SÉRGIO GOMES DA SILVA

ESTUDO DA PLASTICIDADE HIPOCAMPAL INDUZIDA PELO EXERCÍCIO FÍSICO DURANTE O DESENVOLVIMENTO CEREBRAL PÓS-NATAL DE RATOS

Tese apresentada à Universidade Federal de São Paulo para obtenção do título de Doutor em Ciências.

São Paulo

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Orientador: Prof. Dr. Ricardo Mario Arida

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Aos meus pais, Loene Barros da Silva e Ana Maria Gomes da Silva

Ao meu orientador, Prof. Dr. Ricardo Mário Arida

Dedico a vocês este trabalho.

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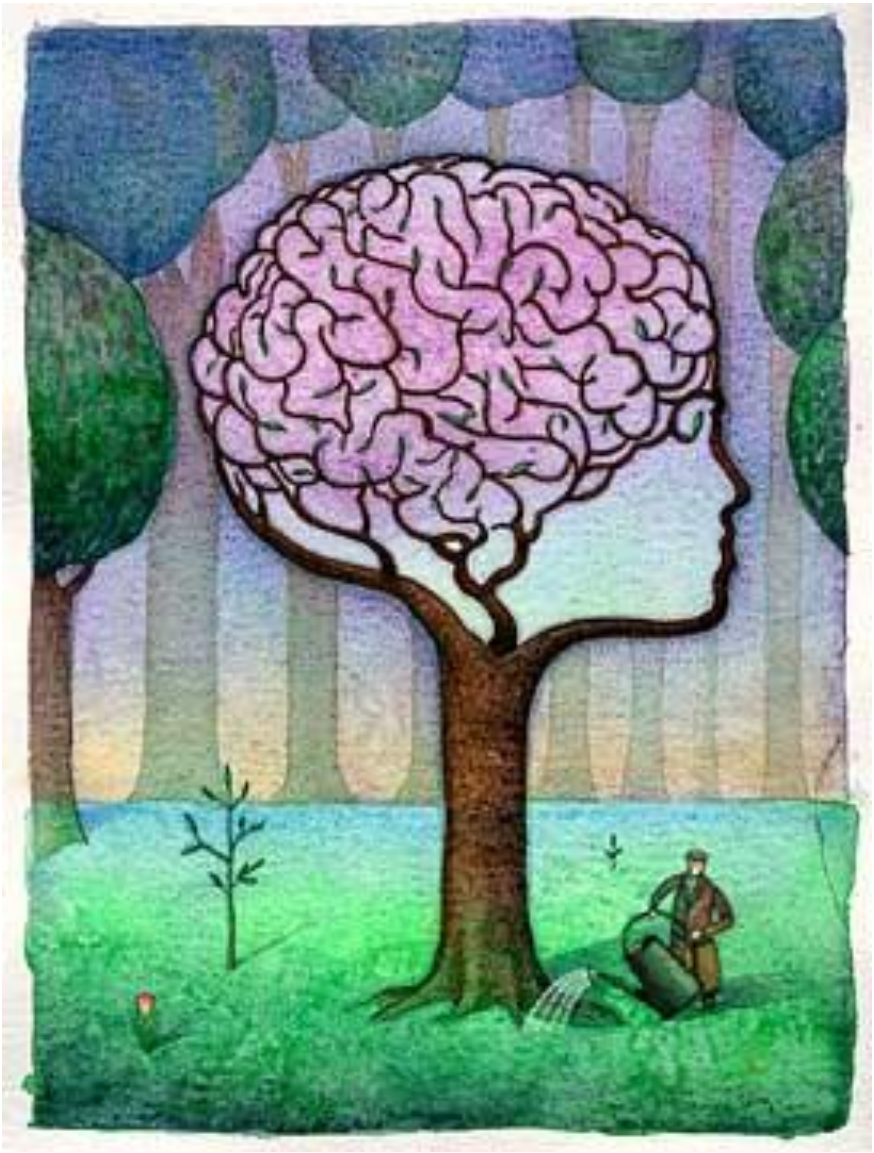

“Brain Tree” – Earl Kenely

É provável que no caso de haver uma grande teoria do funcionamento do cérebro, esta não seja uma frase, mas uma enciclopédia.

"O Breve Lapso entre o Ovo e a Galinha" – Mariano Sigman

SUMÁRIO

RESUMO

Nas últimas décadas, muitos estudos têm se dedicado ao entendimento das bases neurobiológicas do exercício físico para manter e melhorar as funções cerebrais em adultos e idosos. Embora os mecanismos de adaptação neurobiológica ao exercício no cérebro maduro sejam amplamente documentados, a influência do exercício durante o processo de desenvolvimento cerebral permanece pouco explorada. A proposta do presente estudo foi investigar os efeitos do exercício físico sobre o desenvolvimento cerebral pós-natal. Para isso, avaliou-se a plasticidade hipocampal de ratos submetidos a um programa de exercício físico durante o período adolescente (21° ao 60° dias de vida pós-natal). Os resultados mostraram que o exercício físico durante o desenvolvimento pós-natal aumentou a densidade de fibras musgosas e a expressão hipocampal de parvalbumina, fator neurotrófico derivado do encéfalo (BDNF) e receptor tropomiosina quinase B (TrkB), reduziu a expressão hipocampal do receptor canabinóide subtipo 1 (CB1), aprimorou a aprendizagem e a memória espacial, e melhorou a capacidade de evocar as memórias em longo prazo. É importante ressaltar que a intensidade e duração adequada do exercício físico durante o período do desenvolvimento cerebral pós-natal não está bem definida. Enquanto o exercício induz plasticidade hipocampal, efeitos degenerativos poderiam aparecer em condições indevidas de estresse físico e mental. Neste sentido, foi demonstrado que o protocolo de exercício físico utilizado neste estudo não induziu resposta inflamatória e degeneração de neurônios na formação hipocampal de ratos adolescentes. Em resumo, esses achados indicam que o exercício físico pode resultar em mudanças positivas para o cérebro em desenvolvimento pós-natal.

ABSTRACT

In the last decades many studies have dedicated to the understanding of neurobiological bases of physical exercise to the maintenance and improvement of neural function in adults and elderly subjects. Although the effects of exercise are well documented in the mature brain, the influence of exercise in the developing brain has been poorly explored. The purpose of present study was to investigate the effects of physical exercise on postnatal brain development. For this purpose, we evaluated the hippocampal plasticity of rats submitted to an aerobic exercise program during the adolescent period (between 21th and 60th postnatal day-old). The results showed that the physical exercise program during the postnatal development increased the mossy fibers density and hippocampal expression of parvalbumin, brain-derived neurotrophic factor (BDNF) and receptor tropomyosin-related kinase B (TrkB), reduced cannabinoid receptor type 1 (CB1) expression, improved spatial learning and memory, and enhanced the capacity to evoke spatial memories in later stages. It is important to note that the adequate intensity and duration of exercise performed during brain development are not well established. While physical exercise induces hippocampal plasticity, degenerative effects could appear in undue conditions of physical or psychological stress. In this regard, we showed that the exercise protocol used in our study did not induce inflammatory response and degenerating neurons in the hippocampal formation of adolescent rats. Our findings demonstrate that physical exercise results in positive changes in postnatal brain development.

1

Introdução

1. INTRODUÇÃO

Mudanças estruturais e funcionais ocorrem no sistema nervoso durante toda a vida. Os mecanismos pelos quais estas modificações são implementadas constitui um processo conhecido como plasticidade (do grego plaistikos, que significa “formar”). A plasticidade permite que o sistema nervoso central adquira novas informações para aprender, reorganizar as redes neuronais, e se recuperar de lesões cerebrais. Os mecanismos básicos que estão envolvidos na plasticidade incluem fatores anatômicos (através da ampliação ou diminuição da superfície dendrítica ou de populações neuronais), neuroquímicos (modificação de neurotransmissores e neuromoduladores), metabólicos (flutuações na atividade metabólica para utilização do oxigênio e da glicose), e eletrofisiológicos (alterações nas propriedades elétricas das células). Estas modificações são adaptativas e benéficas, mas também podem provocar alterações negativas para o cérebro (Trojan e Pokorny, 1999, Johnston, 2009).

Uma região altamente plástica no sistema nervoso central (SNC) é a formação hipocampal. Esta estrutura cerebral é considerada a principal sede da memória e um importante componente do sistema límbico, unidade responsável pela formação das emoções (Sanders et al., 2003; Kesner et al., 2004; Andersen et al., 2007). Seu nome deriva do formato curvado semelhante a um cavalo marinho (do grego *hippo,* que significa *“*cavalo”, e *kampos,* que significa “monstro marinho”). Em humanos, a formação hipocampal é essencialmente uma faixa curva de córtex filogeneticamente primitivo (arquicórtex), de aproximandamante quatro centímetros de comprimento, localizada na porção medial do lobo temporal. Ela estende-se por todo o comprimento do assoalho do corno inferior (ou temporal) do ventrículo lateral (Brodal, 1979). Em

roedores, a formação hipocampal apresenta proporção e localização diferentes (Figura 1), emergindo dos núcleos septais até o córtex temporal (Shepherd, 1998).

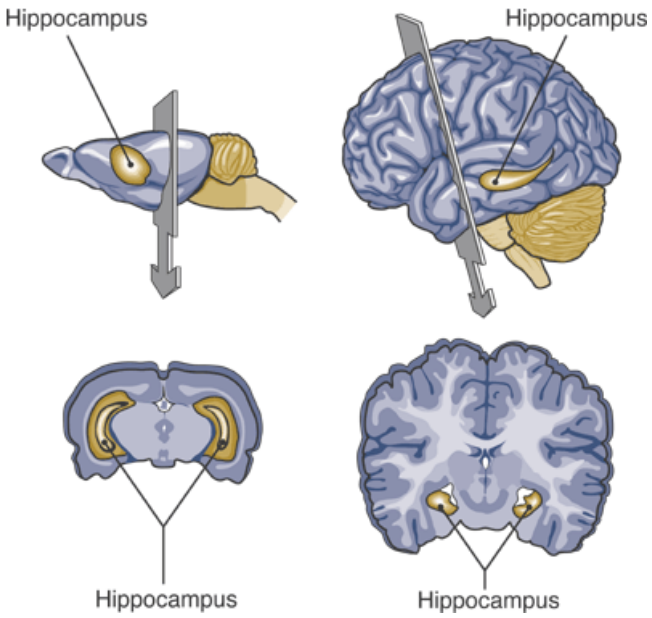

Figura 1. Imagem representativa da proporção e localização anatômica da formação hipocampal em roedores (à esquerda) e humanos (à direita) (Hiller-Sturmhöfel e Swartzwelder, 2004-2005).

Embora a proporção e localização anatômica da formação hipocampal sejam distintas entre humanos e roedores, os circuitos neuronais entre as regiões hipocampais são similares (Bear et al., 2002). De maneira geral, a formação hipocampal é constituída por duas regiões interligadas: o giro dentado e o hipocampo (ou Corno de Ammon). Ambos possuem uma organização interna trilaminada, composta por dois tipos de células principais: as células granulares do giro dentado e as células piramidais do Corno de Ammon (CA), sendo estas divididas nos setores de CA1, CA2 e CA3. Cada uma dessas regiões mantém um padrão organizado de conexões intrínsecas e extrínsecas, sendo que a principal aferência para a formação hipocampal origina-se no córtex entorrinal. As fibras que deixam o córtex entorrinal em

direção a formação hipocampal constituem um feixe de axônios chamados de via perfurante. Esses axônios estabelecem sinapses com os dendritos das células granulares, cujos corpos celulares estão densamente empilhados no estrato granular do giro dentado. As células granulares projetam axônios através das fibras musgosas que fazem sinapses com as células piramidais da região de CA3. Os neurônios de CA3 projetam axônios que se ramificam. Um ramo deixa a formação hipocampal pela via fímbria-fórnix e o outro chamado de colateral de Schaffer, forma sinapses com as células piramidais de CA1, que por sua vez, emitem fibras para as camadas profundas do córtex entorrinal. O circuito córtex entorrinal-giro dentado-CA3-CA1 é tradicionalmente denominado como via trisináptica (Figura 2). As sinapses deste circuito são predominantemente excitatórias, e a inibição se faz principalmente através de interneurônios localizados no hilo do giro dentado e na região do CA. Os principais neurotransmissores envolvidos neste circuito são o glutamato e o ácido gama-aminobutírico (GABA) (Amaral e Witter 1989; Andersen et al., 2007).

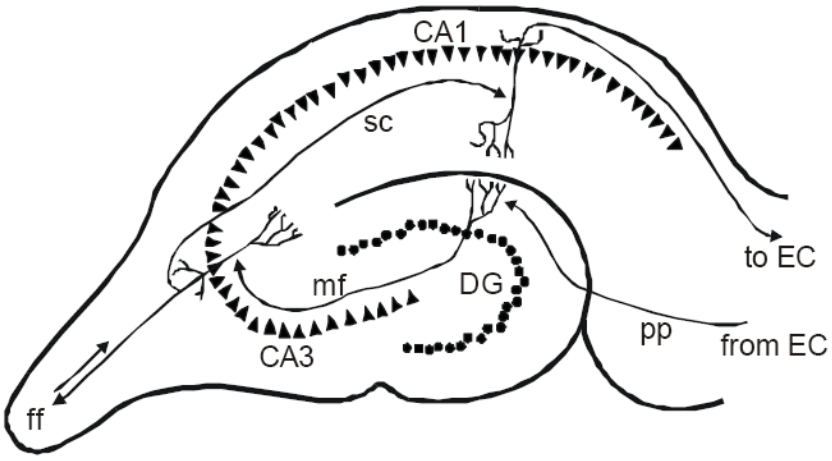

Figura 2. Imagem representativa da via trisináptica hipocampal. EC = córtex entorrinal; DG = células granulares do giro denteado; CA1 e CA3 = células piramidais do Corno de Ammon; pp = via perfurante; ff = via fímbria-fórnix; mf = fibras musgosas; sc = colateral de Schaffer (Ikonen, 2001).

O circuito trisináptico básico da formação hipocampal apresenta contatos sinápticos extremamente plásticos e susceptíveis a modulação tanto por agentes endógenos quanto por exógenos (Cooper e Lowenstein, 2001). Muitos estudos descrevem que a atividade neuronal na formação hipocampal pode ser alterada pela atividade física (Vanderwolf, 1969; Czurko et al., 1999; Van Praag et al., 1999; Farmer et al., 2004; O'Callaghan et al., 2007). Em um trabalho clássico, Vanderwolf (1969) observou que a atividade locomotora em ratos induz na formação hipocampal um padrão de disparo persistente conhecido como ritmo teta, uma atividade elétrica de ondas lentas presente durante o estado de vigília e no sono paradoxal. Em outro estudo, Van Praag e colaboradores (1999) demonstraram que o exercício físico favorece a indução da potenciação de longa duração (long-term potentiation, LTP) em fatias hipocampais de camundongos. A análise deste fenômeno na formação hipocampal tem sido frequentemente utilizada para investigar as bases sinápticas da aprendizagem e memória em vertebrados (Bliss e Collingridge, 1993).

1.1. Exercício Físico e Sistema Nervoso Central

Entende-se por atividade física qualquer movimento corporal produzido por contração muscular que aumente o gasto energético (Howley, 2001). A atividade física inclui desde um programa estruturado de treinamento até atividades como caminhar, correr e dançar. Quando a atividade física é realizada regularmente sua definição muda para exercício físico, pois o organismo adapta-se a este estímulo através de modificações morfológicas e funcionais que podem resultar em desenvolvimento do desempenho físico e benefícios à saúde em geral (Zaryski e Smith, 2005).

Vários estudos na literatura têm documentado os efeitos benéficos da atividade física regular em vários aspectos da função cerebral (Folkins e Sime, 1981; Kramer et al., 2006; Hillman et al., 2008; Martinsen, 2008; Kashihara et al., 2009). O exercício físico contribui para melhora das funções cognitivas (Kramer et al., 2006; Hillman et al., 2008; Kashihara et al., 2009), e na proteção contra os processos neurodegenerativos (Goodwin et al., 2008; Rolland et al., 2008), particularmente durante o envelhecimento. Mudanças de bem-estar emocional com apenas poucas sessões de exercício físico (North et al., 1990; Petruzzello, et al., 1991; Gleniester, 1996; Yeung, 1996) e redução da depressão e ansiedade após um programa de exercício físico regular têm sido constantemente observadas (Farmer, et al. 1988; Gleniester, 1996). Esses efeitos benéficos relacionados à prática de exercício físico podem estar diretamente associados a vários mecanismos capazes de modular a liberação e utilização de neurotransmissores (Herholz et al, 1987; Petruzzello et al., 1991), tal como as monoaminas (Struder et al., 1996), os fatores neurotróficos endógenos (Neeper et al., 1995; 1996; Vaynman et al., 2003), os peptídeos opióides (Arentz et al., 1986) e o sistema endocanabinóide (Sparlin et al., 2003; Fuss e Gass, 2010). Desta forma, o exercício físico ativa cascatas celulares e moleculares que aumentam e mantém a plasticidade cerebral, induz expressão de genes associados à plasticidade, promove sinaptogênese e neurogênese, e aumenta a vascularização e o metabolismo cerebral (Cotman e Berchtold, 2002; Vaynman e Gomez-Pinilla, 2005; Christie et al., 2008).

1.1.1. Exercício Físico e Fatores Neurotróficos

Os fatores neurotróficos constituem um grupo heterogêneo de peptídeos de secreção que regulam os processos de proliferação, desenvolvimento e diferenciação

celular (Skaper, 2008). Estas proteínas são sintetizadas tanto por neurônios como por células gliais e permitem que os neurônios recebam nutrição adequada para crescer, desenvolver ou se regenerar. A ausência destes fatores em níveis fisiológicos faz com que as células neurais diminuam sua atividade metabólica e as conexões sinápticas com as células adjacentes (Lessmann et al., 2003).

Um dos fatores neurotróficos de maior impacto sobre a plasticidade cerebral é o fator neurotrófico derivado do encéfalo (brain derived neurotrophic factor, BDNF) (Lo, 1995; Lu e Chow, 1999; Schinder e Poo, 2000). O BDNF é membro de uma família das neurotrofinas, conhecidas por exercerem um papel fundamental durante o desenvolvimento (Leibrock et al., 1989; Barde, 1994) e por modularem a plasticidade no cérebro maduro (Lo, 1995; Lim et al., 2003). Dentre os vários tipos de plasticidade, o BDNF regula a ramificação e o remodelamento de dendrítos e axônios (Shimada et al., 1998; Lom e Cohen-Cory, 1999; McAllister et al., 1999; Yacoubian e Lo, 2000), a sinaptogênese (Alsina et al., 2001), a eficácia da transmissão sináptica junto com a síntese e liberação de neurotransmissores (Kang e Schuman, 1995; Boulanger e Poo, 1999; Kafitz et al., 1999), a maturação funcional das sinapses excitatórias e inibitórias (Rutherford et al., 1998; Vicario-Abejon et al., 1998; Seil e Drake-Baumann, 2000) e a apoptose celular (Friedman, 2000).

As diversas funções do BDNF são mediadas via duas classes de receptores: o receptor de tropomiosina quinase B (tropomyosin receptor kinase B, TrkB), membro da família dos receptores tirosina-quinase, e o receptor neurotrófico p75 (p75 neurotrophin receptor, p75ntr), membro da superfamília dos receptores de fatores de necrose tumoral (Bibel e Barde, 2000; Kaplan e Miller, 2000; Chao, 2003). Ambos receptores podem estar localizados no mesmo neurônio, porém desencadeiam efeitos celulares distintos quando ativados: os receptores TrkB ativam vias de sinalização intracelular

relacionadas com sobrevivência, proliferação neuronal e plasticidade sináptica (Minichiello, 2009), enquanto os receptores p75ntr ativam vias associadas à morte celular por apoptose (Friedman, 2000) (Figura 3).

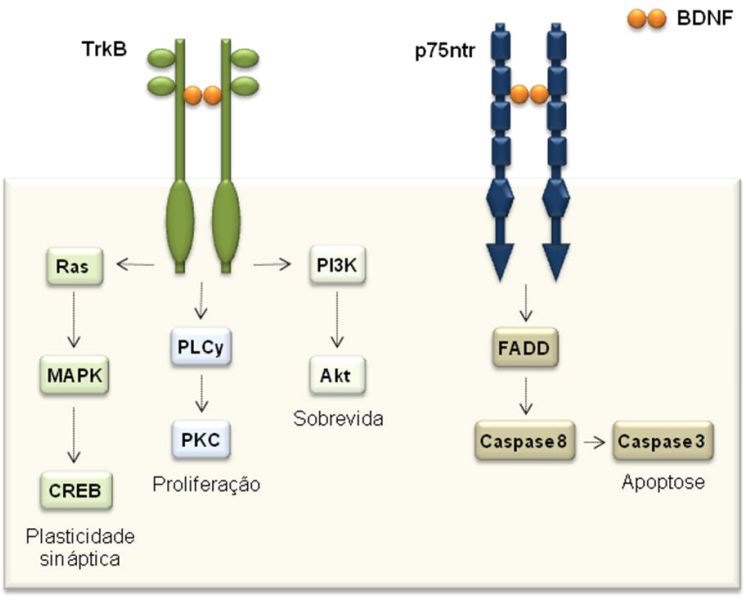

Figura 3. Imagem representativa das vias de sinalização intracelular mediadas pela interação do fator neurotrófico derivado do encéfalo (BDNF) com seus receptores, tropomiosina quinase B (TrkB) e neurotrófico p75 (p75ntr). Quando ativados, os receptores TrkB ativam as vias de sinalização da Ras, da fosfolipase Cγ (phospholipase Cγ, PLCγ) e da fosfatidilinositol 3-quinase (phosphoinositide 3-kinase, PI3K). A Ras funciona como uma proteína G estimulatória, aumentando a atividade da proteína quinase ativada por mitógeno (mitogen-activated protein kinase, MAPK) e da proteína de ligação ao elemento de resposta ao AMP cíclico (cyclic AMP-response element-binding protein, CREB), fatores de transcrição que regulam a síntese de várias proteínas relacionadas à plasticidade sináptica. A sinalização da PLCγ modula a resposta proliferativa pela ativação da proteína quinase C (protein kinase C, PKC), enquanto a sinalização da PI3K promove sobrevivência pela ativação da proteína quinase B (uma serina-treonina quinase também conhecida como Akt) (Minichiello, 2009). Através de um conjunto de proteínas adaptadoras, a união do BDNF com o receptor p75ntr gera uma cascata de reações químicas que leva a apoptose. A proteína Fas associada com domínio da morte (Fas-associated protein with death domain, FADD) ativa a caspase 8, uma caspase iniciadora que, por sua vez, ativa a caspase 3, uma caspase efetora. A caspase 3 ativada então inicia uma cascata de eventos que resulta em morte celular por apoptose. Entretanto, devido às vias antiapoptóticas associadas aos receptores tirosina-quinases, observa-se que a morte celular induzida pela sinalização BDNF/p75ntr ocorre somente quando a sinalização BDNF/TrkB está ausente ou diminuída (Davey e Davies, 1998; Friedman, 2000; Unsain et al., 2009).

Muitos estudos mostram que animais submetidos a diferentes tipos de atividade física apresentam um aumento da concentração cerebral de fatores neurotróficos (Neeper et al., 1995; 1996; Huang et al., 2006; Soya et al., 2007). Por exemplo, níveis elevados de BDNF são detectados no córtex, cerebelo e formação hipocampal de ratos submetidos à atividade física voluntária em roda (Neeper et al., 1995; 1996). Este aumento aparece em poucos dias, e pode ser mantido em quantidades elevadas mesmo após uma semana de exercício (Neeper et al., 1996). Na formação hipocampal, a expressão aumentada de BDNF em animais treinados tem sido observada em neurônios de CA1, CA3, hilo e giro denteado (Neeper et al., 1995) (Figura 4). Embora outros fatores tróficos, como o fator de crescimento neural (nerve growth factor, NGF) e o fator de crescimento fibroblástico 2 (fibroblast growth factor 2, FGF-2) (Neeper et al., 1996; Gomez-Pinilla et al., 1997), sejam também induzidos na formação hipocampal em resposta ao exercício físico, sua expressão tem sido transitória e menos evidente que a do BDNF, sugerindo que o BDNF seja o melhor candidato para mediar os efeitos a longo prazo do exercício no cérebro.

