

## Neither environmental enrichment nor voluntary wheel running enhances recovery from incomplete spinal cord injury in rats

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Received 13 February 2006; accepted 1 April 2006

Available online 8 June 2006

### Abstract

Environmental enrichment and exercise may be neuroprotective or promote recovery after different forms of CNS injury. Here, we tested the possible effects of moderate environmental enrichment and voluntary exercise on the outcome of incomplete spinal cord injury in rats. We provided rats in standard cages with basic environmental enrichment (carton house, nesting material, tube, gnawing sticks). We also analyzed the effect of increased activity by housing spinal-cord-injured rats in cages with or without access to running wheels. In a third experiment, we looked at the possible effect of pre-injury training. In all experiments, a battery of behavior tests were used. Enriched environment provided before, after or both before and after injury did not alter the outcome on any of these tests. Similarly, despite excessive running after injury, no differences in terms of recovery and behavior were found in the running experiment. Similarly, running prior to injury did not significantly decrease the degree of functional deficit caused by the injury. Since there were no effects of further enrichment, above the possible effects of being socially housed, and since exercise did not improve the outcome, we conclude that these forms of increased activity do not render the animals significantly less sensitive to spinal cord injury and do not cause robust improvement when initiated after injury. While these results pose a limit to how helpful environmental and physical training programs may be in rodent impact injury models, they do not contradict the fact that voluntary and guided training can be effective tools in human spinal cord rehabilitation.

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**Keywords:** Spinal cord injury; Wheel running; Spontaneous exercise; Environmental enrichment; Training; Functional recovery

### Introduction

Despite a number of promising novel treatment strategies in experimental spinal cord injury models, the prognosis in terms of functional recovery for patients with spinal cord injury remains poor. The only generally accepted pharmacological treatment after spinal cord injury is the corticosteroid methylprednisolone, given in high doses within 8 h after injury (see Cochrane library for review). However, even this is controversial (Nesathurai, 1998). Physiotherapy and training of patients with spinal cord injury are crucial. Physical activity decreases the incident of complications such as urinary tract infections and pressure sores and improves cardiovascular health and subjective well being (Curtis et al., 1986; Ditor et al., 2005; Hicks et

al., 2003, 2005). In addition to these general health effects, patients trained on a treadmill can also improve both their treadmill and general motor performance. Recently, a robotic device (“The rat stepper”) for training spinal-cord-injured rats was introduced, and improvement of functional recovery after injury reported (de Leon et al., 2002).

Activity-enhanced plasticity in the spinal cord following injury was recently reviewed (Wolpaw and Tennissen, 2001). Both environmental enrichment and training have been associated with positive effects on functional recovery (Engesser-Cesar et al., 2005; Lankhorst et al., 2001; Van Meeteren et al., 2003), with respect to both sensory and motor recovery. Environmental enrichment appears to be particularly beneficial in several other models of central nervous system (CNS) afflictions, such as brain injury and stroke/ischemia. An enriched environment may also increase neurogenesis (van Praag et al., 2000), the behavioral repertoire (Van de Weerd et al., 1997),

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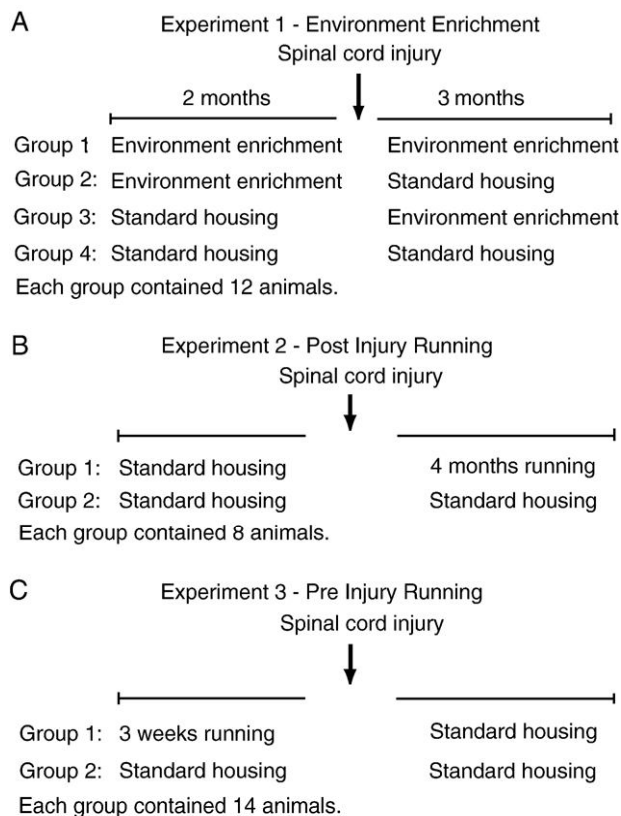


Fig. 1. Study design for the three experiments. Four different enrichment conditions were used for the environmental enrichment experiment. In the second experiment, rats had access to running wheels after spinal cord injury and in the third experiment before injury.

learning and neuronal plasticity (Mohammed et al., 2002). However, environmental enrichment does not appear to change the response in standard behavior tests (Mohammed et al., 2002; Wolfer et al., 2004).

Voluntary running also increases neurogenesis in the brain (Aberg et al., 2005; van Praag et al., 1999). Training post-injury leads to very specific improvements, so that for example cats that were trained to stand improved standing, but not walking, whereas cats trained to walk did not improve their ability to stand (De Leon et al., 1998). This suggests that any functional recovery is closely related to the specific training paradigm. At

the molecular level, exercise has been shown to alter release of neurotrophic factors including BDNF, as well as Synapsin 1 and Gap43, in spinal-cord-injured rats (Gomez-Pinilla et al., 2002; Hutchinson et al., 2004). These proteins are associated with growth and neuronal plasticity, and the increase could be correlated to sensory improvements, a reduction of allodynia and restoration of normal sensation (Hutchinson et al., 2004).

