**OBJECTIVES:** This prospective, randomized, experimental study with rats aimed to investigate the influence of general treatment strategies on the motor recovery of Wistar rats with moderate contusive spinal cord injury.

**METHODS:** A total of 51 Wistar rats were randomized into five groups: control, maze, ramp, runway, and sham (laminectomy only). The rats underwent spinal cord injury at the T9-T10 levels using the NYU-Impactor. Each group was trained for 12 minutes twice a week for two weeks before and five weeks after the spinal cord injury, except for the control group. Functional motor recovery was assessed with the Basso, Beattie, and Bresnahan Scale on the first postoperative day and then once a week for five weeks. The animals were euthanized, and the spinal cords were collected for histological analysis.

**RESULTS:** Ramp and maze groups showed an earlier and greater functional improvement effect than the control and runway groups. However, over time, unexpectedly, all of the groups showed similar effects as the control group, with spontaneous recovery. There were no histological differences in the injured area between the trained and control groups.

**CONCLUSION:** Short-term benefits can be associated with a specific training regime; however, the same training was ineffective at maintaining superior long-term recovery. These results might support new considerations before hospital discharge of patients with spinal cord injuries.

**KEYWORDS:** Spinal cord injury; Rehabilitation; Exercise movement techniques.

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**INTRODUCTION**

Spinal cord injury (SCI) either completely or partially damages the ascending and descending spinal pathways, leading to a loss of adequate stimulus perception and imprecision in the reorganization of motor and sensory behaviors (1). In such cases, physical therapy and training are considered to be the most efficient strategies for inducing neuroplasticity and motor recovery (2). Therefore, recent functional recovery studies in human and animal models focus on new methods of optimizing the sensory information associated with locomotor training (3,4). However, the effect of certain physical therapy and rehabilitation strategies used in human functional recovery remains unclear. Experimental studies with injured animals undergoing a range of training strategies in different environments may facilitate an understanding of SCI recovery mechanisms and may promote the development of improved human rehabilitation programs. This study aimed to assess the therapeutic effects of different types of locomotor training in Wistar rats with moderate contusive spinal cord injury.

**MATERIALS AND METHODS**

**Animals**

A total of 51 male adult Wistar rats (150–300 g) were divided into five groups according to the type of exercise they underwent: control (n = 11), maze (n = 10), ramp (n = 9), runway (n = 10) and sham (n = 11). The sample size was estimated according to the Multicenter Animal Spinal Cord Injury Study (MASCIS) using the New York University (NYU) protocol, which was previously used in studies published by our laboratory (5-7). The runway, maze, ramp, and control groups were subjected to contusive SCI, whereas the sham group was subjected only to the surgical...
procedure without SCI. The project and all of the procedures were approved by the University of São Paulo Ethical Committee (CAPPesq).

Surgical procedure

The rats were anesthetized with diazepam (intraperitoneal (ip), 0.1 ml/100 g) and ketamine hydrochloride (ip 0.2 ml/100 g). Lidocaine hydrochloride was administered as a local anesthetic. The spinal cord contusion surgeries were performed by the biologist in charge of the laboratory (who was blind to the other procedures planned for each rat) and in accordance with MASCIS using the NYU-Impactor. Laminectomy at the T9-T10 thoracic level exposed the spinal cord, and a 10-g rod dropped from a 25-mm height produced a moderate injury (5,8). The antibiotic cefazoline (0.1 ml/100 g) was administered immediately after surgery and once a day for seven days.

Apparatuses

Three locomotor training apparatuses, a runway, a maze, and a ramp (Figure 1), were constructed for this study. Runway: the animals walked on a horizontal plane surface. Maze: the animals walked on a horizontal surface with different textures at every 20 cm (flat, rugose, rough, smooth, pointed, and undulate) and with blockages that lead to deviations from the path. Ramp: the animals walked on an inclined surface (15°, 20°, 25°, or 30°), with the slope depending on the functional recovery.

Training

All of the animals underwent a 10-minute handling to gain familiarity with the researcher two days before the experimental procedures. The maze, ramp, runway, and sham groups were subjected to a 12-minute training procedure twice a week during the two weeks before the SCI to learn the task that they needed to perform in the apparatuses (9). The rats were food-deprived for 24 hours before the first two trainings, and water with sucrose was used as a reward during the trainings to stimulate the animals to accomplish the tasks. After the SCI, twice-a-week training sessions were initiated on the third post-operative day (POd) without food or water deprivation; these sessions also lasted 12 minutes and were continued for five weeks.

