Behavioral study of rats with spinal cord hemisection in the midthoracic level.

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ABSTRACT: Introduction: Various experimental models exist in order to induce a spinal cord lesion and to monitor the potential recovery of the animals, with or without any therapeutic means. From the behavioral point of view, the Basso-Beattie-Bresnahan (BBB) score is the most popular scale, used consistently to evaluate the effect of the traumatism onto the motor behavior of the animals. Although it is well established and it is regarded as the “gold standard” in the literature, in case of partial lesions, with high rate of spontaneous recovery, more elaborate tests may be indicated.Materials-methods: In this study we performed spinal cord hemisection at the midthoracic level in adult Wistar rats, which were evaluated postoperatively with BBB, as well as with a more extended behavioral protocol, comprising grid walking and footprint analysis. Results: Although BBB score improved, grid walking, stride length, step length and limb rotation did not reach statistical significance. Conclusion: More sensitive motor behavior protocols are needed to corroborate the results of the BBB test and to detect subtle defects in sensorimotor coordination. This is especially true in cases of partial lesions, due to a high rate of spontaneous gross motor recovery.

Key words: spinal cord injury, spinal cord hemisection, motor behaviour evaluation,

INTRODUCTION

Spinal cord injury imparts severe functional deficits below the level of the traumatism. Various experimental models are currently applied in order to induce either partial or complete lesions. Cord hemisection is one of the most frequently used. The main features of this model are the completeness of the lesion unilaterally, which permits the study of regeneration, collateral sprouting or other synaptic arrangements and, at the same time, the substantially less dramatic effects than whole cord transection[1].

It is of note, however, that in all these models, and especially in those with partial lesions, spontaneous recovery is observed, which, under certain circumstances, may be impressive. For example, hemisection of the mid- at lower thoracic levels in rats results in an initially marked, but only transient paresis of the ipsilateral hindlimb[2]. It is postulated that incomplete lesions with sparing of a small proportion of supraspinal axons leads to a significant return of locomotor function[3].

Several models exist in order to study the effect of the lesion onto the motor behavior of the experimental animals. The Basso-Beattie-Bresnahan (BBB) score[4] has prevailed in the field of spinal cord injury and is considered as the “gold standard” of the behavioral tests. Though well established, it remains insensitive for monitoring all aspects of recovery. In this study we performed spinal cord hemisection in the midthoracic level and, apart from evaluating the BBB score, we supplemented the study of the recovery of the animals with several other, more complex tests. Our hypothesis was that although the recovery, either spontaneous or induced, that occurs after the lesion manifests itself as an improvement in BBB score, a more elaborate trial would reveal and better delineate the remaining impairment in locomotion. In this way, subtle differences due to administration of therapeutic agents, could be more discernible.

MATERIALS-METHODS

Animals and Animal Care. This project complies with the guidelines for animal use established by the American Physiological Society and was approved by the local ethical committee in accordance with European Union council directive 86/ 609. 10 adult Wistar rats (2 months of age), with an average body weight of 250g, were used. The animals were housed in the animal facility of the Laboratory of Physiology of the Medical School of the Aristotle University of Thessaloniki, under standard conditions of humidity and temperature with a 12-h light/12-h dark cycle. Commercial rat chow and tap water were provided ad libitum. All efforts were made to minimize the number of animals and their suffering in the experiments.

Surgical procedure: Rats were anaesthetized with chloral hydrate (4%, 1ml/100mg body weight). In order to prevent infections, ampicillin was administered

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(150mg/kg BW, i.m.) and was continued postoperatively for 5 days. In addition, the animals received meloxicam (Movatec, 7.5mg/kg BW) and methylprednisolone (Lyo-Drol 30mg/kg BW) preoperatively. The animals were placed on the heating pad in ventral decubitus position. The animal's back was shaved and disinfected (Sterilium). A midline incision was performed over the spinous processes of T9-T10 vertebrae. Paravertebral muscles were laterally displaced and a self retaining retractor was placed. A right hemilaminectomy of T10 was performed, with preservation of the spinous process. The dura mater was identified and cut in the midline, and retracted laterally to expose the dorsal surface of the cord. After the midline vessels were visualized and protected, the right part of the cord was completely transected with an iridectomy scissors. A segment of 1mm was removed by microforceps and any residual fibers were severed after passing a 23G needle from the posterior vertebral surface. Collagen with thrombin (Tachosil) was placed in the gap. The muscles and the subcutaneous tissue were sutured with 4-0 vicryl and the skin with 4-0 nylon. The rats were allowed to fully recover from anesthesia in an incubator (37 ◦C) and were given lactated Ringers solution (5 ml, s.c.) as needed.

