UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
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PROGRAMA DE PÓS-GRADUAÇÃO EM NEUROCIÊNCIAS

EFEITO DO EXERCÍCIO FÍSICO AERÓBICO SOBRE O PADRÃO DE IMUNORREATIVIDADE DA SEROTONINA LOCALIZADA NOS NÚCLEOS DORSAL E MAGNO DA RAFE E NA MEDULA ESPINAL DE RATOS SUBMETIDOS À SECÇÃO DO NERVO CIÁTICO

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Orientadora:
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"Na imensidão da vida, não basta tentar ser, nem querer ser...
... A vida, é bem mais do que um curriculum vitae".

(autor desconhecido)

Resumo

Diversos estudos têm demonstrado que o sistema serotoninérgico participa ativamente da regulação do circuito nociceptivo e locomotor da medula espinal. A atividade física induz analgesia e é um dos tratamentos efetivos para a melhora da função sensorial e motora de indivíduos com lesão nervosa periférica. Estudos demonstram que o exercício físico ocasiona mudanças em diferentes neurotransmissores. Para melhor compreensão da relação entre exercício físico e alterações no sistema serotoninérgico, o presente estudo demonstra os efeitos do treinamento aeróbico em esteira ergométrica sobre o padrão de imunorreatividade à serotonina nos núcleos dorsal e magno da rafe, e na medula espinal lombossacral de ratos submetidos à secção do nervo ciático, mediante emprego de imunoistoquímica e densitometria óptica.

Para isto os animais foram divididos em seis grupos: (1) ratos sem qualquer manipulação experimental e sedentários (NS, n = 5); (2) ratos sem qualquer manipulação experimental e treinados (NT, n = 5); (3) ratos com secção do nervo ciático e treinamento aeróbico (SNTT, n = 5); (4) ratos com secção do nervo ciático e sedentários (SNTS, n=5); (5) ratos com nervo ciático isolado, mas não seccionado (sham) e submetidos ao treinamento (ST, n=5); e (6) ratos com nervo ciático isolado, mas não seccionado, e sedentários (SS , n=5).

Sete dias após o procedimento cirúrgico, os animais dos grupos com treinamento foram adaptados em esteira ergométrica diariamente por 10 minutos, durante 4 dias, a velocidade de 5 m/min. No quinto dia, esses ratos foram submetidos ao teste de esforço máximo, o qual consistiu em exercício graduado na esteira, com acréscimos da velocidade em 5 m/min a cada 3 min, iniciando com velocidade de 5 m/min e tendo como limite a intensidade máxima de cada animal. O valor máximo alcançado foi utilizado para planejamento do programa de treinamento aeróbico dos ratos. Após uma semana da secção do nervo ciático, os animais iniciaram o programa de treinamento aeróbico em esteira ergométrica, o qual teve duração de quatro semanas.

A densitometria óptica revelou aumento da imunorreatividade no citoplasma de neurônios do núcleo magno da rafe (NMR) nos grupos de animais SNTT e SNTS. No núcleo dorsal da rafe (DNR), o acréscimo ocorreu apenas no citoplasma de neurônios de ratos do grupo ST. No corno ventral da medula espinal, apenas o grupo SNTT teve aumento dos valores de densitometria óptica. Apesar de este grupo ter mostrado os maiores valores de densitometria óptica no corno dorsal, este acréscimo não foi estatisticamente significativo. O teste dos filamentos de Von Frey mostrou a presença de analgesia nos grupos de animais com lesão nervosa periférica e treinamento físico. O índice de funcionalidade do nervo ciático indicou recuperação apenas no grupo SNTT. Com base nesses resultados, pode-se sugerir que tanto o treinamento aeróbico em esteira como a lesão nervosa contribuem para o aumento da imunorretividade à serotonina mostrada neste estudo. Todavia, ainda é necessária a realização de estudos mais detalhados relacionando serotonina, exercício físico e lesão de nervo periférico para melhor entendimento das relações funcionais entre estes parâmetros.

Sumário

Lista de Abreviaturas

5-HT: Sertonina
PAG : Substância cinzenta periaquedutal
SNC : .Sistema Nervoso Central
SNP : Sistema Nervoso Periférico

Artigo

Acetyl-COA: Acetyl coenzima A
DRN: Dorsal raphe nucleus
DH: dorsal horn
DTNB: 5,5-dithiobis-(2-nitrobenzoic acid)
E: Experimental
EDTA: Ethylenediamine tetraacetic acid
ERK: Extracellular signal regulated kinase
5-HT: Serotonin
HCl: Hydrochloric acid
ITS: Intermediary toe spread
L4: 4^{th} lumbar vertebra of spinal cord
MET: Maximal exercise test
N: Normal
NIH: National Institutes of Health
NMR: Nucleus raphe magnus
OD: Optical density
PBS: Phosphate buffered saline
PBS-T: Triton X-100 diluted in phosphate buffered saline
PL: Print length
SFI: Sciatic functional index
TS: Toe spread
US: United States
VF: Von Frey

1. Introdução

1.1 Lesão nervosa periférica

Dor trata-se de uma experiência sensorial desagradável onde o estímulo nocivo é codificado como uma mensagem nociceptiva, sendo progressivamente transmitido e processado em centros nervosos superiores. Entretanto, além do componente sensorial, deve ser salientado o aspecto emocional, bem como as respostas vegetativas e reações psicológicas e comportamentais provocadas pela lesão tecidual (Millan, 1999; Byers & Bonica, 2001). Entre as diversas abordagens para o estudo da dor, podem ser citadas a secção de um nervo periférico e a indução de lesão tecidual ou processos inflamatórios locais. A lesão de um nervo periférico pode causar dor neuropática severa.

Além da dor neuropática, a lesão em nervos periféricos ocasiona perda total ou parcial de funções motoras, sensoriais e vegetativas devido à interrupção da continuidade dos axônios, degeneração das fibras nervosas distais à lesão, e eventual morte de neurônios axotomizados. Como conseqüência há um decréscimo na qualidade de vida dos indivíduos em decorrência dos danos em funções motoras e sensoriais (Fredericks, 1996; Vallat & Magy, 2005; Navarro et al., 2007). Existem diversas condições patológicas que afetam os nervos periféricos (Vallat & Magy, 2005). Estas são freqüentemente causadas por traumas agudos como esmagamento por compressão, contusões traumáticas ou secção por artefatos penetrantes (Fredericks, 1996; Xie et al., 2004).

Esta injúria nervosa periférica determina uma série de reações em neurônio sensorial. Ocorre a degeneração Walleriana que leva a liberação de fatores nos tecidos alvos e citocinas, sensibilizando o tecido nervoso adjacente e fibras nervosas não lesionadas, excitando e produzindo descargas ectópicas nos axônios adjacentes (Wall & Devor, 1983; Liu et al., 2002). Após a secção nervosa, além da degeneração Walleriana há formação de um neuroma

a partir do qual ocorre formação de brotos regenerativos de maneira irregular, que podem crescer e formar microneuromas ao longo do tronco nervoso distal em direção ao tecido alvo (Zimmermann, 2001).

A degeneração axonal e subseqüente brotamento após lesão promovem mudanças em conexões sinápticas do corno dorsal da medula espinal, as quais alteram o campo receptivo sensorial desses neurônios. Estas modificações estão envolvidas nos mecanismos da dor neuropática, e não se restringem apenas ao corno dorsal, mas também se propagam às regiões mais superiores do neuroeixo (Romanelli et al., 2004).

A lesão nervosa induz aumento de atividade espontânea em fibras aferentes primárias, levando a acréscimos na excitabilidade dos neurônios de segunda ordem do corno dorsal, sendo esta uma aferência contínua, com origem no neuroma e no corpo neuronal localizado no gânglio da raiz dorsal (Omana - Zapata et al., 1997). Estas mudanças na excitabilidade dos neurônios aferentes nociceptivos resultam na ativação de múltiplas proteínas cinases intracelulares com subseqüente fosforilação de canais iônicos (Romanelli & Esposito, 2004), sendo que as descargas ectópicas ocorrem por alterações em canais iônicos dessas regiões (Mcmahon et al., 1993; Omana - Zapata et al., 1997; Xie et al., 2004; Fuchs et al., 2007).

O aumento das respostas do corno dorsal da medula espinal aos estímulos aferentes, fenômeno denominado sensibilização central, ocorre devido à sensibilização dos neurônios que recebem aferências da região lesada, levando à hiperalgesia primária como também ao aumento da sensibilidade nas áreas intactas adjacentes, ocasionando hiperalgesia secundária (Schaible & Richter, 2004; Rahman et al., 2006).

O corno dorsal da medula espinal é o local da primeira sinapse no circuito nociceptivo quando o estímulo nocivo incide sobre a pele e os músculos dos membros e tronco, e é um poderoso alvo para a regulação desta transmissão, tanto para mecanismos locais como supraespinais (Heinricher et al., 2008). As projeções nociceptivas dos neurônios da medula

espinal projetam para diversas regiões do tronco encefálico e diencéfalo, incluindo o tálamo, núcleos hipotalâmicos, substancia cinzenta periaquedutal (PAG), região parabraquial, lócus ceruleus, formação reticular bulbar, assim como para o sistema límbico (Zimmermann, 2001; Romanelli et al., 2004).

