UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE CIÊNCIAS BÁSICAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM NEUROCIÊNCIAS

ANÁLISE MORFOFUNCIONAL DO MÚSCULO SÓLEO E DO NERVO CIÁTICO DE RATOS SUBMETIDOS AO TREINAMENTO DE EQUILÍBRIO E COORDENAÇÃO APÓS LESÃO POR ESMAGAMENTO DO NERVO CIÁTICO

Dissertação de Mestrado

Leandro Viçosa Bonetti

Porto Alegre
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RESUMO

Diversas formas de exercício físico estão sendo empregadas em estudos experimentais com o intuito de relacionar o exercício a uma regeneração nervosa periférica mais rápida e eficiente após uma lesão nervosa; entretanto, estas pesquisas não avaliam os efeitos de um protocolo de treinamento proprioceptivo ou treinamento de equilíbrio e coordenação específico sobre estes parâmetros. Neste trabalho, um programa de treinamento de equilíbrio e coordenação, e um programa motor controle foram utilizados após a lesão do nervo ciático por esmagamento em ratos, durante 4 semanas, para verificar a influência destas atividades sobre os parâmetros morfométricos do nervo lesionado (área média das fibras de mielina, área média da bainha de mielina, diâmetro médio das fibras de mielina, diâmetro médio axonal e *g ratio*) e do músculo sóleo (área média das fibras); além de verificar sua influência sobre parâmetros sensoriomotores. Os resultados demonstram que o treinamento de equilíbrio e coordenação melhora a regeneração nervosa, pois estes animais tiveram uma melhor maturação das fibras mielínicas do nervo lesionado, que possibilitou reverter/evitar a atrofia do músculo sóleo e melhorar a performance nos testes sensoriomotores após 4 semanas de treinamento. Entretanto, o treino de marcha simples, realizado pelo grupo motor controle, não melhorou a regeneração nervosa, não reverteu/evitou a atrofia muscular e também não melhorou as funções sensoriomotoras, apresentando resultados semelhantes ao grupo com lesão e que não realizou qualquer atividade física. Estes dados provêm evidências de que o treinamento de equilíbrio e coordenação pode promover a melhora sensoriomotora e a melhora de parâmetros morfométricos do músculo sóleo e do nervo ciático após uma lesão traumática experimental, e que o treinamento motor controle não tem qualquer influência sobre estes parâmetros.

SUMÁRIO

LISTA DE ABREVIATURAS

LNP	Lesão nervosa periférica
NM	Neurônio motor
RNP	Regeneração nervosa periférica
SNC	Sistema nervoso central
SNP	Sistema nervoso periférico

ARTIGO

BC	Balance and coordination
BV	Blood vessel
CNS	Central nervous system
Dd	Degeneration debris
HLRWT	Horizontal ladder rung walking test
MC	Motor control
Mf	Myelinated nerve fiber
NBT	Narrow beam test
SE	Sedentary
SEM	Standard error of mean
Sc	Schwann cell
SH	Sham

LISTA DE FIGURAS

ARTIGO

1 INTRODUÇÃO

Problemas em nervos periféricos são comuns e abrangem um amplo espectro de lesões traumáticas, doenças, tumores, etc. A incidência de lesões traumáticas é estimada em mais de 500.000 novos pacientes a cada ano no mundo e levam a perda parcial ou total das funções motora, sensorial e autonômica no segmento corporal envolvido (Rodríguez et al., 2004). As lesões nervosas periféricas (LNPs) resultam em perda do controle neural das funções motora, sensorial e autonômica no território denervado (Valero-Cabré e Navarro, 2002). Perifericamente os alvos musculares perdem sua função, e centralmente, os neurônios motores (NMs) perdem sua função (Johnson et al., 2005); porém, estas perdas podem ser recompensadas graças à recuperação dos neurônios lesionados, fazendo com que os axônios seccionados enviem novos prolongamentos ao coto distal e restabeleçam novas conexões funcionais com os órgãos periféricos apropriados (Valero-Cabré e Navarro, 2002).

Imediatamente após uma lesão nervosa periférica, ocorrem mudanças no sistema nervoso periférico (SNP) e central (SNC) que contribuem para a reorganização, recuperação fisiológica e coordenação (Duff, 2005). Os déficits funcionais causados por estas lesões podem ser compensados por meio de três mecanismos neurais: a reinervação dos alvos denervados pela regeneração dos axônios lesionados, a reinervação por ramificações colaterais de axônios não danificados, e a remodelação da circuitaria do sistema nervoso relacionada às funções perdidas (Navarro et al., 2007).

1.1 Regeneração nervosa periférica e o modelo do nervo ciático

Após uma lesão nervosa periférica (LNP) os axônios e as bainhas de mielina localizadas distalmente à lesão degeneram. Os produtos da degeneração são eliminados pela ação cooperativa das células de Schwann e dos macrófagos. Esta degeneração, chamada de degeneração Walleriana (anterógrada), serve para criar um microambiente favorável ao novo crescimento axonal (Rodríguez et al., 2004) e é uma etapa essencial na regeneração de axônios lesionados (Zhang et al., 2000). A regeneração axonal requer um adequado substrato de fatores trópicos e tróficos das células de Schwann, macrófagos e da matriz extracelular (Rodríguez et al., 2004; Johnson et al., 2005). As células de Schwann e sua matriz extracelular endoneural têm papel fundamental na promoção seletiva da regeneração axonal motora e sensorial (Johnson et al., 2005).

Como em todas as LNPs, na lesão nervosa por esmagamento a regeneração é usualmente realizada com sucesso graças à favorável reação das células de Schwann e à preservação da continuidade dos tubos endoneurais. Estes fatores auxiliam o prolongamento axonal e facilitam a adequada reinervação do alvo (Valero-Cabré e Navarro, 2004), pois as fibras nervosas da porção proximal à lesão e novos prolongamentos se estendem através do tubo distal endoneural para reinervar seus órgãos alvos (Fu e Gordon, 1997). De acordo com Johnson et al. (2005), quando ocorre uma lesão por esmagamento o axônio sofre alterações tanto na porção distal quanto na porção proximal à lesão (Fig. 1), mas estas alterações acontecem principalmente na porção distal, porém, às vezes, as lesões mais extensas ocorrem na fibra nervosa proximal à lesão.

