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EFEITO NEUROPROTETOR DO ÁCIDO HIDROXÂMICO DE SUBEROILANILIDA (SAHA), UM INIBIDOR DE HDAC, EM MODELO DE DOENÇA DE ALZHEIMER INDUZIDA POR INJEÇÃO DO PEPTÍDEO β-AMILÓIDE 1-42

DISSERTAÇÃO DE MESTRADO

Kellen Mariane Athaide Rocha

Itaqui, RS, Brasil.

2017

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

Orientador: Prof. Dr. Cristiano Ricardo Jesse

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Área de concentração: Bioquímica Farmacêutica e Toxicológica

Dissertação defendida em: 14 de julho de 2017

Banca examinadora:

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Prof. Dr. Cristiano Ricardo Jesse (Presidente, orientador)

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Profº César Augusto Brüning (UFPEL)

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PARTE I

RESUMO

Dissertação de Mestrado

Programa de Pós-Graduação em Bioquímica

Universidade Federal do Pampa, RS, Brasil

EFEITO NEUROPROTETOR DO ÁCIDO HIDROXÂMICO DE SUBEROILANILIDA (SAHA), UM INIBIDOR DE HDAC, EM MODELO DE DOENÇA DE ALZHEIMER INDUZIDA POR INJEÇÃO DO PEPTÍDEO β-AMILÓIDE 1-42

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Orientador: Cristiano Ricardo Jesse

Data e Local da Defesa: Itaqui, 14 de Julho de 2017.

A doença de Alzheimer (DA) é uma desordem neurodegenerativa crônica caracterizada clinicamente pela perda progressiva de função cognitiva, distúrbios neuropsiquiátricos e comportamentais. Patologicamente esta doença caracteriza-se pelo acúmulo anormal do peptídeo β-amilóide (Aβ) no córtex e no hipocampo, emaranhados neurofibrilares intracelulares formados por *tau* hiperfosforilada, disfunção progressiva sináptica e, posteriormente perda neuronal. As opções terapêuticas disponíveis melhoram os sintomas, mas não impedem a progressão da doença, portanto, ainda está faltando uma estratégia terapêutica efetiva para DA. Há estudos relacionados à utilização de terapia epigenética para o tratamento da DA, a terapêutica mais desenvolvida é a que envolve a classe dos inibidores das deacetilases (HDACs). Assim, este trabalho tem por objetivo investigar o efeito protetor do inibidor da HDAC ácido hidroxâmico de suberoilanilida (SAHA) em um modelo de DA em camundongos. Para isso, foram utilizados 50 camundongos Swiss adultos, pesando entre 30-35 g, divididos em dois experimentos. No primeiro, os camundongos foram divididos em 6 grupos que receberam uma injeção de $A\beta_{1-42}$ via intracerebroventricular (i.c.v.) no início da experiência (exceto o grupo Sham que foi utilizado como controle) para investigar a atividade das histonas

acetiltransferase (HATs) e HDAC, determinação dos níveis do fator neurotrófico derivado do cérebro (BDNF), expressão do mRNA de BDNF e modulação da via (cAMP/PKA/CREB) em uma curva de tempo (6 horas, 1, 3, 7 e 21 dias). Ao final de cada tempo, os animais foram submetidos ao teste cognitivo e foram eutanasiados. O córtex pré-frontal e o hipocampo foram removidos para posteriores análises. No segundo experimento, os camundongos foram divididos em 4 grupos: Grupo Controle (sham+veículo); Grupo $A\beta_{1-42}$ ($A\beta_{1-42}$ + veículo); Grupo SAHA (25 mg/kg, via intraperitoneal) (sham + SAHA); Grupo Interação ($A\beta_{1-42}$ + SAHA). O peptídeo $A\beta_{1-42}$ ou o veículo foram infundidos por injeção i.c.v. e, um dia depois, iniciou-se o tratamento, por via i.p., durante 21 dias. Ao final do experimento os animais foram submetidos ao teste cognitivo, eutanásiados para retirada das estruturas cerebrais. As amostras foram utilizadas para a determinação dos níveis de BDNF, expressão do mRNA de BDNF, atividade enzimática das histonas (HDAC e HATs) e regulação da via cAMP/PKA/CREB. O presente estudo observou deficiências significativas causadas pela $A\beta_{1-42}$ na memória (Labirinto Aquático de Morris), bem como causou desequilíbrio das enzimas HAT/HDAC, redução de cAMP, PKA e CREB e BDNF no córtex pré-frontal e hipocampo de camundongos. A inibição de HDAC, com SAHA demostrou neuroproteção nas alterações comportamentais e neuroquímicas induzidas por $A\beta_{1-42}$. Estes dados mostram que a acetilação através da inibição do HDAC, desempenha um papel fundamental na mediação da memória e demonstra que SAHA poderá ser uma ferramenta médica promissora na abordagem terapêutica para o tratamento da DA.

PALAVRAS-CHAVE:
Doença de Alzheimer; Epigenética, memória, Histona deacetilase.

ABSTRACT

Dissertation of Master

Program of Post-Graduation in Biochemistry

Federal University of Pampa

NEUROPROTETIC EFFECT OF SUBEROILANILIDA HYDROXAMIC ACID (SAHA), A HDAC INHIBITOR, IN ALZHEIMER'S DISEASE MODEL INDUCED BY INJECTION OF β-AMYLOID PEPTIDE 1-42

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Site and Date of Defence: Itaqui, July 14, 2017.

Alzheimer's disease (AD) is a chronic neurodegenerative disorder characterized clinically by the progressive loss of cognitive function, neuropsychiatric and behavioral disorders. Pathologically this disease is characterized by the abnormal accumulation of β-amyloid peptide (Aβ) in the cortex and hippocampus, intracellular neurofibrillary tangles formed by hyperphosphorylated tau, progressive synaptic dysfunction and, later, neuronal loss. The available therapeutic options improve the symptoms but do not impede the progression of the disease, therefore, an effective therapeutic strategy for AD is still lacking. There are studies related to the use of epigenetic therapy for the treatment of AD, the most developed therapy is that involving the class of deacetylase inhibitors (HDACs). Thus, this work aims to investigate the protective effect of the HDAC inhibitor hydroxamic acid suberoilanilide (SAHA) in an AD model in mice. For this, 50 Swiss adult mice weighing between 30-35 g were used, divided in two experiments. In the first, the mice were divided into 6 groups that received an injection of $A\beta_{1-42}$ via the intracerebroventricular (icv) at the beginning of the experiment (except the Sham group that was used as control) to investigate the activity of histones acetyltransferase (HATs) and HDAC , Determination of brain-derived neurotrophic factor (BDNF), expression of BDNF mRNA, and modulation of the pathway (cAMP/PKA/CREB) in a time curve (6 hours, 1, 3, 7 and 21 days). At the end of each time, the animals were submitted to the cognitive test and were euthanized. The

prefrontal cortex and hippocampus were removed for further analysis. In the second experiment, the mice were divided into 4 groups: Control Group (sham + vehicle); Group $A\beta_{1-42}$ ($A\beta_{1-42}$ + vehicle); SAHA group (25 mg/kg, intraperitoneal route) (sham + SAHA); Interaction Group (Aβ1-42 + SAHA). The $A\beta_{1-42}$ peptide or vehicle was infused by i.c.v. and one day later, the treatment was started i.p. for 21 days. At the end of the experiment the animals were submitted to the cognitive test, euthanasia for removal of the cerebral structures. Samples will be used for determination of BDNF levels, expression of BDNF mRNA, histone enzymatic activity (HDAC and HATs) and regulation of the cAMP/PKA/CREB pathway. The present study observed significant deficiencies caused by $A\beta_{1-42}$ in memory (Morris Aquatic Labyrinth), as well as caused imbalance of HAT/HDAC enzymes, reduction of cAMP, PKA and CREB and BDNF in the prefrontal cortex and hippocampus of mice. Inhibition of HDAC with SAHA demonstrated neuroprotection in behavioral and neurochemical changes induced by $A\beta_{1-42}$. These data show that acetylation through inhibition of HDAC plays a key role in memory mediation and demonstrates that SAHA may be a promising medical tool in the therapeutic approach to AD.

KEYWORDS:

Alzheimer's disease; Epigenetics, memory, Histone deacetylase.

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PARTE II

LISTA DE ABREVIATURAS E SIGLAS

DA = Doença de Alzheimer

APP = *Amyloid precursor protein* = Proteína precursora de amilóide

Aβ = *Amyloid-beta* = Beta-amilóide

Aβ1-42 = isoforma do peptídeo beta-amilóide com 42 aminoácidos

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HDAC = Histona Desacetilase

HAT = Histona Acetiltransferase

DNMT = Metiltransferase

CpG/ MBD = Proteína de ligação a metil-CpG

DNA = Ácido Desoxirribonucleico

SNC = Sistema Nervoso Central

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BDNF = *Brain-derived neurotrophic factor* = fator neurotrófico derivado do cérebro

RNAm = RNA mensageiro

MAPK = Proteínas cinases ativadas por mitógenos

NGF = Fator de crescimento neuronal

NT3 = Neurotrofina-3

NT4/5 = Neurotrofina 4/5

SNP = Sistema Nervoso Periférico

PI3K = Fosfotidilinositide-3-quinases

PKA = Proteína Cinase A

AMPc = Monofostato de adenosina 3,5 cíclico

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GTP = Trifosfato de Guanosina

CREB = Proteína de ligação ao elemento responsivo ao AMPc

CRE = Elemento responsivo ao AMPc

PDE = Fosfodiesterases

AC = Adenil ciclase

AMP = Monofosfato de adenosina

TrkB = Receptor de Tropomiosina B

SUMÁRIO

PARTE I

APRESENTAÇÃO

Os resultados que compõem essa dissertação apresentam-se divididos em 3 partes. Na **parte I** encontram-se a **Introdução** e os **Objetivos**. A **parte II** refere-se aos resultados deste trabalho sob a forma de **Manuscrito** que abrange as seções introdução, materiais e métodos, resultados, discussão dos resultados e referências bibliográficas. O item **Conclusão** encontra-se na **parte III** desta dissertação e apresenta conclusões gerais do manuscrito contido nesse trabalho. O item **Referências** inclui somente as citações que aparecem no item introdução desta dissertação.

1. INTRODUÇÃO

1.1. Doença de Alzheimer

A doença de Alzheimer (DA) é uma desordem neurodegenerativa crônica, sendo a forma mais comum de demência em idosos, o que representa 50 a 75% de todos os casos (Choi et al., 2014). É caracterizada por um comprometimento do sistema colinérgico, o que leva a um quadro clínico de perda progressiva da memória e das funções cognitvas (Suh & Checler, 2002).

A DA foi primeiramente descrita em 1906 pelo médico alemão Alois Alzheimer, durante o 37° Congresso do Sudoeste da Alemanha de Psiquiatria, na cidade de Tübingen. Durante sua conferência intitulada "Eine eigenartige Erkrankung der Hirnrinde" (Uma Doença Peculiar dos Neurônios do Córtex Cerebral), Alzheimer definiu seu achado como uma patologia neurológica, não reconhecida, que cursa com demência, dando destaque aos sintomas de déficit de memória, alterações comportamentais e incapacidade para as atividades rotineiras. Posteriormente, Alzheimer ainda viria a descrever os aspectos anatomopatológicos da doença, cujas principais características eram o acúmulo de placas senis e emaranhados neurofibrilares e a perda neuronal (Medeiros, 2007; Goedert & Spillantini, 2006).

Em 1968, um estudo demonstrou que os achados anatomopatológicos característicos da DA também apareciam em indivíduos com demência senil, em que os sintomas se iniciam aos 65 anos ou após essa idade, sendo então considerados como parte da mesma entidade patológica (Blessed et al., 1968).

A apresentação clínica típica da DA é a perda progressiva da memória e das funções cognitivas (Chen et al., 2015). A DA pode levar à morte dentro de 3 a 9 anos após o seu diagnóstico, sendo a idade o principal fator de risco, com sua incidência dobrando a cada 5 anos após os 65 anos de idade (Souza, 2012).

A susceptibilidade à DA é resultante de múltiplos determinantes ambientais e genéticos que interagem durante a vida. Os fatores de risco relacionados à DA compreendem: baixa escolaridade, traumatismo craniano associado à perda de consciência, sexo feminino, depressão, diabetes mellitus, hipertensão arterial, fumo, hiperinsulinemia, inatividade física, fibrilação arterial, dieta rica em gorduras e fatores genéticos. Porém, dentre os fatores de risco associados a DA, a idade é a que exerce maior influência (Gorelick, 2004; Mattson, 2003).

Duas formas da doença são descritas: a forma da DA familiar (FAD), que é referente a 5% dos casos da doença, caracterizada pela precocidade do quadro clínico,

ocasionada devido a mutações dos genes da proteína precursora amilóide (APP) ou das presenilinas I e II. Os outros 95 % dos casos da doença são referentes ao tipo esporádico e apresenta uma evolução mais lenta, sendo que os sintomas raramente surgem antes do paciente completar 50 anos de idade (Suh & Checler, 2002).

Estimativas indicam que a doença acomete 8-15 % da população com mais de 65 anos. Atualmente, existem em todo o mundo, aproximadamente 25 milhões de pessoas com a DA, o que demonstra sua grande importância. Considerando projeções do crescimento populacional que indicam que o número de indivíduos com mais de 80 anos chegará a 300 milhões em 2050 (Zinser et al., 2007).

Das múltiplas causas associadas ao aparecimento da DA, destacam-se três: O alelo ε4 da apolipoproteína E (apoE), um dos grandes fatores de risco para o aparecimento da DA e também um importante indicador do seu desenvolvimento, uma outra causa para o desenvolvimento desta doença é o acúmulo das placas de beta-amilóide, a terceira causa é a neurodegeneração mediada pela *tau*. Relativamente ao alelo ε4 da apoE, no corpo humano este gene situa-se no braço longo do cromossoma 19 (19q13.2) (Petersen et al., 1995). A informação contida neste gene é importante para a formação da proteína apoE, uma vez que esta proteína é fundamental no mecanismo de redistribuição de triglicerídios e colesterol nos vários tecidos do organismo humano. O segundo órgão de maior importância na síntese de apoE é o cérebro, assumindo um papel específico na formação das placas amilóides, o que sucede é uma interação entre a beta-amiloide (Aβ) solúvel e a apoE, criando um complexo apoE-Aβ que posteriormente é digerido enzimaticamente e origina duas porções, uma é degradada e a outra promove o acúmulo de placas amilóides que irão ser excretadas para o meio extracelular (Ojopi, Bertoncini & Dias Neto, 2004).

A Aβ é o maior constituinte das placas sendo caracterizada como o maior agente da patologia da DA, devido os níveis desta proteína na doença se encontram aumentados, o que ocasiona um aumento extracelular de placas de fibras amilóides de Aβ. Juntamente com as fibras amilóides também se encontram fibras neurocelulares desordenadas. A deposição destas placas amilóides leva a uma grande diminuição de neurônios no cérebro (Sung et al., 2014). Os emaranhados neurofibrilares e as placas senis podem estar presentes nos cérebros normalmente senis, mas em menor quantidade e com distribuição menos extensa (Walsh & Selkoe, 2004).

Os emaranhados neurofibrilares são constituídos primariamente de proteínas *tau* associadas aos microtúbulos. As proteínas *tau* dão estabilidade aos microtúbulos do

citoesqueleto neural, sendo esta função regulada por um processo de fosforilação e defosforilação. Nos neurônios que sofrem degeneração, as proteínas *tau* associadas a microtúbulos tornam-se anormalmente hiperfosforiladas e acumulam-se na forma de filamentos emaranhados helicoidais pareados. Por sua vez, as placas senis são constituídas da deposição de fragmentos amilóides no parênquima cerebral (Haass, 2004; Mattson, 2004).

A Aβ é um fragmento proteolítico formado a partir de uma glicoproteína transmembrânica maior, a APP, que sofre a ação das enzimas proteolíticas β- e γ-secretases. Os mecanismos responsáveis pela neurotoxicidade da Aβ são complexos, mas acredita-se que envolva a quebra da homeostase intracelular do cálcio e potássio, indução de estresse oxidativo e ativação do processo de morte celular e neuroinflamação via caspases (Hardy & Selkoe, 2002).

Avanços recentes na compreensão dos mecanismos patofisiológicos relacionados à doença de Alzheimer apontam para novas estratégias no desenvolvimento de drogas. Os modelos animais têm contribuído consideravelmente para estes avanços e têm um papel ainda maior na avaliação de eventuais drogas com potencial terapêutico, não apenas de aliviar a demência associada com a doença de Alzheimer, mas de modificar o processo da doença (Van Dam & De Deyn, 2006).

O tratamento da doença de Alzheimer inclui estratégias farmacológicas e intervenções psicossociais para o paciente e seus familiares. Inúmeras substâncias psicoativas têm sido propostas para restabelecer ou preservar a cognição do paciente. Estudos recentes sugerem que alterações de natureza epigenética estão associadas à DA. Esta nova área de estudo associa a influência do ambiente sobre a expressão gênica, ou seja a capacidade de fatores ambientais induzirem alterações no fenótipo sem afetarem a sequência de DNA (Mount & Downton, 2006).

1.2. Epigenética

Epigenética é o termo utilizado para descrever acontecimentos que não são explicados através de princípios genéticos. A epigenética é definida, por Conrad Waddington (1942), como “uma área da biologia que estuda as interações entre genes e os seus produtos, o que leva ao início do fenótipo” (Goldberg, Allis & Bernstein, 2007).

As alterações epigenéticas estão presentes desde a fase pré-natal de vida e as mudanças fenotípicas causadas por essas mesmas alterações estão associadas ao processo de envelhecimento, assim como nas doenças neurodegenerativas. Esta nova

área de estudo associa a influência do ambiente sobre a expressão génica, ou seja a capacidade de fatores ambientais induzirem alterações no fenótipo sem afetarem a sequência de DNA (Zhu et al., 2015).

A epigenética pode interferir com o fenótipo através de vários mecanismos. Os dois mecanismos principais centram-se na interferência do mecanismo biológico levando à doença ou servindo como biomarcador da doença ou da exposição ambiental (Goldberg, Allis & Bernstein, 2007). A interferência dos fatores epigenéticos no mecanismo biológico são a nível da metilação do DNA, modificação de histonas, da cromatina e regulação do RNA não codificante (Zhu et al., 2015). Esses mecanismos atuam modificando a acessibilidade da cromatina para a regulação da transcrição local ou globalmente, pelas modificações no DNA e pelas modificações ou rearranjos dos nucleossomos (Jenuwein & Allis, 2001; Jones & Takai, 2001).

Os nucleossomos e as histonas são estruturas associadas ao DNA com a função de organização da cromatina. Esta organização está intimamente ligada à expressão gênica. Mudanças na estrutura da cromatina influenciam a expressão dos genes, sendo que, estão inativos quando a cromatina está compactada e os genes são expressos quando a cromatina estiver aberta, ou seja, descompactada. Esses estados dinâmicos da cromatina são controlados por padrões epigenéticos reversíveis de metilação do DNA e de modificações das histonas (Rodenhiser, 2006; Feinberg, 2001).

