UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

INSTITUTO DE CIÊNCIAS BÁSICAS DA SAÚDE

PROGRAMA DE PÓS-GRADUAÇÃO EM NEUROCIÊNCIAS

EFEITO DO EXERCÍCIO FÍSICO SOBRE A MEMÓRIA AVERSIVA E
PARÂMETROS INFLAMATÓRIOS NO PROCESSO DE ENVELHECIMENTO E
NA ISQUEMIA CEREBRAL GLOBAL

GISELE AGUSTINI LOVATEL

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GISELE AGUSTINI LOVATEL

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Tese apresentada ao Programa de Pós-graduação em Neurociências da Universidade Federal do Rio Grande do Sul como requisito parcial à obtenção do grau de doutor em Neurociências.

Porto Alegre, 2013.

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"Nós, homens do conhecimento, não nos conhecemos;

de nós mesmos somos desconhecidos."

Friedrich Nietzsche

SUMÁRIO

APRESENTAÇÃO

Esta tese é constituída por:

1. Introdução, onde está o embasamento teórico necessário para a compreensão da proposta de trabalho e objetivos.

2. Objetivos, onde se encontram todas as metas a serem desenvolvidas ao longo dos capítulos.

3. Métodos e resultados, constituídos pelos capítulos 1, 2 e 3:

Capítulo 1, onde se encontra o primeiro artigo publicado pertencente a esta tese.

Capítulo 2, onde se encontra o segundo artigo publicado pertencente a esta tese.

Capítulo 3, referente ao terceiro artigo que será submetido à revista Neuoscience.

4. Discussão, que contém uma interpretação dos resultados obtidos relativos aos três capítulos acima, englobando-os em um contexto geral.

7. Conclusões, onde se encontra o fechamento geral da tese.

8. Perspectivas, que aborda as possibilidades futuras de pesquisas relativas à continuação deste trabalho.

9. Referências, onde se encontram listadas todas as referências bibliográficas utilizadas na Introdução e Discussão deste trabalho.

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LISTA DE ABREVIATURAS

Acetil-CoA: Acetil-coenzima A

BDNF: Fator neurotrófico derivado do encéfalo

CaMKII: Ca2+/calmodulina dependente de proteina cinase II

CA: Corno de Amon

COX-2: Ciclooxigenase-2

EP: Receptores prostanóides (EP1, EP2, EP3,EP4)

EROs: Espécies reativas de oxigênio

Erk: Cinase relacionada a sinais extracelulares

Fim: Fímbria

GD: Giro denteado

HAT: Histonas acetiltransferases

HDAC: Histonas desacetilases

IκB: Inibidor do fator kappa-B

IKK: Cinase de IκB

IL-1β: Interleucina-1β

IL-4: Interleucina 4

LTP: Potenciação de longa duração

MF: Fibras musgosas

NF-κB: Fator de transcrição nuclear kappa-B

NPCs: Células precursoras neuronais

PGE$_2$: Prostaglandina E2

POG: Privação de oxigênio e glicose

PP: Via perforante

SC: Via colateral de Schaffer

SNC: Sistema nervoso central

TNF-α: Fator de necrose tumoral alfa

trkB: Receptores tirosina cinase B

RESUMO

O objetivo desta tese foi avaliar o efeito do exercício físico sobre a memória aversiva e parâmetros inflamatórios no processo de envelhecimento e na isquemia cerebral global em ratos Wistar. Para isso foram realizados três experimentos.

No primeiro, ratos de 3 meses de idade foram submetidos a exercício (corrida esteira, 20 minutos por 2 semanas) ou mantidos sedentários. Foram avaliados o desempenho na tarefa de esquiva inibitória e os níveis de COX-2, PGE_2 e de receptores E-prostanóides (EP1-3) no hipocampo de ratos em diferentes tempos após a última sessão de exercício. O exercício induziu alterações tempo-dependentes sobre a via da COX-2, especificamente, aumentou os níveis de COX-2 e dos receptores EP4 e EP2 e diminuiu os níveis de PGE_2. Além disso, uma correlação positiva entre o desempenho no teste da memória aversiva e os níveis de COX-2 foi observada.

No segundo experimento, ratos de 3 e 20 meses foram submetidos ao mesmo protocolo de exercício. Foram analisados o desempenho na tarefa de esquiva inibitória e parâmetros inflamatórios e epigenético (TNF-α, IL1-β, IL-4, NF-κB e acetilação global da histona H4) no hipocampo de ratos em diferentes tempos após a última sessão de exercício. Foi observado um declínio da memória aversiva associado a um estado pró-inflamatório e uma redução da acetilaçao da histona H4 em ratos velhos. O exercício foi capaz de melhorar a memória, diminuir marcadores pró-inflamatórios e aumentar a acetilação de histona em hipocampo de ratos de 20 meses de idade; além disso, aumentou os níveis de IL-4 no hipocampo de ratos de 3 meses de idade.

No terceiro experimento, ratos de 3 meses foram submetidos a isquemia cerebral global e ao mesmo protocolo de exercício. Nós investigamos o efeito do exercício antes e depois da isquemia sobre a sobrevivência celular e a função de células gliais em hipocampo de ratos submetidos à isquemia. Exercício pós-isquemia aumentou a sobrevivência celular e modulou a função das células gliais, especificamente, aumentou a área ocupada por astócitos e diminuiu a área ocupada por células da microglia no giro denteado após isquemia cerebral.

Estes resultados sugerem que o exercício físico de corrida em esteira por 2 semanas pode induzir mudanças tempo-dependentes sobre a memória e parâmetros inflamatórios em hipocampo de ratos. Além disso, as respostas do exercício podem ser influenciadas pelo envelhecimento e pela isquemia cerebral.

ABSTRACT

The aim of this thesis was to study the effect of treadmill exercise on aversive memory and inflammatory parameters in the aging process and global cerebral ischemia in Wistar rats. For this, we made three experiments.

In the first, rats of 3 months of age were divided in exercise (running daily for 20 min for 2 weeks) or non-exercised (sedentary) groups. Were analyzed the performance in the inhibitory avoidance task and COX-2, PGE2 and E-prostanoid receptors levels in the rat hippocampus at different time points after the last training session of treadmill exercise. The treadmill exercise causes time-dependent changes on COX-2 pathways function, specifically increased COX-2 and EP4, EP2 receptors levels and decreased PGE_2 levels. Moreover a significant positive correlation between aversive memory performance and COX-2 levels was observed.

In the second experiment, rats of 3 and 20 months of age were submitted to the same exercise protocol. We analyzed the performance in the inhibitory avoidance task and inflammatory and epigenetic parameters (TNF-α, IL1-β, IL-4, NF-κB and global histone H4 acetylation) in the rat hippocampus at different time points after the last training session of treadmill exercise. It was observed a decline on aversive memory associated to a pro-inflammatory state and reduction on H4 acetylation in aged rats. The exercise ameliorated memory, decreased pro-inflammatory markers and increased histone acetylation in hippocampi of 20-months-old rats; moreover, increased IL-4 levels in hippocampi from young adult rats.

In the third experiment, rats of 3 months of age were submitted to global cerebral ischemia and the same exercise protocol. Here, we investigated the effect of both pre and postischemic treadmill exercise on cell survival and glial cells functions in the hippocampus of rats submitted to ischemia. Postischemic exercise increases cell survival and modulates glial cells functions, specifically increased area occupied by astrocyte and decreased area occupied by microglia in dentate gyrus following cerebral ischemia.

These results suggest that the treadmill exercise for 2 weeks can lead to time-dependent changes on memory and neuroinflammatory parameters in the rat hippocampus. Moreover, the responses to the exercise can be influenced by aging and cerebral ischemia.

Introdução

1.1 Memória

O aprendizado é o processo resultante da interação do individuo com o meio ou de representações internas que possibilitam aquisição de novas habilidades ou informações. A memória, por sua vez, compreende o conjunto de mecanismos que operam no sistema nervoso central (SNC) para que o aprendizado ocorra. Estes mecanismos operam em sequência, iniciando pela fase de aquisição, que corresponde ao período durante a exposição da experiência. Após, estas informações são codificadas e armazenadas, o que constitui a fase de consolidação e por fim, estas informações podem ser acessadas, o que constitui a fase de evocação (McGaugh, 2002; Izquierdo et al., 1985).

A formação da memória requer modificações celulares e moleculares que ocorrem durante a fase da consolidação. Após, a memória é armazenada em um estado estável e durante a evocação, torna-se lábil novamente, necessitando de processos, como a transcrição e a tradução gênica, ativação de receptores entre outros mecanismos necessários para sua manutenção (Lee et al., 2005; Da Silva et al., 2008). A reconsolidação da memória mantém e acrescenta novas informações à memória antiga enquanto que a extinção permite formar nova memória com significado diferente da memória original (Izquierdo, 2002).

As memórias podem ser classificadas utilizando-se diferentes critérios, por exemplo, o tempo de retenção. Considerando este critério, podem ser classificadas em memória de curta duração, a qual dura horas, mantendo a informação enquanto a memória de longa duração está sendo formada e não requer síntese de novas proteínas; ou memória de longa duração que pode durar dias ou anos, garantindo o registro do passado autobiográfico, conhecimento e habilidades do indivíduo (Dalmaz & Netto., 2004; Izquierdo, 2002).

As funções envolvendo o armazenamento de informações emergem como resultado do funcionamento do SNC. O conhecimento a respeito da participação de certas estruturas encefálicas na formação de memórias originou-se a partir da observação de humanos e animais de laboratório com lesões encefálicas específicas. A partir destas observações, o crescente interesse pelo envolvimento das estruturas do sistema límbico na formação da memória, com destaque à elucidação do papel do hipocampo, acrescentou de forma significante o conhecimento a respeito do processamento mnemônico (Izquierdo, 2002; Ofen, 2012).

O hipocampo é uma estrutura subcortical do lobo temporal que compõe o sistema límbico formado por duas regiões principais: o giro denteado e o Corno de Amon (CA), que por sua vez é subdividido nas regiões CA1, CA2 e CA3. Conexões sinápticas do hipocampo incluem a via perforante, as fibras musgosas, e a via colateral de Schaffer. O hipocampo desempenha um papel fundamental na formação de memórias declarativas de curta e longa duração, e diferentes áreas corticais interagem com o hipocampo para regular a aquisição e o armazenamento de novas informações (Kandel, 2000; Izquierdo et al., 1998; Figura 1).
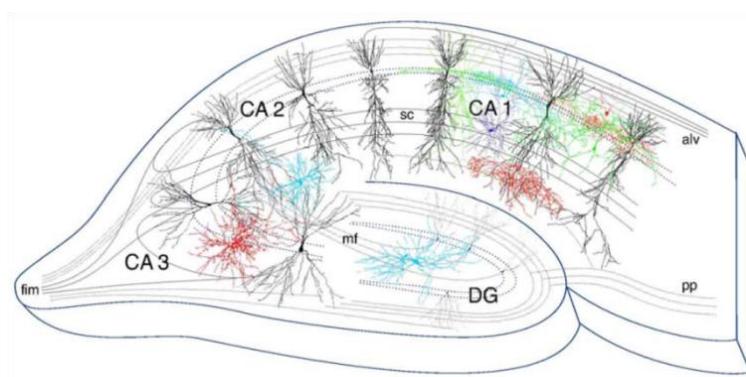
Figura 1. Secção coronal do hipocampo de rato (Adaptado de Pittson et al., 2005).

Para elucidação dos mecanismos envolvidos nos processos de memória, têm-se empregado modelos animais baseados no aprendizado de tarefas comportamentais

simples. Um teste amplamente utilizado para estudar a memória aversiva, a qual é considerada um subtipo de memória declarativa, consiste na tarefa de esquiva inibitória. Nesta tarefa, o animal aprende que ao descer de uma plataforma, recebe um choque. Este aprendizado requer um único treino e envolve a repressão específica da tendência natural dos ratos para explorar o ambiente além da plataforma. Um aumento na latência de descida, no teste, indica melhora da memória aversiva (Netto et al., 1985; Izquierdo et al., 2006). A formação da memória associada à tarefa de esquiva inibitória no rato está associada a ativação de receptores glutamatérgicos, aumento nos níveis intracelulares de Cálcio, ativação de vias de sinalização que resultam em síntese de novas proteínas. Estes processos podem ocorrer no hipocampo, córtex entorrinal e amígdala (Izquierdo et al., 1999; Izquierdo et al., 2006).

1.2 Sistema imune

Nas últimas duas décadas tornou-se evidente que o sistema imune desempenha um papel importante na modulação das funções cognitivas (Yirmiya & Goshen, 2011). Em condições fisiológicas, mecanismos imunes podem ser ativados por estímulos ambientais e modular circuitos neuronais, promovendo a consolidação da memória, potenciação de longa duração (LTP, do inglês *long-term potentiation*) e neurogênese no hipocampo. Estes efeitos benéficos são mediados por interações entre células do SNC com funções imunes (microglia e astrócitos), neurônios e células precursoras neuronais. Estas interações envolvem a capacidade de resposta de neurônios e células gliais à neurotransmissores, hormônios, neurotrofinas e mediadores inflamatórios (Yirmiya e Goshen, 2011).

Durante o aprendizado a liberação glutamatérgica, monoaminérgica e adrenocortical pode ativar neurônios, microglia e astrócitos no hipocampo. A ativação microglial pode

resultar na produção e liberação de interleucina-1 (IL-1) a qual ativa astrócitos e induz a expressão de mediadores envolvidos na plasticidade e memória. Microglia e astrócitos secretam mediadores que influenciam neurônios e células precursoras neuronais (NPCs) envolvidos na neurogênese hipocampal, incluindo fator neurotrófico derivado do encéfalo (BDNF) e fator de necrose tumoral alfa (TNF-α). Tem sido proposto o envolvimento da IL-4 neste circuito, contribuindo para o aprendizado, memória e neurogênese (Yirmiya e Goshen, 2011).
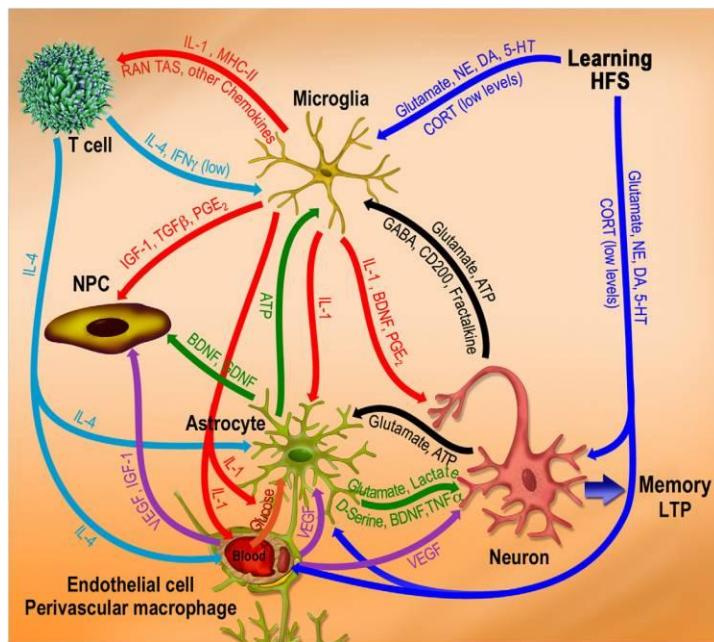
Figura 2. Ação do sistema imune sobre a memória (Adaptado de Yirmiya et al., 2011).

Estudos têm elucidado o papel das citocinas inflamatórias na formação da memória. Neste contexto, um aumento dos níveis de citocinas inflamatórias como IL-1β, TNF-a, foi associado com a melhora da memória em roedores saudáveis (Yirmiya et al., 2002; Song et al., 2003; Gibertini, 1998; McAfoose et al., 2009). Além disso, outros mediadores inflamatórios envolvendo a via da ciclooxigenase-2 (COX-2) parecem desempenhar um papel neste processo. Embora a função da COX-2 no encéfalo, em

condições fisiológicas, tenha sido pouco estudada, foi sugerido seu papel na transmissão sináptica, plasticidade neuronal, memória e neurogênese (Murray & O'Connor et al., 2003; Chen & Bazan 2005; Yang & Chen, 2008; Rall et al., 2003).

Embora tenha sido sugerido que o sistema imune possa exercer efeitos benéficos sobre as funções cognitivas, sua ativação crônica pode levar a alterações patológicas. Estas alterações podem perturbar o delicado equilíbrio necessário para manutenção das funções encefálicas, levando a prejuízos sobre plasticidade, neurogênese, aprendizado e memória. Estas condições podem ocorrer tanto em situações fisiológicas, como durante o envelhecimento, como na presença de distúrbios da circulação cerebral (Jurgens & Johnson, 2010; Yirmiya & Goshen, 2011; Cechetti et al., 2011; Vicente et al., 2009).

1.3 Envelhecimento

O envelhecimento é um processo biológico complexo, caracterizado por alterações estruturais e funcionais das células e tecidos, podendo levar a redução das funções cognitivas e aumentar a susceptibilidade a doenças associadas à idade (Siqueira et al., 2004; Siqueira et al., 2005; Paradies et al., 2011). No Brasil, estima-se que nos próximos 50 anos aproximadamente 30% da população estarão na faixa etária acima dos 65 anos (IBGE, 2008). Estes dados demonstram a importância da pesquisa sobre o envelhecimento normal objetivando investigar sobre as modificações associadas à idade e contribuir para uma melhor qualidade de vida.

Embora estudos sejam conduzidos para investigar os mecanismos celulares e moleculares envolvidos no processo de envelhecimento normal, ainda não está totalmente esclarecido como este processo interfere na redução das funções neuronais e gliais e na disfunção cognitiva. Evidências experimentais têm sugerido que as modificações do microambiente do encéfalo senescente estão associadas ao estresse

oxidativo, aumento da reatividade microglial e inflamação crônica (Siqueira et al., 2005; Jurgens & Johnson, 2010). Recentemente tem sido proposto que alterações epigenéticas também podem estar relacionadas ao processo de envelhecimento (Chung et al., 2009; Peleg et al., 2010; Jurgens & Johnson, 2010).

O encéfalo é particularmente susceptível aos efeitos prejudiciais das espécies reativas de oxigênio (EROs), devido à sua alta taxa metabólica, redução tanto das defesas antioxidantes quanto dos mecanismos de reparo durante o envelhecimento (Andersen, 2004). Além disso, as EROs estão envolvidas na regulação redox das células do sistema imune como por exemplo, as células da microglia que são ativadas sob condições oxidativas (Halliwell, 2007). Essa ativação pode ser mediada pelo sistema da NADPH oxidase resultando num aumento no consumo de oxigênio e consequente produção do radical ânion superóxido ($O2^{-}$). Este radical pode formar peroxinitrito ($ONOO^{-}$), que pode depletar os grupamentos tióis e com isso alterar o balanço redox da glutationa. Este desequilíbrio pode induzir a fosforilação do inibidor kappa-B (IκB) promovendo a ativação do fator de transcrição nuclear kappa-B (NF-κB) através da sua translocação para o núcleo. A ativação do NF-κB leva à transcrição de mediadores inflamatórios, como TNF-α, IL-1β, entre outros (Oktyabrsky & Smirnova, 2007; Figura 3).
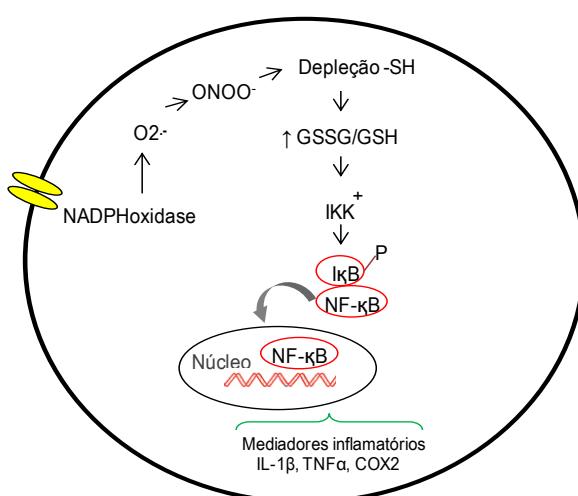
Figura 3: Alterações no estado redox celular (Adaptado de Filippin et al., 2008).

Alterações do sistema imune podem induzir um estado pró-inflamatório no microambiente encefálico senil e desta forma participar no processo do declínio cognitivo relacionado ao envelhecimento (Buchanan et al, 2008; Lynch, 2010). Células da microglia com um fenótipo reativo são normalmente referidas como "sensibilizadas" e apresentam alterações como desramificação e aumento do citoplasma. Neste estado, estas células promovem uma maior liberação de citocinas inflamatórias em comparação com a microglia ramificada, em "repouso", denominada de vigilante. Embora a microglia "sensibilizada" tenha sido associada com doenças crônico-degenerativas, evidências indicam que este fenótipo também está presente no encéfalo durante o envelhecimento normal (Bilbo, 2010). A reatividade microglial associada ao envelhecimento pode contribuir para o dano neuronal assim como para a perda de mecanismos neuroprotetores e neurorregulatórios, podendo resultar em redução das funções cognitivas (Jurgens & Johnson, 2010; Barrientos et al., 2010; Figura 4).

Estudos demonstraram que altos níveis de citocinas pró-inflamatórias podem estar associados a prejuízos na memória em roedores (O'Donnell et al., 2000; Griffinetal, 2006). No entanto, estes estudos utilizam modelos de doenças neurodegenerativas ou administração central e periférica de mediadores pró-inflamatórios para avaliar este processo. O envolvimento de citocinas inflamatórias sobre tarefas comportamentais, como testes de memória espacial e aversiva, no processo do envelhecimento normal, em roedores, tem sido pouco estudado.
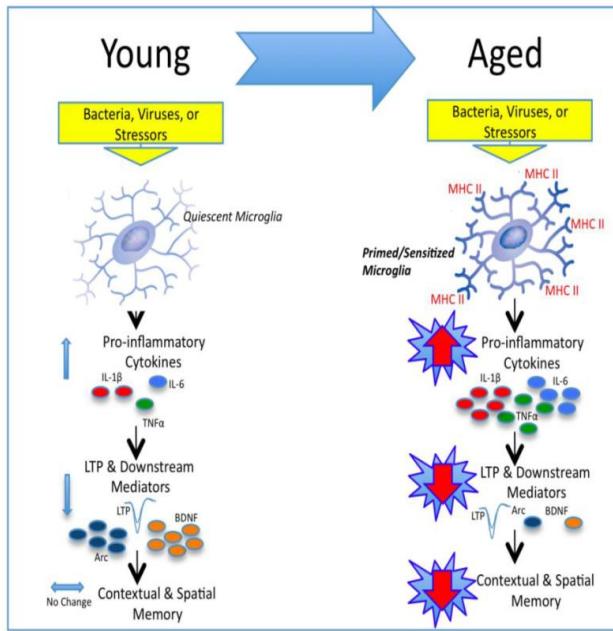
Figura 4: Alterações microgliais associadas ao envelhecimento (Adaptado de Barrientos et al., 2010).

Além dos mecanismos inflamatórios, estudos recentes têm sugerido que o declínio cognitivo, associado ao envelhecimento, pode estar relacionado à modificações epigenéticas. Epigenética refere-se a alterações da expressão gênica induzidas por modificação da conformação da cromatina sem alterar a sequência de DNA (Bird, 2007). Estas modificações podem ocorrer nas caudas N-terminais das histonas e entre as mais estudas destaca-se a acetilação (Kouzarides, 2007). Em geral, a acetilação de histonas facilita o processo de transcrição, enquanto que a desacetilação atenua este processo (Waggoner, 2007; Turner, 2002). A acetilação pode ser regulada por enzimas denominadas histonas acetiltransferases (HAT) e desacetilases (HDAC). As HAT catalisam a adição do grupo acetil da molécula doadora acetil-coenzima A (acetil-CoA) aos resíduos de lisina das histonas, enfraquecendo as interações eletrostáticas entre as histonas e o DNA. Dessa forma, a cromatina adota uma configuração mais aberta, facilitando a transcrição (Waggoner, 2007). Por outro lado, as HDAC desacetilam as histonas, removem o grupo acetil, ligando-se fortemente ao DNA e assim tornando a

estrutura da cromatina mais compacta. Isto atenua o processo de transcrição e contribui para o silenciamento gênico (Turner, 2002; Figura 5).
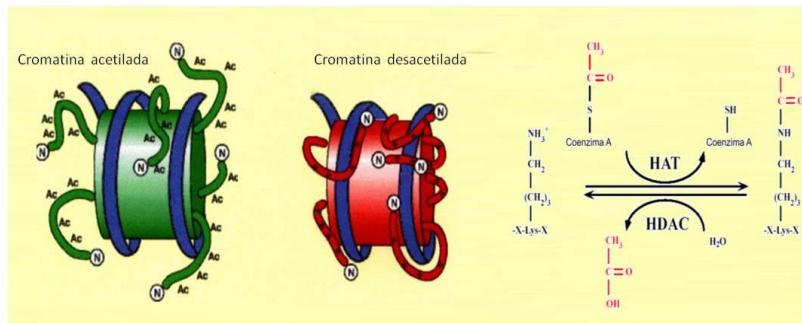
Figura 5: Modificações das histonas. (Adaptado de http://bricker.tcnj.edu/Amb/amble9.html).

É interessante destacar que a formação da memória tem sido associada com aumento dos níveis de acetilação de histonas em roedores (Levenson et al., 2004; Vecsey et al., 2007; Bousiges et al., 2010). Neste contexto, Reolon e colaboradores (2011) demonstraram que a administração de inibidores da HDAC melhorou o declínio de memória associado ao envelhecimento. Além disso, foi proposto que a acetilação de histonas induz a transcrição de BDNF (Bekinschtein et al., 2008). O BDNF liga-se a receptores tirosina kinase B (trkB, do inglês, *BNDF-tyrosine kinase receptor B*) o qual ativa vias intracelulares como a cinase relacionada a sinais extracelulares (Erk) e a proteina cinase dependente de Ca^{2+}/calmodulina II (CaMKII). Esta ativação contribui para o aumento de espinhos dendríticos e plasticidade sináptica (Mendelsohn & Larrick, 2012). Contudo, a redução da acetilação de histonas e da síntese de BDNF podem estar ligadas a prejuízos na plasticidade sináptica observados durante o envelhecimento (Mendelsohn & Larrick, 2012; Figura 6).
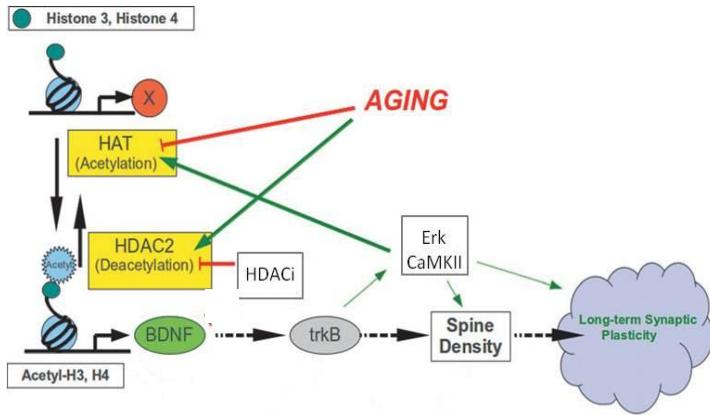
Figura 6: Alterações epigenéticas associadas ao envelhecimento. (Adaptado de Mendelsohn e Larrick, 2012).

