our systematic analysis suggests some characteristics that interventions based in physical training should include in attempt to increase the chances of success in the purpose of improving LTM by exercise.

2 Materials and methods

2.1 Data sources and searches

We analyzed relevant articles published in scientific journals indexed in Medline searched through PubMed and PubMed Central websites. Only articles published as full-text between January 1st 2005 and December 29th 2014 were considered. Comprehensive reviews were performed until 2005(Angevaren et al., 2008; Roig et al., 2013), and this is the main reason we focused on publications from the last decade. The following combinations of keywords and Mesh Terms were used for the searches:

- In the PubMed: “(("long term memory"[MeSH Terms] OR "memory"[All Fields]) AND ("aged"[MeSH Terms] OR "aged"[All Fields] OR "elderly"[All Fields]) AND ("exercise"[MeSH Terms] OR "exercise"[All Fields])) AND (Journal Article[ptyp] AND "2005/01/01"[PDat]: "2014/12/29"[PDat] AND "humans"[MeSH Terms])”
- In the PubMed Central: “(((((("long term memory"[MeSH Terms] OR "memory"[All Fields]) AND ("aged"[MeSH Terms] OR "aged"[All Fields] OR "elderly"[All Fields])) AND ("exercise"[MeSH Terms] OR "exercise"[All Fields])) AND ("2004/01/01"[PrintPubDate]: "2014/"[PrintPubDate])) AND ("men"[MeSH Terms] OR "men"[All Fields])) AND ("women"[MeSH Terms] OR "women"[All Fields])”

2.2 Study identification

Original articles investigating the effects of systematic physical exercise on at least one parameter of LTM in the independent elderly, as define by age 65 or higher, without cognitive impairments were included in this review. Cognitive status was ensured based in the information presented by the authors in the included articles, or by information of mini-mental score higher than 24. To be included, papers should be published as a journal article, involving experiments with humans, written in English language, and discussing the effects of physical exercise training on LTM, not only acute effects of exercise; paper should present information on intensity, weekly frequency, and duration of the sessions performed. Case studies, pilot studies and those papers considering subjects with some neurological impairment that could significantly affect memory processes (for example, Alzheimer disease) were not eligible. All processes of search, selection and review of the papers were conducted by at least two independent authors.

First of all, studies were selected considering their titles and abstracts. In this phase, concordance between reviewers was not mandatory. Afterwards, overlapping papers from different bases were excluded. Inclusion and exclusion criteria were applied for the rest of the papers, and those that fulfilled the criteria aforementioned were included. When reading the abstract did not permit to fully decide by inclusion or exclusion of the paper, the full text was checked. A third author solved any discrepancy between the two independent reviewers.

2.3 Quality assessment

We assessed the quality of individual studies by using a standardized form (for details, see the supplemental file) considering scores ranging from 0 to 1 (1 means better quality). Two independent reviewers performed the initial screening and extraction procedures. Then, another investigator independently assessed all articles satisfying the inclusion criteria. Whenever there was a disagreement, it was solved by collective discussion among the investigators.

2.4 Main outcomes

We extracted information about the clarity of the research purpose, participant details (including sample size, age, gender, health status), recruitment and sampling methods, presence of inclusion and exclusion criteria, control of co-variants in the data analysis, key outcome variables, methodological approach, reliability of the method used, key findings supported by the main results, and practical implications.

3 Results

3.1 Yield

The initial search returned 2,670 articles. After the first round of exclusions, 1,741 articles had the abstract and/or full text read to identify if they filled exclusion and inclusion criteria. In the final yield a total of 8 articles remained (Figure 1).

3.2 Quality analysis

A full description of all aspects of the quality assessment is presented as a supplemental file. Each score corresponds to the average from three independent authors. Scores near to 1 represents a better quality, and near to 0 a poor quality. Additionally, an average score for each study is included in the Table 1. When the quality analysis from each study was merged we observed that the average ± standard deviation for the quality assessment of the included papers was 0.85 ± 0.12.

3.3 Characteristics of the included studies

The main characteristics of the articles included in this systematic review are summarized in the Table 1. Further details on the exercise programs and outcomes related to LTM are presented in the Table 2. Details of the memory tests conducted in these articles can be reviewed in a recent publication (Roig et al., 2013).

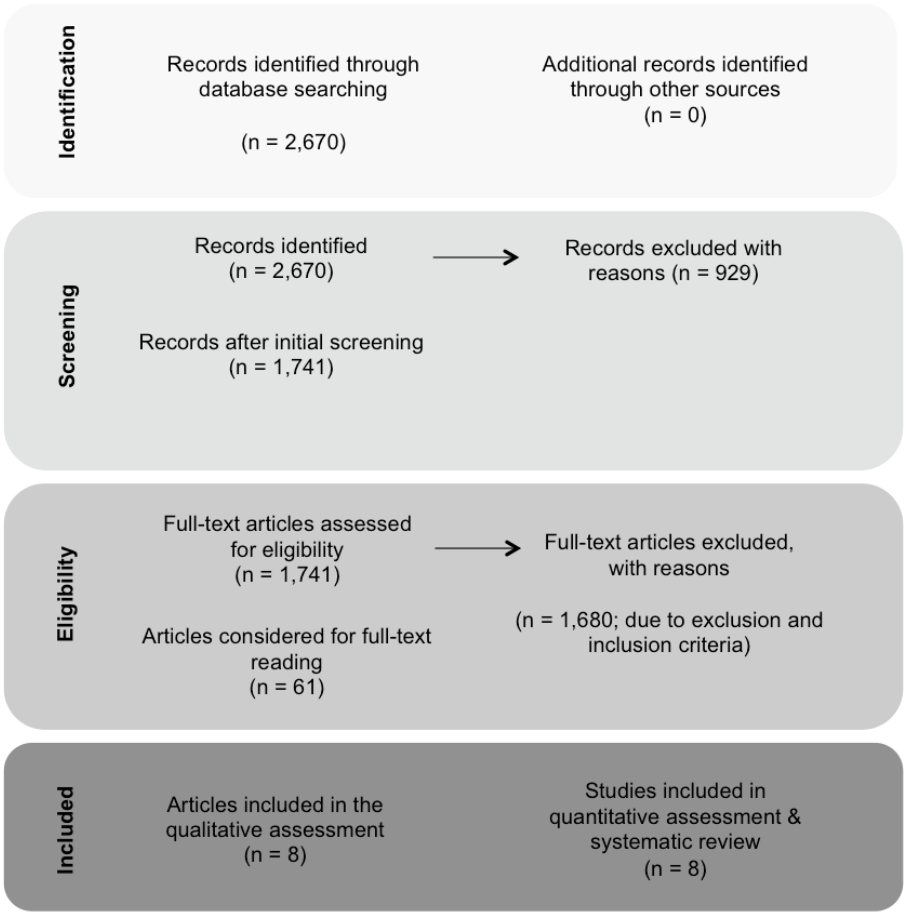

Figure 1. Flowchart of search and selection of the articles included in this systematic review.

Five studies (Erickson et al., 2011; Kamegaya et al., 2012; Langlois et al., 2013; Ruscheweyh et al., 2011; Tseng et al., 2013) found improvements in LTM in response to single physical training, one study (Theill, Schumacher, Adelsberger, Martin, & Jancke, 2013) only observed benefits of physical training when it was combined with a cognitive training, and two studies (McDaniel et al., 2014; van Uffelen, Chinapaw, Hopman-Rock, & van Mechelen, 2009) did not find benefits of physical exercise on LTM in the independent elderly.

Table 1 - Main characteristics of the studies included in this systematic review.

Studies	***Study design***	***Quality assessment***	***Elderly participants***			***Cognitive testing****
		Average score	*n*	*age*	*gender*	
Mc Daniel et al 2014	I	0.92	74	65 ± 6	M, F	Cooking Breakfast, Virtual Week, Memory for Health Information
Tseng et al 2013	C	0.69	24	73 ± 5	M, F	Executive function using Delis-Kaplan Executive Function System, Trail Making Test, Stroop Color Word Test; Declarative memory using California Verbal Learning Test-II, Working Memory, and processing speed and reaction time using Automated Neuropsychological Assessment Metrics Battery
Theill et al 2013	I	0.77	63	72 ± 5	M, F	Cognitive transfer including selective attention, paired-associates learning, executive control, reasoning, memory span, information processing speed; motor-cognitive dual task; verbal working memory.
Langlois et al 2012	I	0.92	83	66 ± 6	M, F	MMSE, abstract verbal reasoning, processing speed, Trail Making Test, modified Stroop Color Word Test, working memory, episodic memory, and executive function
Kamegaya et al 2012	I	0.73	30	74 ± 5	M, F	Five-cog test (attention, memory, visuospatial function, language and reasoning) and Wechsler Digit Symbol Substitution Test
Ruscheweyh et al 2011	I	0.99	62	60 ± 6	M, F	Episodic memory performance Beck's Depression Inventory
Erickson et al 2011	RCT	0.99	120	66 ± 6	M, F	Spatial memory task
Uffelen et al 2009	RCT	0.77	152	75 ± 3	M, F	Mini Mental State Examination, Auditory Verbal Learning Test, Verbal Fluency Test, Digital Symbol Substitution Test, Abridged Stroop Color Word Test

Age is presented for mean ± standard deviation of the elderly included in the study. n: number of participants; I: interventional study; C: cross-sectional; RCT: randomized controlled trial; M: male; F: female; * additional details on the cognitive testing are available in the papers cited in this systematic review.

Table 2 - Main characteristics of the exercises programs described in the studies included in this systematic review.

Studies	***Training programs***	***Weekly Frequency***	***Session Duration***	***Exercise intensity***	***Program duration***	***Effects on memory****
Mc Daniel et al 2014	Treadmill walking or bicycle program combined or not with cognitive training	3	Progressive from 15 to 50 minutes	Progressive from 50-60% to 65-85% of HRR, or Borg's scale from 12-12 to 15-16	6 m	Cognitive training but not aerobic exercise improves prospective memory. Single cognitive or aerobic training did not improve coordinating multiple tasks and retrospective memory
Tseng et al 2013	Endurance training performed for at least 15 previous years	ND	ND	ND	180 m	Long-term training improves executive function and semantic memory processing. Cognitive performance is similar between elderly and young adults
Theill et al 2013	Treadmill walking combined or not with a cognitive task	2	40 minutes	60-80% of maximal heart rate for age (220 minus age)	2.5 m	Simultaneous cognitive and physical training improves cognitive performance. Single exercise cognitive training only improves working memory and executive control tasks
Langlois et al 2012	Aerobic training followed by a short period of strength training	3	60 minutes	Moderate to hard (Borgs' scale)	3 m	Exercise improves processing speed, working memory, and executive function
Kamegaya et al 2012	Body flexibility, strength and balance, and endurance	1	45 minutes	ND	3 m	Exercise improves recall task included in the Five-cog test and the Wechsler Digit Symbol Substitution Test, without improvements in physical conditioning
Ruscheweyh et al 2011	Nordic walking or gymnastics	3-5	50 minutes	Nordic walking at 50-60% of maximal exertion; Gymnastics at 30-40% of maximal exertion	6 m	Increases in physical activity were positively associated with increases in memory score regardless of exercise intensity
Erickson et al 2011	Walking program	ND	Progressive from 10 to 40 minutes	Walking: from 50-60 to 60-75% of the maximal HRR	12 m	Exercise training increases size of the hippocampus, leading to improvements in spatial memory
Uffelen et al 2009	Walking exercise	2	60 minutes	3 MET and aiming at low to moderate intensity	12 m	Exercise at low to moderate intensity improves aerobic conditioning but was not associated with changes in cognitive performance

All exercise programs performed in the included articles involved aerobic activities (walking was the most frequent activity included in the training regimen), and the average weekly frequency was 2-3 days, at least. For those not observing any effect of physical exercise on LTM (McDaniel et al., 2014; van Uffelen et al., 2009), target intensity was described as low to moderate. When exercise was effective for LTM, intensity was described as at least moderate (Erickson et al., 2011; Langlois et al., 2013; Ruscheweyh et al., 2011; Tseng et al., 2013) or eliciting endurance performance (Kamegaya et al., 2012). For two studies we were not able to fully identify the weekly frequency of training (Erickson et al., 2011; Tseng et al., 2013). Average weekly frequency of exercise was 2-3 times per week, and adherence was controlled to reach at least 70% of the sessions.

4 Discussion

In this systematic review 8 articles full filled the requirements to be included in the quality assessment. Their results suggest that LTM can be improved by physical exercise, but the success of interventions will depend on several aspects of the training program. Our review was not able to differentiate effects of different types of exercises since all studies were based in aerobic training. The different conclusions among the included articles raise questions concerning the influence of training configuration on its effects on LTM.

Previous reviews discussed the exercise characteristics that may determine LTM gains. They concluded that there is a lack of evidences to support the assumption that improvements in cognitive function can be attributed to physical exercise due to improvements in cardiovascular fitness although there is a temporal association between

cognitive and fitness gains (Angevaren et al., 2008) as well as acute and long-term exercise may reflect two different strategies to improve memory (Roig et al., 2013).

In general, the duration of exercise sessions increases from the beginning to the end of the training intervention, which permits the participant a proper adaptation to the training workload. For most of cases the frequency was of 2-3 days per week and therefore is was not possible to discriminate effects of exercise in relation to the weekly frequency of exercise. Similarly, training sessions had similar duration between the different studies (up to 50-60 min; for more details, see Table 2).

Longer exercise programs in general resulted in LTM gains, but longer periods of training seemed not mandatory to improve LTM. The longer period of training considered was 15 years and included endurance intensity. In this case, master athletes were evaluated and the main outcomes of the systematic long-term endurance training were an improved executive function and memory, with elderly achieving similar results in comparison to young adults (Tseng et al., 2013). The shortest period of training resulting in LTM gains had duration of 3 months (Kamegaya et al., 2012; Langlois et al., 2013). Contradictory results were found in the different studies in which elderly were trained during 6 (McDaniel et al., 2014; Ruscheweyh et al., 2011) and 12 months (Erickson et al., 2011; van Uffelen et al., 2009). Six months of walking combined or not with a cognitive training (McDaniel et al., 2014) did not result in LTM improvement as observed after similar period of Nordic walking (Ruscheweyh et al., 2011). Also, 12 months of walking at low to moderate intensity (van Uffelen et al., 2009) did not improve LTM as observed after 12 months attending a walking program (Erickson et al., 2011). Although the different results for the same training durations is curious, explanation for such contradictory result may not rely on the duration of the program itself, but the lower exercise intensity for both trainings finding no LTM

improvement after 6 and 12 months of activity. Walking training did not improve physical conditioning for subjects performing at lower intensities (van Uffelen et al., 2009). Exercise programs based in walking seemed to result in controversial results and therefore only walking cannot be ensured as an efficient exercise for cognitive improvement as depicted by LTM in the independent elderly.

Even shorter interventions can provide the elderly with benefits for memory. A short physical training period (20 sessions) combined with a cognitive training promoted good results for LTM in the elderly (Theill et al., 2013). In this case, exercise intensity was higher compared to the other studies. Unfortunately, the authors were not able to describe the single-exercise effect, as such experimental condition was missing in the methods. In addition, varying activities during a short period of physical training may play a role for LTM improvements. Therefore it is not clear if the main task performed during the training period is the actual determinant of its benefits for LTM.

How much exercise is need is a good question that we were able to partially answer. However, in addition to the 2-3 sessions of physical exercise per week for least 2.5 or 3 months, which exercise is performed still questionable. Benefits for LTM were reported after 3 months of training once a week (Kamegaya et al., 2012) in a routine of stretching exercise and activities (which were not fully detailed in the article) aiming at development of body flexibility, strength, balance and endurance performed in elderly groups. It is possible that exercise programs not focused on a specific exercise type can provide similar benefit that those single-activities orientated. Exercises based exclusively in walking programs were not effective to improve memory (McDaniel et al., 2014; Theill et al., 2013; van Uffelen et al., 2009); such result was achieved when walking exercise program had a specific configuration, such as the case of Nordic walking improving LTM (Ruscheweyh et al., 2011).

Not only start a training program, but also keep active on doing this is important. Therefore, it is important to know if missing training session can impair the benefits expected for LTM in the physical training. Most of the studies mentioned controlled adherence to the training session, and take it into account during the data analyses considering only those participants that joined a significant number of sessions (for example, at least 70% of the sessions). However, one study reported that cognition improvement was not significantly associated with session attendance (van Uffelen et al., 2009). However, this study (van Uffelen et al., 2009) did not find association between exercise and cognitive improvement most likely because exercise was performed at low to moderate intensities, or the lack of adherence control may have influenced the elderly performance. Therefore, it seems to be recommendable that adherence to the training be considered when analyzing data, but more importantly to ensure that the elderly can in fact be benefitted by attending the physical training.