A transmissão nociceptiva na medula espinal, por sua vez, está sob o controle de regiões supraespinais, o qual é proveniente de diversas áreas do encéfalo. O bulbo é um dos participantes do sistema de modulação nociceptiva, servindo como uma estação retransmissora, uma via final comum no recebimento e processamento da informação sensorial. Núcleos bulbares com importante papel nessa modulação são os núcleos da rafe e estruturas adjacentes. O bulbo recebe aferências da substância cinzenta periaquedutal e envia projeções para o corno dorsal da medula espinal, as quais finalizam nos funículos dorsolaterais, constituindo o canal eferente do sistema descendente modulador da nocicepção (Fields & Basbaun 1994; Rahman et al., 2006). Estas projeções convergem, em sua maior parte, para a porção dorsal do funículo lateral, terminando nas lâminas I, II, IV e V deste corno, e próximo ao canal central (Byers & Bonica, 2001). Junto com a substância cinzenta periaquedutal, o bulbo dá origem a redes neuronais engajadas na determinação de facilitações e/ou inibições descendentes no controle da nocicepção (Bee & Dickenson, 2007; Vanegas & Schaible, 2004). A PAG, por sua vez, conecta-se com o hipotálamo, estruturas límbicas corticais e amígdala, além de receber diretamente impulsos espinomesencefálicos. Essa região mesencefálica projeta para o núcleo magno da rafe, o qual envia impulsos para as lâminas I e II do corno dorsal da medula espinal (Wall & Melzack`s, 2006; Heinricher et al., 2008). Esta modulação descendente exerce alterações dinâmicas na entrada de estímulos nocivos provenientes de uma dada inflamação periférica. Estudos têm demonstrado que a ativação de regiões bulbares produz analgesia e tais neurônios antinociceptivos estão interligados com neurônios pré-simpáticos e pré-motores do bulbo, sendo possível que a ativação dessa região

possa coordenar respostas somatomotoras, autonômicas e sensitivas a um estímulo nociceptivo (Kerman et al., 2006). Mudanças neuroquímicas no bulbo estão relacionadas com o controle de estímulos provenientes de dor inflamatória. Essas mudanças neuroquímicas centrais parecem envolver o sistema serotoninérgico (Smith et al., 2005).

1.2 Serotonina

Diversos estudos têm demonstrado que o sistema serotoninérgico participa ativamente da regulação do circuito nociceptivo e locomotor da medula espinal (Barbeau & Rossignol, 1990; Crown & Grau, 2005). O aumento da neurotransmissão serotoninérgica facilita o impulso motor e inibe o processamento da informação nociva aferente, suprimindo a nocicepção (Lebars et al., 1988; Jacobs et al., 1997).

A serotonina (5-HT) é uma indolamina sintetizada a partir do aminoácido triptofano principalmente em neurônios localizados próximos à linha média do tronco encefálico, nos chamados núcleos da rafe. Os núcleos situados mais caudalmente no bulbo, denominados núcleos da rafe bulbar e núcleo magno da rafe, possuem projeções que finalizam na medula espinal (corno dorsal e coluna intermédio lateral) e se destinam ao controle do sistema nervoso simpático e da modulação nociceptiva (Auerbach et al., 1985; Hains et al., 2002; Jacobs et al., 2002). Na medula espinal, as maiores concentrações de 5-HT ocorrem nas lâminas I e II, onde finalizam os aferentes primários nociceptivos (Marlier et al., 1991; Rahman et al., 2006; Hains et al., 2002).

Um aspecto do envolvimento da serotonina no controle motor aparece na filogênese, onde em uma variedade de espécies de invertebrados a 5-HT participa de funções integrativas e de funções como controle postural, natação e alimentação, participando do controle em múltiplos níveis de motoneurônios, músculos e padrão de geração central (Jacobs & Fornal, 1995, Fornal et al., 1996; Schmidt & Jordan, 2000).

A atividade neuronal serotoninérgica está tonicamente elevada durante a execução de certos comportamentos, sugerindo que a 5-HT facilita o "comportamento" como um todo e não um ato motor particular ou grupo muscular que participa da ação (Jacobs et al., 2002). A distribuição da inervação do terminal axonal serotoninérgico do tronco encefálico e da medula espinal sugerem maior envolvimento da 5-HT em padrão de movimento grosseiro de músculos esqueléticos (Jacobs et al., 2002). As projeções de neurônios serotoninérgicos espinais emitem colaterais axonais para vários níveis da medula, os quais mostram uma forte relação com o impulso motor (Skagerber & Bjorklund 1985; Jacobs and Fornal, 1995). A atividade da serotonina encefálica possui relação com a atividade motora. Estudos mostram que esta indolamina influencia a atividade reflexa segmentar da medula, predominantemente inibindo motoneurônios (Kissel & Domino, 1950). Estudos prévios, onde foi empregada a administração sistêmica de agentes serotoninérgicos ou de seus precursores que cruzam a barreira hematencefálica, demonstraram efeito facilitador da 5-HT em reflexos monossinápticos de animais com lesões agudas na medula espinal. Agentes serotoninérgicos facilitam o reflexo flexor e este efeito é mais proeminente em ratos com lesão crônica do que com lesão aguda na medula espinal (Schmidt & Jordan, 2000).

O sistema serotoninérgico central tem ainda um importante papel na atividade motora tônica e repetitiva. Neurônios serotoninérgicos projetam densamente do tronco encefálico (núcleo palido e obscuro) para o corno ventral da medula espinal, onde se encontram motoneurônios (Holstege and Kuypers, 1987; Jankowska et al., 1997). Estudos de microscopia óptica mostram que varicosidades contendo 5-HT fazem contato sináptico com motoneurônios de diversos diâmetros (Schmidt & Jordan, 2000). Alguns resultados experimentais sugerem que células serotoninérgicas do núcleo dorsal da rafe participam do controle de vários aspectos da função motora (Veasey et al., 1997).

A 5-HT também está envolvida na modulação da atividade motora de músculos pélvicos. Sua liberação está aumentada no corno dorsal da medula espinal lombar durante atividade locomotora (Gerin et al., 2008). Ela também facilita a ativação de neurônios do trato espinocerebelar (Jankowska et al., 2000). Especula-se que a liberação de serotonina tenha um papel modulador na locomoção, aumentando a eficácia da pré-ativação de resposta motora, já que foram observadas mudanças na concentração extracelular de serotonina do corno dorsal durante atividade motora. (Jankowska et al., 2000; Gerin et al., 2008).

A concentração de serotonina decresce durante o período pós exercício, quando esta foi comparada com o período durante o exercício. Todavia, a concentração de serotonina permanece elevada durante os primeiro 60 minutos pós-exercício, quando este valor foi comparado com os demais obtidos nos períodos pós exercício (Gerin et al., 2008). Neste estudo foi sugerido que o exercício induz uma estimulação prolongada em neurônios do núcleo da rafe.

1.3 Exercício Físico

Diferente do que ocorre no sistema nervoso central (SNC), as lesões axonais do sistema nervoso periférico (SNP) freqüentemente resultam em maior grau de regeneração. Esta maior capacidade regenerativa é determinada principalmente pela presença de um ambiente permissivo ao crescimento axonal. O conhecimento de que as células de Schwann são capazes de permitir e dar suporte a regeneração axonal no SNP tem gerado a falsa noção de que nervos periféricos lesionados regeneram e reinervam com facilidade seus alvos. Entretanto, é clinicamente sabido que a recuperação funcional de pacientes após lesões de nervos periféricos é comummente incompleta ou até mesmo inexistente (Gordon et al., 2003). Terapias empregando programas de exercícios físicos são frequentemente utilizadas na

reabilitação de pacientes com neuropatias periféricas. Os exercícios físicos diminuem as complicações comuns às patologias do SNP, tais como as contraturas e fraqueza dos músculos desnervados, promovendo a recuperação funcional (Linderman et al., 1995).

A atividade física aumenta a função motora após lesão nervosa, tanto clinicamente como experimentalmente. Alguns estudos sugerem que a atividade física pode ser um tratamento efetivo para a melhora da função sensorial. A forma de atividade física comumente investigada é o treinamento de caminhada em esteira, que pode melhorar a função sensorial devido aos seus efeitos no sistema molecular envolvido com a função e transmissão sináptica (Hutchinson et al., 2004).

Na síndrome aguda do nervo ciático, o tratamento conservador consiste em repouso nas duas primeiras semanas após a lesão, administração de drogas antiinflamatórias e a realização de exercícios aeróbicos após este período inicial (Vroomen et al., 2000). Estudos clínicos têm sugerido que muitos pacientes, acometidos por síndrome aguda do nervo ciático, beneficiam-se com a realização de exercício físico (Linderman et al., 1995). A atividade física, aumentando a função motora após lesão nervosa, pode ser um tratamento efetivo para a melhora da função sensorial (Hutchinson et al., 2004).