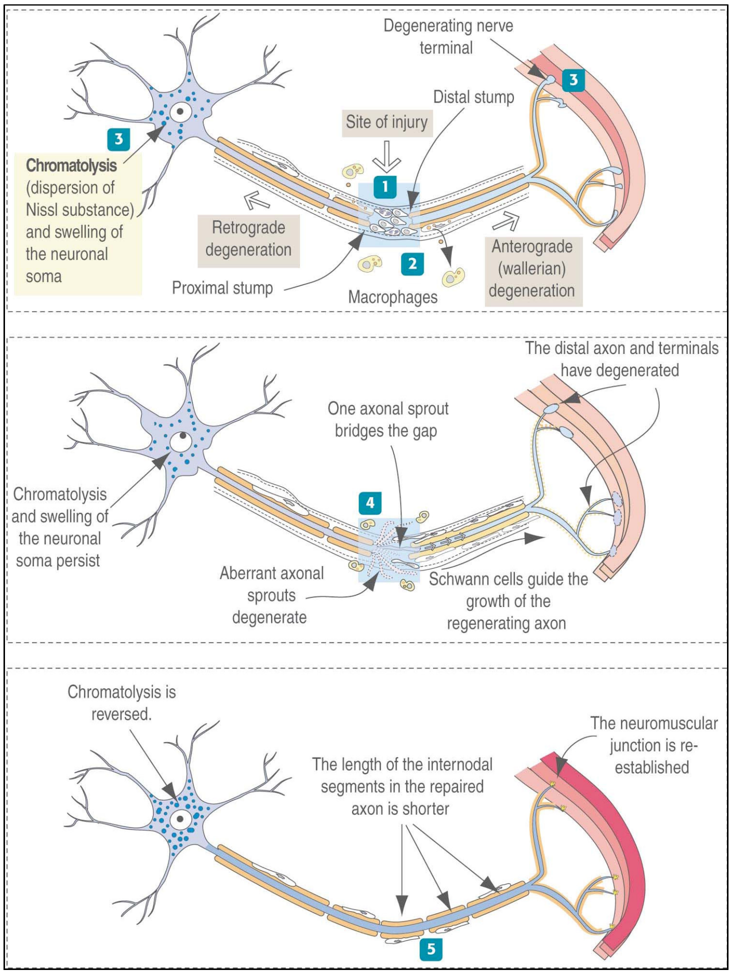

Figura 1. Figura esquemática mostrando resumidamente o processo de degeneração e de regeneração no SNP. Após uma lesão axonal por esmagamento, as células de Schwann sofrem divisão mitótica e preenchem o espaço entre os cotos proximais e distais do axônio (1). Estas células fagocitam a mielina. Gotículas de mielina são excretadas por estas células de Schwann e, em seguida, fagocitadas pelos macrófagos (2). Ocorre cromatólise (3) e é observada a degeneração dos segmentos distal e proximal do axônio (degeneração anterógrada e retrógrada respectivamente). O coto proximal do axônio gera múltiplos brotamentos que avançam por entre as células de Schwann, e estes brotamentos persistem e crescem distalmente para reinervar o músculo (4). Uma vez o axônio regenerado atinge o órgão-alvo, as células de Schwann começam a produzir mielina (5) (modificado de Kierszenbaum, 2008).

As lesões nervosas por esmagamento são apropriadas para a investigação dos mecanismos celulares e moleculares da regeneração nervosa periférica (RNP), e para a avaliação de diferentes mecanismos envolvidos no processo de regeneração (Udina, 2003). Para o estudo da RNP, o modelo do nervo ciático de ratos é o mais freqüentemente utilizado por ter um tronco nervoso, na porção média da coxa, com comprimento adequado para manipulações cirúrgicas e introdução de enxertos. O nervo ciático se divide acima da fossa poplítea em três ramos: nervo tibial, peroneal e sural. Cada um destes ramos leva diferentes proporções de axônios motores, sensórios e autonômicos em direção aos músculos, receptores cutâneos ou veias, localizados em territórios definidos do membro posterior. A lesão em um, mas não em outros ramos ciáticos, leva a paralisia e anestesia apenas de regiões particulares do membro (Valero-Cabré e Navarro, 2002; Rodríguez et al., 2004). Embora as lesões do nervo ciático por si só sejam raras em humanos, este modelo de lesão proporciona um ótimo teste que envolve lesões nervosas com axônios de diferentes tamanhos e tipos, competindo para alcançar os tubos distais endoneurais e reinervar seus alvos (Varejão et al., 2001).

1.2 Informações periféricas e as respostas motoras

Alguns trabalhos têm demonstrado que as informações aferentes cutâneas dos membros posteriores são importantes para a locomoção dos ratos, e que a perda desta informação contribui para um importante déficit postural. O SNC responde às aferências dos músculos, dos tendões, dos ligamentos, das cápsulas articulares e dos receptores cutâneos para gerar uma resposta motora efetiva para ajustes posturais e locomoção (Varejão e Filipe, 2007). O controle postural depende da habilidade de extração de informações sensoriais periféricas, integração desta informação no SNC, e coordenação e execução de uma resposta motora apropriada (Westlake e Culham, 2007), além de informações de sensores visuais, vestibulares e somatosensoriais importantes no controle postural dinâmico e na estabilidade postural (Gauchard et al., 1999).

Cada um destes sistemas não fornece ao SNC um quadro completo da posição e do movimento do corpo para o controle da postura, mas a integração destes três sistemas permite manutenção do equilíbrio e consequentemente a prevenção de quedas (Marigold et al., 2004). Mesmo com estas informações, a contribuição dos aferentes periféricos na atividade locomotora continua sendo estudada, tanto em modelos animais e humanos, e em diferentes cenários experimentais (Varejão e Filipe, 2007). Sabemos que o SNC depende de todas estas fontes de *feedback* sensório para assegurar uma ótima performance dos movimentos, e a performance motora é comprometida quando o *feedback* sensorial é abolido ou quando ocorre algum distúrbio desta informação (Gandevia e Burke, 1992).

As LNPs reduzem o recrutamento e a sensação muscular e perturbam a coordenação através de mudanças que ocorrem no SNC e SNP (Hansson e Brismar, 2003). Déficits no controle sensoriomotor também são experimentados devido a estas mudanças no SNP e SNC (Duff, 2005). Esta informação proprioceptiva anormal também causa problemas significantes na locomoção (Taylor et al., 2005), demonstrada no estudo de Freeman e Wky (1967) sobre os receptores proprioceptivos em terminações nervosas encapsuladas nas articulações de gatos, onde os autores verificaram que gatos ficaram incapazes de andar adequadamente depois que tiveram os receptores aferentes de articulações periféricas cortados do SNC. A diminuição da sensibilidade dinâmica do fuso muscular e a lentidão da velocidade de condução das fibras aferentes indicam depreciação do fuso muscular, bem como depreciação no processamento sensitivo no SNC causando déficits de reflexo e da manutenção do equilíbrio (Miwa et al., 1995).

1.3 Exercícios proprioceptivos e exercícios de equilíbrio e coordenação

O termo propriocepção foi criado pelo fisiologista inglês Charles Sherrington para indicar a “percepção do próprio corpo”, em oposição à exterocepção (percepção dos estímulos externos) e à interocepção (percepção dos estímulos internos, originários das vísceras). Embora o termo não seja ideal pelo simples fato de que utilizamos todos os sentidos para perceber as posições assumidas pelo nosso corpo, é útil por reunir os receptores situados nos músculos e nas articulações e suas conexões com o SNC até o córtex cerebral (Lent, 2004). Hoje em dia, a palavra propriocepção (do latim *proprius*, pertencente a si próprio) engloba a sensação de posição e de movimento dos próprios membros e do corpo sem o auxílio da visão. Existem submodalidades proprioceptivas: a sensação de posição estacionária dos membros (sentido de posição dos membros) e a de movimentação dos membros (cinestesia) (Kandel, Schwartz e Jessel, 2003).

Entre as várias formas de tratamento após uma lesão nervosa, tanto central como periférica, existem os exercícios proprioceptivos, que são importantes na reabilitação destas lesões, pois restaurando o déficit proprioceptivo o corpo manterá a estabilidade e a orientação durante as atividades estáticas e dinâmicas (Laskowski et al., 1997). O treino proprioceptivo consiste em exercícios designados a dar ênfase ao equilíbrio e coordenação (Seidler e Martin, 1997), e o treinamento de equilíbrio é o grupo de exercícios mais utilizados no tratamento proprioceptivo (Laskowski et al., 1997). De acordo com Riemann e Lephart (2002a, 2002b), o uso de superfícies instáveis pode alterar o *input* somatosensorial dos pés em contato com uma superfície instável. Segundo Perrin et al. (1998), o treinamento específico tem sido relatado com uma das melhores maneiras de melhorar o equilíbrio, pois a melhor integração central favorecida pela prática de tais movimentos coordenados parece ser responsável pelas respostas motoras apropriadas.

Em modelos animais, ainda não há descrito na literatura uma modalidade exercícios proprioceptivos, porém, outro tipo de exercício, os exercícios acrobáticos (EA), exige que os animais desenvolvam equilíbrio e coordenação, aprendizado motor (Anderson, Alcantara e Greenough, 1996), habilidades no uso dos membros posteriores e em coordenação com os membros anteriores (Chu e Jones, 2000), além de requerer coordenação motora dos animais (Kleim et al., 1996). Neste tipo de exercício, os animais atravessam uma série de obstáculos elevados como vigas de madeiras de diferentes diâmetros, pontes suspensas, pontes de cordas, etc.; com a dificuldade das trilhas aumentando de acordo com o progresso do treinamento, composta no final por obstáculos mais instáveis e mais difíceis de serem vencidos (Black et al., 1990).