If behavioral measures of LTM are positively affected by exercise, some brain adaptation could be expected. A few studies addressed LTM-related neural adaptations to the physical training. Among the neural adaptations associated with improved LTM there were increases in gray and white matter concentrations, especially in the hippocampus, prefrontal and cingulate cortex extending into occipital, frontal and parietal cortices. Therefore, we could argue that the physical exercise did not influence the different brain regions in the same extent. An increase in the hippocampus volume was observed in the trained elderly (Erickson et al., 2011). In other hand, a control group (performing only stretching exercises at low intensity) reduced hippocampal volume over one-year (Erickson et al., 2011). Both groups (walking and stretching) improved spatial memory, which was positively correlated with physical fitness. Elderly engaged in endurance training for significant period of life (~15 years) presented higher

gray matter concentrations in the right parietal lobe, occipital lobe, and cerebellum than sedentary elderly (Tseng et al., 2013). White matter was also higher in the trained elderly than sedentary ones when looking at the parietal, temporal and occipital lobe (Tseng et al., 2013). Gray matter volume was correlated with physical activity for several cortical areas of elderly trained in the Nordic walking program(Ruscheweyh et al., 2011). Significant correlations between fitness and gray matter volume emerged in the left prefrontal and cingulate cortex extending into occipital cortex (Ruscheweyh et al., 2011). The prefrontal cortex contributes to memory through strategic control over memory retrieval processes within other brain areas as the hippocampus and amygdala. Additionally the hippocampus provides outputs to others cortical areas. These feedback pathways allow the retrieval of detailed memories in the hippocampus that constitute strong recollected experiences and recollection memory (Preston & Eichenbaum, 2013).

Serum brain-derived neurotrophic factor (BDNF) did not differ between elderly trained for walking and controls, but correlated with hippocampal volume in the exercise group (Erickson et al., 2011). In addition, serum BDNF was not correlated with spatial memory (Erickson et al., 2011). It could be related to the fact that the measurements presented are from peripheral BDNF, and, although it can be used as an approximation of central BDNF levels, it does not exactly represents central BDNF levels, because the BDNF can cross the blood-brain barrier (Pan, Banks, Fasold, Bluth, & Kastin, 1998). The measure of BDNF in the brain most likely could provide a better idea of the impact of physical exercise on LTM, since seems clear that physical exercise increases the brain BDNF levels and the BDNF is involved with synaptic plasticity and neurogenesis in neuronal survival (Erickson, Miller, & Roecklein, 2012). However, such measure is limited in humans.

Physical exercise may influence LTM due to the increase of others neurotrophic factors than by BDNF, as the vascular endothelial growth factor (Cotman, Berchtold, & Christie, 2007), which when associated to increased brain blood perfusion (Swain et al., 2003) could explain memory enhance. However, such measures were not performed in the studies included in this systematic review. Moreover, it is still matter of debate whether peripheral measures of neurotrophic factors, for instance, are indicators of central levels. They definitely measure on a different level than neuroimaging techniques and therefore, could not replace them.

In one of the included articles, the catecholamines were positively correlated with physical activity levels. It was observed positive correlations of physical activity with dopamine, but not with epinephrine or norepinephrine levels (Ruscheweyh et al., 2011). Although epinephrine and norepinephrine cannot cross the blood brain barrier, the peripheral release of epinephrine and norepinephrine can activate peripheral β-adrenergic receptors that project to higher areas of the central nervous system involved in the regulation of memory, such as the hippocampus (Skriver et al., 2014). The mechanisms on how physical activity affects LTM are not fully understood. The oxidative stress and inflammation processes, increase of vascularization, release of neurotrophins and catecholamines and increased neurogenesis, especially in the hippocampus, are also mentioned as possible pathways (Ruscheweyh et al., 2011). However, not all of them were investigated in the studies presented in this review.

A tentative representation of the factors most likely related to LTM improvement in response to regular physical training during the aging is illustrated in the Figure 2.

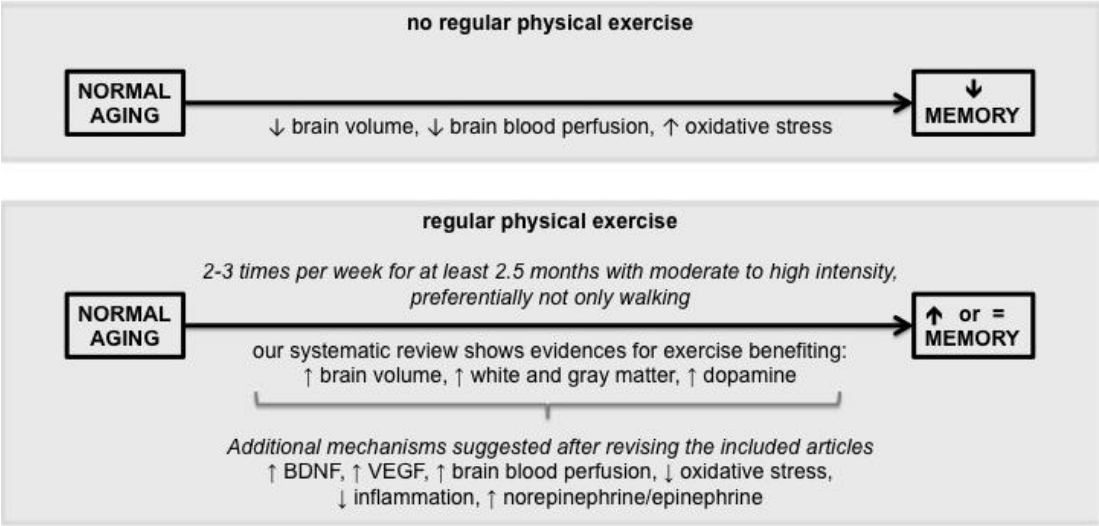

Figure 2. Overview of the exercise-related benefits for long-term memory in the independent elderly, and the possible factors related to LTM improvement in response to physical training.

Education (as quantified by years) seems important to be a controlled covariant when comparing trained and control elderly. When trained elderly had lower education than controls, aerobic training per se was not able to improve LTM (McDaniel et al., 2014), while for trained elderly with higher education than control (Langlois et al., 2013) it resulted in improved LTM. For studies considering participants with similar education, physical exercise was positively associated with LTM performance (Ruscheweyh et al., 2011; Theill et al., 2013; Tseng et al., 2013), but only when physical exercise was performed at low intensity (van Uffelen et al., 2009). Other studies did not discuss whether physical exercises performed alone or in small groups may influence LTM outcomes in different extends. Activities performed in groups most likely can help to promote elderly's adherence to the training, and adherence was shown important in the included articles. In addition, strength exercises, which are very often found as part of fall-prevention programs in the elderly (Cadore et al., 2014; Ko et al.,

2014), have not been addressed considering its impact cognitive performance. Other physical exercises programs that are becoming popular among the elderly (Pilates for instance) have no been discussed considering LTM benefits yet.

5 Conclusion

Exercise programs to improve LTM require strict control of exercise intensity while different training duration and weekly sessions seem effective. Based in the studies included in this systematic review, training based in walking exercise requires further investigations to ensure its efficacy for enhancement of long-term memory.

Conflict of Interest

The authors have no conflict of interest concerning the content of the paper.

References

Angevaren, M., Aufdemkampe, G., Verhaar, H. J., Aleman, A., & Vanhees, L. (2008). Physical activity and enhanced fitness to improve cognitive function in older people without known cognitive impairment. *Cochrane Database Syst Rev*(3), CD005381. doi: 10.1002/14651858.CD005381.pub3

Barnes, J. N. (2015). Exercise, cognitive function, and aging. *Adv Physiol Educ, 39*(2), 55-62. doi: 10.1152/advan.00101.2014

Cadore, E. L., Pinto, R. S., Bottaro, M., & Izquierdo, M. (2014). Strength and endurance training prescription in healthy and frail elderly. *Aging Dis, 5*(3), 183-195. doi: 10.14336/AD.2014.0500183

Cotman, C. W., Berchtold, N. C., & Christie, L. A. (2007). Exercise builds brain health: key roles of growth factor cascades and inflammation. *Trends Neurosci, 30*(9), 464-472. doi: 10.1016/j.tins.2007.06.011

Erickson, K. I., Miller, D. L., & Roecklein, K. A. (2012). The aging hippocampus: interactions between exercise, depression, and BDNF. *Neuroscientist, 18*(1), 82-97. doi: 10.1177/1073858410397054

Erickson, K. I., Voss, M. W., Prakash, R. S., Basak, C., Szabo, A., Chaddock, L., . . . Kramer, A. F. (2011). Exercise training increases size of hippocampus and improves memory. *Proc Natl Acad Sci U S A, 108*(7), 3017-3022. doi: 10.1073/pnas.1015950108

Hernandez, S. S., Sandreschi, P. F., Silva, F. C., Arancibia, B. A., da Silva, R., Gutierres, P. J., & Andrade, A. (2014). What are the Benefits of Exercise for Alzheimer s Disease? A Systematic Review of Past 10 Years. *J Aging Phys Act*. doi: 10.1123/japa.2014-0180

Hogan, C. L., Mata, J., & Carstensen, L. L. (2013). Exercise holds immediate benefits for affect and cognition in younger and older adults. *Psychol Aging, 28*(2), 587-594. doi: 10.1037/a0032634

Izquierdo, I., Barros, D. M., Mello e Souza, T., de Souza, M. M., Izquierdo, L. A., & Medina, J. H. (1998). Mechanisms for memory types differ. *Nature, 393*(6686), 635-636. doi: 10.1038/31371

Izquierdo, M., & Cadore, E. L. (2014). Muscle power training in the institutionalized frail: a new approach to counteracting functional declines and very late-life disability. *Curr Med Res Opin, 30*(7), 1385-1390. doi: 10.1185/03007995.2014.908175

Kamegaya, T., Maki, Y., Yamagami, T., Yamaguchi, T., Murai, T., & Yamaguchi, H. (2012). Pleasant physical exercise program for prevention of cognitive decline in community-dwelling elderly with subjective memory complaints. *Geriatr Gerontol Int, 12*(4), 673-679. doi: 10.1111/j.1447-0594.2012.00840.x

Ko, D. S., Jung, D. I., & Jeong, M. A. (2014). Analysis of Core Stability Exercise Effect on the Physical and Psychological Function of Elderly Women Vulnerable to Falls during Obstacle Negotiation. *J Phys Ther Sci, 26*(11), 1697-1700. doi: 10.1589/jpts.26.1697

Krzeminski, K., Cybulski, G., Ziemba, A., & Nazar, K. (2012). Cardiovascular and hormonal responses to static handgrip in young and older healthy men. *Eur J Appl Physiol, 112*(4), 1315-1325. doi: 10.1007/s00421-011-2069-y

Langlois, F., Vu, T. T., Chasse, K., Dupuis, G., Kergoat, M. J., & Bherer, L. (2013). Benefits of physical exercise training on cognition and quality of life in frail older adults. *J Gerontol B Psychol Sci Soc Sci, 68*(3), 400-404. doi: 10.1093/geronb/gbs069

McDaniel, M. A., Binder, E. F., Bugg, J. M., Waldum, E. R., Dufault, C., Meyer, A., . . . Kudelka, C. (2014). Effects of cognitive training with and without aerobic exercise on cognitively demanding everyday activities. *Psychol Aging, 29*(3), 717-730. doi: 10.1037/a0037363

Nielson, K. A., & Meltzer, M. A. (2009). Modulation of long-term memory by arousal in alexithymia: the role of interpretation. *Conscious Cogn, 18*(3), 786-793. doi: 10.1016/j.concog.2009.06.001

Nielson, K. A., & Powless, M. (2007). Positive and negative sources of emotional arousal enhance long-term word-list retention when induced as long as 30 min after learning. *Neurobiol Learn Mem, 88*(1), 40-47. doi: 10.1016/j.nlm.2007.03.005

Nielson, K. A., Radtke, R. C., & Jensen, R. A. (1996). Arousal-induced modulation of memory storage processes in humans. *Neurobiol Learn Mem, 66*(2), 133-142. doi: 10.1006/nlme.1996.0054

Nielson, K. A., Wulff, L. L., & Arentsen, T. J. (2014). Muscle tension induced after learning enhances long-term narrative and visual memory in healthy older adults. *Neurobiol Learn Mem, 109*, 144-150. doi: 10.1016/j.nlm.2014.01.008

Pan, W., Banks, W. A., Fasold, M. B., Bluth, J., & Kastin, A. J. (1998). Transport of brain-derived neurotrophic factor across the blood-brain barrier. *Neuropharmacology, 37*(12), 1553-1561.

Preston, A. R., & Eichenbaum, H. (2013). Interplay of hippocampus and prefrontal cortex in memory. *Curr Biol, 23*(17), R764-773. doi: 10.1016/j.cub.2013.05.041

Roig, M., Nordbrandt, S., Geertsen, S. S., & Nielsen, J. B. (2013). The effects of cardiovascular exercise on human memory: a review with meta-analysis.

Neurosci Biobehav Rev, *37*(8), 1645-1666. doi: 10.1016/j.neubiorev.2013.06.012

Ruscheweyh, R., Willemer, C., Kruger, K., Duning, T., Warnecke, T., Sommer, J., . . . Floel, A. (2011). Physical activity and memory functions: an interventional study. *Neurobiol Aging*, *32*(7), 1304-1319. doi: 10.1016/j.neurobiolaging.2009.08.001

Skriver, K., Roig, M., Lundbye-Jensen, J., Pingel, J., Helge, J. W., Kiens, B., & Nielsen, J. B. (2014). Acute exercise improves motor memory: exploring potential biomarkers. *Neurobiol Learn Mem*, *116*, 46-58. doi: 10.1016/j.nlm.2014.08.004

Snigdha, S., de Rivera, C., Milgram, N. W., & Cotman, C. W. (2014). Exercise enhances memory consolidation in the aging brain. *Front Aging Neurosci*, *6*, 3. doi: 10.3389/fnagi.2014.00003

Swain, R. A., Harris, A. B., Wiener, E. C., Dutka, M. V., Morris, H. D., Theien, B. E., . . . Greenough, W. T. (2003). Prolonged exercise induces angiogenesis and increases cerebral blood volume in primary motor cortex of the rat. *Neuroscience*, *117*(4), 1037-1046.

Theill, N., Schumacher, V., Adelsberger, R., Martin, M., & Jancke, L. (2013). Effects of simultaneously performed cognitive and physical training in older adults. *BMC Neurosci*, *14*, 103. doi: 10.1186/1471-2202-14-103

Tseng, B. Y., Uh, J., Rossetti, H. C., Cullum, C. M., Diaz-Arrastia, R. F., Levine, B. D., . . . Zhang, R. (2013). Masters athletes exhibit larger regional brain volume and better cognitive performance than sedentary older adults. *J Magn Reson Imaging*, *38*(5), 1169-1176. doi: 10.1002/jmri.24085

van Uffelen, J. G., Chinapaw, M. J., Hopman-Rock, M., & van Mechelen, W. (2009). Feasibility and effectiveness of a walking program for community-dwelling older adults with mild cognitive impairment. *J Aging Phys Act, 17*(4), 398-415.

Weerdesteyn, V., Rijken, H., Geurts, A. C., Smits-Engelsman, B. C., Mulder, T., & Duysens, J. (2006). A five-week exercise program can reduce falls and improve obstacle avoidance in the elderly. *Gerontology, 52*(3), 131-141. doi: 10.1159/000091822

Yau, S. Y., Gil-Mohapel, J., Christie, B. R., & So, K. F. (2014). Physical exercise-induced adult neurogenesis: a good strategy to prevent cognitive decline in neurodegenerative diseases? *Biomed Res Int, 2014*, 403120. doi: 10.1155/2014/403120

CAPITULO V

MANUSCRITO SUBMETIDO

STRENGTH AND RUNNING TRAININGS ELICIT DIFFERENT NEUROPROTECTION OUTCOMES IN A B-AMYLOID PEPTIDE MEDIATED ALZHEIMER MODEL

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Abstract

It is widely accepted that aerobic exercise induces neuroprotection, but few studies have investigated whether strength training does so too. The impact of physical training on disrupted cognition is relevant to neurodegenerative conditions like Alzheimer disease (AD). Here we examine whether effects of running training differ from those of strength training concerning cognitive symptomatology, oxidative stress and cholinergic status in a model of AD-like cognitive impairment induced by intrahippocampal infusion of a mix of β-amyloid peptides (Aβ) in rats. Male Wistar rats were submitted to 8 weeks of daily running exercise (RunEx) (40 min sessions at 70% of indirect VO_2 max, 3 times per week) or strength exercise (StrEx) (3 times/week, 12 repetitions in 8 sets, 2 sets with repetitions at 50%, 2 at 75%, 2 at 90% and 2 at 100% of the maximum load), followed by Aβ infusion in the dorsal hippocampus. Short-term (STM) and long-term memory (LTM) of object recognition (OR), and social recognition memory (SR) were evaluated. Biochemical assays from hippocampus samples determined hippocampal oxidative status by quantification of reactive oxygen species (ROS), lipid peroxidation by thiobarbituric acid reactive substance test (TBARS), total antioxidant capacity by ferric reducing/antioxidant power (FRAP), and acetylcholinesterase enzyme activity (AChE). Aβ infusion is an often-used model of AD-like disease and resulted in STM and LTM deficits, hippocampal oxidative damage and changes in AchE. StrEx caused a better neuroprotection than RunEx by preventing OR and SR memory deficits, and prevented increase in lipid peroxidation and alterations on AChE activity. RunEx was neuroprotective only for SR memory deficits, prevented increase in ROS and lipid peroxidation, and preserved the total antioxidant capacity. While RunEx effects are more related to oxidative status, strength exercise was the only to show potential to influence the cholinergic system.