1.4 Isquemia cerebral

Conforme descrito previamente, além do envelhecimento, distúrbios da circulação cerebral também podem estar relacionados à ativação crônica do sistema imune.

Desordens relacionadas à circulação cerebral contribuem significativamente para o desenvolvimento de danos neurológicos e representam uma importante causa de óbito e dependência (Radavonic, 2000). Entre estas desordens, a isquemia cerebral global é caracterizada por uma redução importante do fluxo sanguíneo cerebral e ocorre em pacientes acometidos por uma variedade de condições clínicas incluindo parada cardíaca, choque e asfixia, e pacientes submetidos à cirurgia cardíaca complexa (Salazar et al., 2001; Bernard et al., 2001; Nussmeier et al., 2002).

O encéfalo é altamente dependente de fluxo sanguíneo contínuo para o suprimento de oxigênio e glicose, sendo mais vulnerável ao dano isquêmico do que os outros tecidos (Siesjo, 1978; Cechetti et al., 2011). Isto se deve a alta taxa metabólica, estoques de energia limitados e uma grande dependência do metabolismo aeróbico da glicose. A redução da taxa de fluxo sanguíneo afeta o microambiente encefálico resultando em alterações bioquímicas e morfológicas associadas à disfunção cognitiva (Rodrigo et al., 2005; Cechetti et al., 2011). Estudos experimentais têm demonstrado que após isquemia

cerebral, ocorre despolarização neuronal, aumento dos níveis de glutamato e Ca^{2+}, produção excessiva de EROs, elevação de mediadores pró-inflamatórios e ativação da sinalização de morte celular (Lo et al., 2003; Figura 7). Estes eventos podem perdurar horas ou dias e levam a morte neuronal (Durukan & Tatlisumak, 2007).
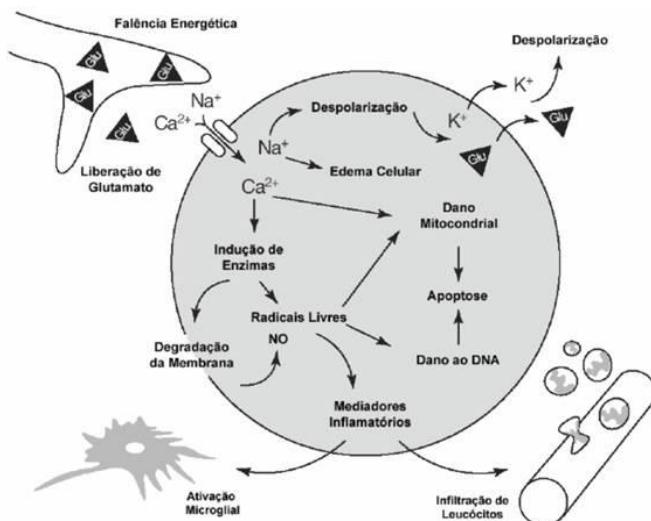
Figura 7: Modificações celulares induzidas pela isquemia cerebral (Dirnagl et al., 1999).

A isquemia cerebral global observada em humanos pode ser reproduzida em modelos animais. Um modelo amplamente utilizado é o de isquemia cerebral induzida pela oclusão dos 4 vasos. Este modelo caracteriza-se por eletrocauterização das artérias vertebrais e oclusão transitória das artérias carótidas em ratos (Pulsinelli & Brierley 1979; Figura 8). A reperfusão ocorre após a restauração do fluxo carotídeo. Este modelo resulta em morte neuronal em regiões específicas do encéfalo, incluindo neurônios piramidais da camada CA1 do hipocampo a qual é obervada até 7 dias após o insulto. Associado a este evento, também se observa um aumento na proliferação celular. Acredita-se que o aumento da proliferação celular está relacionado a um mecanismo compensatório devido à significativa morte neuronal nesta região. Esta proliferação

celular inicia no terceiro dia após o insulto e apresenta um ápice 7 a 10 dias após a isquemia (Wiltrout et al., 2007).
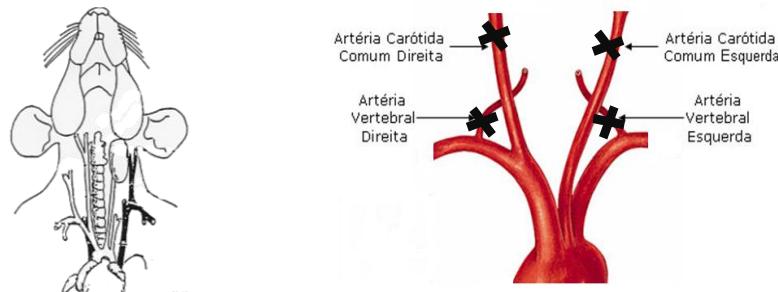
Figura 8: Modelo de isquemia cerebral induzido por oclusão dos 4 vasos.

Outro evento importante que ocorre após o insulto isquêmico é a ativação de células gliais a qual é vista como um fenômeno que pode agravar o dano neuronal e prejudicar a recuperação funcional pós-lesão (Iadecola et al., 2011; Harukuni & Bhardwaj, 2006; Mehta et al., 2007). Esta ativação glial ocorre logo após a isquemia e permanece semanas após o insulto (Marioka et al., 1991; Valentim et al., 1999) podendo influenciar a proliferação e sobrevivência das células progenitoras (Chao et al., 1992).

A ativação de células da microglia está associada à promoção de um estado pró-inflamatório o qual influencia a progressão da lesão isquêmica e agravamento dos déficits neurológicos (Kato et al., 1996; Gehrmann, et al., 1992).

A ativação de astócitos está associada à formação de cicatriz glial podendo prejudicar o crescimento axonal e a função neuronal (Sofroniew, 2009). Entretanto, um estudo utilizando camundongos *knockout* para a proteína glial fibrilar ácida (GFAP; do inglês, *glial fibrilar acid protein*), a qual integra o citoesqueleto astrocitário e está implicada em modificações estruturais e funcionais dos astócitos, demonstrou que esses animais apresentaram pior recuperação funcional após um modelo de trauma encefálico

comparado aos animais lesionados que expressam GFAP (Otani et al., 2006). Adicionalmente, foi demonstrado que astrogliose reativa adjacente à lesão em modelo de isquemia focal pode contribuir para recuperação funcional e influenciar no remodelamento neurovascular (Hayakawa et al., 2010). Portanto, a concepção de que astrogliose reativa é um processo apenas danoso talvez não seja completamente correta e que respostas gliais podem influenciar na melhora estrutural e funcional após a lesão.

1.5 Exercício físico

O exercício físico tem recebido atenção na últimas décadas devido ao seu potencial terapêutico e neuroprotetor. Esta área de investigação tem direcionado seus esforços no esclarecimento do papel do exercício sobre processos de aprendizado e memórias dependentes do hipocampo.

Estudos têm evidenciado que diferentes protocolos como corrida em esteira, natação e exercício voluntário (corrida em roda) podem melhorar a memória em roedores nos testes de *Water Maze* (Berchtold et al 2010; Vaynman et al., 2004; Pietrelli et al., 2012), reconhecimento de objetos (Hopkins et al., 2011) e esquiva inibitória (Kim et al., 2010; Radak et al., 2006; Bem et al., 2010).

 Ênfase tem sido dada sobre os efeitos do exercício físico sobre a bioquímica e a morfologia do hipocampo. Têm sido proposto que o exercício físico pode aumentar os níveis de BDNF (Vaynman et al., 2004; Radak et al., 2006; Berchtold et al., 2010) e promover neurogênese hipocampal (Farmer et al., 2004; van Praag et al., 1999). Também foi observado que exercício físico pode modular mecanismos epigenéticos, alterando a atividade das enzimas HAT e HDAC e assim, podendo induzir acetilação de histonas em hipocampo de ratos jovens (Elsner et al., 2011). Ainda, estudos demonstraram que exercício físico pode alterar os níveis de citocinas inflamatórrias

como TNF-α e IL-1β no encéfalo de roedores, no entanto os resultados são controversos (Ang et al., 2004; Chennaoui et al., 2008; Funk et al., 2011; Ding et al., 2005; Carmichael et al., 2005).

Esta divergência dos resultados pode estar relacionada aos diferentes protocolos utilizados. Neste contexto, por exemplo, Scopel e colaboradores (2006), avaliaram o efeito de diferentes intensidades de exercício físico sobre a suscetibilidade celular ao dano induzido por privação de oxigênio e glicose (POG) em fatias hipocampais de ratos. Foi demonstrado que exercício moderado (corrida em esteira, 20 min por 2 semanas), diminuiu o dano celular induzido e POG, enquanto que o exercício intenso (corrida em esteira, 60 min por 2 semanas) aumentou o dano. Estes dados indicam que a intensidade do exercício pode determinar seus efeitos, e que exercício moderado foi neuroprotetor.

Além disso, poucos estudos têm investigado a duração dos efeitos do exercício após a última sessão de treino. Berchtold e colaboradores (2010) observaram que o exercício voluntário melhorou a memória espacial e aumentou os níveis de BDNF hipocampal em camundongos, sendo que este efeito foi verificado quando avaliado 1 e 2 semanas após o término do exercício. Por outro lado, outros estudos demonstraram que a melhora da memória induzida pelo exercício, avaliada pelos testes de reconhecimento de objetos e esquiva inibitória, não foi observada 2 e 8 semanas após o útimo treino (Hopkins et al., 2011; Radak et al., 2006).

Apesar de estudos demonstrarem que o exercício físico pode melhorar a memória em roedores, seu mecanismo de ação, assim como a duração de seus efeitos após seu término, necessitam ser melhor explorados.

Objetivos

2.1 Objetivo Geral:

O objetivo dessa tese foi avaliar o efeito do exercício físico sobre a memória aversiva e parâmetros inflamatórios no processo de envelhecimento e na isquemia cerebral global.

2.2 Objetivos específicos:

I. Avaliar o efeito do exercício físico sobre a memória aversiva no processo de envelhemento, utilizando o teste de esquiva inibitória em ratos Wistar de 3 e 20 meses.

II. Avaliar o efeito do exercício físico sobre os níveis da enzima COX-2 em hipocampo de ratos Wistar de 3 meses.

III. Avaliar o efeito do exercício físico sobre os níveis de PGE_2 e seus receptores (EP1, EP2 EP3 e EP4) em hipocampo de ratos Wistar de 3 meses.

IV. Avaliar o efeito do exercício físico sobre os níveis de citocinas inflamatórias, TNFα, IL-1β e IL-4, em hipocampo de ratos Wistar de 3 e 20 meses.

V. Avaliar o efeito do exercício físico sobre os níveis do fator de transcrição NF-kB em hipocampo de ratos Wistar de 3 e 20 meses.

VI. Avaliar o efeito do exercício físico sobre os níveis de acetilação global da histona H4 em hipocampo de ratos Wistar de 3 e 20 meses.

VII. Avaliar o efeito do exercício físico sobre a ativação de astrócitos marcados com GFAP (marcador de astrócitos maduros) em hipocampo de ratos Wistar submetidos à isquemia cerebral global.

VIII. Avaliar o efeito do exercício físico sobre a ativacao de microglia marcada com Iba-1 (do inglês, *ionized calcium-binding adaptor molecule-1*, marcador de células da microglia) em hipocampo de ratos Wistar submetidos à isquemia cerebral global.

IX. Avaliar o efeito do exercício físico sobre a sobrevivencia de células BrdU-positivas (do inglês, *bromodeoxyuridine*, marcador de proliferação celular) de ratos Wistar submetidos à isquemia cerebral global.

Métodos e Resultados

3.1 CAPÍTULO 1

TIME-DEPENDENT EFFECTS OF TREADMILL EXERCISE ON AVERSIVE
MEMORY AND CYCLOOXYGENASE PATHWAY FUNCTION
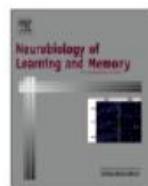
Time-dependent effects of treadmill exercise on aversive memory and cyclooxygenase pathway function

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ARTICLE INFO

ABSTRACT

Exercise induces brain function adaptations and improves learning and memory; however the time window of exercise effects has been poorly investigated. Studies demonstrate an important role for cyclooxygenase-2 (COX-2) pathway function in the mechanisms underlying memory formation. The aim of present work was to investigate the effects of treadmill exercise on aversive memory and COX-2, PGE_2 and E-prostanoid receptors contents in the rat hippocampus at different time points after exercise has ended. Adult male Wistar rats were assigned to non-exercised (sedentary) and exercised (running daily for 20 min, for 2 weeks) groups. The inhibitory avoidance task was used to assess aversive memory and the COX-2, PGE_2 and E-prostanoid receptors (EP1, EP2, EP3 and EP4) levels were determined 1 h, 18 h, 3 days or 7 days after the last training session of treadmill exercise. The step down latency in the inhibitory avoidance, COX-2 and EP4 receptors levels were acutely increased by exercise, with a significant positive correlation between aversive memory performance and COX-2 levels. Increased EP2 content decreased PGE_2 levels were observed 7 days after the last running session. The treadmill exercise protocol facilitates inhibitory avoidance memory and induces time-dependent changes on COX-2 pathways function (COX-2, PGE_2 and EP receptors).

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1. Introduction

Physical exercise has been shown to enhance memory performance and there is evidence that exercise may modulate physiological mechanisms underlying memory formation, especially those related to hippocampal neural plasticity such as increased neurogenesis (Bjørnebekk, Mathé, & Brené, 2005) and long-term potentiation (LTP), a form of synaptic plasticity and model of learning and memory (O'Callaghan et al., 2007). Most studies in rodents have focused on hippocampal-dependent behavior demonstrating that wheel running or treadmill exercise improves spatial learning in the water maze task (Albeck, Sano, Prewitt, & Dalton, 2006; Berchtold, Castello, & Cotmana, 2010; Cassilhas et al., 2011; van Praag, Christie, Sejnowski, & Gage, 1999; Vaynman, Ying, &

Gomez-Pinilla, 2004). In addition, the effects of exercise have been demonstrated in other memory tasks, such as object recognition and inhibitory avoidance (Hopkins & Bucci, 2010, 2011; Radak et al., 2006). Interestingly, the time window of exercise action on physiological and behavioral functions has been poorly investigated. Berchtold and colleagues (2010) demonstrated that the voluntary running improves memory parameters in radial arm water maze, which can persist until 2 weeks after the exercise period. Nonetheless, the object recognition memory was enhanced when rats were tested immediately after voluntary running, but not 2 weeks after last session (Hopkins & Bucci, 2011). Similarly, Radak and colleagues (2006) demonstrated that regular swimming (8 weeks) improves performance in an inhibitory avoidance task when rats were tested one day, but not 6 weeks, after the last training session.

Mechanistic studies have found an increase of neurotrophic factors levels in the hippocampus with a similar temporal profile to cognitive performance after the exercise training in rodents (Berchtold et al., 2010; Hopkins & Bucci, 2011; Radak et al.,

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2006). An association between the improvement in the aversive and declarative memory performance and increased BDNF levels in hippocampus from exercised rats is then suggested.

According to that, our group has recently demonstrated that a single session of treadmill exercise (20 min) acutely modify epigenetic parameters in the rat hippocampus (Elsner et al., 2011), indicating that the time course of the exercise effects on brain functions must be better explored.

It is important to note that different pre and post synaptic events underlying memory formation have been the subject of numerous investigations, such as neurotrophic factors and epigenetic parameters (Bekinschtein, Cammarota, Izquierdo, & Medina, 2008; Levenson & Sweatt, 2005). In this context, a role for cyclooxygenase-2 (COX-2) in memory acquisition and consolidation has been described (Rall, Mach, & Dash, 2003; Teather & Wurtman, 2003). COX-2 is enriched in brain regions involved with the memory formation, such as hippocampus, neocortex, and amygdala (Kaufmann, Andreasson, Isakson, & Worley, 1997; Yamagata, Andreasson, Kaufmann, Barnes, & Worley, 1993). The inhibition of COX-2 induced memory impairment and decreased cell proliferation and LTP (Akaneya & Tsumoto, 2006; Chen, Magee, & Bazan, 2002; Kumihashi et al., 2001; Rall et al., 2003; Teather, Packard, & Bazan, 2002). Consistent with that, Yang and Chen (2008) suggest that an increase of COX-2 expression can enhance synaptic transmission and LTP in hippocampus. Recently, it was described that treadmill exercise increases COX-2 immunoreactivity in hippocampus from Zucher rats, an inbred strain used as a genetic model for research on obesity and hypertension, and also from its derived Zucker diabetic fatty rats (Hwang et al., 2010). Considering that different strains may show variation in their outcomes to exercise, other laboratory animal strains, particularly without metabolic diseases phenotypes must be tested.

It is relevant to describe that previously we demonstrated that the treadmill exercise protocol here used, i.e., 2 weeks of daily 20 min sessions, has neuroprotective properties, since it was able to reduce in vitro ischemic damage in hippocampal slices of Wistar rats (Scopel et al., 2006).

It has been documented that Prostaglandin E_2 (PGE_2) mediates many of the COX-2-induced neurotoxicity. The effects of PGE_2 are related to its binding to four receptors, named EP1, EP2, EP3 and EP4, and activation of EP2 and EP4 receptors is frequently associated with neuroprotection (Akaike et al., 1994; Andreasson, 2010; McCullough et al., 2004), while EP1 and EP3 receptors may mediate neurotoxicity in models of excitotoxicity and cerebral ischemia (Abe et al., 2009; Ahmad, Saleem, Ahmad, & Dore, 2006; Kawano et al., 2006). To our knowledge, there is a lack of studies describing exercise-related modifications on PGE_2 and EP receptors in any brain region.

Our working hypothesis is that the exercise would affect aversive memory formation and cyclooxygenase pathway function, possibly increasing the amounts of the enzyme recently linked to memory, COX-2, as well as, of receptors related to neuroprotective properties, EP2 and EP4. The aim of the present work is to investigate the time course of treadmill exercise protocol (20 min/day during 2 weeks) effects upon aversive memory, and the hippocampus COX-2, PGE_2 and E-prostanoid receptors contents. The correlation between biochemical and behavioral effects was studied.

2. Materials and methods

2.1. Animals

Male Wistar rats aged 3 months were used. Animals were housed five per cage and maintained under standard conditions (12 h light/dark cycle, at 22 ± 2 °C, with free access to food and water). The NIH "Guide for the Care and Use of Laboratory Animals" (NIH publication No. 80-23, revised 1996) was followed in all experiments. The Local Ethics Committee (Comitê de Ética em Pesquisa – UFRGS) approved all handling and experimental conditions (nr. 2007876).

2.2. Exercise training

Rats were randomly divided into sedentary (SED) or exercised (EXE) groups. The exercise training consisted of 2 weeks daily 20 min sessions on an adapted motorized rodent treadmill (INBRAMED TK 01, Porto Alegre, Brazil), with individual Plexiglas lanes, at 60% of animals maximal oxygen uptake (Brooks & White, 1978). Peak oxygen uptake (VO_2) was measured indirectly in all animals before training. Each rat ran on a treadmill at a low initial speed increased of the speed by 5 m/min every 3 min until the point of exhaustion (i.e., failure of the rat to continue running). The time to fatigue (in min) and workload (in m/min) were taken as indexes of exercise capacity, which was in turn taken as VO_2 max (Arida, Scorza, dos Santos, Peres, & Cavalheiro, 1999; Brooks & White, 1978; Cechetti et al., 2007, 2008; Elsner et al., 2011; Scopel et al., 2006; Siqueira et al., 2009). In the first few sessions, rats were adapted to the treadmill by running at 6.7 m/min for the first 2 min, 10 m/min for the next 4 min, 15 m/min for 8 min, 10 m/min for 4 min and 6.7 m/min for the last 2 min. Thereafter, animals ran at 6.7 m/min for the first 4 min, 15 m/min for 12 min and 6.7 m/min for the last 4 min (Elsner et al., 2011). Any animal that initially refused to run were encouraged by gently tapping in their backs; neither electric shock nor physical prodding was used in this study. The SED group was handled exactly as the experimental animals and was left on the treadmill for 5 min without any stimulus to run. Running sessions took place between 14:00 and 17:00 h.

2.3. Inhibitory avoidance

We used the single-trial step-down inhibitory avoidance task as an established model of fear-motivated memory. At step-down inhibitory avoidance training, animals learn to associate a location in the training apparatus (a grid floor) with an aversive stimulus (footshock). The general procedures for inhibitory avoidance behavioral training and retention test were described in previous reports (Amaral, Luft, Cammarota, Izquierdo, & Roesler, 2007; Quevedo et al., 1999). The inhibitory avoidance apparatus was a $50 \times 25 \times 25$ cm acrylic box (Albarsch, Porto Alegre, Brazil) whose floor consists of parallel caliber stainless steel bars (1 mm diameter) spaced 1 cm apart. A 7 cm wide, 2.5 cm high platform was placed on the floor of the box against the left wall.

In the training trial, rats were placed on the platform and their latency to step down to the grid with all four paws was measured with a digital chronometer connected to the control box unit. Immediately after stepping down onto the grid, rats received a 0.6 mA, 3.0 s footshock and were removed from the apparatus immediately after the footshock. The behavioral task was performed 30 min before euthanasia on days 14, 15, 17 and 21, resulting in different groups namely 1 h, 18 h, 3 days or 7 days, respectively (Fig. 1). The test trial took place 24 h after the training one, by placing the rats on the platform and recording their latencies to step down. Retention test latencies were cut off at 180 s and no footshock was delivered. Different groups were submitted to inhibitory avoidance task in different time of day, since running sessions took place between 14:00 and 17:00 h and the time course of exercise effects was evaluated. The acute and delayed effects on memory task were evaluated between 15:00 and 18:00 h, while the inhibitory avoidance training and test was employed between 8:00 and 11:00 h in 18 h group (18 h after the last exercise
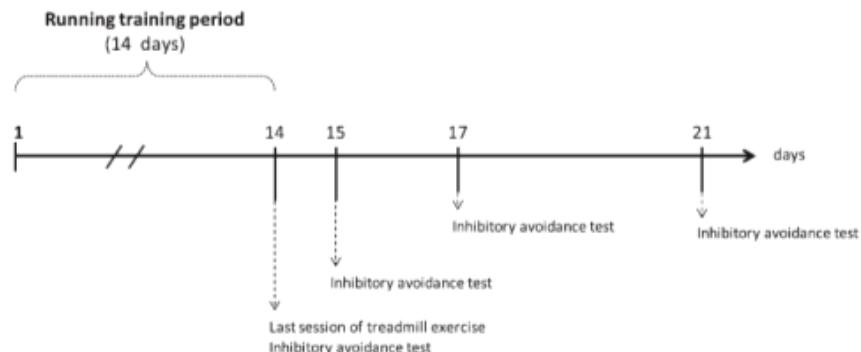
Fig. 1. Diagram showing the timeline of experiments used in the present study. The treadmill exercise protocol (20 min/day during 14 days) was used. The training trial took place 24 h before the test one, by placing the rats on the platform and recording their latencies to step down. The behavioral task was performed 30 min before euthanasia on days 14, 15, 17 and 21, resulting in different groups namely 1 h, 18 h, 3 days or 7 days, respectively.

session). In order to minimize the time of day effect, one control group, SED, was evaluated for every EXE one. The behavioral task was performed 30 min before euthanasia (Fig. 1).

2.4. Sample preparation

Exercised rats were decapitated 1 h (acutely), 18 h, 3 days or 7 days (delayed) after the last session of treadmill exercise (Fig. 1). The hippocampus were quickly dissected out and immediately frozen in liquid nitrogen, then stored at −80 °C until the determination content of COX-2, PGE_2 and E-prostanoid receptors (EP1, EP2, EP3 and EP4). On the day of the assays, hippocampi were homogenized and the lysates were subject to centrifugation in a microcentrifuge tube, supernatant was removed for analysis. Protein concentration of each sample was measured by the Coomassie Blue method using bovine serum albumin as standard (Bradford, 1976).

2.5. PGE_2 enzyme immunoassay

Tissue PGE_2 concentration was evaluated using an enzyme immunoassay kit (Cayman Chemical, EUA). The PGE_2 concentration was determined spectrophotometrically after incubation with tracer and anti-PGE_2 monoclonal antibody on a microplate according to the manufacturer's instructions.

2.6. COX-2 and E-prostanoids receptors determination by Slot Blot

Samples were incubated with denaturing solution and after, were applied on a membrane by a Slot Blot Filtration (Bio-Rad, USA). The membranes were incubated with TBS buffer (Tris–HCl, NaCl) containing blocking solution and later incubated with specific antibody for capture. Each membrane was then incubated with COX-2 antibodies (1:1500) or one type E-prostanoid receptor antibodies (EP1, EP2, EP3 or EP4) (1:1000). Thereafter, membranes were washed with wash buffer and were next incubated with TBS buffer containing Tween 20, blocking solution and the peroxidase-linked detection antibody. After wash, membranes were incubated with color reagent Blots were dried, scanned and quantized with ImageJ (PC version of Macintosh compatible NIH image). The blot had a faint background that was corrected in image analysis.

2.7. Statistical analysis

All results are presented as mean (±S.D.). Results of behavioral testing were expressed in latencies (sec) to step down platform. Data were evaluated by Two-Way Analysis of Variance (ANOVA) followed by the Duncan multiple comparison test when appropriate. Biochemical results were expressed as percentage of control, relative to the sedentary group at each time point. Independent-sample Student t-test was used to compare the difference between
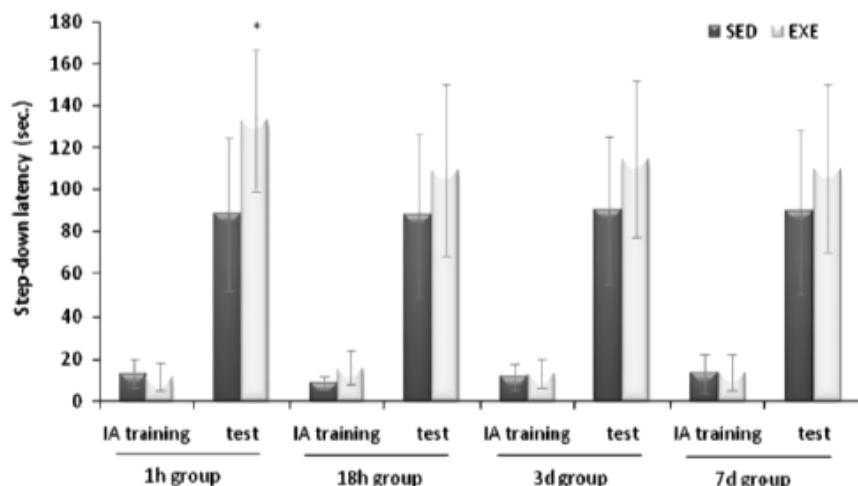

Fig. 2. Exercise effect on step down latency in inhibitory avoidance. Columns represent mean ± S.D. (n = 9–11). ANOVA followed by Duncan. *Values significantly different from those respective sedentary control group ($p < 0.05$).
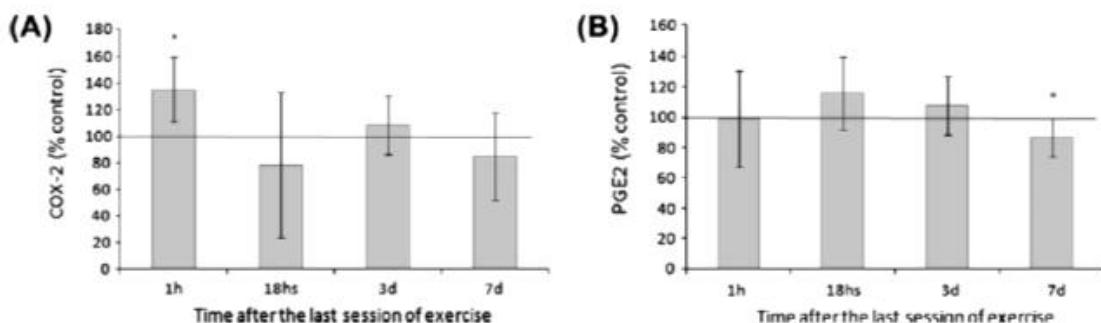
Fig. 3. Effect of treadmill exercise protocol on COX-2 and its product PGE$_2$ levels contents in hippocampus of rats. (A) Effect of exercise on COX-2 content. (B) Effect of exercise on PGE$_2$ levels. Results are expressed as percentage of control. Line represents time-matched sedentary controls results (n = 5–7). Independent-samples Student's t-test. *Values significantly different from those respective sedentary control group (p < 0.05).
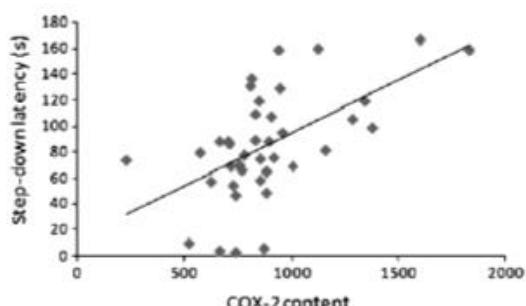
Fig. 4. Correlation between step down latency in inhibitory avoidance test and COX-2 content. Linear regression analysis and Pearson correlation (r = 0.571, p < 0.001).