Estes EA demonstraram significativa melhora na habilidade motora de ratos sem qualquer tipo de lesão, demonstrada pela diminuição no tempo de realização destas tarefas motoras (Black et al., 1990; Kleim et al., 1996; 1997a; 1997b; 1998a; 1998b; 2007). Em ratos com lesão unilateral do córtex sensoriomotor do membro anterior, este tipo de exercício também melhorou a performance no *footfault test* de membros anteriores (Jones et al., 1999) e de membros posteriores (Chu e Jones, 2000); demonstrando uma melhora funcional destes animais após o treinamento acrobático. Os animais do grupo sham também apresentaram melhor performance no *footfault test* de membros anteriores (Jones et al., 1999) e de membros posteriores (Chu e Jones, 2000) quando comparados com o grupo motor controle.

Os EA foram primeiramente utilizados no estudo das modificações morfológicas cerebelares, e um dos pesquisadores pioneiros foi Black et al. (1990) que verificou, devido ao aprendizado de habilidades motoras, um aumento significativo no número de sinapses por célula de Purkinje na camada molecular do lóbulo paramedial do cerebelo. Estudos subseqüentes, utilizando um protocolo de treinamento muito semelhante ao de Black et al. (1990), constataram que o aumento no número de sinapses parece estar primariamente envolvido com o aumento de sinapses nas fibras paralelas das células de Purkinje (Anderson, Alcantara e Greenough,1996; Kleim et al., 1998a). Outros estudos demonstraram que alterações sinápticas, das células de Purkinje no lóbulo paramedial do cerebelo de ratos, persistem após o encerramento do protocolo de treino acrobático (Kleim et al., 1997b), além do aumento do número de astrócitos por célula de Purkinje e aumento do volume da camada molecular por célula da Purkinje de ratos que realizam este tipo de tarefa motora (Kleim et al., 2007). O objetivo principal destes estudos foi demonstrar que atividades motoras complexas, como as realizadas pelo grupo de treinamento acrobático em detrimento às atividades motoras simples de repetição, provocam aumento no número de sinapses no córtex cerebelar (Kleim et al., 1998a).

Após lesão unilateral do córtex sensoriomotor do membro anterior de ratos (nos animais submetidos ao treinamento acrobático quando comparado com animais lesionados que realizaram atividade física controle) houve um aumento de sinapses por neurônio no córtex motor contralateral à lesão (Jones et al., 1999). Na região do córtex sensoriomotor do membro posterior ipsilateral a lesão, este exercício reduziu a perda do volume cortical da região associada à lesão, indicando a recuperação do volume tecidual do córtex próximo à lesão (Chu e Jones, 2000), e aumentou o número de mitocôndrias nas sinpases por neurônio nos animais que realizaram treinamento acrobático (Sakata e Jones, 2003), demonstrando que este tipo de exercício é capaz de influenciar a plasticidade estrutural em regiões cerebrais lesionadas, bem como em regiões intactas.

Na comparação entre exercícios de equilíbrio e coordenação (realizado no Rota-rod) com exercício simples de repetição e seus efeitos em animais com lesão isquêmica e em animais sem lesão verificou-se que o treinamento realizado no Rota-rod melhorou significativamente a função motora destes animais em todos os testes realizados; concluindo que atividades motoras complexas são mais efetivas na recuperação funcional pós-isquemia do que uma atividade motora simples (Ding et al., 2004).

O treinamento de habilidades motoras leva à aquisição e o subseqüente refinamento de novas combinações de seqüência de movimentos (Adkins et al., 2006), e as mudanças na performance motora devido ao treinamento específico ocorrem em resposta a uma variedade de diferentes experiências motoras e parecem ser sustentadas por mudanças na função do sistema motor (Remple et al., 2001).

1.4 Treinamento físico e a recuperação funcional

A atividade física após lesão na medula espinhal melhora a função motora, mas os efeitos após uma LNP são menos claros (Sabatier et al., 2008). Devido a estes e outros fatores, numerosas intervenções terapêuticas e várias formas de treinamento físico estão sendo testadas para melhorar a recuperação funcional, e parâmetros relacionados à função muscular e nervosa estão sendo avaliados (Van Meeteren et al., 1997; Meek et al., 2004; Molteni et al., 2004; Ilha et al., 2008). Porém, apesar do crescente aumento destes estudos a maioria dos trabalhos sobre LNPs enfatiza os aspectos da dor e tendem a ignorar o trauma distal, os déficits motores e outras características que poderiam relacionar a regeneração e recuperação (Hulata et al., 2008).

Após lesão nervosa severa, a recuperação funcional da maioria dos troncos nervosos frequentemente é incompleta e insatisfatória (Johnson et al., 2005) e os mecanismos envolvidos na regeneração axonal destas lesões, não proporcionam uma recuperação funcional adequada (Rodríguez et al., 2004). O melhor entendimento dos mecanismos envolvidos na recuperação da função nervosa e na adaptação dos mecanismos centrais deve ajudar a planejar estratégias terapêuticas mais efetivas para alcançar a melhor recuperação funcional (Meek et al., 2004). Uma maneira de melhorar a função nervosa enquanto ocorre a regeneração neuronal é a implementação de métodos inovadores, como novas formas de tratamento ou novos medicamentos (Duff, 2005).

Alguns resultados demonstram que a velocidade do crescimento axonal é aumentada por protocolos de treinamento específicos (Van Meeteren et al., 1997) e devido a estes e outros fatores, há um interesse crescente de pesquisadores em buscar formas de tratamento que levem a uma melhor regeneração nervosa e, conseqüentemente, uma melhora na funcionalidade; e a influência da atividade física sobre a regeneração nervosa periférica está no centro desta discussão. De acordo com Byl et al. (2003) profissionais que trabalham com reabilitação podem influenciar o processo de reorganização para ajudar os indivíduos a aperfeiçoar o controle sensoriomotor ou a coordenação após lesão específica, através da prescrição de experiências sensoriais e motoras específicas.

2 HIPÓTESE DO TRABALHO

Apesar de existirem vários trabalhos utilizando o exercício físico como tratamento na reabilitação de modelos animais após lesão nervosa periférica por esmagamento, não foram encontrados estudos que utilizassem exercícios proprioceptivos ou exercícios de equilíbrio e coordenação no tratamento de patologias desta natureza.

A grande utilização destes exercícios na prática clínica (tratamento proprioceptivo), aliada à falta de estudos científicos específicos sobre a eficácia desta modalidade terapêutica, nos leva à discussão deste tema. Desta forma, o presente trabalho busca o conhecimento científico dos efeitos do treinamento de equilíbrio e coordenação sobre a morfologia das fibras musculares e das fibras do nervo ciático após lesão nervosa por esmagamento; além de avaliar as funções sensoriomotoras destes animais, através da comparação entre diferentes grupos.

Neste contexto, os resultados obtidos neste estudo poderão influenciar o planejamento terapêutico dos profissionais da área da saúde que trabalham com neuropatias periféricas.

3 OBJETIVOS

3.1 Objetivo geral

Analisar os efeitos de quatro semanas de treinamento de equilíbrio e coordenação sobre a recuperação funcional e morfológica do nervo ciático e do músculo sóleo de ratos machos adultos após lesão por esmagamento.

3.2 Objetivos específicos

- Analisar, morfometricamente, as fibras nervosas do nervo ciático dos ratos com lesão neste nervo dos diferentes grupos experimentais.

- Analisar, morfometricamente, as fibras musculares do músculo sóleo dos ratos com lesão do nervo ciático dos diferentes grupos experimentais.

- Avaliar possíveis melhoras sensoriomotoras dos ratos com lesão do nervo ciático dos diferentes grupos, através de testes sensoriomotores.

