Original Article

Whole-body vibration therapy does not improve the peripheral nerve regeneration in experimental model

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Abstract

Objectives: Whole-body vibration (WBV) is commonly used to improve motor function, balance and functional performance, but its effects on the body are not fully understood. The main objective was to evaluate the morphometric and functional effects of WBV in an experimental nerve regeneration model. **Methods**: Wistar rats were submitted to unilateral sciatic nerve crush and treated with WBV (4-5 weeks), started at 3 or 10 days after injury. Functional performances were weekly assessed by sciatic functional index, horizontal ladder rung walking and narrow beam tests. Nerve histomorphometry analysis was assessed at the end of the protocol. **Results**: Injured groups, sedentary and WBV started at 3 days, had similar functional deficits. WBV, regardless of the start time, did not alter the histomorphometry parameters in the regeneration process. **Conclusions**: The earlier therapy did not change the expected and natural recovery after the nerve lesion, but when the WBV starts later it seems to impair function parameter of recovery.

Keywords: Nerve Crush, Nerve Regeneration, Sciatic Nerve, Therapy, Whole-Body Vibration

Introduction

Traumatic peripheral nerve injury is among the most frequent causes of injuries on the peripheral nervous system[1]. The regeneration capacity of the peripheral nerve is high compared to the central nervous system[2]. However, peripheral nerve injuries cause sensorial and motor function impairments that may result in an incomplete recuperation[3]. In animal models, the sciatic nerve-crush model is commonly used to study functional recovery, histological properties and the subsequent regenerative capacity of peripheral nerve[4].

Therapeutic modalities like physical exercise have been associated to peripheral nervous regeneration and to improve functional recovery[5]. Swimming[6], walking or running on a treadmill[7,8] and balance and coordination training[9,10] have been used for this purpose. All these exercise modalities were able to improve the diameter of myelinated fibers[7-10] and neurotrophic factors[11], to decrease the inflammatory process of nerve degeneration[12]. Also, to accelerate the rate of functional recovery on locomotion[6,8], balance and sensorimotor control[9,10,13]. However, its effects depend on the intensity and exercise types starting few days after injury[6,8].

Regarding the exercise modalities, whole-body vibration (WBV) has been considered an exercise modality used as a potential therapy adjunct for clinical and functional training. WBV is often targeted at individuals who have reduced mobility and difficulty walking. Studies has been showed

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that WBV can be used to improve gait and balance in older adults[14] and in patients with multiple disease conditions, such as, cerebral palsy[15] spinal cord injury[16], multiple sclerosis[17], Parkinson disease[18] and stroke[19]. Overall, these investigations show some evidence for improving balance and mobility outcomes, but the effects are inconclusive. In animal models, the vibration demonstrates controversial effects and shows the neuroprotective function in a Parkinson's disease model[20], or the opposite, neurodegenerative effects in spinal cord models, for example, according to the vibration therapy starting time[21,22].

However, to the best of our knowledge, there are no experimental studies about the effects of WBV therapy on peripheral nerve regeneration. In addition, it is unknown the effect of the WBV therapy starting at different times after injury. For this reason, this study was designed to analyze the effects of whole-body vibration on histological nerve repair and functional recovery after peripheral nerve lesion on a sciatic nerve crush model, starting the therapy at different times after injury.

Materials and methods

Animals

Male Wistar rats, 2.5 months old, weighing 250-350 g were housed in standard plexiglas boxes (3 or 4 rats per box) under a 12-h light/ 12-h dark cycle in a temperature-controlled environment ($20\pm2°C$) with food and water available *ad libitum*. All procedures were approved by the animal ethics committee of Federal University of Rio Grande do Sul (Protocol Nº. 29395), and all animals were handled in accordance with the Arouca Brazilian law (11794/2008).

The animals were randomly allocated into 5 groups: (1) Naive group - sedentary rats without the sciatic crush or surgical procedures (Naive, NA, *n*=10); (2) sham-operated group - sedentary rats with the surgical procedures without sciatic crush (Sham-operated, SH, *n*=10); (3) sedentary rats submitted to the sciatic crush (non-trained, NT, *n*=11); (4) rats submitted to sciatic crush procedure and WBV training starting 3 days after the injury (vibration group 3, V3, *n*=11); (5) rats submitted to sciatic crush and WBV training starting 10 days after the injury (vibration group 10, V10, *n*=11). Three and 10 days were chosen because microenvironment allowing successful regrowth of the proximal nerve fiber ending differs in these time points[1]. According to these authors, two days after nerve injury degenerating myelinated fibers are observed and pre-existing blood vessels are ruptured in injury local and one and two weeks after injury there is presence of macrophages and proliferation of the basal membrane of pre-existing vessels and new vessels growth.

Sciatic nerve crush

Surgical procedure was performed initially under ketamine and xylazine anaesthesia (90 and 15 mg/kg intraperitoneally, respectively). Rats were placed on ventral decubitus position on a thermal plate (Heat Pad – EEF 442, Insight, Brazil) with 37.5°C temperature. The right sciatic nerve was exposed through an incision in the skin from the greater trochanter to the mid-thigh followed by splitting of the overlying gluteal muscle. The nerve crush injury was performed according Ilha et al.[8] using a 1 mm haemostat forceps for 30s, 10 mm above the bifurcation of the tibial and common fibular nerves. The crush injury site was identified through a non-dissolvable 8-0 silk yarn (Shalon, Brazil) suture located just at the edge of the epineurium. The muscle and skin were then closed with 4-0 nylon sutures (Somerville, Brazil), and the animals were placed in their cages to rest.

