

Model of Recovery of Locomotor Ability After Sensorimotor Cortex Injury in Rats

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Abstract

Animal models of locomotor recovery after brain injury provide tools for understanding the basic neurobiological processes that may underlie recovery after stroke in humans. Measurement of the ability of rats to traverse a narrow elevated beam has proven to be a particularly useful test of locomotor function. Repeated measurement of this behavior over time provides a simple method for quantifying the rate and degree of a rat's locomotor recovery after sensorimotor cortex injury and constitutes a tool for studying its mechanisms and possible treatment strategies. The model has proven particularly useful in predicting the effects of drugs on poststroke recovery in humans.

Key Words: brain injury; locomotion; rats; recovery; stroke; trauma

Motor impairments are a major determinant of dependence in activities of daily living following stroke in humans (Lincoln et al. 1989). Locomotor impairments can be particularly devastating. As a result, poststroke rating scales such as the commonly used Rankin Index are heavily weighted to measure ambulatory ability (Rankin 1957). However, it has long been recognized that despite the severity of their initial deficits, many stroke patients recover at least some motor function (Duncan et al. 1992, 1994). Maximal enhancement of functional motor ability is one of the goals of poststroke physiotherapy and other novel interventions. Viable animal models of locomotor recovery after brain injury are of critical importance in that they provide tools for understanding the basic neurobiological processes that may underlie recovery after stroke in humans. In addition, they provide a way of testing putative therapies intended to improve poststroke outcome. Rodent models have proven particularly useful for both purposes.

A variety of tests may be used to assess postbrain injury sensorimotor functions in rodents such as paw print analysis, rotor rod performance, incline plane, grid navigation, analysis of limb use asymmetries, and an elevated peg task (Hruska et al. 1979; Markgraf et al. 1992; Soblosky et al. 1997; Watson and McElligott 1984; Yonemori et al. 1998). The use of a battery of several of these types of tasks can provide a comprehensive assessment of lesion-related deficits. However, measurement of the ability of rats to traverse a narrow elevated beam has proven to be a particularly useful test of locomotor function. A focal, unilateral hindlimb sensorimotor cortex lesion in the rat does not typically cause a significant functional locomotor deficit when the animals are observed walking on a flat surface. Although a lesion in this location may cause changes in toe position and stride, these types of deficits are generally subtle (Gentile et al. 1978). However, the gait deficit becomes obvious when the animals are required to traverse a narrow walkway (Buytendijk 1932; Gentile et al. 1978; Maier 1935), an ability that would have important survival benefit in the wild. Repeated measurement of this behavior over time provides a simple method for quantifying the rate and degree of a rat's locomotor recovery after sensorimotor cortex injury and constitutes a tool for studying its mechanisms and possible treatment strategies. The model has proven particularly useful in predicting the effects of drugs on poststroke recovery in humans (Goldstein 2000).

Methods

Animals

Rats should be maintained in a vivarium with a 12-hr light-dark cycle and controlled temperature and humidity. They may be housed singly or several per cage and in either standard or "enriched" environments depending on the requirements of the experiment. However, housing conditions should be consistent within an experiment unless this condition represents a variable under study. Food and water deprivation is not required and is provided ad libitum. Male Sprague-Dawley rats weighing 250 to 300 g (ages ranging from 51 to 66 days) have been the most commonly employed; however, younger rats (weighing 176 to 200 g (Goldstein and Davis 1990a,b), older rats (e.g., weighing 290-400 g, ages ranging from 60 to 120 days [Held et al. 1985; Sutton and Feeney 1992]), and females (Goldstein and Bullman 1999) may also be used.

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Behavioral Testing

Testing Environment

Behavioral testing is carried out in a soundproof room with subdued lighting (less than 10.76 lumens/m², ambient light). Temperature and humidity should be the same as the housing environment. Ambient noise should be kept to a minimum.

Walkway

The apparatus is constructed by elevating the surface of a 2.5 × 122 cm wooden beam 75 cm above the floor with wooden supports. A 20 × 25 × 24 cm goal box with a 9.5-cm opening is located at one end of the beam.