EXE and SED groups at each time point. Linear regression analysis and Pearson correlation were used to study the association between behavioral and biochemical results. In all tests, p < 0.05 was considered significant.

3. Results

3.1. Step-down inhibitory avoidance

Latencies to step down from the platform during training were similar between all groups (ANOVA, $F_{(1,74)}$ = 1.581, p = 0.213). The aversive memory was evaluated in a test session carried out 24 h after training. Two-way ANOVA showed a main group effect of exercise on step down latencies ($F_{(1,66)}$ = 9.316,

p = 0.003; Fig. 2); the exercise induced an acute increase in the step down latency when compared to the sedentary group (p < 0.05). There was no significant delayed effect in the step down latency, as measured 18 h, 3 days or 7 days after the last session of treadmill exercise.

3.2. COX-2 determination

The exercise protocol acutely augmented the COX-2 content in the rat hippocampus after the last treadmill session (t = −3.06, df = 9, p = 0.014; Fig. 3); a significant positive correlation between inhibitory avoidance test and COX-2 content was revealed (r = 0.571, p < 0.001; Fig. 4).

3.3. PGE$_2$ determination

It was observed a decrease on the PGE$_2$ levels only 7 days after the last session of treadmill in exercised rats, when compared to their respective sedentary control (t = 2.27, df = 12, p = 0.042; Fig. 3).

3.4. EPs receptors determination

The hippocampus of exercised rats showed an increase of EP2 receptor content 7 days after the last treadmill session (t = −2.20, df = 16, p = 0.048; Fig. 5), while an acute increase on EP4 receptor content (1 h after the last training session, t = −2.34, df = 17, p = 0.031; Fig. 5) was also observed. The levels of EP1 and EP3 receptors were not modified by exercise (data not shown).
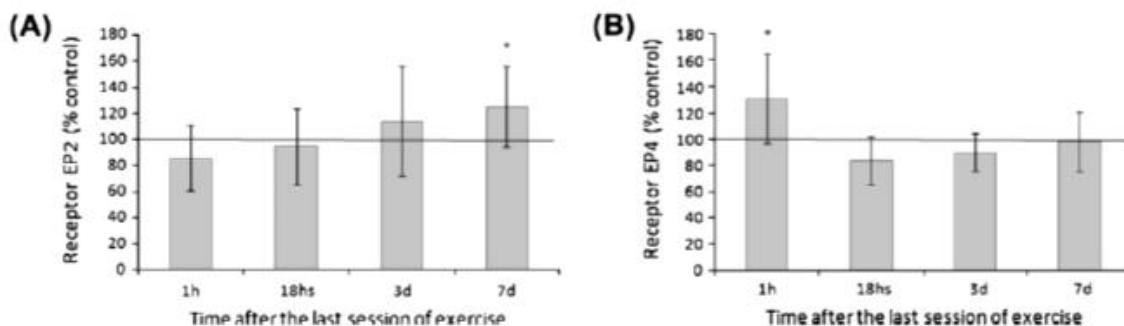
Fig. 5. Effect of treadmill exercise protocol on EP2 receptor content and EP4 receptor content in hippocampus of rats. (A) Effect of exercise on EP2 content. (B) Effect of exercise on EP4 content. Results are expressed as percentage of control. Line represents time-matched sedentary controls results (n = 6–9). Independent-samples Student's t-test. *Values significantly different from those respective sedentary control group (p < 0.05).

4. Discussion

The present study demonstrates that exercise may modulate behavioral and biochemical functions, specifically improving aversive memory performance and modifying the COX-2 pathway with both acute and delayed features. To our knowledge, this is the first evidence of a modulation effect of exercise on the COX-2 pathway function, E-prostanoids receptors and PGE_2 in hippocampus. Although it was previously described that treadmill exercise increases COX-2 immunoreactivity in hippocampus from diabetic and obese rats (Hwang et al., 2010), we here describe the effect of exercise on COX-2 content in the hippocampus of healthy animals.

Our exercise protocol (20 min/day during 2 weeks) transiently improved the inhibitory avoidance aversive memory performance, as shown by longer latencies to step down from the platform compared to sedentary group. This result is not related to exercise-induced changes in locomotor activity (e.g. fatigue), since this effect would have been detected in inhibitory avoidance training latencies (see Fig. 2). Interestingly, Berchtold and colleagues (2010) demonstrated that the cognitive effect of running can persist in a spatial task, the radial arm water maze. In agreement with our results, other studies showed a similar temporal profile of exercise effects on non-spatial memory paradigms, specifically increasing acutely the aversive and declarative memory, respectively, in passive avoidance (Radak et al., 2006) and object recognition tasks (Hopkins & Bucci, 2011). Along with the behavioral effect, exercise caused an acute increase on COX-2 content. Corroborating with the hypothesis that COX-2 is involved with memory formation (Kaufmann et al., 1997; Yamagata et al., 1993), a positive correlation between COX-2 content and inhibitory avoidance performance was observed, so allowing us to suggest that the exercise-induced COX-2 levels may be involved, at least in part, in the memory enhancing effects of exercise.

The exercise-induced COX-2 levels increase could be related to N-methyl-D-aspartate (NMDA) glutamate receptors, since NMDA receptors are involved in the aversive, contextual and spatial aspects of memory (Izquierdo & Medina, 1997; Roesler et al., 2003). NMDA receptor-dependent synaptic activity dynamically regulates the expression of the COX-2 gene in the brain. Interestingly, it was observed that exercise acutely increased the hippocampal COX-2 content without any change on PGE_2 levels; we can suppose that the involvement of COX-2 on memory formation, and its relationship with cognitive effects of exercise, is PGE_2-independent. Although several authors have claimed that PGE_2 is the effector of COX-2-induced synaptic plasticity (Chen & Bazan, 2005; Yang & Chen, 2008), Hein and colleagues (2007) suggested that it is impossible to exclude the role of others PGs. Considering that COX-2-induced neurotoxicity seems be induced by PGE_2 production, the lack of acute effect on PGE_2 levels would exclude the potential toxicity induced by the increase of COX-2.

The behavioral finding here presented might be linked to role of COX-2 on endocannabinoid signaling. Endocannabinoids, endogenous metabolites of eicosanoid fatty acids, namely 2-arachidonoyl glycerol (2-AG) and arachidonoyl ethanolamide (AEA), are substrates for COX-2 (Kozak, Prusakiewicz, & Marnett, 2004; Piomelli, 2003). COX-2 oxygenates 2-AG and AEA, respectively, to prostaglandin glycerol esters and prostaglandin ethanolamides. Then, the augment of COX-2 activity may accelerate the metabolism of endocannabinoids, reducing their levels (Chen & Bazan, 2005; Yang & Chen, 2008), besides it has been well established that cannabinoid blockade can improve memory (Bialuk & Winnicka, 2011; Castellano, Rossi-Arnaud, Cestari, & Costanzi, 2003).

Surprisingly, despite increasing evidence of memory impairment by COX-2 inhibition (Rall et al., 2003; Sharifzadeh et al., 2005; Teather et al., 2002) there are no published clinical studies investigating the role of COX-2 inhibition on normal learning and memory.

Although the time window of memory-enhancing exercise effect was consistent with the increases in COX-2 and EP4 receptor contents, we did not show any correlation between memory and EP4 receptor content. Considering our findings and those of literature, we can correlate COX-2 with memory formation, while EP4 levels might be linked to neuroprotection effects. It is relevant to note that we previously demonstrated that this protocol, i.e., 2 weeks of daily 20 min sessions, reduces *in vitro* ischemic damage in hippocampal slices of Wistar rats (Scopel et al., 2006). EP4 may confer neuroprotection in excitotoxic or hypoxic paradigms *in vitro* and *in vivo* models (Andreasson, 2010). Additionally, selective EP4 agonist protected neurons in hippocampal slices against NMDA-induced excitotoxicity (Ahmad, Ahmad, de Brum-Fernandes, & Dore, 2005).

This exercise protocol has delayed effects on EP2 receptor content, which has been linked to neuroprotection activity. Ahmad and colleagues (2006) demonstrated that the EP2 receptor stimulation has a protective role in a model of striatal excitotoxicity. Therefore, it is reasonable to think that the modulation of EP2 and EP4 prostanoid receptors might be related, at least in part, to the neuroprotective effects of exercise previously described (Scopel et al., 2006).

It is impossible to establish, at this moment, a definite mechanism by which the exercise increases EP2 levels and decreases PGE_2 content in the long term, although it is plausible that modulation of epigenetic modifications that could vary according to specific genes (Szyf, 2009). We might suggest that the exercise was able to reduce PGE2 synthase expression without affecting COX-2 expression. Interestingly, the exercise induced a reduction on PGE_2 levels without affecting COX-2 content at 7 days after the last training session. This result may be related to Candelario-Jalil & colleagues, 2007, which demonstrated that resveratrol, a neuroprotective compound, was able to reduce both PGE_2 levels and PGE_2 synthase without affecting COX-2 content in brain rats.

Summarizing, the treadmill exercise causes time-dependent changes on COX-2 pathways function (COX-2, PGE_2 and EP receptors) and inhibitory avoidance memory. We suggest that the increase of COX-2 is associated to the exercise-induced acute memory enhancing effect; in addition the increase of EP2 and EP4 receptors content, as well as the decrease of PGE_2 levels, may be associated to the neuroprotective effects of this exercise protocol. Further studies are needed to elucidate the modulatory action of exercise on cyclooxygenase pathway function and its relationship with the memory improvement.

Acknowledgments

References

Abe, T., Kunz, A., Shimamura, M., Zhou, P., Anrather, J., & Iadecola, C. (2009). The neuroprotective effect of prostaglandin E2 EP1 receptor inhibition has a wide therapeutic window, is sustained in time and is not sexually dimorphic. *Journal of Cerebral Blood Flow & Metabolism, 29*, 66–72.

Ahmad, A. S., Ahmad, M., de Brum-Fernandes, A. J., & Dore, S. (2005). Prostaglandin EP4 receptor agonist protects against acute neurotoxicity. *Brain Research, 1066*, 71–77.

Ahmad, A. S., Saleem, S., Ahmad, M., & Dore, S. (2006). Prostaglandin EP1 receptor contributes to excitotoxicity and focal ischemic brain damage. *Toxicology Science, 89*, 265–270.

Akaike, A., Kaneko, S., Tamura, Y., Nakata, N., Shiomi, H., Ushikubi, F., et al. (1994). Prostaglandin E2 protects cultured cortical neurons against N-methyl-d-aspartate receptor-mediated glutamate cytotoxcity. *Brain Research, 663*, 237–244.

Akaneya, Y., & Tsumoto, T. (2006). Bidirectional trafficking of prostaglandin E2 receptors involved in long-term potentiation in visual cortex. *Journal Neuroscience, 26*, 10209–10221.

Albeck, D. S., Sano, K., Prewitt, G. E., & Dalton, L. (2006). Mild forced treadmill exercise enhances spatial learning in the aged rat. *Behavior Brain Research, 168*, 345–348.

Amaral, O. B., Luft, T., Cammarota, M., Izquierdo, I., & Roesler, R. (2007). Temporary inactivation of the dorsal hippocampus induces a transient impairment in retrieval of aversive memory. *Behavioral Brain Research, 180*, 113–118.

Andreasson, K. (2010). Emerging roles of PGE$_2$ receptors in models of neurological disease. *Prostaglandins and Other Lipid Mediators, 91*, 104–112.

Arida, R. M., Scorza, F. A., dos Santos, N. F., Peres, C. A., & Cavalheiro, E. A. (1999). Effect of physical exercise on seizure occurrence in a model of temporal lobe epilepsy in rats. *Epilepsy Research, 37*, 45–52.

Bekinschtein, P., Cammarota, M., Izquierdo, I., & Medina, J. H. (2008). BDNF and memory formation and storage. *Neuroscientist, 14*(2), 147–156.

Berchtold, N. C., Castello, N., & Cotmana, C. W. (2010). Exercise and time-dependent benefits to learning and memory. *Neuroscience, 167*, 588–597.

Bialuk, I., & Winnicka, M. M. (2011). AM251, cannabinoids receptors ligand, improves recognition memory in rats. *Pharmacological Reports, 63*(3), 670–679.

Bjørnebekk, A., Mathé, A. A., & Brené, S. (2005). The antidepressant effect of running is associated with increased hippocampal cell proliferation. *International Journal Neuropsychopharmacology, 8*, 357–368.

Bradford, M. M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*, 218–254.

Brooks, G. A., & White, T. P. (1978). Determination of metabolic and heart rate responses of rats to treadmill exercise. *Journal of Applied Physiology, 45*, 1009–1015.

Candelario-Jalil, E., de Oliveira, A. C. P., Gräf, S., Bhatia, H. S., Hüll, M., Muño, E., et al. (2007). Resveratrol potently reduces prostaglandin E2 production and free radical formation in lipopolysaccharide-activated primary rat microglia. *Journal Neuroinflammation, 10*, 4–25.

Cassilhas, R. C., Lee, K. S., Fernandes, J., Oliveira, M. G., Tufik, S., Meeusen, R., et al. (2011). Spatial memory is improved by aerobic and resistance exercise through divergent molecular mechanisms. *Neuroscience, 27*, 309–317.

Castellano, C., Rossi-Arnaud, C., Cestari, V., & Costanzi, M. (2003). Cannabinoids and memory: animal studies. *Current Drug Targets CNS Neurological Disorders, 2*(6), 389–402.

Cechetti, F., Fochesatto, C., Scopel, D., Nardin, P., Gonçalves, C. A., Netto, C. A., et al. (2008). Effect of a neuroprotective exercise protocol on oxidative state and BDNF levels in the rat hippocampus. *Brain Research, 1188*(January), 182–188.

Cechetti, F., Rhod, A., Simão, F., Santin, K., Salbego, C., Netto, C. A., et al. (2007). Effect of treadmill exercise on cell damage in rat hippocampal slices submitted to oxygen and glucose deprivation. *Brain Research, 1157*(July), 121–125.

Chen, C., & Bazan, N. G. (2005). Endogenous PGE$_2$ regulates membrane excitability and synaptic transmission in hippocampal CA1 pyramidal neurons. *Journal Neurophysiology, 93*, 929–941.

Chen, C., Magee, J. C., & Bazan, N. G. (2002). Cyclooxygenase-2 regulates prostaglandin E2 signaling in hippocampal long-term synaptic plasticity. *Journal Neurophysiology, 87*, 2851–2857.

Elsner, V. R., Lovatel, G. A., Bertoldi, K., Vanzella, C., Santos, F. M., Spindler, C., et al. (2011). Effect of different exercise protocols on histone acetyltransferases and histone deacetylases activities in rat hippocampus. *Neuroscience, 29*, 580–587.

Hein, A. M., Stutzman, D. L., Bland, S. T., Barrientos, R. M., Watkins, L. R., Rudy, J. W., et al. (2007). Prostaglandins are necessary and sufficient to induce contextual fear learning impairments after interleukin-1 beta injections into the dorsal hippocampus. *Neuroscience, 150*, 754–763.

Hopkins, M. E., & Bucci, D. J. (2010). Mechanisms underlying exercise-induced improvement in object recognition memory. *Neurobiology of Learning and Memory, 94*, 278–284.

Hopkins, M. E., & Bucci, D. J. (2011). Physical exercise during adolescence versus adulthood: Differential effects on object recognition memory and brain-derived neurotrophic factor levels. *Neuroscience, 194*, 84–94.

Hwang, I. K., Yi, S. S., Yoo, K. Y., Park, O. K., Yan, B., Kim, I. Y., et al. (2010). Effects of treadmill exercise on cyclooxygenase-2 in the hippocampus in type 2 diabetic rats: Correlation with the neuroblasts. *Brain Research, 1341*, 84–92.

Izquierdo, I., & Medina, J. H. (1997). Memory formation: The sequence of biochemical events in the hippocampus and its connection to activity in other brain structures. *Neurobiology of Learning and Memory, 68*(3), 285–316.

Kaufmann, W. E., Andreasson, K. I., Isakson, P. C., & Worley, P. F. (1997). Cyclooxygenases and the central nervous system. *Prostaglandins, 54*, 601–624.

Kawano, T., Anrather, J., Zhou, P., Park, L., Wang, G., Frys, K. A., et al. (2006). Prostaglandin E2 EP1 receptors: downstream effectors of COX-2 neurotoxicity. *Nature Medicine, 12*, 225–229.

Kozak, K. R., Prusakiewicz, J. L., & Marnett, L. J. (2004). Oxidative metabolism of endocannabinoids by COX-2. *Current Pharmaceutical Design, 10*, 659–667.

Kumihashi, K., Uchida, K., Miyazaki, H., Kobayashi, J., Tsushima, T., & Machida, T. (2001). Acetylsalicylic acid reduces ischemia-induced proliferation of dentate cells in gerbils. *Neuroreport, 12*, 915–917.

Levenson, J. M., & Sweatt, D. (2005). Epigenetic mechanisms in memory formation. *Nature Reviews Neuroscience, 6*(2), 108–118.

McCullough, L., Wu, L., Haughey, N., Liang, X., Hand, T., Wang, Q., et al. (2004). Neuroprotective function of the PGE$_2$ EP2 receptor in cerebral ischemia. *Journal of Neuroscience, 24*, 257–268.

O'Callaghan, R. M., Ohle, R., & Kelly, A. M. (2007). The effects of forced exercise on hippocampal plasticity in the rat: A comparison of LTP, spatial- and non-spatial learning. *Behavior Brain Research, 176*, 362–366.

Piomelli, D. (2003). The molecular logic of endocannabinoid signaling. *Nature Reviews Neuroscience, 4*, 873–884.

Quevedo, J., Vianna, M. R., Roesler, R., De-Paris, F., Izquierdo, I., & Rose, S. P. (1999). Two time windows of anisomycin-induced amnesia for inhibitory avoidance training in rats: Protection from amnesia by pretraining but not pre-exposure to the task apparatus. *Learning and Memory, 6*, 600–607.

Radak, Z., Toldy, A., Szabo, Z., Siamilis, S., Nyakas, C., Silye, G., et al. (2006). The effects of training and detraining on memory, neurotrophins and oxidative stress markers in rat brain. *Neurochemistry International, 49*, 387–392.

Rall, J. M., Mach, S. A., & Dash, P. K. (2003). Intrahippocampal infusion of a cyclooxygenase-2 inhibitor attenuates memory acquisition in rats. *Brain Research, 968*, 273–276.

Roesler, R., Schröder, N., Vianna, M. R., Quevedo, J., Bromberg, E., Kapczinski, F., et al. (2003). Differential involvement of hippocampal and amygdalar NMDA receptors in contextual and aversive aspects of inhibitory avoidance memory in rats. *Brain Research, 975*(1–2), 207–213.

Scopel, D., Fochesatto, C., Cimarosti, H., Rabbo, M., Belló-Klein, A., Salbego, C., et al. (2006). Exercise intensity influences cell injury in rat hippocampal slices exposed to oxygen and glucose deprivation. *Brain Research Bulletin, 71*(1–3), 155–159.

Sharifzadeh, M., Naghdi, N., Khosrovani, S., Ostad, S. N., Sharifzadeh, K., Roghani, A., et al. (2005). Post-training intrahippocampal infusion of the COX-2 inhibitor celecoxib impaired spatial memory retention in rats. *European Journal of Pharmacology, 511*(2–3), 159–166.

Siqueira, I. R., Elsner, V. R., Rilho, L. S., Bahlis, M. G., Bertoldi, K., Rozisky, J. R., et al. (2009). A neuroprotective exercise protocol reduces the adenine nucleotide hydrolysis in hippocampal synaptosomes and serum of rats. *Brain Research, 1316*(February), 173–180.

Szyf, M. (2009). The early life environment and the epigenoma. *Biochimica et Biophysica Acta, 1790*(2009), 878–885.

Teather, L. A., Packard, M. G., & Bazan, N. G. (2002). Post-training cyclooxygenase-2 (COX-2) inhibition impairs memory consolidation. *Learning & Memory, 9*, 41–47.

Teather, L. A., & Wurtman, R. J. (2003). Cyclooxygenase-2 mediates platelet-activating factor-induced prostaglandin E2 release from rat primary astrocytes. *Neuroscience Letters, 340*(3), 177–180.

van Praag, H., Christie, B. R., Sejnowski, T. J., & Gage, F. H. (1999). Running enhances neurogenesis, learning, and long-term potentiation in mice. *Proceedings of the National Academy of Sciences, 96*(9), 13427–13431.

Vaynman, S., Ying, Z., & Gomez-Pinilla, F. (2004). Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. *European Journal of Neuroscience, 20*, 2580–2590.

Yamagata, K., Andreasson, K. I., Kaufmann, W. E., Barnes, C. A., & Worley, P. F. (1993). Expression of a mitogen-inducible cyclooxygenase in brain neurons: regulation by synaptic activity and glucocorticoids. *Neuron, 11*, 371–386.

Yang, H., & Chen, C. (2008). Cyclooxygenase-2 in synaptic signaling. *Current Pharmaceutical Design, 14*, 1443–1451.

3.2 CAPÍTULO 2

TREADMILL EXERCISE INDUCES AGE-RELATED CHANGES IN AVERSIVE MEMORY, NEUROINFLAMMATORY AND EPIGENETIC PROCESSES IN THE RAT HIPPOCAMPUS

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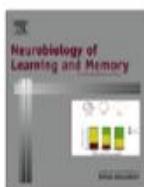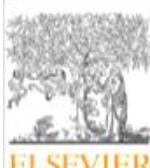
Treadmill exercise induces age-related changes in aversive memory, neuroinflammatory and epigenetic processes in the rat hippocampus

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ABSTRACT

It has been described that exercise can modulate both inflammatory response and epigenetic modifications, although the effect of exercise on these parameters during the normal brain aging process yet remains poorly understood. Here, we investigated the effect of aging and treadmill exercise on inflammatory and epigenetic parameters specifically pro and anti-inflammatory cytokines levels, activation of NF-kB and histone H4 acetylation levels in hippocampus from Wistar rats. Additionally, we evaluated aversive memory through inhibitory avoidance task. Rats of 3 and 20 months of age were assigned to non-exercised (sedentary) and exercised (running daily for 20 min for 2 weeks) groups. The effect of daily forced exercise in the treadmill was assessed. The levels of inflammatory and epigenetic parameters were determined 1 h, 18 h, 3 days or 7 days after the last training session of exercise. It was observed an age-related decline on aversive memory, as well as aged rats showed increased hippocampal levels of inflammatory markers, such as TNFα, IL1-β and NF-kB and decreased IL-4 levels, an anti-inflammatory cytokine. Moreover, lower levels of global histone H4 acetylation were also observed in hippocampi from aged rats. Interestingly, there was a significant correlation between the biochemical markers and the inhibitory avoidance test performance. The forced exercise protocol ameliorated aging-related memory decline, decreased pro-inflammatory markers and increased histone H4 acetylation levels in hippocampi 20-months-old rats, while increased acutely IL-4 levels in hippocampi from young adult rats. Together, these results suggest that an imbalance of inflammatory markers might be involved to the aging-related aversive memory impairment. Additionally, our exercise protocol may reverse aging-related memory decline through improving cytokine profile.

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1. Introduction

Several studies have pointed out that both normal aging process and neurodegenerative diseases are associated with chronic neuroinflammatory response (de Magalhaes, Curado, & Church, 2009; Lynch, 2010; Salminen et al., 2008). It has been suggested that alterations on pro-inflammatory cytokines levels, such as interleukin-1β (IL-1β) and tumor-necrosis factor alpha (TNF-α), and anti-inflammatory cytokines, such as interleukin-4 (IL-4) and interleukin-10 (IL-10), may contribute to aged-related decline of brain functions (Buchanan, Sparkman, Chen, & Johnson, 2008; Griffin et al., 2006; Lynch, 2010; Nolan et al., 2005; O'Donnell et al., 2000).

Interestingly, a great body of evidences has shown that cytokines can alter memory formation and synaptic plasticity, where the both the over-expression and absence of IL-1β and TNF-a may directly influence the long-term potentiation (LTP) maintenance, an animal model for learning and memory. However, the involvement the inflammatory cytokines on hippocampal-dependent learning and memory behavioral tasks; i.e., spatial memory, object recognition and contextual fear conditioning has been poorly studied. There are no studies, to our knowledge, reporting the association of inflammatory cytokines with aversive memory formation in the aging process.

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The neuroinflammation is a dynamic process subject to aging process and several factors, including physical activity, seem to modulate inflammatory signaling (Wannamethee et al., 2002). Treadmill exercise can decrease TNF-α and IL-1β levels in different brain regions from healthy young rodents (Ang, Wong, Moochhala, & Ng, 2004; Chennaoui, Drogou, & Gomez-Merino, 2008; Funk et al., 2011), however Ding et al. (2005) showed controversial results. We recently demonstrated that the treadmill exercise alters some neuroinflammatory parameters, such as cyclooxygenase-2 (COX-2), prostaglandin E2 and E-prostanoid receptors levels in young adult rats (Lovatel et al., 2012). Besides, the aversive memory enhancing effects by exercise seems be related to higher COX-2 (Lovatel et al., 2012).

In addition, the aging process has been associated with increased activation of the nuclear factor-kappaB (NF-kB), an inducible transcription factor complex that regulates the expression of inflammatory molecules (Baeuerle & Baichwal, 1997; Manning & Anderson, 1994). NF-kB activation is commonly short-lived, but this pattern may be different during the aging process (Yu & Chung, 2006). Interestingly, the NF-κB-signaling pathway may be regulated by histone acetylation, an important epigenetic mechanism that can control the expression of specific genes with opposite directions depending on the cell type (Chen, Fischle, Verdin, & Greene, 2001; Jenuwein & Allis, 2001; Viatour et al., 2003; Yamamoto, Verma, Prajapati, Kwak, & Gaynor, 2003). There are evidences indicating that histone acetylation status is implicated with the inflammatory responses. However, these findings were mostly based in hematopoietic cell, monocytes and macrophages (Bode et al., 2007; Rahman, Marwick, & Kirkham, 2004; Roger et al., 2011). However, studies reporting the relationship between NF-kB or histone acetylation status and neuroinflammation cytokines during aging process are lack.