Behavioral assessment

The Basso, Beattie, and Bresnahan (BBB) Open Field Locomotor Scale was used for the motor recovery assessment (10). The BBB test has a 21-point rating scale: complete hindlimb paralysis is scored 0, and normal locomotion is scored 21 (1,8). This scale assesses hindlimb movement and position, dorsal or plantar stepping, forelimb-hindlimb coordination, toe clearance and trunk and tail position, among other features of motor functional recovery. An open field (90 cm × 90 cm) with a non-slippery floor and two video cameras were used for the locomotor assessment. All of the animals were evaluated for four minutes on the 1st, 8th, 15th, 22nd, 29th, and 36th POd by three observers who were blinded to the allocation of the animals. The videos were recorded for further analysis in case of divergence in the BBB scores assigned by the observers. If there was any divergence, the evaluators discussed the case until they reached a consensus.

Histology

On the 45th POd, all of the animals were anesthetized with ketamine overdose and were then perfused transcardially with saline and 4% buffered paraformaldehyde. The spinal cords were dissected, and a 2-cm segment (centered on the lesion epicenter) was removed. The tissue was post-fixed overnight in the same fixative, immersed for one day in PBS and then embedded in paraffin.
Transverse 7-µm serial sections were cut using a Leica RM 2155 microtome. Slices were selected every 0.5 cm rostral and caudal from the injury epicenter (slices from levels +1.0 cm; +0.5 cm; epicenter; -0.5 cm; -1.0 cm) and were stained with cresyl violet.

Spinal cord morphometry was conducted in five randomly chosen animals from each group (ten slices per animal, two slices for each level). The sections were examined at low magnification (5X), and the images were captured with a Canon Power Shot A620 digital camera coupled to an Axio Scope 40 microscope (Carl Zeiss, Jena, Germany). The outer borders of the white and gray matter were spared, and the entire spinal cord section (Figure 2) was manually traced using AxioVision 4.5 software to determine its area (µm²). The accuracy of the tracing was verified by viewing the delineations at a higher magnification.

Statistical analysis

The locomotor scores for the hindlimbs were averaged together for each rat. All of the parametric data were analyzed using Statistical Package for Social Sciences (SPSS) software. The open field locomotor scores were compared using an analysis of variance (ANOVA) with the factors group (between-subjects) and POd (within-subjects and repeated measure factor). A one-way analysis was used to examine the differences in the between-subjects factor. A post hoc Bonferroni test was used when necessary to examine specific differences between the groups.

One-way ANOVA was used for between-group comparisons of the spared tissue. Pairwise post hoc comparisons were made with Bonferroni-corrected t tests.

RESULTS

Behavioral assessment

As expected, the sham group achieved the highest BBB score (21 points) from the first day after the surgical procedure without spinal cord injury. The other groups showed no significant differences on the 36th POd (F3,30 = 0.34, p = 0.790, Figure 3), indicating a contusive injury consistency with no movements or slight movement of one or two hindlimb joints (mean locomotor BBB scale ranging between 0 to 0.4).

The results showed a significant main effect for post-operative day (F5,130 = 95.32, p < 0.001), a significant POd × group interaction (F(5,130) = 2.57, p = 0.002), and a marginally significant main effect for group (F(3,31) = 2.85, p = 0.054). On the 36th POd, there were no significant differences (e.g., the rats had similar spinal cord lesions), and soon after the training intervention, on the 8th POd, the maze and ramp groups achieved better motor control performances (as verified by BBB scores) compared with the control and runway groups (F(3,30) = 7.468, p = 0.001). Whereas the control and runway groups achieved BBB scores of 1.8 and 2.1, respectively, the maze and ramp groups achieved BBB scores of 5.8 and 7.4, respectively. Functionally, there is a significant difference between these scores because up to 2 points, the animals have extensive movement in one joint and slight movement in another one, whereas from 3 to 7 points, animals exhibit extensive movement of all three joints. Thus, there were practically no improvements in the control and runway groups, which resulted in a delay of functional recovery among these animals compared with the others.

