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COMPARAÇÃO DOS EFEITOS NEUROPROTETORES DO ENRIQUECIMENTO AMBIENTAL, DO EXERCÍCIO FÍSICO E DA SOCIALIZAÇÃO EM UM MODELO ANIMAL DE DOENÇA DE ALZHEIMER

DISSERTAÇÃO DE MESTRADO

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Uruguaiana, RS, Brasil
2017

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Por

Mariza Garcia Prado Lima

Dissertação apresentada ao Programa de Pós-Graduação em Bioquímica da Universidade Federal do Pampa (UNIPAMPA, RS), como requisito parcial para a obtenção do grau de **Mestre em Bioquímica**

Orientadora: Profª. Drª. Pâmela Billig Mello Carpes

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Como requisito parcial para a obtenção do grau de
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2017

“O que prevemos raramente ocorre; o que menos esperamos geralmente acontece.”

Benjamin Disraeli

DEDICO

Dedico este trabalho à minha família
que me apoiou incondicionalmente na
conquista de meus objetivos.

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A Deus, que todos os dias me deu forças para nuca desistir.

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RESUMO

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Resumo

A Doença de Alzheimer (DA) é uma enfermidade incurável que causa perda das funções cognitivas (memória, orientação, atenção e linguagem). As lesões cerebrais são características dessa doença, sendo as principais alterações: as placas senis decorrentes do depósito de proteína beta-amiloide (βa) anormalmente produzida, e os emaranhados neurofibrilares. Atualmente, muitas abordagens são propostas para tratar ou prevenir a DA, mas os estudos geralmente usam protocolos que dificilmente permitem o estabelecimento da relação causa-efeito, pois envolvem mais de uma variável que poderia ter benefícios no cérebro com DA. O objetivo deste trabalho foi avaliar e isolar os efeitos neuroprotetores do enriquecimento ambiental, do exercício físico anaeróbio, e do enriquecimento social, em déficits de memória relacionados à neurotoxicidade induzida pela beta-amiloide (βa) em um modelo animal. Para isto, foram utilizados ratos Wistar submetidos às intervenções propostas por 8 semanas, e, logo após, à cirurgia estereotáxica para a injeção de βa no hipocampo. A memória foi avaliada pelos testes de reconhecimento de objetos e reconhecimento social, considerando memória de curta e de longa duração. O estado de oxidativo do hipocampo (níveis de espécies reativas de oxigênio, peroxidação lipídica e capacidade antioxidante total - ROS, TBARS e FRAP) e a atividade da enzima acetilcolinesterase (AChE) também foram verificados. Os dados mostram que a injeção de βa resultou em déficits de memória e danos oxidativos no hipocampo. O enriquecimento ambiental e o exercício físico

evitaram todos os déficits de memória e a peroxidação lipídica (TBARS) hipocampal induzida por βa. O enriquecimento social evitou apenas o déficit de memória de reconhecimento social induzido pela beta-amiloide e aumentou a capacidade antioxidante total (FRAP).

Palavras chave: Alzheimer, Treinamento físico, Enriquecimento ambiental, Reconhecimento de objetos, Reconhecimento social, Estresse Oxidativo.

ABSTRACT

Masters Dissertation
Graduation Program in Biochemistry
Federal University of Pampa

COMPARISON OF NEUROPROTETIC EFFECTS OF ENVIRONMENTAL ENRICHMENT, PHYSICAL EXERCISE AND SOCIALIZATION IN AN ALZHEIMER'S DISEASE ANIMAL MODEL

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Site and date: Uruguaiana, July 12, 2017

Abstract

Alzheimer's Disease (AD) is an incurable disease that causes loss of cognitive functions (memory, orientation, attention, and language). Brain lesions are characteristic of this disease, in which main alterations being: senile plaques arisvel from the deposition of abnormally produced beta-amyloid protein (βa) and neurofibrillary tangles. Many approaches have been proposed to treat or prevent AD, but studies generally use protocols that becouse difficult to attribute a cause-effect relationship because they involve more than one variable that could be benefical in observed changes. The objective of this work was to evaluate and isolate the neuroprotective effects of environmental enrichment, anaerobic physical exercise, and social enrichment on memory deficits related to beta-amyloid neurotoxicity in an animal model. For this, Wistar rats were submitted to 8 weeks of intervention, and soon thereafter underwent stereotactic surgery for the injection of βa into the hippocampus. The memory was evaluated by object recognition and social recognition memory tests, considering short and long term memory. The oxidative state of the hippocampus (ROS, TBARS and FRAP) and acetylcholinesterase (AChE) activity are also verified. The data show that the injection of βa resulted in memory deficits and oxidative damage in the hippocampus. Environmental enrichment and exercise avoided all memory deficits and hippocampal lipid peroxidation (TBARS) induced by βa. Social enrichment avoided only the social

recognition memory deficit and avoided the total antioxidant capacity (FRAP) decrease induced by βa.

Key-words: Alzheimer, Physical training, Environmental enrichment, Object Recognition, Social Recognition, Oxidative stress.

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LISTA DE ABREVIATURAS

APP – Proteína precursora da amiloide, do inglês *Amyloid Precursor Protein*

βA – Beta-amiloide

CA1 – Região CA1 do hipocampo dorsal, do latim *Cornu ammonis* (Corno de Ammon)

Cu – Cobre

DA – Doença de Alzheimer

EA – Enriquecimento ambiental

EROs – Espécies reativas de oxigênio

Fe – Ferro

Nrf2 – Fator nuclear eritróide 2, do inglês *nuclear factor erythroid 2-related factor 2*

Zn – Zinco

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APRESENTAÇÃO

A presente dissertação é organizada em três partes.

A primeira parte é composta pela introdução, na qual estão descritos os temas que fundamentam este estudo, bem como sua justificativa e os seus objetivos. A segunda parte é composta pelos métodos, resultados e discussão do estudo, apresentados na forma de um manuscrito científico, a ser submetido para publicação em um período internacional. A terceira parte é composta por uma discussão geral dos resultados, conclusões e perspectivas.

Ao final, encontram-se as referências bibliográficas citadas na primeira e na terceira parte da dissertação, já que as da segunda estão inclusas no manuscrito.

PARTE I

1. INTRODUÇÃO

1.1. Doença de Alzheimer

Durante o envelhecimento surgem diversas alterações macroscópicas e microscópicas no encéfalo, incluindo alterações no peso e volume do órgão, no volume dos ventrículos, no aspecto dos giros e sulcos, no tamanho e no número dos neurônios, no número de espinhos e de sinapses, na extensão das ramificações dendríticas, aparecimento de placas senis e enovelamentos ou emaranhados neurofibrilares (MARTIN, 2006; MATTSON, 2007; SCAHILL et al., 2003).

Diversas das modificações que ocorrem no cérebro durante o processo de envelhecimento são fisiológicas, mas é nesta fase que as demências têm maior probabilidade de se desenvolver. Dentre elas existem as demências degenerativas e as não degenerativas. As demências classificadas como não degenerativas são decorrentes de outras condições patológicas, como os processos infecciosos, deficiências nutricionais, tumores e traumatismos. Já as demências degenerativas têm sua origem predominantemente cortical, com início insidioso, caráter progressivo e comprometimento da memória episódica (FORNARI et al., 2010; ALLEGRI et al., 2001).

A neurodegeneração pode estar associada a diversos fatores e processos, que culminam em uma cascata de sinalização que resulta na morte celular (HARDY & HIGGINS, 1992). Estas perdas celulares no sistema nervoso central podem se manifestar como disfunções comportamentais, tais como déficits cognitivos. Há manifestações que levam a alterações celulares e fisiológicas ditas “normais”, que acompanham o processo de envelhecimento, mas podem ter seus efeitos pronunciados, dificultando o dia a dia do idoso (TROULINAKI & TAVERNARAKIS, 2005). Apesar do avanço científico, alguns

dos mecanismos celulares e moleculares que representam a interface entre envelhecimento e as patologias neurodegenerativas continuam confusos.

Dentre as doenças neurodegenerativas, a mais comum é a Doença de Alzheimer (DA), que foi descrita pelo neurologista alemão Alois Alzheimer em 1906 (HARMAN, 1996). Apesar de muitas pesquisas se debruçarem sobre esta doença, na tentativa de melhor entender seus mecanismos patológicos e encontrar tratamentos mais adequados, ela permanece até os dias atuais sem perspectiva de cura, juntamente com o aumento da expectativa de vida e da população idosa (FOX & ALDER, 2012).

Atualmente, o diagnóstico da DA é baseado na avaliação clínica, ou seja, nos sintomas clínicos apresentados pelos pacientes, o que resulta em baixa acurácia no diagnóstico da doença (NITRINI et. al., 2005; BERTOLLUCCI, 2000; WALDEMAR et. al., 2007). Além disso, com o diagnóstico dependente dos sintomas do paciente, o que dificulta sua exatidão, pois se trabalha com processo de eliminação e exclusão, este costuma ocorrer mais tardiamente; do mesmo modo, o progresso da doença varia muito entre os indivíduos (MOONEY, 2011). A progressão da doença ocorre em três estágios principais: o estágio pré-clínico (assintomático), o estágio pródromo (quando há manifestação de sintomas moderados como, por exemplo, alterações de memória episódica), e, por último, o estágio sintomático com presença de demência (DUBOIS et. al., 2007).

A DA está geralmente associada ao avanço da idade, e as manifestações cognitivas resultam em uma deficiência progressiva e uma eventual incapacitação, gerando perda de memória, diminuição do desempenho nas atividades diárias, seguida pela completa demência (ZHAO & TANG, 2002; JANUS & WESTAWAY, 2001). Ela é caracterizada pela presença de placas senis no cérebro, contendo deposição extracelular do peptídeo Beta-amilóide (βA) derivado da proteína precursora de amiloide (APP, do inglês *Amyloid Precursor Protein*, figura 1), e de emaranhados neurofibrilares intracelulares contendo proteína Tau hiperfosforilada (GÓMEZ et al., 1996; WANG et al., 2006; SCHUFF et al., 1997; CHANTAL et al., 2002).

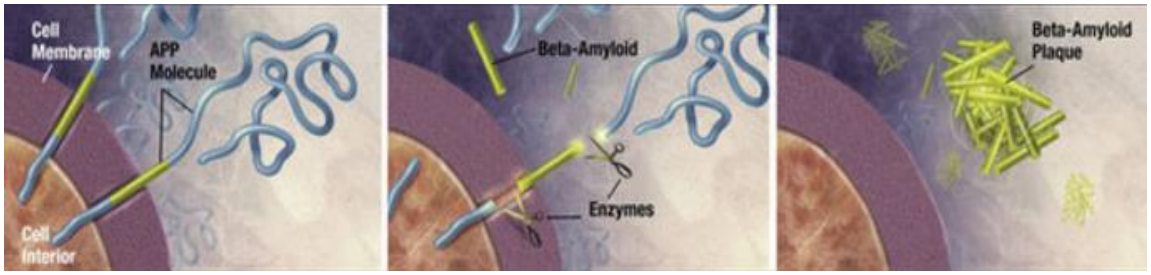


Figura 1. Processo de formação das placas senis. No cérebro de indivíduos com a Doença de Alzheimer ocorre fragmentação da proteína precursora de amiloide (APP) por meio de enzimas. A produção excessiva destes fragmentos beta-amilóide culmina na formação das placas senis no espaço extracelular. Fonte: National Institute on Aging (2012).

A proteína precursora beta-amilóide, constituída de 40 a 42 aminoácidos, é uma glicoproteína localizada parcialmente no interior e parcialmente no exterior da membrana plasmática (figura 1). Os peptídeos beta-amilóides oriundos dela facilitam a produção de oxiradicais, podendo ser diretamente tóxicos para os neurônios e células da glia, por agirem na peroxidação lipídica da membrana celular, desregulando a homeostase do cálcio (KAWASUMI et al., 2002; CUMMINGS, 2003; CORRÊA, 1996; MATTSON & MATTSON, 2003). Ainda, as placas senis formadas pelos peptídeos beta-amilóides ativam as células da glia, como a micróglia e os astrócitos, que estão envolvidos na fagocitose na área em degeneração e em processos de inflamação (SELKOE et al., 1995; PALMER, 1996; NANN, 1996). Os emaranhados neurofibrilares também possuem propriedades neurotóxicas, e sua ação compromete a integridade das células neuronais (NANN, 1996; MACCIONI et al., 2001). A degeneração neurofibrilar é um processo rápido, e os neurônios expostos à proteína beta-amilóide têm mostrado aumento da vulnerabilidade a excitotoxicidade que leva à morte neuronal.

Todas estas alterações degenerativas geradas pela doença de Alzheimer ocorrem no giro denteado e na sub-região CA1 do hipocampo, no neocórtex de associação e no córtex entorrinal, em todos os estágios da doença (GÓMEZ et al., 1996; WANG et al., 2006; SCHUFF et al., 1997; CHANTAL et al., 2002). Estudos mais recentes sugerem que a DA poderia iniciar em regiões mais baixas do Sistema Nervoso Central, como *Locus Coerulleus* e Área Tegumentar Ventral, no entanto, nesta fase, o paciente não apresenta

sintomas, o que impede a identificação da doença (EHRENBERG et. al., 2017; ANDRÉS et. al., 2017; TRILLO et. al., 2013; NOBILI et. al., 2017).

O hipocampo, localizado no lobo temporal, tem um papel importante para o aprendizado e a consolidação inicial da memória e, lesões nesta região impedem a formação e consolidação de novas memórias; entretanto, outros tipos de memória permanecem intactas, ao menos nos primeiros estágios da doença (ENNACEUR et al., 1988; CLARK et al., 2000; BAKER & KIM, 2002). Assim, a deterioração das memórias de curto e longo prazo, pelo impedimento de sua adequada consolidação, é uma das principais características iniciais da doença, seguida da afasia[1], agnosia[2] (depois de vários anos de acometimento pela doença) e apraxia[3] (FOX & ALDER, 2001).

1.1.1. Doença de Alzheimer e Estresse Oxidativo

Para a função celular normal, é essencial que haja equilíbrio entre a produção de espécies reativas de oxigênio (ERO's) e a de defesas antioxidantes. No entanto, na doença de Alzheimer, em função de toda alteração patológica que ocorre, a atividade das enzimas antioxidantes está alterada, contribuindo para o acúmulo de dano oxidativo (KIM et al., 2006).

Quando ocorre desequilíbrio que gera uma superprodução de ERO's combinada com uma insuficiente defesa antioxidante ocorre o estresse oxidativo. Evidências demonstram que o dano mitocondrial que resulta em aumento da produção de ERO's contribui para os estágios iniciais da DA, antes mesmo do início dos sintomas clínicos e da presença patológica de βA (UTTARA et al., 2009). Ainda, marcadores de estresse oxidativo, incluindo altos níveis de proteínas oxidadas, produtos glicosilados, peroxidação lipídica extensa, formação de álcoois, aldeídos, carbonilas livres, cetonas, colestenona e modificações oxidativas no RNA e DNA nuclear e mitocondrial, foram

[1] Perda da linguagem causada por lesão no sistema nervoso central.
[2] Incapacidade de reconhecer e/ou identificar objetos usuais, apesar de funcionamento sensorial intacto.
[3] Incapacidade de executar atividades motoras, apesar de um funcionamento sensorial intacto.

encontrados no tecido cerebral de pacientes com DA pós-morte, e em sistemas periféricos, incluindo células e mitocôndrias isoladas de pessoas em estágios pré-clínicos ou iniciais da DA (SULTANA et al., 2011; SMITH et al., 1995; MIGLIORE et al., 2005).