Whole body vibration therapy

The whole-body vibration training was performed on a vibration platform (TBS 100 A Total Image Fitness Inc, Canada), producing spiral vibrations and was adapted for animal's use. The rats were placed into two acrylic boxes (21 x 23.5 cm) attached on the vibration platform. The animals were conditioned in the WBS-chambers before surgery for one week and could move freely inside the boxes during the procedures.

Every WBV session comprised 5 sequential bouts each of 1 min at 15 Hz (g~0.9 m/s^2) vibration followed by 2 min at 30 Hz (g~3.6 m/s^2); between the bouts, a rest time interval of 1 min 30 s was adopted. The 5 bouts comprised a total of 15 min of WBV therapy. These settings were chosen according to the motoneuron discharges frequencies[21,22]. The amplitude used was 1 mm. The therapy was performed 5 days per week until the end of the fifth week after nerve injury. These parameters are common values used in physical training with vibration in humans[23] and animal studies[22].

The accuracy of the vibration frequencies of the equipment was measured using a 3D gyroscope, model GDM-3X2 (MicroStrain, USA) and through the Fourier transform analysis the equipment's frequencies were checked. The accelerometer was fixed on the central part of the block approximately at the position of the animal's boxes. To measure the amplitude of the equipment, a laser pointer was attached on the top of the block; after a projection on the wall, a displacement of 2 mm peak-to-peak was detected. The acceleration peak (a_{Peak}) was calculated through the formula: $D=a_{Peak}/(2 \times \pi^2 \times f^2)$, where "D" is the peak-to peak displacement and "f" is the frequency[24].

Locomotor, sensorimotor and balance performances

For measuring the locomotor, sensorimotor and balance performances after peripheral nervous injury, were performed, respectively, the Sciatic Functional Index (SFI)[25,26], the Horizontal Ladder Rung Walking Test (HLRWT)[9,10] and the Narrow Beam Test (NBT)[9,10]. These tests were realized weekly, starting in second week after nerve injury. The start was choosing because functional and nociceptive responses change slowly after surgery in rats with sciatic nerve crush[27]. Before the sciatic crush surgery, the animals were trained to the test apparatus for one week.

The SFI is an index based on measurements of the footprints of the rats during walking. This is a quantitative and reproducible method of the functional condition of rats after sciatic injury. The rats were trained to walk over a white sheet of paper covering the bottom of a 100 cm long and 8.5 cm wide track, which ended in a dark box. Afterwards, the animals had the plantar surfaces of their hind paws painted with dark dye, and they were placed on the track to walk. With a millimetre ruler, the rat footprints were used to determine the following measurements: distance from the heel to the third toe [print length (PL)]; distance from the first to the fifth toe [toe spread (TS)]; and distance from the second to the fourth toe [intermediary toe spread (ITS)]. These 3 measurements were obtained from the experimental (E) and normal (N) paws. Several prints of each foot were obtained on each track, but only three prints of each foot were used to determine the mean measurements in the E and N sides. These mean measurements were then included in the sciatic functional index-formula: SFI= -38.3 (EPL–NPL)/NPL+109.5 (ETS−NTS)/NTS+13.3 (EIT−NIT)/NIT−8.8. The result obtained was considered a functional index of the sciatic nerve, where 12 to -12 represents excellent function; -13 to -37, good function; -38 to -62, average function; -63 to -87, unsatisfactory function; - 88 to -112, complete deficit; and -113 to -137, worse than complete deficit[25,26].

The HLRWT apparatus was 100 cm long and 5 cm wide with horizontal parallel metal track (3 mm in diameter) was inserted 30 cm above the floor to create a horizontal ladder with a minimum distance of 1 cm between rungs which had an irregular pattern that was changed on each test day. In the NBT, the animals were required to walk along a 100 cm long, 2.6 cm wide flat surface beam that was elevated 30 cm above the floor. In both tests, a small dark box was at the end of the apparatus. The pathway was filmed from the side during 3 trials and the number of slips from the injured paw (right hind limb) in each trial was counted and these values were used for analysis[9,10].

The function tests were conducted at the same time of day by the same researcher, starting with the SFI test followed by the HLRWT test and NBT test. These tests were always performed fourth-eight hour after end each week training. The behavioral test protocols were highly standardized and maintained over time.

Histological and morphometric analysis

Thirty six hours after the end of vibration therapy, animals were anesthetised with sodium thiopental (50 mg/kg intraperitoneally; Cristália, Brazil), injected with 1000 IU of heparin (Cristália, Brazil), and transcardially perfused with 300 ml of saline solution followed by 300 ml of 4% paraformaldehyde (Reagen, Brazil) in 0.1 M phosphate buffer (PB, pH 7.4), at room temperature. A short segment (~2 mm) of the right sciatic nerve 1 mm distal to the crush injury site was dissected carefully from the surrounding tissue. The specimens were post-fixed by 2.5% glutaraldehyde (Sigma Chemical Co., USA) at 4°C until processed. The samples were then washed in 0.1 M PB and post-fixed in 1% OsO_4 (Sigma Chemical Co., USA) in 0.1 M PB for 30 min. The samples were washed again in 0.1 M PB, dehydrated in a graded series of acetone, embedded in resin (Durcupan ACM-Fluka, Switzerland), and polymerized at 60°C. Semi-thin cross-sections (1 µm) were obtained using an ultramicrotome (MT 6000-XL; RMC, USA) and stained with 1% toluidine blue (Merck, Germany) in 1% sodium tetraborate (Ecibra, Brazil)[26].