By the end of the experiment, the rats subjected to training on the ramp apparatus showed 11.4 BBB punctuation, which means that these animals had frequent to consistent weight-supported plantar steps (10). This motor behavior was not achieved by any other group, even occasionally. However, there was no significant difference among the groups at the 36th POd.

Notably, the control group showed motor recovery similar to that of the runway group from the beginning of the experiment. This result might be due to the lower effort required by the runway task compared with the training in the maze and ramp apparatuses. However, it should be remarked that these rats could move freely in their cages as well as the other groups. There was no significant functional difference between the control and runway groups, even in the first week; thus, exercising on the runway or moving freely in the cage result in the same, functionally. The highest BBB score obtained by these groups was 7.5, which corresponds to the capacity to move all three joints of the hindlimbs extensively but without any body weight support. This score was the same as that achieved by the ramp group on the 8th POd. Although these differences could be noted, at the end of the experiment, there were no significant differences among any group.

Histological assessment

Histological analysis showed that spinal cord weight-drop contusion resulted in cavities and loss of gross tissue structure, which could be observed as alterations in both white and gray matter at the injury epicenter and throughout several millimeters rostrally and caudally in all of the injured animals. Large cystic cavities were observed in regions previously occupied by the gray matter, as illustrated in Figure 4. The spared tissue was lightly stained and appeared similar to partial demyelination, and a microcystic zone was observed. It could be noted in many spinal cord slices that a small region of gray matter was spared in the superficial dorsal horns (Figure 4). Otherwise, as expected, the sham group histology revealed intact spinal cords, as demonstrated in Figure 2.

The results showed a significant main effect for distance (F4,80 = 12.357, p < 0.001), a non-significant distance × group interaction (F12,80 = 0.816, p = 0.634) and a non-significant

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**Figure 2** - Sham spinal cord transverse slice, 7 µm thick, with indication of total spinal cord and gray matter areas (µm²). The white matter area was calculated by subtracting gray matter area of the total spinal cord area.
main effect for group ($F_{3,20} = 0.913, p = 0.452$). A post hoc test showed that the lesion was more severe at the epicenter than at the extremities (Figure 5).

DISCUSSION

In this study, training sessions in the apparatuses were initiated on the third day after injury. This early intervention appears to have led to more efficient motor recovery of the maze and ramp animals, as these groups exhibited performance improvement from the beginning compared to the control and runway groups. Certain authors (11,12) suggest that a small interval (two weeks, subchronic phase) between generation of the lesion and intervention could be more favorable for axonal regeneration and behavioral recovery. More recently, Smith et al. (13) also indicated that swimming training initiated acutely might be detrimental to functional recovery due to the exacerbation of the acute phase inflammatory processes.

Many authors agree that early intervention is an efficient strategy for rehabilitation (14-16). They support the idea that the regeneration capacity decreases over time in not only humans but also experimental models due to many factors, such as axonal demyelination, increase in the lesion area, loss of neurons and glial scar formation, among others.

The training sessions on the ramp and maze apparatuses were more effective for performance improvement soon after the SCI. This fact is critical when considering that it is desirable for injured persons to acquire functional independence as soon as possible. According to Norrie et al. (15), the early training session facilitates the synapses that are more plastic before the development of aberrant reflex connections. Thus, rehabilitation programs should initiate as rapidly as possible in an attempt to minimize secondary damage caused by injuries to achieve functional recovery through the motor features that remain after SCI.

However, the beneficial aspects of maze and ramp training observed in the early stage after the contusion did not persist in the late phase. The stimulus provided by these apparatuses was not sufficient to generate a better recovery than that achieved by the runway or control groups. It is possible that the training load should vary over time for the acquisition of new motor features rather than merely maintaining those already gained.

Although 24 minutes of training per week could appear insufficient to cause motor control improvement, recently, Smith et al. (13) reported that only eight minutes of swimming per week is sufficient to induce a degree of functional recovery of hindlimb movement.
Training in spinal cord injury

Notably, the control group displayed motor recovery without being subjected to any type of training. These data indicate that there is spontaneous recovery after experimental spinal cord injury, supporting Leung and Wrathall (17), who claimed that a level of functional recovery could be reached without intervention. This recovery is mediated by a structural reorganization of the residual motor system, which may develop over weeks in animal models of SCI and over many months in humans (18). Although there is a spontaneous recovery, this recovery is delayed by the limited capacity of the mammalian central nervous system to reestablish functional neuronal connections (16). This result is in accordance with our data: until the 8th POD, the control motor recovery was less than that of the maze and ramp groups. In a study of rats with spinal injuries, Siegenthaler et al. (19) also verified that animals trained on a running wheel showed greater improvement until the second week after injury, whereas the peak improvement of sedentary animals was delayed by one more week.