O exercício induz analgesia. Isto tem sido demonstrado mediante o uso de uma diversidade de estímulos dolorosos, como calor, frio, pressão, estimulação elétrica e isquemia. A magnitude e a duração do efeito analgésico são variáveis. O efeito parece mais plausível após exercício de alta intensidade e quando estímulos nocivos permanecem fortes após a execução do exercício (O'Conner & Cook, 1999). O exercício físico com duração e intensidade elevadas determina analgesia, principalmente 30 min após sua realização (Hoffman et al., 2004).

Existe um decréscimo significativo na "dor" relatada pelos pacientes 5 minutos após a realização de 30 minutos de exercícios de alta intensidade. Nenhum efeito significativo foi

encontrado após exercício de baixa intensidade. Esse resultado apóia a hipótese de que existem alterações na percepção dolorosa após exercícios aeróbicos de alta intensidade e duração (Droste et al., 1991).

Uma hipótese amplamente aceita para explicar o mecanismo da analgesia induzida pela realização de exercício físico é a participação de substâncias opióides centrais nesse processo. Este envolvimento tem sido demonstrado mediante administração de um antagonista opióide, o naloxona, o qual bloqueia os efeitos analgésicos da atividade física (Bement & Sluka, 2005). Alguns outros estudos mostraram que o tratamento com naloxona não alterou o limiar nociceptivo após a realização de exercício físico (Droste et al., 1991; Koltyn, 2002). Nessas condições, estes autores postularam que o exercício físico induz analgesia não somente pela ação central de opióides, mas também mediante a ativação proprioceptiva. Os aferentes musculares ativados inibiriam o circuito central relacionado com a dor, modulando vias inibitórias descendentes. Todavia, parece ser necessário um período e uma determinada intensidade de atividade física, geralmente exercícios de alta intensidade e longa duração, para que esses impulsos aferentes possam agir (Meeusen, 1995; Hoffman, 2004; Ruble et al., 2005).

Está demonstrado ainda que o exercício físico altera a neurotransmissão central, inclusive a serotoninérgica (Meeusen & De Meirler, 1995). Como a 5-HT participa ativamente da regulação do circuito nociceptivo e locomotor da medula espinal (Barbeau & Rossignol, 1990; Crown & Grau, 2005), e o efeito analgésico do exercício físico não parece decorrer apenas da atividade de peptídeos opióides (Droste et al., 1991; Koltyn, 2002), o presente estudo mostra a possível relação entre exercício físico, sistema serotoninérgico e lesão do nervo ciático. Apesar da existência de muitos estudos envolvendo serotonina e seu papel na modulação nociceptiva e motora, é de grande importância à associação de estudos envolvendo lesão de nervo periférico, serotonina e exercício físico. O aprofundamento deste

conhecimento trará mais subsídios para o entendimento dos efeitos analgésicos do exercício físico após lesão de nervo periférico e sua associação com o efeito antinociceptivo da serotonina.

2. OBJETIVOS

2.1 Objetivo Geral

O presente trabalho teve como objetivo analisar os efeitos do treinamento aeróbico em esteira ergométrica sobre o padrão de imunorreatividade à serotonina nos núcleos dorsal e magno da rafe, e na medula espinal lombossacral de ratos submetidos à secção do nervo ciático, empregando para isto a técnica de imunoistoquímica e densitometria óptica.

2.2 Objetivos específicos:

-Determinar as alterações da sensibilidade mecânica, mediante emprego do teste dos filamentos de *Von Frey*, em ratos submetidos à secção do nervo ciático e ao treinamento aeróbico em esteira ergométrica.

-Determinar a recuperação funcional, através do índice de funcionalidade do nervo ciático, em ratos submetidos à secção do nervo ciático e ao treinamento aeróbico em esteira ergométrica.

-Determinar o padrão de imunorreatividade a serotonina nos núcleos da rafe (dorsal e magno), e nos cornos dorsal e ventral da medula espinal lombossacral de ratos submetidos à secção do nervo ciático e ao treinamento aeróbico em esteira ergométrica, mediante utilização de técnica de imunoistoquímica e quantificação por densitometria óptica.

3 Resultados

Este trabalho resultou no manuscrito do artigo científico que segue em anexo. Este artigo será submetido ao corpo editorial da revista *Neurochemical Research* para análise.

3.1 Effect of Treadmill Exercise on Medullary Rafe Nuclei and Spinal Cord Serotonin Immunoreactivity Following Sciatic Nerve Transection in Rats

Arthiese Korb[a,b], Leandro Viçosa Bonetti[a,b], Sandro Antunes da Silva[a,b], Simone Marcuzzo[a,b], Jocemar Ilha[a,b], Wania Partata[a,c], Maria Cristina Faccioni-Heuser[a,b]

[a] Programa de Pós-Graduação em Neurociências, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, RS, Brazil

[b] Departamento de Ciências Morfológicas, Instituto de Ciências Básicas da Saúde, UFRGS, Porto Alegre, RS.

[c] Departamento de Fisiologia, Instituto de Ciências Básicas da Saúde, UFRGS, Porto Alegre, RS.

Running head: Serotonin immunoreactivity in lesioned rats submitted to treadmill exercise

Maria Cristina Faccioni-Heuser(✉)

Departamento de Ciências Morfológicas, Instituto de Ciências Básicas da Saúde, UFRGS, Rua Sarmento Leite, 500. 90050-170 Porto Alegre, RS, Brazil

e-mail: heuser@ufrgs.br

Tel.: +55-51-3303599/3789

Fax: +55-51-33083092

Abstract

Serotoninergic system modulates nociceptive and locomotor spinal cord circuits. Exercise improves motor function and changes dopaminergic, noradrenergic, and serotonergic central systems. However, the direct relationship among serotonin, peripheral nerve lesion and aerobic treadmill exercise has not been studied. Using immunohistochemistry and optic densitometry this study showed that the sciatic nerve transection increased the serotoninergic immunoreactivity in neuron cytoplasm of the Magnus Raphe Nuclei of the trained- and sedentary-rats. In Dorsal Raphe Nuclei the increase only occurred in sedentary and sham-operated rats. In the spinal cord, the trained- transected-rat ventral horn showed significant changes, while the changes in dorsal horn was not significant. Von Frey's test indicated analgesia in all exercise-trained rats. The sciatic nerve functional index indicated recovery in trained group. Thus, the aerobic treadmill exercise training and nervous lesion appears contribute to changes of serotonin immunoreactivity.

Key words: peripheral nervous lesion; serotonin; aerobic exercise; pain; analgesia; functional recovery.

Introduction

Therapies using exercise are frequently indicated for peripheral neuropathy patients under rehabilitation. Exercise training improves motor function after clinical and experimental peripheral nervous lesion, and it can be considered an effective treatment of sensorial function [1, 2, 3, 4].

Studies with different noxious stimuli, such as heat, cold, pressure, electric stimulation, and ischemia, have showed that exercise induces analgesia. The analgesia intensity and duration are variable. It seems to be enhanced after high intensity exercise and with persistent noxious stimuli after exercise [5, 6].

It was recently demonstrated that endorphin levels are increased during exercise [7]. Other previous studies have shown that the treatment with naloxone, an antagonist opioid, has not changed the nociceptive threshold after exercise [8, 9]. Indeed, it is known that aerobic exercise changes dopaminergic, noradrenergic, and serotonergic central systems [10].

Serotonergic system modulates nociceptive and locomotor spinal cord circuits [11, 12]. Increased serotonergic neurotransmission may facilitate the motor impulse and inhibit the afferent information processing, which suppress nociception [13, 14]. The medulla is an important element and has a key role on nociception modulation system. It works as a retransmission station and a common end pathway of receiving and processing the sensorial information. Some medulla nuclei exert a key role on information processing such as the raphe nuclei and its adjacent structures [15]. Medulla sends projections to the dorsal horn of spinal cord, which ends in the dorsolateral funiculus on laminae I and II, consisting one afferent channel of the descending nociceptive modulation system [15, 16]. The increase of encephalic serotonin activity is also associated with the motor activity [17]. In addition,

serotoninergic brain stem neurons are densely projected through the ventral horn of spinal cord, ending in contact with spinal motoneurons [18, 19].

Although there are many studies considering serotonin in nociceptive [20,21,22,23] and motor [24,25,26] modulations, and exercise-induced analgesia [8,10,5,6,27,28], it is important to demonstrate the possible changes of spinal and supraspinal serotonergic nerves activity in animals submitted to peripheral nervous lesion associated with aerobic exercise training. Thus, this study showed the aerobic treadmill exercise training effects on serotonin immunoreactivity patterns in magnus and dorsal raphe nuclei and lumbosacral spinal cord of rats under sciatic nerve transection.

Experimental procedure

Animals

Experiments were conducted in adult male Wistar rats weighing 200–250g. All animal procedures were approved by the Ethics Committee of the Federal University of Rio Grande do Sul. Under anaesthesia (ketamine 80 mg/Kg and xylazine 2 mg/Kg) and sterile conditions, the right sciatic nerve was exposed and transected at mid thigh level. In order to expose the sciatic nerve in sham rats all surgical procedures involved in the experimental group were used except transection. For further comparisons a naïve group was included in which the animals did not undergo surgical manipulation.

The animals were randomly divided in 6 groups: (1) naïve and sedentary (NS, n =5); (2) naïve and trained (NT, n =5); (3) rats with sciatic transected and trained (SNTT, n =5); (4) rats with sciatic transected and sedentary (SNTS, n=5); and (5) rats under sciatic manipulation (sham) and trained (ST, n=5); (6) rats under sciatic manipulation and sedentary (SS , n=5).