A metilação do DNA é indispensável para as funções do genoma dos vertebrados e está relacionada com processos de regulação gênica, estabilidade cromossômica e *imprinting* parental. A metilação do DNA ocorre quando um grupo metil (CH3) é adicionado no carbono 5 de citosinas situados adjacentes a guanina (Sítio CpG). As partes de sequências de DNA com uma elevada concentração de CpG são referidos como 'ilhas' CpG, são localizadas, em sua maioria, nas regiões promotoras de genes e apresentam tamanho igual ou superior à 200 pares de bases (pb) sendo que há pelo menos 10 vezes mais metilação nessa região do que em outras regiões do genoma com CpG (Illingworth & Bird, 2009; Bird, 2002). O processo de metilação é mediado, ao menos, por três DNA metiltransferases (DNMT1, DNMT3a, DNMT3b), estas enzimas catalizam e transferem o grupamento metil da S-adenosyl-L-metionina (doador de metil) para as bases de citosina ou adenina na molécula de DNA. A metilação de DNA dentro da região promotora de um gene pode ser a explicação do porque essa modificação tem um efeito tão profundo na expressão gênica, podendo afetar a transcrição impedindo fisicamente a ligação de fatores de transcrição nos genes e/ou

recrutamento, através da ação das proteínas de ligação a metil-CpG (MBDs), componentes epigenéticos ao locus, como por exemplo histonas desacetilases e outras proteínas com ação sobre a remolodeção da cromatina, ocasionando uma compactação da cromatina (Robertson, 2005).

O DNA nuclear encontra-se associado à proteínas chamadas histonas. Ambos encontram-se sob a forma da estrutura básica de condensação do DNA, o nucleossomo. Este é composto por dois complexos idênticos, cada um constituído de 4 proteínas histonas, que formam um octâmero. As proteínas histonas presentes em cada nucleossomo são: a H2A, H2B, H3 e H4 (Pruss, Hayes & Wolffe, 1995). A modificação de histonas normalmente é pós- transcricional e pode ocorrer por vários processos, como por exemplo, acetilação, metilação, fosforilação e ubiquitinação. A acetilação de histonas é um processo reversível e necessita de um equilíbrio dinâmico entre duas enzimas principais que atuam no processo: as acetiltransferases (HATs) e as deacetilases (HDACs) (Van Den Hove, Kenis & Rutten, 2014).

Acredita-se que a acetilação das histonas H3 e H4 nas caudas N-terminais seja um sinal predominante para a ativação da cromatina, aumentando a acessibilidade para que ocorra a transcrição. Esse sinal é removido pela ação das histonas deacetilases (HDAC), que promovem a condensação da cromatina. As HATs atuam na transcrição do genoma em zonas específicas, acetilando resíduos de lisina, enquanto que as deacetilases atuam removendo os grupos acetil, no qual gera o silenciamento de genes (Strahl, 2000).

A falta de modificações da cromatina associada ao DNA metilado será rapidamente corrigida pelo recrutamento de enzimas modificadoras de cromatina (Nan, Cross & Bird, 1998; Nan et al., 1998). E a diminuição de metilação do DNA será corrigida pelo recrutamento de DNMts pela cromatina modificada (D´Alessio & Szyf, 2006). Existe uma interação entre as enzimas DNMTs e HDAC e também entre as DNMTs e metiltransferases de histonas para a manutenção de alguns padrões de metilação do DNA que marcam a cromatina inativa (Fuks et al., 2003; Rountree, Bachman & Baylin, 2000).

A acetilação das histonas atua como fator de demetilação do DNA e os mecanismos pelos quais isso ocorre são os seguintes: em uma visão mais simplista do processo epigenético, as histonas com caudas não acetiladas impediriam o acesso das DNAs metiltransferases para a cromatina compactada, e assim evitariam a metilação do DNA (Balch et al. 2005). Um segundo mecanismo propõe que a demetilação não

ocorreria imediatamente após a acetilação das histonas, mas seria dependente da interação com a enzima de transcrição RNA polimerase II com o promotor metilado do gene (D´Alessio & Szyf, 2006). Na DA a alteração mais comum são acetilações ou fosforilações das histonas (Portela & Esteller, 2010).

Na DA a acetilação de histonas no córtex temporal encontra-se diminuída, existindo também uma diminuição da acetilação da H3, esta diminuição leva a uma redução da expressão do regulador da atividade pós-sináptica (Millan, 2014). A H3 é um marcador de silenciamento de genes e regulador na compactação da cromatina, os níveis de histonas fosforiladas encontram-se aumentados em indivíduos com DA, afetando a distribuição da *tau* fosforilada. A modificação de histonas está também relacionada com o processo de hidrólise da APP em Aβ e na transcrição de genes reguladores da acetilação das histonas. A epigenética consegue integrar mecanismos de entrada diferentes e gerar os de saída mais longos e moldáveis, fornecendo assim um substrato para a localização da memória e por combinar a hipótese de interação entre o gene e o ambiente neste tipo de doença (Sanchez-Mut & Gräff, 2015).

Esta nova vertente da genética tem um grande impacto na DA, que se verifica na regulação da expressão gênica quando existe um estímulo ambiental exterior que afeta o SNC. São vários os estímulos ambientais que podem dar origem a alterações epigenéticas que levam ao surgimento e desenvolvimento da DA. Estudos recentes sugerem que estes estímulos e eventuais alterações epigenéticas são causas possíveis para o desenvolvimento da DA de início tardio, uma vez que essa forma da doença não tem nenhuma causa genética e por isso as alterações acima descritas podem ter uma grande influência a nível dos mecanismos pelos quais surge a DA (Maloney et al., 2012).

As modificações epigenéticas são uma das causas da doença de Alzheimer podendo, no entanto, ser utilizadas no seu tratamento. O desenvolvimento desta nova área da genética na DA parece trazer alguns avanços em nível de tratamento desta doença. São vários os estudos que apontam a epigenética como uma área de intervenção terapêutica. A interindividualidade relativamente à resposta a fármacos é uma dessas áreas. As classes mais utilizadas são os inibidores das DNMT e das HDACs, que têm como objetivo reverter as alterações epigenéticas (Cacabelos & Torrellas, 2014; Adwan & Zawia, 2013).

A terapia mais estudada é a que envolve a classe dos inibidores das HDACs. Estas enzimas na sua função normal são responsáveis pela acetilação das histonas,

quando estão inibidas regulam o nível de acetilação e melhoram a capacidade de memória e aprendizagem de um indivíduo. Na DA esta classe afeta a atividade da proteína Aβ e da *tau* (Adwan & Zawia, 2013, Lovatel et al., 2013).

1.3 BDNF

O fator neurotrófico derivado do cérebro (BDNF) é um intermediário dos processos dependentes da atividade no cérebro e tem um impacto muito grande no desenvolvimento neuronal e na plasticidade (Karpova, 2014). O BDNF desempenha um efeito protetor nos neurônios (Numakawa et al., 2010).

O BDNF é um gene-alvo da ativação de CREB, pertencente à família de neurotrofinas polipepitídicas que inclui o fato de crescimento neuronal (NGF), a neurotrofina-3 (NT3) e a neurotrofina 4/5 (NT4/5). É sintetizado inicialemente como um precursor com 32Kda, o pró BNDF, após a quebra intracelular origina a proteína madura de 14Kda, BDNFm (Mowla et al., 2001), podendo formar dímeros não-covalentes estáveis (Chao, 2003). Há duas vias de secreção de BDNF nos neurônios, a via dependente de regulação e a constitutiva. Na secreção pela via dependente de regulação, o BDNF é estocado em vesículas grandes que fundem-se à membrana plasmática em resposta do aumento do cálcio intracelular. Na via constitutiva, é armazenado em vesículas menores e gradativamente liberado por um processo de fusão independente de cálcio, o qual ocorre na ausência de qualquer evento específico. A liberação da neurotrofina pela via dependente de regulação requer o aumento do cálcio intracelular. A PKA pode regular a cinética de liberação vesicular do BDNF. Os estímulos capazes de liberar o BDNF são diversos, incluindo glutamato, estimulação elétrica, cafeína, entre outros, e essa heterogeneidade nas fontes de cálcio e de estímulos sugerem que diversas modalidades de secreção da neurotrofina podem co-existir (Kuczewski et al., 2009).

Esta proteína é importante não somente para o desenvolvimento normal do SNP e SNC, mas também pode desempenhar um papel fundamental na sobrevivência neuronal, plasticidade sináptica, orientação axonal, morfologia celular, formação de memória e cognição no cérebro adulto (Nettiksimmons et al., 2014; Balaratnasingman, 2012; Binder & Scharfman, 2004). A maior concentração do BDNF se encontra no sistema nervoso. O BDNF produzido em neurônios cerebrais age localmente em sinapses, e não é liberado e transportado do cérebro para a periferia (Partridge, 2002). O BDNF circulante nas plaquetas é produzido a partir de megacariócitos e armazenado em

grânulos α, que não recebem o seu conteúdo a partir de fontes externas (Chacón-Fernández et al., 2016).

A transdução do sinal de BDNF é mediada pela ligação a dois receptores transmembrana diferentes: o receptor de alta afinidade tirosina quinase B (TrkB), que reconhece espeficicamente o BDNF, e o receptor de neurotrofina de baixa afinidade p75, que reconhece outras neurotrofinas (Rex et al., 2007) As vias MAPK, PI3K, Cálcio-Calmodulina e AMPc-PKA regulam CREB, e esta ativação por sua vez leva a transcrição de BDNF (Tao et al., 1998).

O BDNF e seu receptor TrkB podem facilitar a formação de memória e a potencialização a longo prazo (Kemppainen et al., 2012). Diminuição da sinalização BDNF-TrkB resulta em uma memória espacial declinada (Minichiello, 2009), enquanto a sobreexpressão do TrkB de comprimento total aumenta a aprendizagem e a memória (Koponen et al., 2004).

Estudos mostram o BDNF está envolvido na potencialização de longo prazo (LTP) tanto na fase inicial quanto na tardia, na modulação da transmissão sináptica, na síntese de proteínas dendríticas, na formação de espinhas dendríticas e em modelos de animais de aprendizagem e memória (Heldt et al., 2007; Soule, Messaoudi & Bramham, 2006).

Especula-se que a variação do BDNF circulante pode refletir parcialmente a variância da secreção de BDNF no cérebro humano e os níveis reduzidos de BDNF no cérebro podem estar associados a níveis mais baixos de BDNF circulante. A redução da concentração de BDNF no hipocampo e no córtex cerebral pode induzir um processo neurodegenerativo no cérebro humano (Laske et al., 2006).

A concentração sérica de BDNF foi relatada como sendo significativamente reduzida em pacientes com demência grave comparando-se aos indivíduos controle (Schindowski, Berlardi & Buee, 2008; Laske et al., 2006). Adicionalmente, maiores níveis séricos de BDNF foram associados com melhor funcionamento neuropsicológico em idosos saudáveis (Driscoll et al., 2012; Gunstad et al., 2008). Alguns pesquisadores descobriram uma diminuição nas concentrações séricas de BDNF na DA (Zuccato et al., 2008). Além disso, em DA, há uma redução significativa do RNA mensageiro de BDNF (mRNA) (Zuccato et al., 2008; Holsinger et al., 2000; Howells et al., 2000). Verificou-se que os níveis de BDNF estavam significativamente associados negativamente aos escores na memória e função cognitiva (Fahnestock, 2011).

Outros estudos mostraram que os níveis séricos de BDNF se correlacionam positivamente com o desempenho em testes neuropsicológicos que investigam o funcionamento e a atenção (Costa et al., 2015). Além disso, o BDNF foi reconhecido como um inibidor potente da morte celular mediada por apoptose e da degeneração (Ziebel et al., 2012).

Os mecanismos exatos da regulação negativa do BDNF na DA não foram estabelecidos. Entretanto, foi demonstrado que a Aβ diminui a expressão de BDNF (Rosa & Fahnestock, 2015; DaRocha-Souto et al., 2012; Garzon & Fahnestock, 2007). Os níveis diminuídos de BDNF e os níveis diminuídos de fosforilação de CREB estão entre os biomarcadores mais frequentemente validados do transtorno depressivo (Autry & Monteggia, 2012). A sinalização BDNF é um modulador fundamental da formação de memória e pode estar envolvida na patologia da DA, que está associada com a produção, acumulação e agregação de Aβ (Fukumoto et al., 2014). As modificações epigenéticas no BDNF podem ser consideradas como um mecanismo de modulação do processo de formação de memória que é usado para ler a consolidação da memória em subtipos de genes específicos (Lubin et al., 2008).

1.4 Via cAMP/PKA/CREB

O cAMP (Monofosfato de Adenosina 3,5 cíclico) foi descoberto em 1958 por Earl Sutherland, da Case Western Reserve, como um "segundo" mensageiro intracelular que é ativado em resposta a certos hormônios - os "primeiros" mensageiros. O cAMP medeia quase todas as suas ações através de uma quinase - uma enzima que fosforila proteínas - chamada proteína quinase dependente de cAMP ou proteína quinase A (PKA) (Kandel, 2012).

A via de sinalização PKA é caracterizada por diversos tipos celulares e a PKA fosforila substratos que podem controlar vários fenômenos celulares. PKA é uma quinase serina/treonina que na sua forma inativa consiste em um tetrâmero composto por 2 subunidades regulatórias (R) e 2 subunidades catalíticas (C). Cada subunidade R contem 2 sítios de ligação para o cAMP. Após a ligação do cAMP ao sítio regulatório a dissociação das subunidades R e C ocorre e as 2 subunidades C são liberadas, possibilitando que elas catalisem a fosforilação de proteínas em resíduos regulatórios. Após ativar o receptor acoplado proteína G, a proteína trimerica Gαβγ dissocia-se na subunidade ativa Gα, ligada ao GTP. A Gα ligada ao GTP ativa a adenil ciclase (AC), a qual gera cAMP a partir do ATP. Na sequência, cAMP se liga às subunidades R da

PKA e provoca dissociação da holoenzima. Então as subunidades C podem fosforilar seus substratos. As fosfodiesterases (PDE) hidrolizam cAMP em AMP (Tasken & Aandahl, 2004).

Tanto a subunidade R quanto a subunidade C têm propriedades físicas e biológicas distintas, sendo diferentemente expressas e capazes de formar diferentes formas de PKA. Duas classes de isoenzimas da PKA, denominadas como tipo I e tipo II, foram identificadas e diferenciam-se no conteúdo das subunidades R, chamadas RI e RII, respectivamente. As subunidades R apresentam afinidades de ligação ao cAMP diferentes, com distintos limiares para ativação. A PKA tipo I possui maior afinidade de ligação para o cAMP, podendo ser ativada com concentrações menores. As subunidades R são expressas de maneira específica em diferentes células e tecidos e possuem capacidade de formar homodímeros e heterodímeros produzindo um elevado número de combinações, os quais contribuem para especificidade e diversidade da via de sinalização do cAMP (Tasken & Aandahl, 2004; Diviani & Scott, 2001). A ativação da PKA pode afetar uma ampla variedade de eventos celulares por fosforilação de proteínas citoplasmáticas e nucleares (Kopperud et al., 2003).

A regulação da expressão de genes de citocinas tem sido relacionada com a via cAMP/PKA. Os níveis elevados de cAMP intracelulares resulta em estimulação da PKA, fosforilação da proteína de ligação ao elemento responsivo ao cAMP (CREB) e ligação do CREB ao sítios do elemento responsivo ao cAMP (CRE) (Tan et al., 2007). Tem sido relatado que a via cAMP/PKA/CREB regulam a resposta inflamatória mediada pelo fator nuclear kappa B (Park et al., 2013).

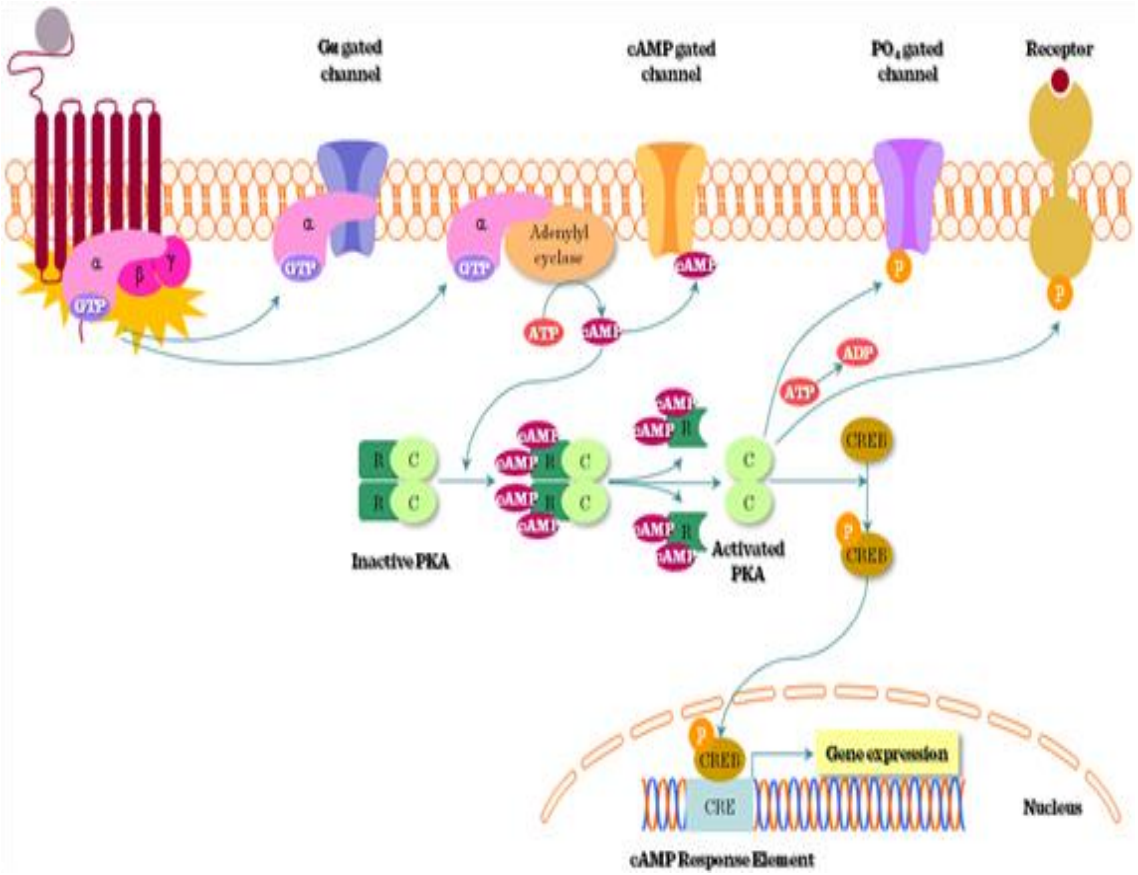

Figura 1. Ativação do CREB pela via AMPc/PKA.

Fonte:http://www.tankonyvtar.hu/hu/tartalom/tamop425/0011_1A_Jelatvitel_en_book/ch02s04.html

O CREB é um fator de transcrição ubíquo e constitutivamente expresso que tem sido amplamente envolvido na plasticidade sináptica de longa duração subjacente à aprendizagem e à memória (Kandel, 2012), bem como à sobrevivência neuronal (Walton & Dragunow, 2000). A proteína CREB tem múltiplos papéis em diferentes áreas cerebrais (Carlezon, Duman & Nestler, 2005), e sua sinalização tem sido estudada em ambos os aspectos afetivo e neurodegenerativa (Schmidt & Duman, 2010). O CREB é induzido por uma variedade de fatores de crescimento e sinais inflamatórios e medeia a transcrição de genes que contem o elemento responsivo ao cAMP (CRE) (Saura & Valero, 2011).

Para ativar a expressão de um gene alvo, a CREB requer fosforilação no resíduo de serina crítico 133, representando um ponto em que convergem várias vias de sinalização principais. Foi relatada a fosforilação em múltiplos locais de CREB por uma variedade de proteínas-cinases. A fosforilação do CREB aumenta sua ligação aos coativadores CBP (proteína de ligação CREB) e p300 (Vo & Goodman, 2001).