It is important to note that histone acetylation is controlled by histone acetyltransferases (HAT) and histone deacetylases (HDAC) enzymes (Choi & Howe, 2009; Kouzarides, 2007). It has been widely recognized that histone acetylation is associated with enhanced transcriptional activity whereas deacetylation is typically associated with transcriptional repression (Kimura, Matsubara, & Horikoshi, 2005; Kouzarides, 2007). Interestingly, several groups have demonstrated that memory formation is associated with increased levels of histone acetylation (Barrett & Wood, 2008; Mikaelsson & Miller, 2011). Evidences have indicated that histone deacetylase inhibitors (HDACi) are able to improve memory performance. Studies demonstrated that administration of HDACi improved memory in object recognition (Reolon et al., 2011), conditioned context (Vecsey et al., 2007), LTP (Levenson et al., 2004) and water maze (Peleg et al., 2010; Ricobaraza, Cuadrado-Tejedor, & Garcia-Osta, 2011; Ricobaraza et al., 2009). We previously showed that a single session of treadmill exercise increased HAT activity, in addition decreased HDAC activity in hippocampi from young adult rats, suggesting that this exercise protocol can induce histone acetylation (Elsner et al., 2011). Accordingly, several studies have demonstrated that exercise improves performance in different memory and learning tasks in aged and young rodents (Berchtold, Castello, & Cotmana, 2010; Radak et al., 2006; van Praag, Shubert, Zhao, & Gage, 2005). Despite these findings, to our knowledge there are no studies reporting the impact of exercise on global histone acetylation in rat brain during normal aging process.

Our working hypothesis was that memory behavioral tasks performance is correlated to neuroinflammation and epigenetic markers, as well forced exercise would be able to reverse the aging effects. Thus, the aim of this study was to investigate the effects of treadmill exercise protocol (20 min/day during 2 weeks) on TNFα, IL-1β, IL-4, NF-kB and histone H4 acetylation levels in hippocampi from 3 and 20-months-old Wistar rats. Moreover, we also investigated the time course of the exercise effects, specifically, 1 h, 18 h, 3 days and 7 days after the last session of treadmill exercise.

2. Methods

2.1. Animals

Male Wistar rats of different ages, 3 and 20-months-old were used. The animals were provided by Centro de Reprodução de Animais de Laboratório (CREAL) at Universidade Federal do Rio Grande do Sul (UFRGS) and were maintained under standard conditions (12-h light/dark, 22 ± 2 °C) with food and water *ad libitum*. The NIH "Guide for the Care and Use of Laboratory Animals" (NIH Publication No. 80-23, revised 1996) was followed in all experiments. The Local Ethics Committee (CEUA de Ética em Pesquisa – UFRGS) approved all handling and experimental conditions (nr. 21449).

2.2. Training

Rats were randomly divided into sedentary (SED) or exercised (EXE) groups. EXE were submitted to exercise protocol consisted on 20 min running session each day for 2 weeks (Fig. 1). SED was handled exactly as the experimental animals and was left on the treadmill for 5 min without any stimulus to run. The exercise training consisted of running sessions in an adapted motorized rodent treadmill (INBRAMED TK 01, Porto Alegre, Brazil), with individual Plexiglas lanes, at 60% of the animals maximal oxygen uptake (Brooks & White, 1978). Peak oxygen uptake (VO₂) was measured indirectly in all animals before training. Each rat ran on a treadmill at a low initial speed with the speed being increased by 5 m/min every 3 min until the point of exhaustion (i.e., failure of the rat to continue running). The time to fatigue (in min) and workload (in m/min) were taken as indexes of exercise capacity, which was in turn taken as VO₂ max (Arida, Scorza, dos Santos, Peres, & Cavalheiro, 1999; Brooks & White, 1978; Cechetti et al., 2007, 2008; Elsner et al., 2011; Scopel et al., 2006). Rats of 3-months-old were adapted to the treadmill by running, in the first few sessions, at 6.7 m/min for the first 2 min, 10 m/min for the next 4 min, 15 m/min for 8 min, 10 m/min for 4 min and 6.7 m/min for the last 2 min. Thereafter, animals ran at 6.7 m/min for the first 4 min, 15 m/min for 12 min and 6.7 m/min for the last 4 min. Rats of 20-months-old were adapted to the treadmill by running, in the first few sessions, at 4.2 m/min for the first 2 min, 6.3 m/min for the next 4 min, 9.5 m/min for 8 min, 6.3 m/min for 4 min and 4.2 m/min for the last 2 min. Thereafter, animals ran at 4.2 m/min for the first 4 min, 9.5 m/min for 12 min and 4.2 m/min for the last 4 min. Animals that initially refused to run were encouraged by gently tapping their backs. Neither electric shock nor physical prodding was used in this study and all the procedures took place between 14:00 and 17:00 h.

2.3. Inhibitory avoidance test

The inhibitory avoidance apparatus was consisted on 50 × 25 × 25 cm acrylic box (Albarsch, Porto Alegre, Brazil) whose floor consists of parallel caliber stainless steel bars (1 mm diameter) spaced 1 cm apart. A 7 cm wide, 2.5 cm high platform was placed on the floor of the box against the left wall. We used the single-trial step-down inhibitory avoidance conditioning as an established model of fear-motivated memory. At step-down inhibitory avoidance training, animals learn to associate a location in the training apparatus (a grid floor) with an aversive stimulus (footshock). The general procedures for inhibitory avoidance behavioral
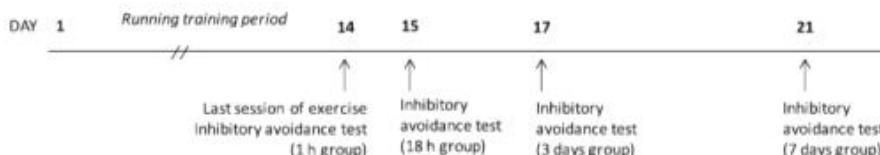
Fig. 1. Schematic diagram showing the design of the experiments used in the present study. The test trials of inhibitory avoidance were conducted at 14, 13, 17 or 21th days. The rats were decapitate 30 min after test trial, resulting in different groups 1 h, 18 h, 3 days or 7 days, related to the end of exercise period, along with time-matched sedentary controls.

training and retention test were described in previous reports (Amaral, Luft, Cammarota, Izquierdo, & Roesler, 2007; Quevedo et al., 1999). Previously, in the training trial, rats were placed on the platform and their latency to step down to the grid with all four paws was measured with a digital chronometer connected to the control box unit. Immediately after stepping down on the grid, the rats received a 0.6 mA, 3.0 s footshock and were removed from the apparatus immediately after the footshock. The test trial took place 24 h after training, by placing the rats on the platform and recording their latencies to step down. Retention test latencies measurements were cut off at 180 s and no footshock was delivered. The inhibitory avoidance test were conducted on the 14, 15, 17 or 21th days after exercise protocol started (Fig. 1). In order to minimize the time of day effect, one control group (SED) was evaluated for each exercised one. The behavioral test was performed 30 min before euthanasia.

2.4. Preparation of samples

In order to verify the acute and delayed effects of exercise, animals were decapitated 1 h (acutely), 18 h, 3 days or 7 days (delayed) after the last session of treadmill exercise. In addition, one sedentary group was used for each exercised animal. The whole hippocampi were quickly dissected out and immediately frozen in liquid nitrogen, then stored at −80 °C.

2.5. Determination of the inflammatory cytokines levels

The levels of TNF-α, IL-1β and IL-4 were determined using Rat ELISA Assay kits (Colorimetric Detection, catalog number 88-7340; 88-6010 eBioscience Ready-SET-Go, USA; 555198, BD OptEIA, USA, respectively) according to the manufacturer's instructions. Briefly, 96-well plates were coated with specific monoclonal antibody. The plates were then blocked for non-specific binding using Assay Diluent. The hippocampi were homogenized with specific kit lyses buffer, lysates were centrifuged and the supernatant and standards curve were incubated with the capture antibody followed by detection antibody. The plates were incubated with Detection enzyme Avidin-HRP followed by Substrate Solution. The Stop Solution was added and the absorbance was measured on a microplate reader (450 nm). Protein concentration of each sample was measured by Lowry method (Peterson, 1977). The cytokines levels were expressed as pg/ml.

2.6. Determination of the NF-kB levels

The NF-κB levels were determined using the NF-kB p65 (Total and Phosphorylated) InstantOne ELISA Assay kit (Colorimetric Detection, catalog number 85-86083, eBioscience Ready-SET-Go, USA) according to the manufacturer's instructions. Briefly, the hippocampi were homogenized with specific kit lysis buffer, lysates were centrifuged and the supernatant was removed for analysis. Sample, negative control and positive control were incubated with antibody cocktail followed Detection Reagent. The Stop Solution was added and the absorbance was measured on a microplate

reader (450 nm). The Pierce BCA Protein Assay kit was used to determine the protein concentration of each sample. The NF-κB levels were expressed as relative optical density/mg protein.

2.7. Global histone H4 acetylation levels

The global histone H4 acetylation levels were determined using the Global Histone H4 Acetylation Assay Kit (Colorimetric Detection, catalog number P-4009, EpiQuik USA) according to the manufacturer's instructions. The hippocampi were homogenized with specific kit lyses buffer for nuclear extraction followed by the histone extraction. After incubations TCA, HCl and acetone and few centrifugations, the pellet was used for the H4 acetylation detection. The samples were incubated with the capture antibody followed by detection antibody. After, the samples were incubated with developing solution. The Stop Solution was added and the absorbance was measured on a microplate reader (450 nm). The Pierce BCA Protein Assay kit was used to determine the protein concentration of each sample. The global histone H4 acetylation levels were expressed as ng/mg protein.

2.8. Statistical analysis

All results were expressed as mean ± S.D. The results were analyzed by Three-Way Analysis of Variance (ANOVA) with age, exercise and time points after the exercise as factors. Pearson correlation and linear regression analysis were used to study the relationships between behavioral and biochemical results. In all tests, $p < 0.05$ was considered to indicate statistical significance.

3. Results

The latencies to step down from the platform during training were similar in all groups ($p > 0.05$). Step down latencies of the test session at different time-points after the exercise has ended are illustrated in Fig. 2. Three-way ANOVA showed the effects of both
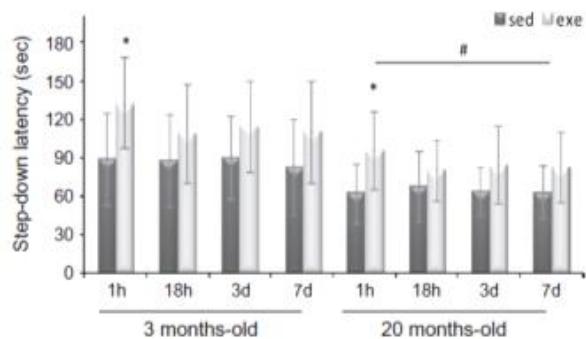

Fig. 2. Effect of aging and exercise on step down latency in inhibitory avoidance. Columns represent mean ± S.D. (n = 9–11). ANOVA followed by Duncan. *Values significantly different from those respective sedentary control group; #significantly different from the 3-months-old groups ($p < 0.05$).

factors, age ($F_{(1,159)} = 29.57$; $p < 0.001$) and exercise ($F_{(1,159)} = 26.16$; $p = 0.003$). Aged rats exhibited impairment inhibitory avoidance performance when compared to young rats. Duncan post hoc test indicated that the exercise increased acutely the step down latency after the last session, without any delayed effect ($p < 0.05$; Fig. 2).

Fig. 3 highlights the TNF-α levels. Three-way ANOVA revealed a significant effect of the age factor ($F_{(1,95)} = 29.33$; $p < 0.001$). It was observed an increase on hippocampal TNF-α levels in the 20 months-old rats when compared to the young group. Moreover, there was a significant interaction between age and exercise factors ($F_{(1,95)} = 8.02$, $p = 0.006$; Fig. 3B). Duncan post hoc test indicated aged rats exhibited lower TNF-α levels (about 25%) when compared to the sedentary group 1 h after the last exercise session ($p < 0.05$).

The IL-1β levels are illustrated in Fig. 4. Similarly, three-way ANOVA demonstrated effect of the age factor ($F_{(1,79)} = 30.67$, $p < 0.001$), since hippocampi from aged rats showed higher IL-1β levels compared to the young group. In addition, a significant interaction between age and exercise factors was observed ($F_{(1,79)} = 4.65$, $p = 0.035$; Fig. 4). Duncan post hoc analysis revealed that exercise induced acute and delay (18 h) reduction on IL-1β levels (about 30%) in hippocampi from aged rats after the last treadmill session ($p < 0.05$; Fig. 4).

Three-way ANOVA showed the effect of the age factor on Fig. 5 ($F_{(1,79)} = 60.85$; $p < 0.001$). Likewise the cytokines IL-1β and TNF-α, aged rats have higher hippocampal NF-kB levels. Moreover, Duncan post hoc test showed that exercise protocol decreased NF-kB levels acutely in hippocampus from aged rats ($p < 0.05$). Interestingly, it was observed a significant negative correlation between inhibitory avoidance test and IL-1β ($R = -0.29$, $p = 0.010$; Fig. 4B) and NF-kB content ($R = 0.277$, $p = 0.012$; Fig. 5B).

Three-way ANOVA showed the effect of both factors, age and exercise on IL-4 levels ($F_{(1,79)} = 12.59$; $p = 0.001$; $F_{(1,79)} = 11.56$; $p = 0.001$, respectively, Fig. 6). Hippocampi from aged rats have lower levels of IL-4 compared to the young group ($p < 0.05$). The exercise protocol increased acutely IL-4 levels in hippocampi from 3 months-old rats (approximately 55%, $p < 0.001$; Fig. 6A), without any effect in the aged group. Furthermore, it was observed a significant positive correlation between inhibitory avoidance test and IL-4 levels ($R = 0.38$, $p = 0.001$; Fig. 6B).

Three-way ANOVA showed a significant effect of both factors, age and exercise on global histone H4 acetylation levels ($F_{(1,81)} = 17.75$, $p < 0.001$; $F_{(1,81)} = 7.63$, $p = 0.007$, respectively, Fig. 7). It was observed that aged rats have lower levels of this parameter comparing to the young group (Fig. 7A). Duncan post hoc analysis revealed that the exercise protocol acutely increased H4 acetylation levels in hippocampi from aged rats, without any effect in the young group (about 30%; $p < 0.05$; Fig. 7A). Moreover, it was observed a significant positive correlation between inhibitory avoidance test and H4 acetylation levels ($R = 0.251$, $p = 0.011$; Fig. 7B).

4. Discussion

In the current study, we provide the first evidence for relationship between age-related aversive memory impairment and the imbalance of inflammatory and epigenetic parameters. A novel finding that emerged from our study involves a potential interaction between aging and exercise on inflammatory parameters. Interestingly, the forced exercise modulated inflammatory cytokines content in an age-dependent manner, increasing anti-inflammatory cytokine content in young hippocampus, while the exercise was able to reverse the increases of pro-inflammatory cytokines levels induced by aging process.

Our data support the idea that the changes of inflammatory cytokines may be involved, at least in part, to the aging-related impairment, since we observed an increase on TNF-α and IL-1β levels, pro-inflammatory cytokines, associated with decreased levels of IL-4, anti-inflammatory cytokines, in hippocampus from 20 months of age rats.

There was a negative correlation between IL-1β levels and the performance on aversive memory test. Although we cannot determine that this phenomenon is causally related to cognitive deficit,
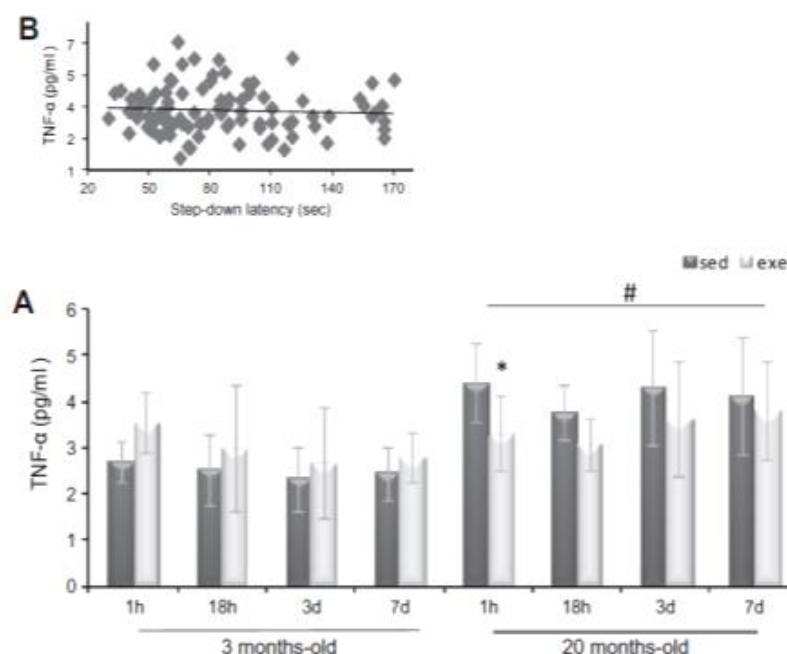
Fig. 3. (A) Effect of aging and treadmill exercise on TNF-α levels in rat hippocampus. Columns represent mean ± S.D. ($n = 5–7$). Three-way ANOVA followed by Duncan test, *significantly different from the respective sedentary control group; #significantly different from the 3-months-old groups ($p < 0.05$). (B) Correlation between inhibitory avoidance test performance and TNF-α levels. Linear regression analysis and Pearson correlation.
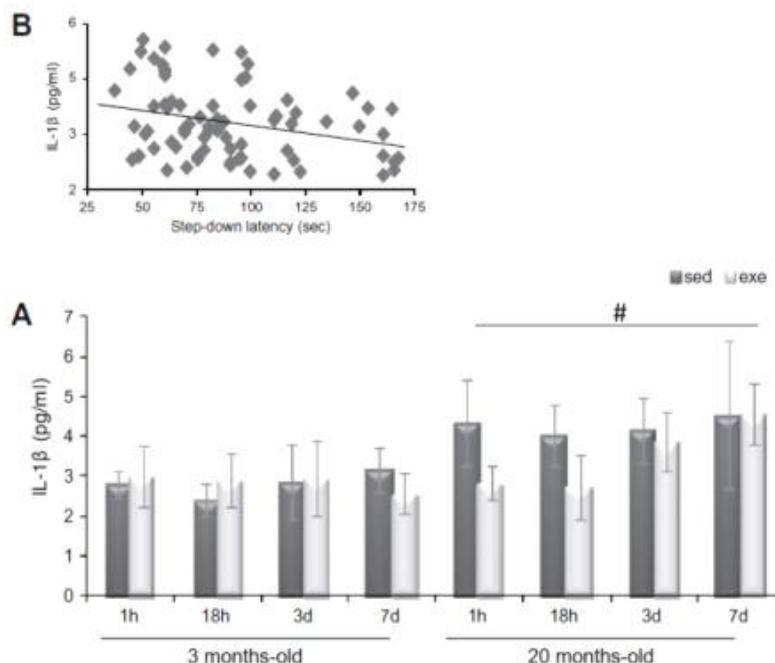
Fig. 4. (A) Effect of aging and treadmill exercise on IL-1β levels in rat hippocampus. Columns represent mean ± S.D. (n = 5–7). Three-way ANOVA followed by Duncan test, *significantly different from the respective sedentary control group; #significantly different from the 3-months-old groups (p < 0.05). (B) Correlation between inhibitory avoidance test performance and IL-1β levels. Linear regression analysis and Pearson correlation.
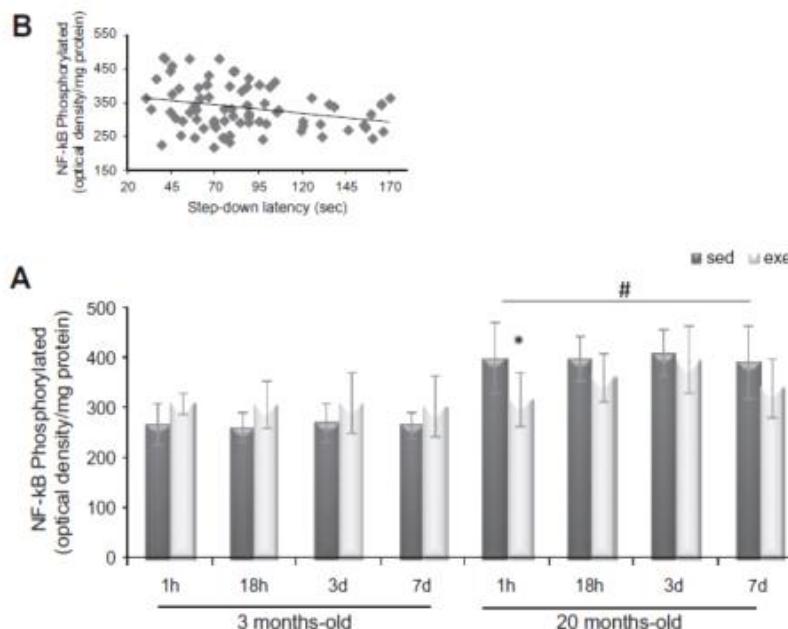
Fig. 5. (A) Effect of aging and treadmill exercise on NF-kB levels in rat hippocampus. Columns represent mean ± S.D. (n = 5–7). Three-way ANOVA followed by Duncan test, *significantly different from the respective sedentary control group; #significantly different from the 3-months-old groups (p < 0.05). (B) Correlation between inhibitory avoidance test performance and NF-kB levels. Linear regression analysis and Pearson correlation.

several studies have demonstrated that cognitive deficits can be induced by increases on IL-1β levels in young rodents (Barrientos et al., 2004; Moore, Wu, Shaftel, Graham, & O'Banion, 2009; Murray & Lynch, 1998). The correlation of aversive memory and IL-1β levels is in agreement with those obtained with LTP and spatial memory in aged rodents (Buchanan et al., 2008; Griffin et al., 2006; O'Donnell et al., 2000). Our findings can be related to experimental studies demonstrating that increased levels of pro-inflammatory cytokines, induced by central administration of IL-1β, peripheral infection and overexpression of IL-1β, may be associated with cognitive deficit (Barrientos et al., 2004; Moore, Wu, Shaftel, Graham, & O'Banion, 2009). In addition, Depino et al. (2004) showed that IL-1 blockade in the dorsal hippocampus has a facilitatory effect in inhibitory avoidance task.
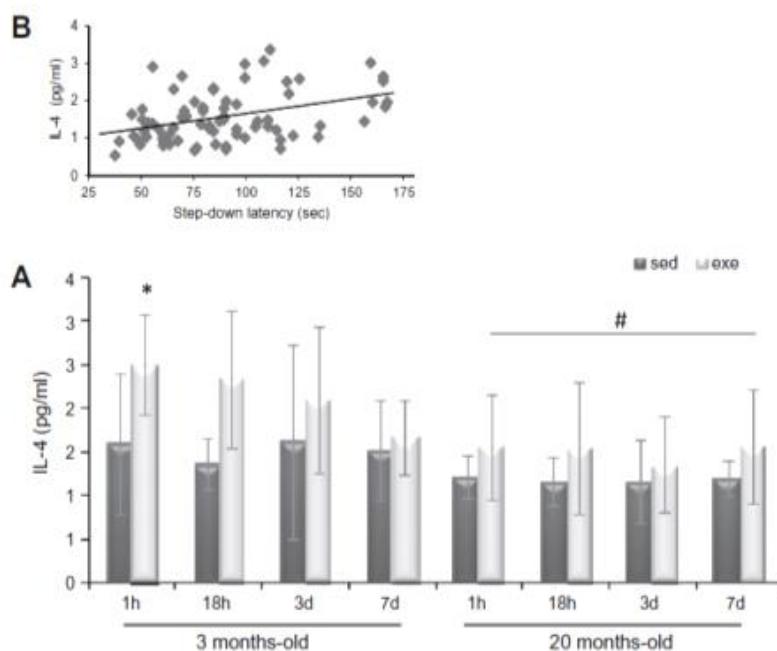
Fig. 6. (A) Effect of aging and treadmill exercise on IL-4 levels in rat hippocampus. Columns represent mean ± S.D. ($n = 5-7$). Three-way ANOVA followed by Duncan test, *significantly different from the respective sedentary control group; #significantly different from the 3-months-old groups ($p < 0.05$). (B) Correlation between inhibitory avoidance test performance and IL-4 levels. Linear regression analysis and Pearson correlation.
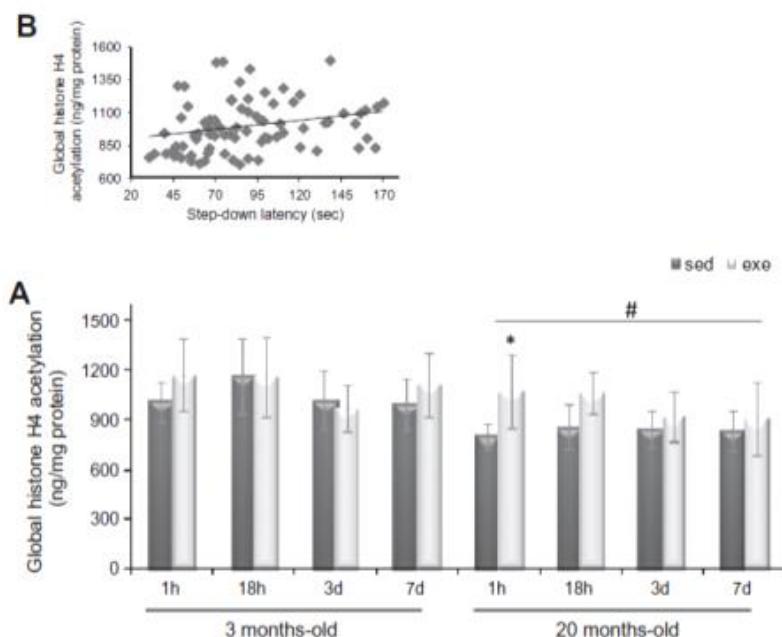

Fig. 7. (A) Effect of aging and treadmill exercise on histone acetylation levels in rat hippocampus. Columns represent mean ± S.D. ($n = 5-7$). Three-way ANOVA followed by Duncan test, *significantly different from the respective sedentary control group; #significantly different from the 3-months-old groups ($p < 0.05$). (B) Correlation between inhibitory avoidance test performance and histone acetylation levels. Linear regression analysis and Pearson correlation.

Moreover, hippocampi from aged rats have higher levels of TNF-α, supporting the idea that TNF-α plays a central role in the inflammatory cascade in aged brain. This finding may be related to susceptibility to excitotoxicity events and disturbed energy metabolism, both situations commonly found in age-related neurological disorders.

Although considering that we did not observe a significant correlation between TNF-α and the aversive memory performance, even with higher levels of TNF-α, it is reasonable to speculate that the TNF-α could not modulate the aging-related aversive memory decline in rats. It is interesting to note that studies demonstrating the effects of TNF-α on cognitive function have been contradictory.

Transgenic mice expressing murine TNF-α showed an impairment of inhibitory avoidance (Fiore et al., 1996), while an opposite pattern was demonstrated by Baune, Ponath, Golledge, et al. (2008) and Baune, Ponath, Rothermundt, et al. (2008) that mice deficient for TNF-α under non-inflammatory conditions have poor memory. Yet, this group suggests that TNF-α is essential for normal functions of memory and learning in healthy elderly humans (Baune, Ponath, Golledge, et al., 2008; Baune, Ponath, Rothermundt, et al., 2008).