Movement behaviour

Behavioural assessment was performed preoperatively and for 2 weeks postoperatively. Movement behaviour was examined by performing 3 kinds of tests. All tests were performed at the same day, while on the previous day, the animals became accustomed to the trials.

1. The open field test (BBB test in which the rodent is left to walk on the open field and a series of parameters regarding the movement of joints as well as the general locomotion are evaluated. The rats were left free to move around for 5 minutes and afterwards were evaluated by two independent observers. This locomotor rating scale is designed to measure the amount of locomotor function on a 21-point rating scale, with a score of 21 representing normal movement and 0 representing complete paralysis of the hindlimb. It is based on the normal recovery observed after a mild SCI and involves detailed analysis of movement, including the motion degree of particular joints of the hindlimbs, trunk stability during movement, weight-bearing capabilities and the placement of each paw of the hind limbs. Experiments were videotaped and analysed offline.

2. Grid test: rats are placed on a grid with fixed parallel bars at regular interbar intervals and the number of errors during a fixed period is measured. With this test one can assess deficits of descending motor control, even if these deficits are not evident during the normal locomotion[5]. The errors (falls of hindlimbs) during 2 minutes were measured.

3. Footprint analysis: The footprint analysis was performed according to previous research[6,7,8] in order to evaluate hindlimb walking patterns. Briefly, the rats had to walk on strips of paper through a walk away (1 m long, 6 cm wide). Their hindpaws were dipped in blue fountain pen ink. Three series of at least one stepping cycle per side (four sequential steps) were performed per experimental day. The parameters examined (Fig.1) were: stride length (distance between two successive placements of the same foot), step length (distance by which the named foot moves forward in front of the other one), limb rotation (angle between a virtual line through the third digit and the centre of the palm and a virtual line parallel to the walking direction) and step width (distance between feet of the left and right stepping cycle).

Statistical analysis

Analysis was performed using SPSS 21.0 software for Windows. Non-parametric tests were applied. The differences between the two limbs were evaluated with Mann-Whitney U test and the repeated measures with Wilcoxon signed rank test. Statistical significance was set to $p<0.05$.

RESULTS

Morbidity-mortality-general appearance: 1 animal died during the course of the study. Death was attributed to profound spinal shock. Feeding behavior was normal. No loss of body weight was noted. Pressure ulcers were not observed. Sphincter function was restored in 2-3 days and no manual evacuation was therefore required. Spinal shock lasted for several hours. Initially, contralateral limbs were also affected and on the second postoperative day movement was nearly normal on the left side and paretic on the right, with the right hindlimb in a position of flexion, external rotation and abduction. Rats improved progressively and during the next two postoperative weeks, differences with the control side became less observable.

Open field: Rats presented with a slight difficulty in forelimb-hindlimb coordination. As it is shown in table 1, the BBB score showed an improvement (from 17.14±2.67 to 19±1.73, values are expressed as mean±SD) which was statistically significant. On grid walking, this defect in sensorimotor coordination was more pronounced and the animals, albeit with satisfactory BBB score, ended up with a significant number of mistakes, not exhibiting statistical significant difference, compared to the immediate postoperative state (Fig 2,3).

Footprint analysis: In all studied parameters (table 2), the right hindlimb was impaired compared to the left one, although only the angle of limb rotation differed significantly. The stride was similar in the two limbs (as it is usual the case, unless the animal performs a circular route), but the right step length was clearly less than the left one (4.48±1.98 vs 6.09±2.23, Fig.4) confirming the observation in the open field that the animals drag their right hindlimb during forward movement. The latter adopts a position of external rotation as well, which is reflected in the angle of rotation (25.43±5.88 right vs 17.86± 5.67 left, Fig.5). Concerning the evolution of the parameters, the improvement which was observed at the second trial was not statistically significant.