Keywords: aerobic exercise, anaerobic exercise, dementia, learning and memory, neurodegeneration, training.

1 Introduction

A healthy lifestyle protects against numerous medical disorders (Kivipelto and Solomon, 2008). Regular physical exercise is part of a healthy lifestyle and enhances cognitive function as well as delays the onset of dementias like Alzheimer's disease (AD) (Rolland, Abellan van Kan, and Vellas, 2010). AD is a progressive, chronic neurodegenerative disorder, reaching up to 100 million cases worldwide by the year 2050 (Qiu, Kivipelto, and von Strauss, 2009). AD has no cure and its causes are still not fully elucidated, but contributing risk factors are known to include aging and associated chronic diseases, for example diabetes and dyslipidemia (Kivipelto and Solomon, 2008).

AD increases formation of intracellular neurofibrillary tangles and extracellular amyloid plaques, which increase production of reactive oxygen species and inflammatory mediators (Kang, Lee, and Lee, 2017). The oxidative stress and the neuroinflammation lead to synaptic losses, selective death of neuronal cells, and reduction of CNS specific-neurotransmitters, which explain one of the main symptoms of AD: the memory deficits (Da Mesquita, Ferreira, Sousa, Correia-Neves, Sousa, and Marques, 2016; Schimidt, Garcia, Martins, Mello-Carpes, and Carpes, 2017). Memory is defined as the ability to acquire, retain and recall information, being fundamental to perform daily activities, and dependent on the integrity of the hippocampus and associated structures (Izquierdo, 2018). To deal with these cognitive impairments related to AD, physical exercise has been widely investigated considering different configurations (Abd El-Kader and Al-Jiffri, 2016; Brown, Peiffer, and Martins, 2012; Kim, Shin, Kim, Baek, Ko, and Kim, 2014).

Neuroprotection induced by exercise is related to neurogenesis and improved antioxidant capacity in the brain, especially in the hippocampus, which is very sensitive to oxidative stress (Ozbeyli, Sari, Ozkan, Karademir, Yuksel, Cilingir Kaya, and Kasimay Cakir, 2017). Most of the research on exercise and neuroprotection addresses the role of aerobic exercise, and only a few studies focused on neuroprotection resulting from strength exercise (Abd El-Kader and Al-Jiffri, 2016; Ozbeyli et al., 2017; Pietrelli, Lopez-Costa, Goni, Brusco, and Basso, 2012). Strength exercise is important in the context of AD since aging is associated with sarcopenia and gradual losses of muscle mass (Saji, Arai, Sakurai, and Toba, 2016). Such losses account for a marked decrease in the levels of physical activity and amount of contractile tissue (Menant, Weber, Lo, Sturnieks, Close, Sachdev, Brodaty, and Lord, 2016). Furthermore, strength training is effective to maintain muscle mass and increase muscle strength in the elderly (Cechetti, Worm, Elsner, Bertoldi, Sanches, Ben, Siqueira, and Netto, 2012). However, little is known about the effects of strength exercise on cognition.

Strength training seems to increase the levels of brain-derived neurotropic factor (BDNF), insulin-like growth factor I (IGF-1) and antioxidant activity (Eduardo Matta Mello, Poliane Gomes Torres, Renata, Eduardo, Renato Sobral, Sergio, and Andrea Camaz, 2015). The neurotropic factors have an important role on modulation of neurogenesis and reduction of amyloid deposition (Cassilhas, Viana, Grassmann, Santos, Santos, Tufik, and Mello, 2007). Therefore, an effect of an AD-like condition could be hypothesized. While there are many studies addressing effects of aerobic training mostly by running exercise (Lopez, Pinto, Radaelli, Rech, Grazioli, Izquierdo, and Cadore, 2017; Loprinzi, Frith, and Edwards, 2018; Nokia, Lensu, Ahtiainen, Johansson, Koch, Britton, and Kainulainen, 2016), there is a lack of evidences regarding effects of strength training on neuroprotection. In order to differentiate the

neuroprotection from different types of physical exercises, here we compared the effects of running training and strength training on cognitive symptomatology, hippocampal oxidative stress and cholinergic system in a AD-like model induced Aβ toxicity in rats. As our main outcome, we found strength exercise can be at least as neuroprotective as the running exercise, and present results of particular interest regarding specific memory types and the activity of the cholinergic system.

2 Material and methods

2.1 Animals and experimental design

Two month-old male Wistar rats were bought from a registered vivarium (DAR O NOME) and housed three per cage under controlled light and environmental conditions (12 h light/dark cycle at 23 ± 2°C and humidity 50 ± 10%) with food and water ad libitum. Experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH, 1996), and were approved by University's Animal Care and Use Committee (IRB #012015). Initially, the rats were randomly assigned into 3 groups: (a) no intervention (NI); (b) strength exercise (StrEx); and (c) running exercise (RunEx). After 8 weeks, surgeries were performed to inject Aβ or saline in the hippocampus (see details below) and groups were reorganized (n = 8-12/group), as follow:

- Group 1 – Control: rats not submitted to any intervention that underwent control surgery for intrahippocampal injection of saline;
- Group 2 – Control + StrEx: rats submitted to strength training and control surgery for intrahippocampal injection of saline;

- Group 3 – Control + RunEx: rats submitted to running training and control surgery for intrahippocampal injection of saline;
- Group 4 – Aβ: rats submitted to surgery for intrahippocampal injection of Aβ;
- Group 5 – Aβ + StrEx: rats submitted to strength training and surgery for intrahippocampal injection of Aβ ;
- Group 6 – Aβ + RunEx: rats submitted to running training and surgery for intrahippocampal injection of Aβ.

Ten days after surgery, the time necessary for beta-amyloid aggregation and plaque formation (Ghasemi, Zarifkar, Rastegar, Maghsoudi, and Moosavi, 2014), rats were submitted to behavioral tests and then euthanized. Biochemical analyses were performed in samples from the bilateral hippocampi to determine the levels of reactive oxygen species (ROS) by DCFH, lipid peroxidation by thiobarbituric acid reactive substances (TBARS), the total antioxidant capacity by ferric reducing/antioxidant power (FRAP), and the acetylcholinesterase enzyme activity (AChE). Figure 1 depicts the experimental design.

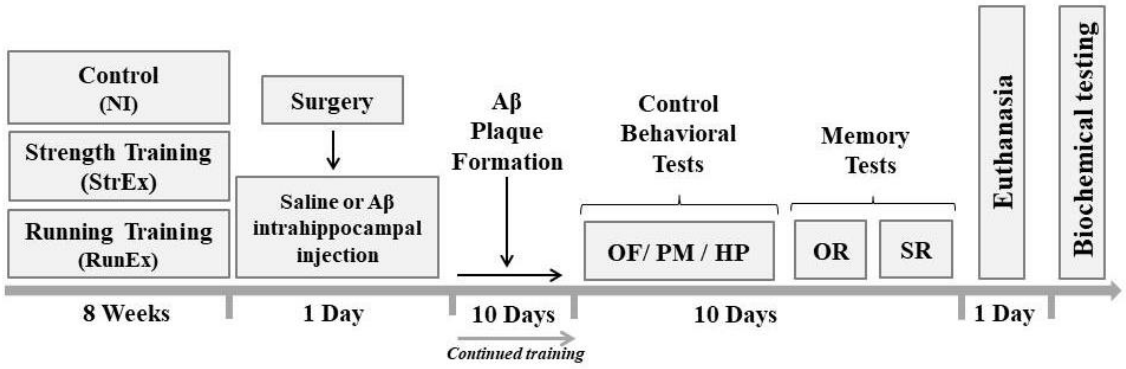

Figure 1 - Experimental design. Rats were maintained in standard conditions (Control/NI – no intervention) or submitted to strength (StrEx) or running training (RunEx) during 8 weeks. After the 8 weeks they were submitted to stereotaxic surgery and intrahippocampal injection of Aβ or saline. Behavioral tests started 10 days after surgery. Euthanasia was 20 days after surgery. Biochemical testing was the last step of the study. OF – open field; PM – plus maze; OR – object recognition memory test; SR – social recognition memory test.

2.2 Running exercise protocol

RunEx training lasted 8 weeks in a motorized treadmill built for rodents (Insight Ltda, SP/Brazil). RunEx was performed at intensity of 60–70% maximal indirect oxygen uptake (VO2) in sessions lasting 40 min, 3 times a week, always in the same period of the day, in light time period (Cechetti et al., 2012). In the week before the start of intervention, rats performed a treadmill running for ten minutes to habituate before the first maximal VO2 test. Maximal VO2 was determined indirectly during a running trial to determine time to fatigue (min) and the work volume (m/min). This is considered as an indirect measure of maximum VO2 (test started at a low velocity, increasing 5 m/min every 3 min until the rat was no longer able to run) (Brooks and

White, 1978). Each rat had the exercise intensity adjusted four weeks after training started by performing an additional indirect VO2 maximal running test.

2.3 Strength exercise protocol

StrEx training was performed using a vertical ladder custom made in wood and iron (1.1×0.18 m, 2 cm grid, 80° inclination) with a house chamber (20×20×20 cm) placed at the top. Rats were familiarized with the exercise of climbing the ladder during 3 days, with four trials per day. Once they climbed to the house chamber they could rest inside the chamber during 120 seconds. Strength training started one week after familiarization. On the first week the load used was 50% of the rat's body mass. In the second week of training the maximal carrying load (MCL) was determined. To determine the MCL, rats started climbing while carrying a load of 75% of the body mass, and for each additional climb, 30 g were added until the rat was no longer able to climb the entire ladder. The highest load successfully carried was considered the MCL. The MCL was determined on the first day of every training week. The training sessions consisted of eight ladder climbs being 2 repetitions for each loads of 50%, 75%, 90%, and 100% of the rat's previous MCL resulting in eight sets of 8-12 repetitions with a 1 min of rest interval among the repetitions (Barone, Bellafiore, Leonardi, and Zummo, 2009; Souza, Leite, de Souza Lino, de Cássia Marqueti, Bernardes, de Araújo, Bouskella, Shiguemoto, de Andrade Perez, and Kraemer-Aguiar, 2014). The strength training was conducted 3 days/week (one day for MCL test and 2 days for training), during 8 weeks.

2.4 Preparation of Aβ 25-35

Amyloid beta (Aβ) peptide 25-35 (Sigma Aldrich; Product Number: A4559) was dissolved in saline (vehicle) at a concentration of 100 μM. Before intrahippocampal injection, the Aβ was incubated at 37°C during 4 days to induce Aβ 25-35 aggregation. The Aβ 25-35 fragment shares with Aβ 1-42 the ability to self-aggregate and induce neurotoxicity (Ghasemi et al., 2014).

2.5 Stereotaxic surgery

The stereotaxic surgeries for intrahippocampal injection of 2 μl Aβ 25-35 or vehicle (saline) were performed after exercise training. Rats were anesthetized with ketamine and xylazine (i.p. 75 mg/kg and 10 mg/kg, respectively). When confirmed the anesthetic plan, the rats were mounted into a stereotaxic frame, and CA1 region of the dorsal hippocampus was located based in the Paxinos and Watson brain atlas (AP - 4.2, LL ± 3.0, VM - 2.0 mm) (Paxinos, Watson, and Emson, 1980). Bilateral infusions were performed using a Hamilton syringe and an infusion bomb. After surgery, rats were returned to their cages and monitored during 10 days, period required to surgery recovery and to induce the aggregation of Aβ protein in the hippocampus (Zussy, Brureau, Keller, Marchal, Blayo, Delair, Ixart, Maurice, and Givalois, 2013).

2.6 Control behavioral tasks

Exploratory and locomotor activity of the rats was analyzed 10 days after surgery to ensure that any procedure impaired such behaviors, altering the memory tests results. Rats were placed on the left quadrant of a 50 x 50 x 50 cm open field made with wood painted in white. Black lines divided the floor into 12 equal quadrants. Crossing and rearing, as measures for locomotor activity and exploration, respectively, were measured over 5 min (Bonini, Bevilaqua, Zinn, Kerr, Medina, Izquierdo, and Cammarota, 2006). To evaluate anxiety state, rats were exposed to an elevated plus maze and the time spent and the numbers of entries into the open arms were recorded over a 5 min session (Pellow, Chopin, File, and Briley, 1985).

2.7 Memory behavioral testing

2.7.1 Object recognition memory test (OR)

Training and testing in the object recognition (OR) task were carried out in an open-field arena (50 x 50 x 50 cm) built with wooden painted in white (Ennaceur and Delacour, 1988). Rats were first habituated individually in the apparatus and left to freely explore it for 20 min during 4 consecutive days before the training. For training session, two different objects (A and B) were placed in the apparatus and rats were allowed to freely explore them during 5 min. The objects were made of metal, glass, or glazed ceramic. Exploration was defined as sniffing or touching the objects with the nose and/or forepaws. Sitting on or turning around the objects was not considered an exploratory behavior. After 3 h and 24 h, in the short-term memory (STM) and long-term memory (LTM) test sessions, one of the objects was randomly exchanged for a

novel object (C and D, respectively) and the rats were reintroduced into the apparatus during 5 min. To avoid confounds by lingering olfactory stimuli and preferences, the objects and the arena were cleaned with 70% ethanol after testing each animal. The time spent exploring the familiar and the novel object was recorded in video.

2.7.2 Social recognition memory test (SR)

The task was completed in three days. On the first day the rats were placed in an arena (the same size and characteristics previously described to OR) with two small cages during 20 min for free exploration (habituation day). On the second day, training was performed with inclusion of an unfamiliar rat in one of the small cages for 1 hour of free exploration. After 24 hours, testing was performed when the same rat of training (the familiar rat) was placed in one small cage and a new rat was placed in another; the exploration was monitored for 5 minutes, when the time spent exploring a new and the familiar rat were recorded (Prado Lima, Schimidt, Garcia, Dare, Carpes, Izquierdo, and Mello-Carpes, 2018). Exploration was defined as sniffing or touching the small cages with the nose and/or forepaws.

2.8 Biochemical testing

2.8.1 Tissue preparation

Rats were euthanized 24 h after the behavioral experiments. The brain was removed and bilateral hippocampus were quickly dissected out and homogenized in 50 mM Tris HCl, pH 7.4. Afterwards, samples were centrifuged at 2400 g for 20 min, and supernatants were used for assay.

2.8.2 Reactive oxygen species (ROS) levels

ROS content was assessed by a spectrofluorimetric method using 20,70-dichlorofluorescein diacetate (DCFH-DA) as a probe (Loetchutinat, Kothan, Dechsupa, Meesungnoen, Jay-Gerin, and Mankhetkorn, 2005). The supernatant was incubated in darkness with 5 μL DCFH-DA (1 mM). The oxidation of DCHF-DA to fluorescent dichlorofluorescein (DCF) was measured for the detection of intracellular ROS. The formation of the oxidized fluorescent derivative (DCF), measured by DCF fluorescence intensity, was recorded at 520 nm (480 nm excitation) 30 min after the addition of DCFH-DA to the medium. Results were expressed as AU (arbitrary units).

2.8.3 Detection of lipid peroxidation (TBARS)

Lipoperoxidation was evaluated by the thiobarbituric acid reactive substance (TBARS) test (Ohkawa, Ohishi, and Yagi, 1979). One aliquot of supernatant was incubated with a 0.8% thiobarbituric acid solution, acetic acid buffer (pH 3.2) and sodium dodecyl sulfate solution (8%) at 95ºC for 2 h, and the color reaction was measured at 532 nm. Results were expressed as nmol of malondialdehyde (MDA) per mg protein.

2.8.4 Ferric reducing/antioxidant power (FRAP) assay

The working FRAP reagent was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution, and 2.5 ml FeCl3.6H2O solution. 10 µL of supernatant was added in the 300 µL working FRAP reagent in microplate (Benzie and Strain, 1996). Additionally, a standard curve was used with 10 μL Trolox concentrations of 15, 30, 60, 120 e 240 mM more 300 µL working FRAP reagent. The microplate was incubated at 37º C for 15 min before the read at 593 nm in a SpectraMax M5 Microplate Reader.