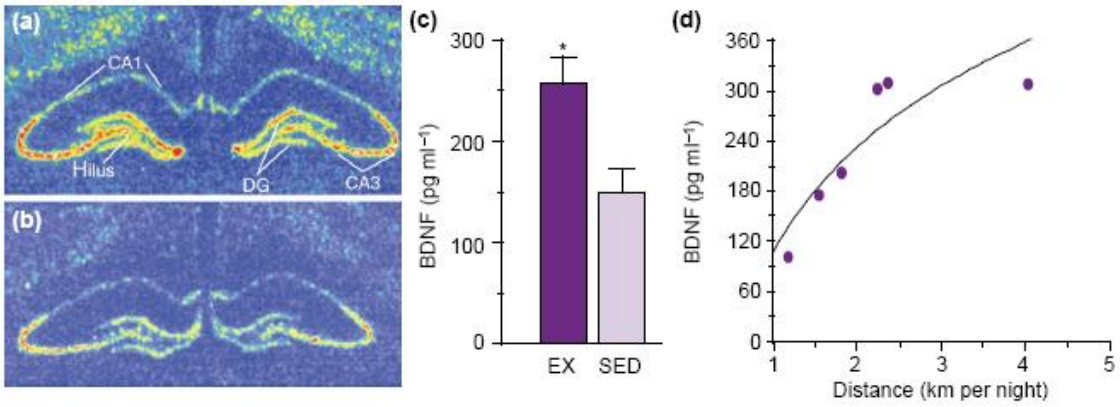

Figura 4. Efeito do exercício físico sobre a expressão hipocampal de fator neurotrófico derivado do encéfalo (BDNF). A expressão do gene (a e b) e os níveis de proteína (c) BDNF aumentam na formação hipocampal de animais com atividade física em roda (a e c EX) quando comparados aos animais sedentários (b e c SED). Nota-se também uma correlação positiva entre os níveis de proteína BDNF e o volume diário de exercício (distância corrida por noite) (d) (Cotman e Berchtold, 2002).

O exercício físico também estimula as vias de sinalização associadas ao BDNF (Tong et al., 2001; Molteni et al., 2002; Vaynman et al., 2003). A expressão aumentada do gene PKC e de muitos componentes da cascata da MAPK, como a MAPKI e MAPKII, tem sido observada após curtos períodos de exercício (Molteni et al., 2002). Em adição à MAPK, o exercício também aumenta a expressão do fator de transcrição CREB (Molteni et al., 2002; Vaynman et al., 2003). Estes achados são de grande importância, uma vez que as vias de sinalização da PKC, MAPK e CREB estão envolvidas com a integração de múltiplos sinais extracelulares, proliferação celular e plasticidade sináptica (Minichiello, 2009) (Figura 3). Além do mais, a via da CREB é necessária para a formação de vários tipos de memória (Silva et al., 1998), e parece exercer um papel importante na resistência neuronal a insultos (Walton et al., 1999).

Muitos genes associados à plasticidade sináptica apoiam a função do BDNF induzido pelo exercício físico na comunicação celular (Tong et al., 2001; Molteni et al., 2002). Isto tem sido observado com a expressão de ácido ribonucléico mensageiro (messeger ribonucleic acid, mRNA) para sinapsina I e sinaptogamina (Molteni et al., 2002), proteínas que exercem uma importante função na fusão das vesículas sinápticas com a membrana celular (Jovanovic et al., 2000; Augustine, 2001). A síntese de sinapsina I aumenta predominantemente em curtos períodos de exercício (3 e 7 dias), enquanto a síntese de sinaptogamina aumenta progressivamente ao longo dos dias de treinamento (3, 7 e 28 dias) (Molteni et al., 2002). Existem fortes evidências de que o aumento das proteínas associadas às vesículas sinápticas está ligada à sinalização BDNF/TrkB: o exercício aumenta a expressão cerebral de BDNF, que por sua vez, promove um aumento da fosforilação da sinapsina I via receptores TrkB no terminal pré-sináptico (Gomez-Pinilla et al., 2002; Vaynman et al., 2003). O resultado final da ativação de sinapsina I via BDNF/TrkB é a liberação de neurotransmissores na

fenda sináptica (Jovanovic et al., 2000). Considerando que a liberação aumentada de neurotransmissores favorece a indução de LTP e a memória (Medina e Izquierdo, 1995), é bem provável que os efeitos benéficos do exercício nas funções cognitivas estejam diretamente relacionados ao aumento dos níveis de BDNF e TrkB no cérebro.

1.1.2. Exercício Físico e Sistema Endocanabinóide

Como citado previamente, existem vários mecanismos associados à neuroplasticidade induzida pelo exercício físico, no entanto, destacaremos neste estudo a influência do exercício físico no sistema canabinóide.

O sistema endocanabinóide está presente em todos os invertebrados e vertebrados (Lutz, 2002). A presença deste sistema durante a evolução reflete a importância dos receptores canabinóides e seus ligantes endógenos para o desenvolvimento e viabilidade das espécies (Elphick e Egertová, 2001). O receptor canabinóide subtipo 1 (CB1) é o receptor acoplado à proteína G inibitória mais presente no cérebro (Pertwee e Ross, 2002), sendo encontrado no córtex cerebral, núcleos da base, cerebelo, formação hipocampal (Glass et al., 1997; Ameri, 1999; Iversen, 2003) e em vários locais do sistema periférico (Lynn e Herkenham, 1994). O receptor canabinóide subtipo 2 (CB2), por outro lado, está localizado preferencialmente no tecido periférico, principalmente em células do sistema imunológico (Makie, 2008).

A descoberta de receptores canabinóides no SNC foi fundamental para a localização dos circuitos neuronais nos quais os canabinóides exercem sua ação fisiológica. Em 1992, Devane e colaboradores identificaram no cérebro do porco um composto lipídico que se ligava especificamente aos receptores CB1 e que produzia efeitos fisiológicos semelhantes ao Δ^9-tetrahidrocanabinol (THC) e outros canabinóides

sintéticos. Essa molécula recebeu o nome convencional de araquidonoiletanolamida em função da sua estrutura química, mas foi batizada por seus descobridores como anandamida (da palavra *anand*, que significa “êxtase”). O segundo composto endógeno ligante ao receptor canabinóide identificado foi o 2-araquidonoil glicerol (2-AG) (Mechoulam et al., 1995).

Devido a sua natureza lipofílica, os endocanabinoides não são armazenados em vesículas sinápticas, mas são sintetizados pelos neurônios pós-sinápticos após a despolarização da membrana e aumento de cálcio intracelular (Freund et al., 2003; Piomelli, 2003). Uma vez liberados na fenda sináptica, os endocanabinóides agem em direção retrógrada para ativarem receptores CB1 pré-sinápticos (Freund et al., 2003). A ativação dos receptores CB1 em neurônios pré-sinápticos gera dois efeitos imediatos que bloqueiam a transmissão de informação de um neurônio ao outro de forma transitória. Um desses efeitos é o bloqueio da abertura dos canais de cálcio no terminal pré-sináptico, impedindo a liberação de neurotransmissores (Nestler et al., 2001; Galante e Diana, 2004). Outro efeito imediato é a abertura de canais que permitem a saída de íons positivos de potássio. Essa saída de cargas positivas neutraliza o efeito elétrico da entrada de sódio, suprimindo o potencial de ação (Mackie e Hille, 1992; Szabo e Schlicker, 2005) (Figura 5). Por este mecanismo, os endocanabinóides podem inibir a liberação de uma variedade de neurotransmissores e influenciar a plasticidade sináptica de curta e longa duração (Chevaleyre et al., 2006).

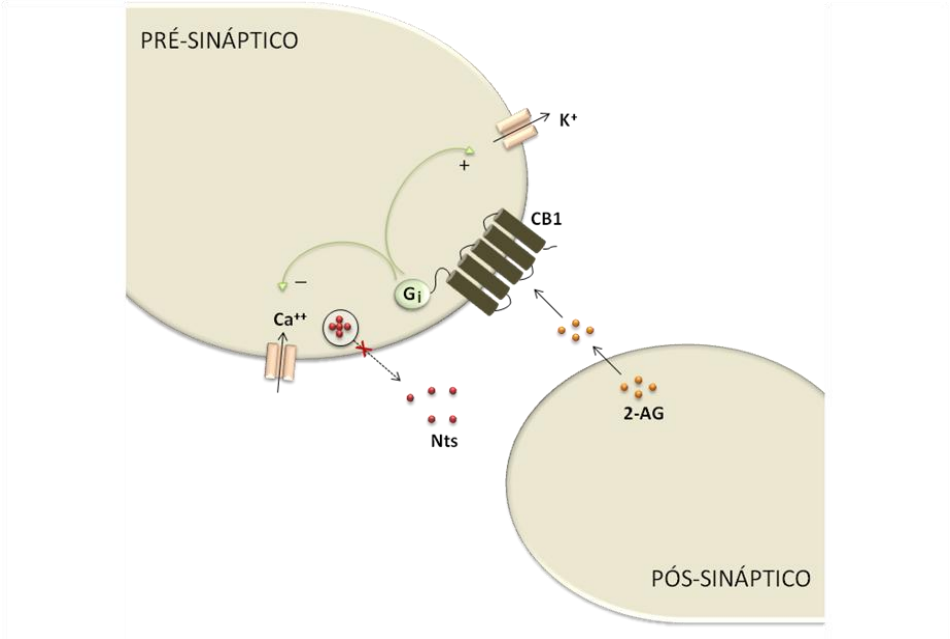

Figura 5. Imagem representativa da sinalização retrógrada mediada por endocanabinóides. A despolarização do neurônio pós-sináptico estimula a síntese de 2-araquidonoil glicerol (2-AG) a partir de componentes da própria membrana celular. O 2-AG produzido desta forma se difunde até a membrana do neurônio pré-sináptico para ativar os receptores canabinóides subtipo 1 (CB1). Uma vez ativados, os receptores desencadeiam uma cascata de reações químicas que leva ao desacoplamento da proteína G inibitória (G_i), fechamento de canais de cálcio (Ca^{++}), abertura de canais de potássio (K^+) e inibição da liberação de neurotransmissores (Nts) na fenda sináptica (Mackie e Hille, 1992; Galante e Diana, 2004; Szabo e Schlicker, 2005).

Os endocanabinóides produzem estados psicológicos similares às experiências relatadas pelo estado “runner’s high” (Fuss e Gass, 2010). Esta condição é um estado de euforia vivenciado por muitos atletas durante provas de longa duração. Comparada com a analgesia dos opióides, a analgesia produzida pelos endocanabinóides é mais consistente (Bushlin et al., 2010). A ativação dos endocanabinóides produz sedação, sensação de bem estar, capacidade de atenção reduzida e dificuldade de estimação do tempo (Iversen, 2003). Este perfil de comportamento é similar às experiências de corredores de longa distância. Ainda, a estrita interação dos endocanabinóides com a dopamina mostra que eles apresentam uma função no sistema de recompensa do cérebro (Dalton et al., 2009). Sparlin e colaboradores (2003) mostraram que o exercício

físico de longa duração ativa o sistema endocanabinóide, sugerindo um novo mecanismo induzido pelo exercício físico nas alterações do estado mental. Uma vez que os endocanabinóides reduzem a sensação de dor (Richardson, 2000) e alteram os processos emocionais e cognitivos (Chaperon e Thiebot, 1999), este sistema pode também ter uma participação nos efeitos psicológicos induzidos pelo exercício físico.

1.2. Exercício Físico e Desenvolvimento Cerebral

O desenvolvimento cerebral é caracterizado por uma série de etapas críticas, e cada uma delas deve ser corretamente cumprida para que, no final, o cérebro configure sua estrutura normal. A etapa inicial do desenvolvimento cerebral ocorre a partir de um grupo de células especializadas que se localizam na região dorsal do embrião (Bayer, 1989). Essas células se proliferam para gerar tanto neurônios imaturos quanto células gliais (Corbin et al., 2008). Após esse processo de proliferação e diferenciação celular, inicia-se um movimento migratório que leva os neurônios imaturos aos seus locais de destino (Gleeson, 2001). Estimativas indicam que esses neurônios migram uma distância equivalente a 1.000 vezes o tamanho de seu corpo celular (Gleeson et al., 1998). Quando os neurônios chegam ao seu destino final, ou mesmo durante a migração, um axônio emerge como um prolongamento celular. Este axônio apresenta na sua extremidade um aparato especializado chamado cone de crescimento, que conduz o crescimento axonal a partir de moléculas do meio extracelular (Dickson, 2002). Em seguida, a formação de contatos entre os axônios em crescimento e suas respectivas células-alvo inicia um processo de desenvolvimento seletivo de sinapses, durante o qual alguns contatos sinápticos são fortalecidos e outros são eliminados (Changeux e Danchin, 1976; Innocenti, 1981).

Em muitas espécies de mamíferos, incluindo o homem, uma parte considerável do desenvolvimento cerebral ocorre após o nascimento (Winick e Nobel, 1965; Dobbing e Sands, 1973; Watson et al., 2006). O cérebro humano cresce rapidamente de 400 gramas ao nascimento para 1.000 gramas no primeiro ano e para 1.400 gramas na maioridade (kinney, 1988). No rato, o peso cerebral sextuplica do nascimento até a vida adulta (Bandeira et al., 2009). Concomitantemente, alterações importantes ocorrem na reestruturação morfológica e funcional de neurônios, sinapses e circuitos neurais (Rice e Barone, 2000; Spear, 2000; Zhang e Poo, 2001; Levitt, 2003; Tau e Peterson, 2010). As sinapses formadas nos estágios iniciais começam a estabelecer uma maquinaria molecular que permite um padrão apropriado de atividade neuronal (Zhang e Poo, 2001), em geral fornecido pela estimulação ambiental (Wiesel, 1982). A ramificação dendrítica aumenta significativamente para ajudar a estabelecer conexões entre as células. Durante esse período, tipos especiais de gliócitos formam uma bainha isolante de mielina que aumenta a velocidade da condução axonal. Pela maior complexidade dos dendritos e axônios, os neurônios passam a ter maior superfície sináptica. Quando a densidade sináptica máxima é atingida, ocorre um mecanismo de eliminação de sinapses e de neurônios conhecido como “poda” (do inglês, “pruning”) (Purves e Lichtman, 1980; Huttenlocher, 1984; Herschkowitz, 1988; Huttenlocher, 1990; Low e Cheng, 2006). A poda sináptica e neuronal ocorre em momentos diversos e em partes diferentes do cérebro (Huttenlocher e Dabholkar, 1997; Bandeira et al., 2009). Alguns neurocientistas explicam esse processo por meio de uma analogia: para obter a forma final de uma escultura, os artistas clássicos eliminam os pedaços desnecessários do mármore. Desta forma, o sistema nervoso seleciona as conexões apropriadas e remove as impróprias. É provável que este mecanismo ocorra para permitir que o cérebro imaturo selecione as conexões sinápticas mais adequadas ao ambiente

(Changeux e Danchin, 1976). Uma vez que a influência do ambiente normalmente é mais intensa durante o desenvolvimento pós-natal do que na fase adulta (Williams et al., 2001; Lores-Arnaiz et al., 2007), eventos que ocorrem durante este período de alta plasticidade podem ser críticos para o desenvolvimento normal do cérebro (Andersen, 2003; Linkenhoker et al., 2005).

Existem evidências que o exercício físico durante a infância e adolescência pode ser favorável para o desenvolvimento cerebral (Sibley e Etnier, 2003; Hillman et al., 2005; Buck et al., 2008; Aberg et al., 2009; Hillman et al., 2009). Em uma meta-análise conduzida sobre 16 estudos, Sibley e Etnier (2003) detectaram uma correlação positiva entre atividade física e níveis de aprendizagem e inteligência em crianças em idade escolar. Hillman e colaboradores (2009) observaram que uma única sessão de exercício moderado (caminhada) em crianças com 9 e 10 anos de idade pode alterar a atividade eletrencefalográfica e melhorar o raciocínio em testes de desempenho acadêmico. O exercício físico aeróbio aumentou o estado de atenção nas avaliações, com melhores resultados nas tarefas e compreensão mais clara da leitura (Hillman et al., 2009). Em um trabalho anterior, o mesmo grupo de pesquisadores havia mostrado que pré-adoslecentes (crianças entre 7 e 12 anos de idade) que realizavam atividade física regularmente apresentavam um processamento cognitivo mais rápido (Buck et al., 2008). A partir desses resultados, Aberg e colaboradores (2009) investigaram a relação entre condição física (cardiovascular) e desempenho cognitivo durante o período adolescente. O estudo acompanhou um milhão e duzentos mil adolescentes na faixa etária de 18 anos alistados no serviço militar na Suécia. Os adolescentes que apresentavam uma melhora cardiovascular entre 15 e 18 anos exibiram melhor rendimento nos testes de inteligência do que aqueles com condição física mais baixa no mesmo período. Para verificar se os resultados poderiam refletir uma influência

genética ou meio familiar, os pesquisadores analisaram na amostra 3.147 pares de gêmeos, nos quais 1.432 eram idênticos. Observou-se que os fatores ambientais, e não genéticos, exerceram uma influência nessa relação (Aberg et al., 2009).

Salientando os efeitos positivos da atividade física durante o desenvolvimento, Dik e colaboradores (2003) observaram que o exercício aeróbio durante a infância pode aumentar a resiliência do cérebro em etapas posteriores. O estudo avaliou a associação entre atividade física no início da vida e cognição em 1.241 indivíduos com idade entre 62 e 85 anos. Os resultados indicaram uma correlação positiva entre a prática de atividade física durante os 15 e 25 anos e a velocidade de processamento de informações na idade avançada (Dik et al., 2003). Esses dados interessantes sugerem que o exercício durante o desenvolvimento pós-natal pode promover efeitos benéficos para as funções cerebrais ao longo da vida. Entretanto, os mecanismos pelos quais o exercício físico exerce tal função não estão bem definidos.

Desde que a capacidade da plasticidade cerebral diminui com o avançar da idade (Akopian e Walsh, 2006), é importante verificar se a atividade física pode influenciar a estrutura e função do cérebro em processo de formação. Neste contexto, o presente estudo teve como objetivo principal investigar os efeitos do exercício físico sobre o desenvolvimento cerebral pós-natal. A primeira etapa deste projeto foi verificar se um programa de exercício físico durante o desenvolvimento pós-natal seria capaz de modificar a plasticidade hipocampal de ratos em desenvolvimento. Para isso, a expressão hipocampal de parvalbumina foi avaliada em ratos submetidos a um protocolo de exercício físico progressivo durante o período adolescente (21° ao 60° dia de vida pós-natal). A parvalbumina é uma proteína ligante de cálcio localizada em uma população de células que formam um grupo heterogêneo de neurônios não piramidais no córtex e na formação hipocampal, particularmente uma população de interneurônios

inibitórios com alta taxa de disparo e elevado metabolismo oxidativo (Nitsch et al., 1990; Baimbridge et al., 1992; Heizmann, 1993). Muitos estudos utilizam a expressão dessa proteína para visualizar mudanças fisiológicas e patológicas no SNC (Nitsch et al., 1990; Baimbridge et al., 1992; Heizmann, 1993). Por exemplo, uma redução da expressão cerebral de parvalbumina tem sido observada em distúrbios neurológicos como a epilepsia, esquizofrenia e distúrbio bipolar (Sloviter, 1989; Abdul-Monim et al., 2007; Andrioli et al., 2007; Pantazopoulos et al., 2007; Lodge et al., 2009). Os nossos resultados mostraram que animais submetidos a um programa de exercício físico durante a adolescência apresentam na vida adulta um aumento da expressão hipocampal de parvalbumina. Esses achados foram publicados na revista Brain & Development (Artigo 01).

A segunda etapa deste projeto foi avaliar a expressão cerebral do receptor CB1 em ratos treinados durante o período adolescente. Independente da função fisiológica e psicológica dos endocanabinóides durante o exercício físico, este sistema exerce função importante na plasticidade cerebral. O sistema endocanabinóide está presente no SNC desde os primeiros estágios do desenvolvimento cerebral e tem um papel relevante na organização cerebral durante a vida pré e pós-natal (Fernandez-Ruiz et al., 2000). Vários trabalhos mostram a presença dos receptores CB1 e seus ligantes endógenos (anandamida e 2-AG) em regiões cerebrais relacionadas com proliferação e migração de células, processos de alongamento axonal e sinaptogênese (Rodriguez de Fonseca et al., 1993; Berrendero et al., 1999; Fernandez-Ruiz et al., 2000; 2004; Fride, 2008). Além disso, os receptores CB1 têm sido encontrados em células progenitoras neuronais e neuroblastos (Pacher et al., 2006; Galve-Roperh et al., 2007). Estes achados indicam que os receptores CB1 podem exercer um papel importante na modulação da proliferação e diferenciação celular (Galve-Roperh et al., 2008). Em

favor da idéia, observa-se que a deficiência do receptor CB1 diminui a neurogênese no giro denteado e na zona subventricular de camundongos (Jin et al., 2004; Aguado et al., 2005). Outros estudos também mostram que a administração de antagonistas do CB1 durante o desenvolvimento pode modificar o processo normal de maturação cerebral e resultar em alterações comportamentais tardias (Trezza et al., 2008). Baseado no fato que o exercício físico durante a infância é capaz de aprimorar as funções cerebrais e tendo em vista o papel do sistema endocanabinóide no desenvolvimento cerebral, nós analisamos a expressão cerebral do receptor CB1 após um programa de exercício físico durante o desenvolvimento pós-natal. Foi observada uma diminuição na expressão do receptor CB1 no hipocampo e estriado em ratos treinados na adolescência. Esses resultados foram publicados na revista Neurochemistry International (Artigo 02).

A última etapa deste projeto foi dedicada à avaliação da expressão hipocampal de BDNF e seus receptores e à análise comportamental dos animais treinados durante a adolescência. A expressão do BDNF durante este período é estritamente controlada. Estudos em animais mostram que alterações na modulação dos níveis de BDNF e de seus receptores podem causar desenvolvimento morfológico e funcional anormal do cérebro (Bibel e Barde, 2000; Huang e Reichardt, 2001; Chao, 2003; Bernd, 2008). Por exemplo, a mutação gênica do BDNF resulta em déficit de aprendizagem em camundongos (Gorski et al., 2003). Por outro lado, trabalhos recentes mostram que o exercício físico durante o desenvolvimento fetal pode aumentar a expressão genética de BDNF e melhorar o desempenho cognitivo dos filhotes após o nascimento (Parnpiansil et al., 2003; Lee et al., 2006; Kim et al., 2007). Parnpiansil e colaboradores (2003) observaram que filhotes de ratas submetidas ao exercício físico durante a gestação apresentam no 40° dia de vida pós-natal índices melhores de aprendizagem

espacial do que os filhotes de ratas não submetidas ao exercício físico. Ao mesmo tempo, um aumento do mRNA para BDNF após o nascimento (Parnpiansil et al., 2003) e em estágios posteriores (Lee et al., 2006; Kim et al., 2007) foram detectados na formação hipocampal dos filhotes de ratas treinadas durante a gestação. Esses achados sugerem que a expressão aumentada de BDNF induzida pelo exercício durante o desenvolvimento pode resultar em aprimoramento das funções cerebrais. Para melhor compreender os efeitos neuroplásticos induzidos pelo exercício físico durante o desenvolvimento cerebral pós-natal, os níveis hipocampais de BDNF e de seus receptores foram analisados em ratos submetidos a um programa de exercício físico no período adolescente. Os resultados mostraram que animais treinados na adolescência apresentam na vida adulta um aumento da expressão hipocampal de BDNF e de seu receptor TrkB. Adicionalmente, observou-se que o exercício durante o este período foi capaz de aumentar a densidade hipocampal de fibras musgosas, de aprimorar a memória espacial e de melhorar a capacidade de evocar as memórias em longo prazo. É importante ressaltar que enquanto o exercício induz plasticidade hipocampal, efeitos degenerativos poderiam aparecer em condições indevidas de estresse físico e mental. Neste sentido, nossos resultados mostraram que o protocolo de exercício físico utilizado neste estudo não induziu resposta inflamatória e degeneração neuronal na formação hipocampal de ratos adolescentes. Em conjunto, esses resultados indicam que o exercício pode resultar em mudanças positivas para o cérebro em desenvolvimento pós-natal. Esses achados foram aceitos para publicação na revista Hippocampus (Artigo 03).