DISCUSSION

In the present study we performed spinal cord hemisection in the midthoracic cord in adult rats and we followed their evolution, concerning their motor behavior. As it has already been pointed out, the advantage of this model is the completeness of the lesion on the one side, without the devastating complications of the complete cord section[2]. After an initial period of spinal shock, followed by bilateral and eventually unilateral defect in hindlimb movement, the animals finally succeeded in acquiring a slightly uncoordinated hindlimb walking. The rate of recovery of locomotion, following this kind of traumatism, was notable, after taking into consideration the degree of the lesion onto the spinal cord. This is in accordance with other studies, who confirmed the immediate total ipsilateral and occasionally bilateral paralysis of the hindlimbs[9,10]. In a clinical analogue, following a Brown-Séquard syndrome, the ipsilesional hindlimb demonstrated a remarkable degree of locomotor performance in most parameters up to 28 days after the lesion in spite of large initial deficits[11].

This high rate of spontaneous recovery is frequently observed in partial lesions. As it is expected, the degree of recovery is directly proportional to the transverse as well as to the rostrocaudal extent of the lesion. It is very important to ensure that no part of the hemicord is spared and this is usually accomplished by a complementary procedure, usually by aspiration of a part of the hemicord. For example, in the model described by Choi et al.[12], their outcomes were definitely worse than the ones presented in our paper and this may be ascribed to the removal of 3mm of neural tissue in their model, instead of 1mm in our study.

Concerning the mechanism of recovery, several compensatory processes are postulated to be activated[13]. These include, in general, reinnervation from collateral sprouts of intact contralateral fibers or synaptic plasticity with reorganization of local circuits[10]. This is the reason that partial lesions are often criticized as their restorative mechanisms do not allow for an isolated study of the organization of the spinal circuits below the lesion. One should take into account, however, that in most clinical applications the lesions are partial and, in particular, of the contusion type, with the aim to render the spinal cord amenable to various therapeutic interventions.

Quantitative evaluation of locomotion after spinal cord injury is usually performed by the BBB score, on which most researchers rely in order to evaluate the recovery from spinal cord injury, either spontaneously or after implementation of a treatment plan. Although the use of this test is well documented and is considered indispensable in behavioral studies of this kind, it may not be adequate to fully evaluate the motor recovery as a sole test. In the current study, although the improvement in hindlimb locomotion was statistically significant, judging by the BBB score, motor deficits continued to be evident and to hinder the animal locomotion, as it was revealed in the series of the other tests. The latter were regarded as more efficient at detecting deficits in sensorimotor coordination. Their inclusion in the study was deemed to provide a more comprehensive view of locomotor behavior.

Current approaches to behavioural assessment, following spinal cord injury, often fail in delineating the exact nature of motor deficits and, subsequently, in evaluating the return of lost functions. Recovery of specific capabilities such as locomotion may entail the recovery of multiple independent features of the underlying neuromotor control. It has been shown that the evolution of recovery of different physiological and mechanical metrics of locomotor ability varies in a predictive manner[14].

Similar results have been drawn by other researchers,[14,15] who conclude that, in addition to the assessment of gross motor behaviour, it is often appropriate to use complex tests to evaluate the masked deficits in the evaluation of functional recovery after spinal cord. Under no circumstances, however, should one underscore the value of the BBB scale, which remains the mainstay of behavioral research. It is just that through application of more elaborate trials, differences during the course of experimental trials could be more prominent.

CONCLUSIONS

Spinal cord hemisection is accompanied by a high rate of functional recovery. The global observation of gross motor behavior and the implementation of the BBB test alone is not sufficient to follow the evolution of the experimental animals. In order to be complete, a behavioral study should entail further, more elaborate, locomotor testing methods. In this way, several factors, which might be beneficial in minor traumatisms, and are underestimated at present, could possibly be exploited.

