



# Functional Recovery in Rats With Chronic Spinal Cord Injuries After Exposure to an Enriched Environment

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## Abstract

**Background/Objective:** The objective of this study was to determine the effect of environmental enrichment on the sensorimotor function of rats with chronic spinal cord injuries.

**Design:** Adult Sprague-Dawley rats received a contusive injury of moderate severity at vertebral level T8 using a weight-drop device. Three months after injury, 1 randomized group ( $n = 16$ ) of rats was placed in an enriched environment, whereas the control group ( $n = 16$ ) remained housed in standard laboratory cages (2/cage).

**Methods:** Animals were placed in an enriched environment for 4 weeks beginning at 3 months after injury. The enriched environment consisted of a large cage (5–6 rats/cage) with access to items such as tubes, ramps, and running wheel, with items changed daily.

**Main Outcome Measures:** Functional evaluation consisted of the open field Basso, Beattie and Bresnahan (BBB) locomotor test and the tests that form the combined behavioral score (CBS). The CBS includes motor score, toe spread, placing, withdrawal, righting, inclined plane, hot plate, and swim tests. Behavioral testing was repeated 7 times before and after the period of intervention.

**Results:** The group placed in the enriched environment scored significantly better on the BBB (ANOVA repeated-measures,  $P < 0.01$ ) test and CBS (ANOVA repeated-measures,  $P < 0.01$ ).

**Conclusions:** Environmental enrichment results in significant functional improvement in animals with spinal cord injury even with a substantial delay in initiating treatment after injury. The features of an enriched environment that may be responsible for the improvement include social interactions, exercise, and novel items in an interesting environment. These findings suggest a continued plasticity of the chronically injured rat spinal cord and a possible therapeutic intervention for people with spinal cord injury.

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**Key Words:** Spinal cord injuries; Rats; Motor score; Locomotor testing; Enriched environment; Exercise; Rehabilitation

## INTRODUCTION

In 1947, Hebb (1) first defined and explored the long-term benefits of an enriched environment in experimental animal studies. An enriched environment provides social interactions and novel items that encourage sensory and motor activity. Numerous studies support the view that the enriched environment induces plasticity in the brain of normal animals (2–4). Changes induced by

environmental enrichment include increases in brain weight (5,6), cortical thickness (7), dendritic branching (8), number of spines (9), and synapses (10). Possible mediators of this induced plasticity are increased levels of neurotrophic factors (11,12), increased neurogenesis and/or survival/differentiation of newly generated cells (13,14), and upregulation of immediate early genes and their products (15,16).

Environmental enrichment also produces improved outcome after neural injury (17) as noted in several experimental animal models of brain damage, including ablative lesions (18,19), focal or global ischemia (20–22), anoxia (23), lateral fluid percussion injury (24), teratologic agents (25), genetic defects (26), aging (27), and diseases (28). When enrichment is provided before and

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after cortical lesions, rats regain normal kinematics during locomotion (19). Enrichment after brain damage caused increased neurogenesis (29), increased levels of neurotrophic factors (30) and transcription factors (31), and decreased apoptotic cell death (32) that suggest that enrichment is inducing plasticity in the brain. One possible mediator of the effects of an enriched environment is insulin-like growth factor (IGF). Blocking this growth factor decreased the effectiveness of enrichment after spinal cord injury (33). Environmental enrichment seems to be particularly effective in combination with cellular/tissue transplants (34).

The discovery that the spinal cord is capable of extensive plasticity (possibly learning) independent of the brain and that plasticity in the brain might greatly influence spinal cord function after injury led to the application of enriched environment conditions to spinal cord injury (SCI) (35). Lankhorst et al (36) found improvement in the Basso, Beattie and Bresnahan (BBB) locomotor test (37), BBB subscale, Gridwalk test, CatWalk test, and thoracolumbar height test in animals placed in an enriched environment immediately after SCI.