Another important finding that emerged from this study is that the decrease in IL-4 levels can significantly contribute to the impairment in inhibitory avoidance model in healthy aged rats, supporting the previous findings obtained with LTP (Maher, Nolan, & Lynch, 2005; Nolan et al., 2005) and water maze model (Kawahara et al., 2012).

The involvement of NF-κB pathway in pro-inflammatory cytokines transcription during aging process has been described (Chung et al., 2009); accordingly, NF-κB phosphorylated, which indicates an activation of NF-kB, was increased in hippocampus from 20 months-old rats. Our findings support the hypothesis that a shift from a homeostatic balance of inflammatory mediators toward a pro-inflammatory state, modulated by NF-kB activation, is related to aversive memory impairments.

It has been demonstrated that epigenetic modifications, such as acetylation of histone and non-histone proteins, are involved in NF-κB-signaling pathway (Aguilera, Hoya-Arias, Haegeman, Espinosa, & Bigas, 2004; Viatour et al., 2003; Yamamoto et al., 2003), although these findings have been contradictory, pointing to a rather complex relationship to link NF-κB activation with histone acetylation. The H4 histone hyperacetylated state induced by HDAC inhibitors can activate NF-kB pathway in both murine macrophage and human embryonic kidney 293 cells (Saccani & Natoli, 2002; Chen et al., 2001), while in bone marrow derived macrophages, HDAC inhibitors did not affect NF-kB nuclear translocation (Roger et al., 2011).

We also infer that the H4 histone hipoacetylation in hippocampi from aged rats would indicate a global acetylation state in cells, what can influence the function of other acetylated substrates, such as coactivators, components of the basal transcription machinery, and proteins involved in nuclear import (Bannister, Miska, Gorlich, & Kouzarides, 2000; Imhof et al., 1997). It is important to note that the acetylation of different lysines in NF-kB subunits, p65 and p50, also controls its transcriptional activity and DNA- binding affinity (Calao, Burny, Quivy, Dekoninck, & Van, 2008). NF-kB was able to interact directly with both enzymes related to histone acetylation, HAT and HDAC. It has been reported that acetylation of p65 subunit plays a critical role in NF-kB-transcriptional activity, Kiernan et al. (2003) suggest that acetylation of p65 regulates negatively transcriptional activity of NF-kB by lowering binding to DNA, promoting its export to the cytoplasm. Taken together, we can suppose that our findings obtained in hippocampi from aged rats would be related to hipoacetylation of NF-kB subunits, blocking its removal to the cytoplasm.

Besides, the aversive memory performance here observed might be linked to a role of H4 histone acetylation, since we observed a positive correlation between both parameters. These results are in agreement with previous studies reporting that aging-related memory decline in spatial memory paradigms and LTP can be related to altered histone acetylation (Peleg et al., 2010; Zeng et al., 2011). Indeed, decreased histone acetylation was observed in several cellular and in vivo neurodegeneration models (Rouaux, Loeffler, & Boutillier, 2004; Saha & Pahan, 2006; Sleiman et al., 2009).

Our exercise protocol (20 min/day during 2 weeks) improved transitorily the inhibitory avoidance aversive memory performance in both young and old rats. In agreement, other studies showed a temporal profile of exercise effects on aversive and declarative memory (Hopkins, Nitecki, & Bucci, 2011; Lovatel et al., 2012; Radak et al., 2006).

The present study is the first evidence reporting the effects of forced exercise on neuroinflammatory and epigenetic parameters in healthy rats of different ages. This exercise protocol decreased pro-inflammatory markers, specifically TNF-α, IL-1β and NF-kB phosphorylated in hippocampus from healthy aged rats. Our data can be related to other findings where the exercise was able to reduce TNF-α and IL-1β levels in transgenic model of Alzheimer Disease (Leem, Lee, Son, & Lee, 2011; Nichol et al., 2008). Taken together, these results suggest that exercise may restore the balance of inflammatory status, improving the aging-related memory decline in rats.

In contrast to the data obtained from the aged group, the exercise paradigm did not modify pro-inflammatory cytokines and NF-kB levels in hippocampi from 3 months-old rats, showing different profiles between young and aged rats. It is impossible at this moment to establish the types of cells are expressing studied cytokines. The proinflammatory cytokines are usually produced in the CNS by activated microglia (Giulian, Baker, Shih, & Lachman, 1986; Hetier et al., 1988) and astrocytes (Chung & Benveniste, 1990; Lieberman, Pitha, Shin, & Shin, 1989), although it has been suggested that hippocampal neurons may produce the cytokines (Bandtlow et al., 1990). However, it has been postulated an age-associated increase in microglial reactivity (Chen et al., 2008). In accordance, Buchanan et al. (2008) observed that a stress model increased hippocampal IL-1β expression associated with a cognitive impairment in aged mice, without any effect in adult mice. Moreover, the microglia activation also impacts on anti-inflammatory cytokines in an age-depend manner, since activated microglia from young rat brain releases anti-inflammatory cytokines, which is considered a protective response (Perry, Cunningham, & Holmes, 2007; Perry, Matyszak, & Fearn, 1993). However microglia activation in middle-aged rats produces IL-1β, leading to a harmful phenotype (Perry, Newman, & Cunningham, 2003). Taken together, we can suppose an exercise-induced shift in the state of microglia from activated to non-activated in aging process, maintaining the homeostasis. This modulation on neuroinflammation process can be related to neuroprotective effect.

Consistent with the idea that exercise can impact inflammatory cytokines content in an age-dependent manner, the exercised young group showed higher IL-4 levels compared to the sedentary group. In addition, it has been proposed that IL-4 may exert neuroprotective effect through to the inhibition of the expression and release of the IL-1β and TNF-α (Brown & Hural, 1997; Chao, Molitor, & Hu, 1993; Loane et al., 2009).

Recently, our group described the modulation of exercise on both inflammatory and epigenetic parameters in hippocampus from young rats (Elsner et al., 2011; Lovatel et al., 2012). Lovatel et al. (2012) demonstrated that the time window of memory-enhancing exercise effect was consistent with the increases in COX-2 and EP4 receptor contents, correlating COX-2 with memory formation, while EP4 levels might be linked to neuroprotection effects.

The results here showed that this exercise protocol had no effect on H4 acetylation levels in hippocampus from 3-months-old rats. Accordingly, our previous studies demonstrated that this protocol did not alter HAT and HDAC activities hippocampal histones H3 and H4 in young adult rats (Elsner et al., 2011). On the other hand, our protocol exercise increased the H4 acetylation levels in 20 months-old rats, this finding led us to hypothesize that exercise may attenuate age-related changes on chromatin structure.

Taken together, our results support the involvement of imbalance of inflammatory cytokines in the aging-related impairment of aversive memory. Furthermore, additional studies are necessary to test whether this phenomenon is causally related to normal

aging-induced impairments. Finally, our data indicate that exercise may ameliorate aging-related memory decline in rats through epigenetic mechanism involving histone acetylation and restoring the homeostatic balance of inflammatory cytokines.

Disclosure

The authors declare that the research was conducted in absence of any commercial or financial relationships that could be construed as potential conflicts of interest. The Local Ethics Committee (CEUA de Ética em Pesquisa – UFRGS) approved all procedures with the animals (nr. 21449).

Acknowledgments

Reference

Aguilera, C., Hoya-Arias, R., Haegeman, G., Espinosa, L., & Bigas, A. (2004). Recruitment of IkappaBalpha to the hes1 promoter is associated with transcriptional repression. Proceedings of the National academy of Sciences of the United States of America, 101, 16537–16542.

Amaral, O. B., Luft, T., Cammarota, M., Izquierdo, I., & Roesler, R. (2007). Temporary inactivation of the dorsal hippocampus induces a transient impairment in retrieval of aversive memory. Behavioural Brain Research, 180, 113–118.

Ang, E. T., Wong, P. T. H., Moochhala, S., & Ng, Y. K. (2004). Cytokine changes in the horizontal diagonal band of broca in the septum after running and stroke: A correlation to glial activation. Neuroscience, 129, 337–347.

Arida, R. M., Scorza, F. A., dos Santos, N. F., Peres, C. A., & Cavalheiro, E. A. (1999). Effect of physical exercise on seizure occurrence in a model of temporal lobe epilepsy in rats. Epilepsy Research, 37, 45–52.

Baeuerle, P. A., & Baichwal, V. R. (1997). NF-kappa B as a frequent target for immunosuppressive and anti-inflammatory molecules. Advances in Immunology, 65, 111–137.

Bandtlow, C. E., Meyer, M., Lindholm, D., Spranger, M., Heumann, R., & Thoenen, H. (1990). Regional and cellular codistribution of interleukin 1b and nerve growth factor mRNA in the adult rat brain: Possible relationship to the regulation of nerve growth factor synthesis. Journal of Cell Biology, 111, 1701–1711.

Bannister, A. J., Miska, E. A., Gorlich, D., & Kouzarides, T. (2000). Acetylation of importin-alpha nuclear import factors by CBP/p300. Current Biology, 10, 467–470.

Barrett, R. M., & Wood, M. A. (2008). Beyond transcription factors: The role of chromatin modifying enzymes in regulating transcription required for memory. Learning and Memory, 15, 460–467.

Barrientos, R. M., Sprunger, D. B., Campeau, S., Watkins, L. R., Rudy, J. W., & Maier, S. F. (2004). BDNF mRNA expression in rat hippocampus following contextual learning is blocked by intrahippocampal IL-1beta administration. Journal of Neuroimmunology, 155, 119–126.

Baune, B. T., Ponath, G., Golledge, J., Varga, G., Arolt, V., Rothermundt, M., et al. (2008). Association between IL-8 cytokine and cognitive performance in an elderly general population—The MEMO-study. Neurobiology of Aging, 29, 937–944.

Baune, B. T., Ponath, G., Rothermundt, M., Riess, O., Funke, H., & Berger, K. (2008). Association between genetic variants of IL-1beta, IL-6 and TNF-alpha cytokines and cognitive performance in the elderly general population of the MEMO-study. Psychoneuroendocrinology, 33, 68–76.

Berchtold, N. C., Castello, N., & Cotmana, C. W. (2010). Exercise and time-dependent benefits to learning and memory. Neuroscience, 167, 588–597.

Bode, K. A., Schroder, K., Hume, D. A., Ravasi, T., Heeg, K., Sweet, M. J., et al. (2007). Histone deacetylase inhibitors decrease Toll-like receptor-mediated activation of proinflammatory gene expression by impairing transcription factor recruitment. Immunology, 122, 596–606.

Brooks, G. A., & White, T. P. (1978). Determination of metabolic and heart rate responses of rats to treadmill exercise. Journal of Applied Physiology, 45, 1009–1015.

Brown, M. A., & Hural, J. (1997). Functions of IL-4 and control of its expression. Critical Reviews in Immunology, 17, 1–32.

Buchanan, J. B., Sparkman, N. L., Chen, J., & Johnson, R. W. (2008). Cognitive and neuroinflammatory consequences of mild repeated stress are exacerbated in aged mice. Psychoneuroendocrinology, 33, 755–765.

Calao, M., Burny, A., Quivy, V., Dekoninck, A., & Van, L. C. (2008). A pervasive role of histone acetyltransferases and deacetylases in an NF-kappaB-signaling code. Trends in Biochemical Sciences, 33, 339–349.

Cechetti, F., Fochesatto, C., Scopel, D., Nardin, P., Gonçalves, C. A., Netto, C. A., et al. (2008). Effect of a neuroprotective exercise protocol on oxidative state and BDNF levels in the rat hippocampus. Brain Research, 1188, 182–188.

Cechetti, F., Rhod, A., Simão, F., Santin, K., Salbego, C., Netto, C. A., et al. (2007). Effect of treadmill exercise on cell damage in rat hippocampal slices submitted to oxygen and glucose deprivation. Brain Research, 1157, 121–125.

Chao, C. C., Molitor, T. W., & Hu, S. (1993). Neuroprotective role of IL-4 against activated microglia. Journal of Immunology, 151, 1473–1481.

Chen, J., Buchanan, J. B., Sparkman, N. L., Godbout, J. P., Freund, G. G., & Johnson, R. W. (2008). Neuroinflammation and disruption in working memory in aged mice after acute stimulation of the peripheral innate immune system. Brain, Behavior, and Immunology, 22, 301–311.

Chen, L., Fischle, W., Verdin, E., & Greene, W. C. (2001). Duration of nuclear NF-kappaB action regulated by reversible acetylation. Science, 293, 1653–1657.

Chennaoui, M., Drogou, C., & Gomez-Merino, D. (2008). Effects of physical training on IL-1beta, IL-6 and IL-1ra concentrations in various brain areas of the rat. European Cytokine Network, 19, 8–14.

Choi, J. K., & Howe, L. J. (2009). Histone acetylation: truth of consequences? Biochemistry and Cell Biology, 87, 139–150.

Chung, I. Y., & Benveniste, E. N. (1990). Tumor necrosis factor-a production by astrocytes: Induction by lipopolysaccharide, IFN-g, and IL-1B. Journal of Immunology, 144, 2999–3007.

Chung, H. Y., Cesari, M., Anton, S., Marzetti, E., Giovannini, S., Seo, A. Y., et al. (2009). Molecular inflammation: Underpinnings of aging and age-related diseases. Ageing Research Reviews, 8, 18–30.

de Magalhaes, J. P., Curado, J., & Church, G. M. (2009). Meta-analysis of age-related gene expression profiles identifies common signatures of aging. Bioinformatics, 25, 875–881.

Depino, A. M., Alonso, M., Ferrari, C., del Rey, A., Anthony, D., Besedovsky, H., et al. (2004). Learning modulation by endogenous Hippocampal IL-1: Blockade of endogenous IL-1 facilitates memory formation. Hippocampus, 14, 526–535.

Ding, Y. H., Young, C. N., Li, X. L., Rafols, J. A., Clark, J. C., McAllister, J. P., et al. (2005). Exercise preconditioning ameliorates inflammatory injury in ischemic rats during reperfusion. Acta Neuropathologica, 109, 237–246.

Elsner, V. R., Lovatel, G. A., Bertoldi, K., Vanzella, C., Santos, F. M., Spindler, C., et al. (2011). Effect of different exercise protocols on histone acetyltransferases and histone deacetylases activities in rat hippocampus. Neuroscience, 29, 580–587.

Fiore, M., Probert, L., Kollias, G., Akassoglou, K., Alleva, E., & Aloe, L. (1996). Neurobehavioral alterations in developing transgenic mice expressing TNF-alpha in the brain. Brain, Behavior, and Immunity, 10, 126–138.

Funk, J. A., Gohlke, J., Kraft, A. D., McPherson, C. A., Collins, J. B., & Harry, G. J. (2011). Voluntary exercise protects hippocampal neurons from trimethyltin injury: Possible role of interleukin-6 to modulate tumor necrosis factor receptor-mediated neurotoxicity. Brain, Behavior, and Immunity, 25, 1063–1077.

Giulian, D., Baker, T. J., Shih, L. C. N., & Lachman, L. B. (1986). Interleukin-1 of the central nervous system is produced by amoeboid microglia. Journal of Experimental Medicine, 164, 594–604.

Griffin, R., Nally, R., Nolan, Y., McCartney, Y., Linden, J., & Lynch, M. A. (2006). The age-related attenuation in long-term potentiation is associated with microglial activation. Journal of Neurochemistry, 99, 1263–1272.

Hetier, E., Ayala, J., Denefle, P., Bousseau, A., Rouget, P., Mallat, M., et al. (1988). Brain macrophages synthesize interleukin-1 and interleukin-1 mRNAs in vitro. Journal of Neuroscience Research, 21, 391–397.

Hopkins, M. E., Nitecki, R., & Bucci, D. J. (2011). Physical exercise during adolescence versus adulthood: Differential effects on object recognition memory and brain-derived neurotrophic factor levels. Neuroscience, 194, 84–94.

Imhof, A., Yang, X. J., Ogryzko, V. V., Nakatani, Y., Wolffe, A. P., & Ge, H. (1997). Acetylation of general transcription factors by histone acetyltransferases. Current Biology, 7, 689–692.

Jenuwein, T., & Allis, C. D. (2001). Translating the histone code. Science, 293, 1074–1080.

Kawahara, K., Suenobu, M., Yoshida, A., Koga, K., Hyodo, A., Ohtsuka, H., et al. (2012). Intracerebral microinjection of interleukin-4/interleukin-13 reduces β-amyloid accumulation in the ipsilateral side and improves cognitive deficits in young amyloid precursor protein 23 mice. Neuroscience, 207, 243–260.

Kiernan, R., Brès, V., Ng, R. W., Coudart, M. P., El Messaoudi, S., Sardet, C., et al. (2003). Post-activation turn-off of NF-kappa B-dependent transcription is regulated by acetylation of p65. Journal of Biology and Chemistry, 278, 2758–2766.

Kimura, A., Matsubara, K., & Horikoshi, M. (2005). A decade of histone acetylation: Marking eukaryotic chromosomes with specific codes. Journal of Biochemistry, 138, 647–662.

Kouzarides, T. (2007). Chromatin modifications and their function. Cell, 128, 693–705.

Leem, Y. H., Lee, Y., Son, H., & Lee, S. (2011). Chronic exercise ameliorates the neuroinflammation in mice carrying NSE/htau23. Biochemical and Biophysical Research Communications, 406, 359–365.

Levenson, J. M., O'Riordan, K. J., Brown, K. D., Trinh, M. A., Molfese, D. L., & Sweatt, J. D. (2004). Regulation of histone acetylation during memory formation in the hippocampus. Journal of Biological Chemistry, 279, 40545–40559.

Lieberman, A. P., Pitha, P. M., Shin, H. S., & Shin, M. L. (1989). Production of tumor necrosis factor and other cytokines by astrocytes stimulated with lipopolysaccharide or a neurotrophic virus. *Proceedings of the National academy of Sciences of the United States of America, 86*, 6348–6352.

Loane, D. J., Deighan, B. F., Clarke, R. M., Griffin, R. J., Lynch, A. M., & Lynch, M. A. (2009). Interleukin-4 mediates the neuroprotective effects of rosiglitazone in the aged brain. *Neurobiology of Aging, 30*, 920–931.

Lovatel, G. A., Bertoldi, K., Elsner, V. R., Vanzella, C., Moysés, F. S., Spindler, C., et al. (2012). Time-dependent effects of treadmill exercise on aversive memory and cyclooxygenase pathway function. *Neurobiology of Learning and Memory, 98*, 182–187.

Lynch, M. A. (2010). Age-related neuroinflammatory changes negatively impact on neuronal function. *Frontiers in Aging Neuroscience, 1*, 6.

Maher, F. O., Nolan, Y., & Lynch, M. A. (2005). Down-regulation of IL-4-induced signalling in hippocampus contributes to deficits in LTP in aged rat. *Neurobiology of Aging, 26*, 717–728.

Manning, A. M., & Anderson, D. C. (1994). Transcription factor NF-kappaB: An emerging regulator of inflammation. *Annual Reports in Medicinal Chemistry, 29*, 235–244.

Mikaelsson, M. A., & Miller, C. A. (2011). The path to epigenetic treatment of memory disorders. *Neurobiology of Learning and Memory, 96*, 13–18.

Moore, A. H., Wu, M., Shaftel, S. S., Graham, K. A., & O'Banion, M. K. (2009). Sustained expression of interleukin-1 in mouse hippocampus impairs spatial memory. *Neuroscience, 164*, 1484–1495.

Murray, C. A., & Lynch, M. A. (1998). Evidence that increased hippocampal expression of the cytokine interleukin-1 beta is a common trigger for age- and stress-induced impairments in longterm potentiation. *Journal of Neuroscience, 18*, 2974–2981.

Nichol, K. E., Poon, W. W., Parachikova, A. I., Cribbs, D. H., Glabe, C. G., & Cotman, C. W. (2008). Exercise alters the immune profile in Tg2576 Alzheimer mice toward a response coincident with improved cognitive performance and decreased amyloid. *Journal of Neuroinflammation, 5*, 13.

Nolan, Y., Maher, F. O., Martin, D. S., Clarke, R. M., Brady, M. T., Bolton, A. E., et al. (2005). Role of interleukin-4 in regulation of age-related inflammatory changes in the hippocampus. *Journal of Biology and Chemistry, 280*, 9354–9362.

O'Donnell, E., Vereker, E., & Lynch, M. A. (2000). Age-related impairment in LTP is accompanied by enhanced activity of stress-activated protein kinases: Analysis of underlying mechanisms. *European Journal of Neuroscience, 12*, 345–352.

Peleg, S., Sananbenesi, F., Zovoilis, A., Burkhardt, S., Bahari-Javan, S., & Agis-Balboa, R. C. (2010). Altered histone acetylation is associated with age-dependent memory impairment in mice. *Science, 328*, 753–756.

Perry, V. H., Cunningham, C., & Holmes, C. (2007). Systemic infection and inflammation affect chronic neurodegeneration. *Annual Review of Immunology, 28*, 161–167.

Perry, V. H., Matyszak, M. K., & Fearn, S. (1993). Altered antigen expression of microglia in the aged rodent CNS. *Glia, 7*, 60–67.

Perry, V. H., Newman, T. A., & Cunningham, C. (2003). The impact of systemic infection on the progression of neurodegenerative disease. *Nature Reviews Neuroscience, 4*, 103–112.

Peterson, G. L. (1977). A simplification of the proteinassay method of Lowry et al. which is more generally applicable. *Analytical Biochemistry, 83*, 346–356.

Quevedo, J., Vianna, M. R., Roesler, R., De-Paris, F., Izquierdo, I., & Rose, S. P. (1999). Two time windows of anisomycin-induced amnesia for inhibitory avoidance training in rats: Protection from amnesia by pretraining but not pre-exposure to the task apparatus. *Learning and Memory, 6*, 600–607.

Radak, Z., Toldy, A., Szabo, Z., Siamilis, S., Nyakas, C., & Silye, G. (2006). The effects of training and detraining on memory, neurotrophins and oxidative stress markers in rat brain. *Neurochemistry International, 49*, 387–392.

Rahman, I., Marwick, J., & Kirkham, P. (2004). Redox modulation of chromatin remodeling: Impact on histone acetylation and deacetylation, NF-kappaB and pro-inflammatory gene expression. *Biochemistry Pharmacology, 68*, 1255–1267.

Reolon, G. K., Maurmann, N., Werenicz, A., Garcia, V. A., Schröder, N., Wood, M. A., et al. (2011). Posttraining systemic administration of the histone deacetylase inhibitor sodium butyrate ameliorates aging-related memory decline in rats. *Behavior Brain Research, 221*, 329–332.

Ricobaraza, A., Cuadrado-Tejedor, M., & Garcia-Osta, A. (2011). Long-term phenylbutyrate administration prevents memory deficits in Tg2576 mice by decreasing Abeta. *Frontiers in Bioscience, 1*, 1375–1384.

Ricobaraza, A., Cuadrado-Tejedor, M., Perez-Mediavilla, A., Frechilla, D., Del Río, J., & García-Osta, A. (2009). Phenylbutyrate ameliorates cognitive deficit and reduces tau pathology in an Alzheimer's disease mouse model. *Neuropsychopharmacology, 34*, 1721–1732.

Roger, T., Lugrin, J., Le Roy, D., Goy, G., Mombelli, M., Koessler, T., et al. (2011). Histone deacetylase inhibitors impair innate immune responses to Toll-like receptor agonists and to infection. *Blood, 117*, 1205–1217.

Rouaux, C., Loeffler, J. P., & Boutillier, A. L. (2004). Targeting CREB-binding protein (CBP) loss of function as a therapeutic strategy in neurological disorders. *Biochemistry and Pharmacology, 68*, 1157–1164.

Saccani, S., & Natoli, G. (2002). Regulated inducible inflammatory genes dynamic changes in histone H3 Lys 9 methylation occurring at tightly. *Genes & Development, 16*, 2219–2224.

Saha, R. N., & Pahan, K. (2006). HATs and HDACs in neurodegeneration: A tale of disconcerted acetylation homeostasis. *Cell Death & Differentiation, 13*, 539–550.

Salminen, A., Huuskonen, J., Ojala, J., Kauppinen, A., Kaarniranta, K., & Suuronen, T. (2008). Activation of innate immunity system during aging: NF-kB signaling is the molecular culprit of inflamm-aging. *Ageing Research Review, 7*, 83–105.

Scopel, D., Fochesatto, C., Cimarosti, H., Rabbo, M., Belló-Klein, A., & Salbego, C. (2006). Exercise intensity influences cell injury in rat hippocampal slices exposed to oxygen and glucose deprivation. *Brain Research Bulletin, 71*, 155–159.

Sleiman, S. F., Basso, M., Mahishi, L., Kozikowski, A. P., Donohoe, M. E., Langley, B., et al. (2009). Putting the 'HAT' back on survival signalling: The promises and challenges of HDAC inhibition in the treatment of neurological conditions. *Expert Opinion on Orphan Drugs, 18*, 573–584.

van Praag, H., Shubert, T., Zhao, C., & Gage, F. H. (2005). Exercise enhances learning and hippocampal neurogenesis in aged mice. *Journal of Neuroscience, 25*, 8680–8685.

Vecsey, C. G., Hawk, J. D., Lattal, K. M., Stein, J. M., Fabian, S. A., Attner, M. A., et al. (2007). Histone deacetylase inhibitors enhance memory and synaptic plasticity via CREB: CBP-dependent transcriptional activation. *Journal of Neuroscience, 27*, 6128–6140.

Viatour, P., Legrand-Poels, S., Van Lint, C., Warnier, M., Merville, M. P., & Gielen, J. (2003). Cytoplasmic IkBa increases NF-kB-independent transcription through binding to histone deacetylase (HDAC) 1 and HDAC3. *Journal of Biological Chemistry, 278*, 46541–46548.

Wannamethee, S. G., Lowe, G. D., Whincup, P. H., Rumley, A., Walker, M., & Lennon, L. (2002). Physical activity and hemostatic and inflammatory variables in elderly men. *Circulation, 105*, 1785–1790.

Yamamoto, Y., Verma, U. N., Prajapati, S., Kwak, Y. T., & Gaynor, R. B. (2003). Histone H3 phosphorylation by IKKa is critical for cytokine-induced gene expression. *Nature, 423*, 655–659.

Yu, B. P., & Chung, H. Y. (2006). Adaptive mechanisms to oxidative stress during aging. *Mechanisms of Ageing Development, 127*, 436–443.

Zeng, Y., Tan, M., Kohyama, J., Sneddon, M., Watson, J. B., Sun, Y. E., et al. (2011). Epigenetic enhancement of BDNF signaling rescues synaptic plasticity in aging. *Journal of Neuroscience, 31*, 17800–17810.

