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Overground gait training promotes functional recovery and cortical neuroplasticity in an incomplete spinal cord injury model

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ABSTRACT

Aim: Evidence suggests that task-specific gait training improves locomotor impairments in people with incomplete spinal cord injury (SCI); however, plastic changes in brain areas remain poorly understood. The aim of this study was to examine the possible effects of a task-specific overground gait training on locomotor recovery and neuroplasticity markers in the cortex, cerebellum, and lumbar spinal cord in an experimental model of incomplete-SCI.

Main methods: Using a blind, basic experimental design, 24 adult Wistar rats underwent a surgical procedure and were allocated into sham, non-trained SCI (SCI), and trained SCI (Tr-SCI) groups. On postoperative day 14, trained animals started a 4-week overground gait training program. All groups were subjected to weekly assessment of locomotor recovery of the hind limbs. On postoperative day 40, brain and lumbar spinal cord structures were dissected and processed for biochemical analysis of the synaptophysin, microtubule-associated protein 2 (MAP-2), and brain-derived neurotrophic factor (BDNF).

Key findings: Tr-SCI group showed greater locomotor function recovery compared with non-trained SCI from the postoperative day 21 ($p < 0.05$). The training was able to improve the neuroplasticity markers synaptophysin, MAP-2, and BDNF expressions in motor cortex ($p < 0.05$), but not in the cerebellum and in the spinal cord for trained SCI group compared to non-trained.

Significance: Task-specific overground gait training improves locomotor recovery in a rat model of incomplete thoracic-SCI. Furthermore, training promotes motor cortex plasticity, evidenced for increasing expression of the neuroplasticity markers that may support the functional recovery.

1. Introduction

The spinal cord injury (SCI) is a neurological condition that promotes disability, severe mobility limitations, as well as reduces the level of physical activity and leads to secondary complications that affect the health and quality of life in people living with this condition [1,2]. Improvement of walking ability is one of the main goals for people with incomplete-SCI and the most challenging aim for neurologic physical therapy [3].

Among therapeutic approaches for people with incomplete-SCI, the locomotor training is one of the most widely used and studied in the clinical literature [4–8]. Although it is known that high doses of repetitive locomotor training associated with appropriate sensory stimuli can trigger spinal cord neural circuits and facilitate the expression of normal gait patterns, the gait training-dependent brain reorganization after SCI remains poorly understood [9–12].

Following a SCI, plastic changes occur in several neural circuits, not only in the spinal cord but also in brain structures [13,14]. It is known that the enhancement of motor skills occurs in association with activity-dependent neuroplasticity that may involve structural changes in

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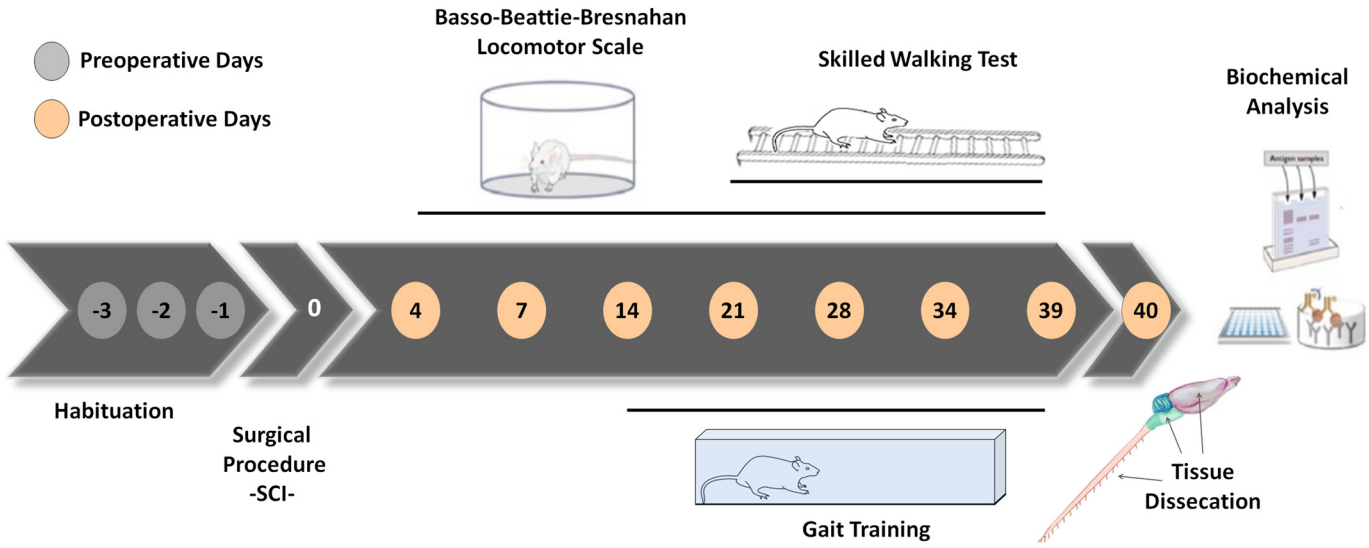

Fig. 1. Flowchart of the experimental timeline.

neuron and synapse remodeling in the motor cortex [15]. Then, it seems rational to think that enhancement in gait performance after incomplete-SCI is probably dependent not only on the spinal cord changes but also on brain plasticity to support skilled motor learning.