4 MÉTODOS E RESULTADOS

4.1 Artigo - Leandro V. Bonetti, Arthiese Korb, Sandro A. da Silva, Jocemar Ilha, Simone Marcuzzo, Matilde Achaval, Maria Cristina Faccioni-Heuser. **Balance and coordination training improves sensorimotor function and sciatic nerve regeneration after peripheral nerve injury model.**

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Balance and coordination training improves sensorimotor functions and sciatic nerve regeneration after peripheral nerve injury model

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Footnotes to the title: Balance and coordination training on sciatic nerve regeneration

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Abstract

Numerous therapeutic interventions have been tested to enhance functional recovery after peripheral nerve injuries, and various forms of exercise training have shown beneficial effects in various muscle and nerve function-related parameters in animal models of nerve injury. In these work, we test the possible effects of balance and coordination training program and motor control training program (for 4 weeks) on sciatic nerve regeneration after crush lesion in rats using sensorimotor analyses and soleus muscle and nerve morphometric analyses. Our results show that the animals in the balance and coordination group and in the sham group had fewer hindlimb slips than the animals in the sedentary and motor control groups in both sensory motor tests. In the analyses of the morphometric parameters, the mean area of muscle fibers was larger in the animals performing coordination and balance exercise than in the sedentary and motor control groups and concerning average myelin sheath thickness and *g* ratio of the distal portion of the sciatic nerve, the balance and coordination group showed significantly better values than the sedentary and motor control groups. The findings indicate that balance and coordination training improves sciatic nerve regeneration after an experimental traumatic injury, as trained animals showed a greater degree of myelinated fiber maturation, so as to make it possible to revert/prevent soleus muscle atrophy and improve performance in sensorimotor tests after 4 weeks of training.

Keywords: Sciatic nerve crush; Balance and Coordination training; Motor control training; Sensorimotor analyses; Soleus muscle analyses; Peripheral nerve regeneration.

Introduction

Peripheral nerves problems are common and encompass a large spectrum of traumatic injuries, diseases, tumors and iatrogenic lesions. The incidence of traumatic injuries is estimated as >500,000 new patients annually. Injuries to peripheral nerves result in partial or total loss of motor, sensory and autonomic functions in the involved segments of the body (Rodríguez et al., 2004). Peripheral nerve injuries reduce muscular recruitment and sensation and can disrupt coordination through the changes that occur in the peripheral and the central nervous system (CNS) (Hansson and Brismar, 2003; Johnson et al., 2005). In addition, deficits in sensorimotor control are experienced immediately after nerve injury (Duff, 2005).

Numerous therapeutic interventions have been tested to enhance functional recovery after peripheral nerve injuries. A range of forms of exercise training have shown beneficial effects in various muscle and nerve function-related parameters in animal models of nerve injury (Van Meeteren et al., 1997; Meek et al., 2004; Molteni et al., 2004; Ilha et al., 2008). For example, in rats, swimming and prolonged wheel running decreased the denerved muscle atrophy (Irintchev et al., 1991). Furthermore, the animals with sciatic nerve crush that had their hindlimb unloading, during regenerative time post-lesion, had worse nerve regeneration compared to animals that supported their weight, showing that hindlimb weight bearing facilitates recovery of neural function and muscle weight (Matsuura et al., 2001). Moreover, a previous study in our laboratory showed that endurance training improves sciatic nerve regeneration after traumatic nerve injury, although resistance training and the combination of two strategies delayed functional recovery and did not alter sciatic nerve fiber regeneration (Ilha et al., 2008).

Although these studies showed that motor and sensorial stimuli are important factors during peripheral nerve regeneration, there are no works in animal models exploring balance and coordination training (proprioceptive training) in the treatment of peripheral nervous lesions. For this reason, the present work was designed to compare the effects of balance and coordination training versus motor control training on sensorimotor recovery, and to carry out a morphometric analysis of the sciatic nerve and soleus muscle fibers after nerve injury through sciatic nerve crushing.

Materials and methods

Experimental design and surgical procedures

The experiment was performed on 23 three-month-old, male Wistar rats weighing 280-330 g (initial age and weight) from a local breeding colony (ICBS, Universidade Federal do Rio Grande do Sul, Brazil). Rats were housed in standard Plexiglass boxes, under a 12:12 h light/dark cycle in a temperature-controlled environment (20 ± 1 °C) with food and water *ad libitum*. All the procedures were approved by the Ethical Committee at the Federal University of Rio Grande do Sul and all the animals were handled in accordance with the Brazilian laws. Animals were randomly divided in four groups: (1) sham-operated rats, without sciatic crush and unexercised, sham (SH, n = 5); (2) rats with sciatic crush and unexercised, sedentary (SE, n = 6); (3) rats with sciatic crush and motor control training (MC, n = 6); and (4) rats with sciatic crush and balance and coordination training (BC, n = 6). Before the surgical procedures, animals were adapted for 5 days in each training program protocol. For the surgical procedures, animals were anesthetized using ketamine and xylazine (90 and 15 mg/kg, i.p., respectively; Vetbrands, Brazil) and the right sciatic nerve was exposed through a skin incision extending from the greater trochanter to the mid-thigh followed by splitting of the overlying gluteal muscle. The nerve crush injury was performed with 1 mm hemostatic forceps for 30 seconds (as previously described by Bridge and coworkers (1994)), 10 mm above the bifurcation into the tibial and common peroneal nerves. The muscle and skin were then closed with 4-0 nylon sutures (Somerville, Brazil), and the animals were put in their cages to rest. Forty-eight hours after the surgery, the animals from the MC and BC groups began specific training for 4 weeks, while SH and SE animals were put in the same location as the MC and BC animals for a few minutes in order to equal as much as possible the handling of all groups, but they did not perform any kind of motor activity.

Motor control training

The motor control training program was adapted from Kleim et al. (1996) and was performed on a flat obstacle-free runway 100 cm long and 8.5 cm wide ending in dark box. Each rat of this group crossed this flat obstacle-free runway 25 times, walking 2.500 cm per day of training. The motor control training program included 5 sessions per week for 4 weeks.

Balance and coordination training

The balance and coordination training program was adapted from the acrobatic training used by Black et al. (1990), Anderson, Alcantara and Greenough (1996) and Kleim et al. (1996). Animals were required to traverse 5 different elevated obstacles per day, such as suspension bridges, rope bridges, parallel bars, etc. (each 100 cm long) ending in dark box. These obstacles require motor learning, balance and coordination from these animals, and each rat of this group crossed these obstacles 25 times, walking 2.500 cm each day of training. The difficulty of the tracks was increased as the training progressed, eventually including more unstable and more challenging obstacles than in the first training week. The balance and coordination training program comprised 5 sessions per week for 4 weeks.

Sensorimotor studies

At the end of the training period, the horizontal ladder rung walking test (HLRWT) and the narrow beam test (NBT) were used to examine hindlimb sensorimotor function. In the last training day, the animals were adapted to the tests apparatus, and the sensorimotor tests were performed one day after the end of the training programs. For each test, the animals were filmed 3 times from a lateral view. The HLRWT apparatus was 100 cm long and 5 cm wide, with horizontal parallel metal rungs (3 mm in diameter) which could be inserted so as to create a floor with a minimum distance of 1 cm between rungs, elevated 30 cm above the floor, and a little dark box at the end. In this test, animals are required to walk along a horizontal ladder and an irregular pattern (the distance of the rungs varied from 1 to 5 cm), which was changed from the adaptation phase to the testing so as to prevented the animal from learning the pattern. The number of hindlimb step slips was counted by two blinded observers. The NBT consisted of walking along a 100-cm-long, 3-cm-wide flat surface beam (for the adaptation) and a 100-cm-long, 2.6-cm-wide surface beam (for the test) elevated 30 cm above the floor, to reach a little dark box at the end. The number of hindlimb step slips was counted by two blinded observers.