Images of the right sciatic nerve (ipsilateral to injury) were then captured and digitized (100X magnification and further amplified 200% for analysis) using an optical microscope (Eclipse E-600; Nikon, Japan) coupled to a Complementary metal-oxide semiconductor (CMOS) camera (Accu-Scope Inc., USA) and processed using Image Pro Plus 6.0 software (Media Cybernetics, USA).

For morphometric evaluation a set of 6 images from distal nerve portion, were chosen through random sampling, 3 random images from the periphery and 3 random images from the center of the nerve[26]. The total area evaluated for the distal segment of the sciatic nerve was the sum of six randomly selected areas of interest (AOI) with 4742.4 μm^2 in total. The morphometric measurements of area of myelinated fiber (μm^2), area of axon of myelinated fiber (μm^2) and myelin sheath thickness (µm) was estimated using the measurement tools of Image Pro Plus 6.0 software after spatial calibration performed on each image acquire. The diameters of the axons and fibers were estimated from the area of the corresponding axons and fibers, where their area was converted to the diameter of a circle with the equivalent area. Its values were used for calculation of degree of myelination (g-*ratio*) (the quotient axon diameter/fiber diameter)[26]. By adding together all the myelinated fiber areas it was possible to arrive at an estimate of the total area occupied by myelinated fibers and calculate its percentage from the total analyzed area (100%). By deducting this percentage of area from the total analyzed area it was possible to estimate the percentage of area occupied by the endoneurium connective and debris tissue, blood vessel and unmyelinated nerve fibers[26].

Statistical Analysis

Data were analyzed by two independent researchers; one was blind to treatment. All data are reported as mean ± standard error of the values of 10 or 11 animals. Morphometric analysis was performed through one-way ANOVA (factor: lesion and treatment) and Bonferroni post hoc ($p<0.05$). The locomotor and sensorimotor tests were analyzed through Generalized Estimating Equations (GEE) followed by Bonferroni post hoc ($p<0.05$). Data were analyzed using SPSS 22.0 software (Statistical Package for the Social Sciences Inc., USA).

Results

Locomotor, sensorimotor and balance performances

In the Sciatic Functional Index (SFI; Figure 1), all injured groups (NT, V3, V10) showed significantly lower values than
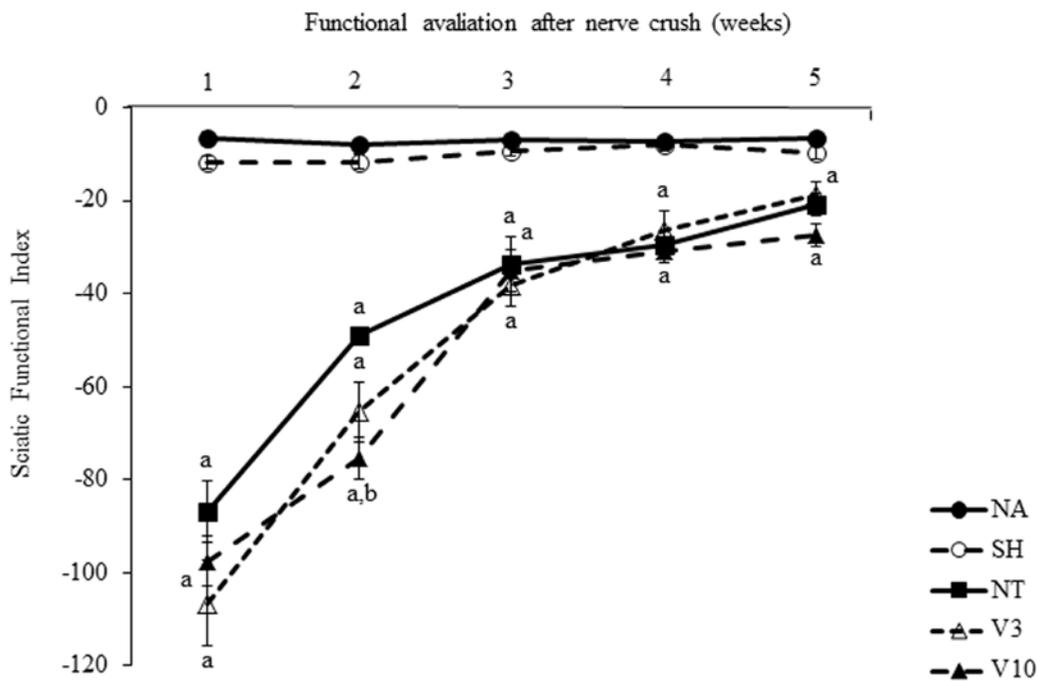
Figure 1. Assessment of Sciatic Functional Index (SFI) in rats without (NA and SH) or with sciatic nerve crush (NT, V3 and V10 groups). Data were expressed as means ± standard errors of the mean. a: p<0.05 vs NA and SH groups. b: p<0.05 vs NT group (Generalized Estimating Equations followed by Bonferroni post hoc test). NA, naive group; SH, sham-operated group, NT, untrained injured rats; V3, trained with WBV starting 3 days after crush injury; V10, trained with WBV starting 10 days after crush injury.

uninjured groups (NA and SH) along the 5 weeks assessments (p<0.05). In the 2nd week after surgery, there was no significant difference between injured groups, however, in the 3rd week after surgery, the V10 group showed significantly lower values than NT group (p<0.05). There were no significant differences in values between V3 group (p>0.05) and the other injured groups at this time point. In the 4th and 5th week assessments, there were no significant differences between injured groups (p>0.05) and these groups showed values significantly lower than uninjured groups (p>0.05).