Seven days after the transection, all animals were adapted on the treadmill during 10 minutes at 5 m/min for 4 days; on the fifth day, they were submitted to a Maximal Exercise Test (MET). The test consisted of a graded exercise on the treadmill, with speed increments of 5 m/min every 3 minutes, starting at 5 m/min and continuing up to the maximal intensity attained by each rat. The values attained in the MET were used to plan the endurance training program. Then, 1 week after the sciatic nerve transected, the animals of the endurance trained, groups began the exercise training.

Endurance Training

The endurance training program was performed on a treadmill designed for human use (Runner, Brazil) and modified for use by rats. This training program consisted of running on the treadmill for 20 min on the first day, this period was progressively increased every day up to 50 min on the fifth day and 60 minutes in the next 4 weeks. Each training session included a warm-up period of 5 minutes running at 30% of the maximal speed reached in the MET (5.5 m/min), 10 to 50 minutes running at 45% to 55% (~9 m/min) and 5 minutes recovery at 30% again (5.5 m/min), 5 sessions per week, once a day during 4 weeks. This training program was considered a moderate-intensity endurance regime, because the animals ran for a long time at 45% to 55% of the maximal speed reached in their MET, that is, about 9 m/min.

Analysis of Hindlimb Motor Function

All animals were submitted to a series of motor activity 24h after transected, 48h after transected, 1 day before (pretraining), and 1, 2, 3 and 4 weeks after completing the physical training program. It should be noted that the first evaluation (pretraining) was performed 2 weeks after the sciatic nerve transection. Recovery of right hindlimb locomotor activity was

considered proof of adequate muscle reinnervation and functional recovery post nerve lesion and was monitored by analysis of the free-walking pattern. This method was originally proposed in 1982 and describes an index based on measurements of the footprints of walking rats, which provides a reliable and easily quantifiable method of evaluating the functional condition of the sciatic nerve. For this test, the rats were trained to walk over a white sheet of paper covering the bottom of a 100-cm-long, 8.5-cm-wide track ending in a dark box. Afterward, the animals had their ventral hind feet painted with dark dye, and then they were placed on the track to walk. The rats' footprints were used to determine the following measurements: (1) distance from the heel to the third toe, the print length (PL); (2) distance from the first to the fifth toe, the toe spread (TS); and (3) distance from the second to the fourth toe, the intermediary toe spread (ITS) (Ilha et al., 2007). The 3 measurements were obtained from the experimental (E) and normal (N) sides. Several prints of each foot were obtained on each track, but only 3 prints of each foot were used to determine the mean measurements on the experimental and normal sides. Then, these means were included in the Sciatic Functional Index.

Sciatic Functional Index (SFI) Formula

$$SFI = -38.3\ (EPL - NPL) / NPL + 109.5\ (ETS - NTS) / NTS + 13.3\ (EIT - NIT) / NIT - 8.8$$

The result obtained was considered an index of the functional conditions of the sciatic nerve, where zero (±11) represents normal function and about –100 represents the loss function

Mechanical Hypersensitivity Analysis

All animals were subjected to a series of sensibility assessments 1 day before transected , 1 day before (pretraining) and 1, 2, 3 and 4 weeks after completing the physical training programs. Responses of the ipsilateral hind paw to a range of applied innocuous Von Frey Filaments (VF) (North Coast Medical, Inc, USA). Following a period of acclimation in individual, clear, acrylic cubes, mechanical sensory thresholds were determined by paw withdrawal to VF 1g, 6g, 8g and 15g. Each filament was applied to the plantar surface of the paw with enough force to cause buckling, and for each animal this was repeated 10 times on each paw. The number of lifts in response to each of the filaments was noted for each paw. Paw withdrawal threshold was defined as the minimum force in grams required to elicit a withdrawal reflex of the paw. Only robust and immediate withdrawal responses produced by stimulus were counted. The number of responses obtained was transformed in percentage through the formula [(number of responses obtained ÷ number of trials) x100] and, in the end, a mean per each group was calculated.

Immunohistochemical Procedure

The rats of the different groups were intracardically perfused with a brief saline flush, the soleus muscle were rapidly excised, weighed, and frozen in liquid nitrogen, then they were intracardically perfused with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The lumbosacral spinal cord and brain stem were quickly dissected out, immersed in the same fixative solution for 4 h at room temperature and then cryoprotected in 15% and 30% sucrose solutions in phosphate buffer at 4º C. Coronal sections (50 µm) were obtained on a cryostat (Leitz Digital 1720) and collected in cold phosphate-buffered saline (PBS). The sections were then treated with 3% hydrogen peroxide in10% methanol for 30 min, washed with PBS for 30 min and incubated for 30 min in 3% normal goat serum in PBS containing 0.4% Triton X-100 (PBS-T). The sections were incubated overnight with gentle stir at 48h in 4^{o} C in a polyclonal

antibody to rabbit 5-HT (diluted 1:3500, Sigma USA). The primary antibody was then removed and the sections washed in PBS-T for 30 min. Then, the sections were immersed in a secondary antibody (anti-lgG, Sigma), diluted 1:100 in PBS-T, for 1 hour at room temperature with gentle stirring. After washing with PBS-T for 30 min, a soluble complex of horseradish peroxidase rabbit anti-horseradish peroxidase diluted 1:100 was applied for 2 hours at room temperature. The samples were then washed in PBS, incubated in a solution of 3,3,diaminobenzidine tetrahydrochloride (60 mg/100 mL, Sigma USA) and 0.005% v/v hydrogen peroxide in PBS. The sections were washed, mounted onto gelatinised slides, dehydrated, cleared and covered with Entellan (Merck) and coverlips. Specific immunostaining was abolished when the primary antibody was omitted from the staining sequence. Sections were examined and photographed with a Nikon Optiphot-2 microscope equipped with a Nikon FX-35DX camera.

Optical Densitometry

In order to measure the intensity of the reaction product of 5-HT immunohistochemistry (semi quantitative analysis), the regional and intracellular optic densitometry was made [29]. A Nikon Eclipse E 600 (40OX) microscope coupled to a USB 2.0 Digital Camera Eyepiece (DCE-2, China), was employed. All images were saved as Tiff files and analysed using ImageJ 1.40 software (Wayne Rasband, National Institutes of Health, USA). Images, RGB (24 bit) color images (640x480 pixels) were converted to 8-bit grayscale images (0–255 gray levels). All lighting conditions and magnifications were held constant and the investigator was unaware of the experimental groups. A reference image of an empty field was recorded and ImageJ's Calculator Plus Plugin 'divide' operation was used for correction of unequal illumination (shading correction). The optical densities of the areas of interest (AOI) were measured in the form of uncalibrated optical density [10/log(255/255-pixel value)].

Background staining was subtracted from optical density measurements using averaged values of tissue sections where primary antibody was omitted. The measurements were done by placing a 8053.87 mμ² area square (AOI) that delimitated a small region of interest in the I and II laminae in dorsal horn of spinal cord. This square has also delimitated a region in the IX lamina of the spinal cord ventral horn. Twenty measures were used for the right side and more 20 for the left side for each rat in the two regions cited before. For dorsal and magnus raphe nuclei measurements, a 3.25 mμ² area square (AOI) was placed in four different regions of each immunoreactive neurons cytoplasm. Ten neurons of each nucleus per rat were analyzed. The rat atlas Paxinos and Watson (1986) was used as a reference for anatomical classification. The results of the animals were compared between groups. The results obtained represent the mean of all pixels in the selected area.

Citrate Synthase Enzyme Activity

Citrate synthase activity, an index of oxidative capacity, was determined in soleus muscle by measuring groups with 5,5-dithiobis-(2-nitrobenzoic acid; DTNB). Approximately 100 mg muscle was homogenized in PBS and centrifuged for 10 minutes at 1000 x *g*, 4 ^{o} C. The medium assay contained 1 mmol/L DTNB dissolved in 10 mL of 1 mol/L Tris-HCl (pH 8.1), 10 mmol/L Acetyl-CoA and solution containing 1 mmol/L EDTA/Triton X 100 0.05%, pH 7.4. Citrate synthase reaction was started by the addition of 0.5 mmol/L oxaloacetate dissolved in 0.1 mol/L Tris/HCl. The absorption was read spectrophotometrically at 412 nm, and the results are expressed as nmol/min/mg protein.

Statical Analysis

Mechanical hypersensitivity (Von Frey Filaments) and Hindlimb Motor Function (IFC) were analysed using ANOVA for repeated analyses followed *post-hoc* Duncan. The immunohistochemistry mensuraments and the citrate syntase activity were analysed using two way ANOVA followed by Duncan *post-hoc*. Data were run on Statistica software package, with significance set at $P \leq .05$. All means are presented ± standard error of the mean (SEM).

Results

The groups NS, NT, SS, ST, SNTT, and SNTS were statistically compared. Since the results obtained in NS, NT, SS, and ST were not significantly different, the figures demonstrate only SS, ST, SNTT, and SNTS groups, permitting an adequate visualization of the results.