3.3 CAPÍTULO 3

POSTISCHEMIC EXERCISE MODULATES DIFFERENTLY GFAP AND IBA-1
EXPRESSION IN HIPPOCAMPAL DENTATE GYRUS FOLLOWING GLOBAL
CEREBRAL ISCHEMIA IN WISTAR RATS

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Postischemic exercise increases cell survival and modulates differently astrocyte and microglia functions in hippocampal dentate gyrus following global cerebral ischemia in Wistar rats

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ABSTRACT

The effect of exercise on glial cells activation and cell survival after global cerebral ischemia yet remains poorly understood. Here, we investigated the effect of both pre and postischemic treadmill exercise protocols (20 min/day during 2 weeks) on glial cells immunostaining and survival of BrdU+ cells in the hippocampus of Wistar rats submitted to global ischemia. It was observed an increase of area occupied by astrocytes in both dentate gyrus and stratum radiatum induced by global ischemia. Moreover, a synergistic effect between ischemia and exercise on area occupied by astrocytes was showed. Postischemic exercise partially reversed the ischemia-induced increase on area occupied by microglia. It was showed an increase of the survival of BrdU-positive cells in the in dentate gyrus induced by cerebral ischemia and the postischemic exercise potentiated this phenomenon. In conclusion, postischemic exercise increases cell survival and modulates differently astrocyte and microglia immunostaining in hippocampal dentate gyrus following global cerebral ischemia in Wistar rats.

1. INTRODUCTION

Epidemiological studies (Lee and Paffenbarger, 1998; Sacco et al., 1998) have shown neuroprotective effects of moderate physical activity. Several studies have demonstrated that pre-ischemic treadmill exercise can reduce infarct volumes induced by focal ischemia model (Wang et al., 2001; Guo et al., 2008; Ding et al. 2004; 2005; 2006; Jia et al., 2009). Moreover, it has been suggested that pre-ischemic exercise is able to inhibit inflammatory injury, evaluated by expression of inflammatory mediators and accumulation of leukocytes, besides induces overexpression of neurotrophins (Ding et al., 2006; 2004). We demonstrated that daily moderate intensity exercise (20 min once a day for two-weeks) reduces the damage in hippocampal slices from Wistar rats submitted to in vitro ischemia (Scopel et al., 2006). Indeed, pre-ischemic voluntary exercise in running wheel reduces the lethality induced by global cerebral ischemia in Mongolian gerbils (Stummer et al., 1994). In addition, these exercised gerbils submitted to ischemia show reduced neuronal damage in striatum, cortex and hippocampus (Stummer et al., 1994). In is interesting to note that postischemic treadmill exercise reverses the global ischemia-induced increase on apoptotic neuronal cell death modulating the expression of neurotrophic factors (Sim et al., 2004; Lee et al., 2003). To our knowledge there are no studies comparing pre and postischemic exercise on cerebral ischemia.

The mechanisms by which physical activity alters brain function are unclear, but it has been suggested that exercise might activate cellular and molecular pathways that contribute to neuroprotection. There are several evidences describing that exercise per se, particulary running, may increase cell proliferation and survival in the hippocampal dentate gyrus of adult rodents (Kim et al.,2002; Trejo et al., 2001; van Praag et al.,

1999; Lee 2003, Sim et al., 2004; Llorens-Martín et al., 2010). Moreover, some studies have investigated the effects of exercise on cell proliferation and survival in rodents after cerebral ischemia, although contradictory findings have been found. Yagita and colleagues (2006) demonstrated that postischemic voluntary wheel running decreases survival of newborn cells in dentate gyrus from Wistar rats submitted to global ischemia, whereas this exercise protocol promotes newborn cells survival in dentate gyrus from mice submitted to focal ischemia (Luo et al., 2007).

A growing body of data demonstrates that glial cells play a multifaceted and complex role in ischemia outcomes; astrocyte and microglia can have both harmful and supportive effects on cell proliferation and survival (Ekdahl et al., 2009). Astrocytes become reactive, known as astrogliosis, what can be characterized as proliferation, hypertrophy of nuclei, cell body and cytoplasmic processes (Wang et al., 2006; Petito and Halaby 1993). Astrogliosis is recognized as a remarkable cellular response following a wide variety of insults to the CNS, such as global ischemia (Valentim et al., 1999; Petito and Halaby 1993), however, it is remarkable to cite that physical exercise itself can induce this phenomenon in different brain areas, including cortex, striatum and hippocampus (Li et al. 2005; Uda et al. 2006; Saur et al., 2012). Besides, the global ischemia has greater microglial response on postischemic days 4-7 days in both dentate gyrus and area CA1 of rats submitted to global ischemia (Marioka et al., 1991; Gehrmann et al., 1992); however, to our knowledge there are no studies reporting the long term effects. Lambertsen and colleagues (2011) suggest that reactive microglia in ischemic rats are ascribed from resident microglia. Divergent results about the influence of exercise per se on microglia activity have been found; voluntary running did not alter staining of microglia cells even increasing its proliferation (Olah et al., 2009), while Kohman et al (2012) observed that wheel running enhanced survival of new neurons

and reduces the proportion of new microglia in aged. Despite these findings, to our knowledge, there are no studies reporting the impact of exercise on staining of microglia and astrocytes in hippocampus of Wistar rats submitted to global ischemia.

Thus, the aim of this study was to investigate the effects of both pre and postischemic treadmill exercise protocols (20 min/day during 2 weeks) on glial cells immunostaining and survival of BrdU+ cells in the hippocampus of Wistar rats submitted to global ischemia.

2. METHODS

2.1 Animals

Male Wistar rats of 3 months-old were used. The animals were provided by Centro de Reprodução de Animais de Laboratório (CREAL) at Universidade Federal do Rio Grande do Sul (UFRGS) and were maintained under standard conditions (12-h light/ dark, 22±2°C) with food and water *ad libitum* at Unidade de Experimentação Animal do Hospital de Clínicas de Porto Alegre (UEA/HCPA). The NIH "Guide for the Care and Use of Laboratory Animals" (NIH publication No. 80-23, revised 1996) was followed in all experiments. The Local Ethics Committee (Comissão de Ética no Uso de Animais - CEUA/HCPA, n° 09-630) approved all handling and experimental conditions.

2.2 Surgery

Transient global cerebral ischemia was induced in Wistar rats using the four-vessel occlusion method described by Pulsinelli in 1982, with minor modifications (Netto et al., 1993). The vertebral arteries were permanently electrocoagulated under halothane

anesthesia, and silastic ties were placed around each common carotid artery without the interruption of blood flow. On the next day, the ties were tightened and clamped for 10 min. Sham animals were operated, but the ties were not tightened. The body temperature was maintained at 37± 0.5 °C during ischemia and reperfusion by means of a rectal probe and heating blanket.

2.3 Training

Rats were randomly divided into sedentary, exercised pre-ischemic or exercised postischemic groups. Pre-ischemic exercised group was submitted to exercise protocol consisting on 20 min running session each day for 2 weeks before global cerebral ischemia, while postischemic exercised group was submitted to exercise 2 days after global cerebral ischemia (Figure 1). The exercise training consisted of running sessions in an adapted motorized rodent treadmill (INBRAMED TK 01, Porto Alegre, Brazil), with individual Plexiglas lanes, at 60% of the animals maximal oxygen uptake (Brooks and White, 1978). Peak oxygen uptake (VO2) was measured indirectly in all animals before training. Each rat ran on a treadmill at a low initial speed with the speed being increased by 5 m/min every 3 min until the point of exhaustion (i.e., failure of the rat to continue running). The time to fatigue and workload were taken as indexes of exercise capacity, which was in turn taken as VO2 max (Arida et al., 1999; Brooks and White, 1978; Scopel et al., 2006; Lovatel et al., 2012). Rats were adapted to the treadmill by running, in the first few sessions, at 6.7 m/min for the first 2 min, 10 m/min for the next 4 min, 15 m/min for 8 min, 10 m/min for 4 min and 6.7 m/min for the last 2 min. Thereafter, animals ran at 6.7 m/min for the first 4 min, 15 m/min for 12 min and 6.7 m/min for the last 4 min. Animals that initially refused to run were encouraged by gently tapping their backs. Sedentary group was handled exactly as the experimental

animals and was left on the treadmill for 5 min without any stimulus to run. Neither electric shock nor physical prodding was used in this study and all the procedures took place between 14:00 and 17:00 h.

2.4 BrdU administration

To study survival of newborn cells in the dentate gyrus of hippocampus, the animals received 4 doses of BrdU (5-bromo-2'-deoxiuridine), an indicator of cell proliferation, DNA synthesis marker, a synthetic analogue of thymidine (Sigma, 100mg/kg, i.p., dissolved in 0,1M NH4OH, 20 mg/mL; 24h apart) (Nowakowski et al., 1989; Taupin, 2007; Veena et al., 2009b) on 7th – 10th days after ischemic insult (Figure 1). This protocol was used since the global ischemia induces an increase cell proliferation peaking at 7-10 days (Yagita et al., 2001; Iwai et al., 2002). In order to evaluate the survival of BrdU-labeled cells in dentate gyrus, the animals were perfused at 28 days after last BrdU administration.

2.5 Histological procedures

Twenty-eight days after the last BrdU injection, animals were anesthetized with ketamine (90 mg/Kg i.p.) and xylazine (10 mg/Kg i.p). Heparin (Cristalia, Brazil) was injected into the cardiac ventricle, and the animals were transcardially perfused (Milan, Brazil) with of saline solution, followed by a fixative solution 4% paraformaldehyde (Synth, Brazil). The brains were dissected, post-fixed and cryoprotected by immersion in sucrose solution (Synth, Brazil). The brains were frozen in isopentane (Merck, Germany) cooled in liquid nitrogen and kept in a freezer (-70 °C) for further analyses. Coronal sections (40μm) of the dorsal hippocampus were obtained using a cryostat (Leica, Germany) and serially collected on gelatin coated slides. This area corresponded

to a distance of approximately 2.30 to 4.52 mm posterior to the bregma (Paxinos and Watson, 1982). Every sixth section (240μm apart) was processed for each immunostaining (Malberg et al., 2000; Veena et al., 2009a).

2.6 BrdU Immunohistochemistry

Sections were fixed in ice cold acetone, followed by washing in ice cold phosphate buffer saline (PBS). Antigen retrieval was performed by heating sections in 0.01 M sodium citrate buffer (pH 6.0) in a thermostatic bath at 92 °C for 20 minutes. The tissues were washed in PBS and pre-incubated with 1% bovine serum albumin (BSA; Sigma, USA) in PBS, containing Triton X-100 (PBS-Tx). Then, samples were incubated with monoclonal mouse anti-BrdU antibody (1:200 diluted in nuclease, GE Healthcare, Amersham Biosciences) at room temperature for 2 h and at 4 °C overnight. Sections were washed in PBS and the endogen peroxidase was inactivated with 3% hydrogen peroxide (Synth, Brazil) dissolved in PBS during 30 min. After washing in PBS-Tx, sections were incubated with the secondary antibody rabbit anti-mouse IgG conjugated with peroxidase (1:500, Sigma, USA) at room temperature for 2 h. The immunohistochemical reaction was revealed using a solution of 0.06% 3,3-diaminobenzidine (Sigma, USA) and 10% hydrogen peroxide for 5 min. Finally, the sections were rinsed in PBS, dehydrated in ethanol, cleared with xylene and covered with synthetic canada balsam (Chemical Reaction, Brazil) and coverslips. In order to minimize differences in the staining and background, all histological procedures were performed at the same time and using the same solutions.

2.7 Quantification of BrdU labeled cells

BrdU labeled cells in the granule cell layer (GCL) and the subgranular zone (SGZ) of the dentate gyrus, defined as a two-nucleus-wide band between the apparent border of the granule cell layer and the hilus, were counted (Beauquis et al., 2006). It was used an Olympus BX40 microscope (magnification of 400x), omitting cells in the outermost focal plane. From each animal, 4-5 sections were analyzed both left and right side, 4-5 animals per group. The number of BrdU labeled cells per section was multiplied by the section periodicity (6) to obtain the total number of cells per dentate gyrus (Malberg et al., 2000; Veena et al., 2009a).

2.8 Iba-1 and GFAP Immunofluorescence

In the remaining sections, fixation, antigen retrieval and tissue block was performed by described above. Sections were incubated with the primary monoclonal rabbit anti-Iba-1 (Ionized calcium binding adapter molecule 1) antibody to detect microglial cells (1:500; Wako Chemicals, USA) or the primary polyclonal rabbit anti-GFAP (Glial Fibrillary Acidic Protein) antibody to detect astrocytes (1:1000; Dako, UK). Sections were washed in PBS-Tx and were incubated with secondary antibody anti-rabbit Alexa Fluor 488.

2.9 Quantification of Iba-1 and GFAP

A confocal microscope (Zeiss) was used to visualize the fluorescence immunostaining (excitation wavelength of 488 nm) of the sections. Images from the dentate gyrus and stratum radiatum of CA1 of the hippocampus (200x) were acquired by z-axis analysis in a series of stacks (x and y: 320.09 μm, z: 1.00 μm). The total area occupied by soma and processes of astrocytes and microglia was determined to reveal a cellular activation using Image J Software 1.42q. From each animal, 8-10 images were analyzed, 4-5

animals per group (Viola et al., 2009; Ferreira et al. 2011; Mestriner et al., 2011). All images containing the region of interest were turned into binary (black and white) and a single threshold value was established for Iba-1 and GFAP staining. Then, each threshold was kept constant for all animal groups and was used to measure the total percentage area (%) occupied by microglia and astrocytes (Centenaro et al., 2011).

2.10 Statistical analysis

The data were analyzed using Two-way analysis of variance (ANOVA) with cerebral ischemia and exercise as independent variables. When there were statistically significant F values (p<0.05), the Duncan post hoc test was used. All data are represented by the mean ± S.D.

3. RESULTS

The GFAP immunostaining in the dentate gyrus is illustrated in Figure 2. Two-way ANOVA revealed significant effect of both factors, ischemia and exercise, on the quantification of the total percentage area (%) occupied by GFAP+ soma and astrocytic processes ($F_{(1,29)}$=297.73; p<0.001; $F_{(2,29)}$=29.16; p<0.001, respectively). The ischemic insult increased % of GFAP area in dentate gyrus subfield compared to sham groups. Interestingly, there was a significant interaction between ischemia and exercise factors ($F_{(2,29)}$=7.57; p=0.003). The Duncan post hoc test showed a significant increase in the % area occupied by astrocytes in the post-ischemic exercised groups when compared to all groups. Furthermore, this exercise protocol per se increased the area occupied by GFAP+ cells in dentate gyrus (Figure 2), while both exercise protocols did not affect this parameter in the *stratum radiatum* of hippocampus (Figure 3). Two-way ANOVA

revealed an effect of ischemia factor on GFAP+ soma and astrocytic processes in the *stratum radiatum* of hippocampus ($F_{(1,29)}$=39.72; $p<0.001$; Figure 3).

Two-way ANOVA showed the effect of both factors, ischemia and exercise, on the quantification of the total percentage area (%) occupied by Iba-1+ soma and microglial processes in the dentate gyrus (respectively, $F_{(1,27)}$=273.09; $p<0.001$; $F_{(2,27)}$=8.27; $p=$ 0.002, Figure 4). Rats submitted to ischemia showed higher % area occupied by microglia when compared to sham groups. Moreover, there was a significant interaction between ischemia and exercise factors ($F(2,27)$=23.03; $p<0.001$). The Duncan post hoc test revealed a significant decrease in the % area occupied by microglia in the post-ischemic exercise groups when compared to other ischemic groups (Figure 4).

Survival of progenitor BrdU-labeled cells in the GCL and SGZ of the dentate gyrus of the hippocampus is illustrated in Figure 5. It was observed an effect of both factors, ischemia ($F_{(1,29)}$=34.15; $p<0.001$) and exercise ($F_{(2,29)}$=9.24; $p=0.001$). In addition, it was observed an increase on cell survival in the dentate gyrus of the post-ischemia exercise group compared to all groups ($p<0.05$).

Discussion

The present study demonstrates that postischemic exercise may modulate glial functions, specifically increase GFAP expression and decrease Iba-1 staining, in addition, this exercise protocol can increase survival of newborn cells in the dentate gyrus from rats submitted to global ischemia.

An important finding that emerged from this study is that there was a synergistic effect between ischemia and exercise on the % area occupied by astrocytes. It is interesting that treadmill exercise suppressed formation of reactive astrocytes in other CNS insult models, such Parkinson's disease (Dutra et al., 2013) and forebrain traumatic brain

injuries (Seo et al., 2010). Additionally, the exercise-induced astrogliosis here observed in sham groups is supported by previous studies which demonstrated an increase in astrocytic activation and proliferation of GFAP-positive cells, in the subgranular zone of the hippocampus, in healthy exercised rats (Uda et al. 2006; Li et al. 2005; Uda et al. 2006; Saur et al., 2012).

We infer that the robust increase in the % area occupied by astrocytes observed in the exercised ischemic rats may play a role in providing a favorable micro-environment, or even necessary for neuronal growth and restructuring after CNS insult. This possibility is supported by previous study that rehabilitation training (complex environment housing) produces enhanced functional recovery, dendritic growth, synaptogenesis, and increased number of synapses associate with astrocytic activation in the hippocampus after cerebral ischemia (Biernaskie and Corbett 2001; Briones et al., 2006). Indeed, the involvement of astrocytes in angiogenesis has been recognized, contributing to induce a favorable state to neurovascular unit (consisting of microvascular endothelium, astroglia, neurons and the extracellular matrix), what would protect the blood-brain-barrier function following ischemic damage. In this context, Huang and colleagues (2013) suggested that exercise-induced cognitive improvement of middle-aged rats might be associated with increases on the capillaries density in the rat cortex.

Duan and colleagues (2011) demonstrated that postischemic treatment with delta opioid peptide, which induces astrocytic activation, could promote neuronal survival after global ischemia in the rat hippocampus. In this context, our data support the idea that the functional modulation of the astrocytes by exercise may be involved, at least in part, to the cellular survival in the hippocampus after ischemia, since we observed an increase in the survival of BrdU-positive cells in the subgranular zone (SGZ) and granular cell layer (GCL) of the dntate gyrus in the postischemic exercise group.

The increase in the % area occupied by astrocytes in ischemic groups corroborates with previous works (Valentim et al., 1999; Petito and Halaby 1993), indicating a possibly hypertrophy of cell bodies and processes, characteristic of astrocytic activation (Sofroniew and Vinters, 2010). Our findings are in accordance with Valentim and colleagues (1999) that observed an early and late increase in both immunocontent and GFAP phosphorylation rate in hippocampus, pointing to a long-term glial phenomenon. Moreover, Petito and Halaby (1993) demonstrated a persistent increase in GFAP immunocontent until five weeks in the CA1 area after ischemic insult.

An early increased Iba-1 expression was previously described in dentate gyrus and CA1 of ischemic rats (Morioka et al., 1991; Gehrmann et al., 1992), and in our study an increase was observed 5 weeks after the ischemic event, pointing to a long-term microglial phenomenon. Interestingly, postischemic exercise reversed partially the microglia activation. To our knowledge, we provide here, for the first time, evidence of exercise modulation on microglia activation after global cerebral ischemia. This effect can be related to previous described neuroprotective properties, since some studies suggest a detrimental role of microglial action by producing neurotoxic molecules and proinflammatory cytokines (Wang et al., 2007; Kaushal and Schlichter, 2008). Recently, our group described that the exercise decreased pro-inflammatory markers in hippocampus from aged rats without any effect in young rats. Taken together, we can suppose that exercise is able to modulate the pro-inflammatory state against injury conditions. Indeed, the exercise-induced reduction of the Iba-1 staining here observed could be related to decline on proinflammatory cytokines observed in aged rats (Lovatel et al., 2012), once these cytokines are frequently produced by activated microglia (Hetier et al., 1988; Giulian et al., 1986).

The increase of the survival of newborn cells induced by cerebral ischemia in dentate gyrus observed in this study corroborates with previous works (Yagita et al., 2006; Lee et al., 2008). Interestingly, the postischemic exercise potentiated the augment of the cell survival. In agreement with this results, other studies showed an increase in the cell survival induced by forced (Lee et al., 2008) and voluntary (Luo et al., 2007) exercise after focal cerebral ischemia. On the other hand, Yagita and colleagues (2006) showed that running exercise decreased the cell survival after global cerebral ischemia.

We can suggest that the astrogliosys observed in exercised ischemic group can contribute to survival of newborn cells, and could be related to its expression of fibroblast growth factor (FGF-2) (Briones et al., 2006). It is known that FGF-2 can play a significant role in cell survival in vivo (Kuhn et al., 1997; Leker et al., 2007). Besides, it was reported that cyclooxygense-2 (COX2) may alter cell proliferation and survival in the adult hippocampus (Zhu et al., 2004). The administration with COX2 inhibitors reduces cell proliferation after global ischemia in adult mice and COX2 knockout mice showed lower proliferation following global ischemia (Sasaki et al., 2003). Recently, our group described that exercise may induce an acute increase on COX2 content and we can propose that this augment might contribute to cell survival observed in this study.

Interestingly, our pre-ischemic exercise protocol did not affect cell survival in hippocampus of ischemic rats, the divergence between pre and postischemic exercise protocol on cell survival can be associated with our experimental design, since in order to study the survival of newborn cells, all samples were obtained 4 weeks after ischemia, consequently there is a difference time interval in pre and postischemic groups. It is possible that the effects of exercise persist no longer than 23 days (interval between ending exercise in the post-ischemic protocol and sacrifice). Moreover, it was

reported that running exercise exerts contradictory effects on cell proliferation and survival depending on the protocol used (Naylor et al., 2005). Mild or moderate exercise protocols seem to be beneficial, whereas intense exercise can be harmful (Scopel et al., 2006). It was demonstrated that voluntary exercise promotes the survival of progenitor cells in the postischemic dentate gyrus whereas forced swimming dose not (Luo et al., 2007; van Praag et al., 1999). Moreover, short-term running (9 days) increases cell proliferation whereas long-term running (24 days) decreases cell proliferation in rats (Naylor et al., 2005). The postischemic exercise protocol was able to increase the survival of newborn cells after global ischemia, and it is possible to infer that the stimulus induced by this exercise protocol occurred in the moment of cell proliferation peak induced by global ischemia (Yagita et al., 2001).

Summarizing, our results support the hypothesis that exercise increases cell survival associated with a modulation on glial cells functions, specifically postischemic exercise induces an augment on astrocytes concomitantly a decrease on microglia imunostaining in dentate gyrus after global ischemia.

Reference List

Arida RM, Scorza FA, dos Santos N F, Peres, CA, Cavalheiro EA (1999). Effect of physical exercise on seizure occurrence in a model of temporal lobe epilepsy in rats. Epilepsy Research, 37:45–52.

Beauquis J, Roig P, Homo-Delarche F, De Nicola A, Saravia F (2006) Reduced hippocampal neurogenesis and number of hilar neurones in streptozotocin-induced diabetic mice: reversion by antidepressant treatment. Eur J Neurosci. (6):1539-1546.

Biernaskie J & Corbett D (2001) Enriched rehabilitative training promotes improved forelimb motor function and enhanced dendritic growth after focal ischemic injury. J Neurosci 21(14):5272-5280.

Briones TL, Woods J, Wadowska M, Rogozinska M, Nguyen M (2006) Astrocytic changes in the hippocampus and functional recovery after cerebral ischemia are facilitated by rehabilitation training. Behavioural Brain Research 171: 17–25.

Brooks GA & White TP (1978). Determination of metabolic and heart rate responses of rats to treadmill exercise. Journal of Applied Physiology, 45:1009–1015.

Centenaro LA, Jaeger MC, Ilha J, Souza MA, Kalil-Gaspar PI, Cunha NB, Marcuzzo S, Achaval M (2001) Olfactory and respiratory lamina propria transplantation after spinal cord transection in rats: Effects on functional recovery and axonal regeneration. Brain Res 1426: 54 – 72.

Ding Y, Li J, Luan X,Ding YH, Lai Q, Rafols JA, Phillis JW, Clark JC, Diaz FG (2004b) Exercise pre-conditioning reduces brain damage in ischemic rats that may be associated with regional angiogenesis and cellular overexpression of neurotrophin. Neuroscience 124:583–591.

Ding Y, Young CN, Luan X, Li J, Rafols JA, Clark JC, McAllister JP, Ding Y (2005) Exercise preconditioning ameliorates inflammatory injury in ischemic rats during reperfusion. Acta Neuropathol 109: 237–246.

Ding Y, Ding Y, Li J, Bessert DA, Rafols JA (2006) Exercise pre-conditioning strengthens brain microvascular integrity in a rat stroke model. Neurological Research 28: 184–189.

Ding YH, Luan XD, Li J, Rafols JA, Guthinkonda M, Diaz FG, Ding Y (2004a) Exercise-induced overexpression of angiogenic factors and reduction of ischemia/reperfusion injury in stroke. Curr Neurovasc Res 1(5):411-420.

Duan YL, Wang SY, Zeng QW, Su DS, Li W, Wang XR, Zhao Z (2011) Astroglial reaction to delta opioid peptide [D-ala2,D-leu5] enkephalin confers neuroprotection against global ischemia in the adult rat hippocampus. Neuroscience 192: 81–90.

Dutra MF, Jaeger M, Ilha J, Kalil-Gaspar PI, Marcuzzo S, Achaval M (2012) Exercise improves motor deficits and alters striatal GFAP expression in a 6-OHDA-induced rat model of Parkinson's disease. Neurol Sci 33:1137–1144.

Ekdahl CT, Kokaia Z, Lindvall O (2009) Brain inflammation and adult neurogenesis: the dual role of micróglia. Neuroscience 158: 1021–1029.

Ferreira AFB, Real CC, Rodrigues AC, Alves AS, Britto LRG (2011) Short-term, moderate exercise is capable of inducing structural, bdnf-independent hippocampal plasticity. Brain Res 1425:111–122.

Gehrmann I, Bonnekoh P, Miyazawa T, Rossmann K, Kreutzberg GW (1992) Immunocytochemical study of an early microglial activation in ischemia. Journal of Cerebral Blood Flow and Metabolism 12:257-269.

Giulian D, Baker TJ, Shih LC, Lachman LB (1986) Interleukin 1 of the central nervous system is produced by ameboid micróglia. J Exp Med 164(2):594-604.

Guo M, Cox B, Mahale S, Davis W, Carranza A, Hayes K, Sprague S, Jimenez D, Ding Y (2008) Pre-ischemic exercise reduces matrix metalloproteinase-9 expression and ameliorates blood-brain barrier dysfunction in stroke. Neuroscience. 151(2):340-351.

Hetier E, Ayala J, Denèfle P, Bousseau A, Rouget P, Mallat M, Prochiantz A. (1988) Brain macrophages synthesize interleukin-1 and interleukin-1 mRNAs in vitro. J Neurosci Res. 21(2-4):391-397.

Huang CX, Qiu X, Wang S, Wu H, Xia L, Li C, Gao Y, Zhang L, Xiu Y, Chao F, Tang Y. (2013) Exercise-induced changes of the capillaries in the cortex of middle-aged rats Neuroscience 233: 139–145.

Iwai M, Sato K, Omori N, Nagano I, Manabe Y, Shoji M, Abe K (2002) Three steps of neural stem cells development in gerbil dentate gyrus after transient ischemia. J Cereb Blood Flow Metab (4):411-419.

Jia J, Hu YS, Wu Y, Liu G, Yu HX, Zheng QP, Zhu DN, Xia CM, Cao ZJ (2009) Pre-ischemic treadmill training affects glutamate and gamma aminobutyric acid levels in the striatal dialysate of a rat model of cerebral ischemia. Life Sci. 84(15-16):505-511.

Kaushal V, Schlichter LC. (2008) Mechanisms of microglia-mediated neurotoxicity in a new model of the stroke penumbra. J Neurosci 28(9):2221-2230.

Kim SH, Kim HB, Jang MH, Lim BV, Kim YJ, Kim YP, Kim SS, Kim EH, Kim CJ (2002) Treadmill exercise increases cell proliferation without altering of apoptosis in dentate gyrus of Sprague-Dawley rats. Life Sci.71(11):1331-1340.