Além da produção de ERO's mitocondriais, a homeostase anormal de metais bioativos, como o Ferro (Fe), Cobre (Cu), Zinco (Zn), entre outros, pode estar envolvida na produção de radicais livres e no estresse oxidativo que influencia a agregação de βA (BEAL, 2005; GREENOUGH et al., 2013; LIU et al., 2006). Acredita-se que o acúmulo desses metais se origina da homeostase neuronal do metal afetada pelo envelhecimento e exacerbada pelas patologias amiloides, no caso da DA (BOOM et al., 2004; BARNHAM & BUSH, 2014).

Outra fonte de produção de ERO's diretamente mediada pela βA envolve a micróglia ativada no cérebro durante uma resposta inflamatória à deposição de pacas amiloides extracelulares (NAKAJIMA & KOHSAKA, 2001). Além disso, níveis aumentados de βA podem acelerar a produção de ERO's ligando-se diretamente às membranas mitocondriais, alterando a dinâmica e a função mitocondrial, levando ao metabolismo energético anormal e à perda da função sináptica (LAFERLA et. al., 2007). O estresse oxidativo induzido por peptídeos βA perturba o metabolismo do colesterol e da ceramida que, por sua vez, desencadeia uma cascata neurodegenerativa levando a um depósito de beta-amilóide adicional, fosforilação de TAU e à doença clínica (MATTSON et al., 2005; ICHIMURA et al., 2003).

A consequência deste desequilíbrio entre as ERO's e a capacidade antioxidante disponível no cérebro, bem como o acúmulo de proteínas oxidadas, leva à potencialização da neurodegeneração e à redução das funções cognitivas (HARDY and SELKOE, 2002; CHOI et. al., 2012; ANNAHÁZI et. al., 2007). Assim, a DA envolve estresse oxidativo e resposta inflamatória neuronal (Figura 2; CORBETTA et al., 2008; DEVORE et al., 2010; GLASS et al., 2010).

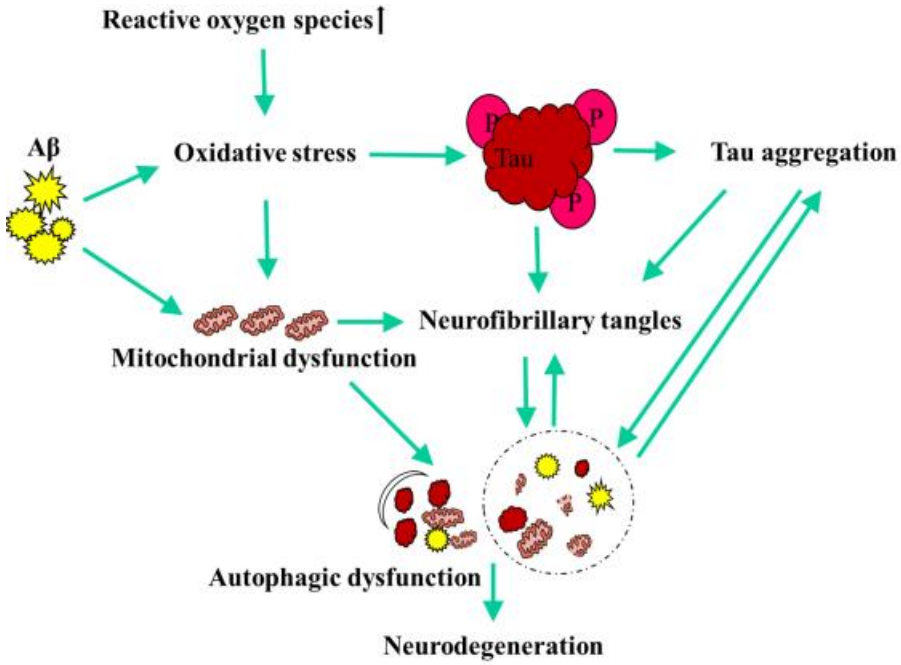


Figura 2. Esquema representativo do processo de formação das placas beta-amilóides e emaranhados neurofibrilares. Ambos geram respostas que envolvem estresse oxidativo e espécies reativas de oxgênio, culminando em neurodegeneração e morte neuronal. Fonte: Liu et. al., 2015.

1.1.2. Doença de Alzheimer, enriquecimento ambiental, exercício físico e socialização

Considerando a gravidade dos sintomas da DA, bem como a velocidade de sua progressão, muitos cientistas estão buscando estratégias para retardar ou impedir o seu desenvolvimento (SHAEFFER, 2009). Pesquisa realizada junto a idosos com déficit cognitivo relacionado ao envelhecimento identificou melhorias no quadro clínico utilizando intervenções que foram consideradas potencialmente promissoras (JEDRZIEWSKI et al., 2014). Os autores demonstraram que os indivíduos que se envolvem em atividades estimulantes podem ter, além de outras alterações cerebrais, aumento no número de neurônios cerebrais, proporcionando-lhes reserva suficiente para realizar tarefas cognitivas sem comprometimento (JEDRZIEWSKI et al., 2014).

É normal encontrarmos, em nossa sociedade, grupos de idosos que promovem atividades deste tipo a fim de manter ou melhorar sua saúde cognitiva. Entretanto, ainda não está claro qual o tipo de atividade que pode ser mais promissora no que diz respeito à intervenção na progressão da DA; isto porque, geralmente, as pesquisas nesta linha realizam propostas que associam um ou mais tipo de intervenção. De todo modo, intervenções como o enriquecimento ambiental, a atividade ou exercício físico, e a interação social, podem ser consideradas estimulantes, mas são bem diferentes entre si no que diz respeito a seus efeitos orgânicos, e, portanto, podem gerar respostas diferentes no cérebro com Alzheimer.

1.1.2.1. Enriquecimento ambiental

O enriquecimento ambiental (EA) envolve a exposição a um ambiente rico em estímulos sensoriais e cognitivos, e, muitas vezes, é combinada com o aumento da interação social e a prática de exercício físico (PEREIRA et al., 2007).

Em modelos animais verificou-se que ratos que experimentam um ambiente enriquecido melhoram o seu bem-estar e suas habilidades cognitivas, especialmente em testes de cognição espacial (LEGGIO et al., 2005). Assim, é uma técnica utilizada para proporcionar estímulos diversos e tentar reverter déficits cognitivos causados por diversos fatores, e, assim como outras estimulações cognitivas e físicas, é uma estratégia que tem sido empregada para melhorar a função cognitiva também na DA (SHAEFFER, 2009). Na DA o EA parece promover o aumento da neurogênese, a plasticidade neuronal hipocampal, a exposição à novidade e o aumento da atividade motora (KUMAR A. et al 2012), consequentemente melhorando o desempenho nas tarefas de memória, sendo capaz de reverter déficits de diversas origens nestas funções (VIVINETTO et al, 2013).

Além disso, Mainardi et al. (2014) demonstraram que a exposição ao EA reduz níveis corticais de oligômeros solúveis de beta-amilóide endógenos e aumenta a expressão de neprilissina, uma das principais enzimas que regulam o catabolismo da proteína amiloide. Estudo prévio também descreveu efeitos

do EA na melhora da resposta imune, como na infecção da influenza tipo A, de forma que animais submetidos ao EA recuperaram-se mais rapidamente do que os animais que viviam em ambiente padrão (JURGENS et al., 2012). Ainda, o EA induziu uma melhora da resposta sistêmica em um modelo de sepsis, provocando aumento do recrutamento de células imunes no local da inflamação (BROD et al., 2017)

Diversas áreas cerebrais podem sofrer modificações após exposição ao EA, como o hipocampo e o neocórtex, promovendo neuroproteção e reduzindo as deficiências de memória e aprendizado (LAMBERT et al., 2005). No entanto, alguns autores acreditam que o exercício ou atividade física frequentemente incluído/a nos protocolos de EA é responsável por estimular a memória e a capacidade cognitiva, principalmente em animais envelhecidos, questionando o fato de que somente o EA não provocaria tais efeitos (LAMBERT et. al., 2005). Ainda, além do aumento do nível de atividade física, em muitos protocolos de EA, especialmente em animais, a interação social também é aumentada significativamente. Isto porque no EA uma variedade de estímulos é disponibilizada ao animal, o que inclui, além de brinquedos e objetos-estímulo, túneis e rodas de atividades, que favorecem o aumento da atividade física (ARAC, 2004; LAMBERT et. al., 2005). Além disso, geralmente os animais são mantidos em caixas moradia maiores, com um número de animais maiores que o padrão, aumentando suas interações sociais.

1.1.2.2. Exercício físico

Estudos que utilizam modelos animais de DA também demonstraram que o exercício físico regular é capaz de alterar diversas características fenotípicas da DA, promovendo, entre outras respostas, o aumento da degradação amiloide, reduzindo assim seu acúmulo no tecido cerebral e, por consequência, a morte neuronal (ADLARD et al., 2005, OGONOVSZKY et al., 2005, GARCIA-MESA et al., 2011, KIM et al., 2014). Ainda, o exercício é capaz de melhorar o desempenho em tarefas de aprendizagem e de memória espacial (DAO et al., 2014).

O exercício aeróbico é uma terapia alternativa de baixo custo e efetiva para distúrbios de ansiedade e transtornos do humor (SALMON, 2001), além disso, este tipo de exercício retarda a progressão do declínio cognitivo (LARSON, 2010), melhora o desempenho em testes de cognição e bem estar psicológico (VOGT et. al., 2010). Em estudo prévio que utilizou o exercício aeróbico, evidenciou-se redução significativa na deterioração induzida pela DA, melhora na capacidade de aprendizagem espacial e memória de curto prazo, através da supressão da apoptose (BAEK & KIM, 2016).

No entanto, a maioria dos protocolos utilizados em pesquisas nesta linha contempla apenas o exercício físico aeróbico, enquanto os efeitos do exercício anaeróbico sobre o sistema nervoso ainda são contraditórios (CASSILHAS et al., 2007). Colcombe e colegas (2006) demonstraram que idosos sem demência que realizam atividade aeróbica apresentaram maiores volumes de matéria cinza e branca. Já um estudo mais recente demonstrou que o treinamento de força promove a neurogênese no hipocampo (NOVAES et al., 2014). Por outro lado, Nokia et al. (2016) evidenciam que o exercício de força não afeta a proliferação, maturação ou a sobrevivência neuronal de novas células no hipocampo adulto, embora uma melhora da força muscular seja evidente.

Dessa forma, é importante discutir o papel das diferentes modalidades de exercício nos distúrbios cognitivos, especialmente o exercício de força, que é importante para os idosos garantirem a saúde e qualidade de vida em outros aspectos, além do cognitivo – evitando tropeços, déficits de equilíbrio, e quedas, por exemplo (HURLEY e HAGBERG, 1998; RUBENSTEIN et al., 2000). Assim, se o mesmo tipo de exercício (anaeróbico/de força) for capaz de gerar benefícios tanto cognitivos quanto motores, será mais fácil envolver os idosos nestas práticas.

1.1.2.3. Socialização

A interação social é um componente importante para o homem e demais animais. Existem crescentes evidências de que as deficiências na interação social são importantes marcadores precoces para desordens do

desenvolvimento neurológico relacionados com fortes componentes genéticos (MOY et al., 2004).

Além disso, o enriquecimento social apresenta efeitos positivos sobre o comportamento social (BRENES et.al., 2016), já o isolamento social gera dano oxidativo, exacerbação aguda de processos inflamatórios crônicos, bem como déficits de cognição (SAPOLSKY et al., 2002; ELUVATHINGAL et al., 2006).

Considerando a DA, alguns estudos de coorte observacional têm demonstrado que uma maior participação social está associada a um menor risco de demência incidente (WANG et al., 2002; BENNETT et al., 2006), e, que se pode reduzir o risco de declínio cognitivo e o início da DA associada ao envelhecimento em sujeitos mentalmente ativos e com ricas conexões sociais (STERN, 2006; SZEKELY et al, 2007). No entanto, embora estas evidências tenham sido observadas, poucos são os trabalhos que se preocupam em entender os mecanismos envolvidos nos efeitos no enriquecimento social sobre o cérebro com DA, especialmente no que diz respeito aos mecanismos pelos quais esta intervenção pode trazer benefícios a estes pacientes.

2. JUSTIFICATIVA

A DA é uma patologia neurodegenerativa progressiva que deteriora funções cognitivas, incluindo a memória de curto e longo prazo (BRAAK et al., 1999; SELKOE, 2002). Muitos estudos têm analisado os efeitos das estimulações físicas, cognitivas e as interações sociais como método de proteção a doença. No entanto, apesar dos resultados promissores, a maioria dos estudos que utiliza estratégias deste tipo, especialmente em modelos animais, não costuma separar adequadamente os efeitos do enriquecimento ambiental, do exercício físico e/ou da socialização, uma vez que o protocolo normalmente utilizado envolvem estes três fatores (figura 3).

Figura 3. Exemplo de um caixa de enriquecimento ambiental para ratos. Na imagem, que representa um aparato semelhante ao utilizado na maioria dos estudos com enriquecimento ambiental, se pode observar que vários animais são mantidos em conjunto. Além disso, verifica-se a presença de uma roda de atividade física. Fonte: Goulart, (2014).

Estudos em modelo animal sugerem que o enriquecimento ambiental apresenta resultados promissores no tratamento da DA, promovendo melhora cognitiva associada à neuroplasticidade aumentada e diminuição da neuroinflamação (STUART et al., 2017; XU et al., 2016). No entanto, o protocolo proposto na maioria dos estudos acaba gerando, também, aumento da interação social, e, estudo prévio demonstra que a interação social, por si

só, pode proteger contra o declínio cognitivo (GREEN et al., 2008). O protocolo de enriquecimento ambiental para roedores também costuma envolver algum grau de atividade ou exercício físico. O exercício físico é uma intervenção que possui um potencial na diminuição do risco no comprometimento cognitivo relacionado com o envelhecimento (JEDRZIEWSKI et al., 2010).

Assim, a translação dos resultados de pesquisas com cobaias para a clínica é comprometida, pois, enquanto nos modelos animais normalmente estas intervenções são realizadas simultaneamente, para o ser humano as três intervenções são processos que podem ser considerados bem diferentes e não necessariamente associados. Um idoso pode estar realizando especificamente um treinamento físico, ou só participar de um grupo de convivência para idosos, o que aumenta sua interação social. Pode, ainda, estar rodeado por um ambiente rico em estímulos cognitivos. Acredita-se que todas estas intervenções possam ter efeitos benéficos sobre a saúde cognitiva, mas, quando se pensa em um idoso vulnerável, é importante termos claro qual delas é a mais indicada, se for o caso de termos que recomendar uma ou outra estratégia. Tal conhecimento é fundamental, também, para o adequado planejamento de estratégias de saúde pública.

Desta forma, é importante a avaliação dos efeitos neuroprotetores específicos do enriquecimento ambiental, do exercício físico e da socialização, bem como o entendimento dos mecanismos envolvidos nestes efeitos. Nesta pesquisa propomos fazer esta investigação, avaliando o envolvimento do estresse oxidativo nos efeitos observados a partir de cada intervenção, já que o estresse oxidativo e um dos mecanismos patológicos envolvidos na DA. Tais resultados ajudarão a identificar os reais benefícios de cada uma das propostas, auxiliando na escolha da melhor estratégia terapêutica junto a idosos (para prevenção da DA) ou a pacientes com DA (para o seu retardamento).