The possible effectiveness of delayed exposure to the enriched environment after injury was more recently explored. When enrichment was delayed until 15 days after focal brain ischemia, Johansson (38) still found improvements in a variety of functional tests. Using a different model of focal ischemia but starting the enrichment with rehabilitative training at 15 days after injury, Biernaskie et al (39) also found functional improvement and enhanced dendritic complexity and length compared with animals in standard caging. When the enrichment was delayed for 30 days after focal ischemia, functional improvement was noted in the ladder-rung walking test, but this improvement took more than 1 month to appear. However, it is not known if the chronically injured spinal cord is also capable of recovery after exposure to an enriched environment. In this study, the effects of an enriched environment starting 3 months after injury are evaluated in rats with a moderate degree of spinal cord injury.

## METHODS

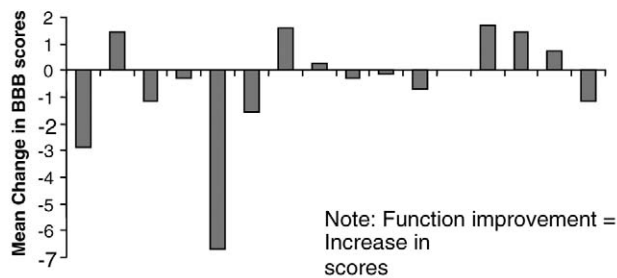
All animal procedures were approved by the Institutional Animal Care and Use Committee at the University of Alabama at Birmingham. Thirty-two adult female Sprague-Dawley rats (200–250 g) were obtained from Charles River Laboratories (Raleigh, NC). To create a contusive SCI, the rats received ketamine (0.25 mL/kg, 100 mg/mL). The back of the animal was shaved and cleaned with Betadine scrub and then Betadine solution. Lacrilube was applied to the eyes. The animal was placed on a water-circulating heating pad. Rats were deeply anesthetized with 1.5% to 1.7% halothane in oxygen (1 liter/min), and a midline incision was made. A laminectomy was performed to expose the spinal cord at T8, and a moderate SCI was created using a weight-drop device,

with a 10-g weight dropped 2.5 cm (40). The weight-drop device was identical (strain-gauged C-ring; maximum force: 18.2 N, impulse, 4.16 N-ms) to that developed by Wrathall and colleagues (40,41), except that the weight was released by shutting off the electricity to an electromagnet as opposed to removing a pin to increase stability during release. After injury, the muscles and the subcutaneous tissue were closed with absorbable sutures. The skin was closed with wound clips. All the rats received daily handling throughout the study to monitor their health. Behavioral deficits were allowed to plateau.

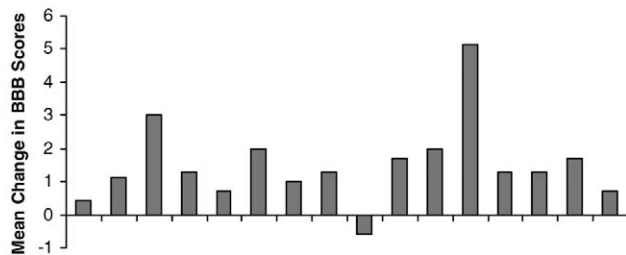
At 3 months after injury, behavioral tests were performed 7 times during a 2-week period to examine residual locomotor and sensory function. Treats such as cereal and corn curls were given to all rats, especially during behavioral testing. The tests used were the BBB locomotor test (37) and Combined Behavioral Score (CBS) (42). The BBB is an open field locomotor test scored on a 21-point scale, with 21 being normal and 0 indicating no movement of the hind limbs. Furthermore, a motor subscore of the BBB test using information from the original BBB scoring sheets was determined to evaluate higher motor function (43). The BBB subscore is a 7-point scale that evaluates toe clearance, paw position, stability, and tail position, with 7 representing normal function. The CBS consists of 8 tests: motor score, toe spread, placing, withdrawal, righting, inclined plane, hot plate, and swim tests. The score is on a scale of 0 to 100 and measures the amount of deficit: a score of 0 is normal and a score of 100 indicates complete paralysis of the hind limbs. Two component tests of the CBS, motor score (not derived from BBB scores) and inclined plane, were additionally analyzed separately. The CBS motor score is a derivative of the 5-point Tarlov score but measures deficits so 0 is normal walking and 45 represents no voluntary movement. For the inclined plane, rats that can maintain their position for 5 seconds on a 45° incline are given 0 points. If a rat only maintains its position on a less steep incline, that rat receives 5 points for a 40° incline, 10 points for a 35° incline, and 15 points for an incline less than 30°.