Von Frey's Test

It was demonstrated through Von Frey's Test that the innocuous stimulus responses on 1g and 4g filaments were not different between experimental groups (Fig 1A). However, in the fourth assessment, which corresponds the second training week, it was observed similar responses between SNTT, SS, and ST groups in 6g (Fig 1B), 8g (Fig 1C), and 15g (Fig 1D) filaments. These values were significantly lower than the SNTS group.

Sciatic Functional Index

After surgery, the SFI values were lower in sciatic nerve transected groups (SNTT and SNTS) compared with sham groups (SS and ST). In SNTT group, the functional index demonstrated high values compared with SNTS after the second and the third training weeks.

The values were not significantly different compared with sham groups. SNTS group has similar values compared with SNTT after the seventh week post peripheral nervous transaction (Fig 2).

Serotonin (5-HT) Immunoreactivity and Optic Densitometry

Spinal Cord

The ventral horn of the spinal cord showed scattered serotonin immunoreactivity in all groups. This reaction was always strong (Fig 3A, B). The densitometric analysis in this region has not detected significant modifications elicited by peripheral lesion in all groups in both ipsi and contralateral sides (Fig 3C). However, SNTT group has significant bilateral increase of 5-HT immunoreactivity when compared with SNTS, SS, and ST groups.

In the dorsal horn of the spinal cord, serotonin immunoreactivity was observed in superficial lamina in all experimental groups (Fig 4A, B). The densitometric analysis of this region has not detected bilateral significant changes induced by peripheral lesion in all groups (Fig 4C). The analysis of optic densitometry results of dorsal horn superficial portion has not shown significant changes in all experimental groups (Fig 4C). However, the SNTT group showed the highest values of the optic densitometric values.

Raphe Nucleus

In NMR, 5-HT immunoreactivity was situated in the neuronal cytoplasm with 14,6 μm average diameter in all experimental groups. This reaction was always strong (Fig 5C); however, the number of immunoreactive neurons was low in all groups (Fig 5A, B). The densitometric analysis has demonstrated the increased immunoreactivity in these neurons'

cytoplasm of SNTT and SNTS groups. The difference was statistically significant when compared with ST and SS groups (Fig 5D).

On the other hand, the DRN, has a higher number of neurons with strongly immunoreactive cytoplasm in all groups (Fig 6A, B, C). These neurons have 15,4 μm in average diameter. The optic densitometry results have shown increased serotonin immunoreactivity in neuronal cytoplasm of ST rats when compared with SS, SNTT, and SNTS groups (Fig 5D).

Citrate Synthase

The citrate synthase enzyme activity in the soleus muscle was increased in both groups submitted to aerobic exercise training (ST and SNTT), and it was statistically elevated compared with sedentary groups (SS and SNTS) (Fig 7).

Discussion

This study has demonstrated the effect of the aerobic treadmill exercise on 5-HT immunoreactivity in magnus and dorsal raphe nuclei and lumbosacral spinal cord of rats submitted to sciatic nerve transection. Many studies have shown modifications of serotonin activity in the central nervous system induced by peripheral nervous injury [11, 30, 31, 12, 32,15]. Moreover, it is known that exercise training modifies 5-HT activity in different central nervous system regions. Brown [33] has observed increased serotonin concentration in rat midbrain and cortex eight weeks after treadmill exercise training. In addition, four weeks swimming training has also increased 5-HT and 5HIAA (main serotonin metabolite) concentrations in the brain stem of trained rats [30]. These substances were also elevated in the spinal cord lateral funiculus, at L4 level, after aerobic exercise [34]. In our study, it was

verified citrate synthase activity increase in exercise-trained groups, and it might suggest that the enhanced serotonin immunoreactivity of lesioned and trained rats in magnus raphe nucleus and spinal cord might be influenced by the exercise effect on this indolamine. According Jacobs et al [17], the analgesia caused by 5-HT in the medulla is not evoked by dolorous stimuli or analgesic medicines, but might be stimulated by motor activity. In this regard, the serotonin-induced analgesia could be secondary to the increased serotoninergic activity associated with the motor activity, which might be the primary neural activity determinant.

It was previously demonstrated that magnus raphe nucleus projects to the dorsal horn of the spinal cord [35, 36, 31]. It could explain the bilateral increase of dorsal horn immunoreactivity in spinal cord. Several studies have shown that the bulbospinal serotoninergic system activation inhibits noxious-stimulated responses [37, 38, 31, 39, 15]. Thus, it is plausible the hypothesis that the serotonin increase observed in the magnus raphe nucleus could be associated with the decreased responses to innocuous stimuli of the animals with sciatic nerve transection. In fact, serotonin immunoreactivity increased in maguns raphe nucleus neurons of the sciatic-transected trained and sedentary groups. It could be indicating that the immunoreactivity change in these neurons is a consequence of the peripheral nervous lesion and it is not evoked by exercise. However, it is interesting the fact that sedentary rats with sciatic nerve transected have not reduced Von Frey's test response. This result can indicate that exercise-induced analgesia might be also related with other mechanisms. Indeed, previous studies have demonstrated that exercise increases endogenous opioids release [10, 7]. These molecules enhance serotonin release in the telencephalon through the selective activation of serotoninergic neurons of dorsal and medium raphe nuclei [40, 41]. Thus, it is possible that this mechanism might be occurring in the animals of our study. If it occurs,

exercise-trained rats analgesia might be evoked by opioids, serotonin, and probably other factors, which can determine the different levels of Von Frey's test sensibility observed here.

The serotonin immunoreactivity was also increased in the dorsal raphe nucleus. However, it was restricted to sham animals submitted to sciatic manipulation and aerobic treadmill exercise training. Considering the results obtained in sham sciatic manipulation groups, it can be suggested that dorsal raphe nucleus serotonin immunoreactivity increase appears associated with the motor activity. Some studies have shown that serotoninergic cells of this specific nucleus are related with some aspects of motor control [24]. It is also demonstrated that dorsal raphe nucleus cells send projections to the basal ganglia, cerebellum, and motor areas of the cortex, which are related with various frontal cortex responses during locomotion [42, 24]. However, it is interesting the fact that dorsal raphe nucleus immunoreactivity was not modified in rats under sciatic nerve transection and aerobic exercise training. If exercise training was the main factor causing this modification, it should also occur in lesioned and exercise-trained animals. At the moment, this result can not be explained. Since it was previously demonstrated that aerobic exercise training modifies dopaminergic, noradrenergic, and serotoninergic central systems [10], these changes probably has a role in this experimental condition.

Interestingly, it was also observed in our study the serotonin immunoreactivity increase in ventral horn of the spinal cord in animals submitted to sciatic nerve lesion and exercise. A recent study has demonstrated that motor activity induces serotonin increase in ventral and dorsal horns of spinal cord [26]. Our results did not shown statistically significant changes in dorsal horn of trained and transected rats. However, the highest optic densitometric values occurred in this region. It needs to be considered that the lack of significant changes in dorsal horn resulted of the animal number and temporal differences. Since dorsal horn had highest value in SNTT rats, it appears plausible to suggest that serotonin immunoreactivity

changes in the dorsal and ventral horns of lesioned and exercise-trained animals can be attributed to the exercise training. On the other hand, if exercise training as the main determinant of this effect, it is interesting the absence of significant changes in these regions of sham animals submitted to the same exercise protocol. In this regard, it is possible that the observed modifications of the spinal cord were also consequences of peripheral nervous lesion. The changes only occurred in lesioned animals. It is known that peripheral axonal lesion induces ectopic discharges in the primary neuron, which is related to changes of ionic channels [43, 44]. In cat spinal cord, the high frequency stimulation of sciatic nerve evoked increase in monoamines concentrations [45].

It is also necessary to consider the hypothesis that regenerative processes might contribute to the modifications observed in the spinal cord. Studies have shown that aerobic exercise can improve peripheral nerves regeneration [46, 47, 48]. It was also demonstrated that treadmill exercise facilitates sciatic nerve regeneration after experimental traumatic lesion [49]. In our study, the SFI revealed functional recovery of this nerve in lesioned animals submitted to treadmill exercise training for four weeks. It is possible that this result was associated with the exercise effect on sciatic nerve regeneration. It can be emphasized that the extremities of transected nerve remained free in the animals with peripheral nervous transection, which has not impeded the nervous regeneration process.

In conclusion, aerobic treadmill exercise training change serotonin immunoreactivity pattern in spinal cord and dorsal and magnus raphe nuclei of rats submitted to sciatic nerve transection. These changes might exert a key role on analgesia effect elicited by the exercise, and it can enhance the sciatic nerve functional recovery. However, the modifications may not be an exclusively consequence of the exercise training. The peripheral nervous lesion also appears contribute to the modifications. It was also difficult to distinguish each mechanism's effect on the modifications observed in these regions of the rat nervous tissue. Thus, further

studies relating serotonin, exercise training, and peripheral nervous lesion are necessary to permit the better understanding of the functional and temporal association of these factors.

