

Disentangling multiple types of recovery from brain injury

T. Schallert^{1,2}, M. T. Woodlee¹, S. M. Fleming¹

Introduction

The success of the large number of interventions currently being advanced for possible treatment of sensorimotor deficits after stroke will depend on whether they can effect meaningful behavioral improvement. Recent progress in the design and implementation of functional outcome tests and in understanding the nature of behavioral changes over time after ischemic injury has been an essential part of the present surge in interest, effort, and hope in stroke research. This chapter describes in considerable detail several behavioral tests that may be useful for screening treatments for acute and long-term clinical potential. It is not enough for a behavioral test to simply be sensitive to ischemic injury; it should also chronically detect treatment-related sparing of tissue or beneficial enhancement of neuroplasticity that would, in clinical trials, recognizably augment performance in people who have suffered a stroke. This requires that the tests specifically target the impairment rather than global sensorimotor function. There is no shortage of tests that have been used to evaluate treatment effects in focal ischemic injury, but not all are equally useful or without serious problems in the interpretation of the clinical significance of their results (see reviews by Devries et al., 2001; Corbett and Nurse, 1998; Schallert et al., 2000; Aronowski et al., 1996).

Processes that potentially affect recovery of function

In the long aftermath of brain injury, one of the most under-appreciated issues with which the stroke model investigator must contend is that there may be different types of processes that partially overlap in time and interact to promote or impede recovery (Schallert et al., 2000a, 2003; Finger and Stein, 1982). For example,

1. **Brain repair.** Besides neuroprotection or replacement of lost tissue, the holy grail of stroke treatment is repairing the injury. Brain repair mechanisms certainly contribute to improved outcome, and these should continue to be identified and amplified with endogenous and exogenous treatment strategies. For example, denervation and injury can alter trophic factor expression, sprouting, synaptic remodeling, mitotic activity, gene expression, receptor sensitivity, neural-glia interactions, cell proliferation, and migration.

¹Institute for Neuroscience, University of Texas at Austin, Austin, TX 78712

²Dept of Neurosurgery, University of Michigan, Ann Arbor, MI
e-mail: Tschallert@mail.utexas.edu;
Tschall@umich.edu

2. **Secondary degeneration.** Subacute and long lasting degenerative events may counteract brain repair mechanisms. Degenerative pathways should continue to be investigated so that treatments can be found to halt their progression. Treatments that might exaggerate degeneration should be avoided. It is not easy to establish the relative influence of brain repair and degenerative processes on functional outcome, as they often counteract each other.
3. **Learned compensation.** When an animal sustains an injury that impairs some function, it can readily learn to cope using compensatory motor strategies, or tricks, of the type that might develop if an animal simply had a peripheral injury such as an accidental limb amputation (LeVere, 1988; Jones and Schallert, 1994; Schallert et al., 2000a). These should be unmasked experimentally if possible because they can obscure the functional status of the primary deficit. Brain injury appears to create particularly rich neurotrophic conditions that permit synaptogenesis and other neural and glial structural changes which promote motor compensation associated with remote structures. Indeed, the functional capacity of non-impaired systems may even surpass that found in sham operated animals with equal extent of training (Bury and Jones, 2002). Also, learned motor strategies are often quite subtle, potentially leading investigators to attribute drug related improvements in function to brain repair. Moreover, the animal being modeled might not adopt totally optimal motor strategies without the aid of impairment-directed rehabilitation therapies and drug interventions that promote motor learning, whereas people with brain injury may be highly motivated to adapt as well as possible to their impairment and thus may not see as much relative benefit from such drugs in standard outcome scales. Thus, a drug that works in animal models, even with concurrent motor rehabilitation, might not show a detectable effect in people who, without the help of drugs, often try to maximize activities of daily living using whatever tricks they can, and who typically receive impairment-specific rehabilitation therapy well before adjunctive drugs are introduced. Only a fraction of patients who already receive motor rehabilitative therapy might show a boost from a drug that works, for example, primarily through its effect on motor learning, memory consolidation, attention, or fatigue.
4. **Mechanisms underlying learning and memory.** After a severe stroke, procedural learning mechanisms needed to cope with a deficit may not work well. The pattern in which mechanisms normally used for motor learning are traumatized would contribute to recovery in a unique way. Theoretically, as problems with these mechanisms resolve, more extensive and efficient learning strategies would be possible. In other words, animals would become more capable of learning new tricks to improve performance. It is important to keep in mind that recovery of the capacity to learn novel strategies for performance in a task, and of the incentive to learn, are independent of, and would precede and overlap with, the learning process itself.
5. **Transient, nonfatal disruption of tissue.** Diaschisis (Feeney, 1991; von Monakow, 1914), edema (Hua et al., 2002), spreading depression (Vinogradova et al., 1991), reduced blood flow, impaired metabolic activity (Schallert and Hernandez, 1998; Gilman et al., 1987; Hovda et al., 1991; Cooper and Thurlow, 1984; Deuel and Collins, 1984; Colle et al., 1986) or related events may transiently depress brain tissue that is not killed. If this tissue is required for residual function, these events may contribute in a major way to the initial behavioral impairment. As these brain regions come back on line, behavioral improvement may be evident but the underlying mechanism of this improvement would not necessarily involve brain repair, new motor strategies, motor learning capacity, or termination of degenerative events. The extent to which dissipation of diaschisis versus brain repair mechanisms might contribute to improvements in motor learning capacity remains unclear.