2

Objetivos

2. OBJETIVOS

A proposta do presente estudo foi investigar a influência do exercício físico sobre o desenvolvimento cerebral pós-natal. Para isso, avaliamos a plasticidade hippocampal de ratos submetidos a um programa de exercício físico aeróbio durante o período adolescente (21° ao 60° dia de vida pós-natal).

2.1. Objetivos Específicos

(a) Para verificar se um programa de exercício físico utilizado neste estudo seria capaz de modificar a plasticidade hipocampal de ratos em desenvolvimento, a expressão de parvalbumina foi investigada em ratos treinados durante a adolescência (Artigo 01);

(b) Tendo em vista o papel do sistema endocanabinóide durante o desenvolvimento, a expressão cerebral do receptor CB1 foi analisada em ratos treinados durante o período adolescente (Artigo 02);

(c) Para melhor compreender os efeitos neuroplásticos induzidos pelo exercício físico durante o desenvolvimento cerebral pós-natal, foi realizada a avaliação da expressão hipocampal de BDNF e seus receptores e à análise comportamental dos animais treinados na adolescência (Artigo 03).

3

Artigos

3.1. Artigo 01

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Original article

Physical exercise during the adolescent period of life increases hippocampal parvalbumin expression

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Abstract

In order to investigate whether physical exercise during development would promote changes the calcium-binding protein parvalbumin (PV) expression in the hippocampal formation, we performed an immunostaining study after an aerobic exercise program in rats during adolescent period of life. Wistar rats were submitted to daily exercise program in a treadmill between postnatal day 21 and 60. Running time and speed were gradually increased during the subsequent days until 18 m/min for 60 min. After the aerobic exercise program, animals of all groups were killed and PV immunostaining procedures were performed. The results showed significant increase of protein level in the hippocampal formation and PV-immunoreactive neurons in CA1 and CA2/CA3 regions of rats submitted to exercise when compared with control rats. This finding indicates that aerobic exercise program during adolescent period promotes neuroplastic changes in hippocampal formation.

Keywords: Physical exercise; Running; Adolescence; Hippocampal formation; Parvalbumin; Plasticity

1. Introduction

In the last decades, studies have dedicated to the understanding of neurobiological bases of physical exercise to the maintenance and improvement of neural function in humans and animals (for review, see [1]). Studies in adult animals demonstrate that physical exercise modifies the expression of neurotrophic factors [2–5], the growth of neuronal processes [4,6] and neurogenesis in the hippocampal formation [7–9], a highly plastic region of the brain important for memory, learning and emotional processes [10,11].

Although the effects of physical exercise on the central nervous system (CNS) of adult animals have been well documented, little is known of its effects in the developing brain. The development of highly organized structures in the CNS is a complex process and stimuli in this period could determine the functional integrity at adult stage. Experience and learning events can modulate the functional maturation of the brain by neuroplastic processes. These stimuli occurring during early postnatal brain development may result in the development of more complex neural circuitry [12]. Since the capacity for neuroplasticity decreases with increasing age [13,14], it is very important to assess how exercise may beneficially regulate neuroplasticity during early life, and to determine the basic mechanism of such effects. A preliminary study conducted by Uysal et al. [15] demonstrated that exercise during the adolescent

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period performed a better spatial memory in Morris water maze and increased cell density in the hippocampal dentate gyrus. Additionally, a recent investigation demonstrated increased hippocampal neurogenesis and gene expression in juvenile rats [16].

Previous studies have used the expression of the calcium-binding protein parvalbumin (PV) as a useful marker to study hippocampal changes in response to physical exercise [17,18]. PV is a low (12 kDa) molecular weight protein expressed in a population of nonpyramidal cells which form a heterogeneous group of neurons in the neocortex and hippocampal formation, particularly a population of inhibitory interneurons that have a high firing rate and a high oxidative metabolism [19–22]. These inhibitory interneurons are essential for information processing and play a crucial role in controlling excitatory transmission in the hippocampal neurons [23]. In our previous study a higher number of PV-immunoreactive (PV-ir) hippocampal neurons in rats submitted to acute physical exercise (voluntary and forced exercise) were observed [18]. As the interference of physical exercise during brain development has been poorly explored, the present investigation was performed to study the occurrence of structural changes in hippocampal formation after physical exercise during the animal development by means of PV immunostaining approach. To test this idea, we used immunohistochemistry and immunoblot analyses in rats submitted to treadmill exercise during adolescent period.

2. Materials and methods

2.1. Animals and protocol of physical exercise

Male Wistar rats at postnatal day 21 (P21) were divided into two groups: exercise group ($n = 10$), control group ($n = 10$). The colony room was maintained at 21 ± 2 °C with a 12-h light/dark schedule, and ad libitum food and water throughout the experiments. Animals of the exercise group were familiarized with the apparatus for three days by placing them on a treadmill (Columbus instruments) for 5 min/day at speed of 8 m/min at 0% degree incline. Electric shocks were used sparingly to motivate the rats to run. To provide a measure of trainability, we rated each animal's treadmill performance on scale of 1–5 according to the following anchors [1, refused to run, 2, below average runner (sporadic, stop and go, wrong direction), 3, average runner, 4, above average runner (consistent runner occasiolly fell back on the treadmill), 5, good runner (consistently stayed at the front of the treadmill)] [24,25]. Animals with a mean rating of 3 or higher were included to the exercise group. The animals which were excluded from the exercise groups did not form the control group. This procedure was used to exclude possible different levels of stress between animals. Subsequently, selected animals were submitted to a physical exercise program performed between P21 and P60, 7 days per week. Each training session started with a 5 min warm-up at 8–10 m/min. Running time and speed gradually increased during the subsequent days, until reach 18 m/min during 60 min (Table 1). Animals of the control group were transferred to the experimental room and handled in the same way as animals of the exercise group (privation of water and food during treadmill exercise). At P60, all animals were killed and prepared to PV immunostaining procedures. All experimental protocols were approved by the ethics committee of the Universidade Federal de São Paulo (UNIFESP).

2.2. Immunoblotting

For immunoblotting, animals' hippocampi (four animals for each group) were removed immediately after decapitation. The hippocampi were homogenized in lysis buffer [125 mM Tris–HCl buffer pH 6.8, containing 2% sodium dodecyl sulfate (SDS), 10% glycerol, 1% mercaptoethanol and 1 mM sodium vanadate] and stored at −80 °C. The analysis for PV expression was performed by mean of immunoblotting. The samples were sonicated, and protein concentration was determined by Bradford's Method [26]. After dilution 40 μg of proteins were applied to Tricine/SDS/Polyacrylamide Gel (7.5 × 5.0 cm; 12% separating gel; 4% stacking gel), according to the methods described by Schägger and Von Jagow [27]. The Gels were blotted in 25 mM Tris, 192 mM glycine, 20% (by vol.) methanol pH 8.3 on 0.2 μm cellulose nitrate sheets (GE). The blots were incubated with antibody against PV (monoclonal 1:1000, Swant) at 4 °C overnight. Peroxidase-conjugated goat anti-mouse IgG (Vectasin) was used according to the manufacturer's instructions. For a revelation was used chemiluminescence detection system (ECL) with exposure to X-ray film (Hyperfirm and ECL Kit, GE). The reprobing of membranes was required for incubation with anti-β-actin immunoglobulins (1:1000, Sigma), it used with internal control. The blots were stripped by incubating with 0.1 M NaOH solution for 5 min at room temperature.

2.3. Immunohistochemistry

Six animals from each group were deeply anesthetized (Tionembutal, 50 mg-kg, i.p.), and perfused transcardially with 0.01 M phosphate-buffered saline (PBS), followed by 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 (PB). The brains were removed, briefly postfixed in 4% paraformaldehyde in PBS, cut coronally with a vibratome in 50 μm-thick sections, which were collected in series (one in each 10 cut sections). A sequence of three sections per animal (Bregma, −2.8/−4.3 mm) was selected for immunocyto-

Table 1
Physical exercise protocol in animals during development.

Postnatal day	Velocity (m/min)	Time (min)
21	8	5
22	8	5
23	8	5
24	9	5
25	9	10
26	10	10
27	10	15
28	11	15
29	11	20
30	12	20
31	12	30
32	13	30
33	13	35
34	14	35
35	14	40
36	15	40
37	15	45
38	16	45
39	16	50
40	17	50
41	17	55
42	18	55
43 ~ 60	18	60

chemistry process. The immunoperoxidase procedure was performed on free-floating sections using antibody against PV (monoclonal 1:7000, Swant, Bellinzola, Switzerland). Paired sections of animals from both groups were processed in the same vial in order to minimize the intergroup differences during the immunohistochemical procedure. The sections were pre-treated with 3% H_2O_2 for 10 min to block endogenous peroxidase activity, rinsed in PBS, preincubated for 45 min in 10% normal serum in PBS with 0.2% Triton X-100, and then incubated in primary antibodies overnight at 4 °C. Sections were then rinsed in PBS, incubated in biotinylated anti-mouse IgG (Vector, Burlingame, USA) at a dilution of 1:200 in PBS (1 h at room temperature), rinsed in PBS, incubated in avidin–biotin peroxidase complex (ABC; Vectastain, Vector) for 1 h, washed several times in PBS, and then incubated in 0.075% diaminobenzidine in 0.002% H_2O_2. Sections were finally washed in PBS, mounted on gelatin-coated slides, dehydrated, and coverslipped.

2.4. Quantification and data analysis

The immunoblotting was performed to determine the expression of PV in the hippocampal formation. The molecular weights of proteins PV and β-actin (10–12 KDa and 42–45 KDa, respectively) were determined by running a prestained protein ladder (Rainbow – GE). The band densities on immunoblots were measured using the Densirag software (Biocom, France). All values were reported (means ± SD) of relative expression for β-actin.

The material processed for immunohistochemistry was performed to analyze the spatial distribution of PV-ir hippocampal neurons. The sections were analyzed quantitatively at the microscope, under bright-field illumination, independently by two investigators. Counts of PV-ir neurons were performed using the magnification of 200× in *stratum pyramidalis* of Cornus Ammonis (subfields CA1, CA2/CA3) and in *stratum granule* of dentate gyrus (DG) of the hippocampal formation. For each animal, the average number of PV-ir neurons in a given region was obtained through counts of three sections (both hippocampi of the each section). The values were expressed as means ± SD.

The statistical analyses between exercise and control groups were performed using Student's *t*-test. Values were considered significant when $p < 0.05$.

3. Results

3.1. Hippocampal PV expression

Quantitative immunoblotting analysis showed that the PV density was significantly enhanced in hippocampal formation of rats submitted to aerobic treadmill exercise (~30%, 1.27 ± 0.1, $p < 0.002$) when compared to the control group (1.0 ± 0.001) (Fig. 1). No difference in β-actin immunoreactivity was detected between the studied groups ($p > 0.05$).

3.2. Hippocampal distribution of PV-ir neurons

PV immunoreactivity in this study was similar to those which have been described before [20]. Briefly, PV-ir neurons in control animals were mostly located within or in the vicinity of the pyramidal cell layer of CA1, CA2 and CA3 and in the stratum oriens and stra-

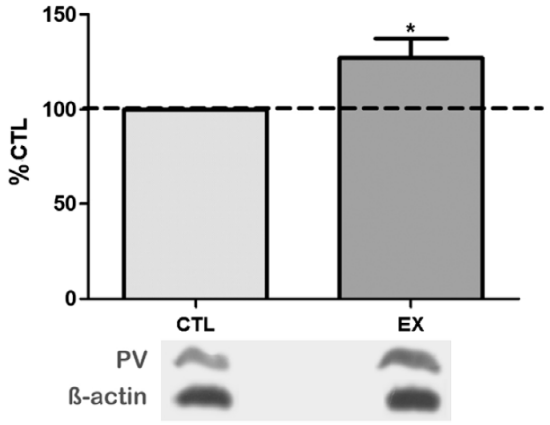

Fig. 1. Immunoblot study of parvabulmin protein (PV) in the hippocampi of rats from exercise group (EX) and control group (CTL). Significant enhance of PV density in the hippocampi of rats submitted to aerobic treadmill exercise during adolescent period ($^*p < 0.002$).

tum radiatum of CA1, CA2 and CA3. In the DG, PV-immunoreactivity was observed in the granule-cell layer (Fig. 2). In trained rats, the pattern of PV-immunoreactivity in the studied regions was similar to control rats. However, the statistical analysis revealed that the aerobic treadmill exercise increased significantly the number of PV-ir neurons in the CA1 (61.9 ± 5.5, $p < 0.0001$) and CA2/CA3 (18.2 ± 2.8, $p < 0.05$) when compared to the control group (CA1 = 42.2 ± 4.3; CA2/CA3 = 14.7 ± 1.75) (Fig. 3). No difference was observed in the DG region between the studied groups (EX = 35.0 ± 5.6; CTL = 34.4 ± 7.1, $p > 0.05$).

4. Discussion

Although a number of studies have investigated the effect of different experience, such as enriched environment in animals during development [28,29], only a few works have applied a programmed physical exercise in adolescent animals [15,16,30]. Our study investigated the effect of a physical exercise program in animals during the adolescent period of life. Immunostaining analyses showed that the aerobic treadmill exercise performed during adolescent period induced

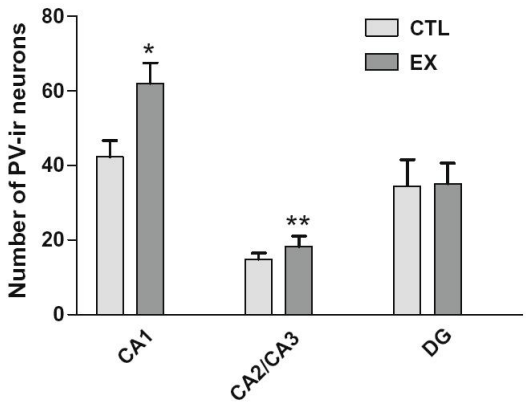

Fig. 3. Number of parvalbumin-immunoreactive (PV-ir) neurons in the hippocampal formation of rats from exercise group (EX) and control group (CTL). An increased of PV-ir neurons in rats trained during adolescent period was detected in the CA1 and CA2/Ca3 regions ($^{*}p < 0.0001$; $^{**}p < 0.05$).

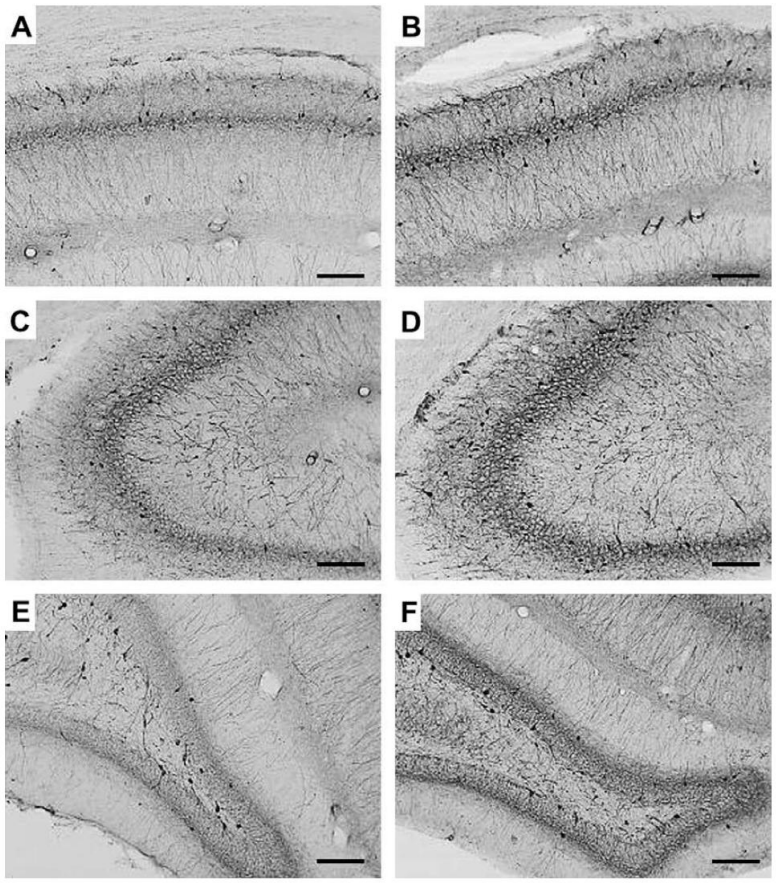

Fig. 2. Photomicrographs of parvalbumin-immunoreactive (PV-ir) neurons in the hippocampal formation of rats from exercise group (B, D and F) and control group (A, C and E) (scale bar = 150 μm). A and B: CA1; C and D: CA2/CA3; E and F: dentate gyrus.

significant changes the hippocampal PV expression. This finding is in accordance with previous observation that shows an influence of regular aerobic exercise on hippocampal formation of adolescent rats [15]. Additionally, we showed that the exercise protocol used was able to induce morphological alteration in brain of adolescent animals. It is important to point out that different intensities of exercise may induce positive and negative changes in brain plasticity. Low and moderate exercises do not alter apoptosis in brain of adult rats [31]. In Uysal and collaborators work animals ran at speed of 8 m/min, 30 min daily, for five consecutive days a week [15]. In our exercise protocol, animals ran at a superior intensity (until 18 m/min during 60 min, 7 days per week). Although easy [15] and moderate intensity (our study) induced positive changes in hippocampal plasticity of rats examined in the adult life, this is a subject that deserves more attention.

Whereas the general structure of the brain is formed before birth, complete development of the complex neural networks depends on postnatal experience. Researches have demonstrated that juvenile stimuli can affect adult behavior [32,33]. Treadmill running by pregnant rats was shown to produce increased the mRNA expression of brain-derived neurotrophic factor (BDNF), enhanced hippocampal cell survival, and improved the short-term memory ability in the rats' pups [34]. In Uysal et al. [15] study, the exercise during the adolescent period performed a better spatial memory in Morris water maze and increased cell density in the hippocampal dentate gyrus. In another study, exercise influenced neurogenesis and mRNA BDNF expression, *N*-methyl-D-aspartate receptor type 1 (NMDAR1) and vascular endothelial growth factor (VEGF) in the hippocampal formation of 5-weeks-old rats trained for one week [16]. Our experiments do not allow identifying whether the increase in PV-ir occurs in new neurons or in pre-existing ones (CA1 and CA2/CA3 regions). It is important to note that neurogenesis induced by early life exercise may have a significant impact on brain structure and functional development. We speculate that the result of this present study could be attributed to cell proliferation and BDNF. The new cell formation in the hippocampal formation is most prevalent in young rats, and an increase cell proliferation in the dentate gyrus has been observed in 4-weeks-old rats trained for five days [30]. To this point, reductions in PV expression in hippocampal formation of BDNF mutant have been reported [35].

The physiological role of PV at the cellular and network levels is less clear. However, several studies have suggested that many biological processes in the CNS linked with calcium ions are regulate via interaction with intracellular calcium-binding proteins [22]. PV acts in neurons as an endogenous calcium buffer that affects temporal-spatial characteristics of calcium transients. In fact, it has been suggested that calcium-binding proteins protect against Ca^{2+} overload, rendering neurons more resistant against excitotoxicity [22]. In this way, several reports have proposed that alterations of the calcium-binding proteins might be involved in numerous disorders of the brain [21,22]. For instance, it has been observed that PV deficient reduces the threshold of seizures and increases the severity of the seizures induced by the convulsant drug pentylenetetrazol [36]. Similarly, mice with a targeted mutation of the gene-encoding urokinase plasminogen activator receptor showed a higher susceptibility to seizure activity and a reduction in interneurons that expression the PV [37]. These studies indicate that a down-regulation of PV could facilitate the development of epilepsy. Additionally, we recently investigated the effect of this treadmill exercise protocol during development on the amygdala kindling process (a valuable model for the agreement of the basic mechanisms of progressive epileptogenesis) [24]. The results showed that the physical exercise training in rats during the adolescent period of life did not retard the amygdala kindling development (stage 5) in the adulthood, but altered the initial stage of kindling (stage 1) [24].

In conclusion, while it is well documented that exercise has beneficial effects of neurons, further studies are needed to explore the mechanisms of exercise improving hippocampal functions in adolescent period of life. We also would like to point out that the main purpose of the present study was not designed to clarify these mechanisms, but primarily focused on morphological findings induced by exercise during brain development. Researches in this topic are relevant for determining optimum exercise strategies for people, particularly for children and teenagers. Future studies may include the impact of early life exercise on the adult brain, duration of exercise effects, and the effects of exercise intensity on cognition in both adolescents and adults.

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3.2. Artigo 02

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Rapid communication

Physical exercise in adolescence changes CB1 cannabinoid receptor expression in the rat brain

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ABSTRACT

Accumulating evidence indicates that the endocannabinoid system plays an essential role in the development and maturation of the central nervous system. Studies also have demonstrated that neural systems that regulate behavioral responses can be influenced by exercise during development. Exercise and endogenous cannabinoid activity have independently been shown to regulate brain plasticity, hence demonstrating a promising field of the endocannabinoid–exercise interaction. In order to investigate whether physical exercise during development would promote changes the brain endocannabinoid system, we investigated the cannabinoid receptor type 1 (CB1) expression in the brain of rats trained during the adolescent period. The results showed that an aerobic exercise program performed during adolescence significantly reduced the CB1 receptor expression in the striatum and hippocampal formation. These findings suggest an important link between the endocannabinoid system and physical training in adolescence.

1. Introduction

Cannabinoid receptors and their endogenous ligands constitute a modulatory system that influence diverse brain functions. The role of the brain cannabinoid system is not fully known; however, cannabinoids have a likely natural role in pain modulation, control of movement and memory (Iversen, 2003). Accumulating evidence also indicates that the endocannabinoid system plays an essential role during neurodevelopment for example in controlling neural progenitor proliferation, lineage segregation and migration (Fernández-Ruiz et al., 1999; Fride, 2008; Harkany et al., 2008). The endogenous cannabinoid system emerges in the early stages of embryonic development, and during pre- and postnatal development (McLaughlin and Abood, 1993; Rodríguez de Fonseca et al., 1993; McLaughlin et al., 1994; Belue et al., 1995). Many of the effects of endocannabinoids are mediated by two G-protein-coupled receptors (CB1 and CB2) (Pertwee, 1997). The CB1 receptor is located in the central nervous system, and it is more densely concentrated on the membrane of neurons located in the cortex, hippocampal formation, amygdala, basal ganglia and cerebellum (Herkenham et al., 1991; Glass et al., 1997). The CB2 receptor, on the other hand, has a more restricted distribution, being found in a number of immune cells and in a few neurons (Mackie, 2008).

Exercise and endogenous cannabinoid activity have independently been shown to regulate brain plasticity hence demonstrating an emerging field of the endocannabinoid–exercise interaction (Fuss and Gass, 2010). For instance, voluntary exercise increases endocannabinoid signaling within the hippocampal formation, and endocannabinoid signaling is required for voluntary exercise to increase proliferation of progenitor cells within the dentate gyrus (Hill et al., 2010). Human findings have also shown that exercise increases serum concentrations of endocannabinoids. The endogenous ligant anandamide is increased in the circulation following exercise in trained male college students (Sparling et al., 2003), demonstrating that exercise activates the endocannabinoid system.

Since the capacity for neuroplasticity decreases with increasing age (Akopian and Walsh, 2006; Lynch et al., 2006), it is very important to assess how exercise may beneficially regulate neuroplasticity during early life, and to determine the basic mechanism of such effects. Recent studies have showed the influence of physical exercise on brain plasticity of adolescent rats (Uysal et al., 2005; Arida et al., 2007; Lou et al., 2008; Gomes da Silva et al., 2010). Rats submitted to a daily exercise program on a treadmill between 21 and 60 days of postnatal life presented in adulthood a significant increase of parvalbumin neurons in the hippocampal formation (Gomes da Silva et al., 2010). Additionally,

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increased neurogenesis, gene expression, density of hippocampal cells and improved spatial memory in the Morris water maze were observed in rats trained on a treadmill during juvenile and adolescent periods (Uysal et al., 2005; Lou et al., 2008). Based on the findings showing the endocannabinoid–exercise interaction and their influence on the brain development, our study investigated the effect of a physical exercise program during the adolescent period on the brain CB1 receptor expression.