2.8.5 Acetylcholinesterase (AChE) activity

AChE is a marker of the cholinergic system activity in the forebrain (Medina, Khachaturian, Rossor, Avila, and Cedazo-Minguez, 2017). The AChE activity in the supernatant was determined by the method of (Ellman, Courtney, Andres, and Feather-Stone, 1961). The reaction mixture was composed of 100 mM phosphate buffer pH (7.4) and 1 mM 5,5′-dithio-bis- 2-nitrobenzoic acid (DTNB). The method is based in formation of a yellow anion, 4,4′-dithio-bis-acid nitrobenzoic after adding 0.8 mM acetylthiocholine iodide. The change in absorbance was measured for 2 min at 30 s intervals at 412 nm (SpectraMax M5 Molecular Devices, CA, USA). Results were expressed as micromoles of acetylthiocholine iodide hydrolyzed/min/mg of protein. Proteins were measured according to Bradford (1976) using bovine serum albumin as a standard.

2.9 Statistical analyses

Normality of data distribution was checked using the Shapiro–Wilk test. Open-field and plus-maze data were compared between groups by one-way ANOVA. Object exploration time in OR and conspecific animal exploration time in SR were converted to percentage of total exploration time, and a one-sample t test was used to compare the percentage of total time of exploration spent on each object or rat considering a theoretical mean of 50%. Biochemical data were compared by two-way ANOVA test with Tukey's multiple comparisons test. Significance level was set at 0.05 for all analyses.

3 Results

3.1 Control behavioral results

Locomotion, exploration, and anxiety behaviors were not influenced by the procedures. There were no differences between groups regarding the total time of exploration in training and testing sessions in OR and SR, open-field and plus-maze performance (Table 1).

3.2 Strength and oxygen uptake (VO2) gain

The strength gain estimate by increased in MCL and the VO_2 maximal gain estimate by indirect VO_2 were determined for each rat during the training periods. Both variables showed a continuous increased towards the end of the training protocols (Figure 2).

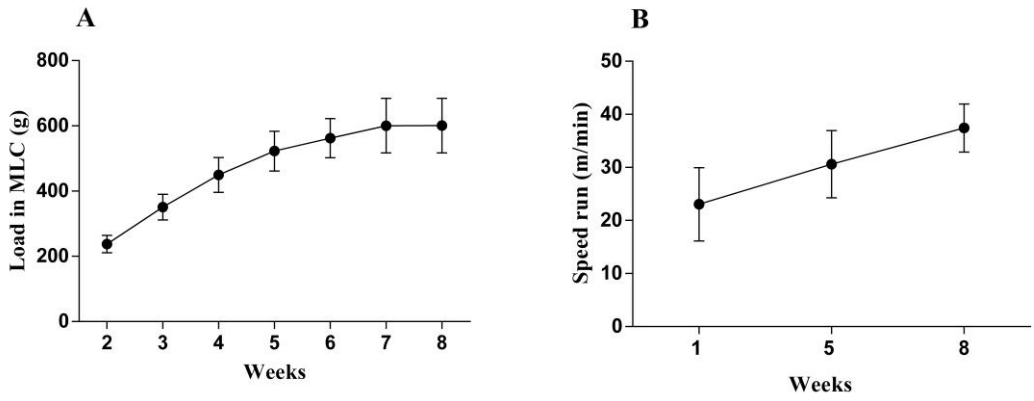

Figure 2 - Progression of exercise intensity determined by the MLC for strength training and indirect VO2 maximal determined by running speed for running training. A: progression of the load (grams) in the MLC in the first, fifth and last week of training. P<0.0001, one-way ANOVA. B: progression of the maximum running speed (m/min) in the indirect VO2 test in the first, fifth and last week of training. p=0<0.0001, one-way ANOVA.

Table 1 - Results of control behavioral tasks expressed as mean (standard deviation). Surgery procedures, Aβ injection, StrEx training and RunEx training did not alter the total exploration time in OR and SR during training and testing sessions, locomotor and exploratory activities in the open field, and anxiety behavior evaluated by plus maze, $p>0.05$, two-way ANOVA test with Tukey's multiple comparisons test; n = 8–10/group.

Behavioral tasks		Control			Abeta		
		NI	StrEx	RunEx	NI	StrEx	RunEx
Exploration time in OR	Total exploration time in training (s)	43.73 (8.30)	38.91 (15.67)	57.96 12.27	41.33 (22.86)	36.05 (12.58)	49.47 (14.29)
	Total exploration time in STM test (s)	42.16 (17.09)	35.64 (8.05)	55.17 16.80	42.09 (13.68)	42.81 (23.84)	45.51 (26.30)
	Total exploration time in LTM test (s)	38.91 (15.67)	36.53 (14.24)	67.55 (21.11)	39.62 (10.55)	35.36 (8.82)	42,49 (8.82)
Exploration time in SR	Total exploration time in SR test (s)	96.21 (22.21)	93.73 (25.06)	110.42 (13.08)	83.10 (34.64)	85.03 (11.15)	103.8 (6.49)
Open field	Crossings (n)	38.75 (2.83)	45.00 (7.59)	46.22 (12.34)	32.88 (3.36)	45.43 (4.49)	45,66 (28.81)
	Rearings (n)	25.20 (5.65)	15.50 (2.51)	19.88 (6.94)	15.53 (2.71)	17.80 (2.38)	21.44 (9.35)
Plus maze	Total entries (n)	22.46 (8.10)	23.38 (7.14)	22.40 (14.30)	19.42 11.12	22.34 (9.14)	23.60 (14.20)
	Time in open arms (s)	25.82 (22.05)	27.09 (8.37)	21.24 (10.05)	27.03 (21.78)	23.14 (19.18)	24.23 (11.04)

3.3 Object recognition memory (OR)

Percentage of total exploration time in training sessions did not differ between objects A and B (mean of all groups: object A = 50.78 ± 8.82%; B = 49.21 ± 8.82%; p = 0.14; $t_{(68)}$ = 47.82). Control rats did not show STM deficits in OR testing (p = 0.02; $t_{(13)}$ = 2.57; Fig. 3A). Control rats trained for strength and running did not show STM deficits in OR testing (p = 0.02, $t_{(9)}$ = 2.56 for StrEx; p = 0.025, $t_{(7)}$ = 2.83 for RunEx; Fig. 3A). Aβ rats showed STM deficits in OR, and time of exploration did not differ between the familiar and the novel object (p = 0.90; $t_{(12)}$ = 0.12; Fig. 4A). StrEx avoided STM deficit in OR memory of Aβ rats (p = 0.009, $t_{(13)}$ = 3.05; Fig. 4A). Running exercise did not avoid STM deficit in OR memory of Aβ rats (p = 0.45, $t_{(7)}$ = 0.78 Fig. 3A).

Control rats did not show LTM deficits in OR testing (p = 0.001, $t_{(11)}$ = 4.10 for NI group; p = 0.003, $t_{(7)}$ = 4.35 for RunEx group; p = 0.02 $t_{(8)}$ = 2.70 for StrEx group; Fig. 3B). Aβ rats presented LTM deficits in OR memory, spending similar time exploring the familiar and the novel object (p = 0.456; $t_{(12)}$ = 0.77; Fig. 43B). StrEx avoided LTM deficit in OR memory of Aβ rats (p =0,026, $t_{(8)}$ = 2.70; Fig. 3B). RunEx did not avoid LTM deficit in OR memory of Aβ rats (p = 0.154; $t_{(7)}$ = 1.59; Fig. 3B).

3.4 Social recognition memory

SR test showed control rats exploring the new rat for longer than 50% of the total exploration time (p < 0.01, $t_{(12)}$ = 3.27 for NI control group; p < 0.01, $t_{(7)}$ = 3.68 for StrEx control group; p < 0.01, $t_{(12)}$ = 4.67 for RunEx control group; Fig. 4). SR memory was impaired in Aβ rats, which explored for a similar time (~ 50%) the familiar and the

new rat (p = 0.08, $t_{(14)}$ = 1.88, Fig. 4). StrEx and RunEx exercises preserved SR memory of Aβ rats (p= 0.003, $t_{(8)}$ = 4.00 for Aβ StrEx; p = 0.0001, $t_{(8)}$ = 6.90 for Aβ RunEx; Fig. 4).

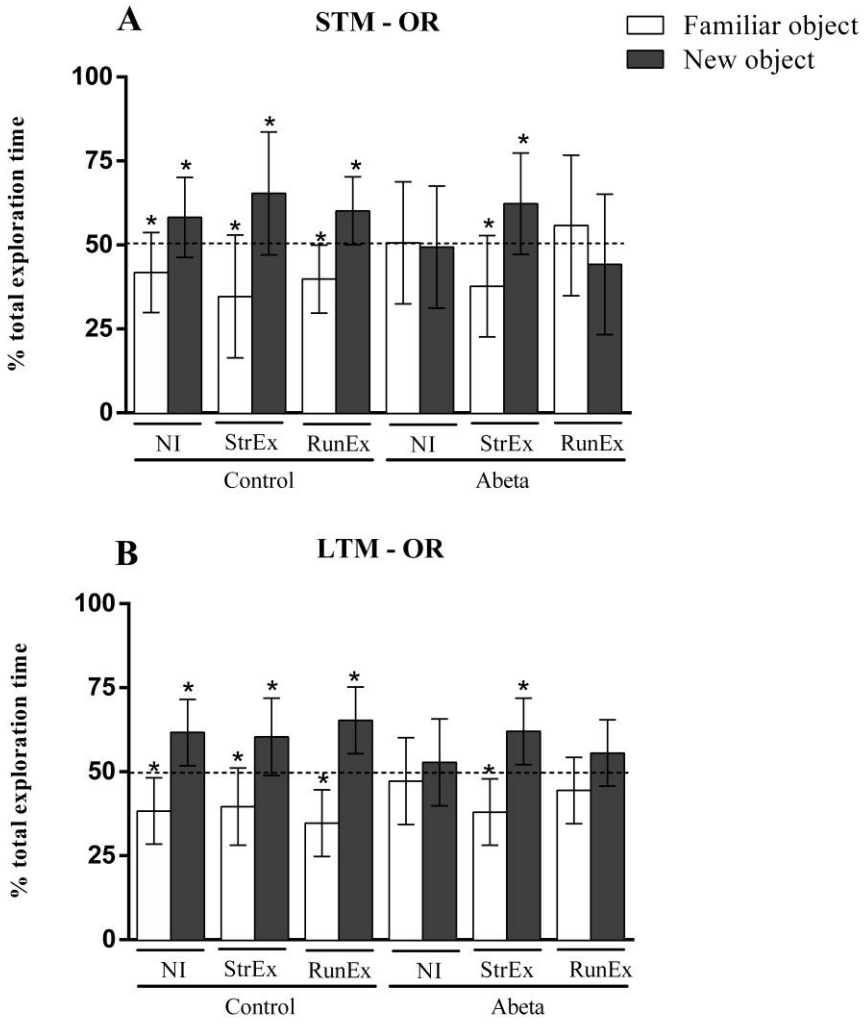

Figure 3 - Aβ impaired short- (STM, A) and long-term (LTM, B) object recognition (OR) memory. Strength exercise prevents these impairments and running exercise not prevent. One-sample *t*-test considering a theoretical mean of 50% (n = 8-14 per group). Data are mean and standard deviation of the percentage of total exploration time (*p < 0.05; NI = intervention. StrEx = Strength Exercise. RunEx = Running Exercise).

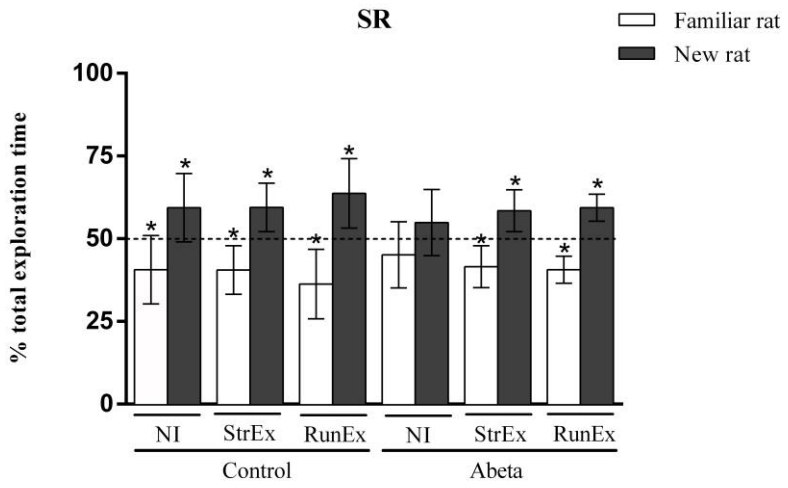

Figure 4 - Aβ impaired social recognition memory. Strength and running exercises showed neuroprotection. Data are mean and standard deviation of the percentage of the total exploration time (*$p < 0.05$, one-sample *t*-test considering a theoretical mean of 50%; n = 8–13 per group). NI = no intervention. StrEx = Strength Exercise. RunEx = Running Exercise.

3.5 Hippocampal oxidative status

ROS levels in the hippocampus showed a main effect of the intervention ($F_{(2, 31)} = 4.515$; $p = 0.01$). RunEx decreased hippocampal ROS levels in Aβ rats ($p = 0.01$, Fig. 5A). Main effects of group ($F_{(1, 35)} = 12.97$; $p = 0.001$) and intervention ($F_{(2, 35)} = 9.51$; $p = 0.0005$) were found in hippocampal lipid peroxidation, with significant interaction between groups and interventions ($F_{(2, 35)} = 6.26$; $p = 0.0047$). Aβ rats presented higher lipid peroxidation than control rats ($p < 0.001$; Fig. 5A). StrEx and RunEx prevented lipid peroxidation in Aβ rats ($p < 0.0001$ for StrEx, and $p < 0.01$ for RunEx; Fig. 5A).

Total antioxidant capacity of hippocampus showed a main effect of group ($F_{(1, 32)} = 22.97$; $p < 0.0001$) and intervention ($F_{(2, 35)} = 5.20$; $p = 0.01$), with significant

interaction between the factors ($F_{(2, 32)}$ = 4.94; p = 0.01). Aβ rats showed lower total antioxidant capacity than control rats (p < 0.01; Fig. 5C). Aβ rats in RunEx presented higher antioxidant capacity than Aβ rats not enrolled in interventions (p = 0.001; Fig. 5C).

Acetylcholinesterase (AChE) activity showed a main effect of the group ($F_{(1, 36)}$ = 5.02; p = 0.03), with significant interaction between the factors ($F_{(2, 36)}$ = 8.71; p = 0.0008). AChE activity was lower in Aβ rats (p < 0.001; Fig. 5D). StrEx prevented the AChE decreases (p < 0.01; Fig. 5D).

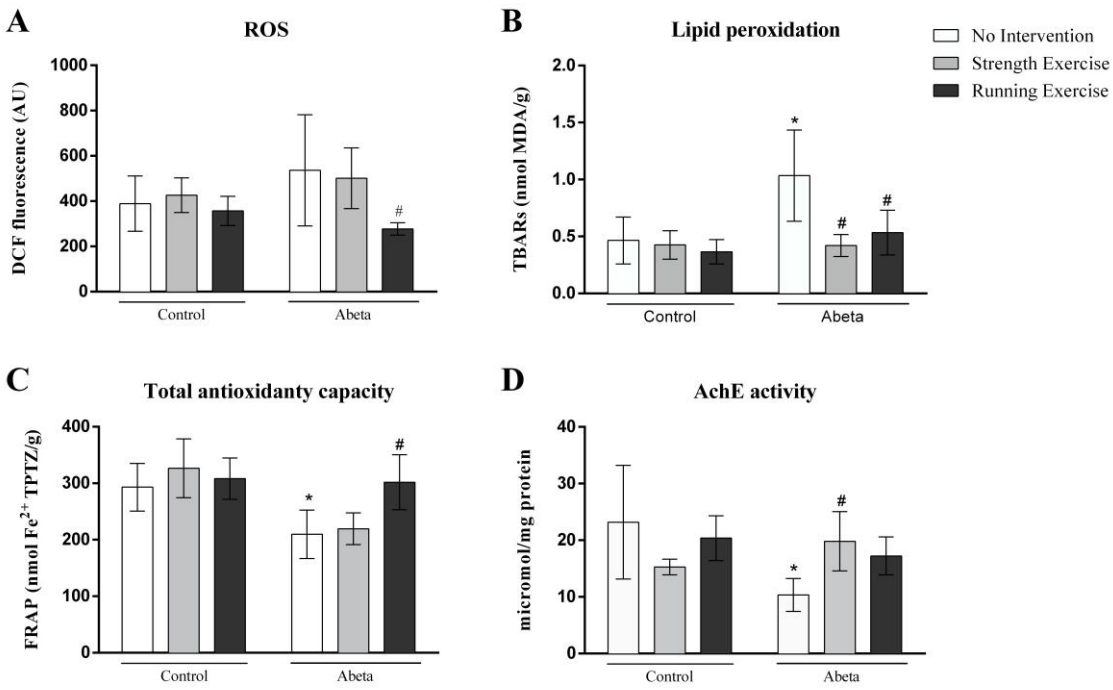

Figure 5 - Effects of Aβ, StrEx and RunEx on hippocampal oxidative status and AChE activity. A: ROS, reactive oxygen species; B: lipid peroxidation; C: total antioxidant capacity; D: AChE activity. Data are mean and standard deviation (* p < 0.05, control NI vs. Abeta NI; # p < 0.05, Aβ NI vs. Aβ + specific intervention; two-way ANOVA with Tukey's post hoc; n = 5–8 per group).