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Legends of Figures

Fig. 1 – Von Frey's test responses of rats submitted to sciatic nerve transection or manipulation and aerobic treadmill exercise training for four weeks. The filaments were calibrated in 1g, 4g (A), 6g (B), 8g (C), and 15g (D). The Y axle shows the test positive response percentage during the ten test executions. In the X axle the following assessments are represented: 1: 24 hours after surgery; 2: 24 hours before the beginning of exercise training protocol; 3: after the first week of aerobic exercise training protocol; 4: after the second week of aerobic exercise training protocol; 5: after the third week of aerobic exercise training protocol; and 6: after the fourth week of aerobic exercise training protocol. SS (sedentary and sciatic nerve manipulation group), ST (exercise-trained and sciatic nerve manipulation group), SNTS (sedentary and sciatic nerve transected group), SNTT (exercise-trained and sciatic nerve transected group). a: indicates the significant difference of SNTS group compared with SS and ST groups; b: indicates significant difference comparing SNTT with SS and ST groups; c: indicates significant difference between SNTS and SNTT groups. Repeated measurement ANOVA followed by Duncan post hoc test, $p<0.05$. Data represent mean ± SEM (n=5/group).

Fig. 2 – Sciatic nerve functional recovery index (SF-Index) in rats submitted to the sciatic nerve transection or manipulation and aerobic treadmill exercise training for four weeks. In the Y axle, the result is expressed in units, and 0 (± 11) indicates normality and –100 the total absence of functionality. In the X axle, the following evaluations are represented: 1: 24 hours after surgery; 2: 48 hours after surgery; 3: one day before the beginning of exercise training protocol; 4: after one week of exercise training protocol; 5: after two weeks of exercise training protocol; 6: in the end of the third week of exercise training protocol; and 7: after the

fourth week of exercise training protocol. SS, ST, SNTS, SNTT, a, b, c (see Fig1 legend). Repeated measurement ANOVA followed by Duncan post hoc test, $p<0.05$. Data represent mean ± SEM (n=5/group).

Fig. 3 – Serotonin immunoreactivity in the lumbosacral ventral horn of spinal cord of SNTT group (A,B) Wistar rats and the immunoreactive optic densitometry measures (C). Note the strong immunoreaction in the soma of motor neurons (B). The optic density is represented in the Y axle and the different groups in the X axle. SS, ST, SNTS, SNTT (see Fig.1 legend). * Significant difference (two-way ANOVA followed by Duncan post hoc test, $p<0.05$). Data represent mean ± SEM (n=5/group). Calibration bar = A: 200 μm, B: 100 μm.

Fig.4 – Serotonin immunoreactivity in superficial laminas of lumbosacral spinal cord dorsal horn of SNTT group (A,B) Wistar rats and immunoreactive optic densitometry measures (C). Note the predominance of the immunoreactivity in the spinal cord superficial laminas (B). The optic density is represented in the Y axle and the different groups in the X axle. SS, ST, SNTS, SNTT (see Fig.1 legend). There was not significant difference with two-way ANOVA test followed by Duncan post hoc test. Data represent mean ± SEM (n=5/group). Calibration bar = A: 200 μm, B: 100 μm.

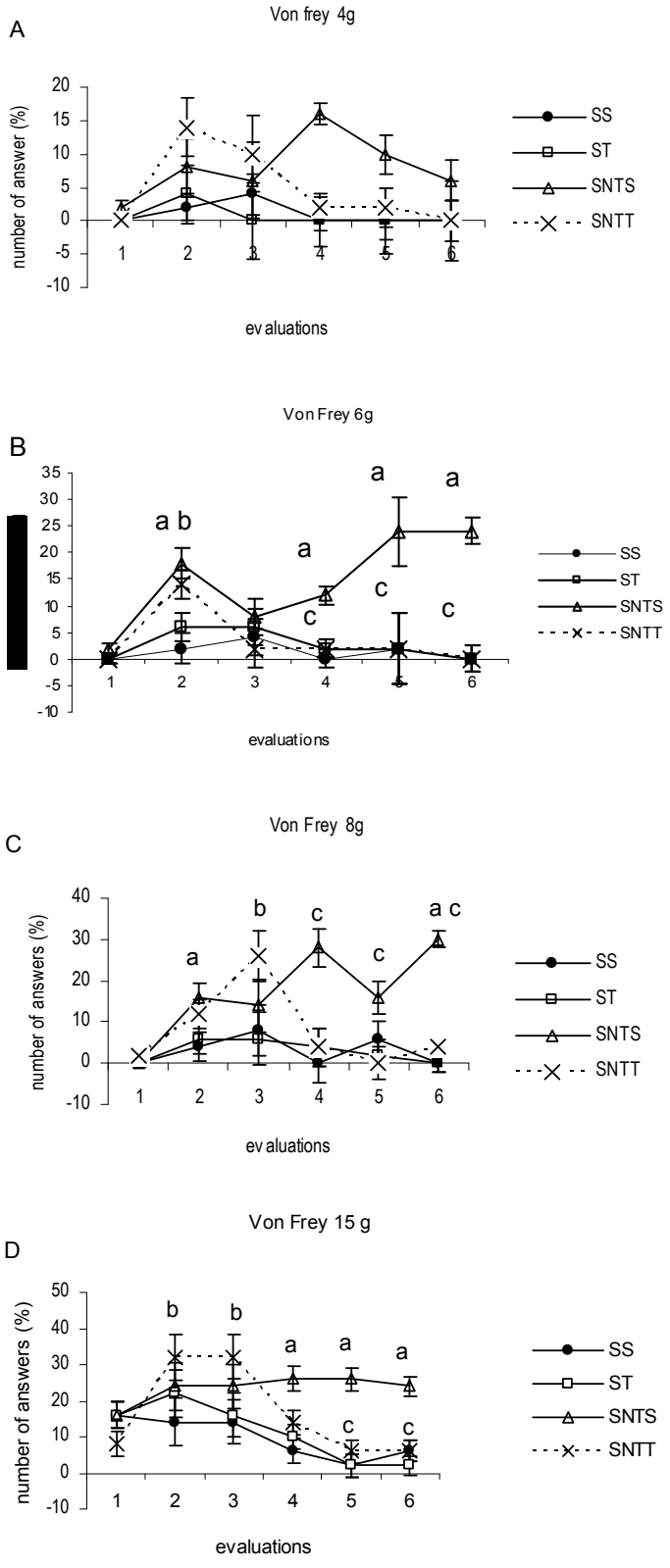
Fig. 5 - Serotonin immunoreactivity in the magnus raphe nucleus of SNTT group (A,B,C) Wistar rats and the immunoreactive optic densitometry measures in the cytoplasm (D). Note the low number of neurons (B) strongly immunoreactives (C) in this nucleus. The optic density is represented in the Y axle and the different groups in the X axle. SS, ST, SNTS, SNTT (see Fig.1 legend). * Significant difference (two-way ANOVA followed by Duncan

post hoc test, $p<0.01$). Data represent mean ± SEM (n=5/group). Calibration bar = A: 200 μm, B: 100 μm, C: 10 μm.

Fig. 6 - Serotonin immunoreactivity in the dorsal raphe nucleus of Wistar rats (A,B,C) and the immunoreactive optic densitometry measures in the cytoplasm (D). Note the high number of neurons (B) strongly immunoreactives (C) in this nucleus. The optic density is represented in the Y axle and the different groups in the X axle. SS, ST, SNTS, SNTT (see Fig.1 legend). * Significant difference (two-way ANOVA followed by Duncan post hoc test, $p<0.05$). Data represent mean ± SEM (n=5/group). Calibration bar = A: 200 μm, B: 100 μm, C: 10 μm.

Fig. 7 – Citrate synthase enzyme activity in Wistar rats soleus muscle (Y axle). The different experimental groups are represented in X axle. SS, ST, SNTS, SNTT (see Fig.1 legend).* Significant difference (two-way ANOVA followed by Duncan post hoc test, $p<0.05$). Data represent mean ± SEM (n=5/group).

Figure 1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evaluations

Figure 2

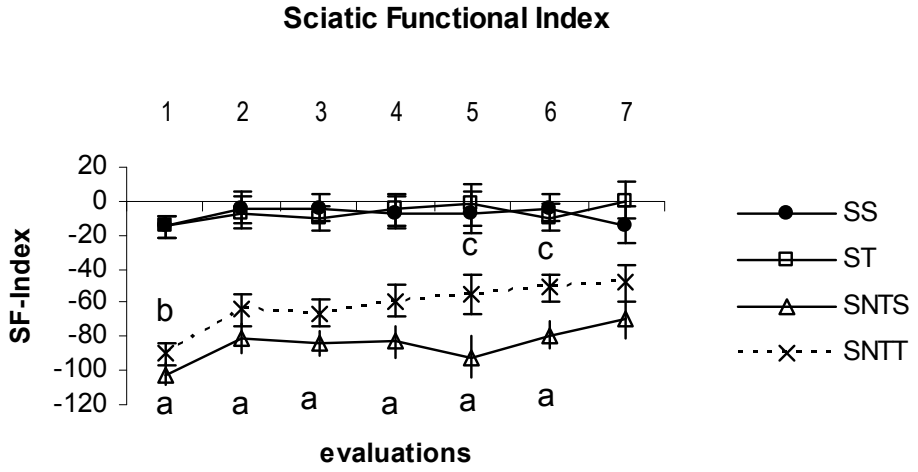
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Figure 3