In this present study, we performed three experiments (Fig. 1). First, we studied how the outcome of mild spinal cord injury in rats might be influenced by environmental enrichment in different phases of the animals' lives. Our enrichment consisted of a gnawing stick, folded paper strips, a PVC tube and a nest-box (Manser et al., 1998; Van Loo and Baumanns, 2004) (Fig. 2B). We analyzed pre-exposure effects and possible imprinting effects by rearing one group of animals before injury from birth onwards in the enriched environment, but removing it after injury, while second group was kept in the enriched environment throughout life and a third group was given enrichment only after injury. This was compared to standard housing, without enrichment (Fig. 2A). In the second and third experiments, we investigated the effects of voluntary wheel running on recovery after spinal cord injury in rats. The rats were given free access to running wheels (Fig. 2C) from 1 week after injury and developed a voluntary running behavior during the next 4 months with up to 20 km per day. In the last experiment, rats had instead access to running wheels before, but not after, injury. This was designed to investigate possible protective effects of being well-trained when injured. Running wheels were selected as the single variable to exclude possible confounding effects of further environmental enrichment.

## Materials and methods

### Experiment 1: environmental enrichment

#### Animals

Five of ten pregnant Sprague–Dawley rats (B&K, Universal, AB Sweden) were randomly selected for an enriched environment and the remaining 5 rats for a non-enriched environment (Fig. 2). Female offspring ( $n = 48$ ; 12/group) weaned on postnatal day 21 were assigned to four groups (I–IV). The animals were housed 4 per cage (Makrolon® type IV, 1800 cm<sup>2</sup>) provided with wood

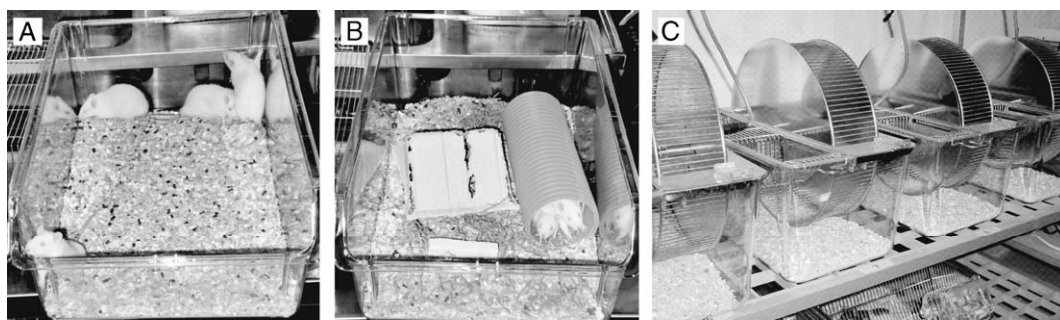


Fig. 2. Housing conditions for the different experiments. (A) Standard conditions, socially housed animals. (B) Enriched housing conditions. Our enrichment consisted of a gnawing stick, folded paper strips, a PVC tube and a shepherd shack. (C) In the second and third experiment, animals were single-housed in cages with running wheels or same cages without. Activity from the running wheels was collected 48 times/day via customized software.

chips (B&K Universal, AB, Sweden) within a 12:12 h dark/light regime, with light on at 6:00 am. Water and food (rodent pellets, Beekay Diets, B&K Universal, AB, Sweden) were available ad libitum. Animals were killed by decapitation after 4 months. Animals that had reached adulthood in standard housing conditions and animals exposed to the enriched environment since birth were subjected to spinal cord injury and half of the animals from each group given enriched environment also after injury, generating four groups with 12 animals in each (Fig. 1).

#### Enrichment conditions

A shepherd shack (egg-box carton  $21 \times 20 \times 10$  cm, Des Res houses for rats, Lillico Biotechnology, Brogaarden, Denmark), an opaque yellow PVC tube (30 cm long, 10 cm wide), folded paper strips as nesting material (Enviro-Dri, Scanbur, BK, Sweden) and two gnawing sticks (aspen wood,  $1 \times 1 \times 5$  cm, Finn Tapvei, Finland) (Fig. 2B).

#### Surgical procedure

Rats were anesthetized with isoflurane, a laminectomy performed at the thoracic level T9, and the rod of the impactor device (NYU impactor) centered at the exposed spinal cord. The rod (weight = 10 g) was then released from a height of 12.5 mm. Muscles and skin were sutured. Rats were allowed to recover on heating pads for 4 days, received analgesic (0.015 mg/kg buprenorphin, Temgesic® i.p.) for 48 h and broad spectrum antibiotics (1 ml/kg Borgal® s.c.) for 1 week. Urinary bladders were emptied twice a day for 2 weeks and then thereafter as necessary. After surgery, they were housed socially in their original groups with or without the environmental enrichment items. Body weight was monitored weekly from weaning until week 16 of the study.

#### BBB locomotor scoring

Behavior analysis was carried out by standardized BBB scoring (Basso et al., 1995), performed by a trained technician in an unbiased, blinded way. The hind limb motor behavior was

observed during a period of 3 min in an open field. The scoring ranges from 0 (flat paralysis) to 21 (normal gait) and observes graded features of limb movement, paw placement, gait and coordination.

#### Grid walk test

This test measures the ability of animals to control hind paw placing and is regarded as an indicator of corticospinal function (Grill et al., 1997). A 1.2 m long grid pathway was used, and animals were allowed to walk three times voluntarily across the pathway. The number of errors (hind limbs stepping through the grid) was counted. The three values were averaged and resulted in one value per animal. Uninjured animals typically made no or one error.