Previous studies using animal models have shown that the microtubule-associated protein 2 (MAP-2) and synaptophysin protein may be involved in molecular processes underlying motor task learning [16,17]. Furthermore, the brain-derived neurotrophic factor (BDNF), one of the most abundant neurotrophins in the central nervous system, has been related to neuroplasticity through the stimulation of dendritic spines and synaptic modulation, and may be modulated by training [18,19]. However, the possible beneficial effects of the overground gait training in brain plasticity following an incomplete-SCI model have not been described in the literature. Then, this study has been conducted using a rat model of the moderate to severe incomplete-SCI in order to clarify the possible beneficial effects of a task-specific overground gait training on locomotor recovery, and neuroplasticity markers in some brain areas involved in the control of movement, as well as in the spinal cord.

2. Materials and methods

2.1. Ethical statement

This study was based on the principle of the Three Rs, to replace, reduce and refine the use of animals used for scientific purposes. For this proposal, the procedures were performed in accordance with EU Directive 2010/63/EU and were approved by the Institutional Animal Care and Committee of the local University (protocol number PP00745). The report was prepared according to the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines [20].

2.2. Animals and study design

This is a basic experimental study performed with 24 female Wistar rats, 2.5 months of age and weighing up 180–250 g (initial age and weight), obtained from a local breeding colony. The animals were housed (2 to 3) in standard plexiglass boxes, under a 12:12-h light/dark cycle, in a temperature-controlled environment (22 °C) with food and water ad libitum over the experiment. The rats were randomically allocated into three groups: (1) Sham (n = 8): animals that underwent surgical procedures without a SCI; (2) non-trained SCI (SCI, n = 8): animals with SCI that did not undergo any training, and (3) trained SCI (Tr-SCI, n = 8): animals with SCI that performed a task-specific overground gait training on flat surface. This study was conducted in one set of the experiment, and all surgical and behavioral procedures were conducted from the beginning of the light cycle.

2.3. Surgical procedures

Animals were deeply anesthetized by intraperitoneal (i.p.) injection of ketamine and xylazine (i.p.; 50 and 10 mg/kg, respectively), as verified by the absence of the tail and paw withdrawal reflex to pinch. First, the back of the rat was shaved and disinfected with povidone-iodine and then a vertebral laminectomy was performed at T8-T9 levels to expose the spinal cord. The animals included at the SCI groups received extradural compression injury during 1 min with an aneurysm clip (Vicca® Brazil) calibrated to deliver 70 g of closing force [21]. The sham-operated control animals underwent the same surgical interventions without the SCI. The surgical procedure was concluded by suturing the muscle plane and skin (6–0 and 4–0 nylon suture lines, respectively; Ethicon, Brazil). During recovery, rats were kept in a warm environment, placed on beds of sawdust. Postoperative treatments included 2.5 mg/kg of enrofloxacin subcutaneously (Baytril Bayer S.A., Brazil) for 14 days to prevent urinary tract infection. Bladders were manually expressed twice daily until reflex bladder emptying returned [21,22], typically by 14 days after injury. Compared to previous studies, this model can be described as a moderate to severe SCI [21,23].

2.4. Task-specific overground gait training program

The training program was performed on a flat, obstacle-free runway 100 cm long, 20 cm high and 8.5 cm wide, ending in a dark box. Before the SCI, animals were familiarized with the training apparatus on three consecutive days. This 3-day habituation period consisted of one set of ten trials a day. In each trial, the animals were placed at the beginning of the runway to explore the place toward a dark box. This procedure was adopted for teaching the rats to walk in the right direction and minimize the interruptions during walking. Afterward, on the post-operative day 14, the trained SCI animals started a 4-week task-specific overground gait training program (Fig. 1).

The training program consisted of regular overground walking on the runway apparatus, i.e. by its self-imposed speed, toward a dark box. Each rat from the trained SCI group crossed the runway 25 times, walking 2500 cm per training day (5 trials without rest interval per day), five sessions per week for 4 weeks. Although the animals taught to

walk on the way before the SCI, if they stopped moving through the course, the investigator occasionally performed a gentle mechanical stimulation on the hindquarters. Animals received a stimulus immediately upon stopping and were stimulated until they began to continue along the runway.

All other animals (sham and non-trained SCI) accompanied the trained rats during their daily training in the experimental room, where they were kept in their cages and handled for ~1 min each training day. No pronounced signs of stress as vocalization, flattening of the nose, and eye squeeze were perceived by the investigators during the training. This training program was chosen because is an easy way to promote an overground gait training and have been used as motor training [24,25].

2.5. Evaluation of functional recovery

The locomotor function recovery of the SCI animals was evaluated using the Basso–Beattie–Bresnahan (BBB) Locomotor Rating Scale [23]. The BBB is a 22-point ordinal scale ranging from 0 (no observed hindlimb movements) to 21 (normal locomotor movements) points, developed to evaluate hindlimb locomotor recovery, including joint movements, stepping ability, coordination, and trunk stability during free open-field locomotion.

Animals were evaluated for 4 min by two blind investigators in the following conditions: (a) before surgery - to assure that all rats have reached maximum score of 21, (b) in the postoperative day 4 - to exclude those animals that reached a score above 2 points (to ensure the injury homogeneity), and (c) weekly to assess locomotor recovery until the end of the experiment. In both trained and non-trained SCI groups, the bladders were emptied just before testing because bladder contraction often accompanies reflex hindlimb activity and may provoke assessment bias.