3. OBJETIVOS

3.1. Objetivo Geral

O objetivo geral deste estudo foi verificar e comparar os efeitos neuroprotetores do enriquecimento ambiental, do exercício físico (anaeróbico) e da socialização sobre déficit de memória e o dano oxidativo hipocampal em um modelo animal de Doença de Alzheimer.

3.2. Objetivos específicos

Os objetivos específicos desta dissertação incluíram:

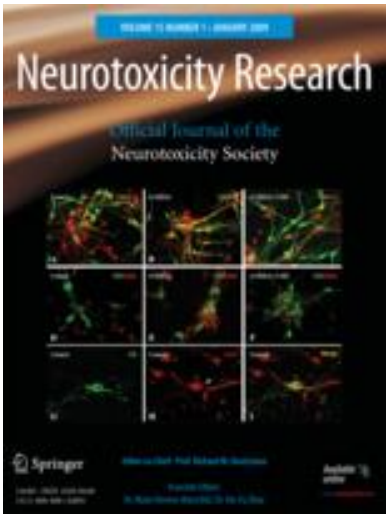
*Avaliar a memória de RO e RS e comparar os efeitos neuroprotetores das diferentes intervenções (enriquecimento ambiental, exercício físico anaeróbico, e socialização) sobre o possível déficit nos diferentes tipos de memória em ratos submetidos a um modelo de DA;

*Avaliar a capacidade antioxidante total, os níveis de espécies reativas de oxigênio, a peroxidação lipídica, e a atividade da enzima acetilcolinesterase e comparar os efeitos neuroprotetores das diferentes intervenções sobre estas no hipocampo de ratos submetidos a um modelo DA;

PARTE II

4. MANUSCRITO

Manuscrito científico escrito nas normas da revista *Neurotoxicity Research* (normas em anexo – ANEXO I).



ISOLATING THE NEUROPROTECTIVE EFFECTS OF ENVIRONMENTAL ENRICHMENT, SOCIAL ENRICHMENT AND PHYSICAL EXERCISE IN MEMORY DEFICITS RELATED TO AMYLOID BETA NEUROTOXICITY

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Abstract

Alzheimer's Disease (AD) is an incurable disease that causes loss of cognitive functions such as memory, orientation, attention, and language. The AD's prevention and treatment are a challenge yet. A lot of non-pharmacological approaches are proposed to treat or prevent the AD, but the studies generally use protocols that difficult to attribute a cause-effect relationship, since they involve more than one variable that could have benefits on AD alterations. The purpose of this work was to evaluate and isolate the neuroprotective effects of environmental enrichment (EE), anaerobic physical exercise (AnPE) and social enrichment (SE) in memory deficits related to amyloid beta (Abeta) neurotoxicity in an animal model for AD. Wistar rats were submitted to EE, AnPE or SE for 8 weeks, and after were submitted to stereotaxic surgery to βa injection on hippocampus. Memory was evaluated by object (OR) and social recognition (SR) test, considering short (STM) and long-term memory (LTM). Hippocampal oxidative status (ROS, TBARS and FRAP) and acetylcholinesterase levels (AChE) are also checked. The data show that Abeta injection resulted in memory deficits and hippocampal oxidative damage. EE and AnPE avoided all memory deficits (OR and SR) and lipid peroxidation (TBARS) induced by Abeta. SE avoided only the SR memory deficits and antioxidant capacity (FRAP) decrease induced by Abeta.

Keywords: Alzheimer's disease. Anaerobic physical exercise. Object recognition. Social recognition. Oxidative stress.

1. Introduction

Alzheimer's disease (AD) is a progressive neurologic disease that affects about 24 million people (Chakrabarti et al., 2015) and results in the irreversible loss of neurons, particularly in the cortex and hippocampus. The pathological hallmarks, besides the neuronal loss, include extracellular senile plaques containing the peptide amyloid beta (Abeta) and neurofibrillary tangles (Mckhann et al., 1984; Clark et al. 1998; Nussbaum and Ellis 2003; Dong et al., 2012).

The Abeta deposition results in neurotoxicity that includes oxidative damage, a central factor in the pathogenesis of AD (Butterfield et al., 2007; Butterfield et al., 2013; Polidori and Nelles 2014). In postmortem AD brains was verified significant lipid, protein and DNA oxidative damage, as well as impaired antioxidant defense (Butterfield et al., 2007; Polidori and Nelles 2014; Butterfield et al., 2013). Some mechanisms seem to be essential for initiating the oxidative damage in the AD brain, but, in particular, Abeta-induced ROS generation has been well documented in a large number of experimental studies using cell culture, experimental animals and cell-free chemical systems (Kadowaki et al., 2005; Chakrabarti et al., 2013).

Some evidences suggest the involvement of enzyme acetylcholinesterase (AChE), indicating that oxidative stress and cholinergic dysfunction were important in the procession of causing AD. Also, a molecular modeling study showed that AChE interacts with the Aβ peptide and promotes amyloid fibril formation (Mesulam, 1986; Racchi et. al., 2001; Ishrat et. al., 2009; De Ferrari et. al., 2001).

Environmental Enrichment (EE) has been studied in human and animal models as a neuroprotective strategy in different models of neurodegenerative diseases (Quattromani et al., 2014; Hase et al., 2017). Previous researches also demonstrated effects of this strategy ameliorating the pathological effects related to Abeta protein oligomers (Xu et al., 2016; Stuart et al., 2017). However, the model of EE used in animal experiments usually includes other variables that can confuse the observed results. In experimental conditions, the animals submitted to EE protocol generally were maintained for a period of time in a large box with a higher number of animals than standard conditions (Vivinetto et al., 2013). In addition, often EE involves, in addition to the object-stimulus, activity wheels and tunnels for animals cross, also toys

that involve some type of physical activity which can be offered in a permanent or rotational way (Fiala et al., 1997; Newberry, 1995).

These models used to investigate EE difficult the correlation of the results with humans models, since in humans EE is very different from social enrichment (SE) or physical exercise practice. Furthermore, evidence demonstrates that enhancements in socialization per se have a positive impact in neurodegenerative conditions, such as Alzheimer's disease (Shea and Rogers, 2014; Quattromani et al., 2014). The effects of regular moderate physical exercise on cognition also improve neural function (Seo et al., 2013; Garland et al., 2011; Adlard et al., 2005). So, here we design a set of experiments looking for isolating the neuroprotective effects of EE, SE and anaerobic physical exercise (AnPE) in memory deficits related to Abeta neurotoxicity. We demonstrated that all these strategies promote neuroprotection, but in different levels and by different mechanisms related to oxidative status.

2. Material and methods

2.1 Animals and experimental design

Male Wistar rats (3-month-old, 350–380 g) were purchased from Central Vivarium of Federal University of Santa Maria (RS/Brazil). During all the experimental period, they were housed four per cage (except in SE groups) and maintained under controlled light and environmental conditions (12 h light/12 h dark cycle; temperature of 23 ± 2 ºC; humidity 50 ± 10%) with food and water ad libitum.

All experiments were conducted in accordance with the ''Principles of laboratory animal care'' (NIH publication nº 80–23, revised 1996) and in agreement with the guidelines established by the Institutional Animal Care and Use Committee of the Local Institution to ensure that number of rats and their suffering were kept to a minimum (protocol #032/2016).

At the age of three months, the animals were randomly assigned to one of the four experimental groups:

(i) Control (cont; n = 20): The rats from this group were maintained in standard lab conditions.

(ii) Environmental Enrichment (EE; n = 20): The rats from this group were submitted to environmental enrichment for 8 weeks;
(iii) Anaerobic Physical Exercise (AnPE; n = 20): The rats from this group were submitted to anaerobic physical exercise for 8 weeks;
(iv) Social Enrichment (SE; n = 20): The rats from this group were submitted to social enrichment for 8 weeks.

After eight weeks of intervention, half of the animals in each group undergo stereotaxic surgery to induce amyloid-beta (Abeta) toxicity - an important characteristic of Alzheimer's disease; another half was submitted to surgery without Abeta injection. After surgery recovery, all rats were submitted to behavioral tests. When behavioral tests were finished, rats were euthanized for posterior brain tissue preparation and biochemical analyses (Fig. 1).

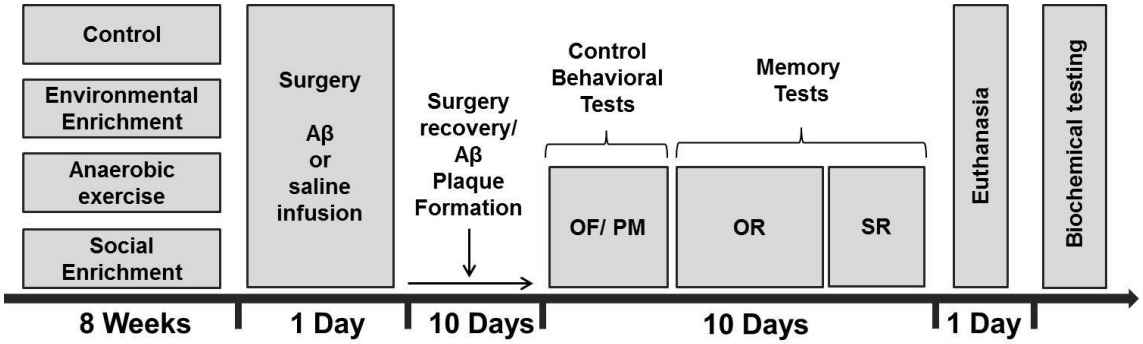

Fig 1. Experimental design. Rats were maintained in standard conditions (control) or submitted to environmental enrichment, anaerobic exercise or social enrichment during 8 weeks. After, they were submitted to stereotaxic surgery with injection of Abeta (Aβ) or saline. Behavioral testing started 10 days after surgery to ensure surgery recovery and Aβ plaque formation. Euthanasia occurred 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 Interventions

2.2.1 Environmental Enrichment (EE)

Rats submitted to EE were housed in large polycarbonate cages (80 × 60 × 60 cm), 4 rats per cage, provided with various objects of different shapes, sizes, colors, textures, and material (wood, plastic, and metal) as plastic tubes, little balls, sound objects and

wooden houses (Bouet et al. 2011; Leger, Bouet et al. 2012). During the 8 weeks of intervention, 3 times a week, 3 hours / day, the objects and their locations were renewed once a week to ensure novelty. No physical activity was used in this intervention.

2.2.2 Social Enrichment (SE)

Rats submitted to SE were housed in large polycarbonate cages (80 × 60 × 60 cm), 10 rats per cage (adapted from Pascual et al., 2006). During the 8 weeks of intervention, 3 times a week, 3 hours / day.

2.2.3 Anaerobic Physical Exercise (AnPE)

The AnPE proposed consisted in a resistance training using a one meter ladder with steps separated by 2 cm from one another, as described by Barone et al. (2009). Rats were familiarized with the exercise (climbing the ladder) for 3 days. In the first week after familiarization, the exercise was performed using the natural load. In order to increase the workload, from the second week upward, increasing weights were attached to the base of the rats' tail. The initial weight was 50% of the rat's body weight and gradually increased throughout the subsequent 8-week training period. The resistance training consisted of 1 set of 8 repetitions with a 1 min of rest interval among the repetitions. The training was conducted 3 days/week, for 8 weeks. When the rats reached the top of the ladder, they were allowed to recover in the resting area.

2.3 Preparation of Amyloid β 25-35

Amyloid beta (Abeta) peptide 25-35 (Sigma Aldrich, São Paulo, Brazil; Product Number: A4559) was dissolved in saline (vehicle) at a concentration of 100 µM. Before intrahippocampal injection, the Abeta was incubated at 37°C during 4 days to induce Abeta 25-35 aggregation (Ghasemi et al., 2014).

2.4 Stereotaxic surgery

The stereotaxic surgeries for intrahippocampal injection of 2 µl Abeta 25-35 or vehicle (saline) were performed after initial interventions. 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 - 3.0 mm) (Paxinos and Watson, 1986). 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 induct the aggregation of Abeta protein in rats' hippocampi (Zussy et al., 2011).

2.5 Control behavioral tasks

To analyze exploratory and locomotor activities and ensuring that any procedure impaired such behaviors, altering the memory tests results, 10 days after surgery rats were placed on the left quadrant of a 50 x 50 x 50 cm open field made with wooden pained white. Black lines were drawn on the floor to divide it into 12 equal quadrants. Crossing and rearing, as measures for locomotor activity and exploration, respectively, were measured over 5 min (Bonini et al., 2006). To evaluate anxiety state rats were exposed to an elevated plus maze (Pellow et al., 1985). The time spent and the number of entries into the open arms were recorded over a 5 min session.

2.6 Memory behavioral testing

2.6.1 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 pained 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 explore them freely 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 3h and 24h, in the short-term memory (STM) and long-term memory (LTM) test session, 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 object and the arena were cleaned after testing each animal with 70% ethanol. The time spent exploring the familiar and the novel object was recorded in video.

2.6.2 Social recognition memory test (SR)

This task is one adaptation of the social interaction test proposed by Kaidanovich et al. (2011). The task was completed in three days. In 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). In the second day, training was performed with inclusion of one unfamiliar rat in the cages for 1 hour of free exploration. After 24 hours, testing was performed when the same rat of training (familiar rat) and a new rat were placed for exploration during 5 minutes. The time spent exploring the new and familiar rat were recorded. Exploration was defined as sniffing or touching the small cages with the nose and/or forepaws.

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. Afterwards, samples were centrifuged at 2400g for 20 min, and supernatants (S1) were used for assay.

2.7.2 Reactive species (RS) levels

RS content was assessed by a spectrofluorimetric method using 20,70-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 RS, specifically reactive oxygen species (ROS) were analyzed. The formation of the oxidized fluorescent derivative (DCF), measured by DCF fluorescence intensity, was recorded at 520 nm (480 nm excitation) 30min after the addition of DCFH-DA to the medium. Results were expressed as AU (arbitrary units).

2.7.3 Detection of lipid peroxidation (TBARS)

Lipoperoxidation was evaluated by the thiobarbituric acid reactive substance (TBARS) test (Ohkawa et al., 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 95ºC for 2 h, and the color reaction was measured at 532 nm. Results were expressed as nmol of malondialdehyde (MDA) per mg protein.

2.7.4 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 homogenate was added in the 300 μL working FRAP reagent in microplate (Benzie and Strain, 1996). Additionally, also

was 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.

2.7.5 Acetylcholinesterase (AchE) activity

AChE is a marker of the loss of cholinergic neurons in the forebrain. The AChE activity was assessed by the Ellman method (Ellman, 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 on the 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.

2.8 Statistical analysis

Data are reported as mean ± SEM. Normality of data distribution was checked using Shapiro-Wilk test. Objects' exploration time in OR and rats' exploration time in SR were converted to percent of total exploration time, and a one-sample t-test was used to compare the percent of total time of exploration spent in each object or rat considering a theoretical default mean of 50%. In OF and PM tests data of all groups were compared using ANOVA one-way. Biochemical results of all groups were compared using Kruskall-Wallis followed by specific t-tests. Significance level was set at 0.05.

3 Results

3.1 Control behavioral results

Locomotor and exploratory behaviors, as anxiety behavior, were not influenced by the procedures (Table1).