6. **Experience and activity.** When a treatment affects behavior, the consequence of the behavioral change per se might impact mechanisms of recovery, e.g., by increasing beneficial neurotrophic factor expression. Injury related or forced behavioral changes can alter neuronal growth and synaptogenesis (Jones and Schallert, 1994; Jones et al., 1996). Likewise, drug or graft treatments that might otherwise improve function might be without effect unless functional rehabilitation is co-administered (Feeney, 1991; Goldstein, 1998; Dobrossy and Dunnett, 2001). Also, forced motor rehabilitation, if too early and intense, may lead to undesirable neurodegeneration of non-fatally compromised but vulnerable tissue that may partially mask the beneficial effects of training (Schallert et al., 2000a; Kozlowski et al., 1996).
7. **Learned non-use.** After injury, animals often fail to use functions that are possible but are difficult or fatiguing to carry out (Taub et al., 2002; Ogden and Franz, 1917; Nudo et al., 2001). Compensatory strategies described above (#3) may permit or even encourage learned non-use by providing alternative motor strategies. Forcing an impaired animal to behave to its maximum capacity with deficit-specific motor therapy has been one useful way to examine the occurrence of learned non-use. However, as noted above, if motor behavior is forced too aggressively early after the injury this can lead in some cases to undesirable neurodegeneration of non-fatally compromised but vulnerable tissue, which might counteract the potential benefits of training (Kozlowski et al., 1996; Bland et al., 2001; Risedal et al., 1999; Farrell et al., 2001).

It is often stated that it might not matter whether a treatment enhances motor compensation (new tricks), the capacity to learn, or neural plasticity that can reverse the deficit itself via brain repair mechanisms or cellular replacement. Rather, it is argued that if the animal gets better, that is the bottom line. For example, if a drug improves the outcome in a rat or a mouse, it might therefore help the human stroke patient as well,

no matter how it does so. However, human stroke patients, their families, and their neurologists are not likely to be fully unaware of the difference between true restoration of function and non-recovery that requires learned tricks. A treatment that is neuroprotective and/or truly restores normal function would be ideal and presumably would be most robustly detectable in clinical trials, assuming that the functional outcome tests used in those trials reflect the function of the tissue that was protected, repaired or replaced (an assumption rarely met, it should be pointed out; see Pineiro et al., 2000). Interventions that only target motor learning may be quite helpful, of course, especially if both the intact and ipsilesional hemispheres are included (Schallert et al., 2003). But, as noted above, it may be that a highly motivated human patient is much less likely to have room for learning-related improvement than an animal whose exposure to the drug co-occurs with its first exposure to performance testing.

A desirable goal is to find ways to enhance all potential avenues for recovery. Functional improvements associated with a treatment that is designed to fix the brain or halt degeneration can be difficult to evaluate when motor learning capacity is recovering at the same time (LeVere, 1988; Jones and Schallert, 1994; Bury and Jones, 2002; see also Robertson and Murre, 1999). If an objective of preclinical research is, for example, to make a stroked upper extremity work better than it otherwise would without treatment, then a battery of functional outcome tests should be designed that can distinguish among all the processes that affect outcome so that the results best reflect the mechanisms mediating the treatment. The test battery described below is not offered as the final solution to disentangling the independent role of the various processes that contribute to functional outcome. The battery includes tests that are reliable, easy to carry out, sensitive to MCAO and other types of injury, and hopefully will help investigators begin to explore how their treatments might be working. Test development is an area in preclinical cerebrovascular research that must remain active, along with other methods, for years to come.