Activating/Avoidance Stimulus

A switch-activated source of bright light (75-watt Tungsten bulb) and white noise (Columbus Instruments International, Columbus, Oh; model 41-A1) (or white noise generator producing 41 dB at 8000 Hz, 58 dB at 4000 Hz, 56 dB at 2000 Hz, 56 dB at 1000 Hz, 58 dB at 500 Hz, and 52 dB at 250 Hz sound pressure level [SPL¹] at the center of the frequency at each octave band) are located at the start-end of the beam and serve as activating/avoidance stimuli. The light intensity is 6.46 × 10³ lumens/m² at the start-end of the beam, 0.45 × 10³ lumens/m² at the center, and 0.21 × 10³ lumens/m² at the goal-end of the beam (measured with a General Electric® type 214 foot-candle meter). The noise stimulus is 80 dB SPL at the start-end and 78 dB SPL at the goal-end of the beam (overall, linear scale measured with a Bruel & Kjaer® type 2203 sound level meter with associated 1613 octave filter set) (Goldstein 1993a). Although the parameters of the activation/avoidance stimulus need not precisely duplicate these characteristics, it is important for each laboratory to standardize internally the methods that are employed. Once rats are trained, the activating/avoidance stimuli are generally not required. Food and water deprivation is not necessary for either training or testing.

Behavioral Training

Behavioral training should be carried out at the same time each day. Rats are allowed to acclimate to the testing room for 1 to 2 hr before each day's training trials. For each training trial, the rat is placed on the beam facing the goal box. If the rat does not begin to traverse the walkway after 10 sec, the light and noise stimuli are activated and continued until the rat enters the goal box or for a total of 80 sec. The rat is allowed to remain in the goal box for 15 sec and

is then returned to its home cage. If the rat does not traverse the beam during the trial, it is placed in the goal box for 15 sec after the termination of the trial. On the first day of training, each rat is given a series of three consecutive approximate trials. For the first approximate trial, the rat is placed 5 cm in front of the goal box. For the second trial, it is placed at the midpoint of the beam with its nose 60 cm from the goal box. For the final approximate trial, the rat is placed at the start end of the beam with its nose 10 to 15 cm from the source of light. Subsequent training consists of one trial on the beam each day until the rat completes the task on 2 consecutive days (Goldstein 1993a). Rats are readily trained to perform the task (generally less than 1% of rats do not achieve training criteria within the first 2 days of training).

Cortex Lesion

The type of cortex injury may be varied depending on the purpose of the experiment. The hindlimb sensorimotor representation in rats is localized to the mesial region of the parietal cortex (Hall and Lindholm 1974). Because of its vascular supply, it is difficult to produce arterial infarction of this area in rats (Salo and Feeney 1987). Experimentally, the hindlimb sensorimotor cortex has most commonly been injured by trauma (Prasad et al. 1995) or suction ablation-type methods. The suction-ablation lesion has several advantages for investigations focused on postbrain injury recovery: (1) it permits highly reproducible lesions of the hindlimb sensorimotor cortex (Goldstein 1993b); (2) because the lesion is the result of actual removal of brain tissue, pathological sequelae such as inflammatory responses and gliosis are limited (Pearlson and Robinson 1981; Szele et al. 1995); (3) lesion size and the accompanying behavioral deficit are maximal at onset; and (4) secondary processes associated with other types of injuries such as ischemia (Pulsinelli et al. 1982; Saunders et al. 1995), concussive trauma (Povlishock et al. 1992), and electrolytic lesions (Szele et al. 1995) are minimized. Thus, by using a suction-ablation lesion, both the size of the lesion and the initial motor deficit can be controlled and those processes related to recovery relatively isolated.