4 Discussion

Here we investigated the neuroprotection resulting from strength (SrtEx) and running (RunEx) exercise trainings in a model of AD-like cognitive impairment induced by Aβ neurotoxicity in rats. This model has been extensively used to reproduce cognitive deficits inherent to AD condition (Ghasemi et al., 2014; Prado Lima et al., 2018). Our study adds important novelties to the field. RunEx and StrEx were different regarding effects on object recognition memory. STM and LTM deficits in object recognition resultant of AD-like condition were preserved in SrtEx group, but not in RunEx. On the other hand, both exercises prevent social recognition memory deficits. Biochemical outcomes also differed between the exercises. SrtEx prevents lipid peroxidation and preserves AChE activity in the hippocampus whereas RunEx prevents the increase in production of free radicals, increase in lipid peroxidation and the decline of total antioxidant capacity in the AD-like condition. These results highlighted that, although both RunEx and SrtEx influence oxidative status, RunEx has more effects on these parameters than SrtEx. Furthermore, SrtEx avoids changes promoted by Aβ in the cholinergic system. Both conditions (oxidative status and cholinergic system) are altered in AD, and the knowledge regarding effects of different types of exercise action mechanisms can help in the choice of the best exercise for each patient.

Our experiments reproduced a main characteristic of AD by injecting Aβ in the dorsal hippocampus, a brain structure with fundamental role for memory (Izquierdo, 2018). Aβ toxicity leads to an oxidative stress condition and consequent loss of synapses and selective death of neuronal cells (Ghasemi et al., 2014). The memory deficit is the main incapacitating and limiting factor of an AD patient (Izquierdo,

Barros, Vianna, Coitinho, deDavid e Silva, Choi, Moletta, Medina, and Izquierdo, 2002). This limitation added to physiological muscle loss through aging, known as sarcopenia (Saji et al., 2016), it accelerates the disability process of an AD patient. The strength training with increasing gradual load is a main recommendation for prevention, maintenance and gain of muscle mass (Lopez et al., 2017; Pizzigalli, Filippini, Ahmaidi, Jullien, and Rainoldi, 2011). Our results support the concept that strength training also has potential to promote important neuroprotection in memory deficits related to AD development.

Much of recent research addresses the role of aerobic exercise on memory (Lopez et al., 2017; Loprinzi et al., 2018; Nokia et al., 2016). In this regard, resistance training has been associated with episodic memory function, and a recent review highlighted the lack of studies trying to understand how strength exercise influences cognition by discussing the availability of only 8 studies with controversial results among them (Loprinzi et al., 2018). One could argue that a determinant factor of our results could be the exercise intensity. However, we consider this may not find support in the literature. Moderate intensity running exercise as performed here (~70% VO2max) is known to result in cognitive benefits in different conditions of brain degeneration (Flores et al., 2014; Schimidt et al., 2014), and such intensity can be equivalent to the intensity experienced by the rats in the StrEx groups. Our strength training protocol had the workload adjusted to the training gains and included repetitions at intensity of 50%, 75%, 90% and 100% of the maximum load, which ensure at least moderate intensity to the StrEx and RunEx groups.

StrEx and RunEx had similar effects on social recognition memory. However, STM and LTM in object recognition task were preserved only in StrEx rats. The benefits of strength training on memory and cognition seem evident, however, studies

are needed to demonstrate the mechanisms involved. In our study, we analyzed the oxidative stress and AChE activity in the hippocampus. A main difference that can help to explain these results relies on the activity of cholinergic system, since the StrEx training rats preserved the hippocampal AChE activity in the AD-like condition. AChE activity is an important marker for cholinergic system functioning and neurotransmission (Medina et al., 2017). Cholinergic input influences hippocampal function, and its dysfunction is related to aging and AD cognitive decline (Bell, Shim, Chen, and McQuiston, 2011). Previously evidences suggest that Aβ may regulate extracellular acetylcholine, altering both its synthesis and degradation (Vijayaraghavan, Karami, Aeinehband, Behbahani, Grandien, Nilsson, Ekdahl, Lindblom, Piehl, and Darreh-Shori, 2013). Considering that acetylcholine is the fast-acting neurotransmitter of the neuromuscular junction (Picciotto, Higley, and Mineur, 2012), and that StrEx requires the recruitment of more motor units than RunEx, we hypothesize that StrEx promotes more activation of peripheral cholinergic system. The relationship between peripheral and central cholinergic system are not well establish, but acetylcholine can act at considerable distances from the cholinergic terminals, and, furthermore, AChE is abundant in extracellular fluids, as plasma and cerebrospinal fluids (Vijayaraghavan et al., 2013). Nonetheless, a relationship between acetylcholine release in the CNS and locomotor activity exists (Day, Damsma, and Fibiger, 1991). These data help us to establish a relationship between motor/peripheral activity and changes in the central cholinergic system (Pepeu and Giovannini, 2004), but more studies addressing this topic are necessary.

It is difficult to conclude whether the oxidative status determines differences between StrEx and RunEx on memory. RunEx prevented the increase in production of reactive oxygen species and preserved the total antioxidant capacity in the AD-like

condition. Aerobic exercise increases mitochondrial density and oxidative function (Egan and Zierath, 2013), and it may explain our results. On the other hand, RunEx and StrEx protected against lipid peroxidation. This finding is very important because lipid peroxidation is an autocatalytic process initiated by free radical attack on the unsaturated (double) bonds of membrane fatty acids (Mattson, 2009). With the effects of running and strength exercises performed in separate and considering that the modern training for elderly advocated for a combined training including both aerobic and anaerobic capacity, it would be valuable to address the role of this combined exercise setup on memory and cognition in comparison to the isolated modalities.

5 Conclusion

Strength and running training protocols impact differently on memory in a model of AD-like brain disturbances induced by Aβ toxicity. While running improves social recognition memory, strength training enhances both social recognition and short- and long-term object recognition memories. Both exercises improved antioxidant capacity, but only strength training also promoted benefits to the cholinergic system. As a conclusion we demonstrate that strength exercises can be as neuroprotective as running exercise in an AD-like model.

References

Abd El-Kader, S. M., & Al-Jiffri, O. H. (2016). Aerobic exercise improves quality of life, psychological well-being and systemic inflammation in subjects with Alzheimer's disease. *African Health Sciences, 16*, 1045-1055.

Barone, R., Bellafiore, M., Leonardi, V., & Zummo, G. (2009). Structural analysis of rat patellar tendon in response to resistance and endurance training. *Scandinavian Journal of Medicine & Science in Sports, 19*, 782-789.

Bell, K. A., Shim, H., Chen, C.-K., & McQuiston, A. R. (2011). Nicotinic excitatory postsynaptic potentials in hippocampal CA1 interneurons are predominantly mediated by nicotinic receptors that contain α4 and β2 subunits. *Neuropharmacology, 61*, 1379-1388.

Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal Biochem, 239*, 70-76.

Bonini, J. S., Bevilaqua, L. R., Zinn, C. G., Kerr, D. S., Medina, J. H., Izquierdo, I., & Cammarota, M. (2006). Angiotensin II disrupts inhibitory avoidance memory retrieval. *Horm Behav, 50*, 308-313.

Brooks, G. A., & White, T. P. (1978). Determination of metabolic and heart rate responses of rats to treadmill exercise. *J Appl Physiol Respir Environ Exerc Physiol, 45*, 1009-1015.

Brown, B. M., Peiffer, J. J., & Martins, R. N. (2012). Multiple effects of physical activity on molecular and cognitive signs of brain aging: can exercise slow neurodegeneration and delay Alzheimer’s disease? *Molecular Psychiatry, 18*, 864.

Cassilhas, R. C., Viana, V. A., Grassmann, V., Santos, R. T., Santos, R. F., Tufik, S., & Mello, M. T. (2007). The impact of resistance exercise on the cognitive function of the elderly. *Med Sci Sports Exerc, 39*, 1401-1407.

Cechetti, F., Worm, P. V., Elsner, V. R., Bertoldi, K., Sanches, E., Ben, J., Siqueira, I. R., & Netto, C. A. (2012). Forced treadmill exercise prevents oxidative stress and memory deficits following chronic cerebral hypoperfusion in the rat. *Neurobiol Learn Mem, 97*, 90-96.

Da Mesquita, S., Ferreira, A. C., Sousa, J. C., Correia-Neves, M., Sousa, N., & Marques, F. (2016). Insights on the pathophysiology of Alzheimer's disease: The crosstalk between amyloid pathology, neuroinflammation and the peripheral immune system. *Neurosci Biobehav Rev, 68*, 547-562.

Day, J., Damsma, G., & Fibiger, H. C. (1991). Cholinergic activity in the rat hippocampus, cortex and striatum correlates with locomotor activity: An in vivo microdialysis study. *Pharmacology Biochemistry and Behavior, 38*, 723-729.

Eduardo Matta Mello, P., Poliane Gomes Torres, V., Renata, S., Eduardo, L., Renato Sobral, M.-J., Sergio, M., & Andrea Camaz, D. (2015). Aging process, cognitive decline and Alzheimer`s disease: can strength training modulate these responses? *CNS & Neurological Disorders - Drug Targets, 14*, 1209-1213.

Egan, B., & Zierath, Juleen R. (2013). Exercise Metabolism and the Molecular Regulation of Skeletal Muscle Adaptation. *Cell Metabolism, 17*, 162-184.

Ellman, G. L., Courtney, K. D., Andres, V., Jr., & Feather-Stone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol, 7*, 88-95.

Ennaceur, A., & Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behav Brain Res, 31*, 47-59.

Ghasemi, R., Zarifkar, A., Rastegar, K., Maghsoudi, N., & Moosavi, M. (2014). Repeated intra-hippocampal injection of beta-amyloid 25-35 induces a reproducible impairment of learning and memory: considering caspase-3 and MAPKs activity. *Eur J Pharmacol, 726*, 33-40.

Izquierdo, I. (2018). *Memória* (3ª ed.).

Izquierdo, L. A., Barros, D. M., Vianna, M. R., Coitinho, A., deDavid e Silva, T., Choi, H., Moletta, B., Medina, J. H., & Izquierdo, I. (2002). Molecular pharmacological dissection of short- and long-term memory. *Cell Mol Neurobiol, 22*, 269-287.

Kang, S., Lee, Y. H., & Lee, J. E. (2017). Metabolism-Centric Overview of the Pathogenesis of Alzheimer's Disease. *Yonsei Med J, 58*, 479-488.

Kim, B. K., Shin, M. S., Kim, C. J., Baek, S. B., Ko, Y. C., & Kim, Y. P. (2014). Treadmill exercise improves short-term memory by enhancing neurogenesis in amyloid beta-induced Alzheimer disease rats. *J Exerc Rehabil, 10*, 2-8.

Kivipelto, M., & Solomon, A. (2008). Alzheimer’s disease — The ways of prevention. *The Journal of Nutrition Health and Aging, 12*, S89-S94.

Loetchutinat, C., Kothan, S., Dechsupa, S., Meesungnoen, J., Jay-Gerin, J., & Mankhetkorn, S. (2005). Spectrofluorometric determination of intracellular levels of reactive oxygen species in drug-sensitive and drug-resistant cancer cells using the 2',7'-dichlorofluorescein diacetate assay. *Radiation Physics and Chemistry, 72*, 323-331.

Lopez, P., Pinto, R. S., Radaelli, R., Rech, A., Grazioli, R., Izquierdo, M., & Cadore, E. L. (2017). Benefits of resistance training in physically frail elderly: a systematic review. *Aging Clin Exp Res*.

Loprinzi, P. D., Frith, E., & Edwards, M. K. (2018). Resistance exercise and episodic memory function: a systematic review. *Clinical Physiology and Functional Imaging, 0.*

Mattson, M. P. (2009). Roles of the lipid peroxidation product 4-hydroxynonenal in obesity, the metabolic syndrome, and associated vascular and neurodegenerative disorders. *Exp Gerontol, 44,* 625-633.

Medina, M., Khachaturian, Z. S., Rossor, M., Avila, J., & Cedazo-Minguez, A. (2017). Toward common mechanisms for risk factors in Alzheimer's syndrome. *Alzheimer's & Dementia : Translational Research & Clinical Interventions, 3,* 571-578.

Menant, J. C., Weber, F., Lo, J., Sturnieks, D. L., Close, J. C., Sachdev, P. S., Brodaty, H., & Lord, S. R. (2016). Strength measures are better than muscle mass measures in predicting health-related outcomes in older people: time to abandon the term sarcopenia? *Osteoporos Int.*

Nokia, M. S., Lensu, S., Ahtiainen, J. P., Johansson, P. P., Koch, L. G., Britton, S. L., & Kainulainen, H. (2016). Physical exercise increases adult hippocampal neurogenesis in male rats provided it is aerobic and sustained. *J Physiol, 594,* 1855-1873.

Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem, 95,* 351-358.

Ozbeyli, D., Sari, G., Ozkan, N., Karademir, B., Yuksel, M., Cilingir Kaya, O. T., & Kasimay Cakir, O. (2017). Protective effects of different exercise modalities in an Alzheimer's disease-like model. *Behav Brain Res, 328,* 159-177.

Paxinos, G., Watson, C. R., & Emson, P. C. (1980). AChE-stained horizontal sections of the rat brain in stereotaxic coordinates. *J Neurosci Methods, 3,* 129-149.

Pellow, S., Chopin, P., File, S. E., & Briley, M. (1985). Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J Neurosci Methods, 14*, 149-167.

Pepeu, G., & Giovannini, M. G. (2004). Changes in acetylcholine extracellular levels during cognitive processes. *Learning & Memory, 11*, 21-27.

Picciotto, M. R., Higley, M. J., & Mineur, Y. S. (2012). Acetylcholine as a neuromodulator: cholinergic signaling shapes nervous system function and behavior. *Neuron, 76*, 116-129.

Pietrelli, A., Lopez-Costa, J., Goni, R., Brusco, A., & Basso, N. (2012). Aerobic exercise prevents age-dependent cognitive decline and reduces anxiety-related behaviors in middle-aged and old rats. *Neuroscience, 202*, 252-266.

Pizzigalli, L., Filippini, A., Ahmaidi, S., Jullien, H., & Rainoldi, A. (2011). Prevention of falling risk in elderly people: the relevance of muscular strength and symmetry of lower limbs in postural stability. *J Strength Cond Res, 25*, 567-574.

Prado Lima, M. G., Schimidt, H. L., Garcia, A., Dare, L. R., Carpes, F. P., Izquierdo, I., & Mello-Carpes, P. B. (2018). Environmental enrichment and exercise are better than social enrichment to reduce memory deficits in amyloid beta neurotoxicity. *Proc Natl Acad Sci U S A, 115*, E2403-E2409.

Qiu, C., Kivipelto, M., & von Strauss, E. (2009). Epidemiology of Alzheimer's disease: occurrence, determinants, and strategies toward intervention. *Dialogues in Clinical Neuroscience, 11*, 111-128.

Rolland, Y., Abellan van Kan, G., & Vellas, B. (2010). Healthy Brain Aging: Role of Exercise and Physical Activity. *Clinics in Geriatric Medicine, 26*, 75-87.

Saji, N., Arai, H., Sakurai, T., & Toba, K. (2016). [Frailty and sarcopenia: a new bridge to dementia]. *Nihon Rinsho, 74*, 505-509.

Schimidt, H. L., Garcia, A., Martins, A., Mello-Carpes, P. B., & Carpes, F. P. (2017). Green tea supplementation produces better neuroprotective effects than red and black tea in Alzheimer-like rat model. *Food Res Int, 100*, 442-448.

Souza, M. V. C., Leite, R. D., de Souza Lino, A. D., de Cássia Marqueti, R., Bernardes, C. F., de Araújo, H. S. S., Bouskella, E., Shiguemoto, G. E., de Andrade Perez, S. E., & Kraemer-Aguiar, L. G. (2014). Resistance training improves body composition and increases matrix metalloproteinase 2 activity in biceps and gastrocnemius muscles of diet-induced obese rats. *Clinics, 69*, 265-270.

Vijayaraghavan, S., Karami, A., Aeinehband, S., Behbahani, H., Grandien, A., Nilsson, B., Ekdahl, K. N., Lindblom, R. P. F., Piehl, F., & Darreh-Shori, T. (2013). Regulated Extracellular Choline Acetyltransferase Activity— The Plausible Missing Link of the Distant Action of Acetylcholine in the Cholinergic Anti-Inflammatory Pathway. *PLoS One, 8*, e65936.

Zussy, C., Brureau, A., Keller, E., Marchal, S., Blayo, C., Delair, B., Ixart, G., Maurice, T., & Givalois, L. (2013). Alzheimer's disease related markers, cellular toxicity and behavioral deficits induced six weeks after oligomeric amyloid-beta peptide injection in rats. *PLoS One, 8*, e53117.