The rats were randomly assigned to either continue to be housed in an opaque standard laboratory cage (2 rats/cage, 19 × 10 × 8 in.) or in an enriched environment (5 rats/cage). This new environment consisted of a large cage measuring 40 × 24 × 10 in. containing a number of items that were changed daily. These items were tunnels for crawling through, a running wheel, a ramp to climb on (which also had a textured surface), a ball, a see-saw-like apparatus that changed position on passing the half-way point, a large jar to nest in, and a box with interchangeable floors that the rats could crawl through. The interchangeable floors were as follows: a plastic grid, a wire grid, or a floor of marbles that moved as the rats walked on them. The same functional tests were repeated every other day (total of 7 times), beginning after 2

A.



B.



**Figure 1.** Changes in the mean BBB scores of individual animals. (A) Difference in the mean BBB scores in individual rats housed in standard cages comparing scores after and before a 4-week period. (B) Differences in the mean BBB scores in individual rats housed in an enriched environment for 4 weeks (mean postscore – mean prescore). *Positive* values represent functional improvement.

weeks of exposure to the enriched environment and ending at 4 weeks.

Data were analyzed using SPSS statistical software. Repeated-measures ANOVAs were used to determine if function had reached a plateau at 3 months after SCI and also used to reveal if there were changes during the repeated testing after exposure to the enriched environment. For the BBB scores, BBB motor subscore, CBS, CBS motor subscore, and inclined plane, repeated-measure ANOVA was used to determine if there were differences in function before and after exposure to an enriched environment. Significance was set at  $P < 0.05$ .

Rats were deeply anesthetized, as described above, and perfused through the heart with saline followed by 4% paraformaldehyde in phosphate-buffered saline (pH 7.2). The spinal cord was removed and placed in fixative overnight. A piece of spinal cord was dehydrated and infiltrated and embedded in polyester wax. Longitudinal sections were cut at 10  $\mu\text{m}$  using a rotary microtome, dewaxed, and stained with cresyl violet. The area and length of the cavity was quantified using digitized photographs (Spot Advanced Software 3.5.5; Diagnostics Instruments, Sterling Heights, MI) of at least 6 sections per animal at regular intervals through the lesion for cavity area and at the epicenter for the length of the

cavity. Cavity length and volume were compared using Student *t*-test.