For suction-ablation lesions, the surgical area is first prepared with a sterile drape. The rats are weighed and then anesthetized with sodium pentobarbital (50 mg/kg i.p.). Additional doses of anesthetic are administered as necessary to maintain anesthesia. Other types of anesthetic agents may also be used. Scalp hair is removed, and the rats are then positioned in a stereotactic apparatus. The incision site is swabbed with Betadine, and a midline skin incision is then made from just caudal to the eyes to 5 to 10 mm caudal to the ears. The periosteum is deflected, and a craniotomy is performed from 2 mm rostral to the coronal suture to 2 mm rostral to the lambdoid suture and from 1 mm lateral to the sagittal suture to the temporal ridge. The dura is then deflected, and the cortex underlying the craniotomy site is

¹Abbreviation used in this article: SPL, sound pressure level.

removed by gentle suction (200-250 mmHg) through a fine glass Pasteur pipet until the underlying white matter is visualized. The skin incision is closed with surgical staples, and the rat is returned to its home cage to recover from the procedure. The rats generally recover quickly and, as indicated above, ambulate without difficulty in their home cages. The procedure is not associated with significant weight loss, and postoperative antibiotics are not necessary. In addition, because pain is minimal, postoperative analgesics are not required (Feeney 1987).

Postlesion Behavioral Testing

An observer blind to the rats' surgical and/or treatment status performs all behavioral assessments using a standardized rating scale (Table 1). For each trial, the rat is placed at the start end of the beam facing the goal box with its nose 10 to 15 cm from the source of light. If the rat does not begin to traverse the beam after 10 sec, the light and noise stimuli are activated and continued until the rat enters the goal box for a total of 80 sec. If the rat traverses the walkway, it is allowed to remain in the goal box for 15 sec and is then returned to its home cage. If the rat does not traverse the beam, it is returned to its home cage at the end of the trial (i.e., score of 1 or 2; Table 1). The rats should not be stimulated during the testing trial because stimulation alone can affect performance (Goldstein and Davis 1990a). For experiments testing the impact of pharmacological agents on recovery, the rats are first tested with a single trial on the beam to assess the initial comparability of the behavioral deficit between groups. The agent of interest is then administered and the effect on recovery measured by subsequent testing.

Ratings of locomotor performance using this scale are quite reliable and focus on the rat's use of the hindpaw contralateral to the cortex injury. For example, Feeney and colleagues found 98% agreement between two observers (Feeney et al. 1982). The disagreements never involved more than one category. Using a slightly modified version

of Feeney's scale (Table 1), we measured interobserver agreement among three pairs of observers with weighted kappa scores. The kappa scores were 0.93, 0.94, and 0.97 indicating "almost perfect" levels of agreement between observers (Goldstein and Davis 1990a).

Data Analysis

The results of a hypothetical experiment comparing two conditions are given in the Figure 1. The initial trial (HO) assesses whether there are comparable postsurgical deficits across groups. Locomotor recovery is measured by repeated testing on the beam over the next 12 days.

To facilitate comparisons with previous work, Figure 1 provides mean (\pm standard error of the mean) beam-walking scores for each group for each trial. To compare treatment effects across groups, the areas under the curves formed when each rat's scores are plotted against time (i.e., area under the time-effect curve) are calculated. The resulting summary data are normally distributed and can be compared with parametric techniques (e.g., *t*-tests for two groups or analysis of variance for more than two groups). However, because an ordinal scale is used to rate beam-walking performance, time point comparisons should generally be analyzed with nonparametric methods.

Discussion

With close attention to animal housing and training and testing conditions, sample sizes of 10 to 20 rats in each experimental group are generally adequate. As described above, although behavioral performance is rated on an ordinal scale, the summary measure of overall recovery provided by the calculation of the areas under the time-effect curves approximates a normal distribution. Using this type of analysis, a sample size of approximately 10 rats in each group is generally sufficient to detect a 20% difference with $\alpha = 0.05$ (two-tailed) and a power of 80% (Colton 1974).

Table 1 Beam-walking scores^a

1. The rat is unable to place the affected hindpaw on the horizontal surface of the beam.
2. The rat places the affected hindpaw on the horizontal surface of the beam and maintains balance for at least 5 sec.
3. The rat traverses the beam while dragging the affected hindpaw.
4. The rat traverses the beam and at least once places the affected hindpaw on the horizontal surface of the beam to aid its step.
5. The rat crosses the beam and places the affected hindpaw on the horizontal surface to aid more than one, but less than half its steps.
6. The rat crosses the beam and places the affected hindpaw on the horizontal surface to aid more than half its steps, but has more than two footslips.
7. The rat crosses the beam with no more than two footslips.