Mouse models. For the most part, the tests that are described below have been developed using rats. However, some of them are adaptable to mice. In particular, the corner turn and adhesive removal tests have been shown to work very well in mouse models (Zhang et al., 2002; Schallert et al., 2003, unpublished data). The sensory asymmetry test can be modified by placing pieces of tape directly on the mouse's feet, which requires rotation of the forelimb for tape removal, a movement that is chronically impaired when the striatum is damaged (Whishaw, 2000). Limb use asymmetry in the cylinder test (using a smaller cylinder, naturally) can be useful early after MCAo in mice if the impairment is sufficiently severe (Schallert, Bland, and Chopp, 2002, unpublished data). We are working now on adapting the forelimb placing test to mice as well. Finally, the ledged tapered beam test has not yet been examined in mice but, with the use of a smaller beam, there is no reason to expect that it would not be useful in that species as well. Note, however, that mice are lightweight, very active, and spend a great amount of time climbing and hanging from the bars of the cage top. Self-regulated enrichment may play a role in the recovery process. Moreover, it remains to be determined whether mice can survive long enough after a loss of striatal and cortical tissue following MCAo comparable to that sustained by rats.

Behavioral tests

The ledged/tapered beam walking test

This test involves scoring foot faults (slips) during traverse of a specially constructed beam. The length of beam used in our experiments is typically 165 cm long and is tapered as shown in Fig. 1. The beam is marked so that the location of the fault can be noted for each limb, with three 45 cm bins of different difficulty along the tapered (scored) section, and untapered 15 cm "loading" and "unloading" sections at the beginning and end of the beam which are not scored. A dark box or the home cage, placed at the end of the beam, can be used

as a reinforcer as long as the animals are allowed to remain there for a few moments rather than being picked up immediately for another trial. The most important feature is that along each side of the beam there are 2 cm wide ledges, located 2 cm below the upper surface of the beam, which allow the animal to place an impaired forelimb or hindlimb off the beam so that it does not fall.

Unlesioned control animals readily learn to walk down the beam on the upper surface, rarely using the ledges. Pretraining the animals to walk spontaneously down the beam requires only about 5 days of training, with 5 trials per day (other regimens, of course, can be implemented satisfactorily). The training begins by placing the animal near the end of the beam and occasionally touching the tail or sides of the animal to encourage it to walk forward without lateral movement. On subsequent trials the animal is placed ever further toward the wide end of the beam. Again, it is important to let the animal stay in the goal area for a while after each trial. Otherwise it learns that getting to the end is without sufficient reward. We often leave sunflower treats for the animals in the goal area as well.

Ledges (2 cm wide) provide a crutch for the animal to use when there is a deficit. An animal with a contralesional limb deficit will use the ledge for weight bearing steps on the same side of the beam as the deficit, apparently as a crutch (see drawing in Fig. 1). Without the ledge crutch, the animal would be forced to alter its posture and weight distribution so that it relies on the non-impaired limbs. Such compensatory behaviors mask the deficit and thus hide from the experimenter any improvements in outcome associated with brain repair mechanisms or treatments. Depending on the extent of the brain damage, a rat with unilateral ischemic sensorimotor cortex or striatal injury typically uses the ledge chronically. As the rat walks down the beam its impaired hindlimb steps on the ledge at the wider section of the beam more than its impaired forelimb (which may step on the ledge only in the narrow section). Meanwhile its intact limbs typically remain on the upper surface of the beam.