The evaluation of skilled walking in SCI animals was performed using the horizontal ladder rung walking test [26,27]. The apparatus consisted of side walls (1 m long and 20 cm high) made of clear Plexiglas and metal rungs (3 mm diameter), which could be inserted 1 cm from the inferior edges of the wall to create a floor with a minimum distance of 1 cm between rungs, elevated 30 cm above the floor, and with a small dark box at the end. For the test trials, animals were required to walk along a horizontal ladder with an irregular pattern (the distance between the rungs varied from 1 to 5 cm), which was changed from adaptation (before each test session) to testing phase to prevent the pattern learning. Five templates of irregular rung patterns were used, so that the same patterns were applied to all animals to standardize the difficulty of the test and enhance comparability of the outcome. Each trial test was recorded by a camera positioned at a slight ventral angle.

The evaluation of the hindlimb placement was performed using a foot fault scoring system, as described by Metz and Whishaw [26,27]. The analysis was made by viewing the video recordings frame-by-frame using Windows Media Player 10. Only consecutive steps of each limb were analyzed. Therefore, the last step before a gait interruption, such as a stop or a foot fault, and the first step after an interruption were not scored. The last stepping cycle performed at the end of the ladder was also excluded from scoring. Foot placement on the rung was rated according to their position and errors that occurred in placement accuracy for each hindlimb. The types of foot placement on the rungs were rated using a 7-point ordinal scale ranging from 0 (total miss) to 6 (correct placement). The data is shown as the mean of the 5 trials for the hindlimbs of each animal. The test was performed only from the postoperative day 21 because to the minimum elapsed time after injury for the animal to cross the horizontal ladder apparatus (results obtained from a pilot–study).

2.6. Biochemical markers of neuroplasticity

On the postoperative day 40, one day after the last training session, the animals from each group were deeply anesthetized with i.p. injection of ketamine and xylazine (90 and 15 mg/kg, respectively) and euthanized by decapitation. After, intact brain and lumbar spinal cord structures were rapidly removed from the bone structures. Bilateral primary motor cortex (M1, approximately +3.0 to 0.0 mm to bregma), cerebellar hemispheres and lumbar spinal cord (approximately at L2–4 level) were dissected en-bloc (left and right) over an ice-cold culture dish. The samples bloc were collected apart in 1.5 mL Eppendorf Tubes®, quickly frozen and stored at −80 °C until they were processed for biochemical analysis through Western blot and Enzyme-linked immunosorbent assays (ELISA) techniques, respectively right and left samples.

To measure synaptophysin and MAP-2 using Western Blot technique, the samples were disrupted and homogenized in an ice-cold RIPA assay buffer containing protease and phosphatase inhibitors (100 mM Tris–HCl–pH 7.4; 2 mM EDTA; 2 μg aprotinin; 0.1 mM PMSF, 200 mM NaF and 2 mM of sodium orthovanadate; Cell Signaling Technology, USA). Then, the homogenate was centrifuged at 10,000 ×*g* for 15 min at 4 °C, the pellet was discarded and the supernatant was collected as a protein-rich fraction. The protein concentration was determined using the Bradford method [28]. Equivalent amounts of proteins (60 μg per sample) were aliquoted in Laemmli buffer (Tris 200 mM, glycerol 10%, SDS 2%, β-mercaptoethanol 2.75 mM and bromophenol blue 0.04%) and boiled for 5 min. Aliquots were stored at −80 °C until required.

Western blot analysis was carried out adapted according Andre et al. [29] and Leal et al. [30]. Proteins were resolved in sodium dodecyl sulfate-polyacrylamide gel by electrophoresis (SDS-PAGE) at 12% for synaptophysin (1:1000; Santa Cruz Biotechnology, CA), and 8% for MAP-2 (1:500; Sigma Aldrich, St. Louis, MO). The α-tubulin antibody (1:1000; Santa Cruz Biotechnology, CA) was used as loading control. These proteins were transferred on to nitrocellulose membranes, saturated with bovine serum albumin (BSA-5%) solution and incubated overnight with primary antibodies and following with their respective adjusted secondary antibody (goat anti-rabbit; rabbit anti-mouse and rabbit anti-goat - 1:3000) for 2 h at room temperature.

Immunoreactive bands were detected by Enhanced Chemiluminescence (ECL; Thermo Scientific Pierce) kit. The semi-quantitative analysis was performed by optical densitometry, using the Image pro-plus 6.0 (Media Cybernetics, Silver Spring, USA). Background light intensity was subtracted from the sum of pixel intensities for each band [31]. The level of total immune content was determined as a ratio of optic densitometry of the protein band over the α-tubulin band that was used as a loading control. The data are expressed as percentage of the Sham-control group of the each membrane.

To measure BDNF levels using ELISA assay, the samples was homogenized with a PBS solution containing: Tween 20 (0.05%), PMSF 0.1 mM, EDTA 10 mM, Aprotinin 2 ng/ml and benzethonium chloride 0.1 mM. Homogenates were transferred to eppendorfs tubes, centrifuged at 3000*g* for 10 min at 4 °C, and the supernatant obtained was stored at −80 °C until required for further analyses. Total protein content was measured in the supernatant using the Bradford method [28]. Sample aliquots of 100 μl were used to measure the BDNF levels using ELISA kits (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. The absorbance for the protein studied was measured using a microplate reader at 450 and 550 nm [32].