#### Hotplate test

The hotplate test was carried out as described elsewhere (Gale et al., 1985). Animals underwent the test 3 months post-injury and were placed on a preheated hotplate (53°C). The latency (in seconds) until the animal showed signs of discomfort (paw licking) was measured. Non-responders were removed from the hotplate after 60 s and given the maximum latency score 60 s. Normal non-injured animals exhibit licking after 10 to 25 s. Values for both fore limbs were averaged as were values for both hind limbs.

#### Open field test

An open field test to assess locomotor and exploratory activities was carried out in a circular arena (diameter 65 cm) using an automated video tracking program (EthoVision, Noldus Technology, The Netherlands; Noldus et al., 2001). The rat was placed in the arena and was allowed to explore it for 10 min. Exploratory activities recorded included total distance traveled, total duration spent on exploring the periphery and the central area, which is usually avoided. These parameters can be used as measurements of stress and anxiety (Meerlo et al., 1996). Mean speed of locomotion and rearing frequency were also measured.

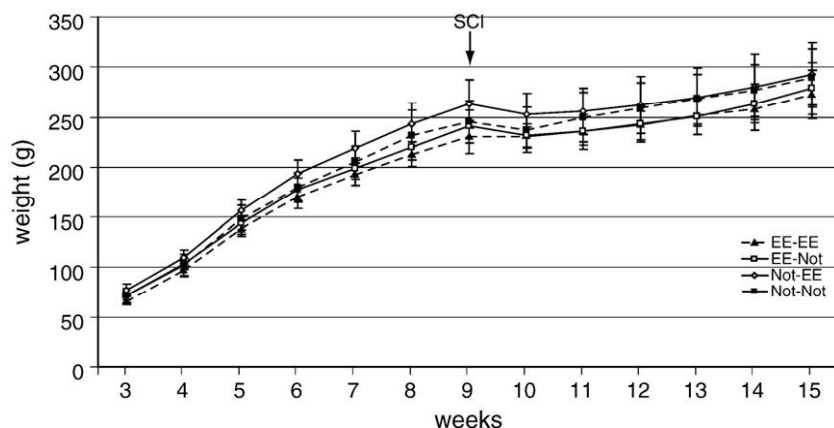


Fig. 3. Development of body weight in the environmental enrichment experiment. Body weight was monitored weekly as a general health parameter. The spinal cord injury clearly caused a weight loss that took the animals some weeks to recover.

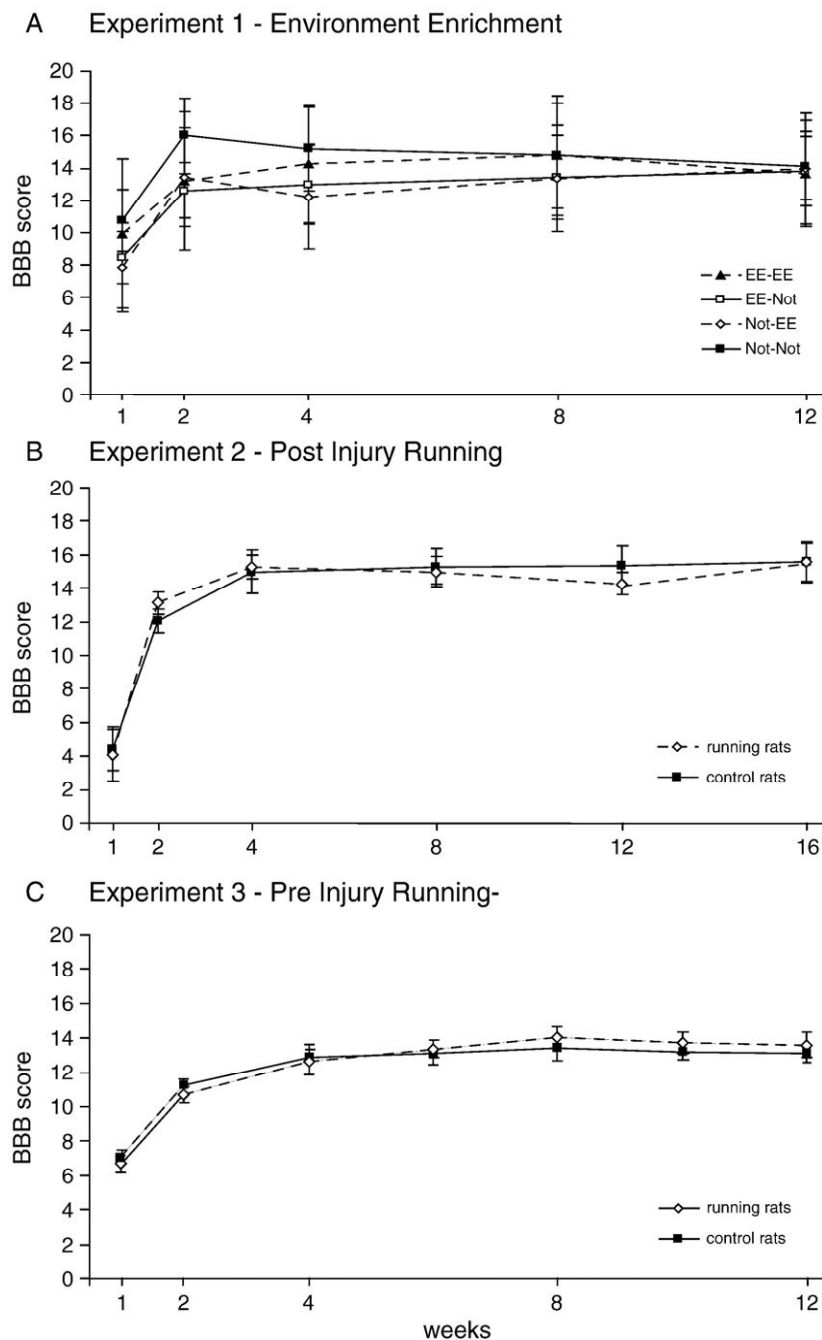


Fig. 4. Functional recovery of animals in the three experiments. Functional recovery was determined with the BBB score. (A) In the environmental enrichment experiment, the BBB scores showed a larger variation but were not significantly different. (B, C) In the running wheel experiments, the scores were very similar. All groups reached a plateau phase after 4 weeks.