CBP/p300, por sua atividade histona acetiltransferase, promovem remodelamento da cromatina e o recrutamento da maquinaria basal de transcrição até os promotores contendo sítios CRE. Apontando a importância desses mecanismos via CREB para a cognição humana (Goodman & Smolik, 2000).

Embora numerosos estudos tenham demonstrado o requisito de transcrição mediada por CREB para consolidação da memória à longo prazo, os mecanismos pelos quais CREB facilita a memória não são totalmente elucidados. No entanto, a capacidade do CREB para induzir a expressão gênica do BDNF é visto como um constituinte chave da mecânica para seus efeitos na plasticidade sináptica e na função cognitiva (Pláteník et al., 2000). Semelhante ao CREB, diversos estudos demonstraram que o BDNF produz efeitos procognitivo através de seus efeitos neurotróficos levando a proliferação da neurite e sinaptogênese melhorada associada à neuroplasticidade (Xue et al., 2016). Como tal, o BDNF mostrou melhorar a aprendizagem em ambos os paradigmas de memória de curto e longo prazo (Thomas & Davies, 2005).

Foi descrito um papel deletério dos distúrbios da via de sinalização de CREB na alteração sináptica na DA. O péptido Aβ altera a plasticidade sináptica dependente do hipocampo e a memória e medeia a perda de sinapse através da via de sinalização CREB (Saura & Valero, 2011). Foi demonstrado que os inibidores de HDACs agem como ativadoras da transcrição regulada por CREB e moduladoras da neuroplasticidade mediada por cromatina (Fass et al., 2013).

2. OBJETIVOS

2.1 Objetivo geral

Investigar o efeito protetor do inibidor de HDAC SAHA em um modelo de DA induzida pela infusão intracerebroventricular (icv) de $A\beta_{1-42}$ em camundongos.

2.2 Objetivos específicos

Compreender a influência da epigenética na DA através do reconhecimento dos seus efeitos neuroprotetores, por meio da:

Análise dos efeitos do SAHA sobre a atividade de HDACs e HAT.

Análise dos efeitos do SAHA em estruturas encefálicas (córtex pré-frontal e hipocampo) de camundongos nos níveis do BDNF.

Análise dos efeitos do SAHA em estruturas encefálicas (córtex pré-frontal e hipocampo) de camundongos sobre modulação da via cAMP/PKA/CREB.

Verificação dos comportamentos cognitivos dos camundongos nos testes do labirinto aquático de Morris.

Neuroprotetic effect of suberoylanilide hydroxamic acid (SAHA), a HDAC inhibitor, in Alzheimer's disease model induced by β-amyloid peptide 1-42 in age mice

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ABSTRACT

Alzheimer's disease (AD) is a chronic neurodegenerative disease characterized clinically by the progressive loss of cognitive function, neuropsychiatric and behavioral disorders. The available therapeutic options improve the symptoms but do not impede the progression of the disease, therefore, an effective therapeutic strategy for AD is still lacking. There are studies related to the use of epigenetic therapy for the treatment of AD, the most developed therapy is that involving the class of histone deacetylase inhibitors (HDAC). This study investigated the neuroprotective effects of suberoilanilide hydroxamic acid (SAHA) on a model of AD induced by β-amyloid peptide 1-42 ($A\beta_{1-42}$) in aged mice. For this, we used Swiss mice that were divided into two experiments. In the first, the mice received an injection of $A\beta_{1-42}$ (icv) to investigate histones activity, neurotrophic factors, and modulation of the cAMP/PKA/CREB pathway in a time curve (6 hours, 1, 3, 7 and 21 days). In the second experiment the mice received SAHA (25 mg / kg, i.p.) for 21 days after administration of $A\beta_{1-42}$. We observed significant $A\beta_{1-42}$ deficiency in memory (MWM), as well as caused enzimatic imbalance of histones, reduced levels of cAMP, PKA, CREB, neurotrophic factors in prefrontal cortex and hippocampus of mice. Inhibition of HDAC through SAHA treatment showed significant neuroprotection against behavioral and neurochemical changes induced by $A\beta_{1-42}$. These data show that acetylation through inhibition of HDAC plays a key role in memory mediation. Thus, SAHA treatment demonstrated a promising medical tool and therapeutic approach for the treatment of AD.

KEYWORDS:

Alzheimer's disease; Epigenetic, memory, Histone deacetylase

Introduction

Alzheimer's disease (AD) was first described by German physician Alois Alzheimer in 1906 and is characterized by accumulation of amyloid plaques, *tau*-mediated neurodegeneration and cognitive impairment in the affected individuals (Petersen et al., 1995). The susceptibility to AD is the result of multiple environmental and genetic determinants that interact during life (Choi et al., 2014). Epigenetic changes are present from the prenatal stage of life and the phenotypic changes caused by these same changes are associated with the aging process, as well as in neurodegenerative diseases, such as AD. The main epigenetic alterations affecting the phenotype are DNA methylation, histone modification, chromatin modification and regulation of non-coding RNA (Zhu et al., 2015). Histone modification is usually post-transcriptional and may occur by various processes, for example, acetylation, methylation, phosphorylation and ubiquitination (Van Den Hove, Kenis and Rutten, 2014).

Histone acetylation is a reversible process and requires a dynamic balance between two main enzymes involved in the process: acetyltransferases (HATs) and deacetylases (HDACs) (Strahl & Allis, 2000). In DA, histone acetylation in the temporal cortex is decreased, and there is also a decrease in HAT acetylation, which leads to a reduction in the expression of the regulator of postsynaptic activity (Millan, 2014). Histone modification is also associated with the hydrolysis of APP in Aβ and the transcription of histone acetylation regulatory genes (Goldberg et al., 2007).

The cyclic AMP signaling pathway (cAMP) has been shown to play a crucial role in long-term memory formation (Lonze and Ginty, 2002). CAMP-dependent protein kinase A (PKA), when activated alosterically by cAMP, can phosphorylate the cAMP response element binding protein (CREB) on serine 133 (Gonzalez and Montminy, 1989). A recent study demonstrated that CREB increases short-term

memory through the regulation of brain-derived neurotrophic factor (BDNF), suggesting that CREB signaling is involved in short- and long-term memory formation (Suzuki et al., 2011) . It has been documented that CREB-mediated expression of BDNF and gene expression are drastically decreased in the hippocampus and in some cortical areas of AD patient and in $A\beta_{1-42}$-treated mice (Fukumoto et al., 2014; Tapia-Arancibia et al., 2008). Recent studies have shown that chromatin remodeling via histone acetylation regulates BDNF transcription. Interactions promoting acetylation are performed by the use of HDAC inhibitors, which increase BDNF levels during in vivo memory formation (Boulle et al., 2012; Lubin et al., 2008).

Thus, the aim of this study was to characterize the mechanisms by which the injection of the $A\beta_{1-42}$ peptide via the intracerebroventricular (i.c.v.) induces the cognitive deficiency and alteration of the neurochemical parameters. For this, the Morris Water Maze (MWM) task behavioral test was performed. We also evaluated the activity of histones HATs and HDAC, neurotrophic factors and regulation of the cAMP/PKA/CREB pathway in the prefrontal cortex and hippocampus of mice in a time curve. The inhibitor of HDAC SAHA (25 mg/kg intraperitoneally (i.p.)) was used to investigate the involvement of epigenetic modifications in the protection of $A\beta_{1-42}$-induced toxicity.

Materials and methods

Animals

Experiments were performed using female Swiss Albino mice (30-35g, 18-22 months old). Animals were maintained at 22-25ºC with free access to water and food, under a 12:12h light/dark cycle, with lights on at 7:00 a.m. All manipulations were carried out during light phase on the day. All efforts were made to minimize animal

suffering and to reduce the number of animals used. The procedures of this study were conducted according to the guidelines of the Committee on Care and Use of Experimental Animals Resources and with the approval of Ethical Committee for Animal Use (CEUA protocol #009/2015) of Federal University of Pampa, Brazil.

Experimental design

Two experiments were conducted. In the first experiment, mice (n=6 animals per group) received an intracerebroventriular (i.c.v.) injection of $A\beta_{1-42}$ to investigate the behavioral disturbances and neurochemical parameters in a time-course curve (times: 6h, 1, 3, 7 and 21 days) (**Fig. 1**).

In the second experiment, mice were divided into four groups (n=6 animals per group): vehicle + PBS (sham-operated); SAHA + PBS; Aβ + vehicle; and Aβ + SAHA. 21 days after the i.c.v. injection of $A\beta_{1-42}$ (peak effect). After the treatments, mice were subjected to behavioral tests. Afterwards, they were euthanized and the prefrontal cortex and hippocampus were removed to neurochemical determinations (**Fig. 2**).

Drug treatment protocol

$A\beta_{1-42}$ (Sigma-Aldrich) was prepared as stock solution at a concentration of 1 mg/ml in sterile 0.1M phosphate-buffered saline (PBS) (pH 7.4), and aliquots were stored at -20º C. $A\beta_{1-42}$ was aggregated by incubation at 37ºC for 4 days before use, as described previously (Souza et al., 2013). The aggregated form of $A\beta_{1-42}$ (400pmol/mouse) was administered by i.c.v. route, using a microsyringe with a 28-gauge stainless-steel needle 3.0 mm long (Hamilton), as described beforehand (Cioanca et al., 2014; Piermatini et al., 2010; Yan et al. 2001). Briefly, mice were anesthetized with intraperitoneal (i.p.) injection of sodium pentobarbital (0.067mg/g) and placed in a stereotaxic apparatus (Insight, Brazil). Under light anesthesia (i.e. just that necessary for

loss of the postural reflex), the needle was inserted unilaterally 1mm to the right of the midline point equidistant from each eye and 1mm posterior to a line drawn through the anterior base of the eyes (used as external reference). $A\beta_{1-42}$ was administered right-unillaterally into the lateral ventricule. The injection volume of 3µl of $A\beta_{1-42}$ or PBS was delivered gradually (1µl/min) using the following coordinates from bregma: anteroposterior (AP) = −0.1 mm, mediolateral (ML) = 1 mm, and dorsoventral (DV) = −3 mm. The sham-operated mice were injected with PBS (3µl/site; i.c.v.). The advantage of this i.c.v. route of administration is the quick distribution of the peptide throughout the brain (Chambon et al., 2011). In order to confirm the accurate placement of the injection site (needle track) at the moment of dissection of the animals, two mice in each group were submitted to dye injection (Evans blue dye, 0,5µl) into the ventricules (Davisson et al., 1998).

SAHA was first dissolved in DMSO at a dose of 50 mg / ml. This initial solution was diluted 1:10 every day in saline (final concentration: 5 mg / ml) prior to intraperitoneal injections at a dose of 25 mg/kg body weight. The injections was administered twice daily at 12-hour intervals, during 21 days after Aβ injection.

Behavioral assessment

During a period of 2 days after i.c.v. injection of $A\beta_{1-42}$ (starting 6 hs, 1, 3, 7 and 21 days after $A\beta_{1-42}$ injection in experiment 1; and starting 21 days after $A\beta_{1-42}$ injection in experiment 2), the animals were submitted to a battery of behavioral paradigms that include Morris Water Maze (MWM) task (day 1). At on day 1, the Morris Water Maze task was also used as the training. All tests were carried out between 9:00 and 16:00h and they were scored by the same trained raters (who were blind to the experimental

treatments) in an observation room where the mice had been habituated for at least 1 h before the beginning of the tests.

Tissue Preparation for neurochemical determinations

After behavioral tests, mice were euthanized with barbiturate overdose (pentobarbital sodium 150 mg/kg; i.p. route) and transcardiacally perfused with 10 ml ice-cold saline via the aorta. The brain dissection was performed according to the method of Spijker (2011), a method to dissect multiple brain regions from a single brain based on existing atlases (Williams, 1999). Prefrontal cortex and hippocampus were bilaterally removed and rapidly homogenized in 50 mM Tris–Cl, pH 7.4. The homogenate was centrifuged at 2,400×*g* for 15 min at 4ºC and a low-speed supernatant fraction (S1) was used for assays.

Morris Water Maze (MWM) task

The apparatus was made of black painted fibreglass, 97 cm in diameter and 60 cm in height. For the tests, the tank was filled with water maintained at 23±2 ◦C. The target platform (10 cm×10 cm) was made of transparent Plexiglas and it was submerged 1–1.5 cm beneath the surface of the water. Starting points for animals were marked on the outside of the pool as north (N), south (S), east (E) and west (W). Four distant visual cues (55 cm×55 cm) were placed on the walls of the water maze room. They were all positioned with the lower edge 30 cm above the upper edge of the water tank and in the standard setting, the position of each symbol marked the midpoint of the perimeter of a quadrant (circle =NE quadrant, square = SE quadrant, cross = SW quadrant and diamond =NW quadrant). The apparatus was located in a room with indirect incandescent illumination. Mice were submitted to a spatial reference memory version of the water maze using a protocol that was described by Prediger et al (2007). The

training session consisted of 10 consecutive trials during which the animals were left in the tank facing the wall and then allowed to swim freely to the submerged platform. The platform was located in a constant position (middle of the southwest quadrant), equidistant from the centre and the wall of the pool. If the animal did not find the platform during a period of 60 s, it was gently guided to it. The animal was allowed to remain on the platform for 10 s after escaping to it and was then removed from the tank for 20 s before being placed at the next starting point in the tank. This procedure was repeated 10 times, with the starting points (the axis of one imaginary quadrant) varying in a pseudo-randomized manner. The test session was carried out 24 h later and consisted of a single probe trial where the platform was removed from the pool and each mouse was allowed to swim for 60 s in the maze. Behavioral data were recorded and analyzed using ANY-maze video tracking software (Stoelting Co., IL, USA). Latency to target platform location and the time spent in correct quadrant (i.e. where the platform was located on the training session) were computed for subsequent analysis.

Brain-derived neurotrophic factor (BDNF) levels

Levels of BDNF were measured using a BDNF E_{max} ImmunoAssay System kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. The supernatants were collected for determination of BDNF levels. The content of BDNF levels was demonstrated as pg/µg total protein in the tissue.

Real-time reverse transcription-PCR

Each frozen brain sample was homogenized, all the RNA was extracted using an RNeasy® total RNA isolation kit (Qiagen, Valencia, CA), and converted into cDNA using a SuperScript III First-Strand Synthesis System for RT-PCR kit (Invitrogen Life

Technologies). The primers used were as follows: BDNF (GenBank accession number BC034862), forward primer, 5′-GCAAACATGTCTATGAGGGTTCG-3′, and reverse primer, 5′-ACTCGCTAATACTGTCACACACG-3′; TaqMan probe, 5′-ACTCCGACCCTGCCCGCCGT-3′, and β-actin (GenBank accession number NM007393), forward primer, 5′-GGGCTATGCTCTCCCTCACG-3′, and reverse primer, 5′-GTCACGCACGATTTCCCTCTC-3′; TaqMan probe, 5′-CCTGCGTCTGGACCTGGCTGGC-3′. PCRs were performed using the Platinum Taq DNA polymerase (Invitrogen). The reaction profile consisted of the first round at 95 °C for 3 min, followed by 40 cycles of denaturation at 95 °C for 10 s, annealing at 60 °C for 34 s, and extension at 72 °C for 1 min, with the final extension reaction carried out at 72 °C for 10 min in an iCycle iQ Detection System (Bio-Rad Laboratories, Hercules, CA). To standardize the quantification, β-actin was amplified simultaneously. Expression levels were calculated by the delta-delta Ct method.

The cAMP assay

cAMP levels were measured using the mouse cAMP ELISA kit (R&D System China Co., Ltd), according to the manufacturer's protocol. Briefly, the tissue samples were rinsed with PBS and homogenized in cold 0.1 N HCl, at a 1:5 ratio (w/v). The homogenates of each sample were centrifuged at 10,000 rpm for 15 min at 4 C to remove the particles. The supernatants were neutralized with 1 N NaOH and then collected for the ELISA assay. Absorbance was measured at 450 nm with a microplate reader.

Determination of Global HDAC Enzyme Activity

The global HDAC enzyme activity was determined using an HDAC Assay Kit (Fluorometric Detection catalog #17-372, Upstate Biotechnology, Temecula, CA, USA) according to the manufacturer's instructions. Briefly, the samples were incubated with the substrate at 30 _C for 45 min before addition of the activator solution. The mixtures were incubated at room temperature for additional 10 min and HDAC enzyme activity was measured on a microplate reader (excitation: 360 nm, emission: 450 nm).

Determination of HAT Enzyme Activity

The HAT enzyme activity was determined using an ELISA HAT Assay Kit (Colorimetric Detection catalog #K332-100, Biovision, Milpitasa CA, USA) according to the manufacturer's instructions. The mix, containing 5 ll of sample, HAT buffer, substrate and NAD generating enzyme, was incubated for 1 h 30 min. The HAT enzyme activity was evaluated in a microplate reader at 440 nm.

Protein determination

Protein concentration was measured by the method of Bradford, using bovine serum albumin as the standard.

Statistical analysis

The data distribution was verified by applying the Kolmogorov-Smirnov test. Results are presented as the mean ± standard error of the mean (SEM). Comparisons between the experimental and the control groups were performed by one-way (time course = independent variable) or two-way ANOVA ($A\beta_{1-42}$ X SAHA treatment = independent variables) followed by Bonferroni post hoc test, when appropriate. A value

of $P < 0.05$ was considered to be statistically significant. All tests were carried out using the GraphPad software 5.0 (San Diego, California, USA).

Results

The i.c.v. injection of $A\beta_{1-42}$ induced desbalance of HAT/HDAC in the prefrontal cortex and hippocampus

In response to $A\beta_{1-42}$ injection a significant increase in HDAC activity was found in the hippocampus when compared to group control. Post hoc comparisons showed that the injection of $A\beta_{1-42}$ increased HDAC activity at 6 hours, 1, 3, 7 and 21 days. The peak effect of HDAC activity occurred at 3, 7 and 21 days time point ($p < 0.001$, $p < 0.001$ and $p < 0.001$, respectively) **(Fig. 3**A). An increase in HDAC activity was also found in prefrontal cortex when compared to group control. Post hoc comparisons revealed that the maximal effect occurred 3, 7 and 21 days after $A\beta_{1-42}$ injection ($p < 0.001$, $p < 0.001$ and $p < 0.001$, respectively) **(Fig. 3**B).

When compared to group control, Aβ-treated mice showed a significant decrease in HAT H3 activity in the hippocampus. Post hoc comparisons showed that the injection of $A\beta_{1-42}$ decreased HAT H3 activity at 6 hours, 1, 3, 7 and 21 days. The peak effect of HAT H3 acitivity occurred at 3, 7, and 21 days ($p < 0.01$, $p < 0.01$ and $p < 0.001$, respectively) **(Fig. 3**C). A similar pattern of HAT H3 activity was found in the prefrontal cortex with a most significant decrease in this brain region in Aβ-treated mice at 3, 7 and 21 days post administration ($p < 0.01$, $p < 0.01$ and $p < 0.001$, respectively) **(Fig. 3**D).

In response to $A\beta_{1-42}$ injection a significant decrease in HAT H4 activity was found in the hippocampus when compared to group control. Post hoc comparisons

showed that the injection of $A\beta_{1-42}$ decreased HAT H4 activity at 6 hours, 1, 3, 7 and 21 days. The peak effect of HAT H4 activity occurred at 3, 7 and 21 days time point ($p < 0.01$, $p < 0.01$ and $p < 0.001$, respectively) **(Fig. 3**E). In similar fashion, a significant decrease in HAT H4 activity was found in the prefrontal cortex at 6 hours, 1, 3, 7 and 21 days, again with maximal effect at 3, 7 and 21 days post $A\beta_{1-42}$ injection ($p < 0.001$, $p < 0.001$ and $p < 0.001$, respectively) **(Fig. 3**F).