2.7. Sample size estimation

The sample size was calculated using G.Power 3.1 software to detect a mean between-group difference of 2.1 points (10%) out of 21 in the BBB scale with 80% power at a two-tailed significance level of 0.05 (ANOVA: repeated measures, between factors). The effect size of 0.61

was derived from a pilot study performed with eight SCI animals (four from non-trained and four from the trained group). The sample to detect the difference between non-trained and trained SCI groups is 7 animals per group. We have decided for 8 animals per group considering some possible missing data, and match the sham group.

2.8. Statistical analysis

Two-way ANOVA for repeated measures was used for BBB locomotor scale and evaluation of skilled walking. Bonferroni post-hoc test was used if necessary. One-way ANOVA was used for statistical analysis of biochemical data, followed Tukey post-hoc test if necessary. In each analysis, the experimental unit was an individual animal. Significance level was set to 95% for all comparisons. Data is presented for each group as mean and standard error or deviation.

3. Results

3.1. Gait training accelerates hindlimb functional recovery after incomplete-SCI

The evaluation of locomotor function recovery was measured with a BBB scale, before the surgery (to ensure the functional integrity of the animals), before the training (in the postoperative day 4, 7 and 14), and once a week over 4-week gait training period (Table 1).

The ANOVA for repeated measures demonstrated significant main effects for groups ($F = 10.27$; $df = 1,84$; $p = 0.006$), for time ($F = 358.7$; $df = 6,84$; $p < 0.001$) and for the interaction between time and group ($F = 4.64$; $df = 6,84$; $p < 0.001$) (Table 1).

Before the surgery, the animals showed maximum scores for hindlimb locomotor function on the BBB scale. On the postoperative day 4, the animals in both SCI groups ranged from 0 (complete absence of movement of the hind limbs) to 2 (extensive movement of one joint, or extensive movement of one joint and slight movement of another joint). The locomotor function recovery of SCI groups occurred gradually and in a similar way until postoperative day 14 (basal data before the training). However, the training promotes an acceleration in recovery, where the Tr-SCI group reaching higher values on BBB scale compared to SCI group from postoperative day 21 ($p = 0.012$) and remained until the last day of training (Table 1).

The skilled walking was assessed only from the postoperative day 21 because to the minimum elapsed time after injury for the animal to perform the test (Table 2).

For foot fault score, the ANOVA for repeated measures demonstrated significant main effects for groups ($F = 9.04$; $df = 1,42$; $p = 0.009$). The Tr-SCI group showed higher score value when compared with the non-trained SCI group from the postoperative days 35 and 39 ($p < 0.05$) (Table 2).

Table 1
Locomotor function evaluation.

Postoperative day	BBB score (Mean ± SD)		Difference between groups (95% confidence interval and p value)
	Tr-SCI	SCI	Tr-SCI × SCI
4	1.12 (0.83)	0.56 (0.67)	0.56 (−0.25 to 1.37); p = 0.16
7	5.62 (0.64)	4.68 (1.73)	0.93 (−0.46 to 2.33); p = 0.17
14	9.62 (1.50)	8.37 (1.86)	1.25 (−0.56 to 3.06); p = 0.16
21	11.37 (1.68)	8.75 (1.96)	2.62 (0.66 to 4.58); p = 0.012
28	12.25 (1.38)	9.50 (1.48)	2.75 (1.20 to 4.29); p = 0.002
35	12.62 (1.57)	10.18 (0.92)	2.43 (1.05 to 3.82); p = 0.002
39	12.75 (1.38)	10.31 (0.96)	2.43 (1.15 to 3.71); p = 0.001

Abbreviation: BBB, Basso–Beattie–Bresnahan locomotor rating scale; Tr-SCI, trained SCI group; SCI, non-trained SCI Group; SD, standard deviation.

Table 2
Skilled walking evaluation.

Postoperative day	Foot fault score (Mean ± SD)		Difference between groups (95% confidence interval and p value)
	Tr-SCI	SCI	Tr-SCI × SCI
21	0.88 (0.84)	0.24 (0.25)	0.63 (−0.10 to 1.37); p > 0.05
28	0.86 (0.62)	0.36 (0.38)	0.49 (−0.24 to 1.23); p > 0.05
35	1.16 (0.73)	0.39 (0.35)	0.76 (0.02 to 1.50); p < 0.05
39	1.14 (0.76)	0.31 (0.26)	0.83 (0.09 to 1.57); p < 0.05

Abbreviation: Tr-SCI, trained SCI group; SCI, non-trained SCI Group; SD, standard deviation.

3.2. Overground gait training promotes motor cortex neuroplasticity

The neuroplasticity was assessed using biochemical techniques to measure the levels of the synaptophysin, MAP-2 and BDNF protein expression in the motor cortex, cerebellum and lumbar spinal cord.

In the motor cortex, the ANOVA demonstrated significant main effects for synaptophysin ($F = 4.08$; $df = 2,14$; $p = 0.044$), for MAP-2 ($F = 13.60$; $df = 2,14$; $p < 0.001$), and for BDNF ($F = 6.27$; $df = 2,17$; $p = 0.027$). At the end of the 4-week task-specific overground gait training program, the Tr-SCI group had higher protein rates of the synaptophysin (129.6 ± 57.9%), MAP-2 (81.2 ± 21.3%) and BDNF level (192.7 ± 16.3 pg/mg protein) in the motor cortex when compared to non-trained (61.4 ± 28.9%, 47.3 ± 9.9%, and 125.5 ± 55.2 pg/mg protein; respectively; $p < 0.05$; Fig. 2).