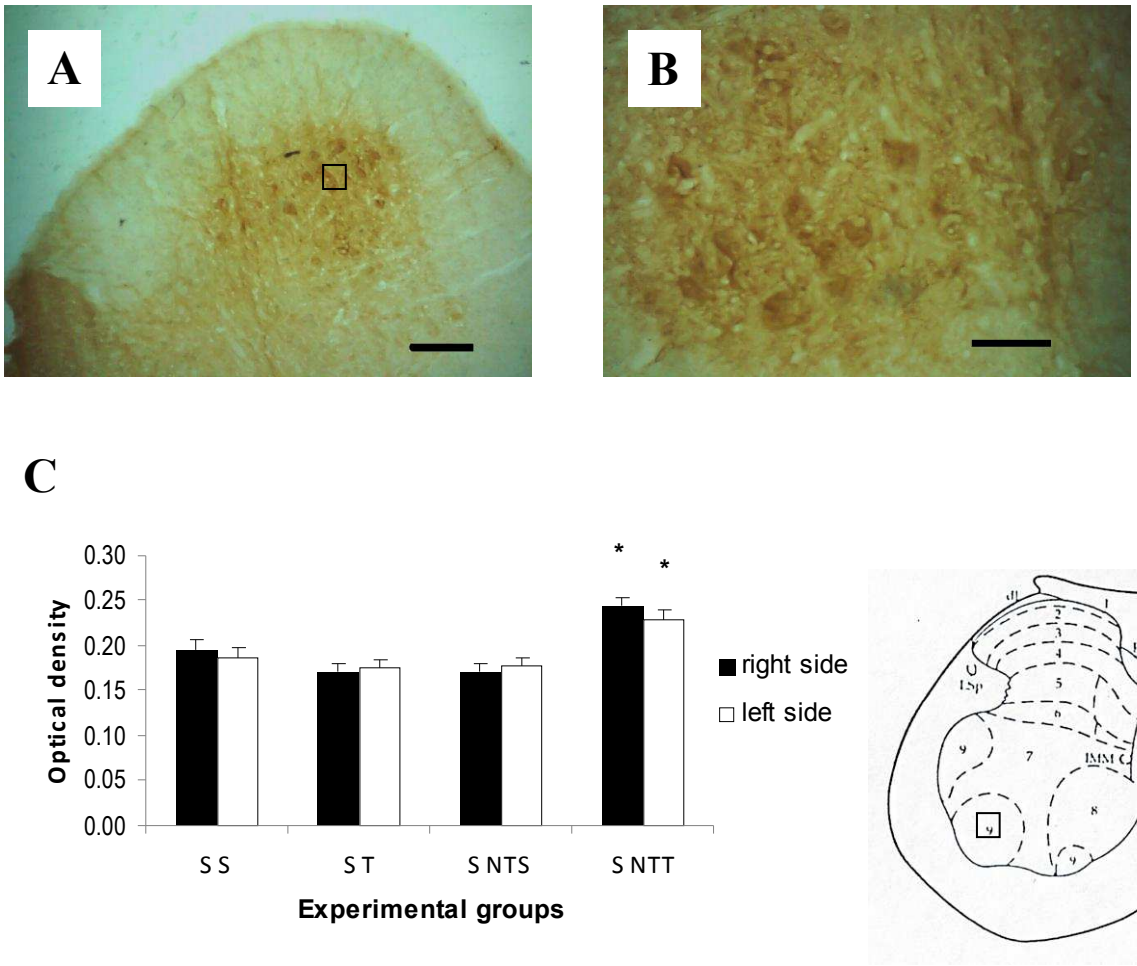
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Figure 4

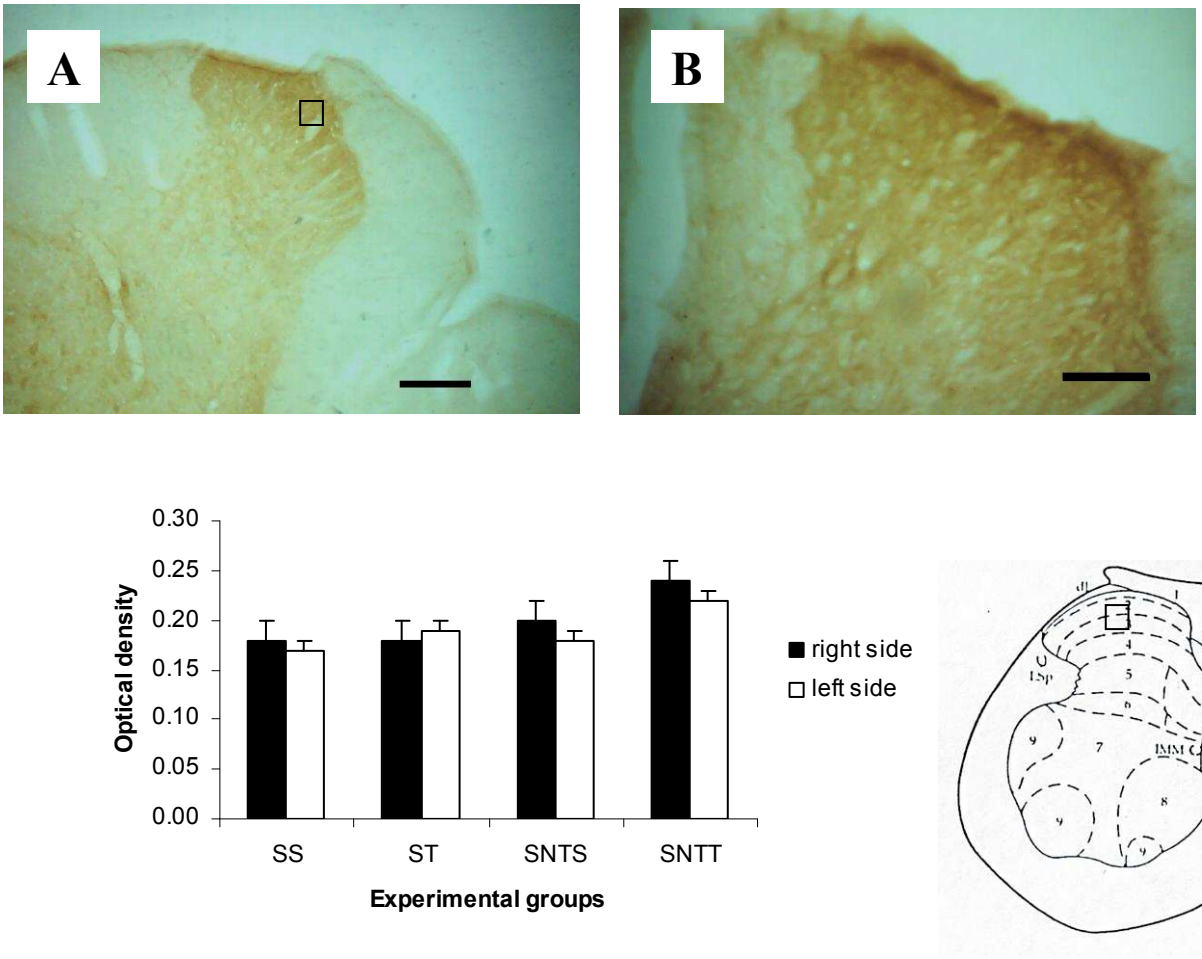
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Figure 5