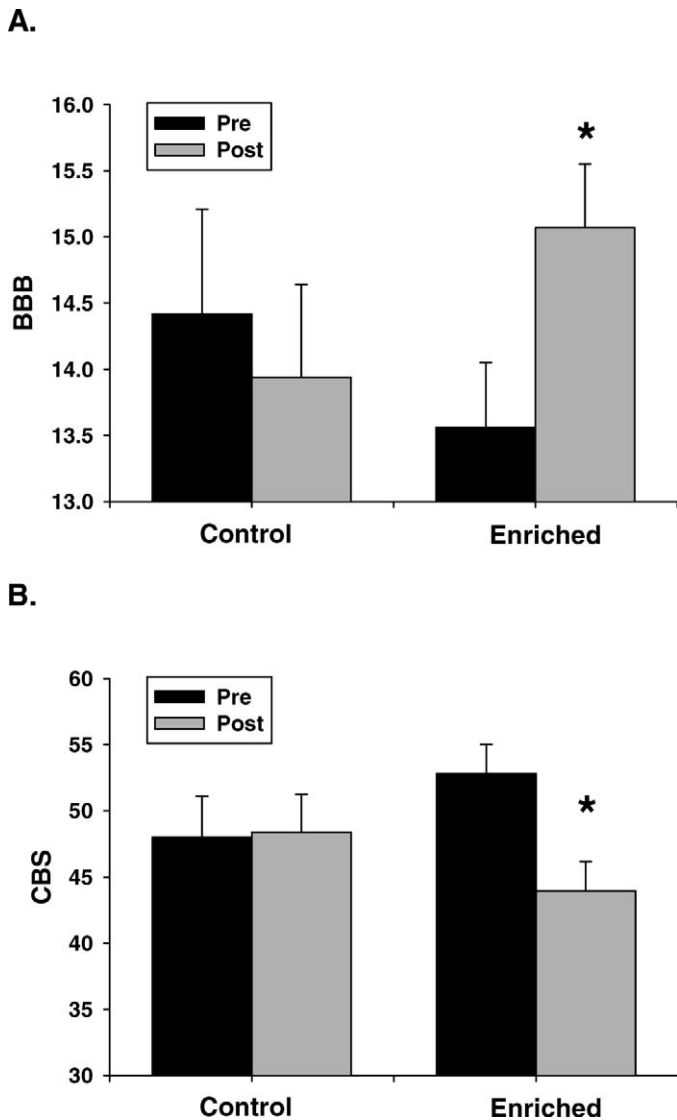
## RESULTS

The animals had only slight to extensive movements in the hind limbs on the first day after injury (BBB range, 1–5) but improved rapidly during first week (mean BBB,  $10.7 \pm 0.37$ ) and less rapidly in the second week after injury (mean BBB,  $12.3 \pm 0.43$ ). The animals reached a plateau in terms of function at about 3 weeks after injury as previously described (44). The mean scores on the BBB and CBS are considered to be “moderate in severity” based on other studies (37,44). All rats were housed in standard cages for 3 months, and neurological testing was repeated 7 times over a 2-week period. No significant change occurred in the behavioral tests before the intervention period (repeated-measures ANOVA). At this time, one half of the rats (experimental group) were randomly assigned to an enriched environment, and one half of the rats (control group) continued to reside in standard cages. Besides the similarity of the groups in mean BBB and CBS scores, the lesion volume (control:  $2.69 \pm 0.07\text{mm}^3$ ; enriched:  $2.72 \pm 0.139\text{mm}^3$ ) and length (control:  $3.70 \pm 0.42\text{mm}$ ; enriched:  $4.01 \pm 0.27\text{mm}$ ) were not significantly different ( $P > 0.1$ ). There was also no significant difference in the body weights of the 2 groups after the treatment period (control pre:  $228.4 \pm 20.5\text{g}$ ; control post:  $229.2 \pm 14.7\text{g}$ ; enriched pre:  $225.6 \pm 3.8\text{g}$ ; enriched post:  $227.6 \pm 6.3\text{g}$ ) that might influence inclined plane results. The rats were evaluated 7 times with the same functional tests for 2 weeks beginning 2 weeks after placement of the experimental group in the enriched environment. At the first testing of the enriched group at 2 weeks after enrichment, there was a significant improvement in function, and no further functional changes occurred (repeated-measures ANOVA).

### BBB Locomotor Test

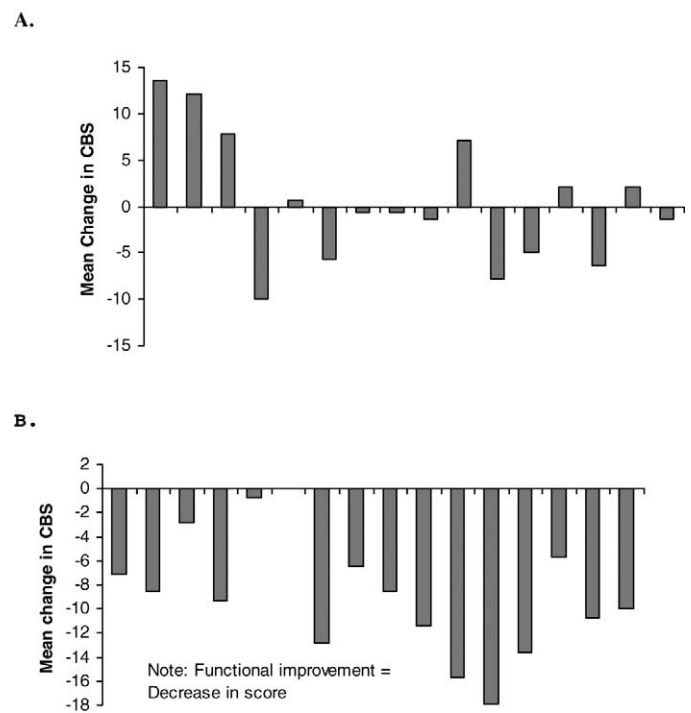
Comparing the change in the mean score before and after the intervention period in individual animals of the control group, the BBB score remained unchanged for about one third of the control rats; one third of control animals became slightly worse (range of decrease, 0.14–6.71, in BBB scale), and one third improved slightly (range of increase, 0.29–1.71, in BBB scale; Figure 1A). The mean pretreatment BBB score was  $14.42 \pm 0.79$  (SE) for the control group. There was a slight nonsignificant decrease ( $-0.43$ ) in the mean BBB scores ( $13.94 \pm 0.70$ ) of the control group comparing the periods before and after the intervention.