### General behavior

Six behavioral categories, locomotion, grooming, rearing, immobility, eating and drinking, were assessed in an automated behavior analysis and registration system (LABORAS®, Laboratory animal behavior observation registration and analysis system, Metris, The Netherlands). With this system, four rats at a time, one from each group, were tested simultaneously on individual sensing platforms for 60 min. Each rat was placed individually in a clean cage (Makrolon® type III, 840 cm<sup>2</sup>) with

bedding, food and water. The cage is placed on top of the sensing platform. The animals' movements were translated into the six behavioral categories and automatically registered by a computer.

### Corticosterone levels

Urine corticosterone levels were measured 1 day before spinal cord injury and weekly 5 times after injury. To obtain urine, the bladder was massaged and urine was collected and stored in polypropylene tubes at −20°C until analysis.



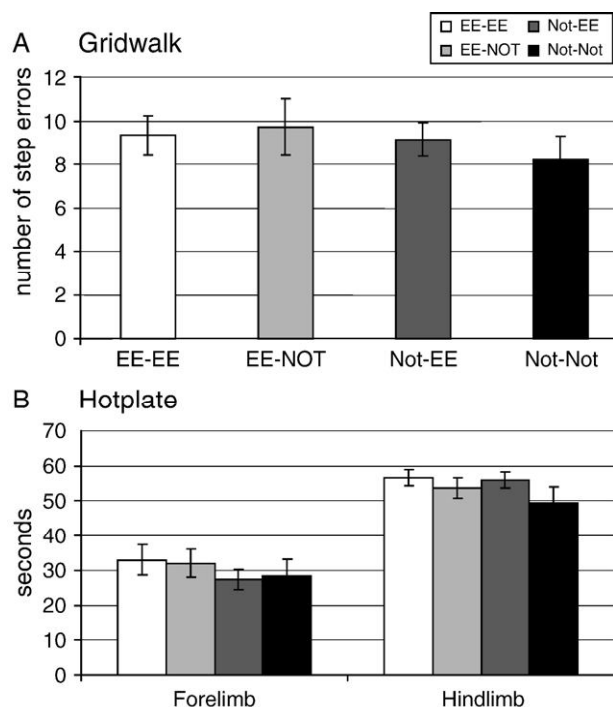


Fig. 5. Sensitivity tests for the environmental enrichment experiment. (A) Grid walk and (B) hotplate test for the different environmental enrichment groups in the first experiment. No significant difference could be found between groups for these tests.

Corticosterone levels were measured using a solid-phase  $^{125}\text{I}$  radioimmunoassay (CAC Rat Corticosterone TKRC1, Diagnostic Products Corporation, Los Angeles, USA). Creatinine concentration, as an indicator for urine dilution, was determined with the use of a commercial test (ABX Diagnostics, Montpellier, France) on a bio auto-analyzer. Corticosterone levels were calculated as ratio corticosterone/creatinine.

#### Experiment 2: running after injury

##### Animals

Sixteen 250 g female Sprague–Dawley rats, kept under the same standard conditions as in experiment 1, were used (Fig. 2A). One week following spinal cord injury, the animals were assessed on the BBB score, randomized into 2 groups. Two outliers (not effectively injured) were excluded before randomization. One group was single-housed in standard cages (Makrolon<sup>®</sup>, type III, 43 × 22 × 20 cm), whereas the second group was single-housed in the same sized cages fitted with free access to running wheels (Fig. 2C; diameter = 34 cm, circumference = 107 cm). Running activity was collected 48 times/day via customized software (Werme et al., 1999). Four months after injury, rats were sacrificed by decapitation. BBB, hotplate and grid walk tests were performed as described above for experiment 1.

##### Sensitivity tests

Mechanical sensitivity was assessed using von Frey hairs (Stoelting, Chicago, USA), which test the withdrawal threshold to

graded mechanical touch/pressure. Von Frey hairs were applied in ascending order on the palmar surface of the fore- and hind paws at a frequency of 0.5–1 per second. The lowest force leading to at least 3 withdrawals in 5 trials was defined as the mechanical threshold. The response rate to cold was tested by spraying ethyl chloride on the palmar surface. The response was scored as 1 (no observable response), 2 (brief withdrawal and licking) or 3 (vocalization, prolonged withdrawal, licking). Heat response was tested with a modification of Hargreaves' method (Hargreaves et al., 1988). Radiant heat was applied to the palmar surface, and the latency to withdrawal of the stimulated paw was measured.

##### Locomotor activity

Animals were placed individually in locomotor cages (25 × 40 × 30 cm). Motility, locomotion and rearing were simultaneously recorded in a computerized multi-cage infrared-sensitive motion detection system (Motor Products, Stockholm, Sweden) (Ogren et al., 1986). Photocells in the cage floor, separated by 4 × 4 cm, detected horizontal motion. Motility was defined as any movement interrupting a photocell beam, i.e. a distance of 4 cm, while movement over eight photocells, or 32 cm, was defined as locomotion. Horizontal infrared beams separated by 4 cm, 10 cm over the cage floor, detected rearing. Rearing, motility and locomotion counts were summed every 10 min.