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Συμπεριφορική μελέτη αρουραίων με ημιεγκάρσια διατομή νωτιαίου μυελού στο μέσο θωρακικό επίπεδο

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Εισαγωγή: Υπάρχουν διάφορα πειραματικά μοντέλα για την πρόκληση βλάβης στο νωτιαίο μυελό, αλλά και για την παρακολούθηση της ανάνηψης των πειραματοζώων, με τη βοήθεια ή όχι ενός θεραπευτικού παράγοντα. Όσον αφορά την κινητική συμπεριφορά, η πιο διαδεδομένη και ευρύτατα χρησιμοποιούμενη κλίμακα για την εκτίμηση του νωτιαίου τραυματισμού είναι η Basso-Beattie-Bresnahan (BBB). Στην περίπτωση ωστόσο των μερικών βλαβών, οι οποίες συνοδεύονται από αυτόματη ανάψηψη, ενδείκνυται η χρήση και άλλων, περισσότερο ειδικών δοκιμασιών. Υλικά-μέθοδοι: Ενήλικοι επίμυες του γένους Wistar, υποβλήθηκαν σε ημιεγκάρσια διατομή του νωτιαίου μυελού στο μέσο θωρακικό επίπεδο και εκτιμήθηκαν μετεγχειρητικά, αφενός μεν με την κλίμακα BBB, αφετέρου με περαιτέρω συμπεριφορικές δοκιμασίες, όπως η βάδιση σε πλέγμα και η ανάλυση αποτυπωμάτων. Αποτελέσματα: Παρά το γεγονός ότι παρατηρήθηκε σαφής βελτίωση στην κλίμακα BBB, αντίθετα η βάδιση στο πλέγμα, το μήκος του διασκελισμού και των βημάτων, καθώς και η στροφή των σκελών, δεν είχαν στατιστικώς σημαντικές διαφορές. Συμπέρασμα: Τα αποτελέσματα της κλίμακας BBB θα πρέπει να συνδυάζονται με περισσότερο ευαίσθητες μεθόδους εκτίμησης κινητικής συμπεριφοράς, έτσι ώστε να αναδεικνύονται καλύτερα ήπια ελλείμματα αισθητικοκινητικού συντονισμού. Αυτό είναι περισσότερο αναγκαίο σε μη-πλήρεις βλάβες, λόγω μεγάλου ποσοστού αυτόματης ανάνηψης της αδρής κινητικότητας

Abbreviations: BBB: Basso-Beattie-Bresnahan

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BBB-GRID RESULTS

	Mean	Std. Error of Mean	Median	Std. Deviation
BBB-1	17,14	1,01	17,00	2,67
BBB-2	19,00	,65	19,00	1,73
GRID-1	8,71	2,43	9,00	6,42
GRID-2	4,43	1,07	4,00	2,82

TABLE 1: Postoperative evaluation of Basso-Beattie-Bresnahan score and errors in grid walking. Only BBB showed a statistically significant difference

FOOTPRINT ANALYSIS RESULTS

	Mean	Std. Error of Mean	Median	Std. Deviation
LEFT STRIDE-1	9,81	,72	10,00	1,90
RIGHT STRIDE-1	9,39	,64	9,50	1,68
STRIDE_DIFF_1	,42	,18	,50	,47
LEFT STEP-1	6,09	,84	4,75	2,23
RIGHT STEP-1	4,48	,75	4,45	1,98
STEP_DIFF_1	1,61	1,12	,30	2,95
LEFT LIMB ROTATION-1	17,86	2,14	15,00	5,67
RIGHT LIMB ROTATION-1	25,43	2,22	25,00	5,88
ROTATION_DIFF_1	-7,57	1,60	-5,00	4,24
LEFT STRIDE-2	9,60	,87	10,20	2,31
RIGHT STRIDE-2	9,74	,84	10,20	2,22
STRIDE_DIFF_2	-,14	,42	,00	1,11
LEFT STEP-2	5,82	,73	5,00	1,93
RIGHT STEP-2	4,64	,36	5,00	,94
STEP_DIFF_2	1,18	,55	,50	1,45
LEFT LIMB ROTATION-2	22,14	2,59	22,00	6,84
RIGHT LIMB ROTATION-2	28,29	3,10	25,00	8,20
ROTATION_DIFF_2	-6,14	1,84	-8,00	4,88
STEP WIDTH-1	3,76	,34	4,00	,90
STEP WIDTH-2	3,79	,21	4,00	,57
STEP_WIDTH_DIFF	-,03	,38	,00	1,01

TABLE 2: Footprint analysis. The difference between the two hindlimbs was compared between the two postoperative time points.