In contrast, all the rats except one placed in the enriched environment showed functional improvement in BBB scores (range of increase, 0.43–3.0, in the BBB scale; Figure 1B). The mean score before enrichment was  $13.56 \pm 0.49$  for the enriched group. The mean BBB score after enrichment was  $15.07 \pm 0.48$ , which



**Figure 2.** Mean functional scores before and after a 4-week exposure to either continued standard housing (control group) or exposure to an enriched environment. (A) Comparison of the mean BBB scores in the control group and enriched group before (pre) and after (post) the time of the intervention. (B) Comparison of the mean CBS score in the control group and enriched group before (pre) and after (post) the time of intervention. \*Significant difference ( $P < 0.01$ ) when analyzed with repeated-measures ANOVA.

represents an average improvement of 1.51 in the BBB scale (Figure 2A). The paw position at initial contact and lift-off in the enriched group went from internal or external rotation to becoming parallel to the body. In addition, toe clearance was more frequent in the enriched group, along with the gain of consistent forelimb-hindlimb coordination. The animals placed in the enriched environment showed a significant improvement in the BBB scores [ $F(7,9) = 13.142$ ;  $P < 0.001$ ]. This improvement occurred at the first test date (2 weeks after



**Figure 3.** Changes in the mean CBS scores of individual animals. (A) Difference in the mean CBS scores in individual rats housed in standard cages comparing scores after and before a 4-week period. (B) Differences in the mean CBS scores in individual rats housed in an enriched environment for 4 weeks (mean postscore – mean prescore). Negative values represent functional improvement.

placement in the enriched environment), and there was no further improvement during the next 2 weeks in the enriched environment based on repeated-measures ANOVA.

In the BBB motor subscore that measures fine motor control, there was no significant change in the control group. The mean pretreatment BBB subscores were  $3.79 \pm 0.21$  for the control group. There was a slight nonsignificant decrease (0.08) in the mean BBB scores ( $3.71 \pm 0.2$ ) of the control group over the 4-week period. In the mean BBB subscore for the enriched group, the mean score before treatment was  $3.65 \pm 0.16$  and after treatment improved to  $4.23 \pm 0.17$ . This decrease (0.58) was a significant change [ $F(5,11) = 5.28$ ;  $P \leq 0.01$ ].

### CBS Tests

In a pattern similar to the results of the BBB locomotor test, about one third of the control rats remained the same, whereas one third of control animals became slightly worse (range of increase of deficits, 0.71–13.57, in the CBS scale) and one third improved (range of decrease of deficits, 0.71–10.0, in the CBS scale) when the mean CBS scores in the 2 weeks before and after the intervention period are compared in individual control

animals (Figure 3A). On the 100-point scale of the CBS, the mean change in the control group was extremely small (0.40) and went from  $47.99 \pm 3.12$  to  $48.39 \pm 2.87$  (Figure 2B).

In comparing the means of each animal in the 2 weeks of scoring before and after the intervention period, all of the rats placed in the enriched environment showed functional improvement (range of decrease of deficits,  $-0.71$  to  $-17.85$ ) in the CBS scale except for one that remained unchanged (Figure 3B). In the enriched group, the mean score before treatment was  $52.81 \pm 2.23$  and after treatment improved to  $43.97 \pm 2.21$  (Figure 2B). This decrease ( $-8.84$ ) was a significant change [ $F(7,90) = 5.77, P < 0.001$ ]. Because the CBS measures deficit, this decrease in score denotes a functional improvement, whereas the increases for the BBB testing observed with enrichment also represent an improvement. The one enriched animal that did not improve on the BBB was different than the only animal that did not improve (no change) on the CBS. Of the 5 animals in the control group that became worse on the BBB scale, 3 of these animals showed decreased function on the CBS and Tarlov score component. Improvement was again present in the CBS scores at the first test date, which was 2 weeks after placement in the enriched environment. There was no further improvement during the next 2 weeks with additional exposure to the enriched environment as determined using repeated-measures ANOVA.