CAPITULO VI

1 DISCUSSÃO

Nesta tese investigamos estratégias de neuroproteção em um modelo de DA induzida pela toxicidade por beta amiloide em ratos, em condição de associação ou não a uma dieta de cafeteria. Para isso, a tese foi dividida em três principais estudos experimentais e um artigo de revisão sistemática.

No primeiro estudo, capitulo II, identificamos o papel de três diferentes chás oriundos da *Camellia Sinensis* nos déficits resultantes do modelo de DA. Observamos um maior potencial neuroprotetor do chá verde quando comparado com o chá vermelho e o chá preto sobre parâmetros de estresse oxidativo no hipocampo e de memória.

O papel neuroprotetor do chá verde vem sendo investigado em nosso grupo de pesquisa em diversos modelos de acometimentos, que têm como consequência déficits cognitivos e de memória. Já confirmamos o efeito protetor do chá verde em um modelo de isquemia e reperfusão (Schimidt, H. L. *et al.*, 2014), de acidente vascular encefálico do tipo hemorrágico (Altermann *et al.*, 2017), em modelo de envelhecimento (Flores *et al.*, 2014) e também de deprivação maternal (Menezes *et al.*, 2017). Em todos esses estudos atribuímos os benefícios do chá verde a presença de catequinas que atuam como potentes antioxidantes.

Mais recentemente, em dois outros estudos investigamos também o papel de distintos chás provenientes da *Camellia sinensis.* Em um modelo de isquemia reperfusão em ratos, investigamos o papel neuroprotetor do chá verde, chá vermelho, chá branco e chá preto, e embora o chá branco e chá vermelho tenham apresentado um efeito positivo sobre a memória de reconhecimento, apenas o chá verde protegeu a

memória espacial e o estresse oxidativo no hipocampo (Martins *et al.*, 2017). Em outro estudo em que colaboramos, o modelo de dieta da cafeteria foi empregada em camundongos fêmeas, e os efeitos neuroprotetores de distintos chás sobre a memória e estresse oxidativo foram investigados. O chá verde e chá vermelho protegeram a memória de reconhecimento e o chá verde e chá preto evitaram o aumento da produção de espécies reativas de oxigênio (Soares, M. *et al.*, 2017). Nesses estudos, e no nosso estudo envolvendo o modelo de toxicidade por beta amiloide, avaliamos a quantidade de quatro principais catequinas na infusão oferecida aos animais. Nessa avaliação realizada por cromatografia líquida de alta eficiência (HPLC) vimos que a infusão de chá verde possuía mais Epigalocatequina galato (EGCG) do que os outros chás. De fato, em estudos in vitro, foi observado que a EGCG tem a maior atividade antioxidante em comparação com as outras catequinas presentes nos chás provenientes da *Camellia sinensis*, isso porque essa catequina tem alta capacidade de dar um elétron atuando como quelante de radicais livres (Legeay *et al.*, 2015; Xicota *et al.*, 2015).

No segundo estudo experimental dessa tese, capitulo III, utilizamos o chá verde, que é o chá com maior efeito protetor no modelo de Alzheimer associado à dieta de cafeteria. O objetivo principal foi combinar fatores de risco da DA com o modelo de toxicidade por beta amiloide e identificar se o chá verde mantinha seu papel neuroprotetor nessa condição. Os resultados nesse estudo não foram de acordo coma a nossa hipótese inicial, aonde a dieta de cafeteria não potencializou os déficits causados no modelo experimental da DA e, consequentemente, não tivemos os fatores de risco associados a DA presentes em nosso modelo. A dieta de cafeteria foi oferecida aos ratos durante oito semanas e era composta por chocolate, biscoito doce, amendoim e ração comercial para roedores. O consumo em quantidade foi rigorosamente controlado e não foram encontradas diferenças significativas na quantidade de ingesta alimentar entre o

grupo com dieta normal e com dieta de cafeteria. No entanto, apesar de os ratos que receberam a dieta de cafeteria terem aumentado significantemente a gordura visceral eles não aumentaram o peso corporal total em relação ao grupo controle. Além disso, os parâmetros sanguíneos como glicose e colesterol não se alteraram com a dieta, apenas o triglicerídeo, que ao contrário do que esperávamos, diminuiu com a dieta no modelo de DA.

O que podemos observar é que a dieta de cafeteria pode induzir obesidade e alterações metabólicas. No entanto, isso pode estar ligado ao modelo experimental empregado. Por exemplo, em camundongos fêmeas, a dieta de cafeteria sozinha provocou essas alterações, seguidas de déficit de memória (Soares, M. *et al.*, 2017). Em um estudo anterior com o objetivo de identificar a eficácia da dieta de cafeteria, também a base de chocolate, amendoim e biscoito, na indução do excesso de peso e da dislipidemia em ratos *Wistar machos,* não foram encontradas diferenças significativas quanto ao índice de massa corporal, colesterol, quantidade dos lipídios totais e glicemia (Almeida *et al.*, 2015).

Em nosso estudo a dieta de cafeteria, além de não atuar de maneira prejudicial, parece ter proporcionado uma proteção sobre estresse oxidativo e memória de reconhecimento em nosso modelo de toxicidade por beta amiloide. Diante desses resultados, acreditamos que a dieta de cafeteria deve ser mais explorada, especialmente em relação aos mecanismos que envolvem hormônios relacionados com o prazer e liberação de neurotransmissores, já que a dieta de cafeteria também é uma dieta palatável. Krolow *et al*, 2010 reportaram que uma dieta palatável preveni o déficit de memória relacionado ao estresse. Porém, esse achado não estava ligado ao controle do dano oxidativo, e também era específico ao sexo, ou seja, isso estava evidente nos modelos com ratas, mas não em modelos com ratos (Krolow *et al.*, 2010).

Durante a elaboração da tese, realizamos uma revisão sistemática da literatura a cerca do papel do exercício físico sobre a memória de longo prazo em sujeitos idosos, capitulo IV. Sabe-se que o envelhecimento fisiológico causa um déficit dessa memória (Flores *et al.,* 2014). Embora a memória de curto prazo seja afetada na DA antes da memória de longo prazo, a memória de longo prazo é importante para que o paciente mantenha o convívio social com seus familiares e consiga realizar atividades de vida diária (Sarkaki *et al.*, 2013). Com nossa revisão de literatura observamos o predomínio de estudos sobre o efeito neuroprotetor do exercício aeróbico. No entanto, com uma variabilidade muito grande de protocolos. O que está claro é que a intensidade da atividade deve ser moderada, e embora muitos estudos mostrem efeitos do exercício agudo, esses efeitos podem ser transitórios (Keyan e Bryant, 2017). Assim, recomenda-se ainda o exercício físico realizado de forma continua para que se obtenha resultados mais consistentes (Flores *et al.*, 2014; Schimidt *et al.*, 2015)

Em nossa revisão apenas oito artigos estiveram de acordo com os critérios de inclusão, e todos envolviam atividades físicas aeróbicas. Por isso, não foi possível comparar o efeito de diferentes modalidades de exercício físico na proteção da memória de longo prazo. Embora o exercício aeróbico seja mais popular na população idosa, cada vez mais o exercício com o uso de carga vem sendo recomendado para essa população, principalmente para a prevenção de distúrbios neuromusculares associados ao envelhecimento, como perda de massa muscular e eficiência neuromuscular (Schimidt, H. L. *et al.*, 2014; Lopez *et al.*, 2017). Alguns estudos começam a inserir nas atividades de grupo com idosos, os exercícios multicomponentes, que compreendem exercício que trabalham força, equilíbrio, coordenação e também o condicionamento aeróbico (Suzuki *et al.*, 2012; Eggenberger *et al.*, 2015). Porém, o papel do exercício de força sobre o cérebro e a memória ainda é pouco explorado, especialmente

considerando populações especiais como pacientes com DA. A partir disso, no nosso terceiro estudo, capitulo V, buscamos verificar o potencial neuroprotetor do exercício físico de força comparado com o exercício de corrida no modelo de DA induzida por toxicidade por beta amiloide em ratos. Neste estudo, identificamos que o exercício de força é tão bom quanto o exercício aeróbico na proteção da memória. No entanto, as duas modalidades parecem ter mecanismos de proteção distintos. Apenas o exercício de força protegeu a memoria de reconhecimento de objetos e as duas modalidades protegeram a memória social. O beneficio do exercício de força parece estar relacionado com o sistema colinérgico, mecanismo esse que também consideramos que deve ser mais explorado em estudos futuros. Enquanto os benefícios do exercício de corrida parece ter a capacidade de proteger frente a uma situação de estresse oxidativo. Para nós, está claro que o exercício de força, por necessitar maior recrutamento de unidades motoras, consequentemente envolverá o sistema colinérgico periférico, o que reflete no sistema colinérgico central. Ao mesmo tempo, o exercício físico aeróbico em intensidade moderada e realizado de maneira crônica, requer um consumo de oxigênio muito grande, e por isso, de forma aguda, aumenta a produção de espécies reativas de oxigênio. Essa exposição a um estresse oxidativo de forma crônica aumenta a capacidade antioxidante endógena, preparando o sistema de defesa antioxidante para atuar frente a situações envolvem o estresse oxidativo, como doenças crônicas e neurodegenerativas (Mello, P. *et al.*, 2008; Gerecke *et al.*, 2013).

Tomando todos os resultados obtidos a partir desta tese, podemos afirmar que o chá verde e o exercício físico, tanto aeróbico como de força, devem ser considerados como estratégias seguras e não farmacológicas para prevenção da DA. Essas estratégias são de fácil acesso e além dos benefícios ao SNC e a DA, também trazem benefícios a saúde de maneira global.

2 CONCLUSÕES

Com base nos nossos resultados podemos concluir que:

1. Diferentes chás oriundos da *Camellia sinensis* apresentam distintos potenciais neuroprotetores;
2. Chás verde e vermelho evitam déficits nas memórias de curto e longo prazo em ratos com DA, mas só o chá verde evita também o dano oxidativo no hipocampo;
3. O chá verde apresenta o maior teor de EGCG;
4. A dieta de cafeteria não prejudica a memória de longo prazo após a injeção de Aβ no hipocampo em ratos *Wistar* machos;
5. A dieta de cafeteria reduz o estresse oxidativo e melhora a capacidade antioxidante no hipocampo após a injeção de Aβ no hipocampo em ratos *Wistar* machos;
6. A dieta de cafeteria, combinada com o chá verde, tem melhores efeitos na memória do que apenas o chá verde após a injeção de Aβ no hipocampo em ratos *Wistar* machos;
7. Os programas de exercícios para melhorar a memória de longo prazo devem ter um controle rigoroso da intensidade;
8. Os protocolos de treinamento de força e de corrida têm impacto diferenciado na memória em um modelo de DA induzida pela toxicidade Aβ;
9. O treinamento de força melhora a memória de reconhecimento social e as memórias de reconhecimento de objeto de curto e longo prazo, enquanto o exercício de corrida melhora apenas a memória de reconhecimento, em um modelo de DA induzida pela toxicidade Aβ;

10. Apenas o exercício de corrida previne a queda da capacidade antioxidante e aumento das espécies reativas, e apenas o treinamento de força promove benefícios ao sistema colinérgico;
11. O exercício de força pode ser tão neuroprotetor quanto o exercício aeróbico, em um modelo de DA induzida pela toxicidade Aβ.

3 PERPECTIVAS FUTURAS

Os resultados desta tese permitiram responder alguns questionamentos e confirmar hipóteses levantadas na literatura contemplando nossos objetivos. No entanto, também ajudou a produzir mais perguntas. Nesse contexto, são perspectivas continuar investigando a temática da dieta, pois os resultados referentes a dieta de cafeteria, estabeleceu muitos questionamentos a cerca de mecanismos envolvidos no potencial de proteção da nossa dieta. Será que existe na dieta um ingrediente que atuou como neuroprotetor? Ou, seria o fato da dieta ser palatável e envolver outros mecanismos que não medimos um determinante dos resultados? Por que a mesma dieta tem um efeito diferente em modelos animais diferentes? E, será que se associada ao exercício físico a dieta potencializa os efeitos de proteção desses?

Quanto ao exercício físico, a partir dos resultados em que observamos os mecanismos diferentes de proteção, é uma perspectiva explorar mais em especial o mecanismo neuroprotetor do exercício de força. E adicionado a isso, já que as duas modalidades são eficientes na neuroproteção, é importante responder se as duas modalidades usadas de maneira combinada teriam seus efeitos potencializados. O efeito do exercício de força no sistema colinérgico e sua relação com a neuroproteção observada também é um fator que merece atenção futura.

Também é uma perspectiva trabalhar com essas estratégias de neuroproteção em um novo modelo de doença neurodegenerativa, um modelo de lesão na estrutura cerebral Locus Coeruleus (LC). Esse modelo estaria mais próximo do que representa a fase inicial da DA, e se de fato isso for verdadeiro, as estratégias de neuroproteçao terão seu potencial mais evidente.

REFERENCIAS

AGUIAR JR., A. S.; PINHO, R. A. Efeitos do exercício físico sobre o estado redox cerebral. Revista Brasileira de Medicina do Esporte, v. 13, p. 355-360, 2007. ISSN 1517-8692. Disponível em: < http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1517-86922007000500014&nrm=iso >.

AHMAD, W. et al. Oxidative toxicity in diabetes and Alzheimer's disease: mechanisms behind ROS/ RNS generation. Journal of Biomedical Science, London, v. 24, p. 76, 2017. ISSN 1021-7770

1423-0127. Disponível em: < http://www.ncbi.nlm.nih.gov/pmc/articles/PMC5606025/ >.

ALBARRACIN, S. L. et al. Effects of natural antioxidants in neurodegenerative disease. Nutr Neurosci, v. 15, n. 1, p. 1-9, 2012/01/01 2012. ISSN 1028-415X. Disponível em: < https://doi.org/10.1179/1476830511Y.0000000028 >.

ALMEIDA, M. E. F.; SIMÃO, A. A.; CORRÊA, A. D. Dieta de Cafeteria com Chocolate, Amendoim e Biscoito: Eficácia na Indução do Excesso de Peso e da Dislipidemia em Ratos. SaBios-Revista de Saúde e Biologia; v. 10, n. 3 (2015), 2015. Disponível em: < http://revista.grupointegrado.br/revista/index.php/sabios2/article/view/1842 >.

ALTERMANN, C. D. C. et al. Short-term green tea supplementation prevents recognition memory deficits and ameliorates hippocampal oxidative stress induced by different stroke models in rats. Brain Res Bull, v. 131, p. 78-84, May 2017. ISSN 1873-2747 (Electronic)

0361-9230 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/28330650 >.

ALVES, C. R. R. et al. Influence of Acute High-Intensity Aerobic Interval Exercise Bout on Selective Attention and Short-Term Memory Tasks. Perceptual and

Motor Skills, v. 118, n. 1, p. 63-72, 2014/02/01 2014. ISSN 0031-5125. Disponível em: < https://doi.org/10.2466/22.06.PMS.118k10w4 >. Acesso em: 2018/05/21.

ANDREEVA, T. V.; LUKIW, W. J.; ROGAEV, E. I. Biological basis for amyloidogenesis in Alzheimer'S disease. Biochemistry (Moscow), v. 82, n. 2, p. 122-139, 2017/02/01 2017. ISSN 1608-3040. Disponível em: < https://doi.org/10.1134/S0006297917020043 >.

ASSUNCAO, M. et al. Chronic green tea consumption prevents age-related changes in rat hippocampal formation. Neurobiol Aging, v. 32, n. 4, p. 707-17, Apr 2011. ISSN 1558-1497 (Electronic)
0197-4580 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19411127 >.

BALOUCH, S.; RUSTED, J. M. Error-monitoring in an everyday task in people with Alzheimer-type dementia: observations over five years of performance decline. J Clin Exp Neuropsychol, v. 36, n. 7, p. 773-86, 2014. ISSN 1744-411X (Electronic)
1380-3395 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25116037 >.

BANERJEE, S.; CHATTERJEE, J. Efficient extraction strategies of tea (Camellia sinensis) biomolecules. J Food Sci Technol, v. 52, n. 6, p. 3158-68, Jun 2015. ISSN 0022-1155 (Print)
0022-1155 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26028699 >.

BARKER, G. R. I.; WARBURTON, E. C. When Is the Hippocampus Involved in Recognition Memory? The Journal of Neuroscience, v. 31, n. 29, p. 10721, 2011. Disponível em: < http://www.jneurosci.org/content/31/29/10721.abstract >.

BIASIBETTI, R. et al. Green tea (-)epigallocatechin-3-gallate reverses oxidative stress and reduces acetylcholinesterase activity in a streptozotocin-induced model of dementia. Behav Brain Res, v. 236, n. 1, p. 186-93, Jan 1 2013. ISSN 1872-7549 (Electronic)

0166-4328 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22964138 >.