Histological and morphometric studies

Two days after the sensorimotor tests the animals were anesthetized with sodium thiopental (50 mg/kg, i.p.; Cristália, Brazil), injected with 1000 IU heparin (Cristália, Brazil) and were transcardially perfused with 300 ml of saline solution, followed by 0.5% glutaraldehyde (Sigma Chemicals Co., St Louis, MO) and 4% paraformaldehyde (Reagen, Brazil) in a 0.1 M phosphate buffer (pH 7.4, PB) at room temperature. The right soleus muscles were carefully dissected free from surrounding tissue and small samples (2x1 mm) of the central part of each soleus muscle were selected (Marcuzzo et al., 2008). Two short segments (~ 2 mm) of the right sciatic nerve were rapidly excised, one from 5 mm before and one after the crush injury site, proximal and distal portions, respectively (Ilha et al., 2008). The specimens were postfixed by immersion in the same fixative solution at 4 °C until processed; after that, the samples were washed in 0.1 M PB and postfixed in 1% OsO4 (Sigma Chemicals Co., St Louis, MO) in 0.1 M PB for 30 min.; washed again in 0.1 M PB; dehydrated in a graded series of acetone, embedded in resin (Durcupan, ACM-Fluka, Switzerlend) and polymerized at 60° C. Cross-semithin sections (1 µm) were obtained using an ultramicrotome (MT 6000-XL, RMC, Tucson, AZ) and stained with 1% toluidine blue (Merck, Germany) in 1% sodium tetraborate (Ecibra, Brazil) (Marcuzzo et al., 2008; Ilha et al., 2008).

Afterwards, images of the muscle soleus of the right hindlimb and of the proximal and distal portions of the right sciatic nerve were captured and digitalized (for muscle 200x and for nerve portions initially 1000x and further amplified by 200% for analysis) using a Nikon Eclipse E-600 microscope (Japan) coupled to a Pro-Series High Performance CCD camera and processed with Image Pro Plus Software 6.0 (Media Cybernetics, USA) (Marcuzzo et al., 2008; Ilha et al., 2008).

For morphometric evaluation of the soleus muscle, a set of 5 images was chosen using random sampling of one slice, which were saved and digitalized. Twenty different muscle fibers of each image were selected (100 fibers of each animal were analyzed). Morphometric measurements of the soleus muscle included the averaged muscle fiber area (μm^2) (Marcuzzo et al., 2008). For the nerve evaluation, both proximal and distal portions of the right sciatic nerves were separately analyzed, a set of 8 images was chosen using random sampling of one slice of each portion. Morphometric measurements of the sciatic nerve included the (1) averaged myelinated fiber area (μm^2); (2) average myelin sheath thickness (µm); (3) averaged myelinated fiber diameter (µm); (4) averaged axon diameter (µm) of the myelinated fiber and

(5) *g* ratio (the quotient axon diameter/fiber diameter, a measure of the degree of myelination). These morphological parameters were chosen to assess the differentiation of regenerating sciatic nerves (Ilha et al., 2008).

The average myelin sheath thickness was estimated using the measurement tools of Image Pro Plus software. The measurements of areas (muscle fiber and nerve fiber areas) was estimated with a point-counting technique (Marcuzzo et al., 2008; Ilha et al., 2008) using grids with point density of 1 point per 59.17 μm^2 and 1.75 μm^2 (for muscle and nerve, respectively) and the following equation:

$$\hat{A}=\sum p.a/p,$$

where $\hat{A}$ is area, $\sum p$ the sum of points, and a/p the area/point value. To estimate the axon and neural fiber diameters, the area of each individual fiber was converted to the diameter of a circle having an equivalent area.

Statistical analysis

Sensorimotor tests and morphometric measurements of the sciatic nerve were analyzed using repeated measures of analysis of variance (ANOVA) and soleus muscles were analyzed using one-way ANOVA. All analyses were followed by post hoc Duncan's. Data were expressed as means ± standard error of the mean (SEM). The significance level was $p<0.05$. Statistical analyses were performed using a statistic software package.

Results

Sensorimotor tests

In the horizontal ladder rung walking test (HLRWT) (Figure 1A), the number of right hindlimb slips was significantly greater than the number of left hindlimb slips in the SE group, a difference which was not observed in the other groups. The SE group (1.8 ± 0.3; $p < 0.05$) showed significantly more right hindlimb slips than the SH (0.5 ± 0.4; $p < 0.05$) and BC groups (0.6 ± 0.3; $p < 0.05$). Apart from that, there were no significant differences in right hindlimb slips between the SH, MC and BC groups, and there were no significant differences in the number of left hindlimb slips across all the groups.

In the narrow beam test (NBT) (Figure 1B), the number of right hindlimb slips did not differ significantly from the number of left hindlimb slips in the SH and BC groups. The BC group (0.7 ± 0.4; $p < 0.05$) showed significantly fewer right hindlimb slips than the SE (2.3 ± 0.4; $p < 0.05$) and MC (2.5 ± 0.4; $p < 0.05$) groups; however there were no significant differences in the right hindlimb slips between the SH and BC groups and in left hindlimb slips between all the groups.

Histological studies

Structural analysis of fiber muscles (Figure 2) revealed differences between the groups. The structure of the muscle fibers in the balance and coordination (BC) group and in the sham (SH) group were similar, presenting most of the large muscle fibers, with polygonal shape and little conjunctive tissue between them. The fibers muscle of sedentary (SE) and motor control (MC) groups are shorter and rounded and had much conjunctive tissue between them.

The structural analysis of regenerating nerves (Figure 3) showed important differences between the sham (SH) and experimental groups (SE, MC and BC). These differences were found in the distal portion of regenerating sciatic. The distal portion of SH group, demonstrated small myelinated fibers and small space existent between the fibers; however, experimental groups showed the predominance of small-diameter thin myelin sheath fibers, increase in endoneurial connective tissue between the nerve fibers and degeneration debris in this portion of the nerve in this portion of nerve regeneration; nevertheless, in the nerve fibers of the this portion, note that in the BC group, myelin sheath thickness appear to be more large than those of the SE and MC groups.

Morphometric studies

In the analysis of morphometric data, the mean area of muscle fibers (Figure 4) was significantly larger in the SH and BC groups (1305.20 ± 77.80 and 1225.50 ± 71.02 μm^2; respectively) than in the SE and MC groups (998.88 ± 71.02 and 1000.49 ± 71.02 μm^2; respectively); however, there was no significant difference in the mean area of muscle fibers between the SH and BC groups, neither between the SE and MC groups

In the nerve morphometry, the mean area of nerve fibers (Figure 5A) showed significant differences between the proximal and distal portion in the SE, MC and BC groups, but no significant difference in the SH group. The mean area of the nerve fibers in the distal portion of the SH group (37.25 ± 2.15 μm^2; $p< 0.05$) was significantly larger than in the SE (10.18 ± 1.76 μm^2; $p< 0.05$), MC (10.89 ± 1.92 μm^2; $p< 0.05$) and BC (12.76 ± 2.15 μm^2; $p< 0.05$) groups.

When the mean thickness of the myelin sheath in the proximal and distal portions of the injured sciatic nerve was compared (Figure 5B), all groups presented statistically different values. In the analysis of the mean thickness of the myelin sheath in the distal portion of this nerve, the SH group (1.22 ± 0.02 μm; $p< 0.05$) had significantly greater values than the SE (0.43 ± 0.02 μm; $p< 0.05$), MC (0.49 ± 0.02 μm; $p< 0.05$) and BC (0.59 ± 0.02 μm; $p< 0.05$) groups, but the BC group had significantly greater values than the SE and MC groups.

In the analysis of the mean diameter of the myelin fibers (Figure 5C), when the mean values of the proximal and distal portions of the sciatic nerve were compared, the SE, MC and BC groups showed significantly different values, while the SH group did not show this difference. In the analysis of the mean diameter of the fibers in the distal portion, the mean value was significantly greater in the SH group (7.10 ± 0.24 µm; $p < 0.05$) than in the SE (3.59 ± 0.20 µm; $p < 0.05$), MC (3.71 ± 0.22 µm; $p < 0.05$) and BC (3.97 ± 0.24 µm; $p < 0.05$) groups.