#### Experiment 3: running prior to injury

##### Animals

Thirty one female Sprague–Dawley rats, 250 g, kept under same standardized conditions as in the other experiments were randomly assigned to standard cages with running wheels (Fig. 2C) (Makrolon<sup>®</sup>, type III, 43 × 22 × 20 cm) or single-housed in the same type of cage without a running wheel for 3 weeks prior to surgery. After spinal cord injury (see above), animals in the running wheel group were transferred to cages without running wheels and group-housed (4 animals per cage).

Animals in experiment 3 were subjected to BBB test, the hotplate test, the grid walk test, sensitivity tests (von Frey, cold response) and locomotor activity. To obtain a BBB score higher than 13, rats must show fore limb–hind limb coordination, a major step in recovery. To take into account other signs of recovery, like paw placement, toe clearance, trunk control and tail position, the BBB subscore system (Lankhorst et al., 1999) was also used.

##### Statistical analysis

All data were analyzed using SPSS statistical software. Data from the BBB, grid walk and hotplate tests in experiment 1 were analyzed by one-way ANOVA, other data by Student's *t* test. Body weights and corticosterone levels were tested by analysis of variance for repeated measures (ANCOVA). LABORAS data were analyzed by ANCOVA when data were normally distributed. When data were not normally distributed, they were analyzed using the nonparametric Friedman test and if significant, also with the nonparametric Wilcoxon test. The EthoVision data were analyzed using the nonparametric

Kruskal–Wallis test and if significant also with the Mann–Whitney  $U$  test. For all data, the  $\alpha$  was set at 0.05.

All experiments were approved by the Stockholm Animal Ethics committee.

## Results

### Experiment 1: environmental enrichment

Body weight decreased in all groups after injury and recovered steadily thereafter. Body weights are shown in Fig. 3. No statistically significant effect of enriched environment on body weight was detected.

Eight days after spinal cord injury, the BBB score (Fig. 4) reached around 8 to 10. The relatively large variation within groups was presumably due to variation in body weight among siblings, when rats were bred and weaned together at our animal

facility, as opposed to animals for the running wheel experiment, which were purchased as adults according to weight. After 4 weeks, all groups reached a plateau phase, with only minor changes. No statistical differences were found among groups.

Hotplate and grid walk tests (Fig. 5) did not reveal any differences between groups. Similarly, we did not detect any difference between the groups using the open field test. Consistent with the lack of effects in other tests, none of the LABORAS parameters was significantly different between the groups. Finally, corticosterone levels did not change during the observed period and no differences were found between the groups (data not shown).

### Running after injury

The rats used the running wheels extensively, and running activity reached peak levels after 2 weeks (Fig. 6A) of more

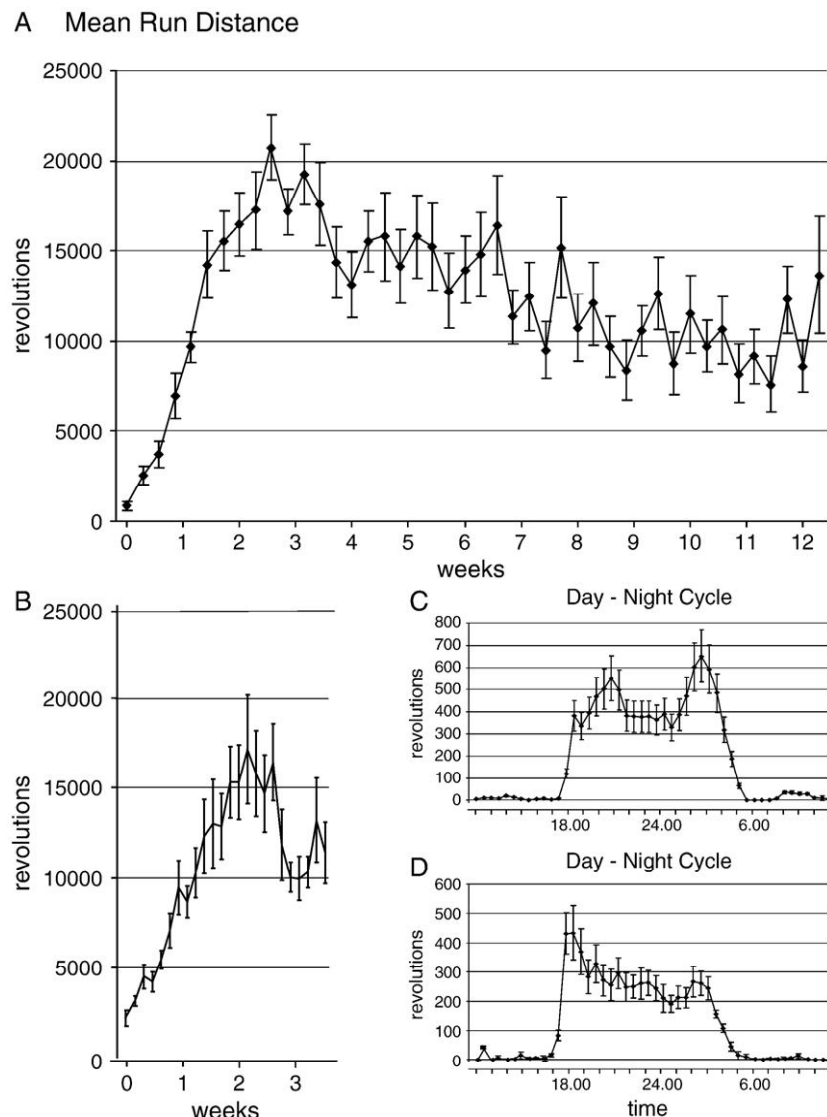


Fig. 6. Mean distance run by the animals with access to running wheels. (A) Animals that had access to running wheels starting with 1 week after injury reached a peak after 2–3 weeks and lowered running activity to a level around 10,000 revolutions a night. (B) Non-injured animals in the third experiment reached a peak after 2 weeks and showed a similar running pattern as injured animals. (C, D) Running activity was strictly performed during the night in both experiments.