In the cerebellum, the ANOVA demonstrated no significant main effects for any neuroplasticity marker assessed (Fig. 3). On the other hand, in lumbar spinal cord, the ANOVA demonstrated significant main effects only for synaptophysin ($F = 21.90$; $df = 2,17$; $p < 0.001$). The trained- and non-trained SCI groups had similar synaptophysin protein rates in lumbar spinal cord (43.9 ± 9.8%, and 42.8 ± 18.6%; respectively); and both had lower rates of this protein expression when compared to Sham-control (100.0 ± 16.0%; $p < 0.05$; Fig. 4).

4. Discussion

The experience is known to be able to change the neural structures and function throughout lifespan. In the context of motor learning and neurological rehabilitation, the specific task practice can provide appropriate movement experience to promote activity-dependent neuroplasticity. The present study was designed to assess the expression of some neuroplasticity markers in the motor cortex, cerebellum, and lumbar spinal cord that contributed to activity-dependent plastic changes paralleling partial recovery of motor function following a 4-week task-specific overground gait training started 14 days after incomplete-SCI in rats.

Consistent with previous works, our results demonstrate that rats with incomplete thoracic-SCI can benefit from a gait training started after the first stage of the injury [33,34]. In our study, the animals with a moderate to severe SCI submitted to a task-specific gait training showed early and better recovery of the locomotor function compared to the non-trained. This functional recovery was evidenced by greater scores on BBB locomotor scale from the postoperative day 21, and on foot fault scoring from postoperative day 35. Additionally, the overground gait training was able to improve the motor cortex neuroplasticity after the SCI as evidenced by the increase in expressions of the synaptophysin, MAP-2, and BDNF in trained animals.

After a SCI in rats, several cortical changes were previously shown due to altered input from the ascending and descending sensory-motor