In the horizontal ladder rung walking test (HLRWT; Figure 2), there were no differences in hind limb slips between the uninjured groups (NA and SH) (p>0.05). All injured groups (NT, V3, V10) showed significantly greater hind limb slips than NA and SH groups along the 5 weeks assessments (p<0.05). However, the limb slips did not differ significantly between injured groups (p>0.05), except in the 2nd week assessment. At this time point, the V10 groups showed significantly greater hind limb slips than the NT group (p=0.018).

On the narrow beam test (NBT; Figure 3), there were no differences on hind limb slips of uninjured groups (NA and SH) (p>0.05). However, the limb slips did not differ significantly between injured groups in the 2nd, 3rd and 4th weeks assessments (p>0.05). In the 5th week assessment, only the V10 group showed significantly greater hind limb slips than NT (p<0.05). In the 6th week assessments the V10

group also showed significantly greater hind limb slips than NT (p<0.05).

Histological and morphometric analysis

Histological analysis of the distal segment of the regenerating sciatic nerves (Figure 4A-D) revealed large myelinated fibers and scant space between these fibers on the uninjured groups. Also, small-diameter, thin myelin sheath fibers, degeneration debris and increased endoneurial connective tissue predominance between the injured groups. The morphometric parameters analyzed confirmed these differences. The average percentages of myelinated fibers area and endoneurial connective tissues, vessels and unmyelinated nerve fibers area of injured groups were, respectively, lower and higher than values from uninjured groups (Figure 5A-B; p<0.05).

Besides that, the injured groups showed a lower area of myelinated fibers (Figure 5C), lower area of axon (Figure 5D), lower myelin sheath thickness (Figure 5E) and a higher degree of myelination (Figure 5F) from uninjured groups (p<0.05). Those results were because of the diameter of axon and myelinated fiber of NT (2.95±0.12; 4.33±0.21, respectively), V3 (2.77±0.18; 4.03±0.23, respectively) and V10 (2.95±0.18; 4.16±0.24) were smaller than NA (4.97±0.08; 7.97±0.14) and SH (4.87±0.17; 7.77±0.17)
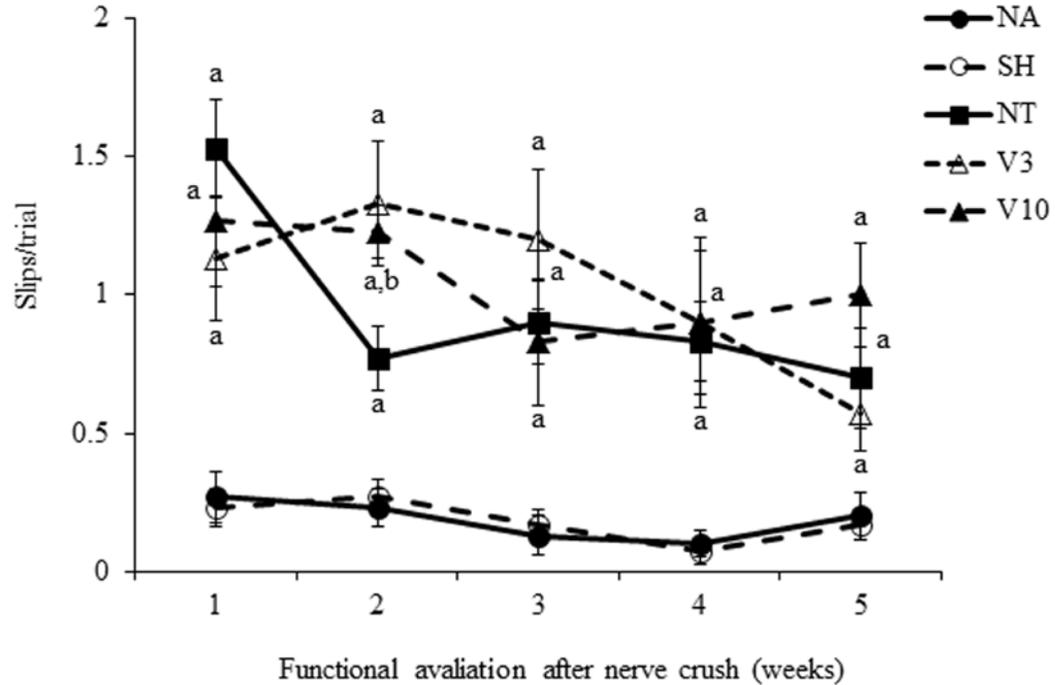
Figure 2. Responses to Horizontal Ladder Rung Test (slips by trial) in rats without (NA and SH) or with sciatic nerve crush (NT, V3 and V10 groups). Data were expressed as means ± standard errors of the mean. a: p<0.05 vs NA and SH groups. b: p<0.05 vs NT group (Generalized Estimating Equations followed by Bonferroni post hoc test). NA, naïve group; SH, sham-operated group, NT, untrained injured rats; V3, trained with WBV starting 3 days after crush injury; V10, trained with WBV starting 10 days after crush injury.
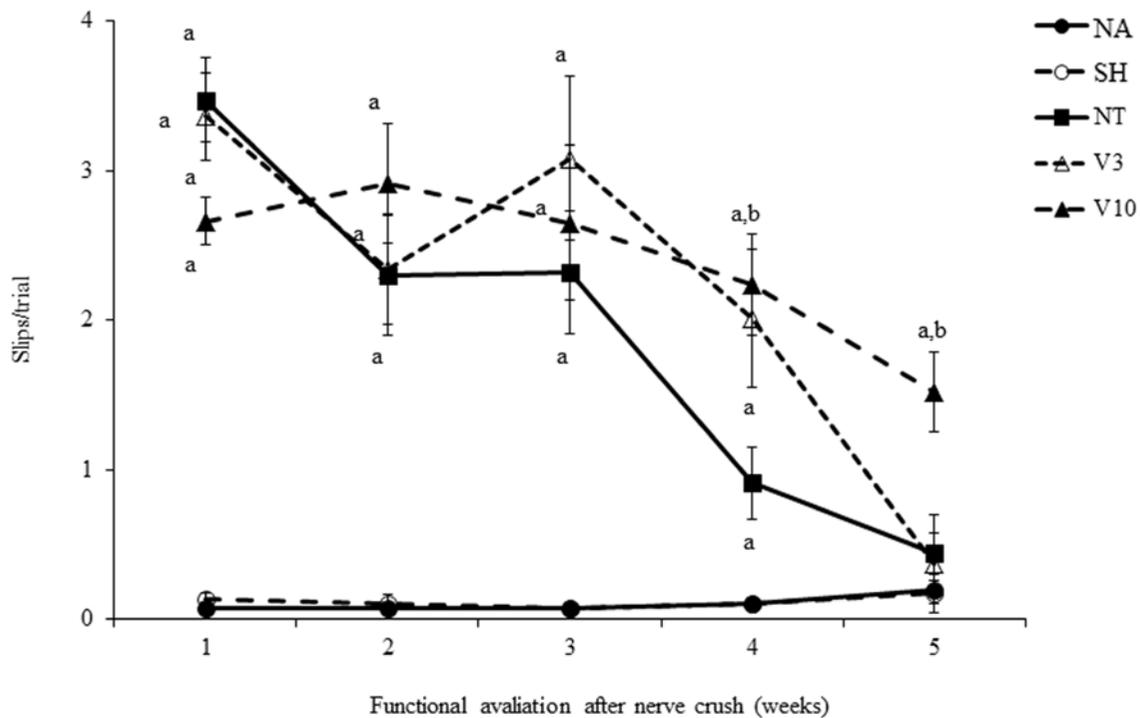