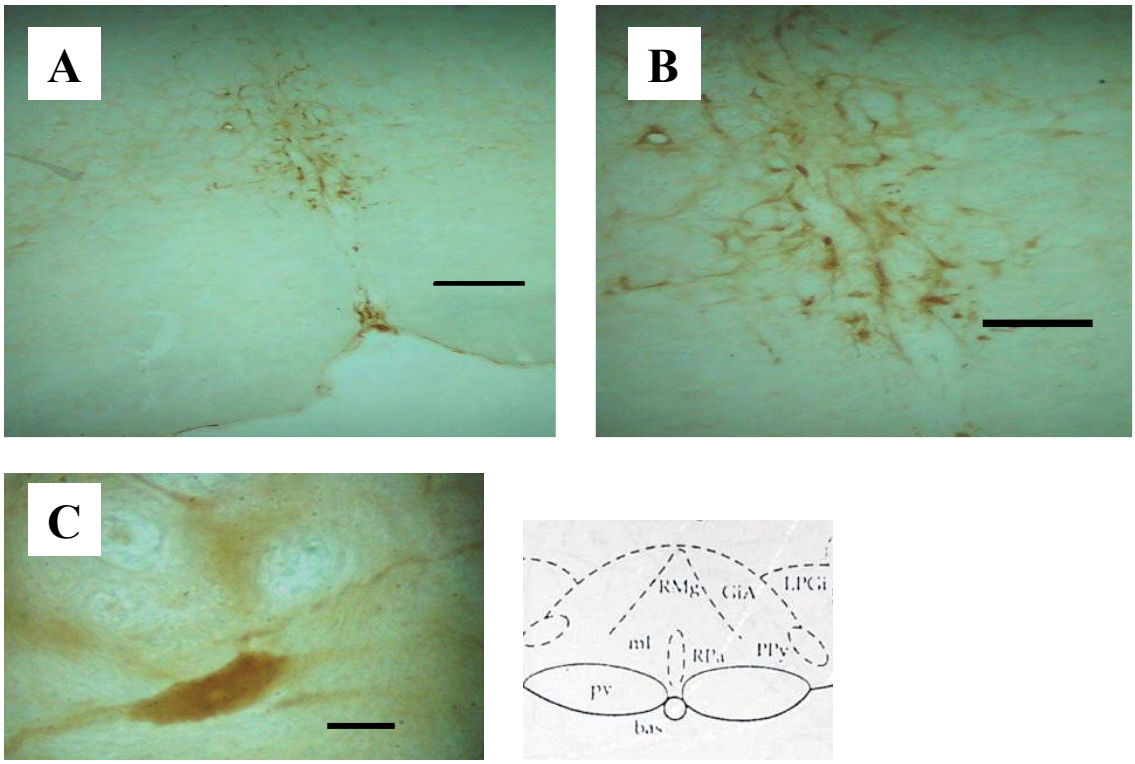
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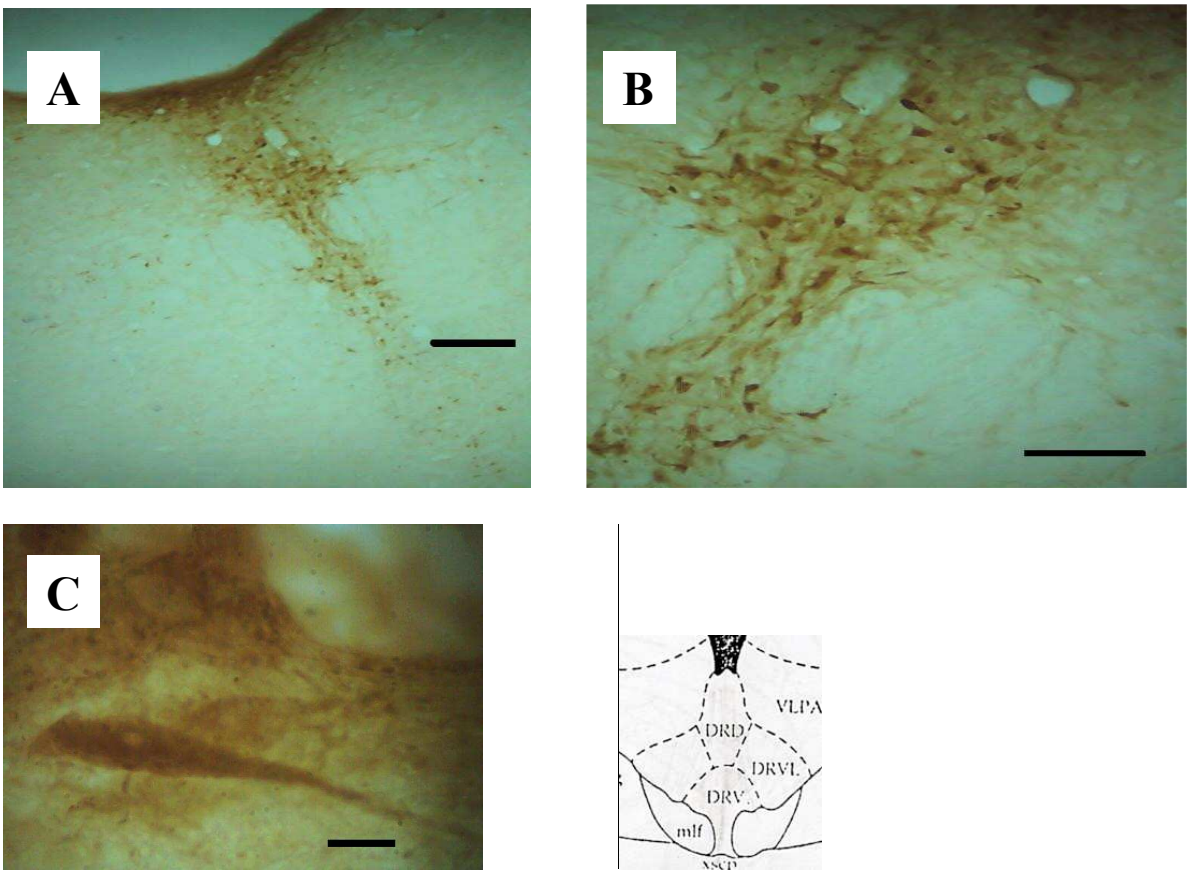
Figure 6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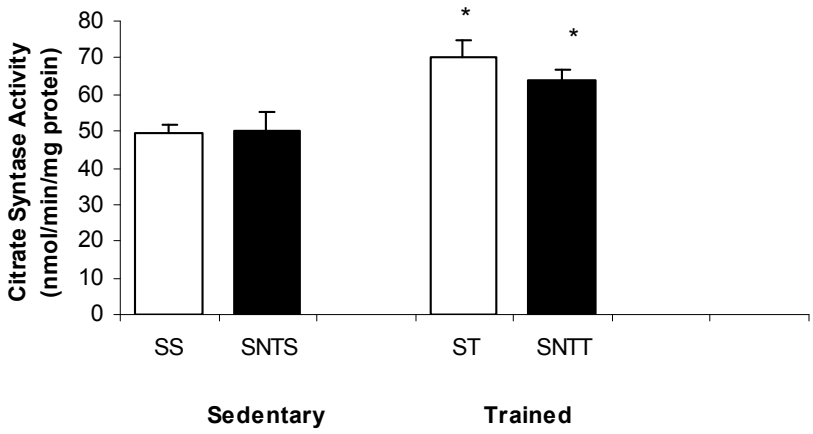
Experimental Groups

Figure 7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4. Conclusões e Perspectivas

Os dados apresentados nesse estudo sugerem que o treinamento aeróbico em esteira durante quatro semanas e/ou a secção do nervo ciático são capazes de aumentar o padrão de imunorretividade da serotonina localizada na medula espinal e nos núcleos magno e dorsal da rafe de ratos. Contudo, não foi possível estabelecer o papel de cada uma dessas condições experimentais nas mudanças observadas nestas regiões do tecido nervoso.

O acréscimo na imunorreatividade da serotonina da medula espinal e dos núcleos da rafe considerados nesse estudo pode estar contribuindo com os efeitos analgésicos do exercício aeróbico em esteira, e este tipo de exercício, por sua vez, parece estimular mecanismos fisiológicos que melhoram os índices de recuperação funcional do nervo ciático lesionado.

Todavia, ainda é necessária a realização de estudos associando serotonina, exercício físico e lesão de nervo periférico para melhor entendimento da relação funcional e temporal destes parâmetros. Nesse contexto propomos a realização de estudos com o objetivo de determinar a relação entre serotonina e opióides no modelo experimental utilizado no presente estudo, bem como o emprego de outros testes para avaliação complementar da sensibilidade dolorosa; e ainda a verificação da expressão e atividade de outros neurotranmissores nesse modelo, como por exemplo, GABA e GLUTAMATO, os quais sabidamente possuem papéis importantes na transmissão da informação nociceptiva.

Para maior compreensão do papel do exercício físico em situações de lesão nervosa periférica, parece importante propor o emprego de outros tipos de exercícios aeróbicos, relacionando seus efeitos sobre o padrão de imunorreatividade da serotonina no sistema nervoso central.

Acreditamos que a realização dos estudos sugeridos permitirá maior compreensão dos efeitos do exercício aeróbico sobre a atividade do tecido nervoso e sua relação com a serotonina localizada neste tecido. Este conhecimento, sem dúvida, trará subsídios para o aprimoramento de terapias empregadas na reabilitação, possibilitando ainda o desenvolvimento de novas estratégias no tratamento de lesões nervosas periféricas e dor.

5. Apresentações em congressos

Arthiese Korb, Leandro Bonetti, Sandro Antunes, Simone Marcuzzo, Jocemar Ilha, Wania Partata, Maria Cristina Faccioni-Heuser

ENDURANCE TRAINING ACELERATES FUNCTIONAL RECOVERY AFTER SCIATIC NERVE TRANSECTION IN RAT

5th World Congress for NeuroRehabilitation - September 24-27, 2008 Brasilia, Brazil

Arthiese Korb, Leandro Viçosa Bonetti, Sandro Antunes da Silva, Simone Marcuzzo, Jocemar Ilha, Wania Partata, Maria Cristina Faccioni-Heuser

Influência do exercício em esteira sobre a recuperação funcional e sobre a dor em ratos com lesão nervosa periférica Neurolatan- I Congresso IBRO/LARC de Neurociências da América Latina, Caribe y Península Ibérica 1 a 4 de setembro de 2008. Búzios-Rio de Janeiro-Brasil

Arthiese Korb, Leandro Viçosa Bonetti, Sandro Antunes da Silva, Simone Marcuzzo, Jocemar Ilha, Wania Partata, Maria Cristina Faccioni-Heuser

INFLUÊNCIA DO EXERCÍCIO EM ESTEIRA ERGOMÉTRICA SOBRE A RECUPERAÇÃO FUNCIONAL E SOBRE A DOR EM RATOS COM LESÃO NERVOSA PERIFÉRICA In: V Oficina de Neurociências. Garibaldi, Brasil.

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