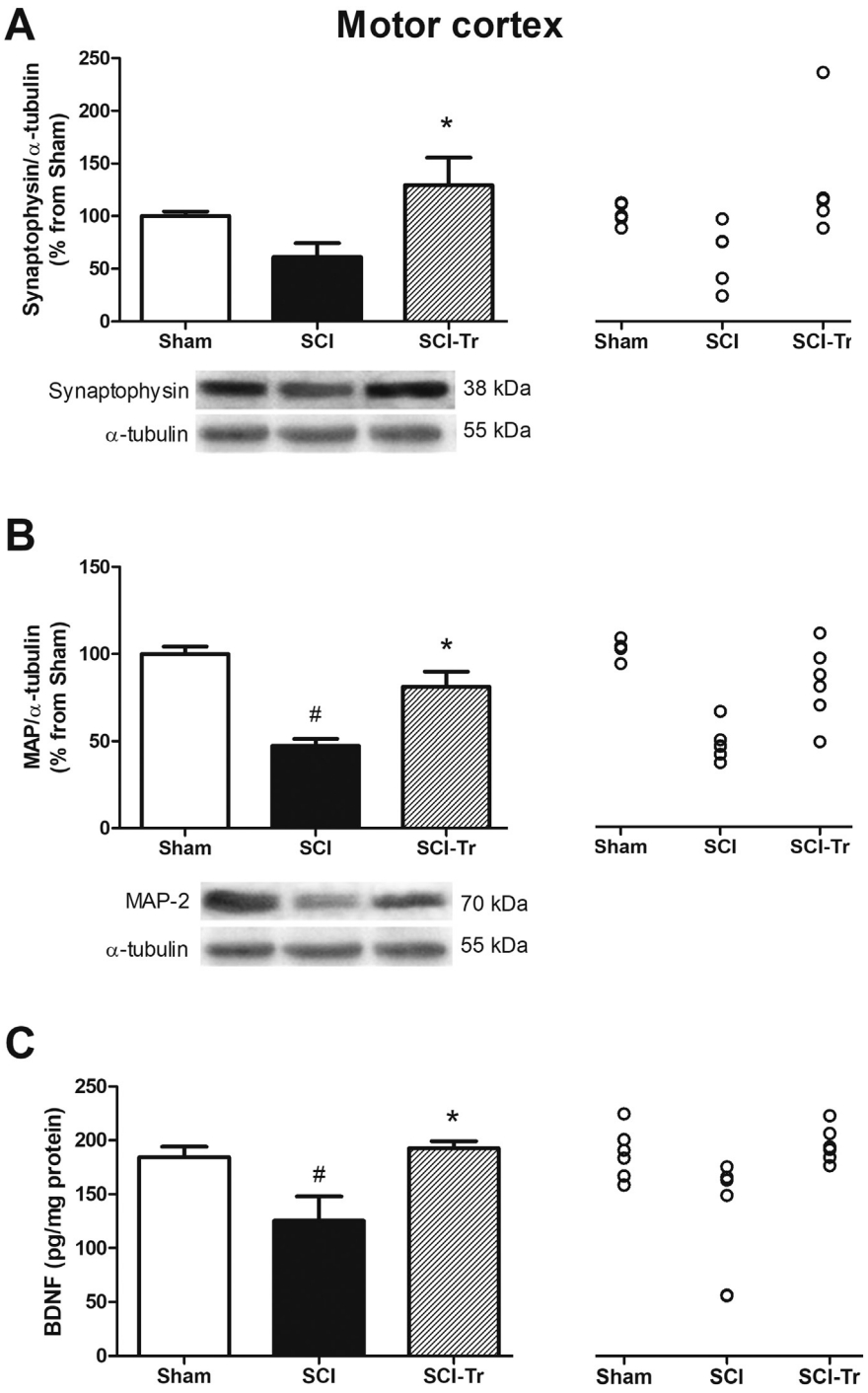

Fig. 2. Effects of the 4-week task-specific overground gait training on the expression of synaptophysin (A), MAP-2 (B), and BDNF (C) proteins in the motor cortex 40 days after thoracic incomplete-SCI. Bar plots show the mean ± standard error of the mean. Dot plots show the individual animal data for each protein. MAP-2, microtubule-associated protein 2; BDNF, brain-derived neurotrophic factor; SCI, spinal cord injury; Tr, trained; #, significant difference ($p < 0.05$) compared with the sham group; *, significant difference ($p < 0.05$) compared with the non-trained SCI.

systems. The synaptic reorganization that occurs in the motor cortex after a SCI can be explained by changes in the morphology and density of dendritic spines. The density of the postsynaptic spines decreases 7 days after the SCI, followed by a partial recovery around the 28 days [14]. Moreover, environmental enrichment and combinatorial treatment with transplants and neurotrophic factor may revert this synaptic alteration in the cortex after SCI in rats [35]. Similarly, our results show that the impaired synaptic changes in the motor cortex can be reversed by a specific motor stimuli, the overground gait training. Animals in the trained-SCI group showed enhancement of the synaptic-related protein markers synaptophysin and MAP-2. These proteins are involved in the motor learning process and may explain the better functional recovery presented by trained SCI animals in our study [16,17]. Moreover, training was able to increase BDNF level in the motor cortex and this may be a responsible factor for training-dependent effects on synaptic remodeling [18,19].

Although previous studies with complete (severe) and incomplete (moderate) SCI in rats have focused on spinal cord plastic changes after repetitive locomotor treadmill training [11,34], our study showed no changes in neuroplasticity-related markers, synaptophysin, and MAP-2