2. Materials and methods

2.1. Animals

Male Wistar rats aged 21 postnatal days-old (P21) were maintained under environmentally controlled conditions (07:00–19:00 h light/dark cycle; 21 ± 2 °C) and permitted free access to food and water throughout the experiment. Rats were bred in our laboratory and the date of birth was considered day 0. The pups were housed with their mother in individual cages until weaning at day 21. Sixteen animals were then divided into two groups: exercise group ($n = 8$), control group ($n = 8$). All experimental protocols were approved by the ethics committee of the Universidade Federal de São Paulo (UNIFESP) and all efforts were made to minimize animal suffering in accordance with the proposals of International Ethical Guideline for Biomedical Research (CIOMS, 1985).

2.2. Exercise paradigm

Animals of the exercise group were familiarized with the apparatus for three days by placing them on a treadmill (Columbus instruments) for 5 min/day at speed of 8 m/min at 0% degree incline. Electric shocks were used sparingly to motivate the rats to run. To provide a measure of trainability, we rated each animal's treadmill performance on scale of 1–5 according to the following anchors [1, refused to run, 2, below average runner (sporadic, stop and go, wrong direction), 3, average runner, 4, above average runner (consistent runner occasionally fell back on the treadmill), 5, good runner (consistently stayed at the front of the treadmill)] (Dishman et al., 1988; Arida et al., 2007). Animals with a mean rating of 3 or higher were included to the exercise group. The animals which were excluded from the exercise groups did not form the control group. This procedure was used to exclude possible different levels of stress between animals. Subsequently, selected animals were submitted to a physical exercise program during the adolescent period as previously described by Gomes da Silva et al. (2010). In brief, animals in the exercise group were submitted to treadmill exercise from P21 to P60. Each training session started with a 5 min warm-up at 8–10 m/min. Running time and speed gradually increased during the subsequent days, until reaching 18 m/min during 60 min. Animals in the control group were transferred to the experimental room and handled in the same way as animals in the exercise group (privation of water and food during treadmill exercise). At P60, animals were killed (1 h after the last exercise session) and prepared for immunoblot and immunohistochemical techniques.

2.3. Immunoblotting

The immunoblotting was performed to determine the expression of CB1 receptor in the cortex, the striatum and the hippocampal formation. After decapitation, each structure of animals from the exercise group and control group ($n = 4$ from each group) was homogenized in lysis buffer [125 mM Tris–HCl buffer pH 6.8, containing 2% sodium dodecyl sulfate (SDS), 10% glycerol, 1% mercaptoethanol and 1 mM sodium vanadate] and stored at −80 °C. Subsequently, the samples were sonicated and protein concentration was determined by Lowry's Method (Lowry et al., 1951). A standard curve was done to determine the linear range of the method. In this line, 35 μg of proteins of each sample were applied to tricine/SDS/polyacrylamide gel (7.5 × 5.0 cm; 10% separating gel; 2% stacking gel). The gels were blotted in 25 mM Tris, 192 mM glycine, 20% (by vol.) methanol pH 8.3 on 0.2 μm cellulose nitrate sheets (Millipore). The blots were incubated with an antibody against CB1 (polyclonal, C-terminal, 1:500, Cayman) at 4 °C overnight. Peroxidase-conjugated goat anti-mouse IgG (Vector) was used according to the manufacturer's instructions. The immunodetection was performed using a chemiluminescence detection system (Millipore) with exposure to X-ray film (Hyperfilm, GE). The incubation with anti-β-actin immunoglobulins (1:1000, Sigma) was performed as internal control. The molecular weights of proteins CB1 and β-actin (123 and 42 kDa, respectively) were determined by running a prestained protein ladder (Amersham Biosciences). The band densities on immunoblots were measured using the densitometer. All values were reported (mean ± SEM) of relative expression for β-actin. The statistical analyses between exercise and control groups were performed using Student's t-test. Values were considered significant when $p < 0.05$.

2.4. Immunohistochemistry

The immunohistochemistry was performed to analyze the spatial distribution of CB1 receptor in the cortex, the striatum and the hippocampal formation. Animals of both the exercise and control groups ($n = 4$ from each group) were deeply anesthetized (Tionembutal, 50 mg kg, i.p.) and perfused transcardially with 0.01 M phosphate-buffered saline (PBS), followed by 4% formaldehyde in 0.1 M phosphate buffer (PB), pH 7.4. The brains were removed, briefly postfixed in 4% paraformaldehyde in PBS, cut coronally with a vibratome in 50 μm-thick sections. A sequence of three sections per animal (Bregma, −1.6/−3.6 mm; Paxinos and Watson, 1998) was selected for the immunocytochemistry process. The immunoperoxidase procedure was performed on free-floating sections using an antibody against CB1 (polyclonal, C-terminal, 1:500, Cayman). Paired sections of each group were processed in the same vial in order to minimize the differences during the immunohistochemical procedure. The sections were pre-treated with 3% H_2O_2 for 10 min to block endogenous peroxidase activity, rinsed in PBS, preincubated for 45 min in 10% normal serum in PBS with 0.2% Triton X-100, and then incubated in primary antibodies for 48 h at 4 °C. Sections were then rinsed in PBS, incubated in biotinylated anti-rabbit IgG (Vector) at a dilution of 1:200 in PBS (1 h at room temperature), rinsed in PBS, incubated in avidin–biotin peroxidase complex (ABC; Vector) for 1 h, washed several times in PBS, and then incubated in 0.075% diaminobenzidine in 0.002% H_2O_2. Sections were finally washed in PBS, mounted on gelatin-coated slides, dehydrated, coverslipped and analyzed under the microscope (Nikon Eclipse 6600) using bright-field illumination. The images were captured using the VideoCap software.

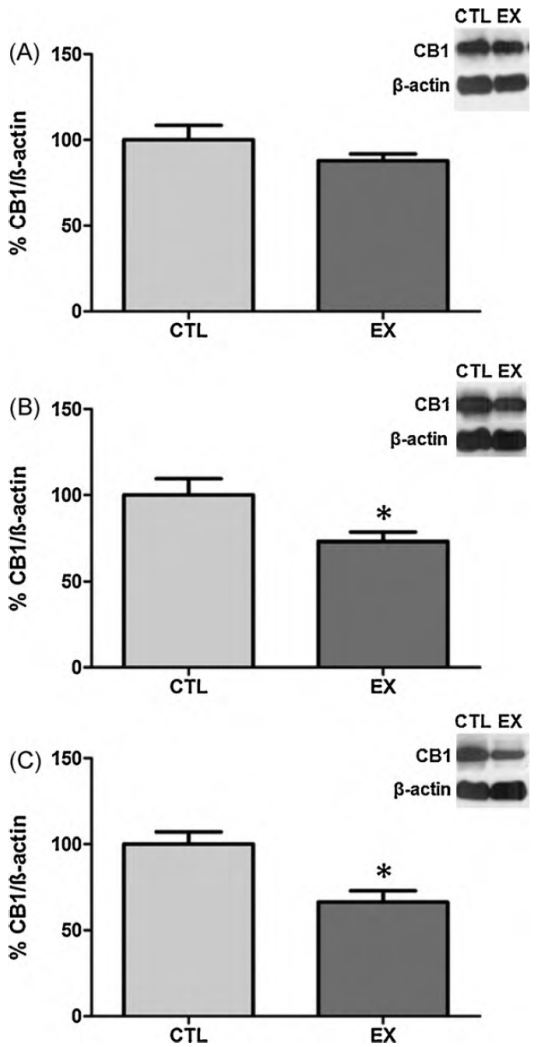

Fig. 1. Immunoblot study of CB1 receptor type 1 (CB1) in the cortex (A), striatum (B) and hippocampal formation (C) of rats from exercise group (EX) and control group (CTL). A significant reduction of CB1 receptor expression was detected in the striatum ($t = 2.448$, df = 6, $p = 0.049$) and hippocampal formation ($t = 4.420$, df = 8, $p = 0.002$) of rats submitted to aerobic treadmill exercise during adolescent period ($^*p < 0.05$).

3. Results

3.1. Body weight

At P60, there was no significant difference in the body weight between the rats from control (204.1 ± 7.02 g) and exercise groups (207.8 ± 6.04 g) ($t = 0.276$, df = 14, $p = 0.786$). This result is in accordance with previous observations showing that regular aerobic exercise in adolescent period does not alter body weight of rats (Uysal et al., 2005).

3.2. Hippocampal CB1 receptor expression

Quantitative immunoblotting analyses of CB1 receptor was performed at 123 kDa band (G-protein-coupled receptor), as previously described (Pettit et al., 1998; Araujo et al., 2010). The results showed a significantly reduced expression of CB1 receptors in the striatum and in the hippocampal formation of rats submitted to the aerobic treadmill exercise (striatum = 73.1 ± 5.4%, $t = 2.448$, df = 6, $p = 0.049$; hippocampal formation = 66.2 ± 6.7%, $t = 4.420$, df = 8, $p = 0.002$) when compared to the control group (striatum = 100 ± 9.5%; hippocampal formation = 100 ± 7.2%). However, no difference in CB1 expression was detected in the cortex between groups (exercise group = 87.8 ± 3.9%; control group = 100 ± 8.4%, $t = 1.303$, df = 6, $p = 0.240$) (Fig. 1).

3.3. Spatial distribution of CB1 receptor

The pattern of antibody immunostaining of the CB1 receptor in this study was similar to those which have been described previously by Egertová and Elphick (2000). In brief, CB1-immunoreactivity (CB1-ir) was observed in fibers surrounding the unstained body cells in cortex (layers II, III and VI of neocortex) and in fibers that encircle unstained compartments of striatum (caudate/putamen). In the hippocampal formation, CB1-ir was mostly located within or in the vicinity of stratum pyramidale of Cornus Ammonis (subfields CA1 – CA4) and in the molecular layer proximal to granule-cell layer of dentate gyrus. Immunohistochemical analysis of the exercise group did not result in visually identifiable qualitative changes in CB1 receptor immunoreactivity in the regions studied (Fig. 2).

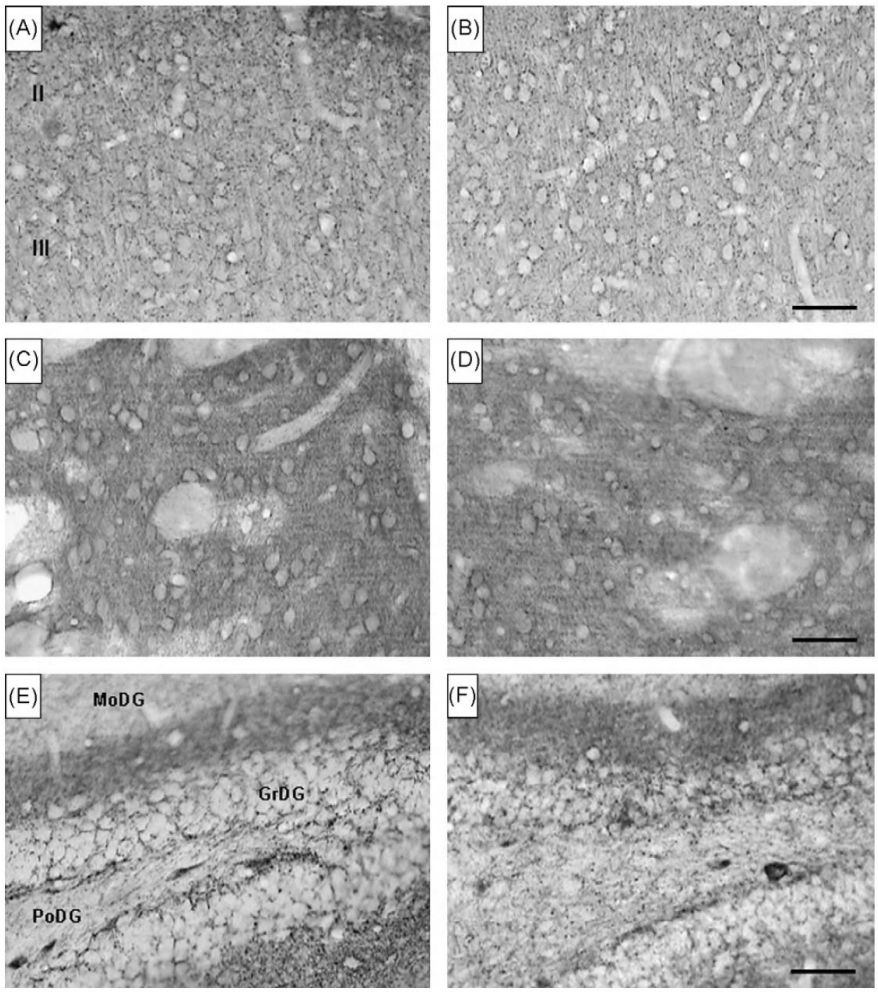

Fig. 2. Photomicrographs of cannabinoid receptor type 1 immunoreactivity (CB1-ir) in the cortex, striatum and hippocampal formation of rats from control group (A, C and E) and exercise group (B, D and F) (scale bar = 50 μm). A and B: layers II and III of neocortex; C and D: putamen; E and F: layers molecular (MoDG), granule (GrDG) and polymorphic (PoDG) of dentate gyrus.

4. Discussion

Our study investigated the effect of a physical exercise program during the adolescent period on the expression of CB1 receptors in the brain. Quantitative immunoblotting analyses showed that the aerobic treadmill exercise performed during adolescence significantly reduced CB1 receptor expression in the striatum and hippocampal formation. These findings are in accordance with previous observations of an influence of regular aerobic exercise on brain plasticity of adolescent rats (Uysal et al., 2005; Arida et al., 2007; Gomes da Silva et al., 2010). In fact, adolescent period is characterized by increased neural plasticity and remarkably sensitive to internal and external stimuli (Andersen, 2003; Chambers et al., 2003; Spear, 2004), such as exposure to cannabinoids (Schneider et al., 2008) and physical exercise (Gomes da Silva et al., 2010). Nevertheless, the mechanisms by which exercise altered endocannabinoid signaling are not clearly elucidated.

Remarkable findings concerning the influence of exercise on the endocannabinoid system have been found. The endogenous ligant anandamide is increased in the circulation following exercise by trained male students (Sparling et al., 2003). Recently, an elegant study conducted by Hill and co-workers (2010) showed that eight days of exercise in voluntary wheel running increases the binding site density of the CB1 receptors in the hippocampal formation of adult rats. In another study, an upregulation of cannabinoid transmission by voluntary exercise was in the striatum of rats after fifteen days of voluntary exercise (De Chiara et al., 2010). A possible explanation for the reduction of CB1 receptor expression in the striatum and hippocampal formation in our study could be attributed to the long-term effects of a physical exercise program, that is, acute stimulus may increase CB1 receptor expression and chronic treatment down-regulate it. Some reports have supported this idea. The long-term effects of repeated administration of an inhibitor of anandamide hydrolysis during adolescence produced a long-lasting significant decrease in CB1 receptor binding in the striatum, nucleus accumbens, ventral tegmental area and hippocampal formation (Marco et al., 2009). Other studies have demonstrated that chronic treatment of rats and mice with a CB1 receptor agonist results in down-regulation of CB1 receptor expression (Breivogel et al., 1999; Sim-Selley and Martin, 2002). Conversely, acute stimuli enhance CB1 receptor expression. Romero et al. (1995) demonstrated that i.p. administration of anandamide daily for 5 days resulted in a significant increase in CB1 receptor density in the hippocampus. Similar findings in studies using physical exercise strengthen this hypothesis. As mentioned above, increased binding site density of the CB1 receptor in the hippocampal formation was observed after short periods of exercise (Hill et al., 2010). Comparable findings with opioid receptors have been observed recently in our laboratory (unpublished data). Seven days of voluntary (wheel) and forced (treadmill) running increased Mu and kappa receptor expression which returned to normal values in animals submitted to 45 days of physical exercise.

Some studies have investigated the maturational processes of the endocannabinoid system during postnatal development (Rodríguez de Fonseca et al., 1993; McLaughlin et al., 1994; Belue et al., 1995). The maximum values of brain CB1 receptor expression have been observed around P30 and P40. Afterwards, CB1 receptor expression seems to decrease during the adolescent period until it reaches adult values (measured on P70) (Rodríguez de Fonseca et al., 1993). These findings coincided with the mechanism of synaptic and axonal pruning observed during infancy and adolescence (Andersen, 2003; Tau and Peterson, 2010), the basic steps of neuromaturation. In this regard, it is possible that the reduction in CB1 receptor following exercise during the adolescent period could also be attributed to an early maturation of the brain endocannabinoid system.

In conclusion, we might suggest that the activation of the endocannabinoid system following repetitive bouts of exercise (40 sessions) might have triggered an over-activation of CB1 receptor expression that led to a down-regulation of this receptor in the striatum and hippocampal formation. Considering previous results showing that the synaptic adaptations of CB1 receptor caused by both exercise and sucrose exposure were reversible after interruption of the treatments (De Chiara et al., 2010), it must be determined in a future study whether the receptor expression also returns to normal after discontinuation of the exercise treatment. Our results suggest an important link between the endocannabinoid system and physical training in adolescence. Taken in account the actions of the endocannabinoid system on learning and memory (Davies et al., 2002), neurotransmitters release (Balázsa et al., 2008; Sidló et al., 2008; Sperlágh et al., 2009), cell proliferation (Aguado et al., 2005), interaction with several neurotrophic systems (Williams et al., 2003; Khaspekov et al., 2004; Aso et al., 2008), and the rewarding properties of running (Keeney et al., 2008), future studies can explore the extent to which exercise interferes with the endocannabinoid system during the adolescent period.

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3.3. Artigo 03

Hippocampus

Hippocampus

Early exercise promotes positive hippocampal plasticity and improves spatial memory in the adult life of rats

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Keywords:	exercise, brain, development, plasticity, hippocampus

EARLY EXERCISE PROMOTES POSITIVE HIPPOCAMPAL PLASTICITY AND IMPROVES SPATIAL MEMORY IN THE ADULT LIFE OF RATS

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ABSTRACT

There is a great deal of evidence showing the capacity of physical exercise to enhance brain health and plasticity. Although the effects of exercise are well documented in the mature brain, the influence of exercise in the developing brain has been poorly explored. Therefore we investigated the morphological and functional hippocampal changes in adult rats submitted to daily treadmill exercise during the adolescent period. Male Wistar rats aged 21 postnatal days old (P21) were divided into two groups: exercise and control. Animals in the exercise group were submitted to daily exercise on the treadmill between P21 and P60. Running time and speed gradually increased over this period, reaching a maximum of 18 m/min for 60 min. After the aerobic exercise program, histological and behavioral analyses were performed. The results show that early-life exercise increased mossy fibers density and hippocampal expression of brain-derived neurotrophic factor and its receptor tropomyosin-related kinase B, improved spatial learning and memory, and enhanced capacity to evoke spatial memories in later stages. It is important to point out that while physical exercise induces hippocampal plasticity, degenerative effects could appear in undue conditions of physical or psychological stress. In this regard, we also showed that the exercise protocol used here did not induce inflammatory response and degenerating neurons in the hippocampal formation of developing rats. Our findings demonstrate that exercise results in positive changes for the brain during the maturational process.

Keywords: exercise; brain; development; plasticity; hippocampus; memory.

1. INTRODUCTION

Evidence indicates that physical exercise can modify structure and hence function of certain brain regions (Cotman and Berchtold, 2002; Vaynman and Gomez-Pinilla, 2005). The ability of brain to change its structure and function in response to physical stimulus is a process known as plasticity. Brain plasticity is characterized by the occurrence of reversible and long-term changes. In general, these changes include anatomical, neurochemical and metabolic manifestations which may trigger positive or negative alterations in the brain regions where they were established (Trojan and Pokorný, 1999). Clinical and animal studies have frequently described the influence of physical exercise on the adult brain plasticity (Cotman and Berchtold, 2002; Vaynman and Gomez-Pinilla, 2005). Exercise improves cognitive functions (Kramer et al., 2006; Kashihara et al., 2009), reduces anxiety and depression (Martinsen, 2008), and protects the brain against neurodegenerative disorders (Goodwin et al., 2008, Rolland et al., 2008; Honea et al., 2009). In addition, studies aimed to understand the neurobiological bases of these benefits have demonstrated that exercise modifies neuronal activity (Vanderwolf, 1969; Czurko et al., 1999; Van Praag et al., 1999), enhances neurotrophic factor expression (Neeper et al., 1995; Gomez-Pinilla et al., 1997), formation of synapses (Dietrich et al., 2008), growth of blood vessels (Van der Borght et al., 2009), and cell proliferation in hippocampal formation (Van Praag et al., 1999), a brain region linked to learning, memory and emotional processes (Sanders et al., 2003; Kesner et al., 2004) and highly susceptible to damage in neurodegenerative disease (Harry and D'hellencourt, 2003). Although the effects of physical exercise in the mature brain are well documented, its influence in the developing brain has been little explored.

Brain development is a complex process and stimuli during this period could determine brain's functional integrity in adulthood. Events that occur during early postnatal development may modulate the functional maturation of the brain and result in the development of a more complex neural circuitry (Linkenhoker et al., 2005). In humans, many reports have shown that exercise induces significant cognitive improvement throughout brain development (Sibley and Etnier, 2003; Hillman et al., 2005). A meta-analysis conducted on 16 studies found a positive relationship between physical activity and learning and intelligence scores in school-age children (Sibley and Etnier, 2003). In addition, it has been observed that aerobic exercise in childhood might increase the resilience of the brain in later life. Indeed, a positive correlation between physical activity in the age range 15–25 years and information processing speed in older men (62–85 years) has been described (Dik et al., 2003).

Since brain plasticity decreases with age (Akopian and Walsh, 2006; Lynch et al., 2006), it is very important to assess how exercise may regulate plasticity during early life, and to determine the basic mechanism of such effects. The aim of the present study was to examine the influence of an aerobic exercise program on postnatal brain development. For this purpose, we evaluated the hippocampal plasticity of adult rats submitted to daily treadmill exercise during the adolescent period.

2. MATERIAL AND METHODS

2.1. Exercise paradigm

Male Wistar rats aged 21 postnatal days old (P21) were used in this study. The colony room was maintained at 21 ± 2°C with a 12 h light/dark schedule, and *ad libitum* food and water throughout the experiments. Rats were bred in our laboratory and the date of birth was considered day 0. The pups were housed with their mother in individual cages until weaning at day 21. The rats were then divided into two groups: exercise (n=27) and control (n=27) groups. Animals in the exercise group were familiarized with the apparatus for three days by placing them on a treadmill (Columbus instruments) for 5 min/day at speed of 8 m/min at 0% degree incline. Electric shocks were used sparingly to motivate the rats to run. To provide a measure of trainability, we rated each animal's treadmill performance on scale of 1–5 according to the following anchors [1, refused to run, 2, below average runner (sporadic, stop and go, wrong direction), 3, average runner, 4, above average runner (consistent runner occasionally fell back on the treadmill), 5, good runner (consistently stayed at the front of the treadmill)] (Dishman et al., 1988; Arida et al., 2007). Animals with a mean rating of 3 or higher were included in the exercise group. If any animal was excluded from the exercise group it would not form the control group. This procedure was used to exclude possible differences in stress levels between animals. Subsequently, selected animals were submitted to a physical exercise program during the adolescent period as previously described by Gomes da Silva et al. (2010). In brief, animals in the exercise group were submitted to treadmill exercise from P21 to P60. Each training session started with a 5 min warm-up

at 8-10 m/min. Running time and speed were gradually increased, reaching a maximum 18 m/min for 60 min. Animals in the control group were transferred to the experimental room and handled in the same way as animals in the exercise group (privation of water and food during treadmill exercise). All experimental protocols were approved by the ethics committee of the Universidade Federal de São Paulo (UNIFESP) and all efforts were made to minimize animal suffering in accordance with the proposals of International Ethical Guideline for Biomedical Research (CIOMS, 1985).