The i.c.v. injection of $A\beta_{1-42}$ elicited a down-regulation of neurotrophic factors in the prefrontal cortex and hippocampus

When compared to group control, Aβ-treated mice showed a significant decrease in BDNF levels in the hippocampus. Post hoc comparisons showed that the injection of $A\beta_{1-42}$ decreased BDNF levels at 6 hours, 1, 3, 7 and 21 days of the time points. The peak effect of BDNF levels occurred at 3, 7 and 21 days ($p < 0.01$, $p < 0.001$ and $p < 0.001$, respectively) **(Fig. 4**A). A similar pattern of BDNF levels was found in the prefrontal cortex with a most significant decrease in this brain region in Aβ-treated mice at 3, 7 and 21 days post injection ($p < 0.01$, $p < 0.01$ and $p < 0.001$, respectively) **(Fig. 4**B).

In response to $A\beta_{1-42}$ injection, expression of BDNF mRNA was significantly reduced in the hippocampus when compared to group control. Post hoc comparisons showed that the injection of $A\beta_{1-42}$ decreased expression of BDNF mRNA at 6 hours, 1, 3, 7 and 21 days. The peak effect of expression of BDNF mRNA occurred at 21 days time point ($p < 0.05$, $p < 0.05$ and $p < 0.01$, respectively) **(Fig. 4**C). In similar fashion, in the prefrontal cortex, reduced of expression of BDNF mRNA was found at 21 days post administration ($p < 0.05$, $p < 0.01$ and $p < 0.01$, respectively) **(Fig. 4**D).

The i.c.v. injection of Aβ$_{1-42}$ elicited a down-regulation on the pathway cAMP /PKA/ CREB in the prefrontal cortex and hippocampus

When compared to group control, Aβ-treated mice showed a significant decrease in CREB levels in the hippocampus. Post hoc comparisons showed that the injection of Aβ$_{1-42}$ decreased CREB levels at 6 hours, 1, 3, 7 and 21 days. The peak effect of CREB levels occurred at 3, 7 and 21 days ($p < 0.01$, $p < 0.01$ and $p < 0.01$, respectively) (**Fig. 5**A). A similar pattern of CREB levels was found in the prefrontal cortex with a most significant decrease in this brain region in Aβ-treated mice at 3, 7 and 21 days post administration ($p < 0.05$, $p < 0.01$ and $p < 0.01$, respectively) **(Fig. 5**B).

In response to Aβ$_{1-42}$ injection a significant decrease in cAMP levels was found in the hippocampus when compared to group control. Post hoc comparisons showed that the injection of Aβ$_{1-42}$ decreased cAMP levels at 6 hours, 1, 3, 7 and 21 days. The peak effect of cAMP levels occurred at 3, 7 and 21 days time point ($p < 0.05$, $p < 0.01$ and $p < 0.01$, respectively) **(Fig. 5**C). An decrease in cAMP levels was also found in prefrontal cortex when compared to group control. Post hoc comparisons revealed that the maximal effect occurred 1, 3, 7 and 21 days after Aβ$_{1-42}$ injection ($p < 0.05$, $p < 0.05$, $p < 0.001$ and $p < 0.001$, respectively) **(Fig. 5**D).

When compared to group control, Aβ-treated mice showed a significant decrease in PKA levels in the hippocampus. Post hoc comparisons showed that the injection of Aβ$_{1-42}$ decreased PKA levels at 6 hours, 1, 3, 7 and 21 days of the time points. The peak effect of PKA levels occurred at 3, 7 and 21 days ($p < 0.01$, $p < 0.01$ and $p < 0.01$, respectively) (**Fig. 5**E). A similar pattern of PKA levels was found in the prefrontal

cortex with a most significant increase in this brain region in Aβ-treated mice at 3, 7 and 21 days post injection ($p < 0.01$, $p < 0.01$ and $p < 0.01$, respectively) **(Fig. 5**F).

The i.c.v. injection of $A\beta_{1-42}$ induced behavioral disturbances

Injection of Aβ1-42 21 days before the MWM, reduced of time spent in correct quadrant when compared to group control. Post hoc comparisons demonstrated that the injection of $A\beta_{1-42}$ decreased of time spent in correct quadrant at 3, 7 and 21 days of the time points. The peak effect occurred at 3, 7 and 21 days ($p < 0.05$, $p < 0.001$ and $p < 0.001$, respectively) **(Fig. 6**A). A similar pattern of escape latency to target platform location most significant decrease in Aβ-treated mice at 3, 7 and 21 days post injection ($p < 0.001$, $p < 0.001$ and $p < 0.001$, respectively) **(Fig. 6**B).

Enzymatic activity of histones

HDAC actitivity

Statistical analysis of HDAC activity in hippocampus revealed a $A\beta_{1-42}$ × SAHA interaction ($F_{1,24} = 0.45$, $p < 0.60$) and a significant effect of SAHA ($F_{1,24} = 15.91$, $p < 0.001$) and $A\beta_{1-42}$ ($F_{1,24} = 11.88$, $p < 0.004$) (**Fig. 7**A). Post hoc comparisons demonstrated that $A\beta_{1-42}$ significantly increase HDAC activity in hippocampus of mice compared to control group. SAHA treatment reversed the increase of HDAC activity induced by $A\beta_{1-42}$.

Two-way ANOVA of HDAC activity in prefrontal cortex revealed a $A\beta_{1-42}$ × SAHA interaction ($F_{1,24} = 9.32$, $p < 0.01$) and a significant effect of SAHA ($F_{1,24} =$

25.69, $p < 0.001$) and $A\beta_{1-42}$ ($F_{1,24} = 40.10$, $p < 0.001$). Post hoc comparisons demonstrated that $A\beta_{1-42}$ significantly increase HDAC activity in prefrontal cortex of mice compared to control group. Treatment with SAHA promoted the attenuation of the increase of HDAC levels induced by $A\beta_{1-42.}$ SAHA decreased the HDAC activity in prefrontal cortex of effect *per se* (**Fig. 7**B). Pearson's correlation tests demonstrated significant positive correlation (Time in correct quadrant X HDAC activity in the HP and Latency to target plataform location X HDAC activity in the PFC and in the HP) and showed negative correlation (Time in correct quadrant X HDAC activity in the PFC) (Table 1).

HAT H3 activity

Statistical analysis of HAT H3 activity in hippocampus revealed a $A\beta_{1-42}$ × SAHA interaction ($F_{1,24} = 6.27$, $p < 0.02$) and a significant effect of SAHA ($F_{1,24} = 32.71$, $p < 0.001$) and $A\beta_{1-42}$ ($F_{1,24} = 23.71$, $p < 0.001$) (**Fig. 7**C). Post hoc comparisons showed that $A\beta_{1-42}$ significantly decrease HAT H3 activity in prefrontal cortex of mice compared to control group. SAHA treatment prevented the decrease of HAT H3 activity induced by $A\beta_{1-42.}$

Two-way ANOVA of HAT H3 activity in prefrontal cortex revealed a $A\beta_{1-42}$ × SAHA interaction ($F_{1,24} = 8.98$, $p < 0.01$) and a significant effect of SAHA ($F_{1,24} = 37.70$, $p < 0.001$) and $A\beta_{1-42}$ ($F_{1,24} = 42.60$, $p < 0.001$). Post hoc comparisons yield that $A\beta_{1-42}$ significantly decrease HAT H3 activity in prefrontal cortex of mice compared to control group. Treatment with SAHA protected the decrease of HAT H3 activity induced by $A\beta_{1-42.}$ SAHA increased the HAT H3 activity in prefrontal cortex of effect *per se* (**Fig. 7**D). Pearson's correlation tests demonstrated significant positive correlation

(Time in correct quadrant X HAT H3 activity in the PFC and in the HP and Latency to target plataform location X HAT H3 activity in the HP) and showed negative correlation (Latency to target plataform location X HAT H3 activity in the PFC) (Table 1).

HAT H4 activity

Statistical analysis of HAT H4 activity in hippocampus revealed a significant $A\beta_{1\text{-}42}$ × SAHA interaction ($F_{1,24} = 10.01$, $p < 0.008$) and a significant effect of SAHA ($F_{1,24} = 19.59$, $p < 0.001$) and $A\beta_{1\text{-}42}$ ($F_{1,24} = 24.68$, $p < 0.001$) (**Fig. 7**E). Post hoc comparisons demonstrated that $A\beta_{1\text{-}42}$ significantly decrease HAT H4 activity in prefrontal cortex of mice compared to control group. SAHA treatment protected the decrease of HAT H4 activity induced by $A\beta_{1\text{-}42.}$

Two-way ANOVA of HAT H4 activity in prefrontal cortex revealed a significant $A\beta_{1\text{-}42}$ × SAHA interaction ($F_{1,24} = 6.91$, $p < 0.02$) and a significant effect of SAHA ($F_{1,24} = 87.38$, $p < 0.001$) and $A\beta_{1\text{-}42}$ ($F_{1,24} = 150.90$, $p < 0.001$). Post hoc comparisons showed that $A\beta_{1\text{-}42}$ significantly decrease HAT H4 activity in prefrontal cortex of mice compared to control group. Treatment with SAHA reversed the decrease of HAT H4 levels induced by $A\beta_{1\text{-}42.}$ SAHA increased the HAT H4 activity in prefrontal cortex of effect *per se* (**Fig. 7**F). Pearson's correlation tests demonstrated significant positive correlation (Time in correct quadrant X HAT H4 activity in the PFC and in the HP) and showed negative correlation (Latency to target plataform location X HAT H4 activity in the PFC and in the HP) (Table 1).

Biomarkers of neurotropic protection

BDNF levels

Statistical analysis of $A\beta_{1\text{-}42}$ × SAHA interaction ($F_{1,24}$ = 6.48, p < 0.02) and a significant effect of SAHA ($F_{1,24}$ = 63.83, p < 0.001) and $A\beta_{1\text{-}42}$ ($F_{1,24}$ = 171.08, p < 0.001) in BDNF levels in hippocampus of mice was observed. Post hoc comparisons demonstrated that $A\beta_{1\text{-}42}$ significantly decreased BDNF levels in hippocampus of mice compared to control group. Treatment with SAHA protected against the decrease of BDNF levels caused by $A\beta_{1\text{-}42}$ (**Fig. 8**A).

Two-way ANOVA of BDNF levels in prefrontal cortex yielded a significant $A\beta_{1\text{-}42}$ × SAHA interaction ($F_{1,24}$ = 0.15, p < 0.70) and a significant effect of SAHA ($F_{1,24}$ = 10.03, p < 0.008) and $A\beta_{1\text{-}42}$ ($F_{1,24}$ = 63.39, p < 0.001). Post hoc comparisons showed that $A\beta_{1\text{-}42}$ significantly decreased BDNF levels in prefrontal cortex of mice compared to control group. SAHA treatment prevented against the decrease of BDNF levels caused by $A\beta_{1\text{-}42}$. SAHA increased BDNF levels in prefrontal cortex of effect *per se* (**Fig. 8**B). Pearson's correlation tests demonstrated significant positive correlation (Time in correct quadrant X BDNF levels in the PFC and in the HP) and yielded negative correlation (Latency to target plataform location X BDNF levels in the PFC and in the HP) (Table 1).

Expression of BDNF mRNA

A significant $A\beta_{1\text{-}42}$ × SAHA interaction ($F_{1,24}$ = 0.17, p < 0.63) and a significant effect of SAHA ($F_{1,24}$ = 12.82, p < 0.003) and $A\beta_{1\text{-}42}$ ($F_{1,24}$ = 22.37, p < 0.001) in mRNA BDNF expression in hippocampus of mice was observed. Post hoc comparisons

demonstrated that $A\beta_{1-42}$ significantly decreased expression of BDNF mRNA in hippocampus of mice compared to control group. SAHA treatment protected against the decrease of expression of BDNF mRNA caused by $A\beta_{1-42}$ (**Fig. 8**A).

Two-way ANOVA of mRNA BDNF expression in prefrontal cortex yielded a significant $A\beta_{1-42}$ × SAHA interaction ($F_{1,24} = 1.32$, $p < 0.27$) and a significant effect of SAHA ($F_{1,24} = 17.94$, $p < 0.001$) and $A\beta_{1-42}$ ($F_{1,24} = 57.78$, $p < 0.001$). Post hoc comparisons demonstrated that $A\beta_{1-42}$ significantly decreased expression of BDNF mRNA in prefrontal cortex of mice compared to control group. SAHA treatment protected against the decrease of expression of BDNF mRNA caused by $A\beta_{1-42.}$ SAHA itself increased expression of BDNF mRNA in prefrontal cortex of effect *per se* (**Fig. 8**B). Pearson's correlation tests demonstrated significant positive correlation (Time in correct quadrant X expression of BDNF mRNA and in the PFC and in the HP) and showed negative correlation (Latency to target plataform location X expression of BDNF mRNA in the PFC and in the HP) (Table 1).

cAMP/PKA/CREB pathway

CREB levels

Statistical analysis of CREB levels in hippocampus revealed a significant effect of SAHA ($F_{1,24} = 72.57$, $p < 0.003$) and $A\beta_{1-42}$ ($F_{1,24} = 80.71$, $p < 0.001$) (**Fig. 9**A). Post hoc comparisons demonstrated that $A\beta_{1-42}$ significantly decrease CREB levels in prefrontal cortex of mice compared to control group. SAHA treatment prevented the decrease of CREB levels induced by $A\beta_{1-42.}$

Two-way ANOVA of CREB levels in prefrontal cortex revealed a $A\beta_{1-42}$ × SAHA interaction ($F_{1,24} = 8.80$, $p < 0.01$) and a significant effect of SAHA ($F_{1,24} = 5.50$, $p < 0.03$) and $A\beta_{1-42}$ ($F_{1,24} = 44.87$, $p < 0.001$). Post hoc comparisons demonstrated that $A\beta_{1-42}$ significantly decrease CREB levels in prefrontal cortex of mice compared to control group. SAHA treatment reversed the decrease of CREB levels induced by $A\beta_{1-42}$. SAHA itself increased CREB levels in prefrontal cortex of effect *per se* (**Fig. 9**B). Pearson's correlation tests demonstrated significant positive correlation (Time in correct quadrant X CREB levels in the PFC and in the HP) and yielded negative correlation (Latency to target plataform location X CREB levels in the PFC and in the HP) (Table 1).

cAMP levels

Statistical analysis of cAMP levels in hippocampus revealed a significant $A\beta_{1-42}$ × SAHA interaction ($F_{1,24} = 14.92$, $p < 0.002$) and a significant effect of SAHA ($F_{1,24} = 10.59$, $p < 0.006$) and $A\beta_{1-42}$ ($F_{1,24} = 9.16$, $p < 0.01$) (**Fig. 9**C). Post hoc comparisons demonstrated that $A\beta_{1-42}$ significantly decrease cAMP levels in prefrontal cortex of mice compared to control group. And the tretament with the inhibitor of HDAC attenuated the decrease of cAMP levels induced in the model of AD.

Two-way ANOVA of cAMP levels in prefrontal cortex revealed a $A\beta_{1-42}$ × SAHA interaction ($F_{1,24} = 20.98$, $p < 0.001$) and a significant effect of SAHA ($F_{1,24} = 11.68$, $p < 0.005$) and $A\beta_{1-42}$ ($F_{1,24} = 8.00$, $p < 0.01$). Post hoc comparisons demonstrated that $A\beta_{1-42}$ significantly decrease cAMP levels in prefrontal cortex of mice compared to control group. Treatment with SAHA reversed the decrease of cAMP levels induced by $A\beta_{1-42}$. SAHA itself increased cAMP levels in prefrontal cortex of effect *per se* (**Fig.**

9D). Pearson's correlation tests demonstrated significant positive correlation (Time in correct quadrant X cAMP levels in the PFC and in the HP) and showed negative correlation (Latency to target plataform location X cAMP levels in the PFC and in the HP) (Table 1).

PKA levels

A significant $A\beta_{1\text{-}42}$ × SAHA interaction ($F_{1,24}$ = 18.24, p < 0.001) and a significant effect of SAHA ($F_{1,24}$ = 7.68, p < 0.01) and $A\beta_{1\text{-}42}$ ($F_{1,24}$ = 6.30, p < 0.02) in PKA levels in hippocampus of mice was observed. Post hoc comparisons demonstrated that $A\beta_{1\text{-}42}$ significantly decreased PKA levels in hippocampus of mice compared to control group. Treatment with SAHA protected against the decrease of PKA levels caused by $A\beta_{1\text{-}42}$ (**Fig. 9**E).

Two-way ANOVA of PKA levels in prefrontal cortex yielded a $A\beta_{1\text{-}42}$ × SAHA interaction ($F_{1,24}$ = 15.69, p < 0.001) and a significant effect of SAHA ($F_{1,24}$ = 12.31, p < 0.004) and $A\beta_{1\text{-}42}$ ($F_{1,24}$ = 6.77, p < 0.02). Post hoc comparisons demonstrated that $A\beta_{1\text{-}42}$ significantly decreased PKA levels in prefrontal cortex of mice compared to control group. SAHA treatment showed protective effect against the decrease of PKA levels caused by $A\beta_{1\text{-}42}$. SAHA itself increased PKA levels in prefrontal cortex of effect *per se* (**Fig. 9**F). Pearson's correlation tests demonstrated significant positive correlation (Time in correct quadrant X PKA levels in the PFC and in the HP) and yielded negative correlation (Latency to target plataform location X PKA levels in the PFC and in the HP) (Table 1).

Spatial memory in MWM test

Spatial memory was measured in a probe test conducted 24h after the last training trial. During this test the platform was removed and the escape latencies and time in correct (target) quadrant was computed.

Two-way ANOVA of time spent in correct quadrant showed a significant $A\beta_{1-42}$ × SAHA interaction ($F_{1.24} = 8.08$ $p < 0.001$) and a significant effect of SAHA ($F_{1.24} = 8.35$; $p < 0.001$) and $A\beta_{1-42}$ ($F_{1.24} = 5.06$; $p < 0.004$). Post hoc comparisons revealed that $A\beta_{1-42}$ significantly decreased the time spent in correct quadrant of Aβ-treated mice compared to control group. Treatment with SAHA totally reversed the decrease in time spent in correct quadrant caused by $A\beta_{1-42}$ (**Fig. 10**A).

Statistical analysis of scape latency of training sessions showed that $A\beta_{1-42}$ caused a spatial learning impairment in Aβ-treated mice compared to control group. On the other hand, treatment with SAHA caused an acquisition improvement in Aβ-treated mice (**Fig. 10**B).

Two-way ANOVA of escape latency to target platform location yielded a significant $A\beta_{1-42}$ × SAHA interaction ($F_{1,24} = 5.69$, $p < 0.04$) and a significant effect of SAHA ($F_{1,24} = 31.23$, $p < 0.001$) and $A\beta_{1-42}$ ($F_{1,24} = 34.80$, $p < 0.001$). Post hoc comparisons demonstrated that $A\beta_{1-42}$ significantly increased the escape latency of Aβ-treated mice compared to control group. Treatment with SAHA significantly protected against the increase in scape latency elicited by $A\beta_{1-42}$ (**Fig. 10**B).