Kohman RA, DeYoung E, Bhattacharya TK, Peterson LN, Rhodes JR (2012) Wheel running attenuates microglia proliferation and increases expression of a proneurogenic phenotype in the hippocampus of aged mice. Brain, Behavior, and Immunity 26: 803–810

Kuhn HG, Winkler J, Kempermann G, Thal LJ, Gage FH (1997) Epidermal growth factor and fibroblast growth factor-2 have diferente effects on neural progenitors in the adult rat brain. J. Neurosci 17: 5820–5829.

Lambertsen KL, Deierborg T, Gregersen R, Clausen BH, Wirenfeldt M, Nielsen HH, Dalmau I, Diemer NH, Dagnaes-Hansen F, Johansen FF, Keating A, Finsen B (2011) Differences in origin of reactive microglia in bone marrow chimeric mouse and rat after transient global ischemia. J Neuropathol Exp Neurol. 70(6):481-494.

Lee IM & Paffenbarger RS Jr (1998) Life is sweet: candy consumption and longevity. BMJ. 7174:1683-1684. No abstract available.

Lee S, Kim Y, Kim Y, Yoon B (2008) Enforced physical training promotes neurogenesis in the subgranular zone after focal cerebral ischemia. Journal of the Neurological Sciences 269:54–61

Leker RR, Soldner F, Velasco I, Gavin DK, Androutsellis-Theotokis A, McKay RD (2007) Long-lasting regeneration after ischemia in the cerebral cortex. Stroke 38:153–161.

Li J, Ding Y, Rafols JÁ, Lai Q, McAllister JP, Ding Y (2005) Increased astrocyte proliferation in rats after running exercise. Neuroscience Letters 386: 160–164.

Llorens-Martín M, Torres-Alemán I, Trejo JL (2010) Exercise modulates insulin-like growth factor 1-dependent and -independent effects on adult hippocampal neurogenesis and behaviour. Mol Cell Neurosci 44(2):109-117.

Lovatel GA, Bertoldi K, Elsner VR, Vanzella C, Moysés Fdos S, Spindler C, Funck VR, Pereira LM, de Oliveira CV, Oliveira MS, Netto CA, Siqueira IR (2012) Time-dependent effects of treadmill exercise on aversive memory and cyclooxygenase pathway function. Neurobiol Learn Mem. 2:182-187.

Luo CX, Jiang J, Zhou QG, Zhu XJ, Wang W, Zhang ZJ, Han X, Zhu DY (2007) Voluntary exercise-induced neurogenesis in the postischemic dentate gyrus is associated with spatial memory recovery from stroke. J Neuros Res 85:1637–1646.

Malberg JE, Eisch AJ, Nestler EJ, Duman RS (2000) Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J Neurosci 20: 9104-9110.

Mestriner RG, Pagnussat AS, Boisserand LSB, Valentim L, Netto CA (2011) Skilled reaching training promotes astroglial changes and facilitated sensorimotor recovery after collagenase-induced intracerebral hemorrhage. Exp Neuro 227: 53–61.

Morioka T, Kalehua AN, Streit WJ (1991)The microglial reaction in the rat dorsal hippocampus following transient forebrain ischemia. Journal of Cerebral Blood Flow and Metabolism.

Naylor M, Bowen KK, Sailor KA, Dempsey RJ, Vemuganti R (2005). Preconditioning-induced ischemic tolerance stimulates growth factor expression and neurogenesis in adult rat hippocampus. Neurochem. Int.47:565–572.

Netto CA, Hodges H, Sinden JD, Peillet E, Kershaw T, Sowinski P, Meldrum BS, Gray JA. (1993) Effects of fetal hippocampal field grafts on ischemic-induced deficits in spatial navigation in the water maze. Neuroscience 54: 69–92.

Nowakowski RS, Lewin SB, Miller MW (1989) Bromodeoxyuridine immunohistochemical determination of the lengths of the cell cycle and the DNA-synthetic phase for an anatomically defined population. J Neurocytol. 3:311-318.

Olah M, Ping P, Haas AH, Brouwer N, Meerlo P, Van Der Zee EA, Biber K, Hendrikus W, Boddeke GD (2009) Enhanced hippocampal neurogenesis in the absence of microglia T cell interaction and microglia activation in the murine running wheel model. Glia 57:1046–1061.

Paxinos G, Watson C (1982) The rat brain in stereotaxic coordinates. Sydney: Academic Press.

Petito CK & Halaby IA (1993) Ischemic neuronal necrosis to astrocyte expression of glial fibrillary acidic protein. Int. J. Devl Neuroscience 11(2):239-247.

Pulsinelli WA, Brierley JB, Plum F (1982) Temporal profile of neuronal damage in a model of transient forebrain ischemia. Ann Neurol 11:491-498

Seo TB, Kim BK, Ko IG, Kim DH, Shin MS, Kim CJ, Yoon JH, Kim H (2010) Effect of treadmill exercise on Purkinje cell loss and astrocytic reaction in the cerebellum after traumatic brain injury. Neurosci Lett 481(3):178-182.

Sacco RL, Gan R, Boden-Albala B, Lin IF, Kargman DE, Hauser WA, Shea S, Paik MC (1998) Leisure-time physical activity and ischemic stroke risk: the Northern Manhattan Stroke Study. Stroke. 2:380-387.

Sasakia T, Kitagawaa K, Sugiuraa S, Omura-Matsuokaa E, Tanakaa S, Yagitaa Y, Okanob H, Matsumotoc M, Horia M (2003) Cyclooxygenase-2 is involved in increased proliferation of neuronal progenitor cells in the ischemic mouse hippocampus. International Congress Series 1252:445– 450.

Saur L, Baptista PPA, Senna PN, Paim MF, Nascimento P, Ilha J, Bagatini PB, Achaval M, Xavier LL (2012) Physical exercise increases GFAP expression and induces morphological changes in hippocampal astrocytes. Brain Struct Funct.

Scopel D, Fochesatto C, Cimarosti H, Rabbo M, Belló-Klein A, Salbego C, Netto CA, Siqueira IR. (2006) Exercise intensity influences cell injury in rat hippocampal slices exposed to oxygen and glucose deprivation. Brain Res Bull 71(1-3):155-159.

Sim YJ, Kim SS, Kim JY, Shin MS, Kim CJ(2004) Treadmill exercise improves short-term memory by suppressing ischemia-induced apoptosis of neuronal cells in gerbils. Neurosci Lett. 372(3):256-261.

Sofroniew MV & Vinters HV (2010) Astrocytes: biology and pathology. Acta Neuropathol. 119(1):7-35.

Stummer W, Weber K, Tranmer B, Baethmann A, Kempski O (1994) Reduced Mortality and Brain Damage After Locomotor Activity in Gerbil Forebrain Ischemia. Stroke 25:1862-1869.

Taupin P (2007) Brdu immunohistochemistry for studying adult neurogenesis: Paradigms, pitfalls, limitations, and validation. Brain Res Rev 53: 198 –214.

Trejo JL, Carro E, Torres-Aleman I (2001) Circulating insulin-like growth factor I mediates exercise-induced increases in the number of new neurons in the adult hippocampus. J Neurosci 21(5):1628-1634.

Uda M, Ishido M, Kami K, Masuhara M (2006) Effects of chronic treadmill running on neurogenesis in the dentate gyrus of the hippocampus of adult rat. Brain Res 1104:64–72.

Valentim LM, Michalowski CB, Gottardo SP, Pedroso L, Gestrich LG, Netto CA, Salbego CG, Rodnight R (1999) Effects of transient cerebral ischemia on glial fibrillary acidic protein phosphorylation and immunocontent in rat hippocampus. Neuroscience 91(4):1291-1297.

van Praag H, Kempermann G, Gage FH (1999) Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. Nat Neurosci 2(3):266-270.

Veena J, Srikumar, BN, Mahati K, Bhagya V, Raju TR, Rao BSS (2009) Enriched environment restores hippocampal cell proliferation and ameliorates cognitive deficits in chronically stressed rats. J Neurosci Res 87: 831–843.

Viola GG, Rodrigues L, Américo JC, Hansel G, Vargas RS, Biasibetti R, Swarowsky A, Goncalves CA, Xavier LL, Achaval M, Souza DO, Wessels AM, Scheltens P, Barkhof F, Heine RJ (2009) Morphological changes in hippocampal astrocytes induced by environmental enrichment in mice. Brain Res 1274: 47–54.

Wang K, Bekar LK, Furber K, Walz W (2004) Vimentin-expressing proximal reactive astrocytes correlate with migration rather than proliferation following focal brain injury. Brain Res.1024(1-2):193-202.

Wang MJ, Lin SZ, Kuo JS, Huang HY, Tzeng SF, Liao CH, Chen DC, Chen WF (2007) Urocortin modulates inflammatory response and neurotoxicity induced by microglial activation. J Immunol 179(9):6204-6214.

Wanga R, Yanga Y, Yub S (2001) Protective effects of treadmill training on infarction in rats. Brain Research 922: 140–143.

Yagita Y, Kitagawa K, Sasaki T, Terasaki Y, Todo K, Matsuoka E, Matsumoto M, Hori M (2006) Postischemic exercise decreases neurogenesis in the adult rat dentate gyrus. Neuroscience Letters 409: 24–29.

List of Legends

Figure 1. Schematic representation of the experimental design.

Figure 2. Effects of cerebral ischemia and exercise on quantification of GFAP+ astrocyte in the dentate gyrus of the hippocampus. (A) Digital images of coronal

sections of the dentate gyrus stained for GFAP immunofluorescence. (B) % of area occupied by astrocytes in the dentate gyrus in different groups. Columns represent mean ±S.D. (n=4–5). Two-way ANOVA followed by Duncan test; # significantly different from the sham groups; * significantly different from the sedentary ischemic and pre-ischemic exercise. Scale bar: 50 μm.

Figure 3. Effects of cerebral ischemia and exercise on quantification of GFAP+ astrocyte in the stratum radiatum (SR) of the hippocampus. (A) Digital images of coronal sections of the SR stained for GFAP immunofluorescence. (B) % of area occupied by astrocytes in the SR in different groups. Columns represent mean ±S.D. (n=4–5). Two-way ANOVA revealed an effect of ischemia factor; # significantly different from the sham groups. Scale bar: 50 μm.

Figure 4. Effects of cerebral ischemia and exercise on quantification of Iba-1+ microglia in the dentate gyrus of the hippocampus. (A) Digital images of coronal sections of the dentate gyrus stained for Iba-1 immunofluorescence. (B) % of area occupied by microglia in the dentate gyrus in different groups. Columns represent mean ±S.D. (n = 4–5). Two-way ANOVA followed by Duncan test; # significantly different from the sham groups; * significantly different from the sedentary ischemic and pre-ischemic exercise. Scale bar: 50 μm.

Figure 5. Effects of cerebral ischemia and exercise on survival of BrdU-labeled cells in the dentate gyrus of the hippocampus. Quantitative analysis of the immunocytochemistry for BrdU in the subgranular zone (SGZ) and granular cell layer (GCL) of the dentate gyrus of the hippocampus in different groups. Columns represent mean ±S.D. (n = 4–5). Two-way ANOVA followed by Duncan test; # significantly different from the sham groups; * significantly different from the sedentary ischeimic and pre-ischemic exercise.

Figure 1

Figure 2

Figure 3

A

B

Figure 4

Figure 5

Discussão

Essa tese teve como objetivo geral avaliar o efeito do exercício físico sobre a memória aversiva e parâmetros inflamatórios no processo de envelhecimento e na isquemia cerebral global.

A necessidade do entendimento dos mecanismos pelos quais o exercício físico exerce seus efeitos benéficos sobre processos cognitivos, tanto em condições fisiológicas, como em patológicas, torna-se cada vez mais importante uma vez que na atualidade o exercício físico regular se encontra indissociável dos conceitos de saúde e de qualidade de vida (Scliar, 2007).

No primeiro capítulo apresentado nessa tese, mostramos os efeitos do exercício físico de corrida em esteira por duas semanas sobre a memória aversiva e função da via COX-2. Neste trabalho evidenciamos que o exercício físico é capaz de melhorar a memória aversiva e modificar de forma tempo-dependente o conteúdo da COX-2, PGE_2 e receptores prostanóides (EP1, EP2, EP3 e EP4) em hipocampo de ratos jovens saudáveis.

A melhora da memória aversiva induzida pelo exercício físico foi transitória, observada 1 h após a última sessão de exercício, mas não a longo prazo (18 h, 3 dias e 7 dias após o último treino). Este resultado está de acordo com estudos prévios que demonstraram que os efeitos benéficos do exercício sobre a memória não são mantidos 2 e 3 semanas após o último treino (Radak et al., 2006; Hopkins et al., 2011). Associado ao efeito comportamental, este protocolo resultou em um aumento do conteúdo de COX-2. Nossos resultados demonstraram uma correlação positiva significativa entre o conteúdo da COX-2 e o desempenho no teste da esquiva inibitória. Desta forma, podemos sugerir que o aumento dos níveis da COX-2, induzido pelo exercício, pode estar relacionado, pelo menos em parte, com a melhora da memória aversiva. Esse resultado contribui com outros estudos que relacionaram o papel da COX-2 na plasticidade sináptica e memória

(Murray e O'Connor et al., 2003; Chen e Bazan 2005; Yang & Chen, 2008) apoiando a ideia de que o sistema imune, em condições fisiológicas, pode participar da formação da memória.

O aumento da COX-2 não foi acompanhado pelo aumento do seu produto, a PGE_2. Assim, sugerimos que o papel da COX-2 na formação da memória aversiva induzida pelo exercício não depende, neste caso, da PGE_2. Embora alguns estudos tenham observado que os efeitos da COX-2 sobre a plasticidade estão associados a PGE_2 (Chen & Bazan 2005; Yang & Chen, 2008), Hein e colaboradores (2007) descreveram que outras prostaglandinas podem estar envolvidas neste processo.

Além disso, considerando que uma neurotoxicidade induzida pela COX-2 está ligada a produção de PGE2, a ausência do aumento da PGE_2 observada neste estudo poderia excluir um possível efeito tóxico do aumento da COX-2.

Outro resultado interessante deste estudo foi que o exercício físico induziu um aumento agudo no conteúdo de receptores EP4 associado a uma redução tardia no conteúdo de receptores EP2. Considerando que estudos têm associado estes receptores prostanóides com neuroproteção (Andreasson 2010), nós sugerimos que a modulação destes receptores pelo exercício pode estar associada a um efeito neuroprotetor deste protocolo de exercício demonstrado previamente (Scopel et al., 2006). Estes dados renovam a importância da prática de exercício físico em condições fisiológicas, com o objetivo de melhorar a memória e promover modificações bioquímicas que possam contribuir para a função cognitiva.

Considerando que o exercício físico exerce um papel sobre o sistema imune e assim melhora a memória aversiva, em condições fisiológicas, nós também investigamos se este protocolo de exercício poderia influenciar em condições onde ativação crônica do sistema imune está presente, como por exemplo, no processo de envelhecimento.

Desta forma, no segundo capítulo desta tese, mostramos que o exercício físico induz mudanças dependentes da idade na memória aversiva, associadas à modulação de processos inflamatórios e epigenéticos em ratos de diferentes idades.

Neste estudo observamos que o perfil inflamatório pode influenciar o declínio cognitivo associado ao envelhecimento, uma vez que observamos um aumento das citocinas pró-inflamatórias como IL-1β e TNF-α, acompanhado por uma redução da interleucina anti-inflamatória IL-4 em hipocampo de ratos senescentes. Uma correlação negativa entre os níveis de IL-1β e o desempenho no teste da esquiva inibitória foi observada. Este achado corrobora com estudos prévios que demonstraram uma relação entre IL-1β com LTP e com memória espacial no processo de envelhecimento (Buchanann et al., 2008; Griffin et al., 2006; O'Donell et al., 2000). Além disso, o aumento da IL-1β, induzido por administração central ou por infecção periférica, foi associado a déficits cognitivos em roedores (Barrientos et al., 2004;Moore et al., 2009). Ainda, o bloqueio da IL-1β apresentou efeito sobre a memória, melhorando o desempenho no teste da esquiva inibitória em roedores jovens (Depino et al., 2004).

Além disso, o hipocampo de ratos senescentes apresentou um aumento nos níveis de TNF-α, apoiando a ideia de que o TNF-α tem um papel central na cascata inflamatória durante o envelhecimento. Considerando que não foi observada uma correlação significativa entre TNF-α e o desempenho no teste da esquiva inibitória, nós podemos inferir que o TNF-a poderia não estar diretamente envolvido no declínio da memória aversiva relacionado com o envelhecimento. Cabe destacar que estudos envolvendo TNF-α e funções cognitivas têm apresentado resultados contraditórios. Neste contexto, foi demonstrado que camundongos transgênicos, apresentando um aumento de TNF-α, demonstraram um prejuízo na memória aversiva (Fiore et al., 1996). Enquanto que

Baune e colaboradores (2008) observaram que camundongos transgênicos, que apresentam deficiência de TNF-α, apresentaram pior desempenho no teste de memória.

O estado pró-inflamantório, observado durante o envelhecimento, pode estar relacionado ao aumento no NF-kB, o principal fator de transcrição de moléculas pró-inflamatórias (Chung et al., 2009). No presente estudo foi observado um aumento dos níveis de NF-kB em hipocampo de ratos senescentes, sugerindo que o estado pró-inflamatório, observado nestes animais, pode estar associado à ativação do NF-kB. Esse resultado corrobora com estudos prévios que demonstraram que roedores senescentes apresentaram aumento da ativacao do NF-kB (Baeuerle & Baich, 1997)

Além disso, foi sugerido que processos epigenéticos, especificamente acetilação de histonas, podem estar ligados a ativação do NF-kB, no entanto os resultados são contraditórios (Saccani & Natoli, 2002; Chen et al., 2002; Roger et al., 2012).

No presente estudo observamos um aumento dos níveis de NF-kB e uma redução da acetilaçao da histona H4 em ratos velhos, sugerindo que a transcrição mediada pelo NF-kB parece não envolver a acetilação desta histona.

Nossos resultados demonstraram uma diminuição dos níveis de acetilação da histona H4 em ratos de 20 meses de idade, sugerindo um papel desta alteração epigenética no declínio da memória aversiva associada ao envelhecimento. Este resultado corrobora com outros autores que demonstraram uma associação entre alteração de histonas com declínio de LTP e memória espacial (Peleg et al., 2010; Zeng et al., 2011).

O protocolo de exercício utilizado neste estudo (corrida, 20 min por 2 semanas) melhorou transitoriamente a memória aversiva em ratos jovens e senescentes. Este resultado corrobora com outros estudos que demonstraram efeitos benéficos da memória sobre a memória, com um perfil temporal (Hopkins et al., 2011; Radak et al., 2006). Além disso, o exercício físico foi capaz de diminuir níveis de citocinas pró-

inflamatórias em ratos senescentes, sugerindo um papel na restauração do equilíbrio destes mediadores e assim melhorando o desempenho no teste de memória. Nossos resultados corroboram estudos prévios que observaram efeito anti-inflamatório, induzido pelo exercício físico, em modelos de doença de Alzheimer (Leem et al., 2011; Nichol et al., 2008).

Outro resultado que emerge deste estudo está relacionado a alterações epigenéticas. O exercício aumentou os níveis de acetilaçao da histona H4 em ratos de 20 meses de idade. Este resultado é a primeira evidencia de que o exercício físico pode alterar acetilação de hitonas em ratos velhos. Isto suporta a ideia de que o exercício pode atenuar as mudanças relacionadas ao envelhecimento sobre a estrutura da cromatina e desta forma contribuir para a função cognitiva.

Estes dados reforçam o papel benéfico do exercício físico durante o processo de envelhecimento, com o objetivo de melhorar a memória e influenciar no equilíbrio neuroquímico.

Considerando que ativação crônica do sistema imune parece estar associada ao prejuízo da memória aversiva observado em ratos senescentes, e que o exercício físico apresentou efeito benéfico sobre este processo, nós também investigamos o papel do exercício físico sobre o modelo de isquemia cerebral global em ratos, uma vez que este modelo representa outra condição relacionada à ativação crônica do sistema imune.

Desta forma o terceiro capítulo dessa tese, aborda os efeitos do exercício físico sobre a sobrevivência celular e a função das células gliais após isquemia cerebral global. Além disso, neste estudo, comparamos dois protocolos de exercício físico, um realizado antes do evento isquêmico, para avaliar o efeito neuroprotetor e outro realizado após a isquemia para avaliar o seu papel na recuperação.

Neste estudo foi observado um aumento da ativação de astrócitos e microglia induzidos pelo evento isquêmico, 5 semanas após a isquemia cerebral global. Estes resultados corroboram com estudos prévios que demonstraram aumento de marcadores astrocitários e microgliais no encéfalo de roedores após isquemia cerebral (Petito et al., 1993; Valentin et al., 1999; Cechetti et al., 2011; Morioka et al., 1991; Gehrmann et al., 1992).

Além disso, nós demonstramos que o exercício após a isquemia modificou a ativação de células gliais, especificamente, aumentou a atividade de astrócitos e reduziu a atividade de células microgliais. Embora o efeito benéfico do exercício após evento isquêmico tenha sido descrito previamente (Cechetti et al., 2012; Cechetti et al., 2008; Scopel et al., 2006), esta é a primeira evidência, de nosso conhecimento, do efeito do exercício físico sobre células gliais após o modelo de isquemia cerebral global em ratos.

O aumento dos astrócitos induzido pelo exercício nos animais após a isquemia pode contribuir para o microambiente encefálico favorecendo o reparo do dano, uma vez que foi demonstrado que ativação de astrócitos pode participar do remodelamento neurovascular e contribuir para a recuperação funcional após isquemia cerebral focal (Hayakawa et al., 2010). Adicionalmente, um aumento de astócitos, induzido por um treinamento de reabilitação, foi associado à recuperação funcional, crescimento dendrítico e sinaptogênese no hipocampo após lesão isquemica (Biernaskie e Corbett 2001; Briones et al., 2006). Ainda, o envolvimento de astrócitos na angiogênese tem sido reconhecido, contribuindo para um estado favorável à unidade neurovascular e desta forma protegendo a função da barreira hematoencefálica após a lesão isquêmica. Neste contexto, Huang e colegas (2013) sugeriram que a melhora cognitiva induzida pelo exercício em ratos de meia-idade podem estar associada ao aumento da densidade vascular no córtex cerebral de ratos.

Duan e colaboradores (2011) demonstraram que ativação de astrócitos, induzida por tratamento com delta opióide, pode estar associada à sobrevivência de neurônios hipocampais após isquemia cerebral global. Neste contexto, nossos resultados apoiam a ideia de que a modulação funcional da atividade dos astrócitos, induzida pelo exercício físico, pode estar relacionada, pelo menos em parte, com a sobrevivência celular no hipocampo após isquemia cerebral.

Outro resultado demonstrado neste estudo foi que exercício realizado após a isquemia reduziu a ativação das células da microglia. De nosso conhecimento, esta é a primeira evidência de que o exercício físico modular a atividade destas células após isquemia cerebral global em ratos. Esse efeito pode estar relacionado a uma ação protetora uma vez que estudos sugerem um papel prejudicial da ação da microglia através da ativação de moléculas neurotóxicas e citocinas pró-inflamatórias (Wang et al., 2007; Kaushal and Schlichter, 2008).

Foi demonstrado no segundo capítulo desta tese que o protocolo de exercício físico de corrida em esteira por 2 semanas, foi capaz de reduzir os níveis de citocinas pró-inflamatórias em hipocampo de ratos senescentes sem alterar nos jovens.

Este resultado pode também ser relacionado ao resultado do terceiro capítulo desta tese que demonstrou uma redução da ativação da microglia em ratos observado após isquemia cerebral global. Considerando que liberação de citocinas pró-inflamatórias é frequentemente associada à ativação da microglia, podemos sugerir que o protocolo de exercício aqui estudado possa ter um papel benéfico, sobre o sistema imune, apenas em condições onde se encontra um estado pró-inflamatório presente (por exemplo, no processo de envelhecimento e na isquemia cerebral). Esta ideia corrobora com outros estudos que observaram diferentes respostas em roedores jovens e velhos submetidos ao

mesmo estímulo (vírus, bactéria ou modelo de estresse; Jurgens & Johnson, 2010; Barrientos et al., 2010).

O protocolo de exercício físico realizado após a isquemia cerebral global influenciou a sobrevivência celular, uma vez que animais exercitados após isquemia cerebral global apresentaram um aumento no número de células BrdU-positivas no giro denteado no hipocampo. Por outro lado, ratos exercitados antes da isquemia cerebral não apresentaram diferença nestes parâmetros comparados ao grupo sedentário.

Considerando o papel das células gliais no microambiente encefálico após a isquemia cerebral e que o exercício pode modular sua atividade, podemos sugerir que o momento em que o exercício é realizado é crucial para determinar seus efeitos. Neste contexto, cabe destacar que sete dias após a isquemia cerebral é observado um pico de proliferação celular, desta forma podemos sugerir que o protocolo de exercício físico, realizado após a isquemia, coincidiu com este momento de maior proliferação celular e por este motivo pode ter apresentado efeitos distintos do protocolo realizado antes do evento isquêmico.

O aumento da sobrevivência celular induzida pelo exercício corrobora com outros estudos prévios que utilizaram protocolos de exercício forçado (Lee et al., 2008) e voluntário (Luo et al., 2007) após modelo de isquemia cerebral focal. Por outro lado, nossos resultados discordam de Yagita e colaboradores (2006) que demonstraram que corrida em esteira diminuiu a sobrevivência celular após modelo de isquemia cerebral global.

Estudos prévios demonstraram que exercício físico pode exercer efeitos contraditórios sobre a proliferação e sobrevivência celular dependendo do protocolo utilizado (Naylor et al., 2005). Foi demonstrado que exercício voluntário promoveu sobrevivência de células progenitoras no giro denteado enquanto que exercício de natação não apresentou

nenhum efeito sobre esse parâmetro (Luo et al., 2007; van Praag et al., 1999). Além disso, corrida em esteira por 9 dias aumentou a proliferação celular enquanto que corrida 24 dias diminuiu esse fenômeno (Naylor et al., 2005).

Por fim, os resultados deste estudo dão suporte à hipótese de que o exercício aumenta a sobrevivência de células progenitoras associada a uma modulação da função de células gliais no giro denteado do hipocampo de ratos após isquemia cerebral global.

Conclusão

Os resultados apresentados nesta tese nos permitem concluir que:

- O exercício físico de corrida por 2 semanas melhorou transitoriamente a memória aversiva em ratos jovens (3 meses de idade) e senescentes (20 meses de idade).

- O exercício físico foi capaz de modificar de forma tempo-dependente o conteúdo da COX-2, PGE_2 e receptores prostanóides EP2 e EP4 em hipocampo de ratos jovens.

- Um declínio da memória aversiva associado ao aumento dos níveis de IL-1β, TNF-α e NFkB e redução da acetilação da histona H4 foi observado em ratos de 20 meses de idade.

- O exercício físico promoveu a redução dos níveis de citocinas pró-inflamatórias como a IL1β e TNF-α assim como do fator de transcrição NFkB em hipocampo de ratos de 20 meses.

- O exercício físico foi capaz de reverter parcialmente à diminuição da acetilação global da histona H4 induzida envelhecimento.

- O exercício físico aumentou os níveis da interleucina anti-inflamatória IL-4 em hipocampo de ratos jovens.