^aData from Goldstein LB, and Davis JN. 1990. Influence of lesion size and location on amphetamine-facilitated recovery of beam-walking in rats. *Behav Neurosci* 104:318-325. Also adapted from Feeney DM, Gonzalez A, Law WA. 1982. Amphetamine, haloperidol, and experience interact to affect the rate of recovery after motor cortex injury. *Science* 217:855-857.

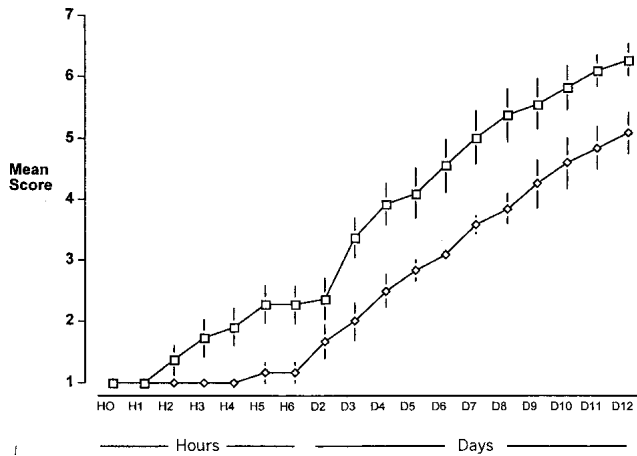


Figure 1 Hypothetical experiment comparing locomotor recovery between two conditions. “H” refers to hours and “D” refers to days. The first trial, “H0,” is given 24 hr after cortex lesion. The symbols represent the mean (\pm standard error of the mean) beam-walking scores for each trial. Motor performance is rated on a 7-point scale as given in Table 1.

Others have used water deprivation as an activating stimulus for the behavioral task (Gentile et al. 1978; Held et al. 1985; Stephens 1986a,b). However, food/water deprivation is clearly not required and may introduce an unnecessary experimental variable.

A variety of variables may influence motor performance. For example, volitional motor activity is influenced by an animal’s experience (Jones et al. 1953). In tests of beam-walking behavior, older male rats tend to walk slower and have poorer motor scores than younger animals (Brailowsky et al. 1986). These tendencies are due to a wider sustentation base in the older animals (Brailowsky et al. 1986) and may be partially related to their larger size. This size/age effect, which can confound longitudinal studies extending over a period of months, makes direct comparisons of the locomotor abilities of rats of differing ages problematic.

Both non-task-specific environmental factors and task-specific postlesion practice can influence locomotor recovery in this paradigm. Rats housed in an environment with the opportunity for extensive locomotor experience have reduced beam-walking deficits when behavioral testing is started 1 mo after surgery (Held et al. 1985). Rats housed singly in standard laboratory cages before surgery and given only postoperative environmental enrichment also have reduced initial deficits and speedier recoveries compared with rats that are housed under environmentally impoverished conditions, both before and after injury. Furthermore, preoperatively impoverished rats that had apparently recovered locomotor ability had abnormal topologies of hindlimb movements compared with those given preoperative environmental enrichment. Therefore, close attention to housing conditions is essential to avoid spurious conclusions.

Because locomotor recovery as measured by this task is highly dependent on pre- and postinjury experience (Feeney et al. 1982; Goldstein and Davis 1990c), training and testing conditions can also have important effects on beam-walking behavior. Increasing the number of training trials, varying the interval between the start of a trial and the activation of the aversive stimuli, varying the number of postinjury testing trials, and altering the intertrial interval can influence performance (Goldstein and Davis 1990a; Stephens 1986a,b). In addition, physical stimulation of the rat during postoperative trials can also affect the rate of locomotor recovery (Goldstein and Davis 1990a; Stephens 1986a,b). As a result, it is also critical to standardize training and testing conditions across groups.

Although qualitative comparisons between experiments are appropriate, because behavioral performance can be affected by the reviewed as well as other factors, it is critical to make direct, quantitative comparisons only within individual experiments and always to include appropriate controls. It is not appropriate to make direct quantitative comparisons between experiments carried out at different times. In this way, valid conclusions regarding the relative effects of different classes of drugs or other interventions on post-brain injury recovery can be reached.

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