One component test of the CBS, the inclined plane, was analyzed separately over a 2-week period before intervention period and a 2-week period beginning the last 2 weeks of the 4-week intervention period. The mean inclined plane score of the control group changed from  $10.54 \pm 1.13$  to  $11.45 \pm 0.79$ , which is a change of 0.94. Over the same time period, the mean scores of the enriched group showed a significant improvement [ $-4.24; F(7,9) = 22.41, P < 0.001$ ] from  $11.53 \pm 0.73$  to  $7.28 \pm 0.75$ . Over the next 2 weeks of testing, there was no further improvement, although the rats were still housed in the enriched environment.

Another component of the CBS, the CBS motor subscore, was also analyzed separately. The mean score before treatment for the control group was  $7.77 \pm 1.64$  and after the treatment period had a nonsignificant decline in function of 1.16 to  $8.93 \pm 1.77$ . For the enriched group, the mean score before treatment was  $9.06 \pm 1.39$  and after treatment improved in function to  $4.51 \pm 1.24$ . This decrease (4.55) was a significant change [ $F(6,10) = 5.93, P < 0.01$ ].

### Functional Effects of Enrichment

Overall, the control group did not change in function, and the enriched group with the moderate degree of SCI had a significant functional improvement in all of the functional tests. These improvements were generally present by 2 weeks after exposure to the enriched

environment, did not increase further, and were observed in almost every animal.

### DISCUSSION

The purpose of the study was to investigate the effects of an enriched environment on rats with chronic SCI. The significant beneficial effects of the enriched environment found in this study are in agreement with the studies on acute SCI (35) and brain-injured animals (17–25,38,46). The degree of improvement in this study was remarkably similar to that observed by Lankhorst et al (36), when enrichment was started immediately after SCI. The improvement in BBB scores (1.51) in this study is slightly greater than that observed with using transplants of NT-3-producing fibroblasts in chronic SCI (47) or Schwann cells transplants in subacute spinal cord injury (48) and slightly less than that observed with subacute delivery of bone marrow cells (49) or acute blocking of Nogo (50). Changes in the CBS in the enriched group were most frequently caused by improvements in the motor subscore and inclined plane results.

Our results and the findings by Lankhorst et al (36) differ markedly from a report by Erschbamer et al (51), who found no functional improvement with enrichment immediately after moderate SCI. However, effects of their limited enrichment conditions (social environment without exercise equipment and without varying the items in the cage) were only compared with another social environment with clear cages. This is in contrast to our enrichment conditions that included varying items in the cage, exercise equipment, and a social environment that was compared to a nonsocial, standard housing environment (2 rats/opaque cage), where animals could not see other cages.

The particular components of the enriched environment responsible for the improvement remain a matter of controversy. An enriched environment provides social interactions and novel items that encourage sensory and motor activity. The particular enriched environment may induce considerable exercise and training and contain a variety of sensory stimulations. Johansson et al (20) found that the combination of physical activity and social interaction was superior to either social interaction or physical activity alone after cerebral infarction. This also seems to be true in the case of enrichment before cortical lesions where enrichment was superior to physical activity alone (52). Several studies assert that social interaction is the most important component (29,31,53), whereas others suggest that exercise (usually wheel-running) is most critical (54–56). Exercise (wheel-running) or environment enrichment after acute SCI produced similar functional improvements (57). However, having similar functional improvement does not necessarily mean that the same mechanism is involved. Both running and the enriched environment in C57BL/6 mice led to a similar number of newly generated neurons 1 month after treatment but through different mechanisms (14).

Running increased the initial proliferation, and enrichment increased cell survival. Another possibility is that novelty is the most important factor of an enriched environment (58,59). Novel items would stimulate the fundamental rodent behavior of exploration that may be mediated by IGF, because IGF stimulates exploratory behavior (60) and blocking IGF lessens the effects of enrichment (33). The enriched environment may also involve learning (61) or training (62). The most comprehensive explanation is that more than one mechanism is responsible for the changes observed with enrichment, and the relative contribution of a specific mechanism depends on the exact conditions used in the enriched environment.