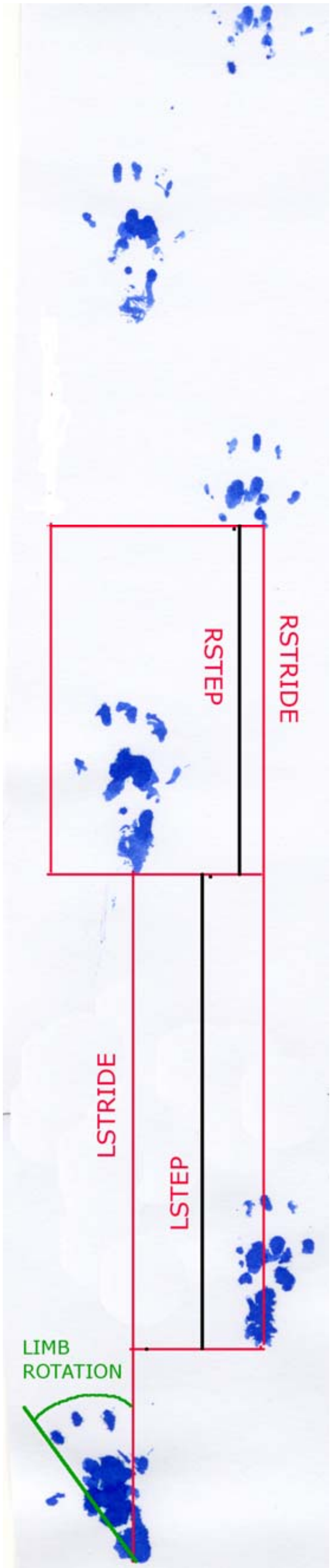

Fig. 1 Footprint analysis. Step length is reduced and limb rotation is increased on the side below the hemisection

Fig 2a. During the stance phase the right hindlimb fully supports the body

Fig 2b. During the beginning of the swing phase, all articulations of the right hindlimb contribute to locomotion

Fig 3 The same animal as in Fig.3 presents its error-prone right hindlimb to fall between the bars in grid walking