Figure 3. Responses to Narrow Beam Test (slips by trial) in rats without (NA and SH) or with sciatic nerve crush (NT, V3 and V10 groups). Data were expressed as means ± standard errors of the mean. a: p<0.05 vs NA and SH groups. b: p<0.05 vs NT group (Generalized Estimating Equations followed by Bonferroni post hoc test). NA, naïve group; SH, sham-operated group, NT, untrained injured rats; V3, trained with WBV starting 3 days after crush injury; V10, trained with WBV starting 10 days after crush injury.
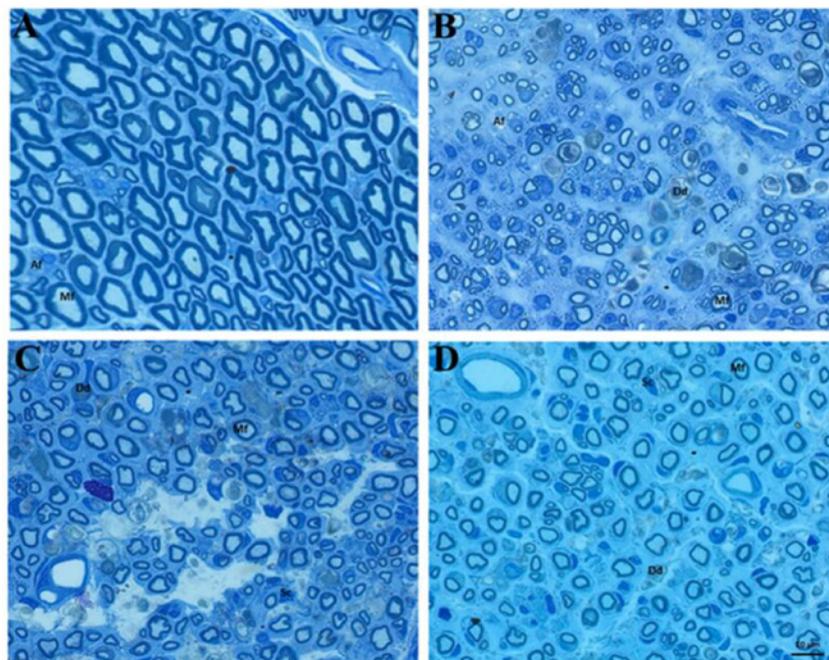
Figure 4. Digitized images of transverse semithin sections of the distal portion of the sciatic nerve stained with toluidine blue of rats without (NA) or with sciatic nerve crush (NT, V3 and V10 groups). A: Naive group (NA); B: untrained injured rats (NT); C: trained with WBV starting 3 days after crush injury (V3); D: trained with WBV starting 10 days after crush injury (V10). Af, unmyelinated nerve fiber; Mf, myelinated nerve fiber; Sc, Schwann cell. *, endoneurium connective tissue; Dd, degeneration debris. Scale bar = 10 μm.

groups. No significant differences were observed between injured groups to all analyzed measurements (p>0.05).