BIRD, C. M. The role of the hippocampus in recognition memory. Cortex, v. 93, p. 155-165, 2017/08/01/ 2017. ISSN 0010-9452. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0010945217301715 >.

BONINI, J. S. et al. Angiotensin II disrupts inhibitory avoidance memory retrieval. Horm Behav, v. 50, n. 2, p. 308-13, Aug 2006. ISSN 0018-506X (Print)
0018-506X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16697382 >.

BOS, M. J. et al. Modifiable etiological factors and the burden of stroke from the Rotterdam study: a population-based cohort study. PLoS Med, v. 11, n. 4, p. e1001634, Apr 2014. ISSN 1549-1676 (Electronic)
1549-1277 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24781247 >.

BROWN, B. M. et al. Intense physical activity is associated with cognitive performance in the elderly. Translational Psychiatry, v. 2, n. 11, p. e191, 2012. ISSN 2158-3188. Disponível em: < http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3565765/ >.

CANCELA, J. M. et al. Effects of a long-term aerobic exercise intervention on institutionalized patients with dementia. Journal of Science and Medicine in Sport, v. 19, n. 4, p. 293-298, 2016/04/01/ 2016. ISSN 1440-2440. Disponível em: < http://www.sciencedirect.com/science/article/pii/S1440244015001218 >.

CARUSO, A. et al. Stress as risk factor for Alzheimer's disease. Pharmacological Research, v. 132, p. 130-134, 2018/06/01/ 2018. ISSN 1043-6618. Disponível em: < http://www.sciencedirect.com/science/article/pii/S1043661817316857 >.

CASSILHAS, R. C. et al. The Impact of Resistance Exercise on the Cognitive Function of the Elderly. Medicine & Science in Sports & Exercise, v. 39, n. 8, p. 1401-1407, 2007. ISSN 0195-9131. Disponível em: < https://journals.lww.com/acsm-msse/Fulltext/2007/08000/The_Impact_of_Resistance_Exercise_on_the_Cognitive.24.aspx >.

CECHETTI, F. et al. Effect of a neuroprotective exercise protocol on oxidative state and BDNF levels in the rat hippocampus. Brain Res, v. 1188, p. 182-8, Jan 10 2008. ISSN 0006-8993 (Print)
0006-8993 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18021756 >.

CHENG, Y.; BAI, F. The Association of Tau With Mitochondrial Dysfunction in Alzheimer's Disease. Frontiers in Neuroscience, v. 12, p. 163, 2018. ISSN 1662-4548
1662-453X. Disponível em: < http://www.ncbi.nlm.nih.gov/pmc/articles/PMC5874499/ >.

CISSE, M.; CHECLER, F. Eph receptors: New players in Alzheimer's disease pathogenesis. Neurobiol Dis, Sep 1 2014. ISSN 1095-953X (Electronic)
0969-9961 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25193466 >.

DA MESQUITA, S. et al. Insights on the pathophysiology of Alzheimer's disease: The crosstalk between amyloid pathology, neuroinflammation and the peripheral immune system. Neurosci Biobehav Rev, v. 68, p. 547-562, Jun 18 2016. ISSN 1873-7528 (Electronic)
0149-7634 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27328788 >.

DA SILVA, W. et al. Effect of green tea extract supplementation on exercise-induced delayed onset muscle soreness and muscular damage. Physiology & Behavior, v. 194, p. 77-82, 2018/10/01/ 2018. ISSN 0031-9384. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0031938418302294 >.

DA SILVEIRA, C. K. et al. The role of histamine receptors in the consolidation of object recognition memory. Neurobiol Learn Mem, v. 103, p. 64-71, Jul 2013. ISSN 1095-9564 (Electronic)

1074-7427 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23583502 >.

EGGENBERGER, P. et al. Does multicomponent physical exercise with simultaneous cognitive training boost cognitive performance in older adults? A 6-month randomized controlled trial with a 1-year follow-up. Clinical interventions in aging, v. 10, p. 1335-1349, 2015. ISSN 1176-9092

1178-1998. Disponível em: < http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4544626/ >.

FERREIRA, S. T. et al. Inflammation, defective insulin signaling, and neuronal dysfunction in Alzheimer's disease. Alzheimers Dement, v. 10, n. 1 Suppl, p. S76-83, Feb 2014. ISSN 1552-5279 (Electronic)

1552-5260 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24529528 >.

FLORES, M. F. et al. Effects of green tea and physical exercise on memory impairments associated with aging. Neurochem Int, v. 78, p. 53-60, Dec 2014. ISSN 1872-9754 (Electronic)

0197-0186 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25195719 >.

FRASCA, D.; BLOMBERG, B. B.; PAGANELLI, R. Aging, Obesity, and Inflammatory Age-Related Diseases. Frontiers in Immunology, v. 8, p. 1745, 2017. ISSN 1664-3224. Disponível em: < http://www.ncbi.nlm.nih.gov/pmc/articles/PMC5725402/ >.

FREUDENBERG-HUA, Y.; LI, W.; DAVIES, P. The Role of Genetics in Advancing Precision Medicine for Alzheimer's Disease—A Narrative Review. Frontiers in Medicine, v. 5, n. 108, 2018-April-24 2018. ISSN 2296-858X. Disponível em: < https://www.frontiersin.org/article/10.3389/fmed.2018.00108 >.

FROTA, N. A. F. et al. Critérios para o diagnóstico de doença de Alzheimer. 2011.

GAWLIK, M.; CZAJKA, A. The effect of green, black and white tea on the level of alpha and gamma tocopherols in free radical-induced oxidative damage of human red blood cells. Acta Pol Pharm, v. 64, n. 2, p. 159-64, Mar-Apr 2007. ISSN 0001-6837 (Print)

0001-6837 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17665865 >.

GERECKE, K. M. et al. Exercise protects against chronic restraint stress-induced oxidative stress in the cortex and hippocampus. Brain Res, v. 1509, p. 66-78, May 6 2013. ISSN 1872-6240 (Electronic)

0006-8993 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23499928 >.

GHASEMI, R. et al. Repeated intra-hippocampal injection of beta-amyloid 25-35 induces a reproducible impairment of learning and memory: considering caspase-3 and MAPKs activity. Eur J Pharmacol, v. 726, p. 33-40, Mar 5 2014. ISSN 1879-0712 (Electronic)

0014-2999 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24418687 >.

GIRALDO, E. et al. Abeta and tau toxicities in Alzheimer's are linked via oxidative stress-induced p38 activation: protective role of vitamin E. Redox Biol, v. 2, p. 873-7, 2014. ISSN 2213-2317 (Electronic)

2213-2317 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25061569 >.

GRANZOTTO, A.; ZATTA, P. Resveratrol and Alzheimer’s disease: message in a bottle on red wine and cognition. Frontiers in Aging Neuroscience, v. 6, p. 95, 2014. ISSN 1663-4365. Disponível em: < http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4030174/ >.

HARAMIZU, S. et al. Catechins suppress muscle inflammation and hasten performance recovery after exercise. Med Sci Sports Exerc, v. 45, n. 9, p. 1694-702, Sep 2013. ISSN 1530-0315 (Electronic)

0195-9131 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23470311 >.

HART, S. A.; SMITH, C. M.; SWASH, M. Recognition memory in alzheimer's disease. Neurobiol Aging, v. 6, n. 4, p. 287-292, 1985/12/01/ 1985. ISSN 0197-4580. Disponível em: < http://www.sciencedirect.com/science/article/pii/0197458085900053 >.

HEBER, D. et al. Green tea, black tea, and oolong tea polyphenols reduce visceral fat and inflammation in mice fed high-fat, high-sucrose obesogenic diets. J Nutr, v. 144, n. 9, p. 1385-93, Sep 2014. ISSN 1541-6100 (Electronic)

0022-3166 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25031332 >.

HENRIQUES, A. D. et al. Fluid and imaging biomarkers for Alzheimer's disease: Where we stand and where to head to. Exp Gerontol, v. 107, p. 169-177, 2018/07/01/ 2018. ISSN 0531-5565. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0531556517304114 >.

HIPPIUS, H.; NEUNDÖRFER, G. The discovery of Alzheimer's disease. Dialogues in Clinical Neuroscience, France, v. 5, n. 1, p. 101-108, 2003. ISSN 1294-8322

1958-5969. Disponível em: < http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3181715/ >.

IZQUIERDO et al. Different molecular cascades in different sites of the brain control memory consolidation. Trends Neurosci, v. 29, n. 9, p. 496-505, 2006/09/01/ 2006. ISSN 0166-2236. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0166223606001482 >.

IZQUIERDO, I. Memória. 2ª. 2011.

IZQUIERDO, I.; MCGAUGH, J. Behavioural pharmacology and its contribution to the molecular basis of memory consolidation. Behavioural pharmacology, v. 11, n. 7-8, p. 517-534, 2000. ISSN 0955-8810.

IZQUIERDO, L. A. et al. Molecular pharmacological dissection of short- and long-term memory. Cell Mol Neurobiol, v. 22, n. 3, p. 269-87, Jun 2002. ISSN 0272-4340 (Print)
0272-4340 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12469870 >.

JACK, C. R., JR. et al. Suspected non-Alzheimer disease pathophysiology - concept and controversy. Nat Rev Neurol, Jan 18 2016. ISSN 1759-4766 (Electronic)
1759-4758 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26782335 >.

KESNER, R.; DIMATTIA, B. Neurobiology of an attribute model of memory. Progress in psychobiology and physiological psychology, v. 12, p. 207-277, 1987.

KEYAN, D.; BRYANT, R. A. Acute physical exercise in humans enhances reconsolidation of emotional memories. Psychoneuroendocrinology, v. 86, p. 144-151, Dec 2017. ISSN 1873-3360 (Electronic)
0306-4530 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/28963883 >.

KISLER, K. et al. Cerebral blood flow regulation and neurovascular dysfunction in Alzheimer's disease. Nature reviews. Neuroscience, v. 18, n. 7, p. 419-434, 2017. ISSN 1471-003X
1471-0048. Disponível em: < http://www.ncbi.nlm.nih.gov/pmc/articles/PMC5759779/ >.

KIVIPELTO, M.; SOLOMON, A. Alzheimer's disease — The ways of prevention. The Journal of Nutrition Health and Aging, v. 12, n. 1, p. S89-S94, January 01 2008. ISSN 1760-4788. Disponível em: < https://doi.org/10.1007/BF02982595 >.

KOZLOV, S. et al. Alzheimer's disease: as it was in the beginning. Reviews in the Neurosciences. 28: 825 p. 2017.

KROLOW, R. et al. Consumption of a palatable diet by chronically stressed rats prevents effects on anxiety-like behavior but increases oxidative stress in a sex-specific manner. Appetite, v. 55, n. 1, p. 108-116, 2010/08/01/ 2010. ISSN 0195-6663. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0195666310000863 >.

KUMAR, A.; TSAO, J. Alzheimer Disease. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing: 2018.

LALKOVIČOVÁ, M.; DANIELISOVÁ, V. Neuroprotection and antioxidants. Neural Regeneration Research, India, v. 11, n. 6, p. 865-874, 2016. ISSN 1673-5374 1876-7958. Disponível em: < http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4962567/ >.

LEFFA, D. D. et al. Effects of palatable cafeteria diet on cognitive and noncognitive behaviors and brain neurotrophins' levels in mice. Metab Brain Dis, v. 30, n. 4, p. 1073-82, Aug 2015. ISSN 1573-7365 (Electronic) 0885-7490 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25998605 >.

LEGEAY, S. et al. Epigallocatechin Gallate: A Review of Its Beneficial Properties to Prevent Metabolic Syndrome. Nutrients, v. 7, n. 7, p. 5443-5468, 2015. ISSN 2072-6643. Disponível em: < http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4517007/ >.

LIGUORI, I. et al. Oxidative stress, aging, and diseases. Clinical interventions in aging, v. 13, p. 757, 2018.

LLORET, A. et al. Vitamin E paradox in Alzheimer's disease: it does not prevent loss of cognition and may even be detrimental. J Alzheimers Dis, v. 17, n. 1, p. 143-9, 2009. ISSN 1387-2877 (Print)
1387-2877 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19494439 >.

LOPEZ, P. et al. Benefits of resistance training in physically frail elderly: a systematic review. Aging Clin Exp Res, Nov 29 2017. ISSN 1720-8319 (Electronic)
1594-0667 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/29188577 >.

MACHADO, S. et al. Alzheimer's disease and implicit memory. Arquivos de Neuro-Psiquiatria, v. 67, p. 334-342, 2009. ISSN 0004-282X. Disponível em: < http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0004-282X2009000200034&nrm=iso >.

MARQUES-ALEIXO., I. et al. Physical exercise as a possible strategy for brain protection: Evidence from mitochondrial-mediated mechanisms. Progress in Neurobiology, v. 99, p. 149-162, 2012. Disponível em: < http://dx.doi.org/10.1016/j.pneurobio.2012.08.002 >.

MARTINS, A. et al. Supplementation with different teas from Camellia sinensis prevents memory deficits and hippocampus oxidative stress in ischemia-reperfusion. Neurochem Int, v. 108, p. 287-295, 2017/09/01/ 2017. ISSN 0197-0186. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0197018617300268 >.

MASTERS, C. L. et al. Alzheimer's disease. Nature Reviews Disease Primers, v. 1, p. 15056, 2015. Disponível em: < http://dx.doi.org/10.1038/nrdp.2015.56 >.

MATSUZAKI, T. et al. Association of Alzheimer disease pathology with abnormal lipid metabolism. The Hisayama Study, v. 77, n. 11, p. 1068-1075, 2011. Disponível em: < http://n.neurology.org/content/neurology/77/11/1068.full.pdf >.

MATTSON, M. P. Roles of the lipid peroxidation product 4-hydroxynonenal in obesity, the metabolic syndrome, and associated vascular and neurodegenerative disorders. Exp Gerontol, v. 44, n. 10, p. 625-633, 2009/10/01/ 2009. ISSN 0531-5565. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0531556509001405 >.

MEDINA, M. et al. Toward common mechanisms for risk factors in Alzheimer's syndrome. Alzheimer's & Dementia : Translational Research & Clinical Interventions, v. 3, n. 4, p. 571-578, 2017. ISSN 2352-8737. Disponível em: < http://www.ncbi.nlm.nih.gov/pmc/articles/PMC5671628/ >.

MELLO, P. et al. Effects of acute and chronic physical exercise and stress on different types of memory in rats. An Acad Bras Cienc, v. 80, n. 2, p. 301-9, Jun 2008. ISSN 0001-3765 (Print)
0001-3765 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18506256 >.

MELLO, P. B. et al. Effects of acute and chronic physical exercise and stress on different types of memory in rats. Anais da Academia Brasileira de Ciências, v. 80, n. 2, p. 301-309, 2008. ISSN 0001-3765.

MENANT, J. C. et al. Strength measures are better than muscle mass measures in predicting health-related outcomes in older people: time to abandon the term sarcopenia? Osteoporos Int, Jul 9 2016. ISSN 1433-2965 (Electronic)
0937-941X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27394415 >.

MENDIOLA-PRECOMA, J. et al. Therapies for Prevention and Treatment of Alzheimer's Disease. Biomed Res Int, v. 2016, p. 2589276, 2016. ISSN 2314-6141 (Electronic). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27547756 >.

MENEZES, J. et al. Green tea protects against memory deficits related to maternal deprivation. Physiology & Behavior, v. 182, p. 121-127, 2017/12/01/ 2017.

ISSN 0031-9384. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0031938417303499 >.

MEREGE FILHO, C. A. A. et al. Influência do exercício físico na cognição: uma atualização sobre mecanismos fisiológicos. 2014. ISSN 1806-9940.

MONACELLI, F. et al. Vitamin C, Aging and Alzheimer's Disease. Nutrients, v. 9, n. 7, p. 670, 2017. ISSN 2072-6643. Disponível em: < http://www.ncbi.nlm.nih.gov/pmc/articles/PMC5537785/ >.

MORRIS, J. K. et al. Aerobic exercise for Alzheimer's disease: A randomized controlled pilot trial. PLoS One, v. 12, n. 2, p. e0170547, 2017. Disponível em: < https://doi.org/10.1371/journal.pone.0170547 >.

NOKIA, M. S. et al. Physical exercise increases adult hippocampal neurogenesis in male rats provided it is aerobic and sustained. J Physiol, Hoboken, v. 594, n. 7, p. 1855-1873, 2016. ISSN 0022-3751
1469-7793. Disponível em: < http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4818598/ >.

OKUYAMA, T. Social memory engram in the hippocampus. Neuroscience Research, v. 129, p. 17-23, 2018/04/01/ 2018. ISSN 0168-0102. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0168010217301864 >.

OZBEYLI, D. et al. Protective effects of different exercise modalities in an Alzheimer's disease-like model. Behav Brain Res, v. 328, p. 159-177, Jun 15 2017. ISSN 1872-7549 (Electronic)
0166-4328 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/28390878 >.