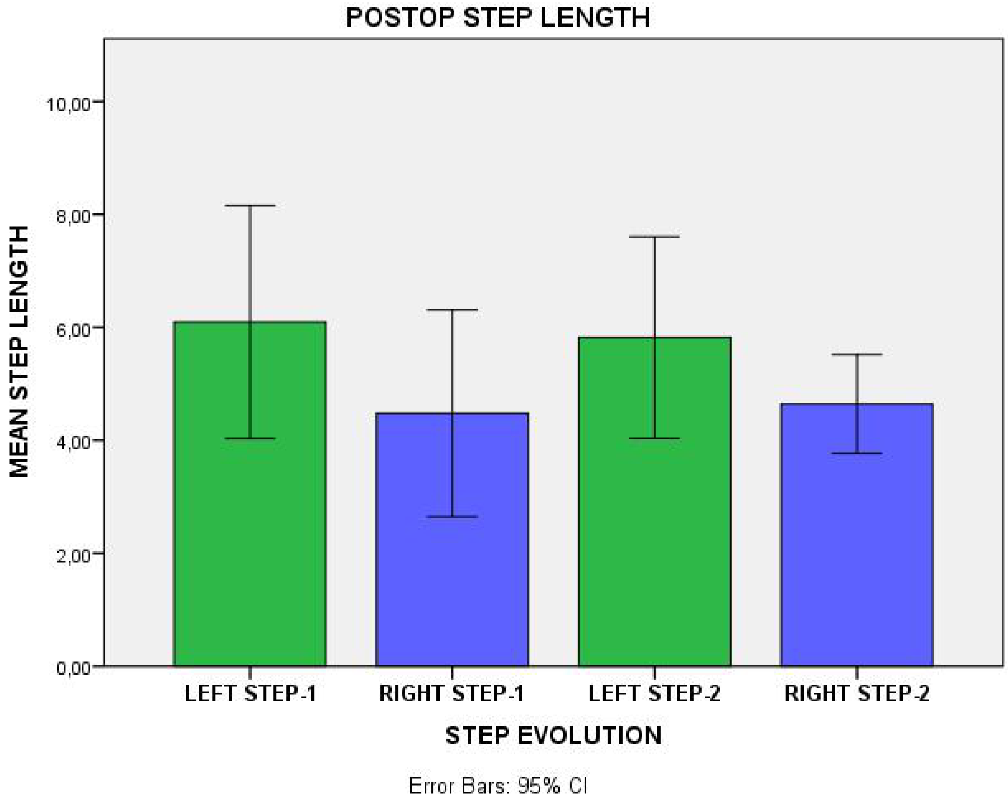

Fig 4. Graph showing the step length of the two hindlimbs. The side below the hemisection continues to show the defect

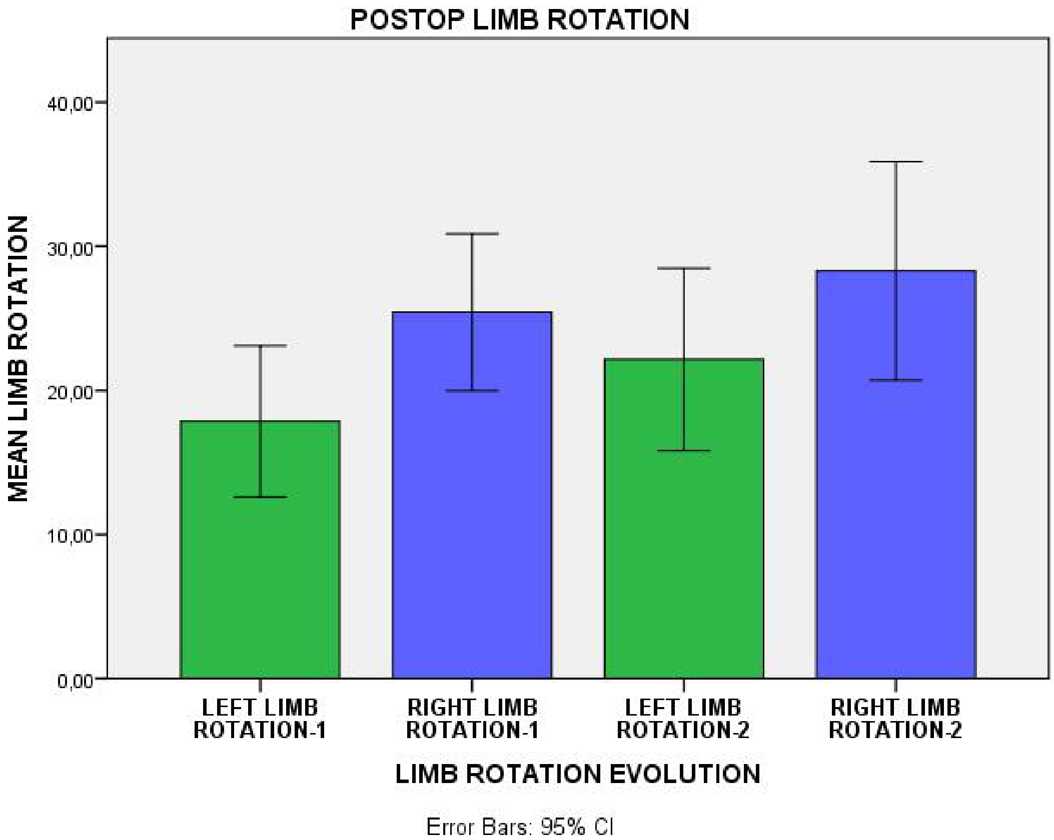

Fig 5. Graph showing the limb rotation of the two hindlimbs. The right hindlimb (homolaterally to the hemisection) adopts and external rotation