When the mean axon diameter was analyzed (Figure 5D), all groups presented significant differences between the mean values of the proximal and distal portions of the injured sciatic nerve. In the analysis of the distal portion of the nerve, the SH group (4.39 ± 0.19 µm; $p < 0.05$) had a greater mean diameter than the other groups [SE (2.71 ± 0.15 µm; $p < 0.05$), MC (2.71 ± 0.17 µm; $p < 0.05$) and BC (2.78 ± 0.19 µm; $p < 0.05$)].

In the comparison of *g* ratios (Figure 5E), only the BC group did not show a significant difference between the mean values of the proximal and distal portions of the right sciatic nerve. In the analysis of the distal portion, the SH group (0.64 ± 0.01; $p < 0.05$) presented significantly lower values than the SE (0.75 ± 0.009; $p < 0.05$), MC (0.73 ± 0.009; $p < 0.05$) and BC (0.69 ± 0.01; $p < 0.05$) groups, but the BC group had significantly lower values than the SE and MC groups. The mean values of the *g* ratio in the SH and BC groups indicate a greater degree of maturation of the myelin fibers in these groups.

Discussion

Crush injuries are appropriate to investigate the cellular and molecular mechanisms of peripheral nerve regeneration and to assess the role of different factors in the regeneration process (Udina, 2003). Rodents, particularly the rat and the mouse, have become the most frequently utilized animal models for the study of peripheral nerve regeneration because of the widespread availability of these animals as well as the distribution of their nerve trunks, which is similar to the human one (Rodríguez et al., 2004; Baptista et al., 2007). Rats appear to use coordinated forelimb movements to guide forward locomotion on the task, whereas hindlimbs appear to be primarily involved in maintaining balance and whole-body postural stability (Chu and Jones, 2000). After a crush lesion, the sensory loss affects hindlimb kinematics (Varejão and Filipe, 2007).

Strategies developed to improve peripheral nerve regeneration require quantitative approaches for evaluating functional outcome, and regeneration can be assessed by numerous methods, including histomorphometry, electrophysiology and gait analysis (Baptista et al., 2007). Although axons in peripheral nerves are known to regenerate better than those in the CNS, methods of accelerating regeneration are needed due to the slow overall rate of growth (Sabatier et al., 2008). The studied approaches for accelerating regeneration after nerve injury, such as treadmill training, resistance training, swimming (Ilha et al., 2008; Sabatier et al., 2008; Irintchev et al., 1991; Van Meeteren et al., 1997) can underestimate the role of balance and coordination deficits in this condition. This study investigated whether balance and coordination exercise training and motor control exercise training, started 2 days after the crush and performed for 4 weeks, could produce different effects in sensorimotor tests and morphological changes in the right hindlimb soleus muscle and in nerve regeneration after a crush lesion of the sciatic nerve. We chose to begin the exercise program as early as two days after the crush in order to show that balance and coordination exercise training can be used in the early phase of rehabilitation, as long as performed carefully and moderately.

Our results show that the animals in the balance and coordination group and in the sham group had fewer hindlimb slips than the animals in the sedentary and motor control groups in both sensory motor tests – horizontal ladder rung walking test (HLRWT) and narrow beam test (NBT). These tests were performed one day after completion of the training protocols, as the HLRWT requires minimal training (Metz and Whishaw, 2002), and in the NBT rats reached maximal performance at the task within a single test session, reducing the need for extensive training and increasing the reliability of the data (Allbutt and Henderson, 2007). These findings show that balance and coordination exercise training improved the performance of animals with nerve injury in motor ability tasks, also showing that the deficit in these motor abilities remains 4 weeks after injury both in the non-trained injured group and in the motor control group. Therefore, the balance and coordination deficits seen in animals with peripheral nerve injury need a specific training to be improved, as the one used in this work. The exercises that demand balance and coordination from the rats require learning various forelimb and hindlimb skills, as well as good coordination of the whole body to perform the activities.

Concerning the morphometric parameters, the mean area of muscle fibers was larger in the animals performing balance and coordination exercise than in the sedentary and motor control groups, while the values in the coordination and balance group were similar to the sham-operated group, showing that 4-week physical training of this type avoided muscle atrophy after nerve crush, whereas motor control exercise did not prevent atrophy by denervation. Denervation resulted in a reduction of soleus muscle fiber size and consequently produced serious muscle atrophy (Sakakima and Yoshida, 2003), which makes it important to use exercises that may revert/avoid this muscle atrophy. The forced use of denervated hindlimb muscle, by contralateral immobilization (Eisen et al., 1973), or overuse induced by maximal stretching to the top of a plexiglass box to reach a water bottle (Van Meeteren et al., 198) also led to an increase in mean fiber diameter and return of sensory function after sciatic nerve crush. The histological analysis showed that the structure of the muscle fibers in the balance and coordination group and in the sham group were similar, presenting most of the large muscle fibers, with polygonal shape and little conjunctive tissue between them, differing from the fibers in the sedentary and motor control groups, which were shorter and rounded and had much conjunctive tissue between them.

The morphometric parameters of nerve fibers (average myelinated fiber area, average myelin sheath thickness, myelinated fiber diameter, axon diameter and *g* ratio) presented a significant difference between the proximal and distal portions of the sciatic nerve in the sedentary, motor control and balance and coordination groups. When these parameters where compared between the groups, the proximal portion of the nerve was similar across all groups. According to Johnson et al. (2005), when there is a crush lesion, the axon undergoes alterations both in the distal and proximal portions to the lesion, but such lesions occur mainly in the distal portion; thus, the morphometric parameters distal to the nerve lesion are more important for the analysis of the influence of exercise in nerve regeneration. In this portion of the nerve, the sham group presented significantly different values from the other experimental groups, which did not show significant difference between each other in most of the analyzed parameters (average myelinated fiber area, myelinated fiber diameter and axon diameter). However, concerning average myelin sheath thickness and *g* ratio, the balance and coordination group showed significantly better values than the sedentary and motor control groups. The greater myelin sheath thickness and smaller *g* ratio values in the balance and coordination-trained animals showed a greater degree of the myelinated fiber maturation (Ilha et al., 2008) compared to sedentary and motor control groups. Exercise training in reaching bottles of water support the suggestion that the speed of axonal growth is increased by this particular training protocol (Van Meeteren et al., 1997); voluntary physical activity can prime adult sensory neurons for enhanced axonal regeneration after subsequent axotomy; neurons from these exercised animals showed overall more robust neurite extension when compared with neurons from the sedentary animals (Molteni et al., 2004), and treadmill training improves sciatic nerve regeneration after an experimental crush injury (Seo et al., 2006; Sabatier et al., 2008; Ilha et al., 2008). All these findings indicate that different types of exercise can influence positively peripheral nerve regeneration.

In summary, our data show that balance and coordination training improves sciatic nerve regeneration after an experimental traumatic injury, as trained animals showed a greater degree of myelinated fiber maturation, so as to make it possible to revert/prevent soleus muscle atrophy and improve performance in sensorimotor tests after 4 weeks of training. On the other hand, a simple walking training, as performed by the motor control group here, does not alter sciatic nerve fiber regeneration, as these animals showed the same results as the sedentary group in all analyzed parameters. In this context, the results reported in this work can influence future studies aimed at relating more effective nerve regeneration to specific physical activities, with different types, lengths and intensity from those proposed here, and can influence the strategies used by health professionals to treat peripheral neuropathies.