2.2. Histological methods

2.2.1. Tissue preparation

At P60, fourteen animals from both the exercise and control groups (seven from each group) were deeply anesthetized (Tionembutal, 50 mg-kg, i.p.) and perfused transcardially with solution of 0.01 M phosphate-buffered saline (PBS), followed by solution containing 4% formaldehyde in 0.1 M phosphate-buffered (PB), pH 7.4. Animals from the exercise group were killed 1 h after the last exercise session. After perfusion, the brains were removed immediately from the skull and postfixed in 4% paraformaldehyde in PB for 24 hours. The brains were then cut coronally with a vibratome (Leica) in 50 µm-thick slices and stored at -20°C in the biological tissue bank in our laboratory (for preservation of tissue). To inhibit the formation of ice crystals that damage the structure of cells, the slices were maintened in an antifreeze solution containing 30% of sucrose, 1% of polyvinylpyrrolidone 40 (PVP-40) and 30% of ethylene glycol in PB (pH 7.2).

2.2.2. Immunofluorescence

Hippocampal slices (bregma –2.8/–3.3mm; Paxinos and Watson, 1996) previously stored in the tissue bank were selected in order to observe whether the protocol of physical exercise during development promotes inflammatory response in the hippocampal formation of rats. For this, the slices were rinsed in PBS and pre-incubated for 20 min in PBS solution containing 0.01% of saponin and 1% of bovine albumin. After this procedure, the slices were incubated for 48 h with the respective primary antibodies [interleukin 6 (IL6; 1:100; IBL), interleukin 10 (IL10; 1:100; R&D), and tumor necrosis factor alpha (TNFα; 1:100; IBL)] previously diluted in solution of 1% albumin and 0.01% of saponin in PBS. For each primary antibody, we used two slices of each animal from both groups (exercise and control). To validate the test, we also incubated in primary antibodies hippocampal slices from an animal with 540 days of life (18 months). These slices were previously stored in the tissue bank (from another project) and were used in this study as positive controls. Subsequently, all slices were rinsed in PBS containing 0.01% of saponin and 1% of albumin and incubated for 30 min with secondary antibodies (1:200) conjugated to AlexaFluor® 488 or 564 diluted in PBS. Finally, the slices were washed in PBS, mounted on slides and coverslipped with Vectashield (Merk). Following this, regions of Ammon's horn (subregions: CA1 and CA3) and dentate gyrus of the hippocampal formation from studied groups were analyzed in a confocal laser scanning system from BioRad 1024UV attached to a Zeiss Axiovert 100 microscope using a 40x 1.2 NA PlanApochromatic water immersion lens.

2.2.3. Fluoro-Jade B

A sequence of three hippocampal slices per animal (bregma –2.8/–3.3mm; Paxinos and Watson, 1996) previously stored in the tissue bank was selected to observe the immunohistochemical staining of Fluoro-Jade B (FJB), a derivative of fluorescein anionic tribasic that selectively labels degenerating neurons (Schmued and Hopkins, 2000). For this, slices from exercise and control groups were mounted on gelatin-coated slides and incubated in a sequence of solutions containing 1% of sodium hydroxide in 80% of ethanol for 5 min, 70% of ethanol for 2 min, distilled water for 2 min and 0.06% of potassium permanganate for 10 min. After these procedures, the slides were rinsed in distilled water and transferred to a stock solution of 0.01% FJB (Chemicon) in 0.01% acetic acid for 20 min. As a positive control, we added to the immunohistochemical procedure a slice of the hippocampal region (from another project) of an animal injected with 350 mg/kg of pilocarpine (a potent cholinergic agonist that induces *status epilepticus* and leads to severe widespread cell loss in several brain areas). Then, hippocampal slices were rinsed in distilled water, mounted on slides, coverslipped and analyzed by confocal microscopy as described above.

2.2.4. Immunohistochemistry

Hippocampal slices from exercise and control groups (bregma –2.8/–3.6mm; Paxinos and Watson, 1996) previously stored in the tissue bank were selected in order to analyze the density of neuronal cells in the hippocampal formation. Three slices per animal were pre-treated with 3% of H_2O_2 for 10 min to block endogenous peroxidase activity, rinsed in PBS, preincubated for 45 min in PBS containing 10% of normal serum and 0.2% of Triton X-100, and then incubated in primary antibody against the neuron-

specific nuclear protein (NeuN; 1:1000; Chemicon) at 4℃ overnight. Paired slices of each group were processed in the same vial in order to minimize the differences during the immunohistochemical procedure. Slices were then rinsed in PBS, incubated in biotinylated anti-rabbit IgG (1:200; Vector) in PBS for 2 h at room temperature, rinsed in PBS, incubated in avidin-biotin peroxidase complex (ABC; Vector) for 1 h, washed several times in PBS, and then revealed in a solution containing 0.075% of diaminobenzidine and 0.002% of H_2O_2. After this sequence of procedures, the slices were finally washed in PBS, mounted on gelatin-coated slides, dehydrated, coverslipped with Entellan (Merk). Subsequently, regions of Ammon's horn (subregions: CA1 and CA3) and dentate gyrus of the hippocampal formation of each animal were digitized with a bright-field microscope (Nikon Eclipse 6600) by quantitative analysis.

2.2.5. Neo-Timm

Neo-Timm is a modification of the traditional Timm's method introduced to improve the specific staining of zinc and thereby produce a more distinct visualization of the mossy fibers (axons of granule cells) (Babb et al., 1991). Animals from the exercise and control groups (five from each group) were deeply anesthetized (Pentobarbital, 75 mg-kg, i.p.) and perfused transcardially with Millonigs's buffer solution (MB) containing 16% of sodium phosphate monobasic, 0.02% of calcium chloride and 4% of sodium hydroxide, followed by solution of 0.1% sodium sulphide in MB, solution of 3% glutaraldehyde in PB, and solution of 0.1% sodium sulphide in MB, pH 7.4. After perfusion, the brains were carefully removed from the skull and postfixed for 24 h in solution containing 3% of glutaraldehyde in PB. The brains were then cut coronally with a vibratome (Leica) in 50 µm-thick slices and mounted on gelatin-coated slides for

revelation in the darkroom. Slides were revealed in solution containing 50% of Arabic gum, 11.7% of citrate buffer, 1% of hydroquinone and 0.15% of silver nitrate at room temperature for 30 min. Hippocampal slices were then dehydrated, coverslipped with Canada balsam, and imaged with a brightfield microscope (Nikon Eclipse 6600) for quantitative analysis.

2.2.6. Quantitative analysis

To analyze the NeuN and Neo-Timm staining, the hippocampal formation of each animal was scanned with a video camera (Sony) connected to a brightfield microscope (Nikon Eclipse E600) (Supplementary material 1A and D). The images were then processed in RGB format with three color frequency bands (red, green and blue), each ranging from 0 (highest luminosity) to 255 (lowest luminosity). Subsequently, the images were compressed to grayscale (Supplementary material 1B and E) to obtain the corresponding histograms (frequency band mean). Afterwards, in order to observe the transition between the background and the staining, the derived of histogram vector values (Supplementary material 2A) was obtained by equation:

$$hist(x)' = hist(x+1) - hist(x)\ ;[0;255)$$

A trend line using the histogram derived values was performed to identify the start of the luminous intensity zone of neuronal cells or mossy fibers (Supplementary material 2B arrow). The significant pixels were then converted into binary matrix (black and white) (Supplementary material 1C and F) and quantified by the black pixels sum per area (i.e.

density of neurons and mossy fibers). The quantification of pixels was carried out by software that allows matrix manipulations (Matlab) and in images with the same resolution (Supplementary material 3). The data were plotted in percentage of neurons and mossy fibers (CTL group = 100%).

2.3. Methods of protein immunodetection

2.3.1. Tissue preparation

At P60, the hippocampal formation of animals from the exercise and control groups (five of each group) was removed immediately after decapitation and homogenized in 0.01M Tris hydrochloride (pH 7.6) containing 5.8% of sodium chloride, 10 % of glycerol, 1% of Nonidet P40 (NP-40), 0.4% of ethylenediamine tetraacetic acid (EDTA) and protease inhibitors. Animals from the exercise group were killed 1 h after the last exercise session. Samples were sonicated, protein concentration was determined by Lowry's Method (Lowry *et al.*, 1951) and samples were stored at -80°C.

2.3.2. Enzyme-Linked Immunosorbent Assay (ELISA)

Brain-derived neurotrophic factor (BDNF) is a neurotrophin that has been shown to mediate the positive effects of exercise on synaptic plasticity and cognitive function (Vaynman et al., 2004). Hippocampal BDNF expression assays were performed using the ELISA kit E-max® (Promega) according to manufacturer's recommendations. Samples from exercise and control groups previously stored at -80°C were centrifuged for 5 min at 14000 rpm at 4°C and the supernatant transferred to a 96-well plate (Corning Costar) coated with anti-BDNF (1:1000) then incubated for 2 h at room

temperature. After this period, the plate was washed with Tris-buffered saline Tween-20 (TBS-T) and incubated with the following antibodies: anti-human (1:500) for 2 h, and conjugate anti-IgY HRP (1:200) for 1 h. After these procedures, color reaction with tetramethyl benzidine was quantified in a plate reader at 450 nm (Quick Elisa). Values were reported as relative expression of total hippocampal protein.

2.3.3. Immunoblotting

The immunoblotting was performed to determine the expression of BDNF receptors, tropomyosin-related kinase B (TrkB) and p75 neurotrophin (p75ntr), in the hippocampal formation of animals from the exercise and control groups. A standard curve was done to determine the linear range of the method. In this line, forty µg of proteins of samples previously stored at -80℃ were applied to Tricine/SDS/Polyacrylamide gel (7.5 × 5.0 cm; 10% separating gel; 2% stacking gel). The gels were blotted in 25 mM Tris, 192 mM glycine, 20% (by vol.) methanol pH 8.3 onto 0.2 µm cellulose nitrate sheets (Millipore). The blots were incubated with the respective primary antibodies [TrkB (1:300; Santa Cruz Biotechnology) and p75ntr (1:300; Santa Cruz Biotechnology)] at 4℃ overnight. Peroxidase-conjugated goat anti-mouse IgG (Vector) was used according to the manufacturer's instructions. The immunodetection was performed using chemiluminescence detection system (Millipore) with exposure to X-ray films (Hyperfilm, GE). The incubation with anti-βIII-tubulin immunoglobulins (1:12000, Abcam) was performed as internal control. The molecular weights of proteins TrkB, p75ntr and βIII-tubulin (145 kDa, 75 kDa and 50 kDa, respectively) were determined by running a prestained protein ladder (Amersham

Biosciences). The band densities on immunoblots were measured by densitometry. All values were reported as relative to the expression of βIII-tubulin.

2.4. Behavioral analysis

To determine the learning and memory of animals from the exercise and control groups (ten from each group) the water maze test similar to that described by Morris (1984) was used. The water maze consisted of a black circular pool (200 cm in diameter) conceptually divided in four equal imaginary quadrants (quadrants 1–4). The water temperature was maintained between 21–25ºC. A black circular platform (12 cm in diameter) was placed 1.5 cm under the water surface in the center of quadrant 3. On the walls of the experimental room were fixed objects (frames, pictures) to be used as reference points. Animals were placed in the water maze for 120 s to find the platform. Over five days (P61–P65), animals performed two trials per day (with a 10 min interval between trials). In each trial, animals began the test at different points (labeled N, S, E and W). A video-camera (Sony) fixed above the water maze recorded all the experiments. The time to reach the platform (latency), the swim path length and the speed of each animal in the maze were analyzed by Ethovision program (Noldus).

A day after the end of the last session in the water maze (P66), the platform was removed for a 120 s probe trial. The time spent in each of four imaginary quadrants was recorded. The analysis of time spent in quadrant 3 was performed to ensure that the animal used the reference points of the experimental room to find the submerged platform (D'Hooge and De Deyn, 2001; Stafstrom, 2002). Thirty days after this test (P96), animals from the exercise and control groups were submitted to a "retest". In the

retest, the platform was re-placed in the center of quadrant 3. From the starting point N, animals performed a single trial (120 s) to find it. The retest was used to assess the long-term memory of the animals studied.

2.5. Statistical analysis

Statistical analysis was conducted by Student's t-test or analysis of variance for repeated measures (ANOVA). Values were considered significant when $p<0.05$. Data are presented as mean and standard error of the mean (±SEM).

3. RESULTS

3.1. Inflammatory response and degeneration of hippocampal neurons

In order to assess whether the physical exercise protocol during the adolescent period of rats could promote negative effects in the hippocampal formation, we used inflammatory and degenerating neuron markers. The pro- (IL6 and TNFα) and anti-inflammatory (IL10) response in positive control was observed in regions of Ammon's horn and in the hilus of the dentate gyrus (Figures 1). The FJB histochemical staining in positive control was observed in neurons located in pyramidal cell layer of CA1 and CA3 and in the hilus of the dentate gyrus (Figure 1). In control and exercise groups, no inflammatory response and degenerating neurons were found in the studied hippocampal regions (Figure 1).

3.2 Density of neurons and mossy fibers in the hippocampal formation

The NeuN and Neo-Timm markers were used to analyze the density of neurons and mossy fibers in the hippocampal formation of developing rats submitted to physical exercise. The NeuN immunoreactivity was observed within or in the vicinity of the pyramidal cell layer of CA1 and CA3 and in the granule-cell layer of the dentate gyrus (Figure 2). No significant differences in neuronal density were detected between the exercise (CA1 = 108 ± 8.7%; CA3 = 104.6 ± 5.1%; dentate gyrus = 103.2 ± 5.2%) and control groups (CA1 = 100 ± 7.1%; CA3 = 100 ± 4.7%; dentate gyrus = 100 ± 5.9%; $p>0.05$) (Figure 2). The Neo-Timm staining was observed throughout the stratum lucidum of CA3 and hilus of the dentate gyrus of both groups (Figure 3). Quantitative analyses revealed that the density of mossy fibers by Neo-Timm staining was significantly higher in the hippocampal formation of the exercise group (119.6 ± 5.4%, $p<0.01$) when compared to the control group (100 ± 3.2%) (Figure 3).

3.3. Hippocampal BDNF expression and its receptors (TrkB and p75ntr)

BDNF has been shown to mediate positive effects of exercise on synaptic plasticity and cognitive function (Vaynman et al., 2004). Therefore, we investigated BDNF expression and its receptors in the hippocampal formation of rats submitted to physical exercise during development. A significant increase in hippocampal BDNF expression (129.3 ± 8.1%, $p<0.01$) and TrkB (160.8 ± 21.5%, $p<0.05$) was noted in the exercise group when compared to the control group (BDNF= 100 ± 1.5%; TrkB= 100 ±

12.1%) (Figure 4 and 5A). No significant difference in p75ntr expression was detected between the studied groups (exercise= 121.2 ± 44.5% versus control= 100 ± 26.7%, $p>0.05$) (Figure 5B).

3.4. Learning and memory

Learning and memory of animals from the exercise and control groups were analyzed in the water maze for five days. ANOVA showed that the latency (day 1= 79.7 ± 4.9s; day 2= 44.2 ± 6.2s; day 3= 40.2 ± 9.3s; day 4= 22 ± 4.1s; day 5= 29.9 ± 6.9s) and the swim path length (day 1= 1827.6 ± 110.6cm; day 2= 1022.9 ± 125.9cm; day 3= 851.7 ± 190.3cm; day 4= 531.4 ± 109.1cm; day 5= 725.2 ± 177.2cm) in the exercise group were significantly lower than in the control group [latency (day 1= 115 ± 4.9s; day 2= 83.6 ± 9.3s; day 3= 48.8 ± 9.8s; day 4= 33.2 ± 7.8s; day 5= 26.2 ± 6.6s) and swim path length (day 1= 2428.7 ± 197.9cm; day 2= 1803.4 ± 243.9cm; day 3= 1009.5 ± 178.5cm; day 4= 689.5 ± 181.1cm; day 5= 593.6 ± 141.6cm)] ($p<0.05$) (Figure 6A and B). These results demonstrate that developing rats submitted to exercise presented a better performance in the water maze when compared to the control rats.

To verify whether the results described above were influenced by the physical conditioning of animals trained during the development, the speed of swimming in the water maze was examined. ANOVA showed no significant difference in mean speed of animals during five days of water maze [exercise group (day 1= 27 ± 0.6cm/s; day 2= 29.7 ± 1.5cm/s; day 3= 26.8 ± 1.3cm/s; day 4= 25 ± 1.3cm/s; day 5= 27.2 ± 1.2cm/s)

versus control group (day 1= 26.7 ± 0.6 cm/s; day 2= 31.3 ± 1.1cm/s; day 3= 25.5 ± 1.1cm/s; day 4= 22.7 ± 1cm/s; day 5= 26.7 ± 1.3cm/s)] ($p>0.05$) (Figure 6C).

At P66, the platform was removed from the water maze to evaluate the time spent in each of four imaginary quadrants. The results showed that both exercise (quadrant 1= 26.2 ± 2.2s; quadrant 2= 17.6 ± 1.2s; quadrant 3= 54.3 ± 2.9s; quadrant 4= 21 ± 3.5s) and control groups (quadrant 1= 29.7 ± 1.4s; quadrant 2= 17.7 ± 2.1s; quadrant 3= 51.8 ± 4s; quadrant 4= 20.3 ± 1.7s) presented a preference for the quadrant where the platform was previously located ($p<0.05$) (Figure 7). The preference for the platform quadrant indicates that animals: (a) used the reference points of the experimental room to find the submerged platform; (b) retained environmental information (spatial memory). This information was important to ensure that the next outcomes would not be influenced by previous problems of learning.

At P96, the platform was re-placed in the water maze to analyze the long-term memory of the animals studied. The results showed that the latency in finding the platform was significantly lower in the exercise group (19.8 ± 4.3s) than in the control group (52.7 ± 9.9s) ($p<0.05$) (Figure 8). This finding suggests that the animals submitted to physical exercise during development present a greater capacity to evoke long-term spatial memories than the control animals.

4. DISCUSSION

The present study demonstrated that an aerobic exercise program undertaken during postnatal brain development increased density of mossy fibers and hippocampal

expression of BDNF and its receptor TrkB, improved spatial learning and memory, and enhanced the capacity to evoke spatial memories in later life stages. It is important to point out that while physical exercise induces hippocampal plasticity, degenerative effects could appear in undue conditions of physical or psychological stress. Forced treadmill running is a type of training that could chronically activate different levels of stress response. Moreover, it has been shown that treadmill exercise can alter expression in the brain of inflammatory cytokines (Colbert et al., 2001; Carmichael et al., 2005, Chennaoui et al., 2008; Carmichael et al., 2010), low molecular weight proteins known to affect the integrity of the blood-brain barrier and induce cell death during development (Hagberg and Mallard, 2005; Deverman and Patterson, 2009). In this regard, we showed that the exercise protocol used in this study did not induce inflammatory response or degenerating neurons in the hippocampal formation of developing rats.

Human and animal studies have demonstrated that exercise in infancy and adolescence can enhance brain health and plasticity (Sibley and Etnier, 2003; Hillman et al., 2005; Uysal et al., 2005; Gomes da Silva et al., 2010; Silva et al., 2010). It is important to note that neurogenesis induced by early-life exercise could have a significant impact on brain structure and functional development. New cell formation in the hippocampal formation is most prevalent in young rats, and an increased cell proliferation in the dentate gyrus has been observed in 4-week-old rats trained for five days compared to animals submitted to exercise at 8 and 62 weeks old (Kim et al., 2004). Thus, exercise-induced hippocampal neurogenesis has been suggested to enhance learning and memory capability (Snyder et al., 2005). In an elegant study, Uysal and collaborators (2005) reported that rats trained during development presented

a significant increase in density of hippocampal cells density using Nissl staining as well as a better spatial memory in the Morris water maze test in adulthood. In our study, treadmill exercise in developing rats improved spatial memory in the Morris water maze but no difference was detected in density of hippocampal neurons. A possible explanation for these results could be related to staining technique performed. We used a specific immunohistochemical procedure for neuronal cells (NeuN staining) while Uysal and collaborators (2005) performed a technique that stains neuronal and glial cells (Nissl staining). The exercise protocol could also influence to these divergent findings. In the study by Uysal and collaborators (2005) animals ran over a period of 8 weeks at speed of 8 m/min, 30 min daily, 5 days per week. In our exercise protocol, animals ran over a period of 6 weeks at a greater intensity (up to 18 m/min over 60 min, 7 days per week). Although low (Uysal et al., 2005) and progressive intensity (our study) of physical exercise during postnatal development have been shown to induce positive changes in spatial memory of rats examined in adult life, changes in neuronal density is a subject that deserves more attention.

In our study the density of mossy fibers was significantly higher in the hippocampal formation of rats trained during development than in untrained rats. Studies using Timm staining in rodents have proposed an interesting correlation between mossy fiber density and performance in hippocampal-dependent tasks. It has been shown that neonatal maternal separation reduced hippocampal mossy fiber density and impaired spatial memory acquisition in the water maze test in the adult life of rats (Huot et al., 2002). On the other hand, mice and rats with larger mossy fiber projections committed fewer reentry errors during radial maze learning (Jamot et al., 1994; Schwegler and Crusio, 1995), showed better relearning after dislocation of the target platform in the

swimming navigation task (Schöpke et al., 1991; Bernasconi-Guastalla et al., 1994), and exhibited better-controlled search behavior in the water maze (Schwegler et al., 1988; Pleskacheva et al., 2000). In view of these observations, we could speculate that increased hippocampal mossy fiber density in our study might contribute, at least in part, to better cognitive performance observed in rats trained during development.

A variety of potential mechanisms could cause exercise-induced increases mossy fibers. Tong and co-workers (2001) examined the hippocampal expression of approximately 5000 genes in rats submitted to 3 weeks of physical exercise and reported changes in a large number of gene transcripts, many of which are known to be associated with neuronal activity, synaptic structure, and neuronal plasticity. Growth-related genes like neurotrophins have been considered the most likely candidates in mediating the effects of exercise on plasticity (Cotman and Berchtold, 2002; Vaynman and Gomez-Pinilla, 2005). Previous studies have demonstrated that a few days of exercise result in a significant upregulation of several neurotrophins including nerve growth factor (NGF) (Neeper et al., 1996), fibroblast growth factor 2 (FGF-2) (Gomez-Pinilla et al., 1997) and BDNF (Neeper et al., 1995, 1996; Russo-Neustadt et al., 1999). In particular, exercise-induced upregulation of BDNF appears to be more robust and long lasting compared to the other neurotrophins. Furthermore, BDNF has been shown to play an important role in mossy fiber outgrowth (Rabacchi et al., 1999). For instance, application of BDNF to cultured rat dentate granule cell explants resulted in marked increases in axon number and extension (Lowenstein and Arsenault, 1996). In addition, BDNF knockout in mice significantly reduced hippocampal mossy fiber sprouting induced by chronic electroconvulsive (Vaidya et al., 1999), suggesting that BDNF

signaling may be necessary for structural plasticity of axons and mossy fibers in particular.

As mentioned above, increased BDNF expression in the hippocampal formation has been observed after short periods of exercise (Neeper et al., 1995, 1996; Russo-Neustadt et al., 1999). Here we showed that an aerobic exercise program during postnatal brain development significantly increased hippocampal expression of BDNF and its receptor TrkB. The importance of exercise-induced increases in BDNF and TrkB expression indicate its potential role in modulation of synaptic plasticity and cognitive function (Vaynman et al., 2004). For instance, in Morris water maze tests, rats submitted to 1 week of voluntary exercise are significantly better at locating the hidden platform than control rats. Significantly, this benefit was eliminated when BDNF-TrkB signaling was inhibited. In their investigation, animals with free access to running wheels but injected with a TrkB-IgG chimera to block the action of BDNF through the TrkB receptor showed no improvement in cognitive performance over control animals (Vaynman et al., 2004). Furthermore, the TrkB-IgG chimera eliminated exercise-driven increases in cAMP response-element-binding protein (CREB) (Vaynman et al., 2004), a molecule that plays a critical role in the formation of long-term memory (Abel and Kandel, 1998).