Behavioral tasks		Control				Abeta			
		Cont	**EE**	**AnPE**	**SE**	**Abeta**	**EE**	**AnPE**	**SE**
Exploration time in OR	Total exploration time in training (s)	43.73 (8.30)	38.91 (15.67)	42.16 (17.09)	32.36 (16.81)	41.33 (22.86)	36.05 (12.58)	41.21 (16.62)	39.71 (15.79)
	Total exploration time in STM test (s)	42.16 (17.09)	35.64 (18.05)	40.27 (25.46)	32.99 (12.85)	42.09 (13.68)	42.81 (23.84)	41.49 (14.02)	33.63 (11.70)
	Total exploration time in LTM test (s)	38.91 (15.67)	36.53 (14.24)	42.84 (15.61)	34.34 (11.99)	39.62 (10.55)	35.36 (8.82)	36.81 (12.31)	35.73 (5.78)
Exploration time in SR	Total exploration time in SR test (s)	96.21 (22.21)	93.73 (25.06)	81.90 (16.53)	86.94 (26.41)	83.10 (34.64)	85.03 (11.15)	84.86 (31.90)	98.93 (25.80)
Open field	Crossings (n)	45.00 (7.59)	45.00 (7.59)	35.21 (3.36)	34.75 (3.77)	32.88 (3.36)	45.43 (4.49)	36.79 (3.88)	49.22 (5.54)

	Rearing (n)	15.50 (2.51)	15.50 (2.51)	15.57 (1.49)	12.33 (2.02)	15.53 (2.71)	17.80 (2.38)	14.64 (1.40)	15.78 (3.20)
Plus maze	Total entries (n)	22.46 (8.10)	23.38 (7.14)	20.47 (9.18)	22.84 (9.05)	19.42 (11.12)	22.34 (9.14)	21.57 (8.17)	24.84 (12.08)
	Time in open arms (s)	25.82 (22.05)	27.09 (18.37)	23.64 (22.01)	27.56 (26.35)	27.03 (21.78)	23.14 (19.18)	26.93 (23.93)	21.41 (11.31)

Table 1. Control behavioral tests results. Abeta, EE, AnPE and SE did not alter the total exploration time on OR and SR training and testing, locomotor and exploratory activities on Open Field, and anxiety behavior evaluated by Pluz Maze ($P > 0.05$; One-way ANOVA; data expressed as mean ± SEM; n = 10-20 per group).

3.2 Object recognition memory

In training session all rats explored each object (A and B) for the a similar percentage of total exploration time (mean of all groups: object A = 50.60 ± 10.02%; B = 49.40 ± 10.02%; P = 0.55; $t_{(94)}$ = 0.58; Fig. 2 tr). Control group did not show deficits in STM OR testing (P = 0.02; $t_{(13)}$ = 2.57; Fig. 2A/Test/cont). Animals submitted to EE, AnPE and SE also did not show deficits in STM OR testing (P < 0.05, Fig. 2A/Test). Abeta rats presented deficits in short-term OR memory, since they spent a similar time exploring the familiar and the novel object (P = 0.60; $t_{(13)}$ = 0.52; Fig. 2A/Test/Abeta). EE and AnPE avoided STM OR deficit in Abeta rats (P = 0.04, $t_{(13)}$ = 2.18 for Abeta+EE; P = 0.009, $t_{(13)}$ = 3.05 for Abeta+AnPE, Fig 2A/test). SE did not avoid STM deficit in Abeta rats (P = 0.28, $t_{(8)}$ = 1.14, Fig 2A/test/Abeta+SE).

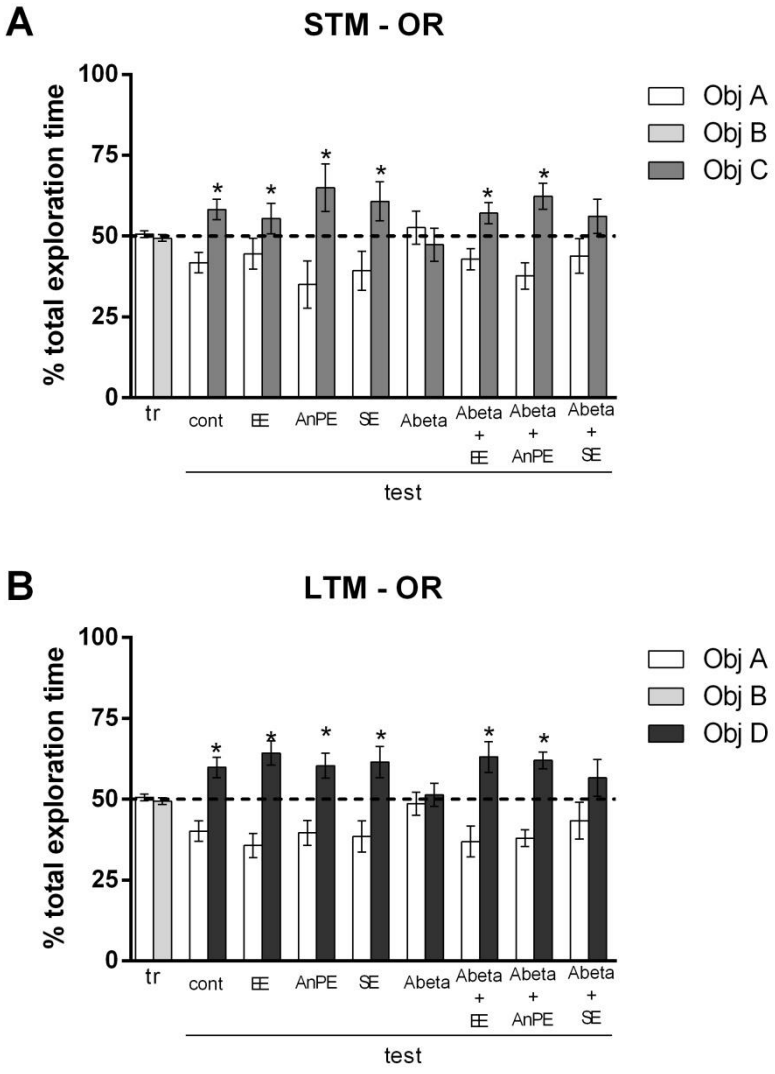

Figure 2. Amyloid beta impairs short (A) and long-term (B) object recognition memory. Environmental enrich
ment (EE) and anaerobic physical exercise (AnPE) for 8 weeks prevents OR memory deficits; social enrichment (SE) does not. Data as present as mean ± SEM of the percent of the total exploration time. *P < 0.05 in one-sample t-test, considering a theoretical mean of 50%; n = 8-14 per group Tr: training; Cont: Control. EE: Environmental enrichment; AnPE: Anaerobic exercise; SE: Social enrichment; Abeta: Amyloid beta.

In LTM test, control group did not show deficits in OR testing (P = 0.009; $t_{(12)}$ = 3.09; Fig. 2B/Test/cont). Animals submitted to EE, AnPE and SE also did not show deficits in STM OR testing (P < 0.05, Fig. 2B/Test). Abeta rats presented deficits in long-term OR memory, since they spent a similar time exploring the familiar and the novel object (P = 0.71; $t_{(13)}$ = 0.37; Fig. 2B/Test/Abeta). EE and AnPE avoided LTM OR deficit in Abeta rats (P = 0.01, $t_{(13)}$ = 2.74 for Abeta+EE; P = 0.0005, $t_{(13)}$ = 4.56 for Abeta+AnPE, Fig 2B/test). SE did not avoid LTM deficit in Abeta rats (P = 0.27, $t_{(8)}$ = 1.16, Fig 2B/test/Abeta+SE).

There are no differences between groups in the total time of exploration on training and testing sessions (table 1).

3.3 Social recognition memory

Control rats explored the new rat for a longer time than the familiar one (P < 0.001; $t_{(16)}$ = 10.23; Fig. 3/Sham). Animals submitted to EE, AnPE and SE presented the same behavior (P < 0.05; Fig. 3). Abeta rats showed impaired social recognition memory, exploring for a similar time the familiar and the new rat (P = 0.07, $t_{(11)}$ = 1.93, Fig. 3/Abeta). EE, AnPE and SE preserved social recognition memory in Abeta rats (P = 0.004, $t_{(12)}$ = 3.52 for Abeta+EE; P = 0.003, $t_{(8)}$ = 4.00 for Abeta+AnPE; and, P = 0.001, $t_{(7)}$ = 4.83 for Abeta+SE; Fig. 3).

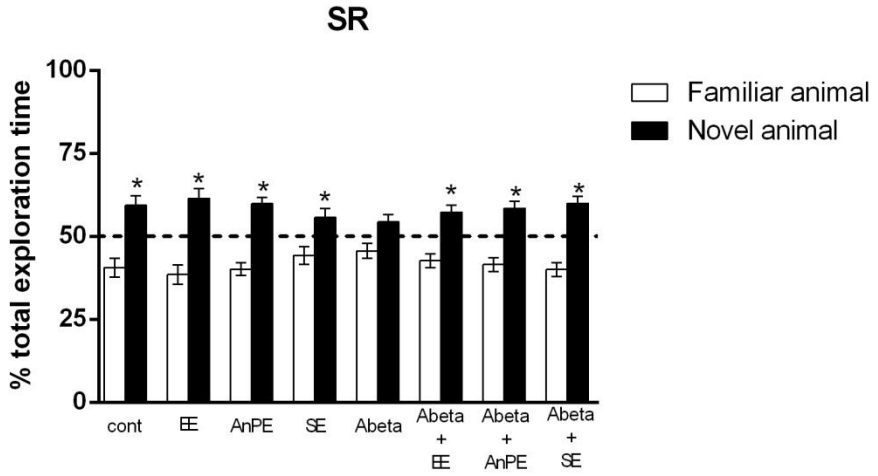

Figure 3. Amyloid beta impairs social recognition memory. Environmental enrichment (EE), anaerobic physical exercise (AnPE) and social enrichment (SE) for 8 weeks prevents SR memory deficits. Data as present as mean ± SEM of the percent of the total exploration time. *P < 0.05 in one-sample t-test, considering a theoretical mean of 50%; n = 8-13 per group Tr: training; Cont: Control. EE: Environmental enrichment; AnPE: Anaerobic exercise; SE: Social enrichment; Abeta: Amyloid beta.

3.4 Hippocampal oxidative status

No significant differences in hippocampal ROS levels were observed between groups ($P = 0.11$, $H_{(8)} = 11.64$; Fig. 4A).

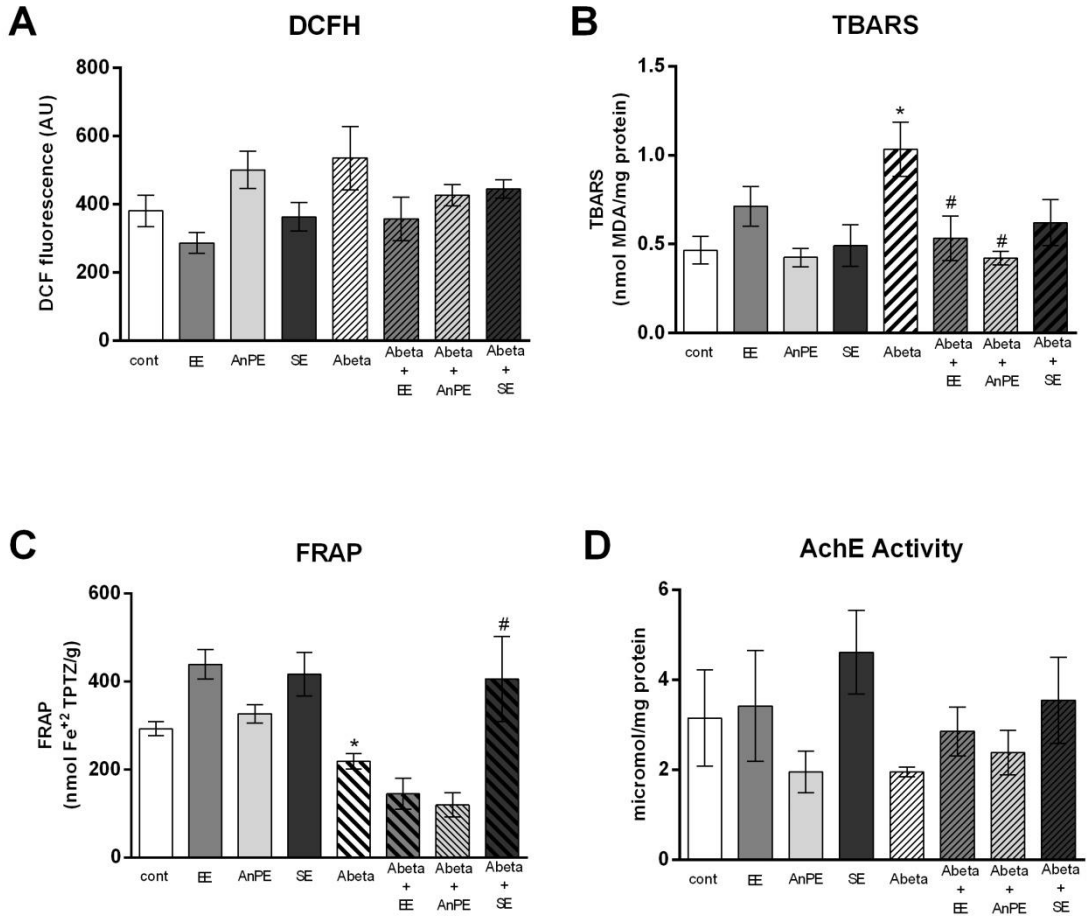

Figure 4. Effects of Abeta and interventions on hippocampal oxidative status and acetylcholinesterase activity. A. Reactive oxygen species (ROS) by DCFH. B. Lipid peroxidation by TBARS (Thiobarbituric Acid Reactive Substance). C. Total antioxidant capacity by FRAP (Ferric reducing/antioxidant power). D. Acetylcholinesterase (AchE) activity. Data as present as mean ± SEM. *P < 0.05, control vs. Abeta; #P < 0.05 Abeta vs. Abeta + specific intervention; Kruskall-Wallis following by t-tests (n = 5-8/group). Cont: Control. EE: Environmental enrichment; AnPE: Anaerobic exercise; SE: Social enrichment; Abeta: Amyloid beta.

A main effect for the treatment was observed in hippocampal lipid peroxidation (TBARS) ($P = 0.03$, $H_{(8)} = 15.20$; Fig. 4B). Abeta rats showed higher lipid peroxidation than control rats ($P = 0.004$; Fig. 4B). Abeta rats submitted to EE and AnPE presented lower lipid peroxidation than Abeta not submitted to any intervention ($P = 0.02$ for EE; $P = 0.01$ for AnE; Fig. 4B).

A main effect for the treatment was observed in hippocampal total antioxidant capacity (FRAP) ($P < 0.0001$, $H_{(8)} = 36.42$; Fig. 4C). Abeta group showed lower total antioxidant

capacity than sham (P = 0.02; Fig. 4C). Abeta rats submitted to SE presented higher antioxidant capacity than Abeta not submitted to any intervention (P = 0.02; Fig. 4C).