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Fig. 1. Comparison of the sensorimotor analysis determined by HLRWT (A) and NBT (B). For both tests, each animal was recorded with a digital video camera crossing three times each test apparatus (HLRWT and NBT) and the number of left and right hindlimb slips was counted. Data are expressed as means and SEM. For HLRWT, letter " a " corresponds to $p < 0.05$ when compared to the SH group and " b " to $p < 0.05$ when compared to the SE group

and for NBT, letter “ a’ ” corresponds to $p < 0.05$ when compared to the SH group, letter “ b’ ” corresponds to $p < 0.05$ when compared to the SE group and letter “ c’ ” corresponds to $p < 0.05$ when compared to the MC group. HLWRT = horizontal ladder rung walking test; NBT = narrow beam test; SH = sham; SE = sedentary; MC = motor-control trained; BC = balance and coordination-trained. White bar = left hindlimb slips; black bar = right hindlimb slips.

Fig. 2. Digitized images of transverse-semithin sections (1 μm) obtained from central part of right soleus muscle after 4 weeks of specific exercise training. A: central part of soleus muscle of the SH group. B: central part of soleus muscle of the SE group. C: central part of soleus muscle of the MC group. D: central part of soleus muscle of the BC. This images show that the structure of the muscle fibers in the BC group and in the SH group were similar, presenting most of the large muscle fibers, with polygonal shape and little conjunctive tissue between them. The fibers muscle of SE and MC groups are shorter and rounded and had much conjunctive tissue between them. SH = sham; SE = sedentary; MC = motor-control trained; BC = balance and coordination-trained. Sections were stained with toluidine blue. Scale bar = 50 μm.

Fig. 3. Digitized images of transverse-semithin sections (1 μm) obtained from regenerating sciatic nerves after 4 weeks of specific exercise training. A and B: proximal and distal portions, respectively, from normal nerves of the SH group. Note the bimodal fiber spectrum, the large and small myelinated fibers and small space existent between the fibers. C and D: proximal and distal portions, respectively, from regenerating nerves of the SE group. Note the small differences between the proximal portions of SE and SH groups; however, the distal portion of SE group show the predominance of small-diameter thin myelin sheath fibers, increase in endoneurial connective tissue between the nerve fibers and degeneration debris in

this portion of the nerve. E and F: proximal and distal portions, respectively, from regenerating nerves of the MC group. Note the small differences between the proximal portions of MC and SH groups. At the distal portion, myelinated fibers appear to be similar to the SE; with predominance of small-diameter thin myelin sheath fibers, increase in endoneurial connective tissue between the nerve fibers and degeneration debris in this portion of nerve. G and H: proximal and distal portions, respectively, from regenerating nerves of the BC group. The proximal portion of the BC group appears to be similar to the others groups; nevertheless, in the nerve fibers of the distal portion, note that the myelin sheath thickness appear to be more large than those of the SE and MC groups. Mf = myelinated nerve fiber; Sc = Schwann cell; * (asterisk) = endoneurial connective tissue; Dd = degeneration debris; BV = blood vessel; SH = sham; SE = sedentary; MC = motor-control trained; BC = balance and coordination-trained; P = proximal portion; D = distal portion. Sections were stained with toluidine blue. Scale bar = 20 μm.

Fig. 4. Effects of specific physical exercise training on the morphometric parameters of central part of right soleus muscle after 4 weeks of specific exercise training. Graphics show the average area of muscle fibers of different groups. Data are expressed as means and SEM. Letter “ a ” corresponds to $p < 0.05$ when compared to the SH group. SH = sham; SE = sedentary; MC = motor-control trained; BC = balance and coordination-trained.

Fig. 5. Effects of specific physical exercise training on the morphometric parameters of regenerating right sciatic nerve fibers after 4 weeks of training. Graphics show the average myelinated fiber area at the proximal and distal portions of the nerves (A); the average myelin sheath thickness at the proximal and distal portions of the nerves (B); the myelinated fiber diameter at the proximal and distal portions of the nerves (C); the axon diameter at the

proximal and distal portions of the nerves (D); and the *g* ratio at the proximal and distal portions of the nerves (E). Data are expressed as means and SEM. Letter “ a ” corresponds to $p < 0.05$ when compared with the proximal nerve portion of the SH group, “ b ” to $p < 0.05$ when compared with the proximal nerve portion of the SE group, “ c ” to $p < 0.05$ when compared with the proximal nerve portion of the MC group, “ a’ ” to $p < 0.05$ when compared with the distal nerve portion of the SH group, “ b’ ” to $p < 0.05$ when compared with the distal nerve portion of the SE group and “ c’ ” to $p < 0.05$ when compared with the distal nerve portion of the MC group. SH = sham; SE = sedentary; MC = motor-control trained; BC = balance and coordination-trained. Black bar = proximal nerve portion; white bar = distal nerve portion.