In our study, no significant difference in hippocampal p75ntr expression was detected in rats trained during development when compared to untrained rats. Although p75ntr expression decreases dramatically by adulthood, it is widely expressed during the developmental stages (Chao, 2003). Interestingly, the activation of the p75ntr receptor facilitates apoptosis during development and after injury in the central nervous system (Chen et al., 2009). However, the activation of p75ntr by BDNF induces cell

death only in the absence of TrkB signaling or when this is decreased (Davey and Davies, 1998; Friedman, 2000).

Previous investigations have shown that experience and learning can modulate the functional maturation of the brain by neuroplastic processes. These stimuli occurring during early postnatal brain development may result in the development of more complex neural circuitry (Linkenhoker et al., 2005). In the present study, we demonstrated that an aerobic exercise program during the adolescent period promotes hippocampal plasticity and improves spatial memory in the adult life of rats. Another important finding in our investigation was that early-life exercise enhanced ability to evoke the spatial memories in later life (when measured at P96), supporting previous findings in humans which show a correlation between physical activity in childhood and cognitive benefits throughout life (Dik et al., 2003). Based on these observations, we can conclude that physical exercise during postnatal development results in positive changes for the brain during the maturational process. This information is relevant for the development of public policies aimed at stimulating physical exercise programs for people, particularly children and teenagers. Moreover, these findings can also have a great therapeutic value for some neurological disorders that emerge during infancy and adolescence (Arida et al., 2010; Gorczynski and Faulkner, 2010).

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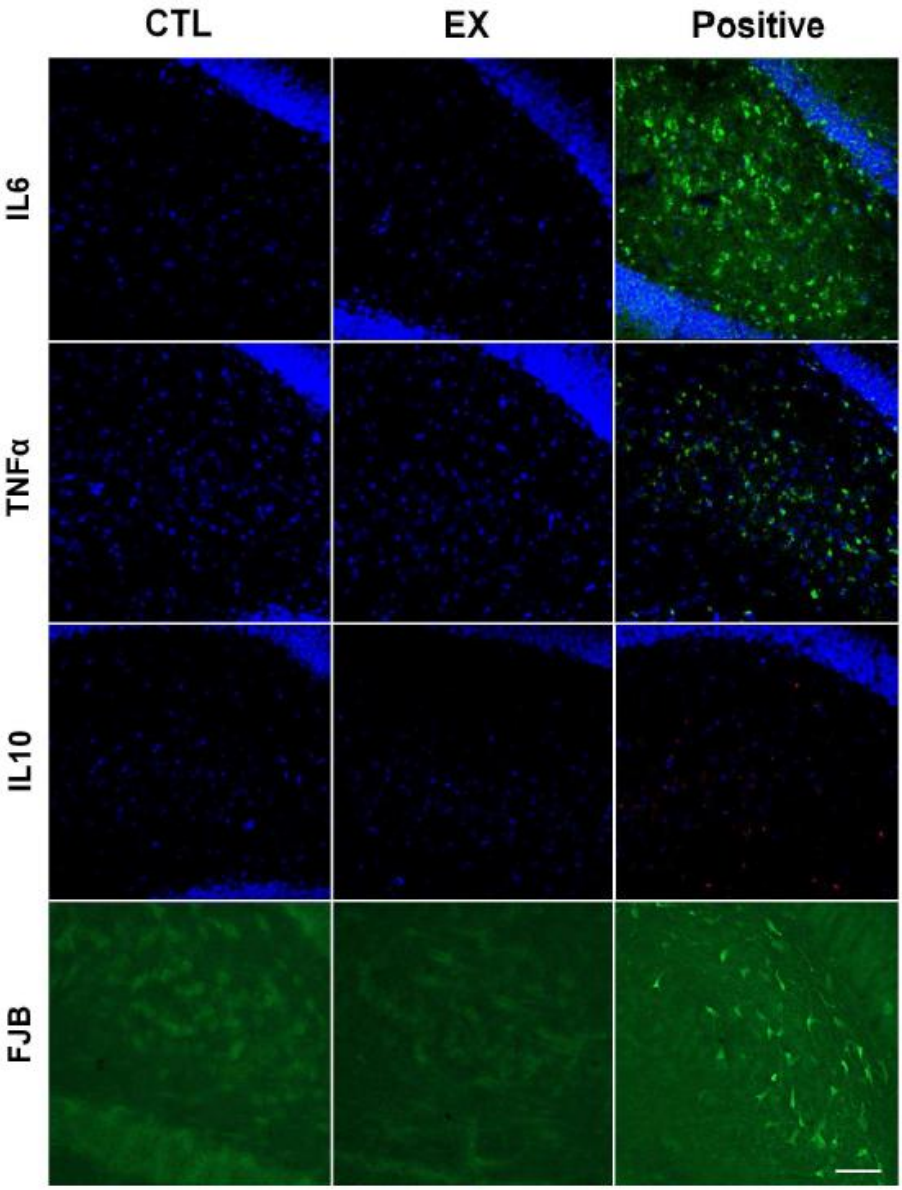


Figure 1. Inflammatory response and degenerating neurons in the dentate gyrus of rats. IL6 (green), TNFα (green) and IL10 (red) immunoreactivity in the hilus of the dentate gyrus (marked with 4'6-diamidino-2-phenylindole; blue) and FJB staining in hilus neurons (fluorescent green) were observed in the positive control group. In control (CTL) and exercise (EX) groups, no inflammatory response or degenerating neurons were detected. Scale bar = 100 µm.

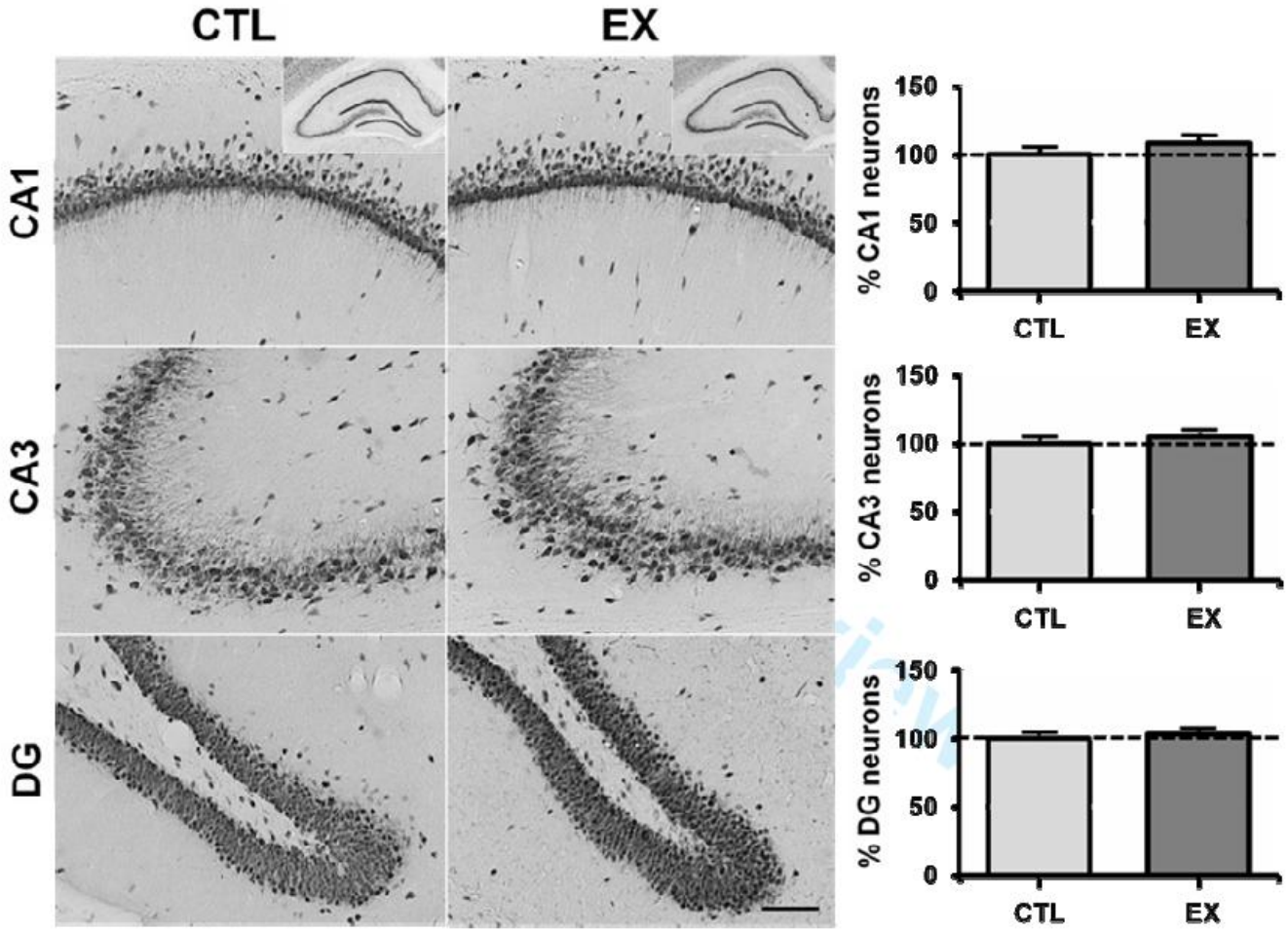


Figure 2. NeuN immunoreactivity and neuronal percentage (%) in regions of CA1, CA3 and dentate gyrus (DG) of rats from exercise group (EX) and control group (CTL). Scale bar = 100 µm.

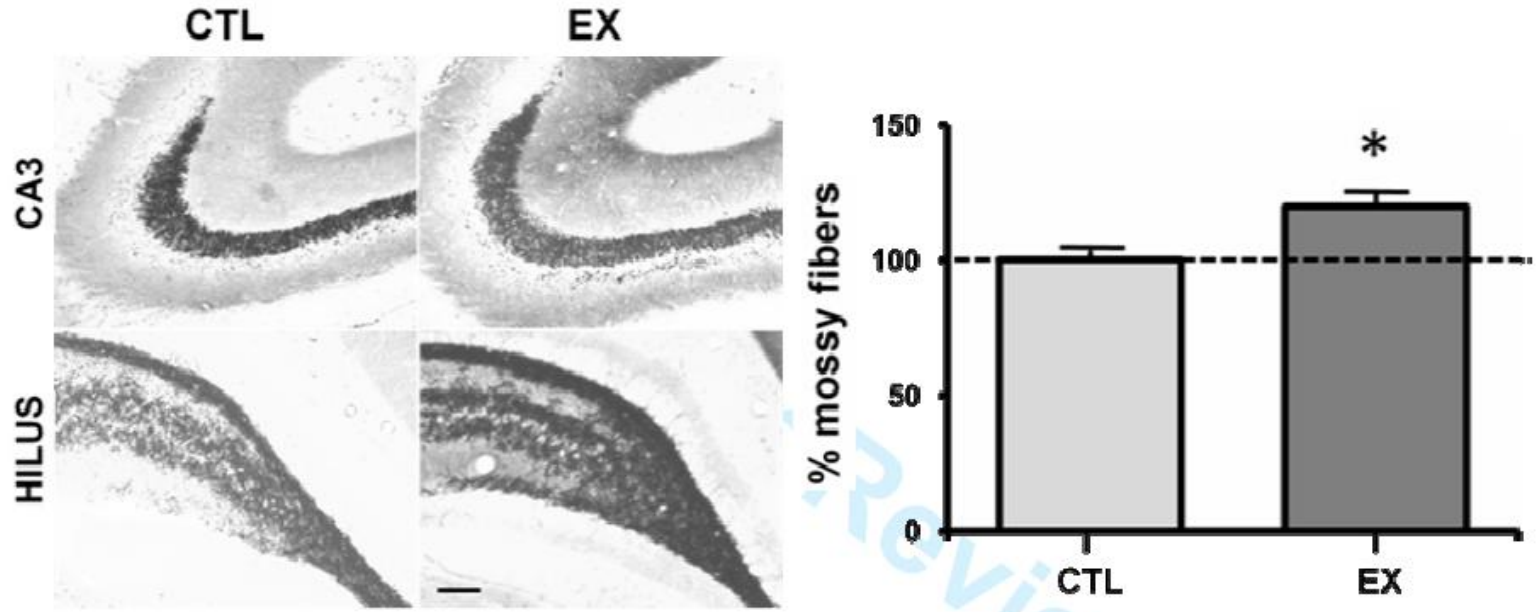


Figure 3. Neo-Timm staining in regions of CA3 and hilus of the dentate gyrus and mossy fibers percentage (%) in rats from the exercise group (EX) and control group (CTL). An increase in density of mossy fibers was detected in the hippocampal formation of rats trained during development (*p<0.01). Scale bar = 150 µm.

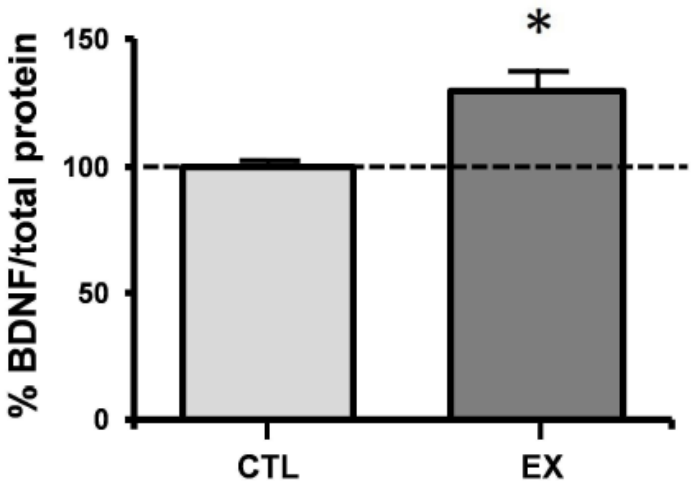


Figure 4. Effect of early-life exercise on hippocampal BDNF expression. An increased of BDNF expression was detected in hippocampal formation of the exercise group (EX) when compared to the control group (CTL) (*p<0.01).

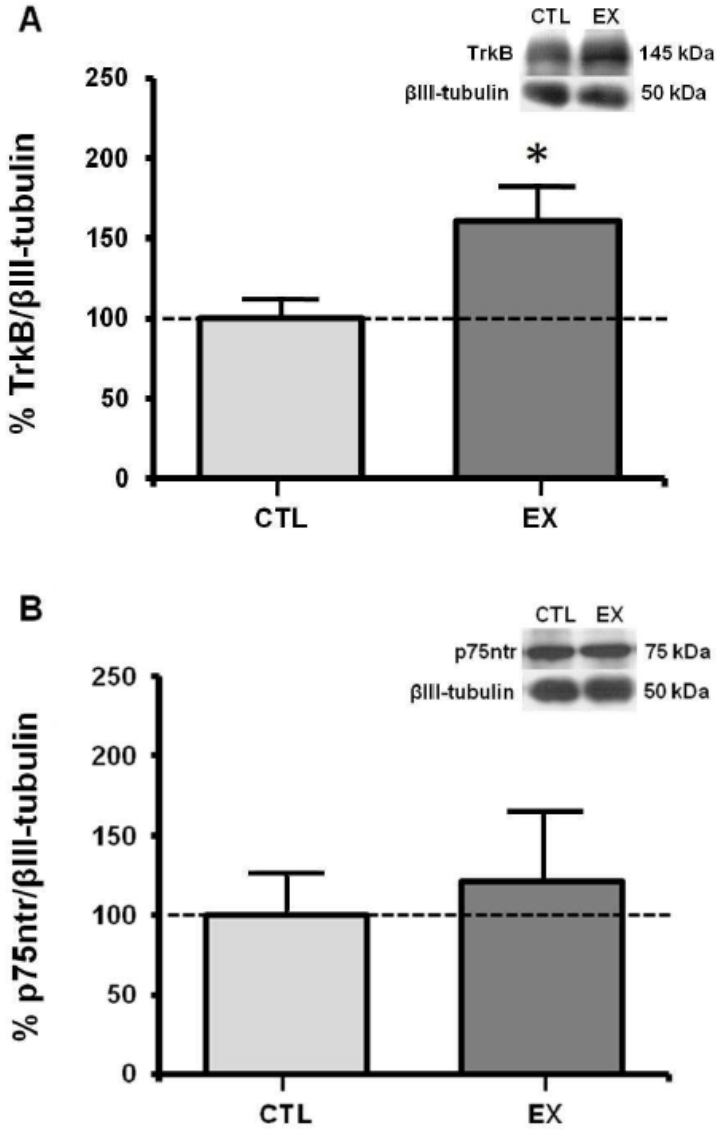


Figure 5. Effect of early-life exercise on hippocampal expression of TrkB (A) and p75ntr (B). The expression of TrkB was significantly higher in the hippocampal formation of the exercise group (EX) than in the control group (CTL) (*$p<0.05$). No difference in p75ntr expression was observed between the studied groups ($p>0.05$).

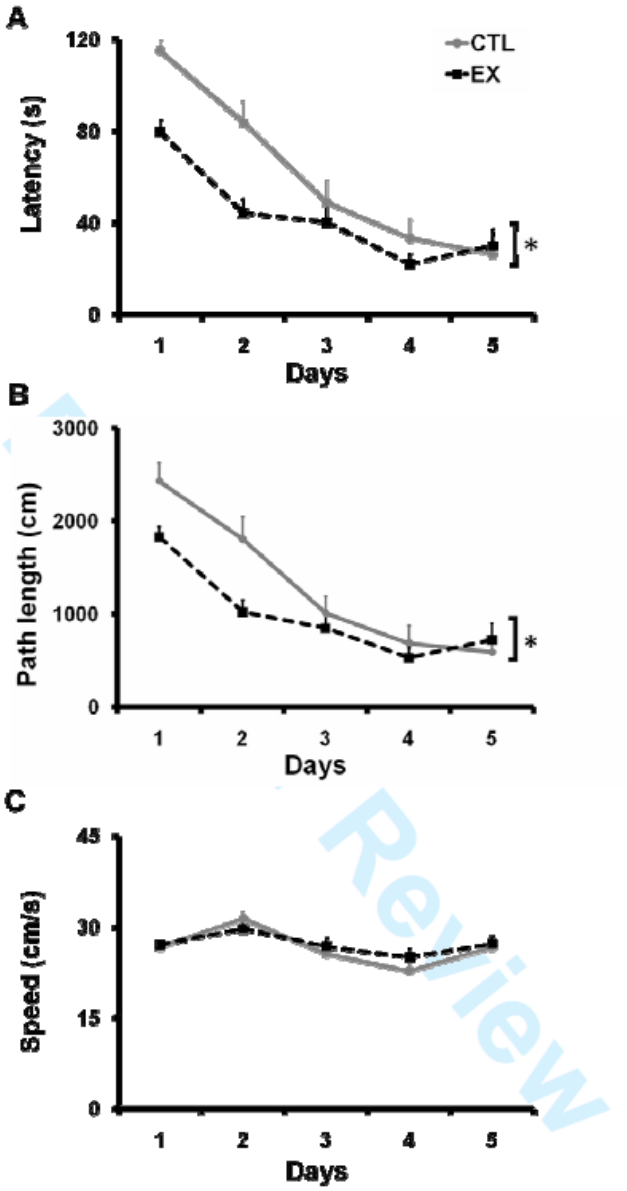


Figure 6. Water Maze learning in rats from exercise group (EX) and control group (CTL). The latency (A) and swim path (B) were significantly lower in rats trained during the development than in control rats (*$p<0.05$). No difference in mean swimming speed (C) was detected between the studied groups ($p>0.05$).

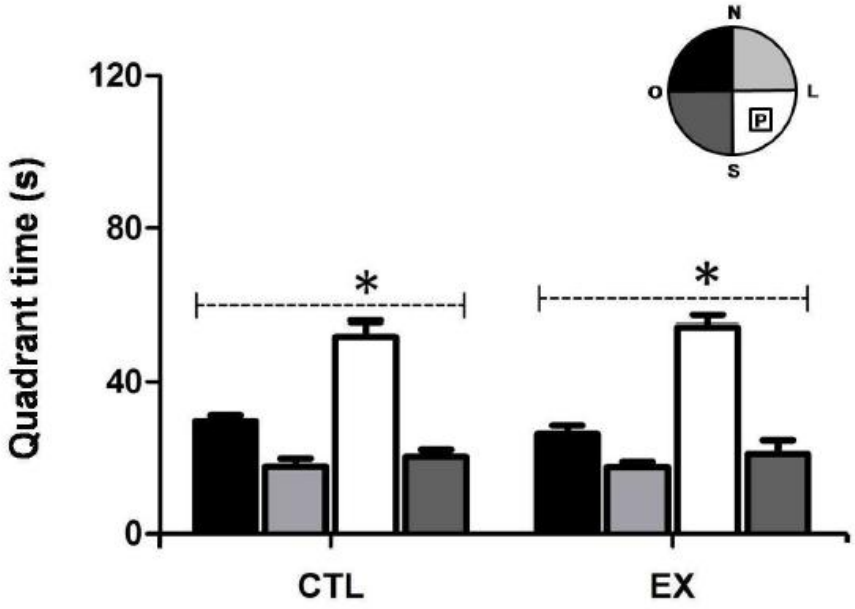


Figure 7. Time spent in four imaginary quadrants of the Water Maze. Rats from both the exercise group (EX) and control group (CTL) presented a significant preference for the platform quadrant (*p<0.05).