An alternate way of viewing the positive effects of an enriched environment is that these functional improvements and anatomical/biochemical changes are caused by the absence of a deprived environment. Standard housing for rats is often 2 rats/cage with nothing in the cage except a water spout (with access to food through grating). This environment provides little sensory or motor stimulation and may be the reason for the dramatic effects often noted with enrichment.

Our findings support the view that the chronically injured spinal cord is capable of plasticity. It is unclear whether plasticity induced after exposure to the enriched environment is occurring in the spinal cord itself and/or in the brain that influences recovery through descending or ascending pathways. Two studies by Goshgarian and colleagues (63,64) on the crossed phrenic phenomenon suggest that the spinal cord is capable of considerable plasticity. Experimental animal studies (65) and various rehabilitative programs (66,67) also indicate that the chronically injured spinal cord is capable of plasticity, possibly through new intraspinal circuits (68). Enrichment could also be a means of overcoming learned nonuse (69). Plasticity may also be occurring through changes in cortical representation or brain stem nuclei (70).

There is a problem in comparing different environmental enrichment studies because of the lack of standardization. Some enriched environments are quite simple, consisting of just 2 items (71), whereas others are superenriched with multiple levels and numerous objects such as toys, nesting material, tubes, ropes, exercise equipment, and other items (61,72). Differences in the degree of enrichment may explain why our findings and others (33,36) differ from the results of Erschbamer et al (51). Enrichment may include multimodal stimulation (olfactory, auditory, visual, and motor) (73,74), rehabilitative training (62), and/or seminatural environment (75). Increasing the complexity of the enriched environment may enhance the benefit. Greater biochemical and anatomic changes are observed in rats exposed to a superenriched as opposed to an enriched environment (61). In a model of traumatic brain injury, superenrich-

ment produced more functional improvement than normal enrichment and smaller lesion volumes (73,74).

Numerous studies suggest that certain transplanted cells or tissue, matrix material, or drugs are effective only in combination with enrichment with little or no effectiveness with either experimental treatment alone. Reduced thalamic atrophy occurred after brain infarction only when neocortical transplants were combined with an enriched environment, and no effect was seen with the transplant or enrichment alone (76). There was a similar finding where only the combination of cell suspensions and enrichment was effective in aspirative brain lesions but not in either intervention alone (77,78). In other studies, enrichment worked as well as the grafts (46) or caused better survival of grafted cells (79). There is a suggestion that a similar effect might be occurring using a matrix treatment. Neurogel in combination with enrichment was effective in chronic severe SCI, but neither treatment alone was effective (80). It may be more difficult for the severely injured rats to interact with novel objects, thus making the enrichment alone ineffective, or the severely injured rats may lack the sufficient anatomical substrate for improvement. In terms of pharmacologic tests, the drug selegiline is only effective after focal brain ischemia when combined with an enriched environment (81). In other cases, environmental enrichment negates the effectiveness of a drug (20).

## CONCLUSION

It is obviously necessary to conduct experimental animal trials in a manner that does not obscure benefits of potential therapies. This conclusion has 2 major implications: (a) standardized enriched housing needs to be developed for experimental animals; and (b) every effort must be made in patient rehabilitation to create an enriched environment that stimulates plasticity and aids in recovery.

These studies may also have implications in the rehabilitation of people with chronically injured spinal cords. It is clear from these studies that functional improvement can occur given appropriate stimulation such as an enriched environment. With enrichment, rats are exposed to a social setting with many new objects, including exercise equipment, and are not forced to interact in any particular manner. There may be substantial benefit from a rehabilitation program in a stimulating environment as opposed to a standard institutional setting. Interesting objects, pictures, sounds, lighting, and smells that are frequently being changed may stimulate recovery. Series of the same repetitive exercises may need to be modified to provide something more interesting. The involvement of the patient in making some choices in the rehabilitative plan may also be vital. Rehabilitation programs may also be improved with group therapy that encourages social interactions. In conclusion, the potential benefits of modifying SCI

rehabilitation with group therapy in a stimulating environment may be worth exploring in a clinical trial.

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