Discussion

In the present study, we demonstrated that SAHA treatment protected against Aβ1-40-induced neurotoxicity in mice. Acute injection of the $A\beta_{1-42}$ peptide induced

cognitive impairment accompanied by histone enzymatic imbalance, reduced levels of BDNF and expression of its mRNA and negative modulation on the cAMP/PKA/CREB pathway in the prefrontal cortex and hippocampus of mice. Treatment with SAHA (25 mg/kg, for 21 days) reversed spatial memory impairment and other damage caused by $A\beta_{1-42}$ exposure, which probably occurred through modulation of the cAMP/PKA/CREB pathway, BDNF and its mRNA expression and regulation in the enzymatic activity of histones.

Synaptic plasticity is fundamental for the formation and storage of memory. Histone acetylation has been implicated in synaptic plasticity and learning and memory. Protein levels and enzymatic activity of HAT and HDAC regulate chromatin status and transcription factor acetylation, and during normal conditions the balance of HAT/HDAC is maintained very stringently (Spindler et al., 2014; Choi and Howe, 2009; Jiang et al., 2003). However, any damage that disrupts this balance, and thus acetylation levels, alters cellular homeostasis. The imbalance of HAT/HDAC represents a critical and decisive factor commonly underlying catastrophic neuronal dysfunction and degeneration (Elsner et al., 2011; Saha and Pahan, 2006). Indeed, decreased histone acetylation has been described in several cellular and in vivo neurodegeneration models (Saha and Pahan, 2006; Rouaux et al., 2003), and this event has been associated with an increase in HDAC, as well as a decrease in HAT activities (Bahari-Javan, Sananbenesi and Fischer, 2014; Saha and Pahan, 2006). Indicating possible beneficial effects resulting from pharmacological treatments that increase the activity of HATs and / or dominate the action of HDACs. In our study, the time course curve showed that injection of $A\beta_{1-42}$ induced a reduction in enzymatic activity of HAT (H3 and H4) and increase the enzymatic activity of HDAC enzymes in the prefrontal cortex and hippocampus, with a peak effect at 21 day time point. Supporting the idea that

neurodegenerative disorders cause imbalance in the enzymatic activity of HATs and HDAC (Lu et al., 2015; Saha and Pahan, 2006). In contrast, the inhibitor SAHA was able to protect against the imbalance in the enzymatic activity of histone HAT and HDAC in the prefrontal cortex and hippocampus of mice caused by the injection of $A\beta_{1-42}$, demonstrating that HDAC inhibitors increase histone acetylation, consequently, potentiating memory and synaptic plasticity and ameliorating cognitive deficits in AD.

Neurotrophic factors are proteins that play an important role in regulating all aspects of the development and function of neural circuits in the mammalian brain, including neuronal survival, synaptic plasticity, neurogenesis, memory consolidation and the pathophysiology of AD (Budni et al., Al., 2015, Sopova et al., 2014). In addition, in AD, there is a significant reduction of BDNF messenger RNA (mRNA) (Zuccato et al., 2008; Garzon & Fahnestock, 2007) that is strongly implicated in memory loss and cognitive function (Fahnestock, 2011). Epigenetic modifications in BDNF can be considered as a modulation mechanism of the memory formation process that is used to consolidate memory into specific gene subtypes (Lubin et al., 2008). In our study, the time course curve showed that i.c.v. injection of $A\beta_{1-42}$ induced a neurotrophic deficiency revealed by reduction of BDNF levels and expression of mRNA in the prefrontal cortex and hippocampus of mice, with a peak effect at 21 days of time. Therefore, we suggest that neurotrophic deficiency in the prefrontal cortex and hippocampus due to the decrease in BDNF levels and mRNA expression. Our results demonstrate a correlation between the low levels of BDNF with spatially allocated memory in response to $A\beta_{1-42}$ injection, as already reported in other studies where the reduction of BDNF levels is related to impairment of the memory and learning in mice treated with $A\beta_{1-42}$ (Fukumoto et al., 2014; Cunha et al., 2009). The SAHA treatament was able to protect against the decrease of BDNF levels and of the expression of BDNF

mRNA in the prefrontal cortex and hippocampus of mice caused by the of $A\beta_{1-42}$ injection. Thus, our results provide primary evidence that SAHA, through the inhibition of the HDAC enzyme, can regulate BDNF levels and mRNA expression in the brain of mice, modulating an important pathway for neuronal and cognitive protection against the neurotoxic effects of Aβ peptide through epigenetic modulations.

The cAMP has been shown to play a crucial role in long-term memory formation (Lonze and Ginty, 2002). CAMP-dependent protein kinase A (PKA), when activated alosterically by cAMP, can phosphorylate the cAMP response element binding protein (CREB) on serine 133 (Gonzalez and Montminy, 1989). CREB is a ubiquitous and constitutively expressed transcription factor that has been extensively involved in the long-term synaptic plasticity underlying learning and memory (Kandel, 2012) as well as neuronal survival (Walton and Dragunow, 2000). It is believed that CREB phosphorylation is responsible for transcriptional activation, leading to the production of numerous gene products, such as BDNF. It has been documented that CREB-mediated expression of BDNF and gene expression are drastically decreased in the hippocampus and in some cortical areas of AD patient and in $A\beta_{1-42}$ treated mice (Fukumoto et al., 2014; Gong et al., 2009; Tapia-Arancibia et al., 2008). In our study, the time course curve showed that i.c.v. injection of $A\beta_{1-42}$ negatively affected the modulation of the cAMP/PKA/CREB pathway revealed by the reduction of cAMP, PKA and CREB levels in the prefrontal cortex and the hippocampus of mice, with a peak effect at 21 days of time. Therefore, we suggest that this negative effect the cAMP/PKA/CREB pathway that occurs in response to $A\beta_{1-42}$ injection is related to the neurotrophic deficiency observed. It has been demonstrated that the pharmacological inhibition of the HDAC enzyme through SAHA treatment was able to protect against decreased levels of cAMP, PKA and CREB in the prefrontal cortex of mice caused by

the injection of $A\beta_{1-42}$. Therefore, these data support the hypothesis that epigenetic modulation through HDAC inhibitors may favor the phosphorylation of CREB through by increasing the access of chromatin-bound CREB to PKA, modulating the cAMP/PKA/CREB pathway important for the synaptic plasticity and memory in which they are affected by the Aβ peptide.

Spatial memory is critical to survival because it relates to its ability to encode, store and retrieve information about locations in the environment (Teixeira, 2013). The MWM is usually accepted as an indicator of spatial learning and reference memory, which reflects long-term memory (Morris, 1984; Souza et al., 2015). The present study demonstrated that following injection of $A\beta_{1-42}$ peptide on day 21 a negative peak in spatial learning and memory impairment was observed during the water labyrinth task. In training trials (acquisition trials), $A\beta_{1-42}$ treated mice exhibited a progressive increase in escape latency to find the platform. $A\beta_{1-42}$ treated mice were observed in the probing assay (retention assay) to show an increase in escape latency for the target platform location and a reduction in the percentage of time spent in the right quadrant, supporting the role of HDAC in the learning and memory dysfunction. We showed that SAHA treatment reversed the impaired learning and memory of mice caused by the injection of $A\beta_{1-42}$ in MWM. The pharmacological inhibition of the HDAC enzyme, thus favoring the histone acetylation, has been shown to prevent the development of spatial memory impairment in $A\beta_{1-42}$ -treated mice. Thus, our data presents the evidence that SAHA may prevent cognitive deficits in an experimental model of AD.

Conclusion

In conclusion, it can be concluded that SAHA, through the inhibition of the HDAC enzyme, avoided the behavioral and neurobiological deficits induced by the $A\beta_{1-42}$ peptide and suggest that these neuroprotective effects probably involve the

hyperacetylation of the HAT enzymes and the positive regulation of BDNF, mRNA of BDNF and the cAMP/PKA/CREB pathway in the brain of mice. Suggesting that epigenetic modulation through HDAC inhibitors may provide a novel therapeutic approach for the treatment of AD.

References

BAHARI-JAVAN, Sanaz; SANANBENESI, Farahnaz; FISCHER, Andre. Histone-acetylation: a link between Alzheimer's disease and post-traumatic stress disorder?. **Frontiers in neuroscience**, v. 8, 2014.

BOULLE, F. et al. Epigenetic regulation of the BDNF gene: implications for psychiatric disorders. 2012.

BUDNI, Josiane et al. The involvement of BDNF, NGF and GDNF in aging and Alzheimer's disease. **Aging and disease**, v. 6, n. 5, p. 331, 2015.

CHOI, Jennifer K.; HOWE, LeAnn J. Histone acetylation: truth of consequences? This paper is one of a selection of papers published in this Special Issue, entitled CSBMCB's 51st Annual Meeting–Epigenetics and Chromatin Dynamics, and has undergone the Journal's usual peer review process. **Biochemistry and Cell Biology**, v. 87, n. 1, p. 139-150, 2009.

CHOI, Y.H.; KWON, H.S.; SHIN, S.G.; CHUNG, C.K. Vaccinium uliginosum L. Improves Amyloid β Protein-Induced Learning and Memory Impairment in Alzheimer's Disease in Mice. **Preventive Nutrition Food Science**, v. 19, p. 343-347, 2014.

CUNHA, Carla et al. Brain-derived neurotrophic factor (BDNF) overexpression in the forebrain results in learning and memory impairments. **Neurobiology of disease**, v. 33, n. 3, p. 358-368, 2009.

ELSNER, V. R. et al. Effect of different exercise protocols on histone acetyltransferases and histone deacetylases activities in rat hippocampus. **Neuroscience**, v. 192, p. 580-587, 2011.

FAHNESTOCK, Margaret. Brain-derived neurotrophic factor: the link between amyloid-β and memory loss. **Future Neurology**, v. 6, n. 5, p. 627-639, 2011.

FUKUMOTO, Kazuya et al. Fingolimod increases brain-derived neurotrophic factor levels and ameliorates amyloid β-induced memory impairment. **Behavioural brain research**, v. 268, p. 88-93, 2014.

GARZON, Diego J.; FAHNESTOCK, Margaret. Oligomeric amyloid decreases basal levels of brain-derived neurotrophic factor (BDNF) mRNA via specific downregulation of BDNF transcripts IV and V in differentiated human neuroblastoma cells. **Journal of Neuroscience**, v. 27, n. 10, p. 2628-2635, 2007.

GOLDBERG, Aaron D.; ALLIS, C. David; BERNSTEIN, Emily. Epigenetics: a landscape takes shape. **Cell**, v. 128, n. 4, p. 635-638, 2007.

GONG, Pingyuan et al. Effect of BDNF Val66Met polymorphism on digital working memory and spatial localization in a healthy Chinese Han population. **Journal of Molecular Neuroscience**, v. 38, n. 3, p. 250-256, 2009.

GONZALEZ, Gustavo A.; MONTMINY, Marc R. Cyclic AMP stimulates somatostatin gene transcription by phosphorylation of CREB at serine 133. **Cell**, v. 59, n. 4, p. 675-680, 1989.

JIANG, Guanchao et al. Testing the position-effect variegation hypothesis for facioscapulohumeral muscular dystrophy by analysis of histone modification and gene expression in subtelomeric 4q. **Human molecular genetics**, v. 12, n. 22, p. 2909-2921, 2003.

KANDEL, Eric R. The molecular biology of memory: cAMP, PKA, CRE, CREB-1, CREB-2, and CPEB. **Molecular brain**, v. 5, n. 1, p. 14, 2012.

LONZE, Bonnie E.; GINTY, David D. Function and regulation of CREB family transcription factors in the nervous system. **Neuron**, v. 35, n. 4, p. 605-623, 2002.

LU, Xi et al. Histone acetylation modifiers in the pathogenesis of Alzheimer's disease. **Frontiers in cellular neuroscience**, v. 9, 2015.

LUBIN, Farah D.; ROTH, Tania L.; SWEATT, J. David. Epigenetic regulation of BDNF gene transcription in the consolidation of fear memory. **Journal of Neuroscience**, v. 28, n. 42, p. 10576-10586, 2008.

MILLAN, Mark J. The epigenetic dimension of Alzheimer's disease: causal, consequence, or curiosity. **Dialogues in Clinical Neuroscience**, v. 16, n. 3, p. 373-393, 2014.

MORRIS, Richard. Developments of a water-maze procedure for studying spatial learning in the rat. **Journal of neuroscience methods**, v. 11, n. 1, p. 47-60, 1984.

PETERSEN, Ronald C. et al. Apolipoprotein E status as a predictor of the development of Alzheimer's disease in memory-impaired individuals. **Jama**, v. 273, n. 16, p. 1274-1278, 1995.

ROUAUX, Caroline et al. Critical loss of CBP/p300 histone acetylase activity by caspase-6 during neurodegeneration. **The EMBO journal**, v. 22, n. 24, p. 6537-6549, 2003.

SAHA, R. N.; PAHAN, K. HATs and HDACs in neurodegeneration: a tale of disconcerted acetylation homeostasis. **Cell Death & Differentiation**, v. 13, n. 4, p. 539-550, 2006.

SOPOVA, Kateryna et al. Dysregulation of neurotrophic and haematopoietic growth factors in Alzheimer's disease: from pathophysiology to novel treatment strategies. **Current Alzheimer Research**, v. 11, n. 1, p. 27-39, 2014.

SOUZA LC. et al. Flavonoid Chrysin prevents age-related cognitive decline via attenuation of oxidative stress and modulation of BDNF levels in aged mouse brain. **Pharmacol. Biochem. Behav**. 134, 22-30 (2015).

SPINDLER, Christiano et al. Treadmill exercise alters histone acetyltransferases and histone deacetylases activities in frontal cortices from wistar rats. **Cellular and molecular neurobiology**, v. 34, n. 8, p. 1097-1101, 2014.

STRAHL, Brian D.; ALLIS, C. David. The language of covalent histone modifications. **Nature**, v. 403, n. 6765, p. 41-45, 2000.

SUZUKI, Akinobu et al. Upregulation of CREB-mediated transcription enhances both short-and long-term memory. **Journal of Neuroscience**, v. 31, n. 24, p. 8786-8802, 2011.

TAPIA-ARANCIBIA, Lucia et al. New insights into brain BDNF function in normal aging and Alzheimer disease. **Brain research reviews**, v. 59, n. 1, p. 201-220, 2008.

TEIXEIRA, Lívia Clemente Motta. **Exercício físico, neurogênese e memória**. 2013. Tese de Doutorado. Universidade de São Paulo.

VAN DEN HOVE, Daniel LA; KENIS, Gunter; RUTTEN, Bart PF. Epigenetic dysregulation in Alzheimer's disease: cause or consequence?. 2014.

WALTON, Marshall R.; DRAGUNOW, Mike. Is CREB a key to neuronal survival?. **Trends in neurosciences**, v. 23, n. 2, p. 48-53, 2000.

ZHU, Y.-P. et al. Epigenetic Modification and Its Role in Alzheimer's Disease. **Integrative Medicine International**, v. 2, n. 1-2, p. 63-72, 2015.

ZUCCATO, Chiara et al. Systematic assessment of BDNF and its receptor levels in human cortices affected by Huntington's disease. **Brain pathology**, v. 18, n. 2, p. 225-238, 2008.

Legends

Fig. 1. Overview of protocol of experimental procedures in a time-course curve.

Fig. 2. Overview of protocol of experimental procedures.

Fig.3. Effect of $A\beta_{1-42}$ (400 pmol; 3µl/site; i.c.v.) on the enzimatic activity of HDAC (A and B), HAT H3 (C and D), HAT H4 (E and F) in the hippocampus (A, C and E) and prefrontal cortex (B, D and F) of mice in groups tested 6h, 1 day, 3 days, 7 days and 21 days after $A\beta_{1-42}$ injection. Values are mean ± S.E.M. (n=6). *P<0.05, **P<0.01, ***P<0.001 compared with the sham group (One-way ANOVA, Tukey post hoc test).

Fig.4. Effect of $A\beta_{1-42}$ (400 pmol; 3µl/site; i.c.v.) on the levels of BDNF (A and B), Relative mRNA expression (C and D), in the hippocampus (A and C) and prefrontal cortex (B and D) of mice in groups tested 6h, 1 day, 3 days, 7 days and 21 days after $A\beta_{1-42}$ injection. Values are mean ± S.E.M. (n=6). *P<0.05, **P<0.01, ***P<0.001 compared with the sham group (One-way ANOVA, Tukey post hoc test).

Fig.5. Effect of $A\beta_{1-42}$ (400 pmol; 3µl/site; i.c.v.) on the levels of CREB (A and B), cAMP (C and D), PKA (E and F) in the hippocampus (A, C and E) and prefrontal cortex (B, D and F) of mice in groups tested 6h, 1 day, 3 days, 7 days and 21 days after $A\beta_{1-42}$ injection. Values are mean ± S.E.M. (n=6). *P<0.05, **P<0.01, ***P<0.001 compared with the sham group (One-way ANOVA, Tukey post hoc test).

Fig.6. Effect of $A\beta_{1-42}$ (400 pmol; 3µl/site; i.c.v.) on recognition in the time in correct quadrant (A), latency to target plataform location (B) in MWM test in mice in groups

tested 6h, 1 day, 3 days, 7 days and 21 days after $A\beta_{1-42}$ injection. Values are mean ± S.E.M. (n=6). *P<0.05, **P<0.01, ***P<0.001 compared with the sham group (Two-way ANOVA, Bonferroni post hoc test).

Fig.7. Effect of SAHA (25mg/kg, i.p.) and injection of $A\beta_{1-42}$ (400pmol/mice, i.c.v.) on HDAC activity (A: hippocampus; B: prefrontal cortex) HAT H3 activity (C: hippocampus; D: prefrontal cortex) and HAT H4 activity (E: hippcocampus; F: prefrontal cortex). Values are mean ± S.E.M. (n=6 per group). *** P<0.05 when compared vehicle/$A\beta_{1-42}$ with vehicle/Sham. * P<0.05 when compared SAHA/Sham with Vehicle/Sham. # P<0.05 when compared SAHA/$A\beta_{1-42}$ with vehicle/$A\beta_{1-42}$ (two-way ANOVA and Bonferroni multiple comparison test).

Fig.8. Effect of SAHA (25mg/kg, i.p.) and injection of $A\beta_{1-42}$ (400pmol/mice, i.c.v.) on BDNF levels (A: hippocampus; B: prefrontal cortex) and relative mRNA expression (C: hippocampus; D: prefrontal cortex). Values are mean ± S.E.M. (n=6 per group). *** P<0.05 when compared vehicle/$A\beta_{1-42}$ with vehicle/Sham. * P<0.05 when compared SAHA/Sham with Vehicle/Sham. # P<0.05 when compared SAHA/$A\beta_{1-42}$ with vehicle/$A\beta_{1-42}$ (two-way ANOVA and Bonferroni multiple comparison test).

Fig.9. Effect of SAHA (25mg/kg, i.p.) and injection of $A\beta_{1-42}$ (400pmol/mice, i.c.v.) on CREB levels (A: hippocampus; B: prefrontal cortex) cAMP levels (C: hippocampus; D: prefrontal cortex) and PKA levels (E: hippcocampus; F: prefrontal cortex). Values are mean ± S.E.M. (n=6 per group). *** P<0.05 when compared vehicle/$A\beta_{1-42}$ with vehicle/Sham. * P<0.05 when compared SAHA/Sham with Vehicle/Sham. # P<0.05

when compared SAHA/Aβ_{1-42} with vehicle/Aβ_{1-42} (two-way ANOVA and Bonferroni multiple comparison test).

Fig.10. Effect of SAHA (25mg/kg, i.p.) and injection of Aβ_{1-42} (400pmol/mice, i.c.v.) on time in correct quadrant (A), latency to target platform location (B), in MWM test in mice. Values are mean ± S.E.M. (n=6 per group). *** P<0.05 when compared vehicle/Aβ_{1-42} with vehicle/Sham. # P<0.05 when compared SAHA/Aβ_{1-42} with vehicle/Aβ_{1-42} (two-way ANOVA and Bonferroni multiple comparison test).