Although there was earlier improvement in the animals subjected to sensorimotor training, more long-term outcomes were not different between the trained and control groups. This result was also observed by Sandrow-Feinberg et al. (20), who used forced exercise as a rehabilitation program after unilateral cervical SCI and verified an early recovery of the trained rats that was not significant compared with that of the control group at the end of the experiment. Andrade et al. (21) also observed a better evolution of the BBB score in injured treadmill-trained rats during the acute period after the injury, but not in the chronic phase.

Soon after SCI, animals develop a flaccid pattern below the lesion level, which means that there are no observable hindlimb joint movements. This flaccidity is the reason that an injured animal’s pattern of stepping remains dorsal. The ramp appeared to be the most efficient in promoting plantar stepping, which can be attributed to the task specificity required by this apparatus. It is believed that the improvement occurred due to the demand imposed by the ramp, in which the animals had to coordinate their steps while going up and down the ramp, actions which require different types of muscle recruitment against a greater load. Multon et al. (22) have also shown that animals subjected to treadmill training after acute incomplete spinal cord compression are able to support their body weight and perform plantar steps. The animals did not exhibit forelimb-hindlimb coordination, which further corroborates our outcomes.

There were no histological differences in the injured area between the trained and control groups. Nevertheless, the animals exhibited functional improvement. These data are in accordance with studies by Siegenthaler et al. (19), who demonstrated no significant difference in the cord area between sedentary and exercised (voluntary wheel running) groups, and Hutchinson et al. (23), who showed no significant differences in the percentages of white matter spared at lesion epicenters among the exercise (treadmill, swimming or standing) and no-exercise groups. Ahmed and Wieraszko (24) verified that the total area of the lesion epicenter and the total spared area were very similar in all of the experimental groups (acrobatic exercised plus magnetic stimulation, acrobatic exercised, magnetic stimulation and control). However, these authors also verified significant improvement in the trained animals’ motor behavior compared to that of the control animals, as in our findings. The absence of histological differences among groups shows that our study groups were homogenous.

The histological analysis revealed that different training types do not significantly change the injured spinal cord area, demonstrating that the nervous system is still able to promote functional recovery when stimulated correctly. Additionally, the histological homogeneity indicates that the functional recovery was likely due to exercising and not to a difference in the severity of the lesions. All in all, the type of training appears to be an important factor in the functional recovery of animals with contusive spinal cord injury.

It is possible that if the training in the apparatus were conducted more frequently (i.e., five times a week instead of two), the results that we observed of greater improvements in the ramp and maze groups in the acute stage after injury could have persisted until the end of the experiment. In addition, other histological analyses could have been performed to identify more specific changes in the spinal cord after rehabilitation procedures and training, along with quantification of neurons or the verification of glial scarring.

Because the training assisted functional recovery in spinal cord injury, this study shows that specific training protocols are crucial to reduce the necessary time for motor behavior recovery in the early period. This observation is extremely relevant to therapy, as the independence of an injured person is one of the primary factors that leads to a better quality of life. However, the training sessions on the ramp and maze apparatuses failed to induce better motor improvement at the end of the experiment. These data...
suggest that different training protocols should be used at different rehabilitation stages, especially when discharging patients from care units, considering their capacity to perform simple daily activities.

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AUTHOR CONTRIBUTIONS

Miranda TA was responsible for the study design, experimental study planning and data collection, data analysis and interpretation, manuscript writing and final reviewing. Vicente JM was responsible for the study design, data collection, data analysis and interpretation, and final reviewing of the manuscript. Marcon RM was responsible for data analysis and interpretation and final reviewing of the manuscript. Cristante AF and Morya E were responsible for data analysis and interpretation, manuscript writing and final reviewing. Valle AC was responsible for the study design, experimental study planning, manuscript writing and final reviewing.

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