Discussion

This study analyzed the effects of WBV on the functional and morphological nerve recovery in the sciatic nerve crush experimental model. According to the SFI results, all injured groups (NT, V3 and V10) presented initially (2nd week after the injury) complete deficit characteristics (values within the range of -88 to -112), and they gradually reached a good function (values within the range of -13 to -37) in the 4th week after injury[25]. At the end of this study, those groups have partial functional recovery, since their SFI values remained different from the uninjured animals. Other studies showed variable results on SFI, presenting total functional recovery at different days like 21[28], 28[12], 35[8] and 49[29] days after the sciatic nerve crush for sedentary animals and from the 14[12], 21 to 28[6], 42[8] days for low-intensity aerobic treadmill running program[12], for swimming protocol[6] and 42 days for resistance training[8]. So, the functional recuperation is also dependent on the therapy protocol type based on this study WBV was not able to promote beneficial effects in this functional parameter.

In the HLRT, the groups NT, V3 and V10 showed progressive recovery over time, but at the end of the study,

similarly, the groups of injured animals had a higher number of slips from the injured hind limb, compared to the uninjured groups (NA and SH). In the NBT, the three groups of injured animals also showed recovery over time, and as in the other functional tests, the NT and V3 groups did not present differences among themselves. Notwithstanding the NA and SH groups achieved a complete functional recovery. In this test, only the V10 group did not reach full recovery. Similarly, in another study, animals with sciatic crush presented functional recovery 35 days after injury in the NBT but no in the HLRT[9]. Thus, there are different recovery rates in two tests. The HRLT allows changing the distance between ladder steps to create an irregular pattern, requiring the animals to adapt their lifts according to the ladder arrangement complexity[30], therefore, demanding a greater challenge in the HLRT execution for animals, resulting in the longer time to complete the recovery. In addition, the NBT is a more sensitive method to check discrete deficits in limb positioning and body balance, while the HRLT is appropriate for assessing sensorimotor coordination of limbs and descending motor control pathways[31].

After the peripheral nerve injury, the muscle spindle fibers are among the ones that recover less their synaptic connexions[5]. Synapsis among Ia fiber and motor neurons are weaker and responsible for remodeling the inhibitory descending neuronal systems. Spinal reflex is highly facilitated at the early stages of reinnervation and reverted
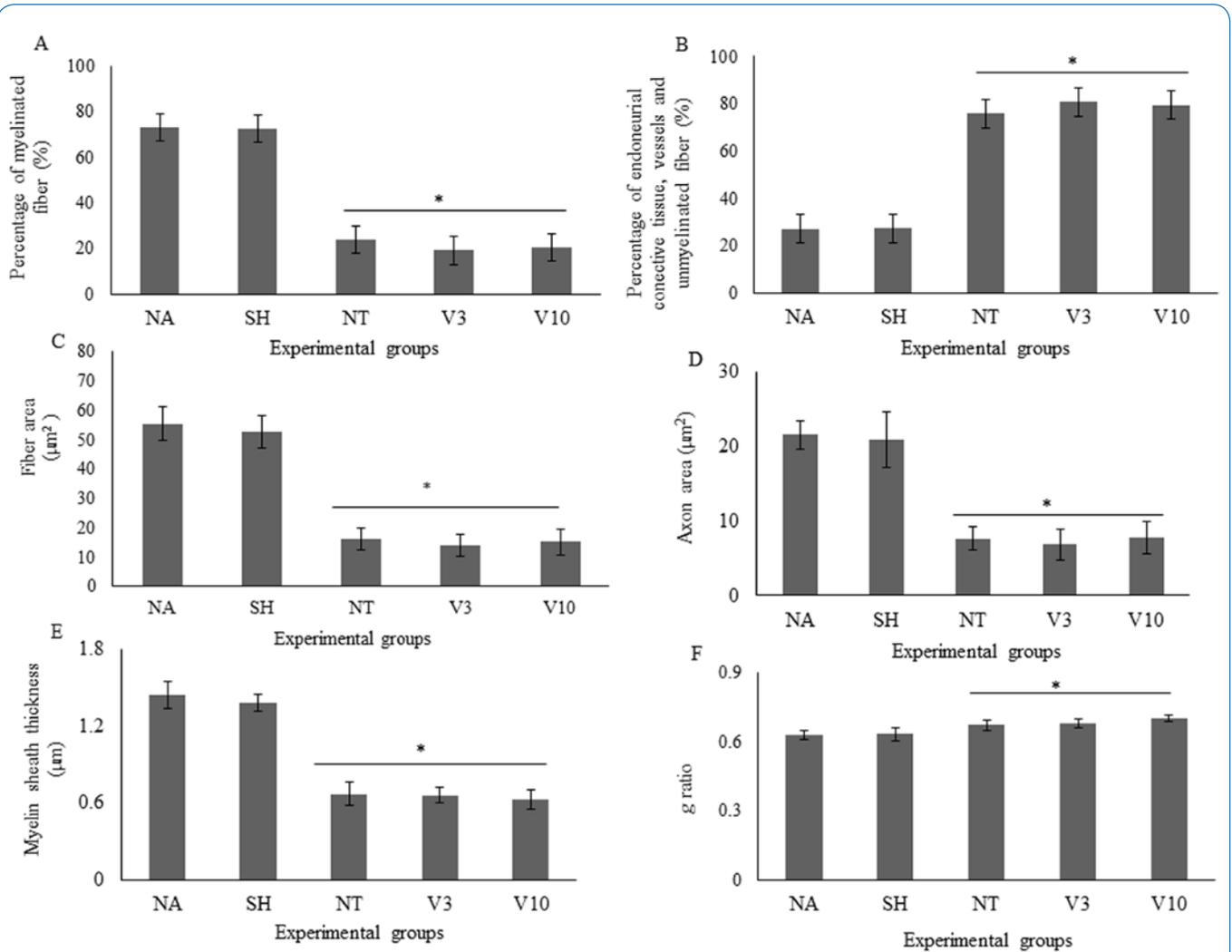
Figure 5. Morphometric parameters of the percentage of area occupied by myelinated fibers (A), percentage of area occupied by endoneurium, blood vessels and unmyelinated fibers (B), mean area of myelinated fibers (C), mean axon area of myelinated fibers (D), mean sheath thickness (E) and mean myelinization degree (F) of the sciatic nerve for the studied groups: Naive (NA), Sham (SH), untrained injured (NT), trained with whole-body vibration starting 3 days after crush injury (V3) and trained with whole-body vibration starting 10 days after crush injury (V10). Data expressed as mean ± standard error of the mean. * p<0.05 vs NA and SH groups (one-way ANOVA followed by Bonferroni post hoc test).