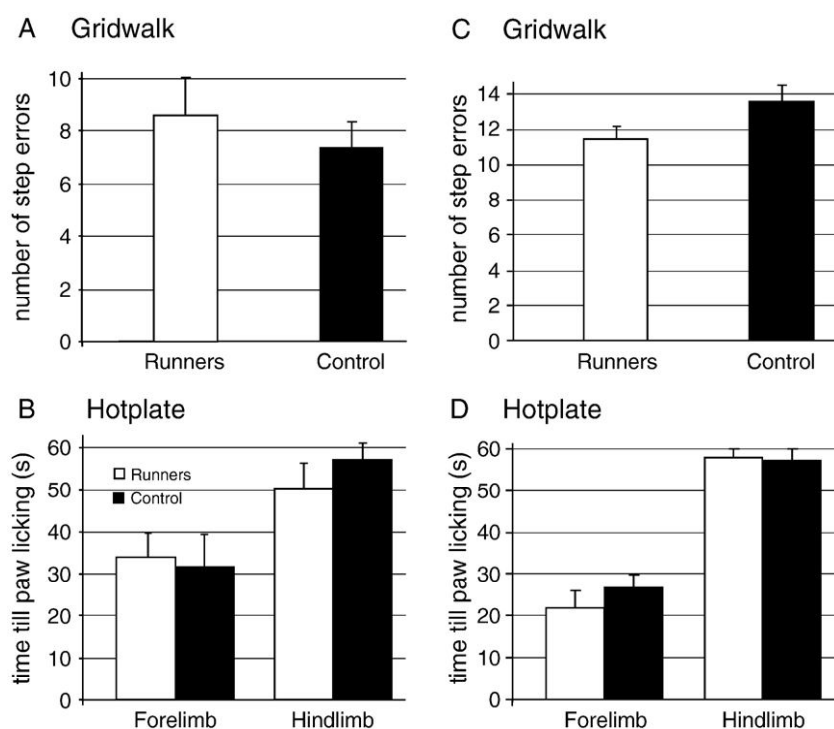


Fig. 7. Behavioral tests for motor recovery for running wheel experiments. (A, B) Grid walk and hotplate test for experiment where rats had access to running wheels after or (C, D) before spinal cord injury: no significant difference was found between groups.

than 20,000 revolutions. Rats were strictly night active (Fig. 6C). In the first weeks of running, we noticed that the rats preferred a position where the body was placed outside the running wheel and the wheels turned by their fore paws. As the

animals recovered, they positioned themselves inside the wheels and produced “classical” wheel running behavior.

Both groups recovered BBB scores in a very similar way, reaching a plateau phase after 4 weeks (Fig. 4B). After 4 weeks,

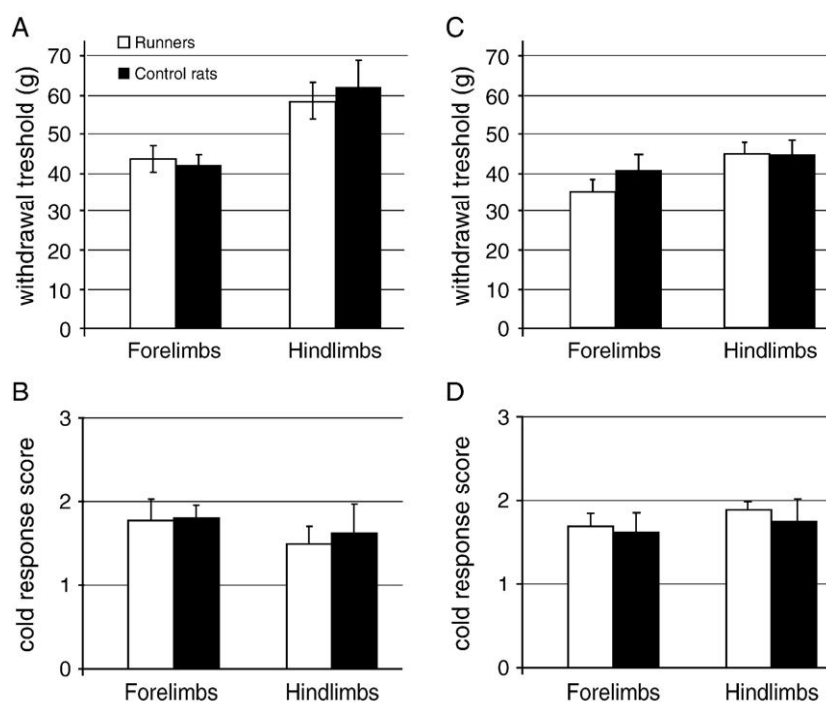


Fig. 8. Sensitivity tests for running wheel experiments. (A, B) von Frey hair and cold response 3 months after injury in rats with access to voluntary wheel running after or (C, D) before injury. Responses for these sensory tests did not differ significantly.