Figure 1

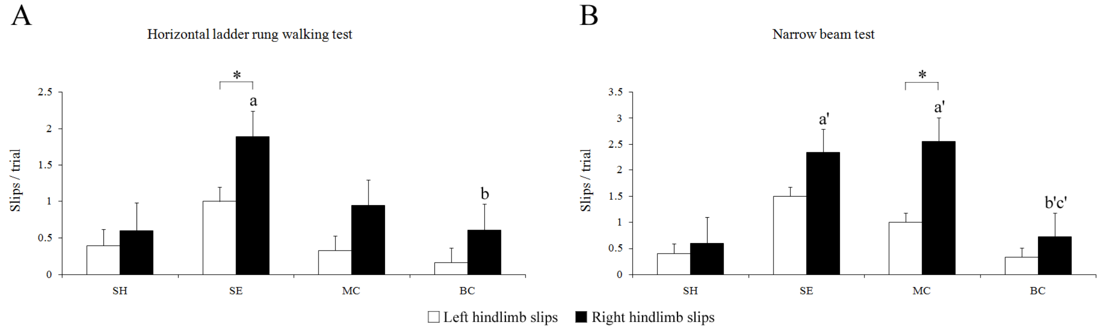

Figure 2

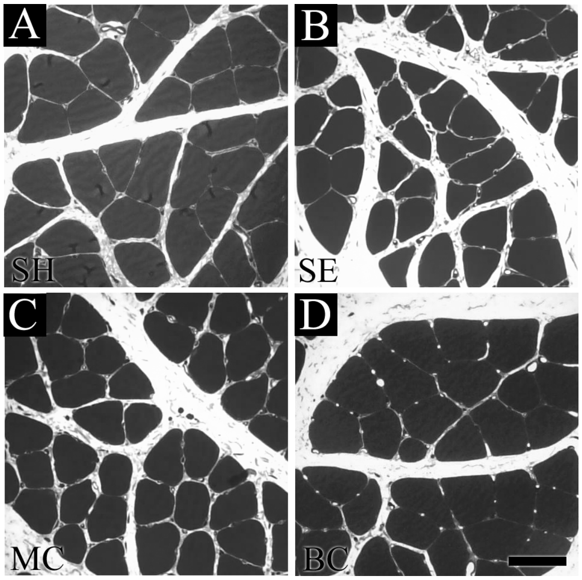

Figure 3

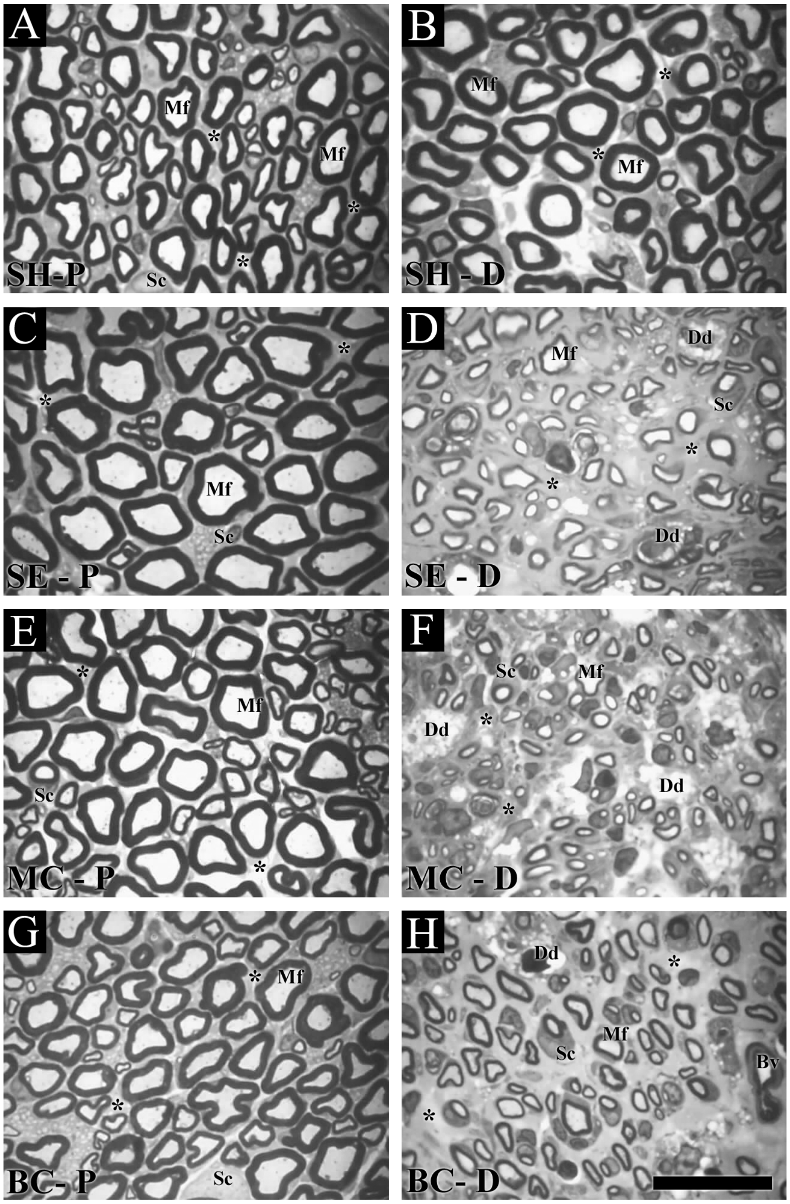
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Figure 4

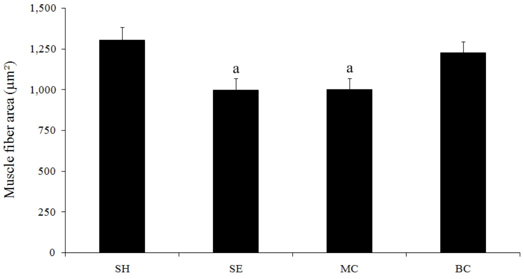
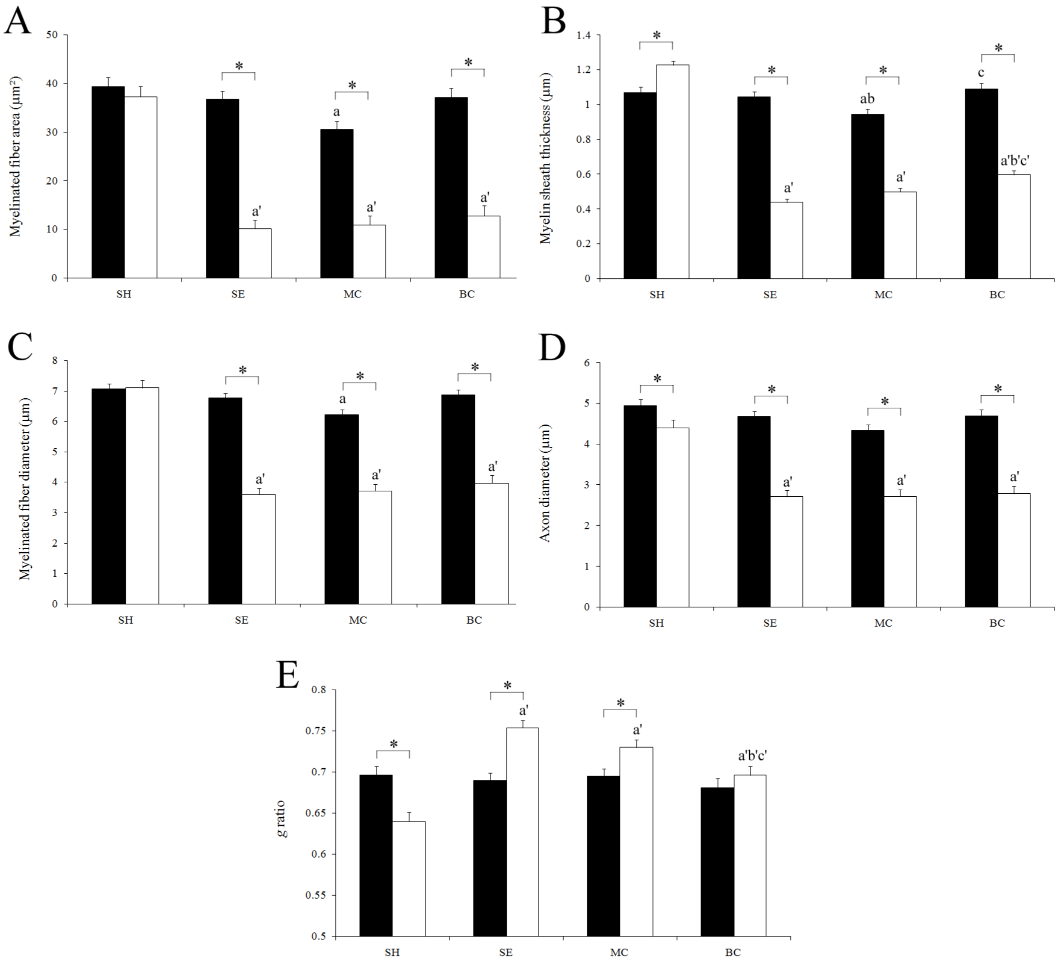


Figure 5



5 CONCLUSÕES E PERSPECTIVAS

Os dados apresentados neste trabalho mostraram evidências de que o treinamento de equilíbrio e coordenação melhora a regeneração do nervo ciático após uma lesão traumática experimental deste nervo, pois os animais deste grupo demonstraram uma melhor maturação das fibras mielínicas do nervo lesionado, que possibilitou reverter/evitar a atrofia do músculo sóleo e melhorar a performance nos testes sensoriomotores após 4 semanas de treinamento.

Por outro lado, um treinamento de marcha simples, realizado pelo grupo motor controle, não demonstrou qualquer influência sobre os parâmetros analisados; não melhorou a regeneração nervosa, não reverteu/evitou a atrofia muscular e também não melhorou as funções sensoriomotoras, apresentando resultados semelhantes ao grupo com lesão e que não realizou qualquer atividade física (grupo sedentário).

Diante disto, os resultados obtidos neste estudo podem interferir no planejamento terapêutico dos profissionais da área da saúde que trabalham com neuropatias periféricas, e podem servir como referência para futuros estudos que tenham o objetivo de relacionar atividades físicas específicas a uma melhor regeneração nervosa periférica. Os efeitos de diferentes tipos de treinamento, tempo de duração e intensidade dos protocolos de exercício ainda necessitam de estudos para a análise de seus efeitos após uma lesão nervosa periférica.

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7 ANEXOS

Apresentação em congressos

Leandro V. Bonetti, Arthiese Korb, Sandro A. da Silva, Jocemar Ilha, Simone Marcuzzo, Maria Cristina Faccioni-Heuser, 2008. **Efeitos do programa de treinamento de equilíbrio e coordenação em ratos após lesão nervosa periférica.** In: I Congresso IBRO/LARC de Nerurociências da América Latina, Caribe e Península Ibérica. Búzios, Brasil.

Leandro V. Bonetti, Arthiese Korb, Sandro A. da Silva, Jocemar Ilha, Simone Marcuzzo, Maria Cristina Faccioni-Heuser, 2008. **Efeitos do treinamento de equilíbrio e coordenação sobre a morfologia do músculo sóleo após lesão do nervo ciático.** In: V Oficina de Neurociências Fronteiras da Investigação. Garibaldi, Brasil.