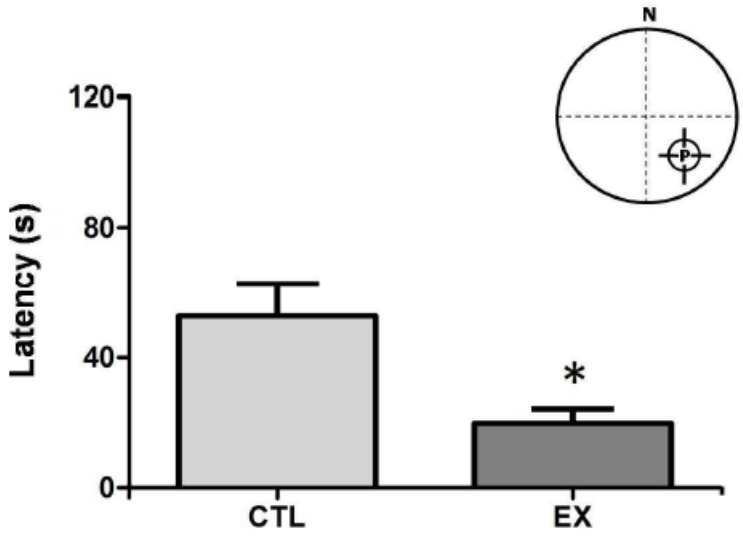
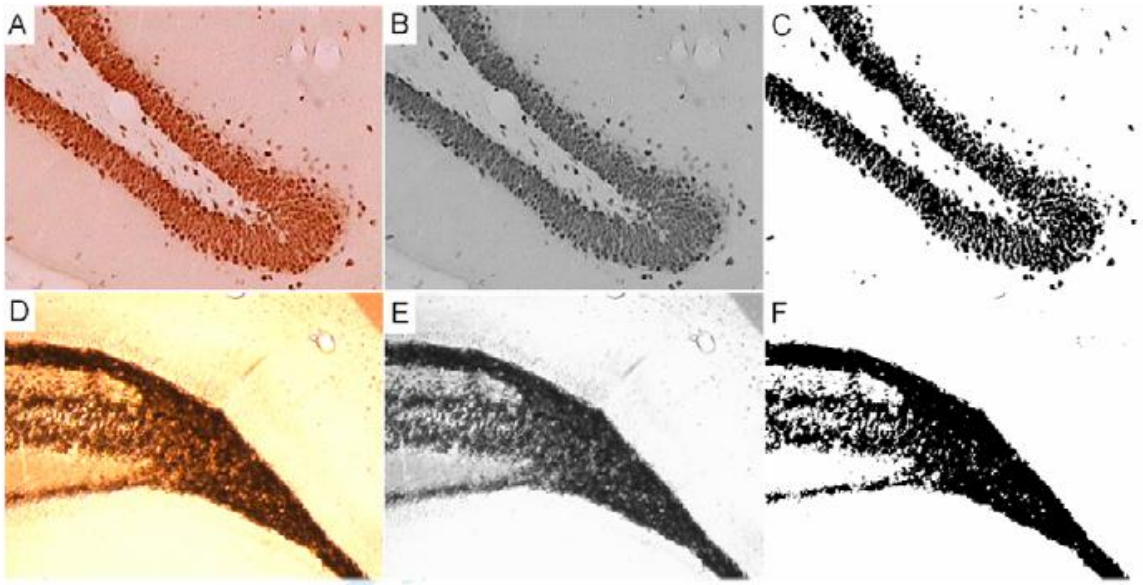
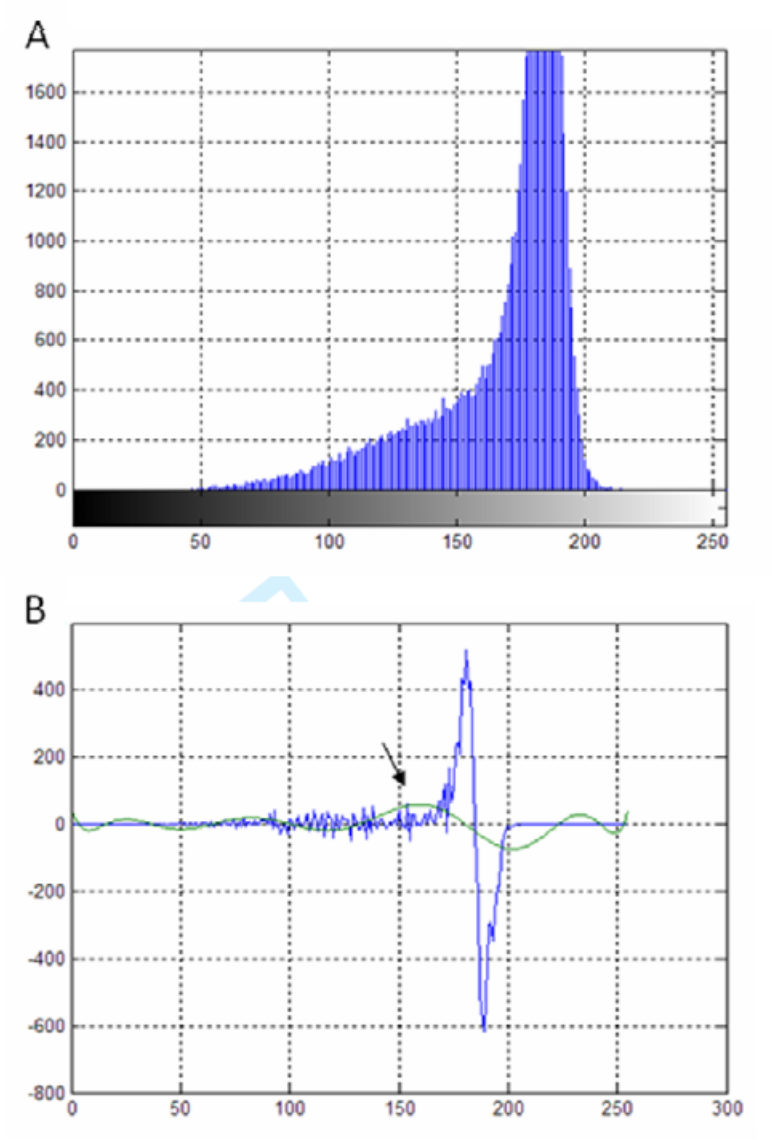


Figure 8. Latency 30 days after Water Maze learning in rats from exercise group (EX) and control group (CTL). Time spent finding the platform was significantly lower in rats trained during development than in control rats (*p<0.05).



Supplementary material 1. Representative illustration of image processing for NeuN-immunoreactive neurons (A–C) and mossy fibers by Neo-Timm staining (D–F).



Supplementary material 2. Histogram (A) and histogram derived under the trend line (B). The arrow indicates the beginning of the luminous intensity of neuronal cells and mossy fibers (maximum point in the trend line).

Supplementary material 3 (Matlab routine)

```
function pixel (arquivo)

    neu      = imread(arquivo); % Reads image
    neu_gray = rgb2gray(neu);   % GrayScale

    [H W] = size(neu_gray);     % Image sizes

    neu_med  = medfilt2(neu_gray, [2 ,2]);  % Remove image noise mask 2x2

    histo = imhist(neu_med); %histogram

    histo_der = 0;
    for i=1:255 % histogram derived
        histo_der(i) = histo(i+1) - histo(i);
    end

    xd = 1:255;

    linearCoef = polyfit(xd,histo_der,20);
    histo_derF = polyval(linearCoef,xd); % polynomial trend line

    cte_der = find(histo_derF == max(histo_derF)); % luminous intensity bound
(grayscale) to distinguish neurons of background
    cte_der = cte_der - 10;
    for i = 1:W
      for j  = 1:H
        if(neu_med(j,i)>cte_der)
          neu_med(j,i) = 255;
        else
          neu_med(j,i) = 0;
        end
      end
    end

    neu_bw = im2bw(neu_med); %binary image

    % Shows histograms, graphs and images
    %figure, imshow(neu)
    %figure, imshow(neu_gray)
    %figure, imshow(neu_med)

    %figure, imhist(neu_gray), title('Histograma CA3'), grid;
    %figure, plot(xd,histo_der,xd,histo_derF), grid;
    %figure, imshow(neu_bw)

    envia = [arquivo,';',num2str(sum(sum(~neu_bw))/(W*H)),';',
num2str(sum(sum(cte_der)))];
    disp (envia); %shows in workspace - file ; Percent of pixels equivalent of
neurons ; constant of contrast
end
```

4

Discussão Geral

4. DISCUSSÃO GERAL

Embora os mecanismos de adaptação neurobiológica ao exercício no cérebro maduro sejam amplamente documentados (Neeper et al., 1995; Kleim et al., 1996; Gomez-Pinilla et al., 1998; Van Praag et al., 1999; Arida et al., 2004; Van Praag et al., 2005; Kramer et al., 2006; Uda et al., 2006; Ploughman et al., 2007; Soya et al., 2007), a influência do exercício durante o processo de desenvolvimento cerebral permanece pouco explorada. A proposta do presente estudo foi investigar os efeitos do exercício físico sobre o desenvolvimento cerebral pós-natal. Para isso, avaliamos a plasticidade hipocampal de ratos submetidos a um programa de exercício físico aeróbio durante o período adolescente (21° ao 60° dia de vida pós-natal).

O primeiro trabalho (Brain & Development) mostrou que o exercício físico durante a adolescência altera a expressão hipocampal de parvalbumina. Na formação hipocampal, a parvalbumina está localizada em uma população de células que formam um grupo heterogênico de neurônios não piramidais, particularmente uma população de interneurônios inibitórios com alta taxa de disparo e elevado metabolismo oxidativo (Nitsch et al., 1990; Baimbridge et al., 1992; Heizmann, 1993). Esses interneurônios inibitórios são essenciais para o processamento de informações e para o controle da transmissão excitatória (Miles e Wong, 1983). O papel da parvalbumina nessas células é manter as concentrações intracelulares de cálcio em equilíbrio, evitando desta maneira a neurotoxicidade produzida pelo excesso de íons cálcio (Baimbridge et al., 1992). Os nossos resultados demonstram que o exercício físico aumenta a expressão hipocampal de parvalbumina e o número de interneurônios imunorreativos à parvalbumina em animais em desenvolvimento. Esses achados corroboram com estudos anteriores do nosso grupo em animais adultos (Arida et al., 2004; 2007).

Existem evidências experimentais de que o exercício físico durante a infância e adolescência pode ser favorável para o desenvolvimento cerebral (Kim et al., 2004; Uysal et al., 2005; Lou et al., 2008). Dependendo da intensidade, o exercício aumenta a neurogênese e a expressão gênica de BDNF e de fator de crescimento vascular endotelial (vascular endothelial growth factor, VEGF) na formação hipocampal de ratos juvenis treinados por uma semana (Lou et al., 2008). O nosso experimento não permite identificar se o aumento de parvalbumina nas regiões de CA1 e CA2/3 ocorre em neurônios novos ou em pré-existentes. É importante notar que a neurogênese induzida pelo exercício no início da vida pode ter um impacto significativo no desenvolvimento estrutural e funcional do cérebro (Kim et al., 2004; Uysal et al., 2005; Lou et al., 2008). Nossos achados sugerem que o aumento de interneurônios parvalbumina em ratos treinados na adolescência poderia ser atribuído ao aumento da neurogênese e de BDNF. Em relação à neurogênege, a formação de novas células na formação hipocampal é mais prevalente em ratos jovens, e um aumento da proliferação celular tem sido observada em ratos com 4 semanas de idade treinados por cinco dias (Kim et al., 2004). Entretanto, os nossos resultados mostram que o exercício físico durante o período adolescente não altera a densidade de neurônios na região hipocampal (veja Artigo 03 - Hippocampus). Estudos futuros podem investigar melhor esse assunto. Considerando a segunda hipótese (BDNF), observa-se que a depleção de BDNF em animais transgênicos reduz o número de células imunorreativas à parvalbumina no cérebro (Jones et al., 1994). Neste sentido, é provável que os nossos achados estejam relacionados ao aumento dos níveis de BDNF observados em animais treinados durante o desenvolvimento pós-natal (veja Artigo 03 - Hippocampus).

O segundo trabalho (Neurochemistry International) mostrou que o exercício físico durante o período adolescente reduz a expressão do receptor CB1 na formação hipocampal e no estriado. Duas hipóteses poderiam explicar esses resultados. A primeira possibilidade poderia ser atribuída ao período de treinamento realizado: estímulos agudos poderiam aumentar a expressão cerebral do receptor e estímulos crônicos poderiam reduzi-la. Em favor desta hipótese, tem sido observado que a administração de anandamida por cinco dias resulta num aumento significante da densidade hipocampal de receptores CB1 (Romero et al., 1995). Por outro lado, Sim-Selley e colaboradores (2002) descrevem que os efeitos tardios da administração de um inibidor da hidrólise de anandamida podem resultar na diminuição do receptor CB1 em várias regiões cerebrais, como os núcleos da base, estriado e formação hipocampal. No nosso laboratório, resultados semelhantes têm sido observados analisando os receptores opióides. Enquanto sete dias de exercício físico em roda ou esteira motorizada aumentam a expressão hipocampal de receptores μ e k, quarenta e cinco dias de exercício causaram uma redução (normalização) da expressão desses receptores na formação hipocampal de animais submetidos a exercício forçado e voluntário (de Oliveira et al., 2010).

A segunda possibilidade que poderia justificar a redução do receptor CB1 no estriado e na formação hipocampal seria o período de estímulo durante o desenvolvimento, ou seja, a adolescência. A expressão do receptor CB1 (Rodriguez de Fonseca et al., 1993) e os níveis de mRNA (McLaughlin e Abood, 1993) aumentam progressivamente no cérebro até o 30° e 40° dias de vida pós-natal. Depois, sua expressão decai durante o período adolescente até alcançar os valores do adulto (medido no 70° dia de vida) (Rodriguez de Fonseca et al., 1993). Esses achados coincidem com as etapas de poda sináptica e axonal observadas durante a infância e a

adolescência (Andersen, 2003; Tau e Peterson, 2010), etapas básicas da neuromaturação. Neste sentido, é possível que os nossos resultados estejam relacionados a uma maturação precoce do sistema endocanabinóide induzido pelo exercício físico nesta fase da vida.

O terceiro trabalho (Hippocampus) demonstrou que o exercício durante a adolescência aumenta a densidade de fibras musgosas e a expressão hipocampal de BDNF e TrkB, aprimora a aprendizagem e a memória espacial, e melhora a capacidade de evocar as memórias em longo prazo. Esses achados estão de acordo com estudos recentes que mostram uma influência positiva do exercício na formação hipocampal de animais em desenvolvimento (Kim et al., 2004; Uysal et al., 2005; Lou et al., 2008). Entretanto, nenhuma diferença na densidade hipocampal de neurônios foi detectada entre animais treinados e não treinados. Esse resultado não corrobora com dados já descritos na literatura (Uysal et al., 2005). Uysal e colaboradores (2005) observaram que animais treinados na juventude apresentam na vida adulta um aumento na densidade de células hipocampais. Adicionalmente, um aprimoramento da memória foi encontrado nesses animais quando testados em labirinto aquático, sugerindo que o aumento da densidade celular na região hipocampal poderia melhorar as funções cognitivas (Uysal et al., 2005). No nosso estudo, os animais treinados durante a adolescência também apresentaram uma melhora da apredizagem e da memória espacial, mas nenhuma mudança na densidade hipocampal de neurônios foi detectada. Uma possível explicação para esses resultados poderia estar relacionada às técnicas de marcação utilizadas. Nós utilizamos um procedimento imuno-histoquímico específico para células neuronais (marcação de NeuN) enquanto Uysal e colaboradores utilizaram um método que marca tanto células neuronais quanto gliais (método de Nissl).

Outra possibilidade para justificar esses resultados divergentes seria os diferentes protocolos de exercício físico realizados. No estudo conduzido por Uysal e colaboradores (2005), os animais em desenvolvimento correram durante 8 semanas com intensidade de 8m/min por 30 min, 5 vezes na semana. No nosso protocolo de exercício físico, os animais correram durante 6 semanas em uma intensidade maior (18m/min por 60 min, 7 vezes na semana). Apesar de intensidades menores (Uysal et al., 2005) e maiores (nosso estudo) de exercício físico durante o desenvolvimento tenham melhorado as funções cognitivas, mudanças na densidade hipocampal de neurônios é um assunto que permanece controverso.

Como o cérebro em desenvolvimento é uma estrutura em processo dinâmico de modificações, deve-se considerar a possibilidade de diferentes respostas ao treinamento físico. Por esse motivo, investigamos se o modelo de exercício físico utilizado neste estudo poderia causar efeitos negativos na formação hipocampal de ratos em desenvolvimento. De forma geral, os métodos mais utilizados para submeter animais a um programa de exercício são os exercícios voluntário em roda e o forçado em esteira motorizada. Embora esses dois métodos tenham variáveis em comum como na função cardíaca, pulmonar e musculo-esquelética, existem também fatores que são únicos de cada método, tal como o desejo psicológico de correr, o medo do manuseio, o comportamento de evitar choque elétrico (stress) e a percepção de dor (Lerman et al, 2002). Para investigar se o nosso programa de exercício físico forçado poderia induzir efeitos degenerativos, nós utilizamos marcadores inflamatórios e de neurônios em degeneração. Os resultados demonstram que o paradigma de exercício físico escolhido não induz resposta inflamatória e degeneração neuronal na formação hipocampal de animais em desenvolvimento.

Em nosso estudo, a densidade das fibras musgosas foi significativamente maior na formação hipocampal de ratos treinados durante o desenvolvimento do que em ratos não treinados. Existem evidências de que a densidade hipocampal de fibras musgosas pode estar relacionada com o desempenho cognitivo de roedores em tarefas dependentes do hipocampo. Por exemplo, a separação materna no período neonatal reduz a densidade hipocampal das fibras musgosas e prejudica a aquisição de memórias na vida adulta de ratos (Huot et al., 2002). Em compensação, roedores com projeções maiores de fibras musgosas cometem menos erros durante a aprendizagem no labirinto radial (Jamot et al., 1994; Schwegler e Crusio, 1995) e apresentam índices melhores de aprendizagem e memória no labirinto aquático (Schwegler et al., 1988; Schöpke et al. 1991, Bernasconi-Guastalla et al., 1994, Pleskacheva et al., 2000). Com base nestas observações, nós especulamos que a melhora do desempenho cognitivo observada no nosso estudo poderia estar correlacionada com o aumento de fibras musgosas em ratos treinados durante o desenvolvimento.

Existe uma grande variedade de mecanismos que causariam o aumento das fibras musgosas induzida pelo exercício. Tong e colaboradores (2001) examinaram a expressão hipocampal de aproximadamente 5.000 genes em ratos submetidos a três semanas de exercício físico, e relataram mudanças em um grande número de genes associados com a plasticidade neuronal e sináptica. Dentre eles, os genes que sintetizam fatores neurotróficos têm sido considerados os candidatos mais prováveis em mediar os efeitos do exercício físico na plasticidade hipocampal (Cotman e Berchtold, 2002; Vaynman e Gomez-Pinilla, 2005). Nós especulamos que o aumento da densidade de fibras musgosas em ratos treinados durante a adolescência poderia ser atribuído ao aumento de BDNF. O BDNF exerce um papel importante no crescimento de axônios (Rabacchi et al., 1999). A aplicação de BDNF em cultura de

células granulares aumenta significativamente o prolongamento e a extensão das fibras musgosas (Lowenstein e Arsenault, 1996). Por outro lado, a depleção de BDNF em camundongos reduz o brotamento de fibras musgosas induzido por eletroconvulsoterapia crônica (Vaidya et al., 1999). Em conjunto, esses achados sugerem que o BDNF parece ser necessário para a plasticidade estrutural de axônios e de fibras musgosas.

As diversas funções do BDNF no cérebro são mediadas via duas classes de receptores: o TrkB e o p75ntr (Bibel e Barde, 2000; Kaplan e Miller, 2000; Chao, 2003). Os nossos resultados demonstram que animais treinados na adolescência apresentam na vida adulta um aumento significativo na expressão hipocampal de BDNF e de seu receptor TrkB. Estes achados são de grande importância porque estão relacionados aos efeitos benéficos do exercício no cérebro (Vaynman e Gomez-Pinilla, 2005). Por exemplo, Vaynman e colaboradores (2004) observaram que ratos submetidos a uma semana de exercício físico apresentaram melhor desempenho no labirinto aquático que ratos controle. No entanto, este benefício foi abolido quando a sinalização BDNF/TrkB foi inibida. Os autores notaram que os animais treinados em roda não apresentavam melhora no desempenho cognitivo e aumento da proteína CREB quando anticorpos que bloqueiam a sinalização intracelular do receptor TrkB eram administrados (Vaynman et al., 2004), indicando que a sinalização BDNF/TrkB pode modular os efeitos positivos do exercício físico nas funções cognitivas.

No nosso estudo, nenhuma diferença significativa na expressão hipocampal do receptor p75ntr foi detectada entre animais treinados e não treinados. Apesar de diminuir drasticamente na vida adulta, o receptor p75ntr está amplamente presente durante as diferentes fases do desenvolvimento (Chao, 2003). É interessante observar que a ligação do BDNF com o receptor p75ntr gera uma cascata de reações químicas

que leva a apoptose tanto no desenvolvimento quanto após lesão do SNC (Chen et al., 2009). Entretanto, devido às vias antiapoptóticas associadas aos receptores tirosina-quinases, observa-se que a morte celular induzida pela sinalização BDNF/p75ntr ocorre somente quando a sinalização BDNF/TrkB está ausente ou diminuída (Davey e Davies, 1998; Friedman, 2000; Unsain et al., 2009).

Evidências indicam que estímulos durante o desenvolvimento cerebral pós-natal podem modular a maturação funcional do cérebro e resultar em circuitos neuronais mais complexos (Linkenhoker et al., 2005). No presente estudo, demonstramos que um programa de exercício aeróbico durante o período adolescente modifica a plasticidade hipocampal e aprimora a memória espacial de ratos na vida adulta. Outro achado importante em nosso estudo foi que exercício físico no início da vida melhora a capacidade de evocar as memórias em estágios posteriores (quando medido no 96° dia de vida), corroborando com estudos em humanos que mostram uma correlação entre a atividade física na infância e benefícios cognitivos ao longo da vida (Dik et al., 2003). Em resumo, os nossos resultados indicam que o exercício físico resulta em mudanças positivas para o cérebro em desenvolvimento pós-natal. Esses achados podem ter grande valor terapêutico para algumas doenças neurológicas que emergem durante a infância e adolescência (Arida et al., 2010; Gorczynski e Faulkner, 2010). Além disso, o conhecimento de tais benefícios pode ser relevante para o desenvolvimento de políticas públicas destinadas a estimular programas de exercícios físicos para a população, especialmente para crianças e adolescentes.

5

Conclusões

5. CONCLUSÕES

(a) O programa de exercício físico aeróbico utilizado neste estudo induz alterações plásticas significativas no cérebro de ratos em desenvolvimento;

(b) O exercício físico durante o período adolescente aumenta a expressão hippocampal de parvalbumina;

(c) O exercício físico reduz a expressão do receptor CB1 na formação hipocampal e no estriado de ratos em desenvolvimento;

(d) O exercício durante a adolescência aumenta a densidade de fibras musgosas e a expressão hipocampal de BDNF e TrkB, aprimora a aprendizagem e a memória espacial, e melhora a capacidade de evocar as memórias em longo prazo;

(e) O programa de exercício físico realizado durante o desenvolvimento pós-natal não altera a densidade hipocampal de neurônios e a expressão do receptor p75ntr;

(f) O paradigma de exercício físico escolhido não induz resposta inflamatória e degeneração de neurônios na formação hipocampal de animais em desenvolvimento.

Em conjunto, esses achados indicam que o exercício físico resulta em mudanças positivas para o cérebro em desenvolvimento pós-natal.

6

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7

Anexos

7. ANEXOS

7.1. Outros trabalhos publicados

ELSEVIER

Physiology & Behavior 90 (2007) 629 – 633

PHYSIOLOGY & BEHAVIOR

Physical training in developing rats does not influence the kindling development in the adult life

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Abstract

The positive effect of physical exercise programs on seizure frequency and severity has been demonstrated both in adult human and animals. However, this investigation during animal brain development has not been examined. To this purpose, the present work was aimed to analyse the effect of physical exercise training in rats after weaning on the kindling process in the adulthood. Thirty rats were divided into 3 groups: the first group (EX = 10) was submitted to daily bout of aerobic exercise (60 min running on the treadmill at 24/26 m/min) between P21 and 60 days of age. After this period of training, animals were submitted to 60 min running at the same speed and kindling stimulated one min post-exercise. The second group (SHAM = 10) was maintained in the treadmill for the same time as the trained group without being submitted to physical exercise. The third group served as control (CTL = 10). The number of stimulations required to reach stage 5 for the EX group was not statistically different from CTL and SHAM groups. However, the EX group spent a longer time and a shorter afterdischarge (AD) in stage 1 compared to the CTL and SHAM groups. The number of stimulations and AD duration in stage 2, 3 and 4 was not statistically different between all the groups. Taken together, our study showed that although forced physical exercise in developing rats does not exert significant influence to reach the stage 5 of amygdala kindling in the adult life its interference during the process of epileptogenesis indicate a positive effect of exercise in developing brain.

Keywords: Kindling; Physical exercise; Treadmill; Developing rats; Brain

1. Introduction

Several investigations on the relationship of physical exercise and epilepsy have been carried out. Clinically, it has been demonstrated that there is a reduction in the number of seizures after physical exercise or physical training programs [1,2], although the mechanisms underlying this protective effect have not been clearly investigated [1,3]. However, experiments on brain electrical activity have shown that abnormal discharges could decrease or even disappear in patients during physical activity that eventually could return at rest [4,5]. Other researches have suggested that physical exercise might raise the seizure threshold and, consequently, could confer a protective effect on people with epilepsy [4,6]. On the other hand, there is little evidence of seizure-induced by physical exercise. Most of previously reported cases had a history of additional seizures independent of exercise [7–9].

In our laboratory we have been investigating the effect of physical exercise in some experimental models of epilepsy [10,11]. Accordingly, we observed that in rats, physical training, performed before and during the kindling procedure, retarded the amygdala kindling development [10]. In addition, we reported that aerobic physical program reduced the frequency of spontaneous recurrent seizures in the pilocarpine model of epilepsy in rats [11]. Metabolic [12] and electrophysiological

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Effects of different types of physical exercise on the staining of parvalbumin-positive neurons in the hippocampal formation of rats with epilepsy

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Abstract

Effects of exercise in animals with epilepsy have been demonstrated. To investigate whether the type of physical activity, voluntary or forced, would promote different morphological changes in hippocampal formation we performed an immunocytochemical study using the parvalbumin (PV) distribution as a marker. Control rats and rats with epilepsy were submitted to a voluntary (wheel running) and forced (treadmill) exercise for 10 days (acute physical exercise) or 45 days (chronic physical exercise). It was observed in normal rats a higher number of PV-positive cells in the hilus of dentate gyrus (DG) in the voluntary and forced exercise groups (acute and chronic physical exercise), when compared to the control group. In animals with epilepsy the number of PV-positive cells and staining intensity of PV-fibers in the hilus was significantly higher only in the acute physical exercise (voluntary and forced). These findings demonstrate that acute physical exercise, both voluntary and forced results in increased number of PV-positive cells and staining intensity of PV-fibers in the hilus of rats with epilepsy and the occurrence of these changes takes place only in the early phase of epilepsy.