3.5 AchE Activity

No significant differences on AchE activity was observed between groups (P = 0.22; $H_{(8)}$ = 9.43; Fig. 4D)

4. Discussion

Here we investigated the effects of EE, AnPE and SE concerning their neuroprotective potential to prevent or minimize memory impairments and hippocampus oxidative stress related to Abeta neurotoxicity. Our results demonstrated that EE and AnPE avoid impairments in object recognition memory (STM and LTM), while social recognition memory deficits were prevented by all interventions, including SE. So, EE and AnPE seem to be more effective in memory protection than SE.

There is previous evidence showing that EE could enhance synaptic plasticity, attenuating cognitive deficits in rodents (Sakalem et al., 2017). Among the several changes caused by EE in the central nervous system, increasing in neurogenesis, neutrophins levels, neuronal survival, synaptogenesis, cellular proliferation, and dendritic arborization in different brain regions were between the benefits previously observed in the brain of animals exposed to EE (Van Praag et al., 2000; Rossi et al., 2006; Kempermann et al., 1997; Mohammed et al., 2002; Faherty et al., 2003). Additionally, a reduction of brain oxidative stress by EE was described in a mice genetic model of AD (Herring et al., 2010). However, how much of these effects observed using EE protocols are related to EE per see, and how much are related to physical exercise and increase of social interaction (social enrichment) associated to these protocols remains poor described.

Recently, investigating the effects of physical and social components of EE after brain ischemia-reperfusion, Chen and cols. (2017) showed that both physical and social enrichment reduce the brain lesion (infarct volume) and increase the astrocytes proliferation and BDNF expression, but their data suggest that physical activity component of EE is the most important factor, being related with positive results in functional, biochemical and histological evaluation. On the other hand, Birch e cols. (2013), demonstrated that EE, in absence of exercise, can increase synaptogenesis

and improve memory in normal rats, and indicated that probably there is a time-dependent cognitive-enhancing effect of EE that is independent of physical activity. We have not found studies that evaluated the effects of each compound of EE in recognition memory and brain oxidative status. Additionally, although there are significant amount of studies relating positive effects of EE on AD, we did not find studies isolating EE, SE and physical exercise effects on models of this disease.

In AD brain, the imbalance of homeostasis leads to the increase of ROS and lipid peroxidation, leading to oxidative stress and degeneration of cholinergic nervous system, which result in impairments of cognition and memory (Chauhan and Chauhan, 2006; Zhu et al., 2007; Melo et. al., 2003). The oxidative damages observed in the hippocampus support the interpretation that oxidative stress in the brain of patients with Alzheimer's disease are related to Abeta deposition and cognitive deficits observed (Butterfield and Lauderback, 2002; Lovell and Markesbery, 2001; Smith et al., 1997; Chauhan and Chauhan, 2006; Mariani et al., 2005; Migliore et al., 2005), so interventions that act avoiding or reducing oxidative stress or increasing antioxidant defenses are an interesting strategy to use in AD/Abeta neurotoxicity. Our results show that AnPE and EE (EE was performed without increasing in physical exercise or socialization levels) can avoid increase on lipid peroxidation observed in Abeta rats' hippocampus. Alterations in ROS were not observed in any group of animals, maybe because their half-life is short. In parallel, these interventions protected all memory parameters evaluated. Probably, this memory protection are related to other protective factors, since EE and AnPE can increase brain neuroplasticity (Neidi et al, 2016; Birch et al., 2013; During and Cao, 2006) but certainly, the avoiding of oxidative damage collaborate to the observed results.

Previous studies have clearly indicating that enhanced level of acetylcholine (ACh) leads to functional improvement of central cholinergic synapses and protection of neuronal degeneration (Wisniewski, 2014), yet elevation of ACh achieved by inhibiting AChE, could improve the cholinergic dysfunction of AD (Yang et. al., 2013). Furthermore, some factors can induce brain impairment by influencing the synthesis, release, and uptake of ACh (Liu et. al., 2013), which may be suggest of neuroprotection caused by the interventions.

One could argue that there are more evidences in the literature regarding aerobic physical exercise neuroprotection, and it is true. Although there is a lack of consensus

regarding physical activities associated with AD prevention or improvement, aerobic balanced training of moderate to severe intensities are considered optimal, and the regular practice of walking, for example, improves cognition in AD (Hill et al., 2009; Venturelli et al, 2011; Winchester et al., 2013; Suttanon et al., 2013; Hurley et al., 2011). On the other hand, strength training (an anaerobic physical exercise) seems to be particularly more effective improving postural and motor function (Granacher et al, 2006; Malling and Jensen, 2015). Here we choose to use anaerobic exercise because we consider that EE protocols used in animal models include more anaerobic exercise (animals climbing ramps and stairs, passing by tunnels, and others) than aerobic continuous training. Yet, instead anaerobic exercise effects on cognition were little investigated, it would be interested if this type of exercise bring neuroprotective effects, since AD are more common in elderly, an age in which strength training are necessary to promote better muscular function, avoiding falls and ensuring better balance and posture (Hurley and Hagberg, 1998; Rubenstein et al., 2000).

Unlike EE and AnPE, in our experimental conditions SE was able only to prevent social recognition memory deficits, and not the object recognition deficits. In humans, an increase in social engagement with the surrounding environment can be correlated with angiogenesis, synaptogenesis, and neurogenesis increasing, important factors for delaying the development of AD and cognitive dysfunctions (Fratiglioni et al., 2004), but a recent study in rats demonstrate that SE had only minor effects on neuroplasticity and cognition (Brenes et al., 2016). On the prevention of AD, including animal research, there is a lack of researches on the effects of social engagement on AD prognosis. Our results indicated that SE memory protection was not so effective as EE and AnPE; it could be related to biochemical observations, considering that SE did not avoid oxidative damage. On the other hand, SE increases total antioxidant capacity, what, despite can be considered a good effect, seems to be not so effective, since the oxidative damage was already present in the hippocampus of these animals. Yet, it is important observe that SE animals present neuroprotection on social recognition memory. It could be attributed to social training, since these animals were habituated to socialize with new rats and Brenes and cols. (2016) demonstrated that SE had positive effects on social behavior.

In conclusion, our results show that Abeta injection resulted in oxidative and memory damage. EE and AnPE avoided all memory deficits (OR and SR) and lipid peroxidation (TBARS) induced by Abeta. SE avoided only the SR memory deficits and antioxidant

capacity (FRAP) decrease induced by Abeta. So, we can resume that environmental and physical training components of EE protocols may be more important than social enrichment in neuroprotection of Abeta neurotoxicity.

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References

Adlard PA, Perreau VM, Pop V, Cotman CW: Voluntary Exercise Decreases Amyloid Load in a Transgenic Model of Alzheimer's Disease. Journal of Neuroscience. 2005; 25(17):4217-4221.

Barone R, Bellafiore M, Leonardi V, Zummo G: Structural analysis of rat patellar tendon in response to resistance and endurance training. Scand J Med Sci Sports. 2009; 19(6):782-9.

Benzie IF, Strain JJ: The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical biochemistry.1996; 239(1):70-6.

Birch AM, McGarry NB, Kelly AM: Short-term environmental enrichment, in the absence of exercise, improves memory, and increases NGF concentration, early neuronal survival, and synaptogenesis in the dentate gyrus in a time-dependent manner. Hippocampus. 2013; 23(6):437-50.

Bonini JS et al.: Angiotensin II disrupts inhibitory avoidance memory retrieval. Hormones and Behavior. 2006; 50:308-313.

Bouet V, Freret T, Dutar P, Billard JM, Boulouard M: Continuous enriched environment improves learning and memory in adult NMRI mice through theta burst-related-LTP independent mechanisms but is not efficient in advanced aged animals. Mech Ageing Dev. 2011; 132(5):240-8.

Bradford MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal. Biochem. 1976; 72:248-254.

Brenes JC, Lackinger M, Höglinger GU, Schratt G, Schwarting RK, Wöhr M: Differential effects of social and physical environmental enrichment on brain plasticity, cognition, and ultrasonic communication in rats. J Comp Neurol. 2016; 524(8):1586-607.

Butterfield DA, Lauderback CM: Lipid peroxidation and protein oxidation in Alzheimer's disease brain: potential causes and consequences involving amyloid beta-peptideassociated free radical oxidative stress. Free Radic. Biol. Med. 2002; 32(11):1050-1060.

Butterfield DA, Reed T, Newman SF, Sultana R: Roles of amyloid beta-peptideassociated oxidative stress and brain protein modifications in the pathogenesis of Alzheimer's disease and mild cognitive impairment. Free Radic. Biol. Med. 2007; 658–677.

Butterfield DA, Swomley AM, Sultana S: Amyloid β-peptide (1–42)-induced oxidative stress in Alzheimer disease: importance in disease pathogenesis and progression. Antioxid Redox Signal. 2013; 19:823–835.

Chakrabarti S, Khemka VK, Banerjee A, Chatterjee G, Ganguly A, Biswas A. Metabolic risk factors of sporadic Alzheimer's disease: implications in the pathology, pathogenesis and treatment. Aging Dis. 2015;6(4):282–299.

Chakrabarti S, Sinha M, Thakurta IG, Banerjee P, Chattopadhyay M: Oxidative stress and amyloid beta toxicity in Alzheimer's disease: intervention in a complex relationship by antioxidants. Curr Med Chem. 2013; 20(37):4648–4664.

Chauhan V; Chauhan A: Oxidative stress in alzheimer's disease. Pathophysiology. 2006; 13(3):195-208.

Chen X, Zhang X, Liao W, Wan Q: Effect of Physical and Social Components of Enriched Environment on Astrocytes Proliferation in Rats After Cerebral Ischemia/Reperfusion Injury. Neurochem Res. 2017; 42(5):1308-1316.

Clark CM, Ewbank D, Lee VM-Y, Trojanowski JQ: Molecular pathology of Alzheimer's disease: neuronal cytoskeletal abnormalities. In: Growdon JH, Rossor MN, eds. The dementias. Vol. 19 of Blue books of practical neurology. Boston: Butterworth–Heinemann, 1998: 285-304.

De Ferrari GV, Canales MA, Shin I, Weiner L.M, Silman I, Inestrosa N.C: A structural motif of acetylcholinesterase that promotes amyloid beta-peptide fibril formation. Biochemistry. 2001;40:10447–10457.

Dong S, Duan Y, Hu Y, Zhao Z: Advances in the pathogenesis of Alzheimer's disease: a re-evaluation of amyloid cascade hypothesis. Transl Neurodegener. 2012; 1(1):18.

During MJ, Cao L: VEGF, a mediator of the effect of experience on hippocampal neurogenesis. Curr Alzheimer Res. 2006; 3(1):29-33.

Ellman GL, Courtney KD, Andres V, Featherstone RM: A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol.1961; 7:88–95

Ennaceur A, Delacour J: A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. Behavioural Brain Research. 1988; 31:47-59.

Faherty C, Kerley D, et. al.: A Golgi-cox morphological analysis of netironal changes induced by environmental enrichment. Developmental Brain Research. 2003; 141(1-2):55-61.

Fiala, B, Snow FM, Greenough WT: "Improverished" rats weigh more than "enriched" rats because they eat more. Developmental Psychobiology, 1997; 10(6):537-541.

Fratiglioni L, Paillard-Borg S, Winblad B: "An active and socially integrated lifestyle in late life might protect against dementia," The Lancet Neurology. 2004; 3(6):343–353.

Garland TJr, Schutz H, Chappell MA, Keeney BK, Meek TH, Copes LE, et al.: The biological control of voluntary exercise, spontaneous physical activity and daily energy expenditure in relation to obesity: Human and rodent perspectives. J Exp Biol. 2011; 15(214(Pt 2)):206–29.

Ghasemi R, Zarifkar A, Rastegar K, Maghsoudi N, Moosavi M. Repeated intra-hippocampal injection of beta-amyloid 25-35 induces a reproducible impairment of learning and memory: considering caspase-3 and MAPKs activity. European journal of pharmacology. 2014; 726:33-40.

Granacher U, Gollhofer A, Strass D: Training induced adaptations in characteristics of postural reflexes in elderly men. Gait Posture. 2006; 24(4):459-66.

Hase Y, Craggs L, Hase M, Stevenson W, Slade J, Lopez D, Mehta R, Chen A, Liang D, Oakley A, Ihara M, Horsburgh K, Kalaria RN: Effects of environmental enrichment on white matter glial responses in a mouse model of chronic cerebral hypoperfusion. Journal of Neuroinflammation. 2017; 14:81.

Herring A, Blome M, Ambrée O, Sachser N, Paulus W, Keyvani K: Reduction of cerebral oxidative stress following environmental enrichment in mice with Alzheimer-like pathology. Brain Pathol. 2010; 20(1):166-75.

Hill KD, LoGiudice D, Lautenschlager NT, Said CM, Dodd KJ, Suttanon P: Effectiveness of balance training exercise in people with mild to moderate severity Alzheimer's disease: Protocol for a randomised trial. 2009; 9:29.

Hurley, BF, Hagberg, JM: Optimizing health in older persons: aerobic or strength training? Exercise and Sports Sciences Reviews. 1998;26: 61-89.

Hurley BF, Hanson ED and Sheaff AK: Strength training as a countermeasure to aging muscle and chronic disease. Sports Med. 2011; 41:289-306.

Ishrat T, Hoda MN., Khan MB, et al.: Amelioration of cognitive deficits and neurodegeneration by curcumin in rat model of sporadic dementia of Alzheimer's type (SDAT) *European Neuropsychopharmacology*. 2009; 19:636–647.

Kadowaki H, Nishitoh H, Urano F, et al.: Amyloid β induces neuronal cell death through ROS-mediated ASK1 activation. Cell Death Differ. 2005; 12(1):19–24.

Kaidanovich-Beilin O, Lipina T, Vukobradovic I, Roder J, Woodgett JR: Assessment of social interaction behaviors. Journal of visualized experiments : JoVE. 2011(48).

Kempermann G, Kuhn HG, et al.: More hippocampal neurons in adult mice living in an environmental enrichment. Nature. 1997; 386(6624:493-495.

Leger M, Bouet V, Freret T, Darmaillacq AS, Dacher M, Dauphin F, Boulouard M, Schumann-Bard P: Environmental enrichment improves recent but not remote memory in association with a modified brain metabolic activation profile in adult mice. Behav Brain Res. 2012; 228(1):22-9.

Liu T, Xia Z, Zhang WW, et al: Bis(9)-(-)-nor-meptazinol as a novel dual-binding AChEI potently ameliorates scopolamine-induced cognitive deficits in mice. *Pharmacology Biochemistry and Behavior.* 2013; 104(1):138–143.

Loetchutinat C, Kothan S, Dechsupa S, Meesungnoen J, Jay-Gerin J, Mankhetkorn S: 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. 2005; 72(2-3):323-31.

Lovell MA, Markesbery WR: Ratio of 8-hydroxyguanine in intact DNA to free 8-hydroxyguanine is increased in Alzheimer disease ventricular cerebrospinal fluid. Arch. Neurol. 2001; 8(3):392-396.

Malling AS, Jensen BR: Motor intensive anti-gravity training improves performance in dynamic balance related tasks in persons with Parkinson's disease. Gait Posture. 2016; 43:141-7.

Mariani E, Polidori MC, Cherubini A, Mecocci P: Oxidative stress in brain aging, neurodegenerative and vascular diseases: An overview. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 2005; 827(1):65-75.

McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM: Clinical diagnosis of Alzheimer’s disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer’s Disease. Neurology 1984; 34:939-44.