Figura 1

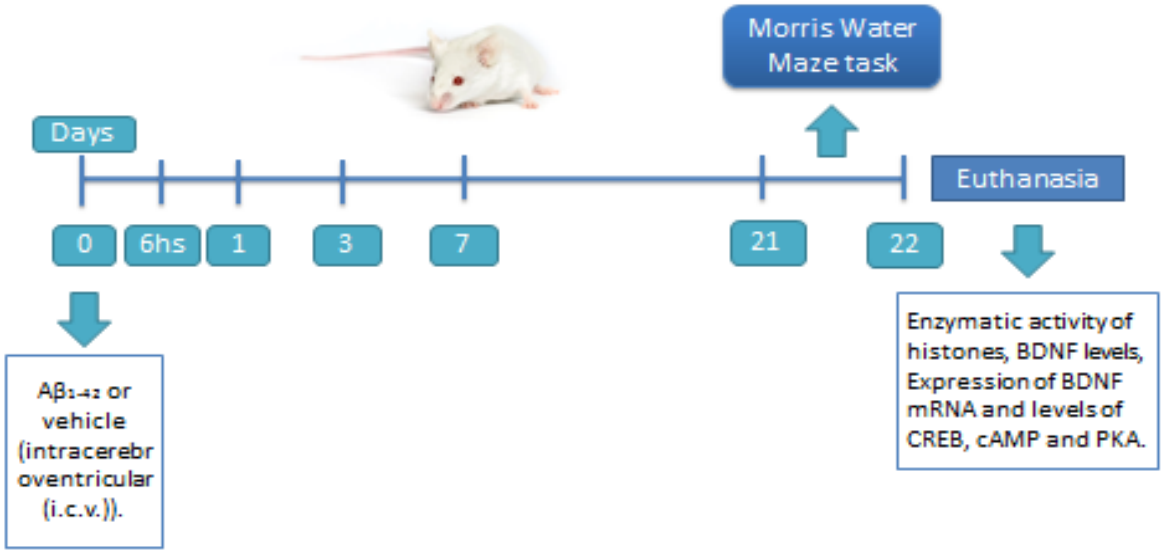
Morris Water Maze task
Days
Euthanasia
0
6hs
1
3
7
21
22
Aβ1-42 or vehicle (intracerebroventricular (i.c.v.)).
Enzymatic activity of histones, BDNF levels, Expression of BDNF mRNA and levels of CREB, cAMP and PKA.

Figure 2

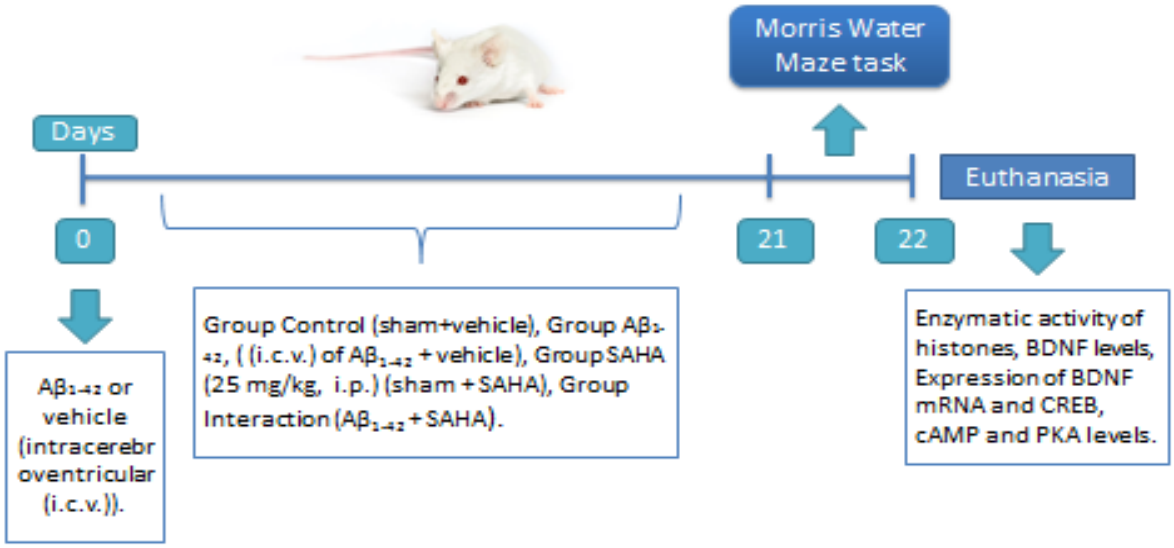
Morris Water Maze task
Days
Euthanasia
0
21
22
Aβ1-42 or vehicle (intracerebroventricular (i.c.v.)).
Group Control (sham+vehicle), Group Aβ1-42, ((i.c.v.) of Aβ1-42 + vehicle), Group SAHA (25 mg/kg, i.p.) (sham + SAHA), Group Interaction (Aβ1-42 + SAHA).
Enzymatic activity of histones, BDNF levels, Expression of BDNF mRNA and CREB, cAMP and PKA levels.

Figure 3

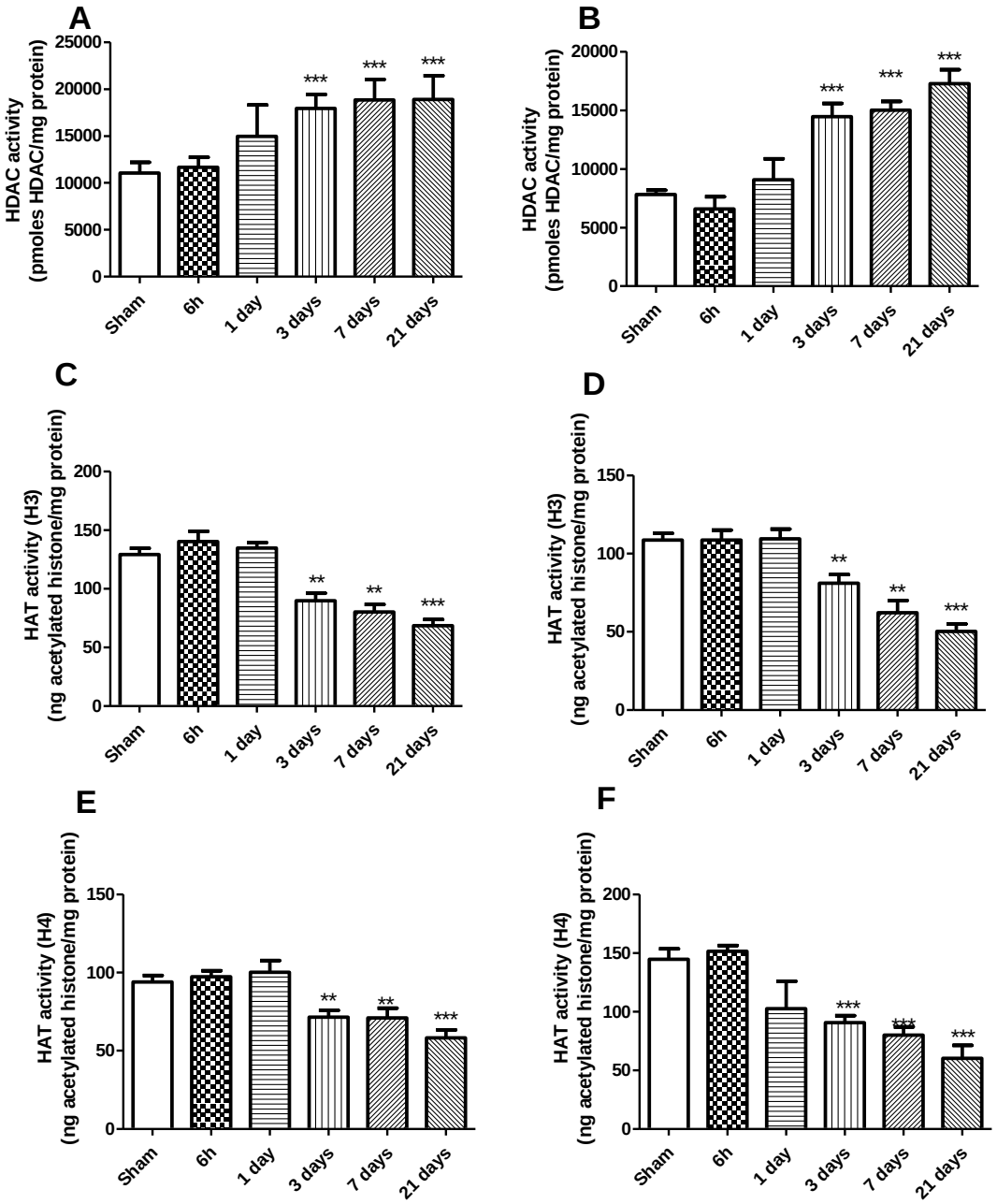
A
HDAC activity
(pmoles HDAC/mg protein)
25000
20000
15000
10000
5000
0
***
***
***
Sham
6h
1 day
3 days
7 days
21 days
B
HDAC activity
(pmoles HDAC/mg protein)
20000
15000
10000
5000
0
***
***
***
Sham
6h
1 day
3 days
7 days
21 days
C
HAT activity (H3)
(ng acetylated histone/mg protein)
200
150
100
50
0
**
**
***
Sham
6h
1 day
3 days
7 days
21 days
D
HAT activity (H3)
(ng acetylated histone/mg protein)
150
100
50
0
**
**
***
Sham
6h
1 day
3 days
7 days
21 days
E
HAT activity (H4)
(ng acetylated histone/mg protein)
150
100
50
0
**
**
***
Sham
6h
1 day
3 days
7 days
21 days
F
HAT activity (H4)
(ng acetylated histone/mg protein)
200
150
100
50
0
***
***
***
Sham
6h
1 day
3 days
7 days
21 days

Figure 4

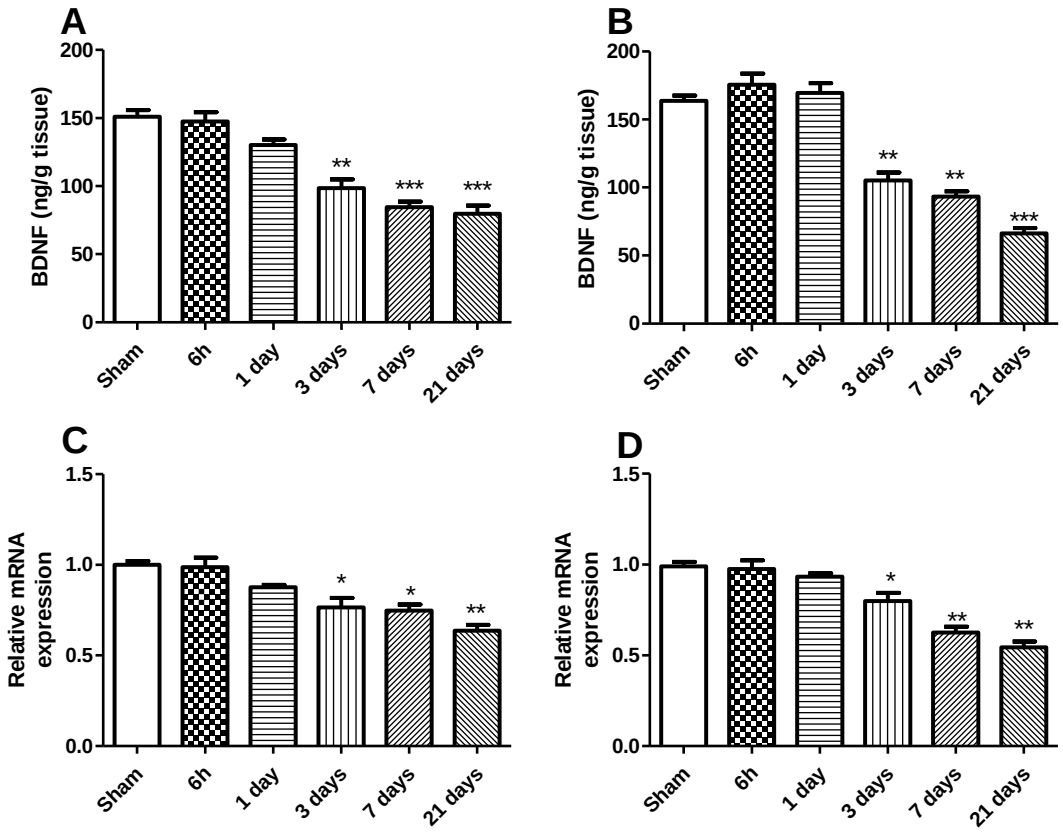
A
BDNF (ng/g tissue)
200
150
100
50
0
**
***
***
Sham
6h
1 day
3 days
7 days
21 days
B
BDNF (ng/g tissue)
200
150
100
50
0
**
**
***
Sham
6h
1 day
3 days
7 days
21 days
C
Relative mRNA expression
1.5
1.0
0.5
0.0
*
*
**
Sham
6h
1 day
3 days
7 days
21 days
D
Relative mRNA expression
1.5
1.0
0.5
0.0
*
**
**
Sham
6h
1 day
3 days
7 days
21 days

Figure 5

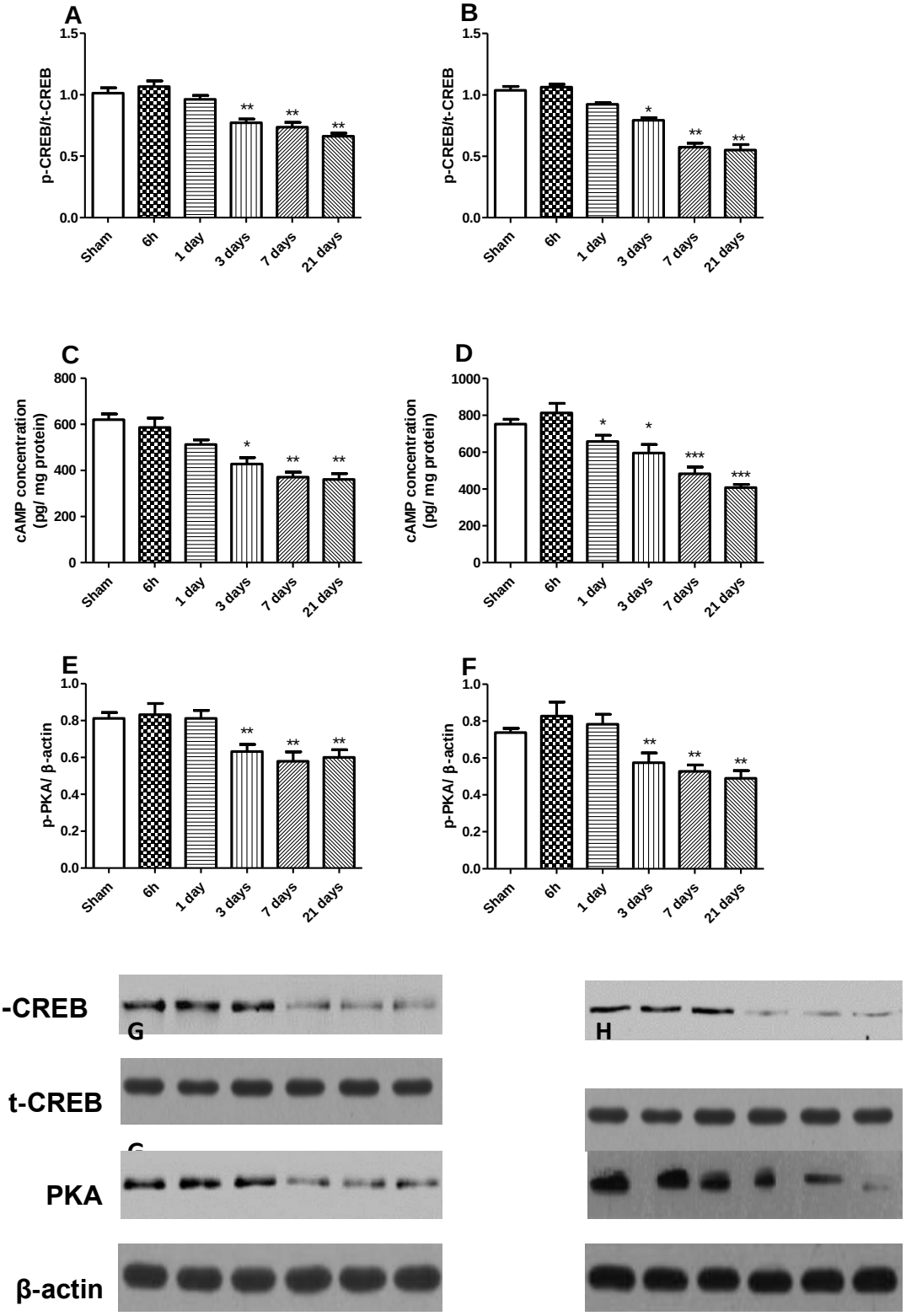
A
p-CREB/t-CREB
1.5
1.0
0.5
0.0
Sham
6h
1 day
3 days
7 days
21 days
B
p-CREB/t-CREB
C
cAMP concentration (pg/ mg protein)
800
600
400
200
0
D
cAMP concentration (pg/ mg protein)
1000
E
p-PKA/ β-actin
F
p-PKA/ β-actin
p-CREB
t-CREB
PKA
β-actin
G
H

Figure 6

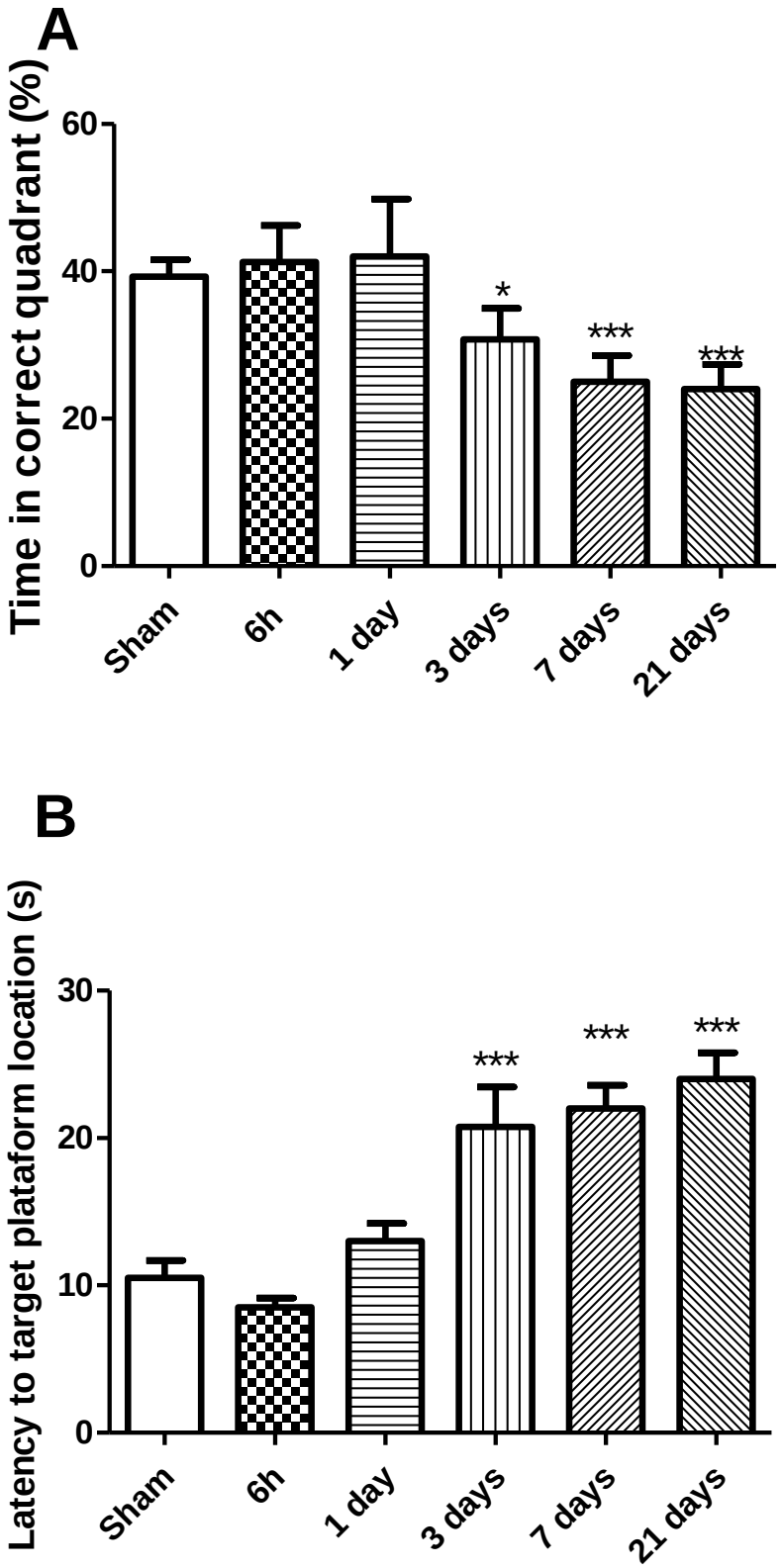
A
Time in correct quadrant (%)
60
40
20
0
*
***
***
Sham
6h
1 day
3 days
7 days
21 days
B
Latency to target plataform location (s)
30
20
10
0
***
***
***
Sham
6h
1 day
3 days
7 days
21 days