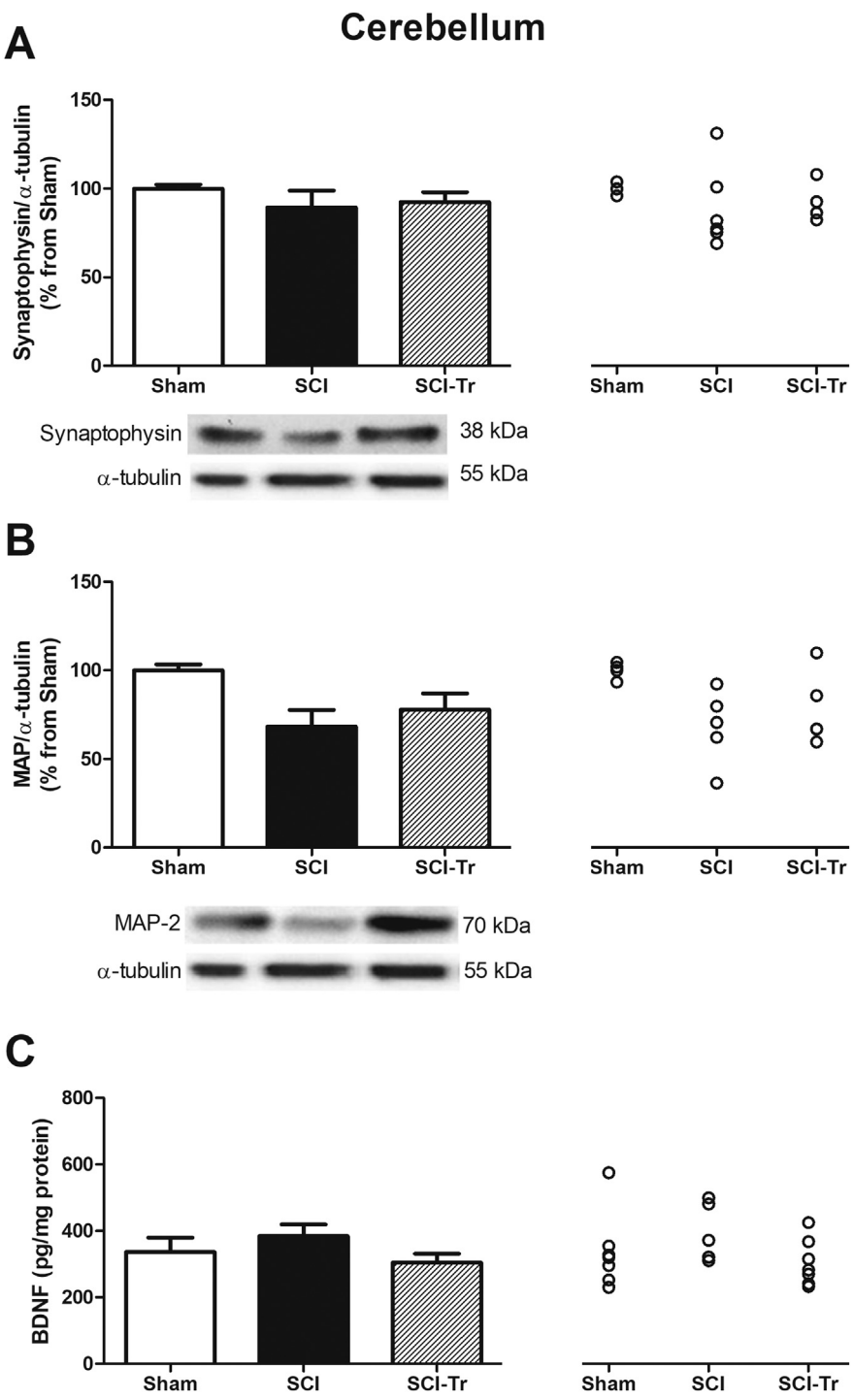

Fig. 3. Effects of the 4-week task-specific overground gait training on the expression of synaptophysin (A), MAP-2 (B), and BDNF (C) proteins in the cerebellum 40 days after thoracic incomplete-SCI. Bar plots show the mean ± standard error of the mean. Dot plots show the individual animal data for each protein. MAP-2, microtubule-associated protein 2; BDNF, brain-derived neurotrophic factor; SCI, spinal cord injury; Tr, trained.

expression, and in BDNF level in the lumbar spinal cord and in the cerebellar hemisphere of the non-trained and trained SCI animals. Similarly to our results, Marques et al. [33] neither found significant differences in lumbar spinal cord BDNF level between trained and non-trained SCI animals nor between injured and non-injured animals. Moreover, they showed improvement in the locomotor recovery in the trained-SCI groups that started the treadmill training in the late stages after SCI (14 and 28 days). On the other hand, the experiment of Wu et al. [36] has shown that treadmill training improves the BDNF concentration in the lumbar segment of rats with moderate SCI in concomitance with improvement in locomotor performance. However, they reported that non-trained injured animals also had a significant increase in BDNF when compared to the sham group, but without the refinement of locomotion. These reports raise two important points of discussion. First, it appears that the SCI is able to increase BDNF concentration regardless of repetitive external stimuli provided by training. Second, the increase in BDNF level is not always associated with improved locomotion. Actually, BDNF may have numerous actions that affect neural circuit function in the injured spinal cord. In a review, Boyce and Mendell [37] have gathered information showing that the