Keywords: Epilepsy; Hippocampal formation; Parvalbumin; Physical exercise; Rat

1. Introduction

Although there is accumulating evidence indicating that physical exercise has beneficial effects on brain function, the implications of epilepsy for physical fitness programs have been subject of controversy. Clinical and experimental studies have analyzed the effect of physical exercise on epilepsy. Previous studies have shown positive effects on seizure frequency and severity (Denio et al., 1989; Eriksen et al., 1994) suggesting that exercise raises seizure threshold and may confer a protective effect on patients with epilepsy (Gotze et al. 1967; Livingston, 1978).

In addition, experimental studies have also demonstrated a positive effect of physical exercise in animals with epilepsy (Arida et al., 1998, 1999, 2004a,b). In the kindling model of

Abbreviations: LTP, long-term potentiation; BDNF, brain-derived neurotrophic factor; NGF, nerve growth factor; FGF, fibroblast growth factor; CaBP, calcium-binding proteins; PV, parvalbumin; DG, dentate gyrus; SE, status epilepticus; SRS, spontaneous recurrent seizures; CT, control rats; V-10, acute voluntary rats; T-10, acute treadmill rats; V-45, chronic voluntary rats; T-45, chronic treadmill rats; V-10 with epilepsy, acute voluntary rats with epilepsy; T-10 with epilepsy, acute treadmill rats with epilepsy; V-45 with epilepsy, chronic voluntary rats with epilepsy; T-45 with epilepsy, chronic treadmill rats with epilepsy; V-35/45 with epilepsy, rats submitted to 10 days of voluntary exercise between the 35th and 45th days, after the second SRS; T-35/45 with epilepsy, rats submitted to 10 days of treadmill exercise between the 35th and 45th days, after the second SRS.

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CLINICAL SCIENCE

EVALUATION OF PHYSICAL ACTIVITY HABITS IN PATIENTS WITH POSTTRAUMATIC STRESS DISORDER

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Assis MA, Mello MF, Scorza FA, Cadrobbi MP, Schooedl AF, Silva SG, de Albuquerque M, da Silva AC, Arida RM. Evaluation of physical activity habits in patients with posttraumatic stress disorder. Clinics. 2008;63:473-8.

OBJECTIVE: In this study, we present data from a survey that aimed to assess the physical activity habits of adult Brazilian patients with Posttraumatic Stress Disorder.
METHOD: Fifty male and female patients with Posttraumatic Stress Disorder participated in this study. The mean age at onset was 37±12 years, and the mean time between diagnosis and follow-up was 3.6±4.2 years.
RESULTS: Substantial changes in physical activity habits were observed following the onset of PTSD. While more than half of the patients participated in physical activities prior to Posttraumatic Stress Disorder onset, there was a significant reduction in their participation afterwards. The justifications for stopping physical activities or sport participation were lack of time and lack of motivation.
DISCUSSION: Several studies have shown that physical exercise decreases reverts symptoms of psychiatric disorders such as depression, anxiety and social isolation. We could therefore hypothesize that patients with Posttraumatic Stress Disorder who exercise should experience the same benefits.
CONCLUSION: Our findings demonstrated that patients with Posttraumatic Stress Disorder have low levels of participation in sports or physical activities.

KEYWORDS: Posttraumatic Stress Disorder. Exercise. Physical activity habits. Patient quality of life.

INTRODUCTION

Posttraumatic stress disorder (PTSD) is a mental disorder that develops in people who are exposed to extremely stressful events such as natural disasters, environmental destruction or violence or who confront life-altering events, such as the death of family members or friends. Although PTSD is a highly prevalent and often chronic condition, the relationship between PTSD, functioning and quality of life remains unclear. It has been associated with a lower quality of life in US war veterans,[1,2] refugees of war,[3] and sexual assault survivors.[4] In addition, PTSD has been associated with poorer mental and physical health,[5] increased violent behavior,[6] marital and family adjustment problems[7] and less favorable performance in work and education.[8]

Studies have consistently found that physical activity is associated with improved psychological well-being, physical health, life satisfaction, cognitive functioning and psychiatric conditions.[9-16] Clinical and epidemiologic studies have also shown an association between physical activity and decreased symptoms of anxiety and depression.[15-17] Few studies, however, have analyzed the impact of physical

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Artigo de Revisão

Epilepsia e atividade física: estudos em humanos e animais

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Resumo: A epilepsia é considerada o distúrbio neurológico crônico mais prevalente no mundo, influenciando negativamente a qualidade de vida de indivíduos com epilepsia. Apesar do efeito favorável do exercício físico sobre a saúde ser inquestionável, a realização de um programa de exercício físico por pessoas com epilepsia ainda é um assunto controverso. Estudos têm mostrado efeitos benéficos do exercício físico na freqüência de crises assim como na qualidade de vida. Entretanto, indivíduos com epilepsia são freqüentemente desencorajados e excluídos da participação em programas de exercício físico pelo medo que tal participação possa precipitar crises epilépticas. Sendo assim, o objetivo desse estudo foi revisar o efeito do exercício físico baseado em dados de estudos clínicos e experimentais de epilepsia.

Palavras-chave: Exercício físico, Epilepsia, Crises, Qualidade de vida.

Epilepsy and physical activity: human and animal studies

Abstract: Epilepsy is the most common chronic neurological disorder in the world that influences negatively the quality of people's life affected by this disease. Although the favorable effect of physical activity on general health is unquestionable, the appropriate physical exercise for people with epilepsy is still controversial. Studies have shown beneficial effect of physical exercise on frequency of seizures as well as on life quality. However, people with epilepsy frequently are discouraged and excluded from participation in physical exercise due to the fear that the participation in a physical exercise program can precipitate epileptic seizures. Therefore, the aim of this study was to review the effect of physical exercise based on clinical and experimental studies of epilepsy.

Key Words: Physical exercise, Epilepsy, Seizures, Quality of life.

Epilepsia

A epilepsia, historicamente, tem sido descrita e registrada por diferentes raças e credos ao longo dos séculos. Por volta de 400 a.C., Hipócrates criticou o caráter de doença sagrada, atribuído à epilepsia, considerando-a como qualquer outra doença. Entretanto, na maioria das culturas ganhou interpretação como algo demoníaco e sobrenatural, devido à forma de manifestação de seus sinais e sintomas (BRODIE; SCHACHTER, 2001). Somente no século XIX que John Hughlings, um neurologista inglês, introduziu o conceito de crise epiléptica como uma atividade elétrica cerebral desordenada originada de áreas discretas do córtex cerebral (camada mais externa do cérebro dos vertebrados, desempenhando papel central em funções complexas como a memória, a atenção, a consciência, a linguagem, a percepção e o pensamento) (ENGEL, 1995; BRODIE; SCHACHTER, 2001).

Atualmente, sabe-se que o termo epilepsia não se refere a uma doença, mas a síndromes completamente distintas do ponto de vista etiológico e fisiopatológico (INTERNATIONAL..., 1985; FISHER, 1989). Uma síndrome epiléptica é

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Review

The potential role of physical exercise in the treatment of epilepsy

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ABSTRACT

The beneficial effects of exercise for people with epilepsy, including reduction of seizure susceptibility, improvement of quality of life, reduction of anxiety and depression, and better social integration, have increasingly been reported. We present data from human and animal studies supporting the role of exercise as a therapy for epilepsy complementary to standard treatments.

1. Introduction

Nonpharmacological therapies, including complementary and alternative medicine (CAM), are often used by patients with epilepsy, frequently without the knowledge of their physicians [1–8]. Exercise is infrequently cited as a complementary therapy, yet the beneficial effects of exercise have been increasingly reported for persons with epilepsy, both for seizure control and for improvement of quality of life.

2. Exercise and effects on seizures and the EEG

Persons with epilepsy are often cautioned to avoid vigorous exercise to prevent seizures. Although there are rare cases of exercise-induced seizures, studies have generally shown that physical activity can decrease seizure frequency, as well as lead to improved cardiovascular and psychological health in people with epilepsy [9–13]. Interictal epileptiform activity recorded by electroencephalograms (EEGs) remains unchanged or decreases in frequency during or immediately after exercise in the majority of patients studied, even in some patients with exercise-associated seizures [11,14–16]. Fewer seizures occur during both mental and physical activity compared with periods of rest [17], suggesting that increased attention and vigilance during physical activity may reduce the occurrence of seizures [15]. Accordingly, some investigators have suggested that exercise may have an anticonvulsant effect on persons with epilepsy [14,18]. One supportive hypothesis proposes that ß-endorphins released during exercise inhibit epileptic discharges [19].

Although only a few studies have evaluated supervised exercise programs for patients with epilepsy; the findings are promising. For instance, Nakken and co-workers [10] reported that 4 weeks of an aerobic training program did not change average seizure frequency. A study in women with medically refractory epilepsy demonstrated that aerobic physical training decreased the number of seizures during the exercise period [12]. Another study showed no impact on seizure frequency after a 12-week exercise program [20].

Epidemiological studies add supportive evidence. A study comparing the exercise habits of people with epilepsy versus controls found that more than half of the patients never had a seizure during exercise, and only 2% of them had exercise-induced seizures (defined as seizures in >50% of the training sessions) [21]. Additionally, Korczyn [22] reported that only 5 of 250 individuals with epilepsy aged 10 years and older experienced a seizure while participating in sport activities. Ninety patients denied ever having a seizure during physical exercise. Seizures during exercise occurred in 6 of 21 patients in the study of Bjorholt et al. [23] and none of the patients in another study [24].

Several animal studies have shown the beneficial effects of exercise on seizure expression [for reviews, see 13,15]. Using the pilocarpine model, investigators demonstrated a significant reduction in seizure frequency during an aerobic training period

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Hippocampal mossy fiber sprouting induced by forced and voluntary physical exercise

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ABSTRACT

Alterations in the function and organization of synapses have been proposed to induce learning and memory. Previous studies have demonstrated that mossy fiber induced by overtraining in a spatial learning task can be related with spatial long-term memory formation. In this work we analyzed whether physical exercise could induce mossy fiber sprouting by using a zinc-detecting histologic technique (Timm). Rats were submitted to 3 and 5 days of forced or voluntary exercise. Rat brains were processed for Timm's staining to analyze mossy fiber projection at 7, 12 and 30 days after the last physical exercise session. A significant increase of mossy fiber terminals in the CA3 stratum oriens region was observed after 5 days of forced or voluntary exercise. Interestingly, the pattern of Timm's staining in CA3 mossy fibers was significantly altered when analyzed 12 days after exercise but not at 7 days post-exercise. In contrast, animals trained for only 3 days did not show increments of mossy fiber terminals in the stratum oriens. Altogether, these results demonstrate that sustained or programmed exercise can alter mossy fiber sprouting. Further Investigations are necessary to determine whether mossy fiber sprouting induced by exercise is also involved in learning and memory processes.

1. Introduction

Plastic events in the central nervous system, particularly alterations in the function and organization of synapses have been proposed to induce learning and memory [1]. These alterations can produce adaptations in the parameters of transmission and permanent synaptic reorganization [2]. The hippocampus plays a crucial role in the performance of spatial tasks, and the activity of its cells has been suggested to be related with spatial representation [3]. In line with these findings, it has been suggested that morphological changes underlie memory formation [4].

One of the most dramatic plastic events that have been associated with learning and memory is mossy fiber sprouting. Alternatively, in the hippocampus, mossy fiber sprouting has also been observed after experimentally induced epilepsy [5–8] as well as after high-frequency stimulation-inducing LTP [9,10]. Within the hippocampus, the mossy fibers have been shown to be critical for spatial learning, as chelation of zinc from hippocampal mossy fibers impairs spatial learning in rats [11]. Timm's staining reveals zinc and it has been used to study changes in the distribution of mossy fiber synapses [12].

Some elegant studies have shown that mossy fiber sprouting occurs in the CA3 hippocampus area after overtraining animals in a Morris water maze task using Timm's staining [13,14]. Recently, Holahan et al. [15,16] provided evidence of structural plasticity induced by learning showing that training rats to locate a hidden platform in a water maze induces growth of hippocampal granule cell mossy fiber terminal fields from the stratum lucidum of CA3 into the stratum oriens and stratum pyramidale. In addition, preliminary findings demonstrate that motor skill learning stimulates synaptogenesis in the cortex [17]. As reported above, the mossy fiber sprouting observed after spatial learning might also be attributed to the locomotor activity induced by the water maze test. In this regard, we analyzed whether physical exercise per se could induce mossy fiber sprouting using two different exercise protocols, voluntary and forced exercise. To establish the time course of mossy fiber sprouting induced by exercise, animals were examined at 7, 12 and 30 days after the last physical exercise session. Furthermore, mossy fiber sprouting distribution throughout the septotemporal axis of the dorsal hippocampus was analyzed in 4 serial hippocampal sections.

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Article
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Evaluation of physical educators' knowledge about epilepsy

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ABSTRACT

People with epilepsy suffer from a considerable lack of physical activity. In addition, an important problem of epilepsy management is the lack of qualified professionals. In this study we present data from a survey which aimed to assess physical educators' general knowledge about epilepsy. One hundred and thirty four physical educators of both sexes answered a questionnaire. Sixty percent of the professionals believe that a seizure is an abnormal electrical discharge of the brain, 13% that epilepsy is a cerebral chronic disease that can not be cured or controlled, 84% that people having convulsions will not necessarily present epilepsy and 5% that people with epilepsy have difficulties of learning. Questions concerned previous professional experience with epilepsy showed that 61% have seen a seizure and 53% have access to some information about epilepsy. Thus, 28% of professionals have a friend or relative with epilepsy, 14% have a student with epilepsy, and 29% helped someone during seizures. Our findings reveal a lack of physical educators' appropriate knowledge about epilepsy. Improvement of this might contribute to the improvement of epilepsy care/management.
Key words: physical activity, physical education, epilepsy, knowledge.

Avaliação do conhecimento de professores de educação física sobre epilepsia

RESUMO

Pessoas com epilepsia apresentam baixa participação em atividades físicas. Um importante problema nos cuidados da epilepsia é a falta de profissionais qualificados. Neste estudo apresentamos dados de uma pesquisa para avaliar o conhecimento de professores de educação física sobre a epilepsia. Um questionário foi respondido por 134 educadores físicos de ambos os sexos. Sessenta por cento dos profissionais acreditam que a crise epilética é uma descarga elétrica anormal do cérebro, 13% que a epilepsia é uma doença crônica cerebral que não pode ser curada ou controlada, 84% que pessoas que têm convulsões não necessariamente apresentam epilepsia e 5% que pessoas com epilepsia têm dificuldade de aprendizado. Em relação à experiência prévia do profissional, 61% presenciaram uma crise epilética e 53% tiveram acesso a alguma informação sobre epilepsia. Além disso, 28% dos profissionais possuíam amigo ou parente com epilepsia, 14% tinham um aluno com epilepsia e 29% já tinham socorrido alguém durante uma crise. Nossos achados revelam uma falta de conhecimento apropriado dos profissionais da área de educação física sobre a epilepsia. A melhora desse conhecimento pode contribuir para um adequado tratamento e cuidado da pessoa com epilepsia.
Palavras-chave: atividade física, educação física, epilepsia, conhecimento.

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The epilepsies are the most common serious neurological condition, affecting cognitive, emotional, and behavioral conditions, ability to work, social functioning, family stability and self-esteem of the patient. They are characterized by spontane-

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RESEARCH ARTICLE

The Use of New World Primates for Biomedical Research: An Overview of the Last Four Decades

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Animal experimentation contributes significantly to the progression of science. Nonhuman primates play a particularly important role in biomedical research not only because of their anatomical, physiological, biochemical, and behavioral similarities with humans but also because of their close phylogenetic affinities. In order to investigate the use of New World primates (NWP) in biomedical research over the last four decades (1966–2005), we performed a quantitative study of the literature listed in bibliographic databases from the Health Sciences. The survey was performed for each genus of NWP that has been bred in the National Center of Primates in Brazil. The number of articles published was determined for each genus and sorted according to the country from which the studies originated and the general scientific field. The data obtained suggests that Brazil is a leader in generating knowledge with NWP models for translational medicine. Am. J. Primatol. 71:1–7, 2010.

Key words: primates; New World primates; experimental models; biomedical research; translational medicine

INTRODUCTION

Interest in nonhuman primates (henceforth referred as "primates"), which can be traced as far back as 2500 BC when Egyptian kings bred baboons for religious purposes, has continued throughout history to the present [Nunes & Catão-dias, 2007]. With the development of modern biomedical research, primates increasingly have served as animal models for laboratory investigations searching for cures of human diseases. The popularity of primates in this research is due to their close biological, behavioral, and phylogenetic relationship to humans [Bantrop, 2001; Goodman & Check, 2002; Hau et al., 2000; Kaup, 2002; King et al., 1988; Sibal & Samson, 2001]. Inevitably, these research activities have impacted wild primate populations in that their scientific use has encouraged their removal from natural habitats. New World primate (NWP) exportation began in the 1940s and reached its peak in 1963, when the first commercial flight between Quito (Peru) and Miami (United State of America-USA) was established. Currently, approximately 30,000 Amazonian monkeys are exported annually for biomedical research [Renctas, 2001].

Primate breeding programs have appeared internationally to produce animals of known origin to meet research demands. Captive primate breeding occurs in a variety of settings, including free-ranging colonies on islands (using wild-caught animals) and caged colonies (using captive-bred animals) [National Research Council, 2003].

Depending on the setting, environmental conditions are controlled to varying degrees to meet species-specific housing and other requirements necessary to maximize the physical and psychological well being of the animals. Controlled breeding programs not only provide animal models for research to improve human health, but these programs also enable us to increase our knowledge of primates in their own right in order to understand their

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GOMES DA SILVA, Sérgio; DONÁ, Flávia; FERNADES, Maria José da Silva; SCORZA, Fúlvio Alexandre; SILVA, Antônio Carlos; ARIDA, Ricardo Mário. Aerobic physical exercise during development increase parvalbumin expression in the hippocampal formation of adult rats. In: **I Congresso IBRO/LARC de Neurociência da América Latina, Caribe e Península Ibérica**, 2008, Búzios - RJ, Brasil.

ABSTRACT: Although the effects of exercise on the central nervous system of adult animals have been well documented, little is known in the developing brain. In our laboratory, the expression of the calcium-binding protein parvalbumin (PV) has been used to visualize the changes in the hippocampal formation following exercise. To investigate whether our protocol of physical exercise would promote morphological changes in the hippocampal formation of rats in development, we performed an immunostaining study using PV. Male Wistar rats aged P21 (postnatal day-old) were divided into two groups: the exercise group, the control group. Animals of the exercise group were submitted to daily exercise in the treadmill between P21 and P60. Running time and speed gradually increased during the subsequent days, until reach 18 m/min during 60 min. After aerobic exercise program, the animals of all groups were sacrificed and Western analysis was performed. The PV density was enhanced significantly in hippocampal formation of rats submitted to aerobic treadmill exercise (~30%, 1.27 $\pm$ 0.1, p>0.0019) when compared to the control group (1.0 $\pm$ 0.001; Student's t-test). No difference in β-actin immunorreactivity was detected among the studied groups (p>0.05). These findings demonstrate that the protocol of physical exercise used in this study promote plasticity in hippocampal formation of adult rats trained during development.

GOMES DA SILVA, Sérgio; SIMÕES, Priscila dos Santos Rodrigues; ARAÚJO, Bruno Henrique Silva; TORRES, Laila Brito; ARRUDA, Renato Mortara; SCORZA, Fúlvio Alexandre; CAVALHEIRO, Esper Abrão; NAFFAH-MAZZACORATTI, Maria da Graça; ARIDA, Ricardo Mário. Influence of age and physical activity on the hippocampal kallikrein 6 expression. In: **XXIV Reunião Anual da Federeção de Sociedades de Biologia Experimental**, 2009, Águas de Lindóia - SP, Brasil.

Objective: Little is known about the physiological functions of kallikreins in normal tissues. However, accumulating evidence indicates that kallikreins might have diverse functions, depending on the tissue and circumstances of its expression. Many reports have proposed that alterations of kallikrein 6 (KLK6) might be involved in diverse diseases, such as certain types of cancer and neurodegenerative disorders. Additionally, it has been observed that KLK6 plays an important role in the progression of inflammatory diseases of the central nervous system. The purpose of present study was to investigate the effects of age and physical activity on the hippocampal KLK6 expression. **Methods**: Male Wistar rats of different ages were used: 8-week-old (n=5); 32-week-old (n=5); 72-week-old (n=5). To investigate whether physical activity would promote changes in hippocampal expression of KLK6, animals of 72-week-old group were submitted to physical exercise in the treadmill (Columbus instruments) during 7 days (14 m/min during 30 min daily). Animals of all groups were killed and samples prepared for immunofluorescence under confocal microscopy. **Results:** No KLK6 immunorreactivity was detected in hippocampus of rats with 8-week-old and 32-week-old. However, a robust KLK6 expression was found in hippocampus of rats with 72-week-old. Interestingly, physical exercise reduced hippocampal KLK6 expression in 72-week-old rats. **Conclusion:** Our findings demonstrated that hippocampal KLK6 expression occurs in old-age rats and this expression was reduced by physical activity. Researches in this topic are relevant for determining optimum exercise strategies, particularly for elderly people.

ARIDA, Ricardo Mário; GOMES DA SILVA, Sérgio; DONÁ, Flávia; FERNADES, Maria José da Silva; SCORZA, Fúlvio Alexandre; CAVALHEIRO, Esper Abrão. Physical exercise in rats submitted to multiple status epilepticus in the early period of life increases hippocampal parvalbumin expression. In: **28th International Epilepsy Congress**, 2009, Budapeste, Hungria.

Purpose: Seizures occurring during development can induce changes in cerebral maturation leading to epilepsy and cognitive deficit. Studies in adult animals have demonstrated a beneficial effect of physical exercise after an epileptic insult. The aim of our work was to study changes in neuronal plasticity in the adult brain following an aerobic exercise program in rats submitted to multiple *status epilepticus* (SE) in the early life using the pilocarpine model. To this purpose, we analyzed, in the adult animal, the occurrence of structural changes in hippocampal formation by means of an immunohistochemical approach utilizing the calcium-binding protein parvalbumin (PV) expression as marker of morphological changes. **Method:** Wistar male pups at postnatal day 7 (P7) were used in this study. The animals were bred in our laboratory and kept with the mother after birth under controlled temperature (21 ± 2°C) and light (12-hour light/dark cycle) conditions. Experiments were conducted according to the ethical rules for animal research at our university. The pups were randomly divided in four groups: exercise group (EX; n=5), control group (CTL; n=5), 3 SE group (SE; n=5) and 3SE exercise group (SEEX; n=5). Animals from 3 SE and 3SE exercise groups were treated with a single intraperitoneal injection of pilocarpine (Sigma, St. Louis, MO, U.S.A.) at 380 mg/kg for three consecutive days (P7 to P9). Control rats were injected with 0.9% saline under the same conditions. After weaning, animals from exercise and 3SE exercise groups were submitted to daily exercise program in a treadmill (between P21 and P60). At P60, animals of all groups were killed and PV immunoblotting procedures were performed. **Results:** Quantitative immunoblotting analysis showed that the PV density was significantly enhanced in hippocampal formation in exercise groups (EX = 1.27 $\pm$ 0.10; SEEX = 1.27 $\pm$ 0.06, $p<0.0006$) when compared to their respectively control groups (CTL = 1.0 $\pm$ 0.01; SE = 1.03 $\pm$ 0.12; ANOVA). **Conclusion:** These findings indicate that aerobic exercise program during early life period promotes neuroplastic changes in hippocampal formation of control animals as well as animals submitted to multiple SE.