Melo JB, Agostinho P, Oliveira CR: Involvement of oxidative stress in the enhancement of acetylcholinesterase activity induced by amyloid beta-peptide. *Neuroscience Research*. 2003; 45:117–127.
Mesulam MM: Alzheimer plaques and cortical cholinergic innervation. Neuroscience. 1986;17:275–276.

Migliore L, Fontana I, Colognato R, Coppede F, Siciliano G, Murri L: Searching for the role and the most suitable biomarkers of oxidative stress in Alzheimer's disease and in other neurodegenerative diseases. Neurobiol. Aging. 2005; 26(5):587-595.

Mohammed AH, Zhu SW, et. al.: Environmental enrichment in brain. Plasticity in the adult brain: from genes to neurotherapy. 2002; 138:109-133.

Neidi R, Schneider A, Bousiges O, Majchrzak M, Barbelivien A, Vasconcelos AP, Dorgans K, Doussau F, Loeffler JP, Cassel JC, Boutillier ALJ: Late-Life Environmental Enrichment Induces Acetylation Events and Nuclear Factor κB-Dependent Regulations in the Hippocampus of Aged Rats Showing Improved Plasticity and Learning. J Neurosci. 2016; 13;36(15):4351-61.

Newberry RC: Environmental Enrichment: Increasing the biological relevance of captive environments. Applied Animal Behaviour Science. 1995; 44(4):229-243.

Nussbaum RL, Ellis CE: Alzheimer's disease and Parkinson's disease. N Engl J Med. 2003; 348(14):1356-1364.

Ohkawa K: Promotion of renewal canes in greenhouse roses by 6-benzyl aminopurine without cutback HortScience. 1979; 5:612-613.

Pascual R et al.: Effects of postweaning social isolation and re-socialization on the expression of vasoactive intestinal peptide (VIP) and dentritic development in the medial prefrontal cortex of the rat. Acta Neurobiol Exp. 2006; 66:7-14.

Pellow S, Chopin P, File SE, Briley: Validation of open : closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. Journal of Neuroscience Methods. 1985; 14:149-167.

Polidori MC, Nelles G: Antioxidant clinical trials in mild cognitive impairment and Alzheimer's disease - challenges and perspectives, Curr. Pharm. Des. 2014; 3083–3092.

Quattromani MJ, Cordeau P, Ruscher K, Kriz J, Wieloch T: Enriched housing down-regulates the Toll-like receptor 2 response in the mouse brain after experimental stroke. Neurobiol Dis. 2014; 66:66–73

Racchi M, Sironi M, Caprera A, Konig G, Govoni S: Shortand long-term effect of acetylcholinesterase inhibition on the expression and metabolism of the amyloid precursor protein. Mol. Psychiatry. 2001; 6:520–528.

Rossi C, Angelucci A, et. al.: Brain-derived neurotrophic fator (BDNF) is required for the enchancement of hippocampal neurogenesis following environmental enrichment> European Journal of Neuroscience. V. 2006; 24(7):1850-1856.

Rubenstein LZ, Josephson KR, Trueblood PR et al.: Effect of a Group Exercise Program on Strength, Mobility, and Falls Among Fall-Prone Elderly Men. J Gerontol Med Sci. 2000;55:317-21.

Sakalem ME, Seidenbecher T, Zhang M, Saffari R, Kravchenko M, Wordemann S, Diederich K, Schwamborn JC, Zhang W, Ambree O: Environmental enrichment and physical exercise revert behavioral and electrophysiological impairments caused by reduced adult neurogenesis. Hippocampus. 2017; 27:36–51.

Seo TB, Cho HS, Shin MS, Kim CJ, Ji ES, Baek SS: Treadmill exercise improves behavioral outcomes and spatial learning memory through up-regulation of reelin signaling pathway in autistic rats. J Exerc Rehabil. 2013; 9:220–9.

Shea TB and Rogers E: Has prenatal folate supplementation established an at-risk population forage-related cognitive decline? J. Alzheimers Dis. 2014; 41:667–669.

Smith MA, Richey Harris PL, Sayre LM, Beckman JS, Perry G: Widespread peroxynitrite-mediated damage in Alzheimer’s disease. J. Neurosci. 1997; 17(8):2653-2657.

Stuart KE, King AE, Fernandez-Martos CM, Dittmann J, Summers MJ, Vickers JC: Mid-life environmental enrichment increases synaptic density in CA1 in a mouse model of Aβ-associated pathology and positively influences synaptic and cognitive health in healthy ageing. J Comp Neurol. 2017; 525(8):1797-1810.

Suttanon P, Hill KD, Said CM, Williams SB, Byrne KN, LoGiudice D, Lautenschlager NT and Dodd KJ: Feasibility, safety and preliminary evidence of the effectiveness of a home-based exercise programme for older people with Alzheimer's disease: A pilot randomized controlled trial. Clin Rehabil. 2013; 27:427-438.

Van Praag H, Kempermann G, et. al.: Neural consequences of environmental enrichment. Nature Reviews Neuroscience. 2000; 1(3):191-198.

Venturelli M, Scarsini R, Schena F: Six-month walking program changes cognitive and ADL performance in patients with Alzheimer. Am J Alzheimers Dis Other Demen. 2011; 26:381-388.

Vivinetto, AL, Suárez MM, Rivarola MA: Neurobiological effects of neonatal maternal separation and post-weaning environmental enrichment. Behavioural Brain Research. 2013; 240:110–118.

Winchester J, Dick MB, Gillen D, Reed B, Miller B, Tinklenberg J, Mungas D, Chui H, Galasko D, Hewett L, et al: Walking stabilizes cognitive functioning in Alzheimer's disease (AD) across one year. Arch Gerontol Geriat. 2013; (56):96-103.

Wisniewski T, Goñi F: Immunotherapeutic approaches for Alzheimer's Disease. *Neuron*. 2015; 85:1162–1176.

Xu H, Gelvana E, Rajsombath M, Yang T, Li S, Selkoe D: Environmental Enrichment Potently Prevents Microglia-Mediated Neuroinflammation by Human Amyloid B Protein Oligomers. The Journal of Neuroscience, 2016; 36(35):9041–9056.

Yang WN, Han H, Hu XD, Feng GF, Qian YH: The effects of perindopril on cognitive impairment induced by d-galactose and aluminum trichloride via inhibition of

acetylcholinesterase activity and oxidative stress. *Pharmacology Biochemistry and Behavior.* 2013; 114-115:31–36.

Zussy C, Brureau A, Delair B, Marchal S, Keller E, Ixart G, Naert G, Meunier J, Chevallier N, Maurice T, Givalois L; Time-course and regional analyses of the physiopathological changes induced after cerebral injection of an amyloid beta fragment in rats. American Journal of Pathology. 2011; 179:315-334.

Zhu X, Lee H, Perry G, Smith MA: Alzheimer disease, the two-hit hypothesis: An update. Biochim. Biophys. Acta. 2007; 4:494-502.

PARTE III

5. Discussão

Os experimentos propostos nesta dissertação buscaram comparar diferentes intervenções utilizando o modelo animal de doença de Alzheimer (DA), investigando as suas capacidades neuroprotetoras e consequente redução dos danos causados na memória e estresse oxidativo devido à neurotoxicidade induzida pela βA.

Os resultados deste estudo demonstraram que o enriquecimento ambiental (EA) e o exercício físico anaeróbico foram capazes de evitar prejuízos na memória de reconhecimento de objetos (RO), tanto em memória de curta duração como na memória de longa duração, bem como na memória de reconhecimento social (RS), evitando o aumento da peroxidação lipídica hipocampal (TBARS). Por outro lado, o enriquecimento social (ou socialização) protegeu somente a memória de reconhecimento social, evitando a diminuição da capacidade antioxidante total no hipocampo.

Estudos prévios comprovaram que o ambiente enriquecido, é capaz de aumentar as taxas de neurogênese (GOULD et al., 1999; SHORS et al., 2001; TASHIRO et al., 2007; DUPRET et al., 2008), e promover um pronunciado efeito positivo tanto na sobrevivência de novas células (VAN PRAAG et al., 2000) quanto na sua proliferação (FABEL & KEMPERMANN, 2008; FABEL et al., 2009). Estudos também evidenciaram que o EA promove o aumento de fatores neurotróficos, colaborando assim na redução dos déficits de memória relacionados ao envelhecimento (KEMPERMANN et al., 2002; MORA, 2013), além de atenuar o estresse oxidativo (SNOW et. al., 2015).

No entanto, o protocolo de EA utilizado nos estudos com modelos animais geralmente abrange não somente o ambiente enriquecido, como também promove uma maior interação social, por incluir maior número de animais do que costuma ser incluído nas caixas moradias de ratos de laboratório, bem como um maior nível de atividade física, por incluir, frequentemente, a roda de

atividades, na qual o animal realiza corrida voluntária, além de túneis e estadas para o animal subir (VIVINETTO et. al., 2013; FIALA et. al., 1997; NEWBERRY, 1995).

Em nosso estudo procuramos isolar os efeitos da socialização, do EA e do exercício físico, de modo que não incluímos roda de atividades em nossas caixas de EA, e o número de animais por caixas era igual para os animais do grupo EA e do grupo controle, mantidos em caixas não enriquecidas. Esta preocupação foi tomada no intuito de tentar isolar os efeitos da cada intervenção, considerando que o EA, a socialização e o exercício físico são propostas diferentes, especialmente para os seres humanos, e é importante conhecer os potenciais terapêuticos de cada uma destas intervenções.

Birch et al. (2013) realizaram uma intervenção com EA sem exercício físico em ratos normais e verificaram melhora da memória e aumento da sinaptogenese. Já Chen e colaboradores (2017) investigaram os efeitos protetores dos componentes social e físico do EA em um modelo de isquemia-reperfusão. Os autores verificaram que ambos os componentes estavam relacionados à diminuição da área de lesão, e aumento de BDNF, no entanto, sugeriram que o componente atividade física é o componente mais importante para garantia dos resultados positivos do EA.

Embora haja muitas referências aos efeitos do exercício físico sobre a cognição, a maioria da literatura documentada evidencia os efeitos do exercício aeróbico, especialmente no tipo de demência estudada neste trabalho. De fato, pessoas que possuem baixos percentis de atividade física são mais propensas a desenvolver demência do que aqueles que praticam com mais frequência (BUCHMAN et al., 2012), informação que corrobora com outro estudo que demonstrou que pessoas que se exercitam três ou mais vezes durante a semana tem menor risco de desenvolver demência, quando comparado aquelas que não realizam a prática (LARSON et. al., 2006). Ainda, Lautenschlager et al. (2008) demonstraram que sujeitos que se exercitaram por seis meses ou mais apresentaram melhorias na cognição.

Por mais que a maioria dos estudos com idosos com e sem DA se detenha em estudar o exercício aeróbico, o exercício anaeróbico, como o treino de força e flexibilidade, mostra-se benéfico em pacientes com DA e idosos, melhorando a função postural e motora (ROACH et. al., 2011; ROLLAND et. al., 2007; GRANACHER et. al., 2006; MALLING, 2015), o que contribui para diminuição do risco de quedas e melhora no desempenho de atividades da vida diária. Assim, é importante reconhecer se o exercício anaeróbico também tem efeito sobre a cognição, pois este traria benefícios motores e cognitivos associados, qualificando o treinamento do idoso, com ou sem demência. Nós demonstramos que o exercício anaeróbico também tem potencial neuroprotetor, evitando o dano oxidativo induzido pela βa, o que corrobora com os achados de Cassilhas et al. (2007), que relatam que adultos cognitivamente normais que realizam exercícios anaeróbicos demostram retardo no declínio cognitivo relacionado à idade, apresentando melhorias significativas nas medidas de memória em comparação com adultos sedentários.

Estudos futuros podem investigar quais os tipos específicos de exercícios ou combinações são mais adequados e produzam um maior benefício à população idosa, especificamente aos pacientes com DA. De toda forma, nossos resultados apontam para um efeito benéfico tanto do EA (sem inclusão do exercício), como do exercício físico anaeróbico/de força, promovendo neuroproteção à neurotoxicidade induzida pela proteína beta-amilóide.

Enquanto o EA e o exercício anaeróbico mostraram efeitos neuroprotetores amplos, o enriquecimento social/aumento da socialização proporcionado a um grupo de animais não teve tamanho efeito. Esta intervenção foi capaz apenas de proteger a memória de reconhecimento social, e, simultaneamente, evitar a diminuição da capacidade antioxidante total promovida pela βa. Em estudos com seres humanos a interação social gerou aumento da angiogênese, sinaptogênese e neurogênese, fatores estes importantes na prevenção das disfunções cognitivas e possível desenvolvimento da DA (FRATIGLIONI et. al., 2004). Contudo, em estudo recente realizado com animais apenas efeitos modestos foram comprovados sobre a neuroplasticidade e cognição (BRENES et. al., 2016). Brenes e cols.

(2016) demonstraram que o enriquecimento social tem efeitos positivos no comportamento social, o que poderia explicar o fato de que neste trabalho ele preservou justamente a memória de reconhecimento social, já que esses animais estavam habituados a se socializar com novos animais.

No cérebro de pacientes com DA, e também de modelos animais da DA, há um desequilíbrio da homeostase, ou seja, a formação de ERO's é maior que a capacidade antioxidante, além da presença da peroxidação lipídica, levando assim ao estresse oxidativo e consequente dano neuronal (CHAUHAN E CHAUHAN, 2006; ZHU ET. AL., 2007). Em nossos resultados demonstramos que o exercício físico anaeróbico e o EA foram capazes de evitar o aumento da peroxidação lipídica no hipocampo de ratos expostos à βa. Nós não observamos redução na formação de ERO's nos animais que receberam injeção de βa, o que poderia ser justificado se considerarmos que a meia vida destas espécies é limitada, portanto, muitas vezes se torna difícil de mensurar em um modelo crônico como este. No entanto, o dano oxidativo observado pelos níveis de TBARS evidencia que ouve estresse oxidativo.

O dano oxidativo observado no hipocampo dos animais que receberam βa foi evitado pelo exercício anaeróbico e pelo EA. Alguns estudos destacam que o treinamento físico crônico pode levar a adaptações favoráveis ao sistema antioxidante (RADAK et. al., 2001; CLARKSON and HUBAL, 2002) e algumas enzimas antioxidantes como a glutationa-peroxidase e glutationa-redutase estã com sua atividade aumentada após seis semanas de treinamento (HELLSEN et. al., 1996), protegendo o tecido do dano oxidativo. Estudos prévios também relatam efeitos antioxidantes relacionados ao EA (SNOW et. al., 2015), incluindo aumento da expressão de genes antioxidantes (GRINAN-FERRE et al., 2016).

Em conclusão, nossos resultados mostram que a injeção da proteína βa no hipocampo de ratos resulta em danos oxidativo e de memória, e que o EA e o exercício físico previnem os déficits de memória e a peroxidação lipídica hipocampal. Já o enriquecimento social protegeu apenas o déficit de memória

de reconhecimento social e a capacidade antioxidante total do hipocampo, o que pode estar relacionado aos seus efeitos sobre o comportamento social.