PARK, J. W. et al. Green tea polyphenol epigallocatechin gallate reduces neuronal cell damage and up-regulation of MMP-9 activity in hippocampal CA1 and CA2 areas following transient global cerebral ischemia. J Neurosci Res, v. 87, n. 2, p. 567-75, Feb 2009. ISSN 1097-4547 (Electronic)

0360-4012 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18752302 >.

PIETRELLI, A. et al. Aerobic exercise prevents age-dependent cognitive decline and reduces anxiety-related behaviors in middle-aged and old rats. Neuroscience, v. 202, p. 252-66, Jan 27 2012. ISSN 1873-7544 (Electronic)
0306-4522 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22183054 >.

PIZZIGALLI, L. et al. Prevention of falling risk in elderly people: the relevance of muscular strength and symmetry of lower limbs in postural stability. J Strength Cond Res, v. 25, n. 2, p. 567-74, Feb 2011. ISSN 1533-4287 (Electronic)
1064-8011 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20838253 >.

PUGAZHENTHI, S.; QIN, L.; REDDY, P. H. Common neurodegenerative pathways in obesity, diabetes, and Alzheimer's disease. Biochim Biophys Acta, v. 1863, n. 5, p. 1037-1045, May 2017. ISSN 0006-3002 (Print)
0006-3002 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27156888 >.

QIU, C.; KIVIPELTO, M.; VON STRAUSS, E. Epidemiology of Alzheimer's disease: occurrence, determinants, and strategies toward intervention. Dialogues in Clinical Neuroscience, France, v. 11, n. 2, p. 111-128, 2009. ISSN 1294-8322
1958-5969. Disponível em: < http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3181909/ >.

RAMÓN Y CAJAL, S. Neue darstellung von historischen bau des zentralnervös system. Archives of Anatomical Physiology,, p. 319-428, 1893.

REZAI-ZADEH, K. et al. Green tea epigallocatechin-3-gallate (EGCG) reduces beta-amyloid mediated cognitive impairment and modulates tau pathology in Alzheimer transgenic mice. Brain Res, v. 1214, p. 177-87, Jun 12 2008. ISSN 0006-8993 (Print)

0006-8993 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18457818 >.

RIEDEL, W. J.; BLOKLAND, A. Declarative Memory. In: KANTAK, K. M. e WETTSTEIN, J. G. (Ed.). Cognitive Enhancement. Cham: Springer International Publishing, 2015. p.215-236. ISBN 978-3-319-16522-6.

RÍOS, J. A. et al. Is Alzheimer's disease related to metabolic syndrome? A Wnt signaling conundrum. Progress in Neurobiology, v. 121, p. 125-146, 2014/10/01/ 2014. ISSN 0301-0082. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0301008214000872 >.

ROLLAND, Y.; ABELLAN VAN KAN, G.; VELLAS, B. Healthy Brain Aging: Role of Exercise and Physical Activity. Clinics in Geriatric Medicine, v. 26, n. 1, p. 75-87, 2010/02/01/ 2010. ISSN 0749-0690. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0749069009000901 >.

SACHDEV, P. S. et al. Progression of cognitive impairment in stroke/TIA patients over 3 years. J Neurol Neurosurg Psychiatry, Mar 21 2014. ISSN 1468-330X (Electronic)
0022-3050 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24659793 >.

SARKAKI, A. et al. Improvement in Memory and Brain Long-term Potentiation Deficits Due to Permanent Hypoperfusion/Ischemia by Grape Seed Extract in Rats. Iran J Basic Med Sci, v. 16, n. 9, p. 1004-10, Sep 2013. ISSN 2008-3866 (Print)
2008-3866 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24171080 >.

SCHIMIDT, H.; MELLO-CAPES, P.; CARPES, F. The role of regular physical exercise for enhancement of long-term memory in the elderly: a review of recent evidences. Pajar, v. 3, n. 2, p. 60-68, 2015.

SCHIMIDT, H. et al. Memory deficits and oxidative stress in cerebral ischemia-reperfusion: neuroprotective role of physical exercise and green tea supplementation. Neurobiol Learn Mem, v. 114, p. 242-50, Oct 2014. ISSN 1095-9564 (Electronic)
1074-7427 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25062645 >.

SCHIMIDT, H. L. et al. Knee extensor force, rate of force development and efficiency asymmetries in different age spans. Revista Brasileira de Cineantropometria & Desempenho Humano, 2014. ISSN 1980-0037.

SILZER, T. K.; PHILLIPS, N. R. Etiology of type 2 diabetes and Alzheimer's disease: Exploring the mitochondria. Mitochondrion, 2018/04/18/ 2018. ISSN 1567-7249. Disponível em: < http://www.sciencedirect.com/science/article/pii/S1567724917303392 >.

SO, J. H. et al. Intense Exercise Promotes Adult Hippocampal Neurogenesis But Not Spatial Discrimination. Front Cell Neurosci, v. 11, p. 13, 2017. ISSN 1662-5102. Disponível em: < http://www.ncbi.nlm.nih.gov/pmc/articles/PMC5281566/ >.

SOARES, M. et al. Catechins are not major components responsible for the beneficial effect of Camellia sinensis on the ovarian delta-ALA-D activity inhibited by cadmium. Food Chem Toxicol, v. 55, p. 463-9, May 2013. ISSN 1873-6351 (Electronic)
0278-6915 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23376781 >.

SOARES, M. et al. Comparative effect of Camellia sinensis teas on object recognition test deficit and metabolic changes induced by cafeteria diet. Nutr Neurosci, p. 1-10, 2017. ISSN 1028-415X. Disponível em: < https://doi.org/10.1080/1028415X.2017.1418726 >.

SOARES, M. B. et al. Comparative effect of Camellia sinensis teas on object recognition test deficit and metabolic changes induced by cafeteria diet. Nutr Neurosci, p. 1-10, 2017. ISSN 1028-415X. Disponível em: < https://doi.org/10.1080/1028415X.2017.1418726 >.

SQUIRE, L.; KANDEL, E. Memória: da mente às moléculas. 2003.

SQUIRE, L.; LARRY, R. Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. Psychological review, v. 99, n. 2, p. 195, 1992. ISSN 1939-1471.

SQUIRE, L. R.; STARK, C. E. L.; CLARK, R. E. The Medial Temporal Lobe. Annual Review of Neuroscience, v. 27, n. 1, p. 279-306, 2004. Disponível em: < https://www.annualreviews.org/doi/abs/10.1146/annurev.neuro.27.070203.144130 >.

STRANGE, B. A. et al. Functional organization of the hippocampal longitudinal axis. Nature Reviews Neuroscience, v. 15, p. 655, 2014. Disponível em: < http://dx.doi.org/10.1038/nrn3785 >.

STUDENSKI, S. et al. Gait speed and survival in older adults. JAMA, v. 305, n. 1, p. 50-8, Jan 5 2011. ISSN 1538-3598 (Electronic)
0098-7484 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21205966 >.

SUN, L.-N.; QI, J.-S.; GAO, R. Physical Exercise Reserved Amyloid-beta Induced Brain Dysfunctions by Regulating Hippocampal Neurogenesis and Inflammatory Response via MAPK Signaling. Brain Res, 2018. ISSN 0006-8993. Disponível em: < https://www.sciencedirect.com/science/article/pii/S0006899318302427 >.

SUN, M. K.; ALKON, D. L. The "memory kinases": roles of PKC isoforms in signal processing and memory formation. Prog Mol Biol Transl Sci, v. 122, p. 31-59, 2014. ISSN 1878-0814 (Electronic)
1877-1173 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24484697 >.

SUTHERLAND, B. A.; RAHMAN, R. M.; APPLETON, I. Mechanisms of action of green tea catechins, with a focus on ischemia-induced neurodegeneration. J Nutr Biochem, v. 17, n. 5, p. 291-306, May 2006. ISSN 0955-2863 (Print)
0955-2863 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16443357 >.

SUZUKI, T. et al. Effects of multicomponent exercise on cognitive function in older adults with amnestic mild cognitive impairment: a randomized controlled trial. BMC Neurology, v. 12, p. 128-128, 2012. ISSN 1471-2377. Disponível em: < http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3534485/ >.

TEIXEIRA-LEMOS, E. et al. Regular physical exercise training assists in preventing type 2 diabetes development: focus on its antioxidant and anti-inflammatory properties. Cardiovascular Diabetology, v. 10, p. 12-12, 2011. ISSN 1475-2840. Disponível em: < http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3041659/ >.

TEWARI, A. et al. Antioxidants: The new frontier for translational research in cerebroprotection. J Anaesthesiol Clin Pharmacol, v. 30, n. 2, p. 160-171, Apr 2014. ISSN 0970-9185 (Print)
0970-9185 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24803750 >.

TOMPOROWSKI, P. D. Effects of acute bouts of exercise on cognition. Acta Psychol (Amst), v. 112, n. 3, p. 297-324, 2003/03/01/ 2003. ISSN 0001-6918. Disponível em: < http://www.sciencedirect.com/science/article/pii/S0001691802001348 >.

VANDAL, M. et al. Insulin Reverses the High-Fat Diet-Induced Increase in Brain Abeta and Improves Memory in an Animal Model of Alzheimer Disease. Diabetes, Jul 9 2014. ISSN 1939-327X (Electronic)
0012-1797 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25008180 >.

VARGAS, L. S. et al. The intrahippocampal infusion of crotamine from Crotalus durissus terrificus venom enhances memory persistence in rats. Toxicon, v. 85, p. 52-58, 2014. ISSN 0041-0101.

VARGAS, L. S. et al. One-single physical exercise session after object recognition learning promotes memory persistence through hippocampal noradrenergic mechanisms. Behav Brain Res, v. 329, p. 120-126, Jun 30 2017. ISSN 1872-7549 (Electronic)
0166-4328 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/28461008 >.

WALKER, J. M. et al. Beneficial effects of dietary EGCG and voluntary exercise on behavior in an Alzheimer's disease mouse model. J Alzheimers Dis, v. 44, n. 2, p. 561-72, 2015. ISSN 1875-8908 (Electronic)
1387-2877 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25318545 >.

WEINBERG, L. et al. A single bout of resistance exercise can enhance episodic memory performance. Acta Psychol (Amst), v. 153, p. 13-9, Nov 2014. ISSN 1873-6297 (Electronic)
0001-6918 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25262058 >.

WEINREB, O. et al. Neurological mechanisms of green tea polyphenols in Alzheimer's and Parkinson's diseases. J Nutr Biochem, v. 15, n. 9, p. 506-16, Sep 2004. ISSN 0955-2863 (Print)
0955-2863 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/15350981 >.

WHITCUP, S. M. Clinical trials in neuroprotection. In: NUCCI, C.;CERULLI, L., *et al* (Ed.). Progress in Brain Research: Elsevier, v.173, 2008. p.323-335. ISBN 0079-6123.

XICOTA, L. et al. Potential Role of (-)-epigallocatechin-3-gallate (EGCG) in the Secondary Prevention of Alzheimer Disease. Curr Drug Targets, Aug 25 2015. ISSN 1873-5592 (Electronic)

1389-4501 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26302801 >.

YANG, X. et al. Pre-Ischemic Treadmill Training for Prevention of Ischemic Brain Injury via Regulation of Glutamate and Its Transporter GLT-1. Int J Mol Sci, v. 13, n. 8, p. 9447-59, 2012. ISSN 1422-0067 (Electronic)

1422-0067 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22949807 >.

ZLATKOVIC, J. et al. Different susceptibility of prefrontal cortex and hippocampus to oxidative stress following chronic social isolation stress. Mol Cell Biochem, Mar 27 2014. ISSN 1573-4919 (Electronic)

0300-8177 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24671494 >.

ZUSSY, C. et al. Alzheimer's disease related markers, cellular toxicity and behavioral deficits induced six weeks after oligomeric amyloid-beta peptide injection in rats. PLoS One, v. 8, n. 1, p. e53117, 2013. ISSN 1932-6203 (Electronic)

1932-6203 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23301030 >.

ANEXO I

Certificado de aprovação na comissão de ética no uso de animais em pesquisa.

MINISTÉRIO DA EDUCAÇÃO
FUNDAÇÃO UNIVERSIDADE FEDERAL DO PAMPA
(Lei nº 11.640, de 11 de janeiro de 2008)

Pró-Reitoria de Pesquisa

COMISSÃO DE ÉTICA NO USO DE ANIMAIS - CEUA

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CERTIFICADO DE APROVAÇÃO DE PROTOCOLO PARA USO DE ANIMAIS EM PESQUISA

Número de protocolo da CEUA: **001/2015**

Título: **ESTUDO DO POTENCIAL NEUROPROTETOR DA CAMELLIA SINENSIS E DO EXERCICÍO FÍSICO EM UM MODELO DE DOENÇA DE ALZHEIMER ASSOCIADO À DIETA HIPERCALÓRICA**

Data da aprovação: **19/03/2015**

Período de vigência do projeto: De: **03/2015** Até: **03/2018**

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Professor Adjunto
Coordenadora da CEUA/UNIPAMPA

ANEXO II

Comprovante de submissão do artigo componente do CAPITULO III desta tese.

Elsevier Editorial System(tm) for Journal of Functional Foods

Manuscript Draft

Manuscript Number:

Title: Effects of cafeteria diet on memory and hippocampal oxidative stress in a rat model of Alzheimer-like disease: neuroprotection of green tea supplementation

Article Type: Full Length Article

Keywords: metabolic diseases; dementia; catechins; neuroprotection; Wistar

Corresponding Author: Professor Felipe P Carpes, PhD

Corresponding Author's Institution: Federal University of Pampa

First Author: Helen Schimidt

Order of Authors: Helen Schimidt; Alexandre Garcia; Alexandre Martins; Marisa Garcia; Melina B Soares; Francielli W Cibin; Pamela B Mello-Carpes; Felipe P Carpes

Abstract: Here we investigate the neuroprotective role of green tea (GT) when cafeteria diet (CAF) is associated with a in a model of β-Amyloid (Aβ) injection that induces impairments related to Alzheimer disease (AD). Wistar male rats were supplemented with GT, CAF, or GT plus CAF during 8 weeks before intra-hippocampal injection of Aβ peptide (2μL of Aβ-25-35, CA1 region). AD-like and sham rats were submitted to memory tests. Oxidative status was quantified in the bilateral hippocampus, and plasmatic triglycerides, total cholesterol, glucose, and AChE activity were determined. CAF per se does not impair object and social recognition memories. GT preserves its neuroprotective role regardless of the CAF combined with AD-like. GT supplementation and CAF, either isolated or combined, avoid oxidative stress and damage in the hippocampus. We conclude that CAF did not influence oxidative damage and memory deficits resultant of β-Amyloid injection when GT is simultaneously ingested.

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ANEXO III

Comprovante de submissão do artigo componente do CAPITULO V desta tese.

Elsevier Editorial System(tm) for Neuroscience

Manuscript Draft

Manuscript Number:

Title: Strength and running trainings elicit different neuroprotection outcomes in a β-amyloid peptide mediated Alzheimer model

Article Type: Research Paper

Section/Category: Behavioral and Cognitive Neuroscience

Keywords: aerobic exercise, anaerobic exercise, dementia, learning and memory, neurodegeneration, training

Corresponding Author: Professor Felipe P Carpes, PhD

Corresponding Author's Institution: Federal University of Pampa

First Author: Helen L Schimidt

Order of Authors: Helen L Schimidt; Alexandre Garcia; Mariza Prado Lima; Ivan A Izquierdo; Pamela B Mello-Carpes; Felipe P Carpes, PhD

Abstract: Aerobic exercise induces neuroprotection, but few studies investigated whether strength training has similar potential. Here we examine whether effects of running training differ from those of strength training concerning cognitive symptomatology, oxidative stress and cholinergic status in a model of AD-like cognitive impairment induced by intrahippocampal infusion of a mix of β-amyloid peptides (Aβ) in rats. Male Wistar rats were submitted to 8 weeks of running exercise (RunEx) (40 min sessions at 70% of indirect VO2 max, 3 times per week) or strength exercise (StrEx) (3 times/week, 12 repetitions in 8 sets, 2 sets with repetitions at 50%, 2 at 75%, 2 at 90% and 2 at 100% of the maximum load), followed by Aβ infusion in the dorsal hippocampus. Short-term (STM) and long-term (LTM) object recognition (OR), and social recognition memory (SR) were evaluated. Hippocampal oxidative status was determined by quantification of reactive oxygen species (ROS), lipid peroxidation by thiobarbituric acid reactive substance test (TBARS), total antioxidant capacity by ferric reducing/antioxidant power (FRAP), and acetylcholinesterase enzyme activity (AChE). Aβ infusion resulted in STM and LTM deficits, hippocampal oxidative damage and changed AchE. StrEx caused a better neuroprotection than RunEx by preventing OR and SR memory deficits, and prevented increase in lipid peroxidation and alterations in AChE activity. RunEx was neuroprotective only for SR memory deficits, prevented increase in ROS and lipid peroxidation, and preserved the total antioxidant capacity. While RunEx effects are related to oxidative status, only StrEx shows potential to influence the cholinergic system.

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