when advancing muscle reinnervation. They must be restored according to the process of reinnervation during the regenerative phase[32]. Deficits in transduction stimulus of muscle spindles, abnormalities reported in spinal reflex facilitation and synaptic excitability, which are correlated with the injury severity, delimit the degree and timing course of neuromuscular control[32].

The vibration protocol of this present study is considered mild with 15 and 30 Hz frequencies in sequential bouts and 2 mm amplitude. According to different training exercise protocols muscle strength has been shown to increase[33], decrease[34] or be unchanged[35] after acute bouts of WBV. Although the specific mechanism underlying adaptations

to WBV remain poorly defined, acute WBV implies that neural mechanisms are likely responsible[36,37]. Among the studies involving WBV, and also in consequence of different in experimental designs, it is a consensus that the responsiveness of the Ia pathway on the acute effect of WBV has yet to be reached[35,37,38].

The stimuli during and after the WBV acts on presynaptic terminals interconnect the primary spindle endings (Ia-afferents) with an α-motoneuron, increasing the synaptic transmitter release prior to spinal motoneurons[39]. Then, immediately following WBV, the responsiveness of the Ia pathway is diminished and contractile function is impaired[37,40] In addition the reduction of spinal cord reflexes (stretch reflex

and Hoffmann´s reflex) occurs[39] and Meissner and Paccini mechanoreceptors sensitivity decreases[41]. These data can suggest that sensitive fibers are more susceptible to the effects of vibration and can contribute to the understanding of the functional results found in this study since the WBV protocol could be unsettled the sub-acute phase of nerve recovery, altering the spinal loop excitability and the neuromuscular control.

The neural mechanisms underlying the improved performance following WBV lie with the alternative hypotheses involving spindle disfacilitation or Golgi afferent modulation[37]. Muscle spindle disfacilitation, either as a result of γ motoneuron fatigue or an adaptation of the spindle apparatus, has been reported following prolonged tendon vibration[42]. The hypothesis that muscle spindle disfacilitation underlies a reduction in Ia pathway efficacy is further supported by the observation that successive reductions in H:M ratio occurred with repeated bouts of WBV similar to the relationship observed between the preceding number of contractions and reduction in muscle spindle afferent firing rate in one previous study[42].

For body balance control, neural feedback of muscles and tendons, spinal motor control in association with visual and vestibular systems provide dynamic updates of self-orientation related to locomotion[43]. In the WBV, animals need to maintain body balance on the moving vibratory platform, requiring a sensory stimulation of skin, muscle and joints receptors. We hypothesized that vibration stimulus also harms the mechanism of regulation of excitatory and inhibitory circuits of spinal cord and supra-spinals structures during the sub-acute phase of nerve regeneration. In cases of chronic pain, central sensitization is established with neuronal hyperexcitability, reduced firing thresholds, and unchecked feedback loops between glia and pro-inflammatory molecules even after the painful stimulus has been removed[44]. Then, central sensitization and mechanical allodynia also can help to understand why the group vibrated later did not reach the full recovery at the end of the experiment. In 10 days after injury of peripheral nerve, without any intervention, the peripheral inflammation is high and when the nerve is exposed to an abnormal situation as peripheral inflammation, noxious stimuli or mild WBV, A^β fibers may signal pain and allodynia is generated disturbing other axon terminals in the dorsal horn of the spinal cord[45].