Figure 7

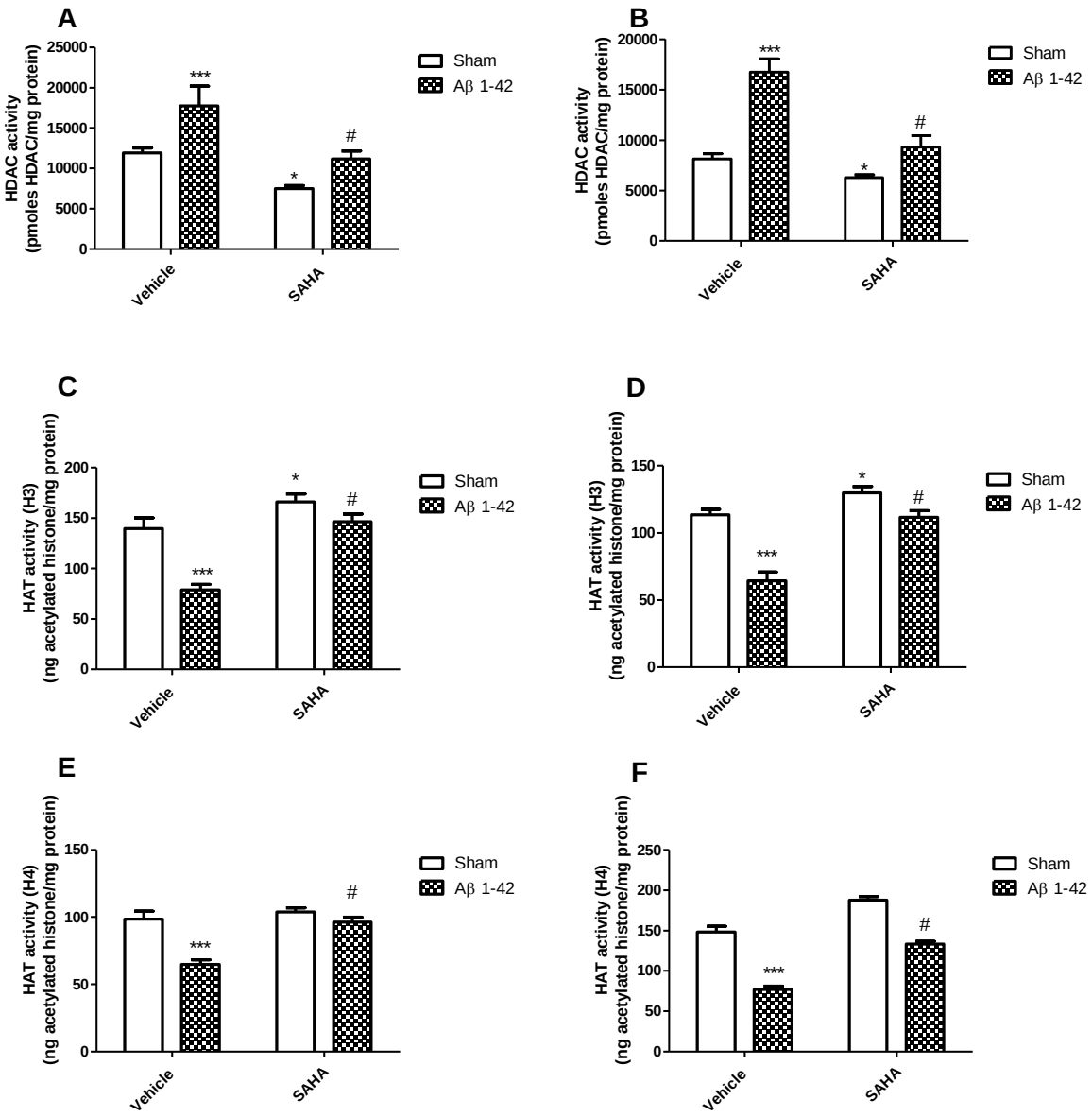
A
HDAC activity
(pmoles HDAC/mg protein)
25000
20000
15000
10000
5000
0
***
*
#
Vehicle
SAHA
Sham
Aβ 1-42
B
HDAC activity
(pmoles HDAC/mg protein)
20000
15000
10000
5000
0
***
*
#
Vehicle
SAHA
Sham
Aβ 1-42
C
HAT activity (H3)
(ng acetylated histone/mg protein)
200
150
100
50
0
***
*
#
Vehicle
SAHA
Sham
Aβ 1-42
D
HAT activity (H3)
(ng acetylated histone/mg protein)
150
100
50
0
***
*
#
Vehicle
SAHA
Sham
Aβ 1-42
E
HAT activity (H4)
(ng acetylated histone/mg protein)
150
100
50
0
***
#
Vehicle
SAHA
Sham
Aβ 1-42
F
HAT activity (H4)
(ng acetylated histone/mg protein)
250
200
150
100
50
0
***
#
Vehicle
SAHA
Sham
Aβ 1-42

Figure 8

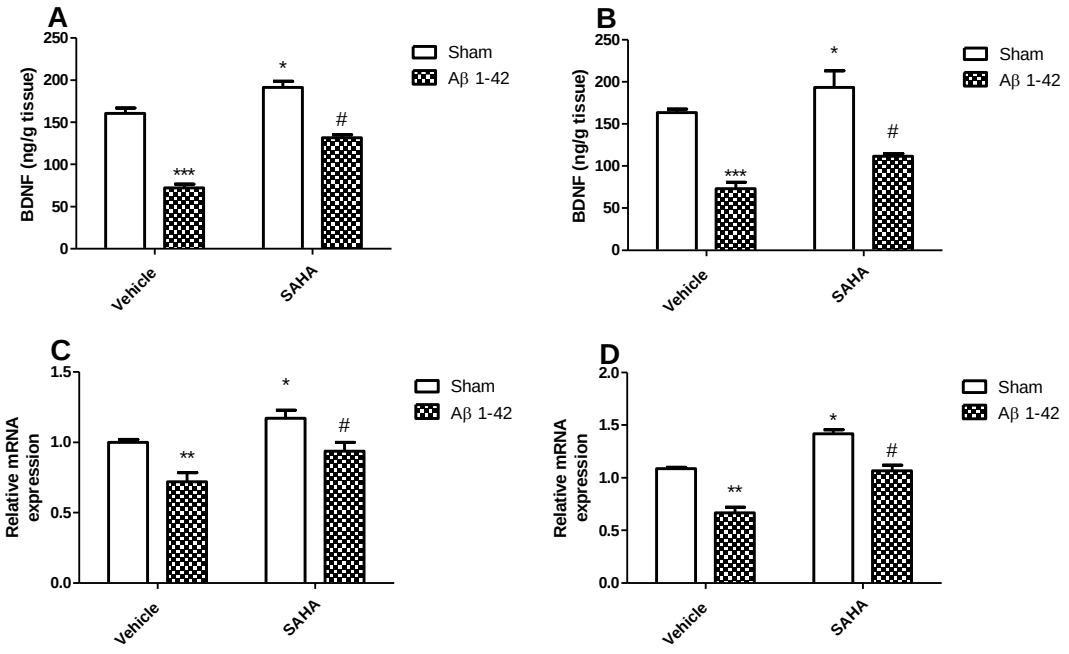
A
BDNF (ng/g tissue)
250
200
150
100
50
0
***
*
#
Vehicle
SAHA
Sham
Aβ 1-42
B
BDNF (ng/g tissue)
250
200
150
100
50
0
***
*
#
Vehicle
SAHA
Sham
Aβ 1-42
C
Relative mRNA expression
1.5
1.0
0.5
0.0
**
*
#
Vehicle
SAHA
Sham
Aβ 1-42
D
Relative mRNA expression
2.0
1.5
1.0
0.5
0.0
**
*
#
Vehicle
SAHA
Sham
Aβ 1-42

Figure 9

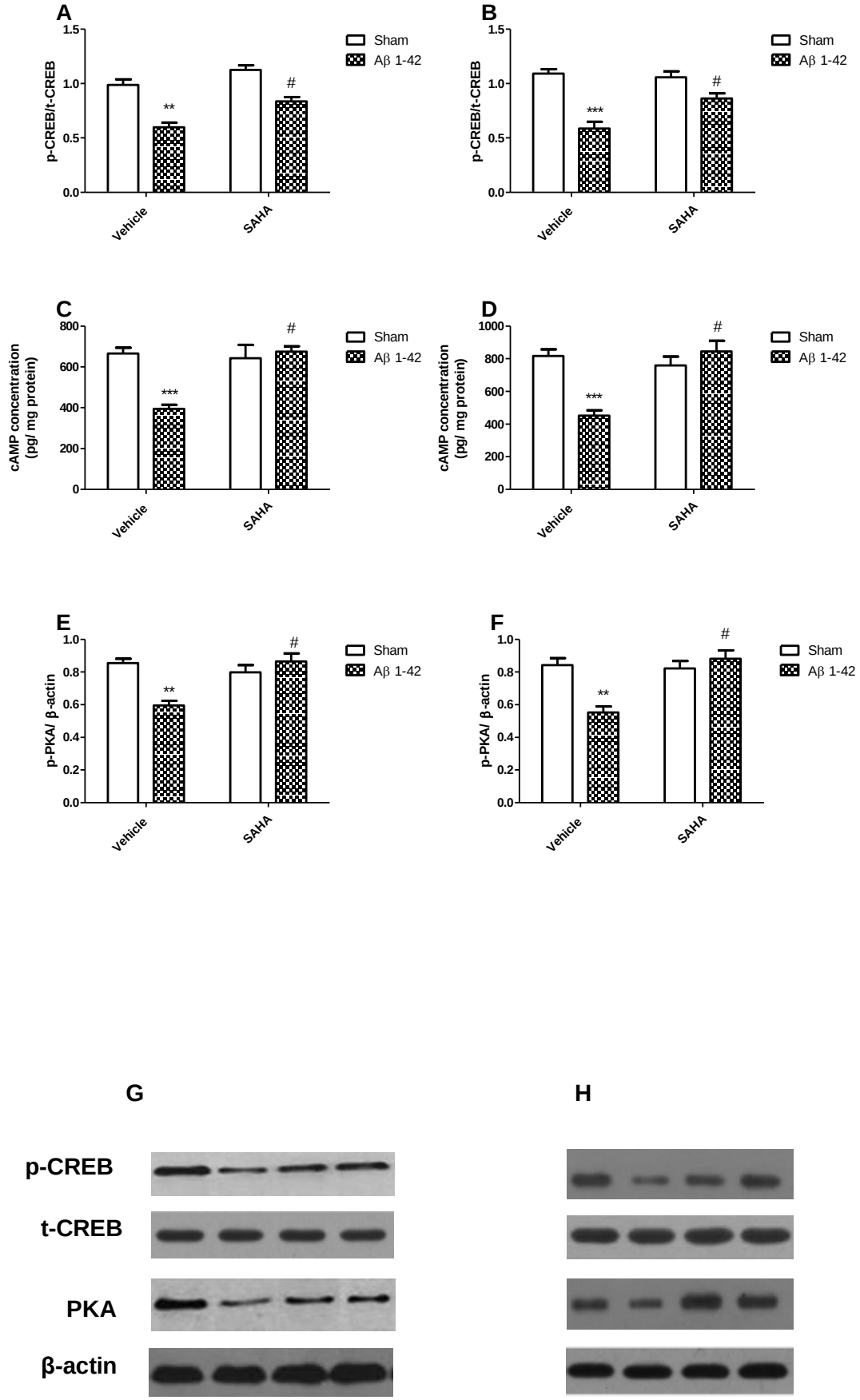
A
p-CREB/t-CREB
1.5
1.0
0.5
0.0
**
#
Vehicle
SAHA
Sham
Aβ 1-42
B
p-CREB/t-CREB
***
#
C
cAMP concentration (pg/ mg protein)
800
600
400
200
0
***
#
D
cAMP concentration (pg/ mg protein)
1000
800
600
400
200
0
***
#
E
p-PKA/ β-actin
1.0
0.8
0.6
0.4
0.2
0.0
**
#
F
p-PKA/ β-actin
**
#
G
H
p-CREB
t-CREB
PKA
β-actin

Figure 10

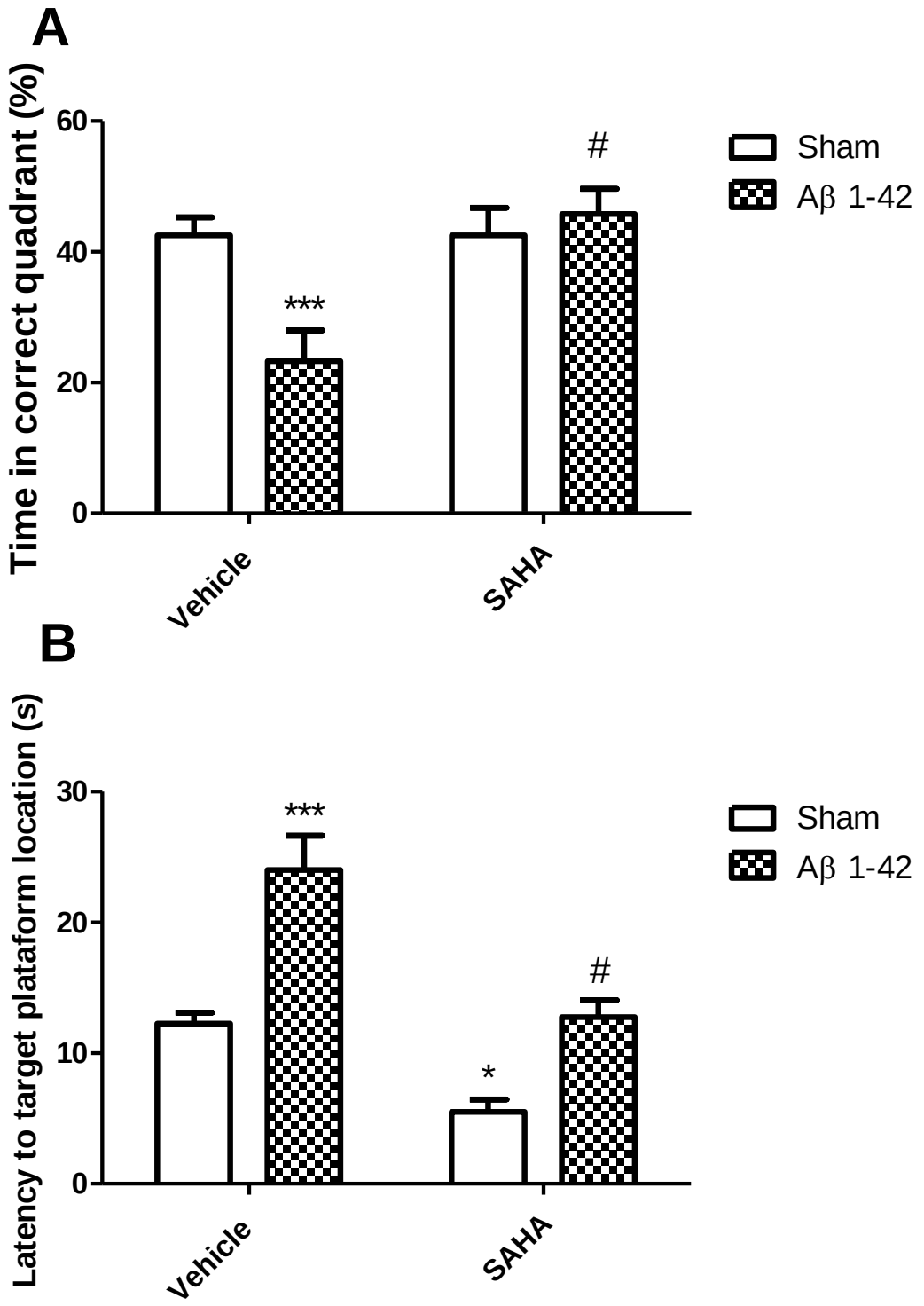
A
Time in correct quadrant (%)
60
40
20
0
***
#
Sham
Aβ 1-42
Vehicle
SAHA
B
Latency to target plataform location (s)
30
20
10
0
***
*
#
Sham
Aβ 1-42
Vehicle
SAHA

Table 1. r values resulting from Pearson's correlation test for neurochemical factors and behavioral parameters

	Time in correct quadrant (%)	**Latency to target plataform location (s)**
HDAC activity	HP= 0.17 PFC= -0.64*	HP= 0.10 PFC= 0.82*
HAT (H3) activity	HP= 0.77* PFC= 0.69*	HP= -0.79* PFC= -0,81*
HAT (H4) activity	HP= 0.61* PFC= 0.55*	HP= -0.78* PFC= -0.91*
BDNF levels	PFC= 0.45 HP= 0.56*	PFC= -0.80* HP= -0.88*
BDNF RNAm	HP= 0.43 PFC= 0.37	HP= -0.90* PFC= -0.65*
CREB	HP= 0.58* PFC= 0.63*	HP= -0.81* PFC= -0.74*
AMPc	HP= 0.62* PFC= 0.82*	HP= -0.69* PFC= -0.66*
PKA	HP= 0.52* PFC= 0.61*	HP= -0.59* PFC= -0.64*

*denoted $p<0.05$.