6. CONCLUSÕES

Com base nos nossos resultados podemos concluir que:

1. A injeção hipocampal de proteína βamiloide promove déficits de curta e longa duração na memoria de reconhecimento de objetos e de reconhecimento social;
2. O exercício físico anaeróbico e enriquecimento social são capazes de evitar déficits de memória de reconhecimento de objetos (tanto memória de curta duração quanto memória de longa duração) e de reconhecimento social, déficits estes relacionados à neurotoxicidade induzida pela βa;
3. O enriquecimento social é capaz de evitar apenas déficits na memória de reconhecimento social, déficits estes relacionados à neurotoxicidade induzida pela βa;
4. A injeção hipocampal de proteína βa promove aumento da peroxidação lipídica e diminuição da capacidade antioxidante total do hipocampo;
5. O exercício físico e o enriquecimento ambiental reduzem a peroxidação lipídica hipocampal, enquanto que o enriquecimento social aumenta a capacidade antioxidante total em um modelo de neurotoxicidade induzida pela βa.

Por fim, podemos concluir que o enriquecimento ambiental e o exercício físico têm efeitos neuroprotetores mais pronunciados que o enriquecimento social na prevenção dos danos causados pela neurotoxicidade induzida pela βa.

7. PERSPECTIVAS

Os resultados dessa dissertação permitiram confirmar algumas hipóteses previamente levantadas pela literatura. Entretanto, ainda há uma escassez de estudos que avaliam o exercício físico anaeróbico em modelos com doença de Alzheimer. Assim, minhas perspectivas futuras envolvem a continuidade de trabalhos de pesquisa em colaboração com o Grupo de Pesquisa em Fisiologia da Unipampa para que possam ser investigadas questões como:

1. Verificar os efeitos neuroprotetores do exercício físico anaeróbico como forma de prevenção e tratamento em outros modelos de doenças neurodegenerativas;

2. Verificar os efeitos das intervenções propostas sobre outros mecanismos que podem estar envolvidos na neuroprotecão observada no modelo propostos, tais como níveis de neurotrofinas, a citar do fator neurotrófico derivado do cérebro (BDNF), e marcadores inflamatórios, como citocinas.

Referências bibliográficas

Adlard P.A., Perreau V.M., Pop V., Cotman C.W.(2005). Voluntary Exercise Decreases Amyloid Load in a Transgenic Model of Alzheimer's Disease. Journal of Neuroscience.25:17;4217-4221.

Allegri R.F., Harris P., Serrano C., Delavald N. (2001). Perfis diferenciais de perda de memória entre a demência frontotemporal e a do tipo Alzheimer. Psicol Reflex Crit.1422:317-24.

Andrés-Benito P., Fernández-Dueñas V., Carmona M., Escobar L. A., Torrejón-Escribano B., Aso E., Ciruela F., Ferrer, I. (2017). Locus coeruleus at asymptomatic early and middle Braak stages of neurofibrillary tangle pathology. Neuropathol Appl Neurobiol.12;386.

Annaházi, A., Mracskó, E., Zoltan, S., Karg, E., Penke, B., Bari, F., Farkas, E. (2007). Pre-tratament and post-tratament whit a-tocopherol attenuates hippocampal neuronal damage in experiment cerebral hypoperfusion, European Journal Prhamacol.571:120-128.

Arac. (2004). Enrichment strategies for rodents in the laboratory.

Barnham K.J., Bush A.I. (2014). Biological metals and metal-targeting compounds in major neurodegenerative diseases. Chem Soc Ver.43:6727–6749.

Baker K.B., Kim J.J. (2002). Hipoccampal plasticity and recognition memory. Learn Mem.9:58-65.

Beal M.F. (2005) Oxidative damage as an early marker of Alzheimer’s disease and mild cognitive impairment. Neurobiol Aging.26:585–586.

Bennett D.A., Schneider J.A., Tang Y., Arnold S.E., Wilson R.S. (2006). The effect of social networks on the relation between Alzheimer’s disease pathology and level of cognitive function in old people: A longitudinal cohort study. Lancet Neurol.5:406-412.

Bertollucci P. (2000). Instrumentos para o Rastreio das Demências. In: Forlenza OV, Caramelli P. Neuropsiquiatria Geriátrica. São Paulo, SP: Atheneu.

Birch AM, McGarry NB, Kelly AM: Short-term environmental enrichment, in the absence of exercise, improves memory, and increases NGF concentration, early neuronal survival, and synaptogenesis in the dentate gyrus in a time-dependent manner. Hippocampus. 2013; 23(6):437-50.

Boom A., Pochet R., Authelet M., Pradier L., Borghgraef P., Van Leuven F., Heizmann C.W., Brion J.P. (2004). Astrocytic calcium/zinc binding protein S100A6 over expression in Alzheimer's disease and in PS1/APP transgenic mice models. Biochim Biophys Acta.1742;161–168.

Braak E., Griffing K., Arai K., Bohl J., Bratzke H., Braak H. (1999). Neuropathology of Alzheimer's disease: what is new since A. Alzheimer? Eur Arch Psychiatry Clin Neurosci.249:14-22.

Bradford MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal. Biochem. 1976; 72:248-254.

Brenes J.C., Lackinger M., Höglinger G.U., Schratt G., Schwarting R.K., Wöhr M. (2016). Differential effects of social and physical environmental enrichment on brain plasticity, cognition, and ultrasonic communication in rats. J Comp Neurol.524:8;1586-607.

Brod, S., Gobbetti, T., Gittens, B., Ono, M;.,Perretti, M;.,D'Acquisto, F. (2017). The impacto f environmental enrichment in the murine inflammatory imune response. JCI Insight. 2:7.

Buchman A. S., Boyle P. A., Yu L., . Shah R. C, R. Wilson S., Bennett D. A. (2012). "Total daily physical activity and the risk of AD and cognitive decline in older adults," Neurology.78:17;1323–1329.

Cassilhas R. C., Viana V. A. R., V. Grassmann et al. (2007). "The impact of resistance exercise on the cognitive function of the elderly," Medicine and Science in Sports and Exercise.39:8:1401–1407.

Chantal S., Labelle M., Bouchard R.W., Braun C.M., Boulanger Y. (2002). Correlation of regional proton magnetic resonance spectroscopic metabolic changes with cognitive deficits in mild Alzheimer disease. Arch Neurol.59:955-62.

Chauhan V; Chauhan A: Oxidative stress in alzheimer's disease. Pathophysiology. 2006; 13(3):195-208.

Chen X., Zhang X., Liao W., Wan Q. (2017.). Effect of Physical and Social Components of Enriched Environment on Astrocytes Proliferation in Rats After Cerebral Ischemia/Reperfusion Injury. Neurochem Res.42:5;1308-1316.

Chen, H., Richard,M., Sandler,D.P., Umbach, D.M., and Kamel,F. (2007). Head injury and amyotrophic lateral sclerosis. Am. J.Epidemiol.166: 810–816.

Clarkson P.M., Tremblay I. (2002). Exercise-induced muscle damage, repair, and adaptation in human. J Appl Physiol.65:1-6.

Choi S.H., Aid S., Kim H.W., Jackson S.H., Bosetti F. (2012). Inhibition of NADPH oxidase promotes alternative and anti-inflammatory microglial activation during neuroinflammation. Journal of Neurochemistry.120:292-301.

Clark R.E., Zola S.M., Squire L.R. (2000) Impaierd recognition memory in rats after damage to the hippocampus. J. Neurosci.20:8853-60.

Colcombe S. J., Erickson K. I., . Scalf P. E. et al. (2006). "Aerobic exercise training increases brain volume in aging humans," Journals of Gerontology.61:11;1166–1170.

Corbetta M., Patel G.H., Shulman G.L. (2008). The reorienting system of the human brain: from environment to theory of mind. Neuron.58:306-324.

Corrêa O.C. (1996). Envelhecimento, depressão e doença de alzheimer. Belo Horizonte;135- 85.

Cummings JL. (2003). Alzheimer's disease: from molecular biology to neuropsychiatry. Semin Clin Neuropsychiatry.8:31-6.

Dao A., Zagaar M., Salim S., Eriksen S., Alkhadi A. (2014). Regular exercise prevents non-cognitive disturbances in a rat model Alzheimer's disease. In J Neuropsychopharmacol.17:4;593-602.

Devore E.E., Grodstein F., Van Rooi J.F.J., Hofman A., Stampfer J., Witteman J.C., Breteler M.M. (2010). Dietary antioxidants an long-term risk of dementia. Arch. Neurol.67:7;819-825.

Dong S., Duan Y., Hu Y., Zhao Z. (2012). Advances in the pathogenesis of Alzheimer's disease: a re-evaluation of amyloid cascade hypothesis. Transl Neurodegener.1:1;18.

Dupret, D., Revest, J.M., Kohel, M., et. al. (2008). Spatial relational memory requires hippocampal adult neurogenisis. PLOS one.3:4;1959.

Ehrenberg A.J., Nguy A.K., Theofilas P.,Dunlop S., Suemoto C.K., Di Lorenzo Alho A.T., Leite R.P., Diehl Rodriguez R. (2017). Quantifying the accretion of hyperphosphorylated tau in the locus coeruleus and dorsal raphe nucleus: the pathological building blocks of early Alzheimer's disease. Neuropathol Appl Neurobiol. 10:1111.

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.

Eluvathingal, T.J.,Chugani ,H.T., Behen,M.E., Juhász,C., Muzik,O.,Maqbool, M., etal.(2006).Abnormal brain connectivity in children after early severe sócio emotional deprivation:adiffusion tensor imaging study. Pediatrics.117,:2093;2100.

Fabel, K., Kempermann, G. (2008). Physical activity and the regulation of neurogenisis in the adult and aging brain. Neuromolecular medicine.10:2;59-66.

Fabel, K., Wolf, A.S., Ehninger, D., et. al. (2009). Additive effects of physical exercise and environmental enrichment on adult hippocampal neurogenesis in mice. Frontiers in neuroscience.3;p.50, 2009.

Fiala, B., Snow F.M., Greenough W.T. (1997). “Improverished” rats weigh more than “enriched” rats because they eat more. Developmental Psychobiology.10:6;537-541.

Fornari L.H.T., Garcia L.P., Hilbig A., Fernandez. (2010). The several faces of dementia syndrome: how to diagnose clinically? Sci Med.202:185-83.

Fox C.M. & Alder R.N. (2012). Mecanismos Neurais do Envelhecimento in Cohen, H. Neurociências para Fisioterapeutas. 2º ed., Ed. Manole, Barueri, SP.17, 344.

Fratiglioni L., Paillard-Borg S., Winblad B. (2004). “An active and socially integrated lifestyle in late life might protect against dementia,” The Lancet Neurology.3:6;343–353.

Garcia-Mesa Y., Lopez-Ramos J. C., Gimenez-Llort L., Revilla S., Guerra R., Gruart A., Laferla F.M., Cristofol R., Delgado-Garcia J.M., Sanfeliu C. (2011). Physical exercise protects against Alzheimer's disease in 3xTg-AD mice. Journal of Alzheimer's disease : JAD.24:421-454.

Garland TJr, Schutz H, Chappell MA, Keeney BK, Meek TH, Copes LE, et al. (2011). The biological control of voluntary exercise, spontaneous physical activity and daily energy expenditure in relation to obesity: Human and rodent perspectives. J Exp Biol. 15;214;206–29.

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. European journal of pharmacology.726:33-40.

Glass C.K., Saijo K., Winner B., Marchetto M.C., Gage F. (2010) Mechanisms Underlying Inflammation in Neurodegeneration. Cell.140:918-934.

Goldman, S.M.,Tanner, C.M., Oakes,D., Bhudhikanok,G.S.,Gupta,A.,and Langston, J.W.(2006). Head injury and parkinson’sdisease risk int wins. Ann. Neurol.60: 65–72.

Gould, E., Beylin., A., Tanapat, P., Reeves, A., Shors, T. J. (1999). Learning enchanges adult neurogenisis in the hippocampal formation. Nature neuroscienc,2:3:260-5.

Gómez-Isla T., Price J.L., McKeel D.W. Jr., Morris J.C., Growdon J.H., Hyman B.T. (1996). Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease. J Neurosci.16:4491-500.

Granacher U, Gollhofer A, Strass D: (2006). Training induced adaptations in characteristics of postural reflexes in elderly men. Gait Posture.24:4;459-66.

Greenough M.A., Camakaris .J, Bush A.I. (2013) Metal dyshomeostasis and oxidative stress in Alzheimer's disease. Neurochem Int.62:540–555.

Hardy J., Selkoe D.J. (2002). The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science.297:353- 6.

Harman D. (1996). A hypothesis on the pathogenesis of Alzheimer's disease. Ann NY.786:152-68.

Hase Y., Craggs L., Hase M., Stevenson W., Slade J., Lopez D., Mehta R., Chen A., Liang D., Oakley A., Ihara M., Horsburgh K., Kalaria RN. (2017). Effects of environmental enrichment on white matter glial responses in a mouse model of chronic cerebral hypoperfusion. Journal of Neuroinflammation.14:81.

Hellsten Y., Apple F.S., Sjödin B. (1996). Effect of sprint cycle training on activities of antioxidant enzymes in human skeletal muscle. J Appl Physiol.81:1484-7.

Hurley, B.F., Hagberg, JM. (1998). Optimizing health in older persons: aerobic or strength training? Exercise and Sports Sciences Reviews.26: 61-89.

Hurley B..F, Hanson E.D. and Sheaff A.K. (2011). Strength training as a countermeasure to aging muscle and chronic disease. Sports Med.41:289-306.

Ichimurra H., Parthasarathi K., Quadri S., Issekutz A.C., Bhattacharya J. (2003). Mechano-oxidative coupling by mitochondria induces proinflammatory responses in lung venular capillaries. J Clin Invest.111;691–699.

Janus C., Westaway D. (2001). Transgenic mouse models of Alzheimer's disease. Physiol Behav.73(5):873-86.

Jedrziewski M. K. et. al. (2014). The Impact of Exercise, Cognitive Activities, and Socialization on Cognitive Function: Results From the National Long-Term Care Survey. American Journal of Alzheimer’s Disease & Other Dementias.29:4;372-378.

Jedrziewski M. K. et. al. (2010). Exercise and cognition: Results from the National Long Term Care Survey. Alzheimer’s & Dementia.6;448–455.

Jurgens, H.Á., Johnson, R,W. (2012). Environmental enrichment attenuates hippocampal neuroinflammation and improves cognitive function during influenza infection. Brain Behav Immun.26:6;1006-1016.

Kawasumi M., Hashimoto Y., Chiba T., Kanekura K., Yamagishi Y. et al. (2002). Molecular Mechanisms for Neuronal Cell Death by Alzheimer’s Amyloid Precursor Protein-Relevant Insults. Neurosignals.11:236-50.

Kempermann, G., Gast, D., GAGE, F.H. (2002). Neuroplasticity in old age: sustained fivefold induction of hippocampal neurogenisis by long-term environmental enrichment. Annals of neurology.52:2;135-43.

Kempermann G, Kuhn HG, et al. (1997). More hippocampal neurons in adult mice living in an environmental enrichment. Nature.386:6624;493-495.

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. Journal of exercise rehabilitation.10:2-8.

Kim T..S, Pae C.U., Yoon S.J., Jang W.Y., Lee N.J., Kim J.J., Lee SJ, Lee C., Paik I.H., Lee C.U. (2006). Decreased plasma antioxidants in patients with Alzheimer’s disease. Int J Geriatr Psychiatry.21:344–348.

Kumar A. et. al. (2012). Influence of late-life exposure to environmental enrichmentor exercise on hippocampal function and CA1 senescent physiology. Neurobiol Aging.33:4;828.e1–828.e17.