In addition, during the sub-acute phase, axon growth and functional recovery of nerve and target organ tissue also depends on microcirculation, trophic factors and inflammatory mechanisms[42]. Experimental studies have demonstrated that vibration therapy can have a dual effect, improving angiogenesis and anti-inflammatory substances[46] or increasing inflammatory[22] response according to the WBV applied time after injury[21,22]. The difference between the NT and V10 groups, according to our hypothesis, is that V10 was exposed to mild WBV during high levels of nerve inflammation, generating allodynia and worsening functional recovery. The early WBV at V3 group, probably, did not cause allodynia, however, did not change the natural recovery course.

According to the histological and morphometric, all the injured groups showed in 38th day after nerve crush, features of incomplete nerve regeneration[32]. Wallerian degeneration, degeneration debris, and small thin myelinated fibers and enlarged endoneurial connective tissue predominance featuring partial recovery found in the injured groups. These features of incomplete nerve regeneration were also identified by other studies with similar methodology, 30 days[6], 32 days[10], 49 days[8] and 119 days[26] after sciatic nerve crush in rats.

During nerve recovery, the myelination is generally much thinner than normal altering nerve conduction velocity represented indirectly by g-ratio. Those are associated with deprivation of the end-organ connections, increased of connective tissue, endoneurium retraction and late effects of neuronal body injury[6]. The higher degree of myelination (g-ratio) obtained by injured groups suggests an indirect representation of lower nerve conduction velocity expected for this type of injury.

Different therapeutic modalities have been used to speed up nerve regeneration after injury crush model. Aerobic[8], balance plus coordination[10] and swimming[6] exercises, for example, promoted higher thickness of the myelin, less amount of connective tissue and a lower rate of myelination[8], lower density[10] and higher diameter of myelinated fibers[6]. However, these benefits to speed up nerve regeneration after injury crush model were not found with endurance and aerobic training combined with resistance training[8]. In our study, similarly WBV presents no direct influence in the nerve regeneration and morphological tissue repair. Therefore, nerve regeneration can be influenced by some therapy modalities.

In the study of Robinson et al.[47], low evidence was identified of WBV therapy to promote improvement in neuropathic pain and balance in patients with diabetic peripheral neuropathy. These data do not support the current use of WBV in patients with diabetic peripheral neuropathy for relieving neuropathic pain, improving balance and plantar tactile sensitivity[47]. It is known that nerve regeneration in diabetes is essential for the reversal of peripheral neuropathy and also promotes the recovery of nerves from injury as a result of acute nerve compression and entrapment.

It´s important to remember that vibration can be dangerous to the body structure when the parameters are extreme (frequencies stronger than physiological resonance frequency of body or long exposition time[48,49]). It is associated to the occupational vibration exposure producing a musculoskeletal, neurobiological, and vascular pathology hand-arm vibration syndrome (HAVS)[50]. This is characterized by damage to myelin and axonal transport and whose early process is related to vascular endothelial cell injury[51]. Persistent hyperalgesia, pro-inflammatory cytokines, interleukin (IL) 6 and tumor necrosis alpha (TNF α) by ergonomic vibration can induce muscle and vessel damage by activation ischemia-reperfusion injury secondary to reflex vasoconstriction and are critical for local expansion of immune regulatory cells[50]. Another study in rabbits used WBV also in extreme conditions, 4.5 Hz, 3h per day

exposition time over a 2-week period showed ultrastructural changes as nuclear clefting and biochemical alterations in dorsal root ganglion neurons[52]. It seems that morphological and several biochemical changes in the vibrated nervous tissue are directly related to the extreme WBV protocols. We believe that the mild vibration protocol used in this study is the one of reason why we did not find morphologic changes in nerve repair in the vibrated groups.

To the best of our knowledge, this was the first study that verified morphological nerve analysis and functional recovery of rats submitted to a WBV protocol after a nerve crush model. Whole body vibration training starting at 3 or 10 days after the sciatic nerve crush appears to present neutral effects on morphological nerve and some functional parameters of locomotor and motor coordination with similar results as the injured and not trained animals. The earlier WBV therapy did not modify the expected and natural (without intervention) recovery time after lesion and the later impaired this functional parameter of recovery. The decrease in Ia pathway's responsiveness, central sensitization and mechanical allodynia appear to be the causes of the poor influence of WBV in the functional parameter of recovery after peripheral nerve injury without interfering in morphological alterations in the regeneration process. Nevertheless, to better elucidate the effects of WBV on peripheral nerve regeneration, it is suggested to carry out further studies that include analysis of vibration about these electrophysiological peripheral and central stimuli, synaptic and tissue repair mechanisms (angiogenesis, pro and anti-inflammatory substances and growth factors, muscle force and resistance) important for functional recovery but not analysed in this study.

This study contributes to the therapeutic understanding of whole-body vibration, analyzing its effects on the morphological and functional recovery after peripheral nerve injury in an experimental model. The earlier whole-body vibration therapy did not modify the expected and natural (without intervention) recovery time after injury and the later impaired this functional parameter of recovery. These data not support the use of whole-body vibration to stimulate peripheral nerve regeneration which is optimized in the treatment of neuropathies and peripheral nerve injuries. Further studies are needed to clarify the effects of this therapeutic alternative on this clinical condition.

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