- O modelo de isquemia cerebral global induziu um aumento na atividade de astrócitos e microglia observado 5 semanas após o evento isquêmico.

- O exercício realizado após a isquemia modulou a atividade de células gliais, aumentando a atividade e astrócitos e diminuindo a ativação microglial em hipocampo de ratos após isquemia cerebral global.

- O exercício realizado após a isquemia aumentou a sobrevivência celular após a isquemia cerebral global.

Esses resultados em conjunto sugerem a ação benéfica de exercício físico sobre a memória e sobre parâmetros inflamatórios no processo de envelhecimento e na isquemia cerebral global.

Perspectivas

Trabalharemos com as seguintes hipóteses futuras:

1. Se o aumento dos níveis de COX-2 induzido pelo exercício físico possa estar envolvido na formação da memória aversiva no envelhecimento e na isquemia cerebral global;

2. Se a modulação de mediadores inflamatórios, induzida pelo exercício nos animais senescentes, também possa ocorrer após a iquemia cerebral global;

3. Se a modulação das células gliais, induzida pelo exercício, também possa ocorrer no processo do envelhecimento;

4. Se outros protocolos de exercício de intensidade moderada, mas com tempo de duração maior, podem exercer efeito mais duradouro sobre a memória aversiva e paramentros morfológicos e bioquímicos.

Referências

Abel, T., Lattal, KM. (2001). Molecular mechanisms of memory acquisition, consolidation and retrieval. Current Opinion in Neurobiology 11(2), 180-187.

Andersen, JK. (2004). Oxidative stress in neurodegeneration:cause or consequence? Nature Medicine 10, 18-25.

Andreasson, K. (2010). Emerging roles of PGE2 receptors in models of neurologicaldisease. Prostaglandins and Other Lipid Mediators, 91, 104–112.

Ang, ET., Wong, PT., Moochhala, S., Ng, YK. (2004). Cytokine changes in the horizontal diagonal band of Broca in the septum after running and stroke: a correlation to glial activation. Neuroscience 129(2), 337-47.

Barrientos, RM., Spruger,DB., Campeu, S., Watkins, LR., Rudy, JW., Maier, SF. (2004). BDNF mRNA expression in rat hippocampus following contextual learning is blocked by intrahippocampal IL-1beta administration. J Neuroimmunology. 155(1-2), 119-26.

Barrientos, R. M., Frank, M. G., Watkins, L. R., & Maier, S. F. (2010). Memory impairments in healthy aging: Role of aging-induced microglial sensitization. Aging and Disease, 1, 212–231.

Baune, BT., Wiede, F., Braun, A., Golledge, J., Arolt, V., Koerner, H. (2008). Cognitive dysfunction in mice deficient for TNF and its receptors Neuropsychiatric Genetics 147(7), 1056-64.

Bekinschtein, P., Cammarota, M., Katche, C., Slipczuk, K., Rossato, Jl., Goldin, A., Izquierdo, I., Medina, JH. (2008). BDNF is essential to promote persistence of longterm memory storage. Procceedings of the National Academy of Sciences 105(7), 2711-6.

Bernar, S.A., Gray, T.W., Buist, M.D. (2002). Treatment of comatose survivors of out-of-hospital cardiac arrest with induced hypothermia. N Engl Journal Medicine 346, 557-63.

Ben, J., Soares, F.M. Scerer, E., Cechetti, F., Netto, C.A., Wyse, A.T.S. (2010). Running exercise effects on spatial and avoidance tasks in ovariectomized rats. Neurobiology of Learning and Memory 94, 312-317.

Berchtold, NC., Castello, N., Cotman, CW. (2010). Exercise and time-dependent benefits to learning and memory. Neuroscience 167(3), 588-97.

Bilbo, SD. (2010). Early-life infection is a vulnerability factor for aging-related glial alterations and cognitive decline. Neurobiology of Learning and Memory 94(1), 57-64.

Biernaskie J & Corbett D (2001) Enriched rehabilitative training promotes improved forelimb motor function and enhanced dendritic growth after focal ischemic injury. J Neurosci 21(14):5272-5280.

Bird, AD., Tan, KH., Olsson, PF., Zieba, M., Flecknoe, SJ., Liddicoat, DR., Mollard, R., Hooper, SB., Cole, TJ. (2007). Identification of glucocorticoid-regulated genes that control cell proliferation during murine respiratory development. Journal of Physiology 585, 187-201.

Bousiges, O., Vasconcelos, AP., Neidl, R., Cosquer, B., Herbeaux, K., Panteleeva, I., Loeffler, JP., Cassel, JC., Boutillier, al. (2010). Spatial memory consolidation is associated with induction of several lysine-acetyltransferase (histone acetyltransferase) expression levels and H2B/H4 acetylation-dependent transcriptional events in the rat hippocampus Neuropsychopharmacology 35(13), 2521-2537.

Briones TL, Woods J, Wadowska M, Rogozinska M, Nguyen M (2006) Astrocytic changes in the hippocampus and functional recovery after cerebral ischemia are facilitated by rehabilitation training. Behavioural Brain Research 171: 17–25.

Buchanan, JB., Sparkman, NL., Chen, J., Johnson, RW. (2008). Cognitive and neuroinflammatory consequences of mild repeated stress are exacerbated in aged mice Psychoneuroendocrinology 33(6), 755-765.

Cechetti, F., Fochesatto, C., Scopel, D., Nardin, P., Gonçalves, C.A., Netto, C.A. Siqueira, I.R. (2008). Effect of a neuroprotective exercise protocol on oxidative state and BDNF levels in the rat hippocampus. Brain Research 1188, 182-188.

Cechetti, F., Pagnussat, A.S., Worm, P.V., Elsner, V.R., Ben, J., da Costa, M.S., Mestriner, R., Weis, S.N., Netto, C.A. (2011). Chronic brain hypoperfusion causes early glial activation and neuronal death, and subsequent long-term memory impairment. Brain Research Bulletin 87, 109-116.

Cechetti, F., Worma, P.V., Elsner, V.R., Bertoldi, K., Sanches, E., Bem, J., Siqueira, I.R., Netto, C.A. (2012). Forced treadmill exercise prevents oxidative stress and memory déficits following chronic cerebral hypoperfusion in the rat. Neurobiology of Learning and Memory 97, 90–96.

Carmichael, Md.,Davis, JM., Murphy, EA., Brown, AS., Carson, JA., Mayer, E., Ghaffar, A. (2005). Recovery of running performance following muscle-damaging exercise: relationship to brain IL-1beta. Brain, Behavior and Immunity 19(5), 445-52.

Chao, CC., Hu, S., Molitor, TW., Shaskan, EG., Peterson, PK. (1992). Activated microglia mediate neuronal cell injury via a nitric oxide mechanism. The Journal of Immunology 15(149), 2736-2741.

Chen, C., Magee, J. C., & Bazan, N. G. (2002). Cyclooxygenase-2 regulates prostaglandin E2 signaling in hippocampal long-term synaptic plasticity. Journal of Neurophysiology, 87, 2851–2857.

Chen, C., Bazan, NG. (2005). Lipid signaling: sleep, synaptic plasticity, and neuroprotection. Prostaglandins Other Lipid Mediators 77(1-4), 65-76.

Chennaoui, M., Drogou, C., Gomez-Merino, D. (2008). Effects of physical training on IL-1beta, IL-6 and IL-1ra concentrations in various brain areas of the rat. European Cytokine Network. 19(1), 8-14.

Chung, HY., Cesari, M., Anton, S., Marzetti, E., Giovannini, S., Seo, AY., Carter, C., Yu, BU., Leeuwenburgh, C. (2009). Ageing Research Reviews 8(1), 18-30.

Da Silva, WC., Bonini, JS., Bevilaqua, LR., Medina, JH., Izquierdo, I., Cammarota, M. (2008). Inhibition of mRNA synthesis in the hippocampus impairs consolidation and reconsolidation of spatial memory. Hippocampus 18(1), 29-39.

Dalmaz, C., Netto, C. A. (2004). A Memória. Ciência e Cultura (SBPC), São Paulo 56 (1), 30-31.

Depino, AM., Alonso, AM., Ferrari, C., del rey, A., Anthony, D., Besedovsky, H., Medina, JH., Pitossi, F. (2004). Learning modulation by endogenous hippocampal IL-1: blockade of endogenous IL-1 facilitates memory formation. Hippocampus. 14(4), 526-35.

Ding, YH., Young, CN., Luan,X., Li, J., Rafols, JA., Clark JC., McAllister, JP 2nd., Ding, Y.(2005). Exercise preconditioning ameliorates inflammatory injury in ischemic rats during reperfusion. Acta Neuropathology 109(3), 237-46.

Dirnagl, U., Iadecola, C., Moskowitz, MA. (1999). Pathobiology of ischaemic stroke: an integrated view. Trends Neuroscience 22(9), 391-7.

Duan YL, Wang SY, Zeng QW, Su DS, Li W, Wang XR, Zhao Z (2011) Astroglial reaction to delta opioid peptide [D-ala2,D-leu5] enkephalin confers neuroprotection against global ischemia in the adult rat hippocampus. Neuroscience 192: 81–90.

Durakan, A., Tatlisumak,T. (2007). Acute ischemic stroke: overview of major experimental rodent models, pathophysiology, and therapy of focal cerebral ischemia. Pharmacology Biochemistry and Behaviour 87(1), 179-97.

Elsner, VR., Lovatel, GA., Bertoldi, K., Vanzella, C., Santos, FM., Spindler, C., de Almeida, EF., Nardin, P., Siqueira, IR. (2011). Effect of different exercise protocols on histone acetyltransferases and histone deacetylases activities in rat hippocampus. Neuroscience 192, 580-7.

Farmer, J., Zhao, Z., van Praag, H., Wodtke, K., Gage, FH., Christie, BR. (2004). Effects of voluntary exercise on synaptic plasticity and gene expression in the dentate gyrus of adult male Sprague-Dawley rats in vivo. Neuroscience 124(1), 71-9.

Filippin, LI., Vercelino, R., Marroni, NP., Xavier, RM. (2008). Influência de processos redox na resposta inflamatória da artrite reumatóide. Revista Brasileira de Reumatologia 48, 17-24.

Fiore M, Probert L, Kollias G, Akassoglou K, Alleva E, Aloe L. (1996). Neurobehavioral alterations in developing transgenic mice expressing TNF-alpha in the brain. Brain, Behaviour and Immunity 10(2), 126-38.

Funk, JA., Gohlke, J., Kraft, AD., McPherson, CA., Collins, JB., Harry, GJ. (2011). Voluntary exercise protects hippocampal neurons from trimethyltin injury: Possible role of interleukin-6 to modulate tumor necrosis factor receptor- mediated neurotoxicity. Brain, Behavior, and Immunity 25, 1063–1077.

Gehrmann, I., Bonnekoh, P., Miyazawa, T., Rossmann, K., Kreutzberg, GW. (1992). Immunocytochemical Study of an Early Microglial activation in ischemia. Journal of Cerebral Blood Flow and Metabolism 12, 257-269.

Gibertini, M. (1998).Cytokines and cognitive behavior. Neuroimmunomodulation 5(3-4), 160-5.

Griffin, R., Nally, R., Nolan,Y., McCartney, Y., Linden, J., Lynch, MA. (2006). The age-related attenuation in long-term potentiation is associated with microglial activation. Neurochemistry 99(4), 1263-72.

Halliwell BJG: Free radicals in biology and medicine. (2007). 4.ed. New York: Oxford University Press 23.

Hayakawa, K. et al., (2010). Inhibition of reactive astrocytes with fluorocitrate retards neurovascular. Journal Cerebral Blood Flow Metabolism 30(4), 871-82.

Harukuni, I., Bhardwaj, A. (2006). Mechanisms of brain injury after global cerebral ischemia. Neurology Clinical 24(1), 1-21.

Hein, A.M., Stutzman, D.L., Bland, S.T., Barrientos, R.M., Watkins, L.R., Rudy, J.W., et al. (2007). Prostaglandins are necessary and sufficient to induce contextual fear learning impairments after interleukin-1 beta injections into the dorsal hippocampus. Neuroscience, 150, 754–763.

Hopkins, ME., Nitecki, R., Bucci, DJ. (2011). Physical exercise during adolescence versus adulthood: differential effects on object recognition memory and brain-derived neurotrophic factor levels. Neuroscience 194, 84-94.

Huang CX, Qiu X, Wang S, Wu H, Xia L, Li C, Gao Y, Zhang L, Xiu Y, Chao F, Tang Y. (2013) Exercise-induced changes of the capillaries in the cortex of middle-aged rats Neuroscience 233: 139–145.

Iadecola, C., Abe, T., Kunz, A., Hallenbeck, J. (2011). Cerebral Ischemia and Inflammation. Stroke (Fith Edition), 138-153.

Izquierdo, I., Izquierdo, LA., Barros, DM., Mello e Souza, T., de Souza, MN., Quevedo, J., Rodrigues, C., Sant'Anna, MK., Madruga, M., Medina, JH. (1998). Differential

involvement of cortical receptor mechanisms in working, short-term and long-term memory. Behavior Pharmacology 9(5-6), 421-7.

Izquierdo, I., Vianna, MR., Izquierdo, LA., Barros, DM., Szapiro, G., Coitinho, AS., Muller, L., Cammarota, M., Bevilaqua, LR., Medina, JH. (2002). Memory retrieval and its lasting consequences. Neurotox Research 4(5-6), 573-593.

Izquierdo, I., Bevilaqua, LRM., Rossatto, JI., Medina, JH., Cammarota, M. (2006). Different molecular cascades in different sites of the brain control consolidation. Trends Neuroscience 29, 496-505.

Izquierdo, I., Netto, C.A., Dalmaz, C., Chaves, M.L., Pereira, M.E., Siegfried, B., Braz, J. (1988). Construction and reconstruction of memories. Medicine Biology Research 21(1),9-25.

Izquierdo, I., Schroder, N., Netto, C.A., Medina, J. (1999). Novelty causes time-dependent retrograde amnesia for one-traial avoidance in rats through NMDA receptor- and CaMKII-dependent mechanisms in hippocampus. European Journal of Neuroscience 11, 3323-3328.

Jurgens, HA., & Johnson, RW. (2010). Dysregulated neuronal-microglia cross-talk during aging, stress and inflammation. Experimental Neurology 233, 40-48.

Kandel, ER. (1997). Genes, synapses, and long-term memory. Journal of Cellular Physiology 173(2), 124-5.

Kato, H., Kogure, K., Liu, X., Araki, T., Itoyama, Y. (1996). Progressive expression of immunomolecules on activated microglia and invading leukocytes following focal. Brain Research 734, 203-212.in the rat

Kaushal V, Schlichter LC. (2008) Mechanisms of microglia-mediated neurotoxicity in a new model of the stroke penumbra. J Neurosci 28(9):2221-2230.

Kim, DH., Kim, TW., Kim, SE., Shin, MS., Kim, KM., Baek, SS. (2010). Treadmill exercise inhibits traumatic brain injury-induced hippocampal apoptosis. Physiology Behavior 101(5), 660-5.

Kouzarides, T. (2007). Chromatin modifications and their function. Cell 128(4), 693-705.

Lee HK, Min SS, Gallagher M, Kirkwood (2005). NMDA receptor-independent long-term depression correlates with successful aging in rats. Nat Neuroscience 12, 1657-1659.

Lee S, Kim Y, Kim Y, Yoon B (2008) Enforced physical training promotes neurogenesis in the subgranular zone after focal cerebral ischemia. Journal of the Neurological Sciences 269:54–61

Leem, Y. H., Lee, Y., Son, H., & Lee, S. (2011). Chronic exercise ameliorates the neuroinflammation in mice carrying NSE/htau23. Biochemical and Biophysical Research Communications 406, 359–365.

Levenson, JM., O'Riordan, KJ., Brown, KD., Trinch, MA., Molfese, DL., Sweatt, JD.(2004). Regulation of histone acetylation during memory formation in the hippocampus. The Journal of Biological Chemistry 279(39), 40545-59.

Lo, CJ., Lin, JG., Kuo, JS., Chaing, SY., Chen.,SC., Lian, ET., Hsieh, CL. (2003). Effect of salvia miltiorrhiza bunge on cerebral infarct in ischemia-reperfusion injured rats. The American Journal of Chinese Medicine 31(2), 191-200.

Luo CX, Jiang J, Zhou QG, Zhu XJ, Wang W, Zhang ZJ, Han X, Zhu DY (2007) Voluntary exercise-induced neurogenesis in the postischemic dentate gyrus is associated with spatial memory recovery from stroke. J Neuros Res 85:1637–1646.

Lynch, AM., Murphy, KJ., Deighan, BF., O'Reilly, JA., Gun'Ko, YK., Cowlev, TR., Gonzalez-Reves, RE., Lynch, MA.(2010). The impact of glial activation in the aging brain. Aging Diseases 1(3), 262-78.

Morioka, T., Kalehua, NA., Streit, WJ. (1991). The microglial reaction in the rat dorsal hippocampus following transient forebrain ischemia. Journal of Cerebral Blood Flow and Metabolism 11, 966-973.

McAfoose, J., Koerner, H., Baune, BT. (2009). The effects of TNF deficiency on age-related cognitive performance. Psychoneuroendocrinology 34(4), 615-9.

McGaugh, JL., McIntyre, CK., Power, AE. (2002). Amygdala modulation of memory consolidation: interaction with other brain systems. Neurobiology Learning and Memory 78(3), 539-52.

Mendelsohn, AR., Larrick,JW. (2012). Epigenetic-mediated decline in synaptic plasticity during aging. Rejuvenation Research 15(1), 98-101.

Moore, AH., Wu, M., Shaftel, SS., Graham,KA., O`Banion, MK. (2009). Sustained expression of interleukin-1beta in mouse hippocampus impairs spatial memory. Neuroscience 164(4), 1484-95.

Murray, Hj., O'Connor, JJ.(2003). A role for COX-2 and p38 mitogen activated protein kinase in long-term depression in the rat dentate gyrus in vitro. Neuropharmacology 44(3), 374-80.

Naylor M, Bowen KK, Sailor KA, Dempsey RJ, Vemuganti R (2005). Preconditioning-induced ischemic tolerance stimulates growth factor expression and neurogenesis in adult rat hippocampus. Neurochem. Int.47:565–572.

Netto, C.A., Dias, R.D., Izquierdo, I. (1985). Interaction between consecutive learnings: inhibitory avoidance and habituation. Behaviour Neural Biology 44(3), 515-20.

Nichol, K. E., Poon, W. W., Parachikova, A. I., Cribbs, D. H., Glabe, C. G., & Cotman, C. W. (2008). Exercise alters the immune profile in Tg2576 Alzheimer mice toward a response coincident with improved cognitive performance and decreased amyloid. Journal of Neuroinflammation 5, 13.

Noa Ofen, N. (2012). The development of neural correlates for memory formation. Neuroscience and Biobehavioral Reviews 36, 1708–1717.

Nussmeier, N.A. (2002). A review of risk factors for adverse neurologic outcomes after cardiac surgery. Journal Extra Copor Techologic 34, 4-10.

Oktyabrsky, ON., Smirnova, GV.(2007). Redox regulation of cellular functions. Biochemistry 72(2), 132-45.

O'Donnell, E., Vereker, E., Lynch, MA.(2000). Age-related impairment in LTP is accompanied by enhanced activity of stress-activated protein kinases: analysis of underlying mechanisms. European Journal of Neuroscience 12(1), 345-52.

Otani, N. et al., (2006). Enhanced hippocampal neurodegeneration after traumatic or kainate excitotoxicity. Journal Clinical of Neuroscience 13(9), 934-8.

Paradies, G., Petrosillo, G., Paradies, V., Ruggiero, FM.(2011). Mitochondrial dysfunction in brain aging: role of oxidative stress and cardiolipin. Neurochemistry International 58(4), 447-57.

Peleg, S Sananbenesi F, Zovoilis A, Burkhardt S, Bahari-Javan S, Agis-Balboa RC, Cota P, Wittnam JL, Gogol-Doering A, Opitz L, Salinas-Riester G, Dettenhofer M, Kang H, Farinelli L, Chen W, Fischer A. Peleg, S. (2010). Altered histone acetylation is associated with age-dependent memory impairment in mice. Science 7(328), 753-6.

Petito, CK., Halaby, IA. (1993). Relationship between ischemia and ischemic neuronal necrosis to astrocyte expression of glial fibrillary acidic protein. International Journal of Developmental Neuroscience 11(2), 239-247.

Pietrelli, A., Lopez-Costa, J., Goni, R., Brusco, A., Basso, N. (2012). Aerobic exercise prevents age-dependent cognitive decline and reduces anxiety-related behaviors in middle-aged and old rats. Neuroscience 202, 252-66.

Pittson, S., Himmel, AM., Maclver, MB. (2005). Multiple synaptic and membrane sites of anesthesic action in the CA1 region of rat hippocampal slices. BMC Neuroscience 5, 52.

Pulsinelli, W.A., Brierley, J.B. (1979). A new model of bilateral hemispheric ischemia in the unanesthetized rat. Stroke 10, 267-272.

Radanovic, M. (2000). Características do atendimento de pacientes com acidente vascular cerebral em hospital secundário. Arquivos de Neuro-Psiquiatria 58, 99-106.

Radak, Z., Toldy, A., Szabo, Z., Siamilis, S., Nyakas, C., Silye, G., Jakus, J., Goto, S.(2006). The effects of training and detraining on memory, neurotrophins and oxidative stress markers in rat brain. Neurochemistry 49(4), 387-92.

Rall, J. M., Mach, S. A., & Dash, P. K. (2003). Intrahippocampal infusion of a cyclooxygenase-2 inhibitor attenuates memory acquisition in rats. Brain Research 968, 273–276.

Reolon, GK., Maurmann, N., Werenicz, A., Garcia, Va., Schroder, N., Wood, MA., Roesler, R.(2011). Posttraining systemic administration of the histone deacetylase

inhibitor sodium butyrate ameliorates aging-related memory decline in rats. Behavior Brain Research 221(1), 329-32.

Rodrigo, J., Fernandez, AP., Serrano, L., Peinado, MA., Martinez, A.(2005). The role of free radicals in cerebral hypoxia and ischemia. Free Radic Biology Medicine 39(1), 26-50.

Roger, T., Lugrin, J., Roy, DL., Goy, G., Mombelli, M., Koessler, T., Ding, XC., Chanson, A., Reymond, MK., Miconnet, I., Schrenzel, J., François, P., Calandra, T. (2011). Histone deacetylase inhibitors impair innate immune responses to Toll-like receptor agonists and to infection. Blood, 117, 1205-1217.

Saccani, S., Natoli, G. (2002). Dynamic changes in histone H3 Lys 9 methylation occurring at tightly regulated inducible inflammatory genes. Genes Developmental 16, 2219-2224.

Salazar, J.D., Wityk, R.J., Grega, M.A. et al., (2001). Stroke after cardiac surgery: short-and long-term outcomes. Ann Thoracic Surgery 72, 1195-201.

Scopel, D., Fochesatto, C., Cimarosti, H., Rabbo, M., Bello-Klein, A., Salbego, C., Netto, Ca., Siqueira, IR. (2006). Exercise intensity influences cell injury in rat hippocampal slices exposed to oxygen and glucose deprivation. Brain Research Bulletin 71(13),155-9.

Scliar, M. História do conceito de saúde. (2007). Physis: Revista de saúde coletiva. 17, 29-41.

Siesjo, BK. (1978). Brain energy metabolism and catecholaminergic activity in hypoxia, hypercapnia and ischemia. Journal of Neural Trasmission Supplementa (14), 17-22.

Siqueira, I.R., Fochesato, C., Andrade, A., Santos, M., Hagenn, M., Belloklein A., Netto., C.A. (2005). Total antioxidant capacity is impaired in different structures from aged rat brain. International Journal of Developmental Neuroscience 23, 663-671.

Siqueira, I.R., Cimarosti, H., Fochesato, C., Salbego, C.G., Netto, C.A. (2004). Age-related susceptibility to oxygen and glucose deprivation damage in rat hippocampal slices. Brain Research 1025(1-2), 226-230.

Sofroniew, M.V. (2009). Molecular dissection of reactive astrogliosis and glial scar formation. Trends Neuroscience 32(12), 638-47.

Song, C., Phillips, AG., Leonard, B. (2003). Interleukin 1 beta enhances conditioned fear memory in rats: possible involvement of glucocorticoids. European Journal of Neuroscience 18(7), 1739-43.

Turner, BM. (2002). Cellular memory and the histone code. Cell 111(3), 285-91.

Valentim, LM., Michalowski, CB., Gottardo, SP., Pedroso, L., Gestrich, LG., Netto, CA., Salbego, CG., Rodnight, R. (1999). Effects of transient cerebral ischemia on glial fibrillary acidic protein phosphorylation and immunocontent in rat hippocampus. Neuroscience 91(4), 1291–1297.

van Praag, H., Kempermann, G., Gage, FH., (1999). Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. Nature Neuroscience 2(3), 266-70.

Vaynman, S., Ying, Z., Gomez-Pinilla, F. (2004). Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. Europen Journal of Neuroscience 20(10), 2580-90.

Vecsey, CG., Hawk, JD., Lattal, KM., Stein, JM., Fabian, SA., Attner, MA., Cabrera, SM., McDonough, CB., Brindle, PK., Abel., Wood, MA. (2007). Histone deacetylase inhibitors enhance memory and synaptic plasticity via CREB:CBP-dependent transcriptional activation. Journal of Neuroscience 27(23), 6128-40.

Vicente, E., Degerone, D., Bohn, L., Scornavaca, F., Pimentel, A., Leite, M., Swarowsky, A., Rodrigues, L., Nardin, P., Vieria L., Souza, D.O., Netto, C.A., Gonçalez, C. A. (2009). Astroglial and cognitive effects of chronic cerebral hypoperfusion in the rat. Brain Research 1251, 204-212.

Waggoner,D., (2007). Mechanisms of disease: epigenesist. Semin Pediatric Neurology 14(1), 7-14.

Wang MJ, Lin SZ, Kuo JS, Huang HY, Tzeng SF, Liao CH, Chen DC, Chen WF (2007) Urocortin modulates inflammatory response and neurotoxicity induced by microglial activation. J Immunol 179(9):6204-6214.

Wilrout, C., Lang, B., Yang, Y., Dempsey, RJ., Vemuganti, R. (2007). Reparing brain after stroke: A review on post-ischemic neurogenesis. Neurochemistry International 50, 1028-1041.

Yang, H., Chen, C. (2008). Cyclooxygenase-2 in synaptic signaling. Current Pharmaceutical Design 14(14), 1443-51.

Yagita Y, Kitagawa K, Sasaki T, Terasaki Y, Todo K, Matsuoka E, Matsumoto M, Hori M (2006) Postischemic exercise decreases neurogenesis in the adult rat dentate gyrus. Neuroscience Letters 409: 24–29.

Yirmiya, R., Winocur, G., Goshen, I. (2002). Brain interleukin-1 is involved in spatial memory and passive avoidance conditioning. Neurobiology Learning and Memory 78(2), 379-89.

Yirmiya, R., Goshen, I. (2011). Immune modulation of learning, memory, neural plasticity and neurogenesis. Brain, Behavior and Immunity 25(2), 181-213.

Zeng, Y., Tan, M., Kohyama, J., Sneddon, M., Watson, JB., Sun, YE., et al. (2011). Epigenetic enhancement of BDNF signaling rescues synaptic plasticity in aging. Journal of Neuroscience 31, 17800–17810.