PARTE III

2. CONCLUSÃO

O presente estudo demonstrou que o tratamento com o inibidor de HDAC SAHA durante 21 dias atuou como neuroprotetor em um modelo da DA. Neste sentido, o tratamento com SAHA foi eficaz em atenuar as seguintes déficits resultantes da exposição dos camundongos à $A\beta_{1-42}$:

- O comprometimento da memória no teste comportamental Labirinto Aquático de Morris;
- O aumento da atividade da enzima HDAC no córtex pré-frontal e no hipocampo;
- Diminuição na atividade enzimática das HATs (H3 e H4) no córtex pré-frontal e no hipocampo;
- Redução dos níveis de BDNF no córtex pré-frontal e hipocampo;
- Diminuição nos níveis do mRNA de BDNF no córtex pré-frontal e hipocampo;
- Regulação negativa da via cAMP/ PKA/ CREB

Em conclusão, os dados demonstram o efeito neuroprotetor do SAHA em um modelo de DA, fornecendo uma nova terapêutica para o tratamento da DA.

3. REFERÊNCIAS

ADWAN, Lina; ZAWIA, Nasser H. Epigenetics: a novel therapeutic approach for the treatment of Alzheimer's disease. **Pharmacology & therapeutics**, v. 139, n. 1, p. 41-50, 2013.

AUTRY, Anita E.; MONTEGGIA, Lisa M. Brain-derived neurotrophic factor and neuropsychiatric disorders. **Pharmacological reviews**, v. 64, n. 2, p. 238-258, 2012.

BALARATNASINGAM, Sivasankaran; JANCA, Aleksandar. Brain derived neurotrophic factor: a novel neurotrophin involved in psychiatric and neurological disorders. **Pharmacology & therapeutics**, v. 134, n. 1, p. 116-124, 2012.

BALCH, Curtis et al. New anti-cancer strategies: epigenetic therapies and biomarkers. **Front Biosci**, v. 10, n. 1, p. 897-931, 2005.

BELROSE, Jillian C. et al. Increased pro–nerve growth factor and decreased brain-derived neurotrophic factor in non–Alzheimer's disease tauopathies. **Neurobiology of aging**, v. 35, n. 4, p. 926-933, 2014.

BINDER, Devin K.; SCHARFMAN, Helen E. Mini review. **Growth factors**, v. 22, n. 3, p. 123-131, 2004.

BIRD, Adrian. DNA methylation patterns and epigenetic memory. **Genes & development**, v. 16, n. 1, p. 6-21, 2002.

BLESSED, Garry; TOMLINSON, Bernard E.; ROTH, Martin. The association between quantitative measures of dementia and of senile change in the cerebral grey matter of elderly subjects. **The British Journal of Psychiatry**, 1968.

CACABELOS, Ramón; TORRELLAS, Clara. Epigenetic drug discovery for Alzheimer’s disease. **Expert opinion on drug discovery**, v. 9, n. 9, p. 1059-1086, 2014.

CARLEZON, William A.; DUMAN, Ronald S.; NESTLER, Eric J. The many faces of CREB. **Trends in neurosciences**, v. 28, n. 8, p. 436-445, 2005.

CHACÓN-FERNÁNDEZ, Pedro et al. Brain-derived neurotrophic factor in megakaryocytes. **Journal of Biological Chemistry**, v. 291, n. 19, p. 9872-9881, 2016.

CHAO, Moses V. Neurotrophins and their receptors: a convergence point for many signalling pathways. **Nature Reviews Neuroscience**, v. 4, n. 4, p. 299-309, 2003.

CHEN, C.; LI, X.; GAO, P.; TU, Y.; ZHAO, M.; LI, J.; ZHANG, S.; LIANG, H. Baicalin attenuates Alzheimer-like pathological changes and memory deficits induced by amyloid β1–42 protein. **Metabolic Brain Disease**, v.30, p. 537–544, 2015.

COSTA, Alberto et al. Brain-derived neurotrophic factor serum levels correlate with cognitive performance in Parkinson's disease patients with mild cognitive impairment. **Frontiers in behavioral neuroscience**, v. 9, 2015.

D'ALESSIO, Ana C.; SZYF, Moshe. Epigenetic tête-à-tête: the bilateral relationship between chromatin modifications and DNA methylation This paper is one of a selection of papers published in this Special Issue, entitled 27th International West Coast Chromatin and Chromosome Conference, and has undergone the Journal's usual peer review process. **Biochemistry and cell biology**, v. 84, n. 4, p. 463-466, 2006.

DAROCHA-SOUTO, B. et al. Activation of glycogen synthase kinase-3 beta mediates β-amyloid induced neuritic damage in Alzheimer's disease. **Neurobiology of disease**, v. 45, n. 1, p. 425-437, 2012.

DIVIANI, Dario; SCOTT, John D. AKAP signaling complexes at the cytoskeleton. **Journal of Cell Science**, v. 114, n. 8, p. 1431-1437, 2001.

DRAGUNOW, Michael et al. c-Jun promotes neurite outgrowth and survival in PC12 cells. **Molecular brain research**, v. 83, n. 1, p. 20-33, 2000.

DRISCOLL, Ira et al. Plasma BDNF is associated with age-related white matter atrophy but not with cognitive function in older, non-demented adults. **PloS one**, v. 7, n. 4, p. e35217, 2012.

FAHNESTOCK, Margaret. Brain-derived neurotrophic factor: the link between amyloid-β and memory loss. **Future Neurology**, v. 6, n. 5, p. 627-639, 2011.

FASS, Daniel M. et al. Crebinostat: a novel cognitive enhancer that inhibits histone deacetylase activity and modulates chromatin-mediated neuroplasticity. **Neuropharmacology**, v. 64, p. 81-96, 2013.

FEINBERG, Andrew P. Cancer epigenetics takes center stage. **Proceedings of the National Academy of Sciences**, v. 98, n. 2, p. 392-394, 2001.

FUKS F. et al. The DNA methyltransferases associate with HP-1 and SUV39H1 histone methyltransferases. **Nucleic Acids Res**, v.31, 2003.

FUKUMOTO, Kazuya et al. Fingolimod increases brain-derived neurotrophic factor levels and ameliorates amyloid β-induced memory impairment. **Behavioural brain research**, v. 268, p. 88-93, 2014.

GARZON, Diego J.; FAHNESTOCK, Margaret. Oligomeric amyloid decreases basal levels of brain-derived neurotrophic factor (BDNF) mRNA via specific downregulation of BDNF transcripts IV and V in differentiated human neuroblastoma cells. **Journal of Neuroscience**, v. 27, n. 10, p. 2628-2635, 2007.

GOEDERT, Michel; SPILLANTINI, Maria Grazia. A century of Alzheimer's disease. **Science**, v. 314, n. 5800, p. 777-781, 2006.

GOLDBERG, Aaron D.; ALLIS, C. David; BERNSTEIN, Emily. Epigenetics: a landscape takes shape. **Cell**, v. 128, n. 4, p. 635-638, 2007.

GOODMAN, Richard H.; SMOLIK, Sarah. CBP/p300 in cell growth, transformation, and development. **Genes & development**, v. 14, n. 13, p. 1553-1577, 2000.

GUNSTAD, John et al. Serum brain-derived neurotrophic factor is associated with cognitive function in healthy older adults. **Journal of geriatric psychiatry and neurology**, 2008.

HAASS, Christian. Take five—BACE and the γ-secretase quartet conduct Alzheimer's amyloid β-peptide generation. **The EMBO journal**, v. 23, n. 3, p. 483-488, 2004.

HARDY, John; SELKOE, Dennis J. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. **Science**, v. 297, n. 5580, p. 353-356, 2002.

HELDT, S. A. et al. Hippocampus-specific deletion of BDNF in adult mice impairs spatial memory and extinction of aversive memories. **Molecular psychiatry**, v. 12, n. 7, p. 656-670, 2007.

HOLSINGER, RM Dalmian et al. Quantitation of BDNF mRNA in human parietal cortex by competitive reverse transcription-polymerase chain reaction: decreased levels in Alzheimer's disease. **Molecular Brain Research**, v. 76, n. 2, p. 347-354, 2000.

HOWELLS, D. W. et al. Reduced BDNF mRNA expression in the Parkinson's disease substantia nigra. **Experimental neurology**, v. 166, n. 1, p. 127-135, 2000.

ILLINGWORTH, Robert S.; BIRD, Adrian P. CpG islands–'a rough guide'. **FEBS letters**, v. 583, n. 11, p. 1713-1720, 2009.

JENUWEIN, Thomas; ALLIS, C. David. Translating the histone code. **Science**, v. 293, n. 5532, p. 1074-1080, 2001.

JONES, Peter A.; TAKAI, Daiya. The role of DNA methylation in mammalian epigenetics. **Science**, v. 293, n. 5532, p. 1068-1070, 2001.

KANDEL, Eric R. The molecular biology of memory: cAMP, PKA, CRE, CREB-1, CREB-2, and CPEB. **Molecular brain**, v. 5, n. 1, p. 14, 2012.

KARPOVA, Nina N. Role of BDNF epigenetics in activity-dependent neuronal plasticity. **Neuropharmacology**, v. 76, p. 709-718, 2014.

KEMPPAINEN, Susanna et al. Impaired TrkB receptor signaling contributes to memory impairment in APP/PS1 mice. **Neurobiology of aging**, v. 33, n. 6, p. 1122. e23-1122. e39, 2012.

KOPONEN, Eija et al. Transgenic mice overexpressing the full-length neurotrophin receptor trkB exhibit increased activation of the trkB–PLCγ pathway, reduced anxiety, and facilitated learning. **Molecular and Cellular Neuroscience**, v. 26, n. 1, p. 166-181, 2004.

KOPPERUD, Reidun et al. cAMP effector mechanisms. Novel twists for an 'old'signaling system. **FEBS letters**, v. 546, n. 1, p. 121-126, 2003.

KUCZEWSKI, Nicola et al. Activity-dependent dendritic release of BDNF and biological consequences. **Molecular neurobiology**, v. 39, n. 1, p. 37-49, 2009.

LASKE, C. et al. Stage-dependent BDNF serum concentrations in Alzheimer's disease. **Journal of neural transmission**, v. 113, n. 9, p. 1217-1224, 2006.

LOVATEL, Gisele Agustini et al. Treadmill exercise induces age-related changes in aversive memory, neuroinflammatory and epigenetic processes in the rat hippocampus. **Neurobiology of learning and memory**, v. 101, p. 94-102, 2013.

LUBIN, Farah D.; ROTH, Tania L.; SWEATT, J. David. Epigenetic regulation of BDNF gene transcription in the consolidation of fear memory. **Journal of Neuroscience**, v. 28, n. 42, p. 10576-10586, 2008.

MALONEY, Bryan et al. Applying Epigenetics to Alzheimer's Disease via the Latent Early–life Associated Regulation (LEARn) Model. **Current Alzheimer research**, v. 9, n. 5, p. 589-599, 2012.

MATTSON, Mark P. Gene–diet interactions in brain aging and neurodegenerative disorders. **Annals of Internal Medicine**, v. 139, n. 5_Part_2, p. 441-444, 2003.,

MEDEIROS, R. A doença de Alzheimer como um processo neuroinflamatório. Florianópolis, 2007, 139 p. Tese (Doutorado em farmacologia). Universidade Federal de Santa Catarina.

MILLAN, Mark J. The epigenetic dimension of Alzheimer's disease: causal, consequence, or curiosity. **Dialogues in Clinical Neuroscience**, v. 16, n. 3, p. 373-393, 2014.

MINICHIELLO, Liliana. TrkB signalling pathways in LTP and learning. **Nature Reviews Neuroscience**, v. 10, n. 12, p. 850-860, 2009.

MOUNT, Claire; DOWNTON, Christian. Alzheimer disease: progress or profit?. **Nature medicine**, v. 12, n. 7, p. 780-784, 2006.

MOWLA, S. Javad et al. Biosynthesis and post-translational processing of the precursor to brain-derived neurotrophic factor. **Journal of Biological Chemistry**, v. 276, n. 16, p. 12660-12666, 2001.

NAN, Xinsheng et al. Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. **Nature**, v. 393, n. 6683, p. 386-389, 1998.

NAN, Xinsheng; CROSS, Sally; BIRD, Adrian. Gene silencing by methyl-CpG-binding proteins. In: **Novartis Foundation symposium**. 1998. p. 6-21.

NETTIKSIMMONS, Jasmine et al. The associations between serum brain-derived neurotrophic factor, potential confounders, and cognitive decline: a longitudinal study. **PLoS One**, v. 9, n. 3, p. e91339, 2014.

NUMAKAWA, Tadahiro et al. BDNF function and intracellular signaling in neurons. **Histology and histopathology**, v. 25, n. 2, p. 237-258, 2010.

OJOPI, Elida P. Benquique; BERTONCINI, Alexandre Bruno; DIAS NETO, Emmanuel. Apolipoproteína E e a doença de Alzheimer. **Archives of Clinical Psychiatry (São Paulo)**, v. 31, n. 1, p. 26-33, 2004.

PARDRIDGE, William M. Blood-brain barrier drug targeting enables neuroprotection in brain ischemia following delayed intravenous administration of neurotrophins. In: **Molecular and Cellular Biology of Neuroprotection in the CNS**. Springer US, 2003. p. 397-430.

PARK, Kyoung-Ha. Trimetazidine effects on restoring endothelial function. **Where is the resistance?**, p. 25, 2013.

PETERSEN, Ronald C. et al. Apolipoprotein E status as a predictor of the development of Alzheimer's disease in memory-impaired individuals. **Jama**, v. 273, n. 16, p. 1274-1278, 1995.

PHILIP B. Risk factors for vascular dementia and Alzheimer disease. **Stroke**, v. 35, n. 11 suppl 1, p. 2620-2622, 2004.

PLÁTENÍK, Jan; KURAMOTO, Nobuyuki; YONEDA, Yukio. Molecular mechanisms associated with long-term consolidation of the NMDA signals. **Life sciences**, v. 67, n. 4, p. 335-364, 2000.

PORTELA, Anna; ESTELLER, Manel. Epigenetic modifications and human disease. **Nature biotechnology**, v. 28, n. 10, p. 1057-1068, 2010.

PRUSS, Dmitry; HAYES, Jeffrey J.; WOLFFE, Alan P. Nucleosomal anatomy–where are the histones?. **BioEssays**, v. 17, n. 2, p. 161-170, 1995.

ROBERTSON, Keith D. DNA methylation and human disease. **Nature Reviews Genetics**, v. 6, n. 8, p. 597-610, 2005.

RODENHISER, David; MANN, Mellissa. Epigenetics and human disease: translating basic biology into clinical applications. **Canadian Medical Association Journal**, v. 174, n. 3, p. 341-348, 2006.

ROSA, Elyse; FAHNESTOCK, Margaret. CREB expression mediates amyloid β-induced basal BDNF downregulation. **Neurobiology of aging**, v. 36, n. 8, p. 2406-2413, 2015.

ROUNTREE, Michael R.; BACHMAN, Kurtis E.; BAYLIN, Stephen B. DNMT1 binds HDAC2 and a new co-repressor, DMAP1, to form a complex at replication foci. **Nature genetics**, v. 25, n. 3, p. 269-277, 2000.

SANCHEZ-MUT, Jose V.; GRÄFF, Johannes. Epigenetic alterations in Alzheimer's disease. **Frontiers in behavioral neuroscience**, v. 9, 2015.

SAURA, Carlos A.; VALERO, Jorge. The role of CREB signaling in Alzheimer's disease and other cognitive disorders. **Reviews in the neurosciences**, v. 22, n. 2, p. 153-169, 2011.

SCHINDOWSKI, K.; BELARBI, K.; BUEE, L. Neurotrophic factors in Alzheimer's disease: role of axonal transport. **Genes, Brain and Behavior**, v. 7, n. s1, p. 43-56, 2008.

SCHMIDT, Heath D.; DUMAN, Ronald S. Peripheral BDNF produces antidepressant-like effects in cellular and behavioral models. **Neuropsychopharmacology**, v. 35, n. 12, p. 2378-2391, 2010.

SOULE, J.; MESSAOUDI, E.; BRAMHAM, C. R. Brain-derived neurotrophic factor and control of synaptic consolidation in the adult brain. 2006.

SOUZA, L. C. Efeito neuroprotetor do exercício físico em um modelo de doença de alzheimer induzido pelo peptídeo β-amilóide1-40 em camundongos. Uruguaiana, 2012, 121 p. Dissertação (Mestrado em bioquímica). Universidade Federal do Pampa.

STRAHL, Brian D.; ALLIS, C. David. The language of covalent histone modifications. **Nature**, v. 403, n. 6765, p. 41-45, 2000.

SUH, Yoo-Hun; CHECLER, Frederic. Amyloid precursor protein, presenilins, and α-synuclein: molecular pathogenesis and pharmacological applications in Alzheimer's disease. **Pharmacological reviews**, v. 54, n. 3, p. 469-525, 2002.

TAN, Kai Soo et al. β 2 Adrenergic receptor activation stimulates pro-inflammatory cytokine production in macrophages via PKA-and NF-κB-independent mechanisms. **Cellular signalling**, v. 19, n. 2, p. 251-260, 2007.

TAO, Xu et al. Ca 2+ influx regulates BDNF transcription by a CREB family transcription factor-dependent mechanism. **Neuron**, v. 20, n. 4, p. 709-726, 1998.

TASKEN, Kjetil; AANDAHL, Einar Martin. Localized effects of cAMP mediated by distinct routes of protein kinase A. **Physiological reviews**, v. 84, n. 1, p. 137-167, 2004.

THOMAS, Kerrie; DAVIES, Alun. Neurotrophins: a ticket to ride for BDNF. **Current Biology**, v. 15, n. 7, p. R262-R264, 2005.

VAN DAM, Debby; DE DEYN, Peter Paul. Drug discovery in dementia: the role of rodent models. **Nature Reviews Drug Discovery**, v. 5, n. 11, p. 956-970, 2006.

VAN DEN HOVE, Daniel LA; KENIS, Gunter; RUTTEN, Bart PF. Epigenetic dysregulation in Alzheimer's disease: cause or consequence?. 2014.

VO, Ngan; GOODMAN, Richard H. CREB-binding protein and p300 in transcriptional regulation. **Journal of Biological Chemistry**, v. 276, n. 17, p. 13505-13508, 2001.

XUE, Wenda et al. PKA-CREB-BDNF signaling regulated long lasting antidepressant activities of Yueju but not ketamine. **Scientific reports**, v. 6, 2016.

WALSH, Dominic M.; SELKOE, Dennis J. Deciphering the molecular basis of memory failure in Alzheimer's disease. **Neuron**, v. 44, n. 1, p. 181-193, 2004.

ZHU, Y.-P. et al. Epigenetic Modification and Its Role in Alzheimer's Disease. **Integrative Medicine International**, v. 2, n. 1-2, p. 63-72, 2015.

ZIEBELL, Morten et al. Striatal dopamine transporter binding correlates with serum BDNF levels in patients with striatal dopaminergic neurodegeneration. **Neurobiology of aging**, v. 33, n. 2, p. 428. e1-428. e5, 2012.

ZINSER, Eva G.; HARTMANN, Tobias; GRIMM, Marcus OW. Amyloid beta-protein and lipid metabolism. **Biochimica et Biophysica Acta (BBA)-Biomembranes**, v. 1768, n. 8, p. 1991-2001, 2007.

ZUCCATO, Chiara et al. Systematic assessment of BDNF and its receptor levels in human cortices affected by Huntington's disease. **Brain pathology**, v. 18, n. 2, p. 225-238, 2008.

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COMISSÃO DE ÉTICA NO USO DE ANIMAIS - CEUA

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CERTIFICADO DE APROVAÇÃO DE PROTOCOLO PARA USO DE ANIMAIS EM PESQUISA

Número de protocolo da CEUA: **025/2014**

Título: **Avaliação dos efeitos e mecanismos de flavonóides e moléculas sintéticas em modelos de depressão em camundongos**

Data da aprovação: **10/07/2014**

Período de vigência do projeto: De: **07/2014** Até: **07/2017**

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