LaFerla, F. M., Green, K. N., Oddo, S. (2007). Intracellular amyloid-beta in alzheimer's disease. Nature Reviews.8:499-509.

Lambert, T. J., Fernandez, S.M., Frick, K.M. (2005). Different types environmental enrichment have discrepant effects os spatial memory and sinaptophysin levels in female mice. Neurobiology os Learning and memory.83:206-216.

Larson E. B., L. Wang, J. D. Bowen et al. (2006). "Exercise is associated with reduced risk for incident dementia among persons 65 years of age and older," Annals of Internal Medicine.144:2:73–81.

Lautenschlager N. T., Cox K. L., Flicker L. et al. (2008). "Effect of physical activity on cognitive function in older adults at risk for Alzheimer disease: a randomized trial," Journal of the American Medical Association.300: 9;1027–1037.

Leger M., Bouet V., Freret T., Darmaillacq A.S., Dacher M., Dauphin F., Boulouard M., Schumann-Bard P. (2012). Environmental enrichment improves recent but not remote memory in association with a modified brain metabolic activation profile in adult mice. Behav Brain Res.228:1;22-9.

Leggio, G.M., Mandolesi, L., Federico, F., Spirito, F., Ricci, B., Gelfo, F., Petrosini, L. (2005). Environmental enrichment promotes improved spatial abilities and enchanced dendritic growth in the rat. Behavioral Brain Researcch.163:1;78-90.

Liu G., Huang W., Moir R.D., Vanderburg C.R., Lai B., Peng Z., Tanzi R.E., Rogers J.T., Huang X. (2006). Metal exposure and Alzheimer's pathogenesis. J Struct Biol.155:45–51.

Liu Z., Li P., Wu J., Wang Y., et. al., (2015). The cascade of oxidative stress and tau protein autophagic dysfunction in Alzheimer's disease. Mental and Behavioural Disorders and Diseases of the Nervous System.

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;323-31.

Lovell M.A., Markesbery W.R. (2001). Ratio of 8-hydroxyguanine in intact DNA to free 8-hydroxyguanine is increased in Alzheimer disease ventricular cerebrospinal fluid. Arch. Neurol.8:3;392-396.

Lye, T.C., and Shores, E.A. (2000). Traumaticbraininjuryasariskfactor for alzheimer'sdisease:areview. Neuropsychol. Rev.10:115–129.

Maccioni B.R., Muñoz .PJ., Barbeito L. (2001). The molecular bases of alzheimer's disease and other neurodegenerative disorders. Archives of Medical Research.32:367-381.

Mainardi, M., Garbo, A., Caleo, M., Berardi, N., Sale, A., Maffei, L. (2014). Environmental enrichment strengthens corticocortical interactions and reduces amyloid-B oligomers in aged mice. Front Aging Neurosci.6:1.

Malling AS, Jensen BR: Motor intensive anti-gravity training improves performance in dynamic balance related tasks in persons with Parkinson's disease. Gait Posture. 2016; 43:141-7.

Mariani E., Polidori M.C., Cherubini A., Mecocci P. (2005). Oxidative stress in brain aging, neurodegenerative and vascular diseases: An overview. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.827:1;65-75.

Martin, L. J. (2006); Mitochondriopathy in Parkinson disease and amyotrophic lateral sclerosis. J Neuropathol.Exp.Neurol.65:1103-1110.

Mattson, M. P. (2007). Calcium and neurodegeneration. Aging Cell.

Mattson P.M., Mattson P.E. (2002). Amyloid peptide enhances nail rusting: novel insight into mechanisms of aging and alzheimer's disease. Ageing Research Reviews.1:327-30.

Mattson P.M. (2003). Methylation and acetylation in nervous system development and neurodegenrative disorders. Aging Research Reviews.2:329-42.

Mattson M.P., Cutler R.G., Jo D.G. (2005) Alzheimer peptides perturb lipid-regulating enzymes. Nat Cell Biol.7:1045–1047.

Migliore L, Fontana I, Colognato R, Coppede F, Siciliano G, Murri L. (2005). Searching for the role and the most suitable biomarkers of oxidative stress in Alzheimer's disease and in other neurodegenerative diseases. Neurobiol. Aging.26:5:587-595.

Mooney, S.F. (2011). Alzheimer: Cuidar de seu ente querido e cuidar de você mesmo. 2ª São Paulo: Paulinas, p.336.

Mora, F. (2013). Successful brain aging: plasticity, environmental enrichment, and lifestyle. Dialogues in clinical neurosciense.15:01;45-52.

Mortimer, J.A.,French, L.R.,Hutton, J.T.,and Schuman,L.M.(1985).Head injury asariskfactorforalzheimer'sdisease. Neurology.35:264–267.

Moy S., Nadler J., Perez A., Barbaro R., Johns J., Magnuson R., Piven J., Crawley J. (2004). Sociability and preference for social novelty in five inbred strains: an approach to assess autistic-like behavior in mice. Genes, Brain and Behavior.3:287–302.

Nann A.M.D. (1996). Pyramidal nerve cell loss in alzheimer's disease. Neurodegeneration.5:423-427.

Nakajima K., Kohsaka S. (2001). Microglia: Activation and their significance in the central nervous system. J Biochem130;169–175.

Newberry R. (1995). Environmental Enrichment: Increasing the biological relevance of captive environments. Applied Animal Behaviour Science.; 44(4):229-243.

Nitrini R., Caramelli P., Bottino C.M.C., et al. (2005). Diagnóstico de doença de Alzheimer no Brasil. Arq Neuropsiquiatr.63:3;713-727.

Nobili A., Latagliata E. C., Viscomi M.T., Cavallucci V., Cutuli D., Giacovazzo G., et. al. (2017). Dopamine neuronal loss contributes to memory and reward dysfunction in a model of Alzheimer's disease.14;727.

Nokia M., Lensu S., Ahtiainen J., Johansson P., Koch L., Britton S., Kainulainen H. (2016). Physical exercise increases adult hippocampal neurogenesis in male rats provided it is aerobic and sustained.doi: 10.1113/JP271552.

Hardy J.A., Higgins G,A. (1992). Alzheimer's disease: the amyloid cascade hypothesis.256:5054;184-5.

Ogonovszky H., Sasvari M., Dosek A., Berkes I., Kaneko T., Tahara S., Nakamoto H., Goto S., Radak Z. (2005). The effects of moderate, strenuous, and overtraining on oxidative stress markers and DNA repair in rat liver. Canadian journal of applied physiology = Revue canadienne de physiologie appliquee.30:186-195.

Palmer M.A. (1996). Neurochemical Studies of Alzheimer's disease. Neurodegeneration.5:381-391

Pereira, L.O., Arteni, N.S., Petersen, R.C., Rocha, A.P., Achaval, M., Netto, C.A. (2007). Effects of daily environmental enrichment on memory déficits na brain injury following neonatal hypoxia-ischemia in the rat. Neurobiology of learning and memory. 87:1;101-8.

Radak Z., Taylor A.W., Ohno H., Goto S. (2001). Adaptation to exercise-induced oxidative stress: from muscle to brain. Exerc Immunol Rev.7:90-107.

Roach K. E., R. M. Tappen, N. Kirk-Sanchez, Williams C. L., D. Loewenstein. (2011). "A randomized controlled trial of an activity specific exercise program for individuals with Alzheimer disease in long-term care settings," Journal of Geriatric Physical Therapy.34:2;50–56.

Rolland Y., Pillard F., Klapouszczak A., et al. (2007). "Exercise program for nursing home residents with Alzheimer's disease: a 1- year randomized, controlled trial," Journal of the American Geriatrics Society.55:2:158–165.

Rubenstein LZ, Josephson KR, Trueblood PR et al. (2000). Effect of a Group Exercise Program on Strength, Mobility, and Falls Among Fall-Prone Elderly Men. J Gerontol Med Sci.;55:317-21.

Sapolsky, R.M., Alberts,S.C., Altmann, J.(2002). Hypercortisolism associated with social subordinance or social isolation among wild baboons. Arch. Gen.Psychiatry.54:1137–1143.

Scahill, R. I., Frost, C., Jenkins, R., Whitwell, J. L., Rossor, M. N., e Fox, N. C. (2003). A longitudinal study of brain volume changes in normal aging using serial registered magnetic resonance imaging. Arch.Neurol.60:989- 994.

Schuff N., Amend D., Ezekiel F., Steinman S.K., Tanabe J., Norman D., et al. (1997). Changes of hippocampal N-acetyl aspartate and volume in Alzheimer's disease. A proton MR spectroscopic imaging and MRI study. Neurology.49:1513-21.

Selkoe D. J. (2002). Alzheimer's disease is a synaptic failure. Science.298:789-91.

Selkoe J.D., Lansbury J.J.R.P. (1995). Biochemistry of Alzheimer's and Prion Disease.949-968.

Shaeffer E. L. (2009). Enriquecimento ambiental como estratégia para promover a neurogênese na doença de Alzheimer: possível participação da fosfolipase A_2. Rev. Psiq. Clín.37:2;73-80.

Smith M.A., Richey Harris P.L., Sayre L.M., Beckman J.S., Perry G. (1997). Widespread peroxynitrite-mediated damage in Alzheimer's disease. J. Neurosci.17:8;2653-2657.

Stuart K.E., King A.E., Fernandez-Martos C.M., Dittmann .J, Summers M.J., Vickers J.C. (2017). Mid-life environmental enrichment increases synaptic density in CA1 in a mouse model of Aβ-associated pathology and positively influences synaptic and cognitive health in healthy ageing. J Comp Neurol.525:8;1797-1810.

Shors, T.J., Miesegaes, G., Beylin, A., et. al. (2001). Neurogenisis in the adult is involved in the formation of the trace memories. Nature.41:6826;372-6.

Snow W. M., Pahlavan P. S., Djordjevic J., McAllister D., Platt E. E., Alashmali S., et al. (2015). Morris water maze training in mice elevates hippocampal levels of transcription factors nuclear factor (erythroid-derived 2)-like 2 and nuclear factor kappa B p65. Front. Mol. Neurosci. 8:7010.

Sultana R., Mecocci P., Mangialasche F., Cecchetti R., Baglioni M., Butterfield D.A. (2011) Increased protein and lipid oxidative damage in mitochondria isolated from lymphocytes from patients with Alzheimer's disease: Insights into the role of oxidative stress in Alzheimer's disease and initial investigations into a potential biomarker for this dementing disorder. J Alzheimers Dis.24:77–84.

Smith M.A., Rudnicka-Nawrot M., Richey P..L, Praprotnik D., Mulvihill P., Miller C.A., Sayre L.M., Perry G. (1995) Carbonyl-related posttranslational modification of neurofilament protein in the neurofibrillary pathology of Alzheimer's disease. J Neurochem.64:2660–2666.

Suttanon P., Hill K.D., Said C.M., Williams S.B., Byrne K.N., LoGiudice D., Lautenschlager N.T. Dodd K.J. (2013). Feasibility, safety and preliminary evidence of the effectiveness of a home-based exercise programme for older people with Alzheimer's disease: A pilot randomized controlled trial. Clin Rehabil.27:427-438.

Tashiro, A., MAKINO, H., GAGE, F.H. (2007). Experience-specific functional modification of the dentage gyrus through adult neurogenisis: a critical period during an immature stage. The jornal of neuroscience: the oficial jornal of the Society for Neuroscience.27:12:3252-9

Taylor, C.A., Saint-Hilaire,M.H., Cupples,L.A., Thomas,C.A., Burchard, A. E., Feldman,R.G.,etal.(1999).Environmental,medicalandfamily history riskfactorsforparkinson'sdisease:anewengland-basedcase control study. Am. J.Med.Genet..88:742–749.

Trillo L., Das D., Hsieh W., Medina B., Moghadam S., Lin B., Dang V., Sanchez M.M., De Miguel Z., Ashford J.W., Salehi A. (2013). Ascending monoaminergic

systems alterations in Alzheimer's disease. translating basic science into clinical care. Neurosci Biobehav Rev.37:8;1363-79.

Troulinaki, K., Tavernarakis, N. (2005). Neurodegenerative conditions associated with ageing: A molecular interplay? Mechanisms of Ageing and Development, v. 126, n. 1, p. 23–33.

Uttara B., Singh A.V., Zamboni P., Mahajan R.T. (2009) Oxidative stress and neurodegenerative diseases: A review of upstream and downstream antioxidant therapeutic options. Curr Neuropharmacol.7:65–74.

Van Praag H, Kempermann G, et. al. (2000). Neural consequences of environmental enrichment. Nature Reviews Neuroscience.1:3;191-198.

Vivinetto, A.L., Suárez M.M., Rivarola M.A. (2013). Neurobiological effects of neonatal maternal separation and post-weaning environmental enrichment. Behavioural Brain Research.240:110–118.

Waldemar G., Dubois B., Emre M., et al. (2007). Recommendations for the diagnosis and management of Alzheimer's disease and other disorders associated with dementia: EFNS guideline. Eur J Neurol.14:e1-e26

Wang H.X., Karp A., Winblad B., Fratiglioni L.(2002). Late-life engagement in social and leisure activities is associated with a decreased risk of dementia: A longitudinal study from the Kungsholmen project. Am J Epidemiol.155;1081-1087.

Wang L., Miller J.P., Gado M.H., McKeel D.W., Rothermich M., Miller M.I., et al. (2006). Abnormalities of hippocampal surface structure in very mild dementia of the Alzheimer type. Neuroimage.30:52-60.

Xu H., Gelvana E., Rajsombath M., Yang T., Li S., Selkoe D. (2016). Environmental Enrichment Potently Prevents Microglia-Mediated Neuroinflammation by Human Amyloid B Protein Oligomers. The Journal of Neuroscience.36:35;9041–9056.

Zhao Q., Tang X.C. (2002). Effects of huperzine A on an acetylcholinesterase isoforms in vitro: comparison with tacrine, donepezil, rivastigmine and physostigmine. Eur J Pharmacol.455 (2-3):101-7.

Zhu X., Lee H., Perry G., Smith M.A. (2007). Alzheimer disease, the two-hit hypothesis An update. Biochim. Biophys. Acta.4:494-502.

ANEXOS

Anexo I – Normas da Revista *Neurotoxicity Research*, disponíveis em: http://www.springer.com/biomed/neuroscience/journal/12640

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Cite references in the text by name and year in parentheses. Some examples:

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  Ideally, the names of all authors should be provided, but the usage of "et al" in long author lists will also be accepted:

  Smith J, Jones M Jr, Houghton L et al (1999) Future of health insurance. N Engl J Med 965:325–329
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- Book

  South J, Blass B (2001) The future of modern genomics. Blackwell, London
- Book chapter

  Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257
- Online document

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Anexo II – Certificado de aprovação do projeto pelo CEUA-UNIPAMPA

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

Pró-Reitoria de Pesquisa, Pós-graduação e Inovação

COMISSÃO DE ÉTICA NO USO DE ANIMAIS - CEUA

Fone: (55) 3413 4321, E-mail: ceua@unipampa.edu.br

CERTIFICADO DE APROVAÇÃO DE PROTOCOLO PARA USO DE ANIMAIS EM PESQUISA

Número de protocolo da CEUA: **032/2016**

Título: **Comparação dos efeitos neuroprotetores do enriquecimento ambiental, do exercício físico e da socialização em um modelo animal de Doença de Alzheimer**

Data da aprovação: **02/12/2016**

Período de vigência do projeto: 02/03/2018

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