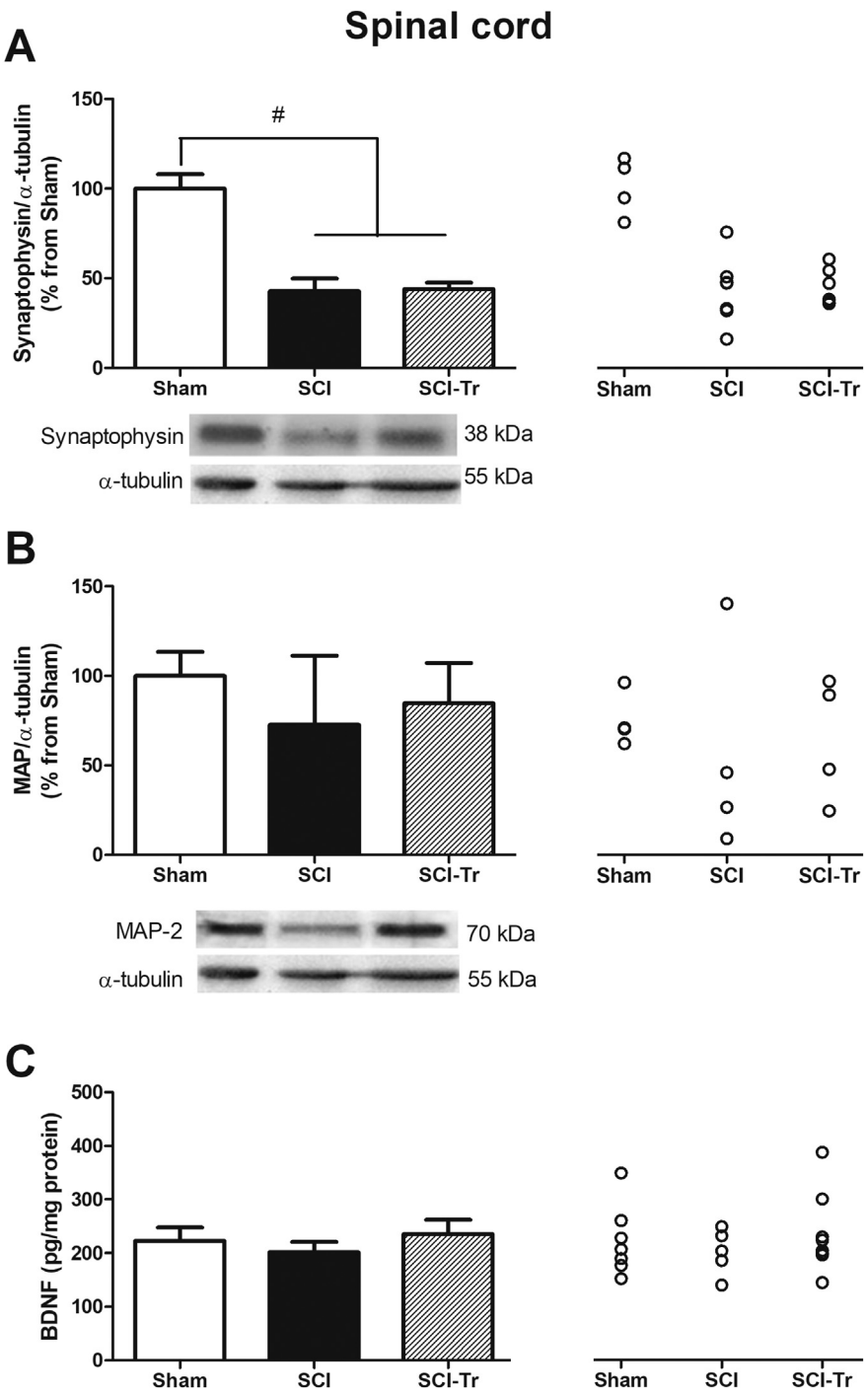

Fig. 4. Effects of the 4-week task-specific overground gait training on the expression of synaptophysin (A), MAP-2 (B), and BDNF (C) proteins in the lumbar spinal cord 40 days after thoracic incomplete-SCI. Bar plots show the mean ± standard error of the mean. Dot plots show the individual animal data for each protein. MAP-2, microtubule-associated protein 2; BDNF, brain-derived neurotrophic factor; SCI, spinal cord injury; Tr, trained; #, significant difference ($p < 0.05$) compared with the sham group.

elevation of the BDNF levels in injured spinal cord enhances the activity in neurons of the locomotor circuit. However, the BDNF level elevation can also result in an increased sensitivity to noxious stimuli. This piece of information raises the question as to whether activating nociceptive circuits is a requirement for BDNF to elicit steps. Although there are no direct studies of this issue, we can speculate that the improvement in hindlimb movements promoted by spinal cord BDNF elevation in SCI animals after training may be, in part, resulted from a reflexive stimulation of the spinal cord circuit instead of voluntary locomotor control recovery.

On the other hand, studies using incomplete SCI models have addressed the potential to neuromuscular activity (training) to enhance the expression of BDNF (gene and protein levels) in active muscle [38,39]. In addition, intact rats trained for 5 days in a treadmill showed increased BDNF mRNA 0, 2 and 6 h after the training in the soleus muscle, but only in 2 h in the lumbar spinal cord [40]. However, the same animals showed an increase in BDNF protein levels in the spinal cord, but not in the soleus muscle. These results suggest that neuromuscular activity can promote retrograde transport of the BDNF from muscle to spinal cord. In fact, retrograde transportation of BDNF is also

showed in the peripheral and central nervous systems, including supraspinal structures [41]. Therefore, it is plausive to think that the same event can occur between the spinal cord and cortex. In other words, the BDNF can be retrogradely transported from the spinal cord to motor cortex and it may induce adaptive cortex plasticity to support functional improvements after incomplete SCI and training.

Furthermore, it has been showed that a contusive injury to the rat thoracic spinal cord leads to a loss of spinocerebellar input and alters cerebellar circuit [42]. Overall, the study showed a reduction in the parallel fibers innervating distal segments of Purkinje cell dendrites and a compensatory increase in a synaptic marker (synapsin-1) on cell bodies in the Purkinje cell layer. Moreover, the study of Kawakami et al. [43] reports no changes in the BDNF level in cerebellum hemisphere after SCI in rats. Thus, it seems that the loss of parallel fiber synaptic contact on distal segments of the Purkinje cell dendritic tree is compensated by the increase of synapses from climbing fibers on the Purkinje cell bodies and proximal dendrites without changes in the BDNF level in cerebellar hemispheres. In this context, it is possible to think that the overground gait training promotes no changes in protein neuroplasticity markers and BDNF level in the cerebellar hemisphere, but has maintained the integrity of the cerebellar circuit in our study.

Once walking ability recovery is one of the main rehabilitation goals for people with SCI [3,44], the locomotor training has been exhaustively investigated as an intervention for gait limitations in this population [4–8]. This intervention approach can be delivered in a variety of ways, such as robotic-assisted training, overground or treadmill training with or without body support and with or without manual step or electrical stimulation assistance, and conventional overground gait training. As the underlying mechanisms of this training modality are based on the consensus that the success in performance is dependent on the repetitive task practice, it may seem reasonable to think that treadmill-based locomotor training offers greater repetition doses and may have better results [45–47]. However, a systematic review conducted by Mehrholz, Kugler and Pohl [48] has concluded that there is no advantage for the treadmill-based approaches compared to conventional overground gait training for improving locomotor function after incomplete-SCI. On the contrary, overground gait training seems better than treadmill training to improve some important gait outcomes, like walking distance [5].

It has already been described that high doses of repetitive locomotor training associated with appropriate sensory stimuli facilitate the expression of normal gait patterns after SCI [5,9,12]. Then it is plausible to think that training in the overground environment offers a challenge most similar to the gait, and allows the subject to learn how to control specific demands of this task, especially by its high cortical demand for voluntary step initiation and forward progression. In this context, the task-specific overground gait training could maximize supraspinal drive to the spinal locomotor circuit, and may be an important approach for improving walking ability after SCI. This information can enhance our understand that locomotor recovery in individuals with incomplete-SCI is not just dependent of the spinal cord retraining to reproduce repetitive and automatic gait movements, but also cortical relearning of the step sequence initiation during overground walking [5,10,49,50].

5. Conclusion and clinical implications

Task-specific overground gait training showed to be a valuable approach for improving locomotor recovery in a moderate to severe rat model of thoracic incomplete-SCI. The training promotes motor cortex plasticity that may support the functional recovery of locomotor function, as evidenced by increased neuroplasticity marker expressions - synaptophysin, MAP-2, and BDNF. This is a basic experimental study and the results may not be fully generalized to humans; however, these findings can provide insight into the underlying mechanisms of the beneficial effects of gait training and promote greater understanding of clinical research results.

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Declaration of Competing Interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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