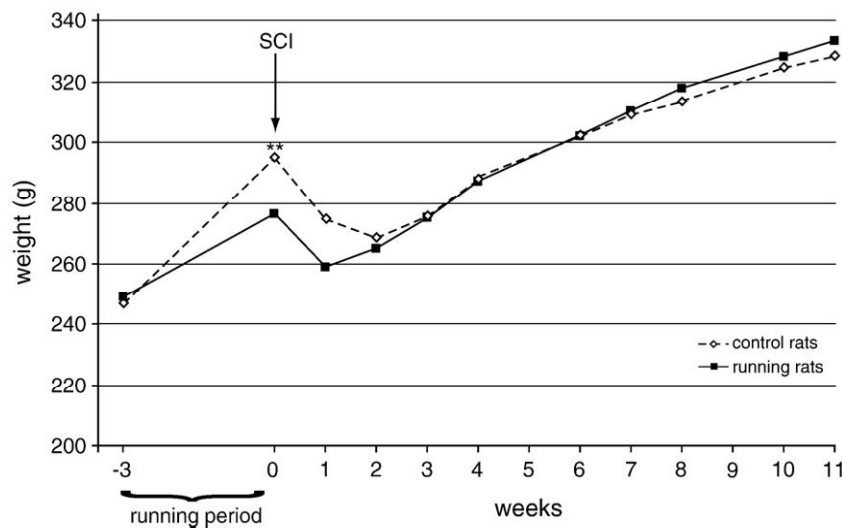


Fig. 9. Weight changes for animals with access to running wheels before injury. Animals that had access to running wheels 3 weeks before injury gained significantly less weight than control rats. However, after spinal cord injury, they lost less weight and recovered faster weight, so that this difference disappeared 2–3 weeks after injury.

virtually, no changes were seen in terms of locomotor recovery. No statistical differences were found among groups. Results for the grid walk and the hotplate tests (Figs. 7A, B) were very similar for both groups.

Neither the von Frey hair test nor the heat or cold test showed any significant differences between groups (Figs. 8A, B). We were unable to detect allodynia, a pathology often seen after spinal cord injury in rats (Yoon et al., 2004). This could be due to differences of age (Gwak et al., 2004a,b) or to strain or sub-strain differences (Bulka et al., 2002).

The locomotor box test also failed to detect differences between the groups. Neither spontaneous horizontal nor vertical locomotor activity differed significantly between groups (data not shown).

#### Running prior to injury

Normal rats made extensive use of the running wheels, and after a week, they ran between 10 and 15 km per night. In comparison to normal control rats, the running rats gained less weight during the 3 weeks running period (27 g vs. 48 g, Fig. 9). However, animals that had been allowed to run lost less weight after injury and reached the pre-injury weight 2 weeks after injury.

We were not able to detect any significant difference of walking behavior between groups using the BBB score (Fig. 4C). Furthermore, running prior to injury did not cause any significant change of the BBB subscore.

In the grid walk test, we found a tendency for animals that had run for 3 weeks prior to injury to perform better after injury

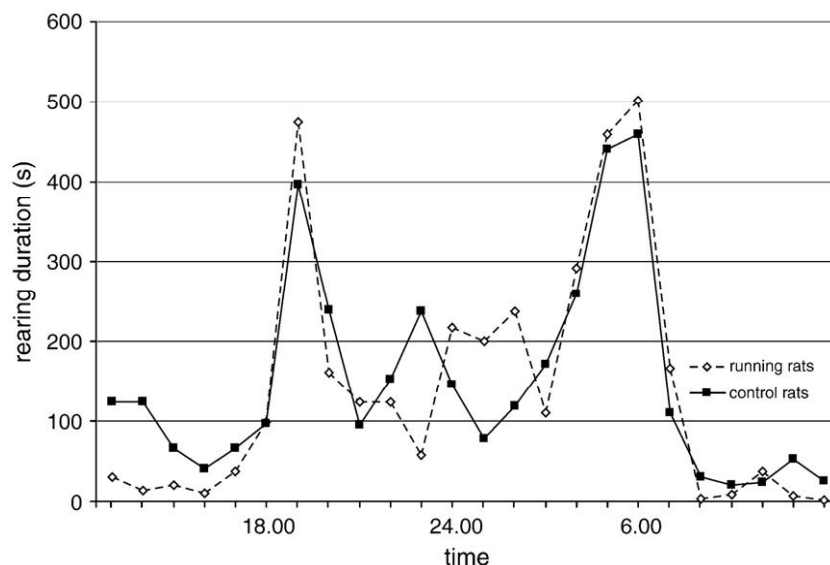


Fig. 10. Rearing activity of animals with access to running wheels before injury. Rearing activity was measured 24 h and compared between groups 3 months after injury. No differences between animals that run before injury versus control animals were found.



than the control group, but this difference did not reach statistical significance ( $P = 0.057$ ) (Fig. 7C). In the hotplate test, animals allowed to run before injury licked their fore paws slightly faster than controls (Fig. 7D). In both sensory tests (von Frey, cold response), runners and controls performed very similarly (Figs. 8D, E).

Rearing is an activity that requires active standing by use of the impaired hind limbs. Injured animals were able to rear after some recovery. Again, we did not find any difference in rearing behavior between groups monitored over a 24-h period (Fig. 10).

## Discussion

Here, we attempted to separate possible effects of various environmental influences, including enriched cage environment and physical training on the recovery from partial spinal cord injury, taking into account both the pre- and the post-injury periods. We show that environmental enrichment and voluntary wheel running do not significantly change recovery of rats from incomplete spinal cord injury, regardless of whether these activating measures were in place before or after injury.

Our results may seem at odds with certain previous studies that report positive effects of both environmental enrichment and running in rodents. However, most experimental animal studies reporting positive outcomes were based on brain interventions, such as stroke, injury or ageing, rather than on spinal cord injury. In experimental spinal cord injury models, most groups report moderate (Engesser-Cesar et al., 2005; Hutchinson et al., 2004; Koopmans et al., 2005; Van Meeteren et al., 2003) or no effects (Fouad et al., 2000; Hicks et al., 1998; Woerly et al., 2001) of environmental enrichment or voluntary wheel running in rodents in terms of functional recovery. In the brain, neurogenesis and plasticity are well described and may be influenced by environmental circumstances and training, while similar effects have not been reported in the spinal cord.

### *Environmental enrichment*

We used basic environment enrichment, not directly stimulating locomotion, but adding some learning experiences and meeting some of the rats' behavioral needs, such as hiding, nesting and gnawing, in order to increase their well-being. Indeed, we observed that the environmental enrichment was used by the animals, but we did not observe improved locomotion. We noticed that, if rats were not raised with environmental enrichment, they were not able to use it adequately; the nest-box was used as shelter only by rats that had been reared with this type of environmental enrichment. Rats who received the shelter after injury (hence in adulthood) just chewed on them, leading to the need of replacement twice a week, which is in line with previous reports (Van Loo and Baumans, 2004). As our enrichment did not enhance physical activity, it may be less surprising that the BBB locomotion scores did not differ between groups (Fig. 4A), as did grid walk and hotplate tests (Fig. 5). Further testing with the LABORAS and open field tests did not reveal any other behavioral effects of the environmental treatments. We tested a battery of parameters, including motion,

speed, rearing, grooming, immobility, eating, drinking, anxiety and stress, without finding any differences between groups. Since place preference is an indicator of stress and anxiety, the lack of effect of various environmental enrichment protocols suggests that the addition of the house, tube, rods and bedding material did not affect the degree of stress and anxiety as reflected in the open field test. Corticosterone levels, another stress parameter, did not vary significantly between groups. However, as the animals were housed in groups, the physical enrichment might not have added much to the existing social enrichment, as found by others (Johansson, 1996). We conclude that our environmental enrichment does not change the behavior of the animals and that such enrichment does not have any observable beneficial effects on recovery from spinal cord injury on top of the possible effects of social housing.

### *Post-injury running*

Presence of the running wheels led to very substantial voluntary physical training, so that animals would increase their use of the wheels within a few weeks to what would correspond to about 20 km/night (Figs. 6A, C). This, however, had no effect on the various measures of recovery from the spinal cord injury (Figs. 4, 7, 8)). We noticed that some animals were not running during the first post-injury period, but instead used only their fore paws to spin the wheel. Animals placed their hind limbs outside the wheels and turned the wheel by their fore paws or just dragged their hind paws. The equipment did not allow discrimination of "real" running from the bipedal form, but the extent of hind limb use increased over time, so that after approximately 1 month, only "real" running was observed. It is probably important to distinguish between voluntary exercise and forced exercise, e.g. applied through a physiotherapist, a robot or a treadmill. In studies using forced exercise, several groups have reported locomotor recovery in both humans (Barbeau, 2003), cats and rats (Fouad and Pearson, 2004). Furthermore, in most experiments where animals were trained on treadmills, the injury was more severe than the injury we used here. A treadmill or the robotic device ("The rat stepper") forces the animals to move in situations where they would otherwise not be able to move at all. Some studies show that trained animals recover quicker and reach a plateau phase in the BBB locomotor score earlier than non-trained animals (Multon et al., 2003). In order to obtain a better recovery, a basic amount of training might be required so that animals that are able to move in their cages already reach this critical threshold of self-training. The amount of recovery can then perhaps not be enhanced above the self-training-induced level by any extensive repetitive exercise. Training effects have been shown to be task-specific (De Leon et al., 1998), so that motor training might be more useful for recovery than repetitive physical exercise (Johansson, 1996; Risedal et al., 2002).

### *Pre injury running*

In this experiment we evaluated the possible neuroprotective effect of physical activity and training. Also in this experiment

animals made extensive use of the running wheels, developing a similar running pattern as injured animals (Figs. 6B, D). There was no initial period of using only the fore paws to spin the wheel, in these healthy rats. After injury the animals were deprived of running. The animals that had run prior to injury recovered a pattern of test results that was very similar to that of non-trained animals (Fig. 4C). The fact that these animals suffered a smaller weight loss and recovered faster weight than control animals may show their more robust body condition (Fig. 9).

The widely used BBB score was similar across the groups, and even when applying the recently introduced BBB subscore, we were not able to find any difference. We found a small improvement in the grid walk test and a slightly faster reaction in the hotplate test, although these differences were not significant (Figs. 7C, D). This might point toward a beneficial effect of voluntary exercise before injury.

## Conclusion

To conclude, we find that rats recover well from moderate spinal cord injury and that this recovery cannot be further enhanced by environmental enrichment or extensive voluntary wheel running. It is not clear to what extent physiotherapy and rehabilitation programs can enhance or accelerate functional recovery after spinal cord injury in patients. Further studies of training in animals with different training paradigms, exposing them to different environmental strategies and combining that with pharmacological interventions, may help understand mechanisms of recovery and the design of better training strategies for patients.

## Acknowledgments

Support: This study was supported by AMF, USPH, Karolinska Institutets fonder and Vetenskapsrådet. Matthias Erschbamer is supported by Karolinska Institutets MD/PhD program.

We would like to thank Stefan Brene and Astrid Bjørnebekk for help and comments with the running wheel experiments, Karin Pernold, Eva Lindqvist and Karin Lundströmer for technical help, Inez Lemmens (Department of Laboratory Animal Science, Utrecht University, Netherlands) for corticosterone analysis.

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