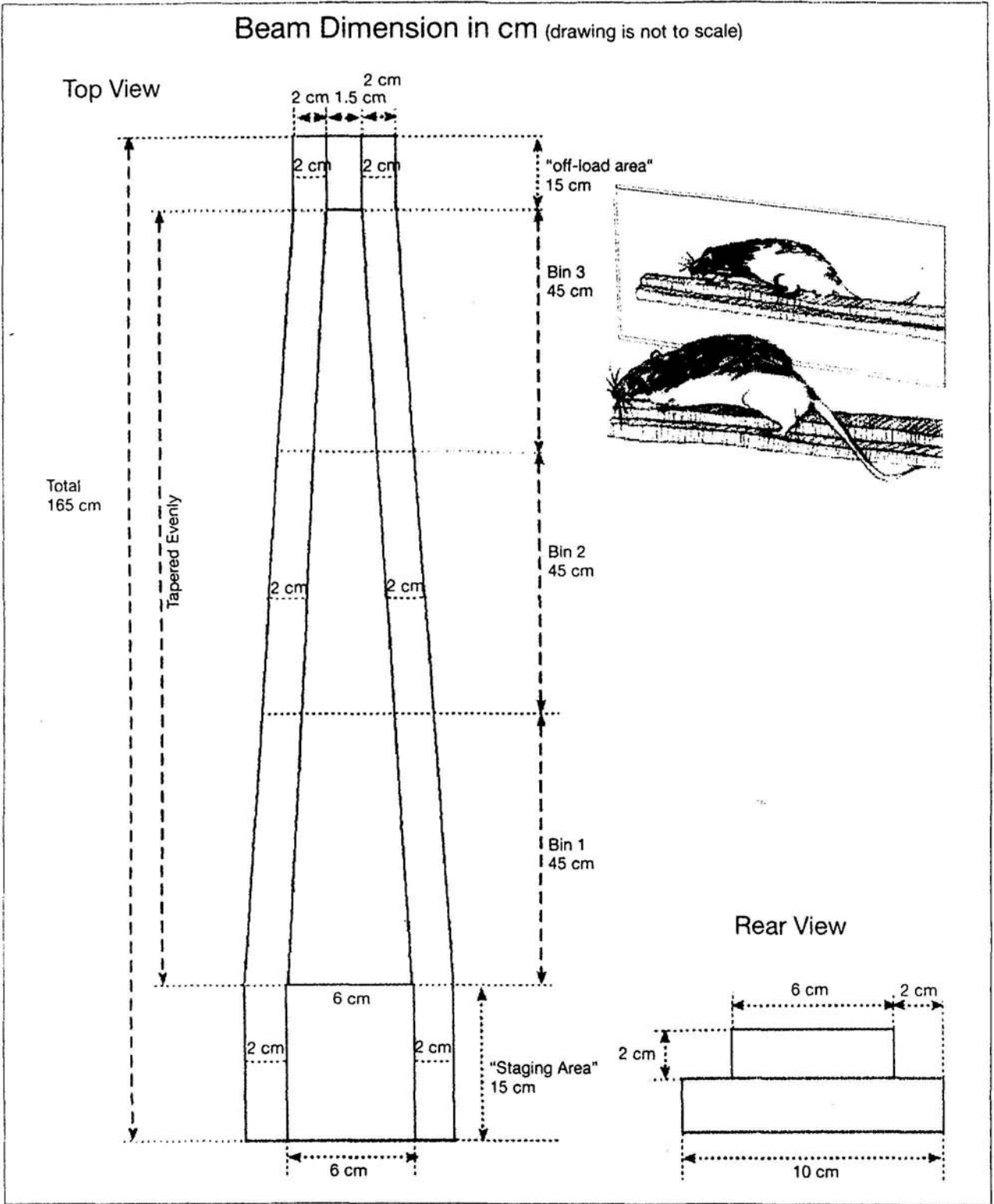


Fig. 1. Dimensions of the ledged tapered beam and drawing of rat displaying a left (but not right) full hindlimb fault on the beam. Upper right: The left (impaired) hindlimb steps on ledge. The mirror image shows that the right (non-impaired) hindlimb steps on the upper surface of the beam and not on the ledge.

On a standard beam without ledges, the typical animal with a unilateral motor cortex injury misplaces the contralateral hindlimb with

increasing frequency as the beam narrows, which causes the animal to either fall or hang clinging to the beam. The animal must learn how to

negotiate the beam skillfully without slipping. Extensive practice speeds the rate of recovery, suggesting that learning is important. Recovery of beam walking likely involves a number of co-occurring processes, including brain repair, easing of diaschisis, and recovery of neural mechanisms that mediate motor learning. However, a major component of recovery is the learning of movements that compensate successfully for chronic impairments, particularly including novel use of the non-impaired limbs, tail deviations toward the impaired limbs to provide the balance needed for leaning on the non-impaired limbs, and other weight bearing adjustments to keep all four limbs on the beam. These behaviors make it difficult to assess how much of recovery is due to restorative brain repair or other mechanisms versus motor learning (practice) effects. Adding the ledges appears to at least partially reduce the need for motor learning, thus revealing the impairment and the extent of other brain recovery mechanisms or treatment effects.

Data from animals with MCAo ($n = 18$) tested at 430 days after ischemic injury are

shown in Fig. 2. These data indicate that this test is reliable for chronic measurement of hindlimb function. Half the animals received motor rehabilitative therapy. The therapy consisted of simply housing them for one week in a modified home cage that had a random grid surface for a floor (Schallert et al., 2000b). The openings in the grid forced the animals to learn to prevent their limbs from slipping through grid openings of varying widths and sizes (rectangular or square openings in the grid had dimensions of 2 cm x 8 cm, 2 cm x 2 cm, 3 cm x 2 cm, 1 cm x 1 cm, 3 cm x 4 cm, or 4 cm x 5 cm scattered randomly in the floor). This therapy is intense in terms of time spent on the task, but is rather specific to limb placing. We have shown that this grid walking therapy has a dramatic beneficial effect on outcome in a ledged grid walking test. The grid used in the assessment had a platform 1 cm below the entire surface of the grid, which, like the tapered beam with ledges, allowed the animal to place the impaired limb down through the grid without slipping. This feature allowed for long-term assessment of impairment despite repeated testing (Schallert

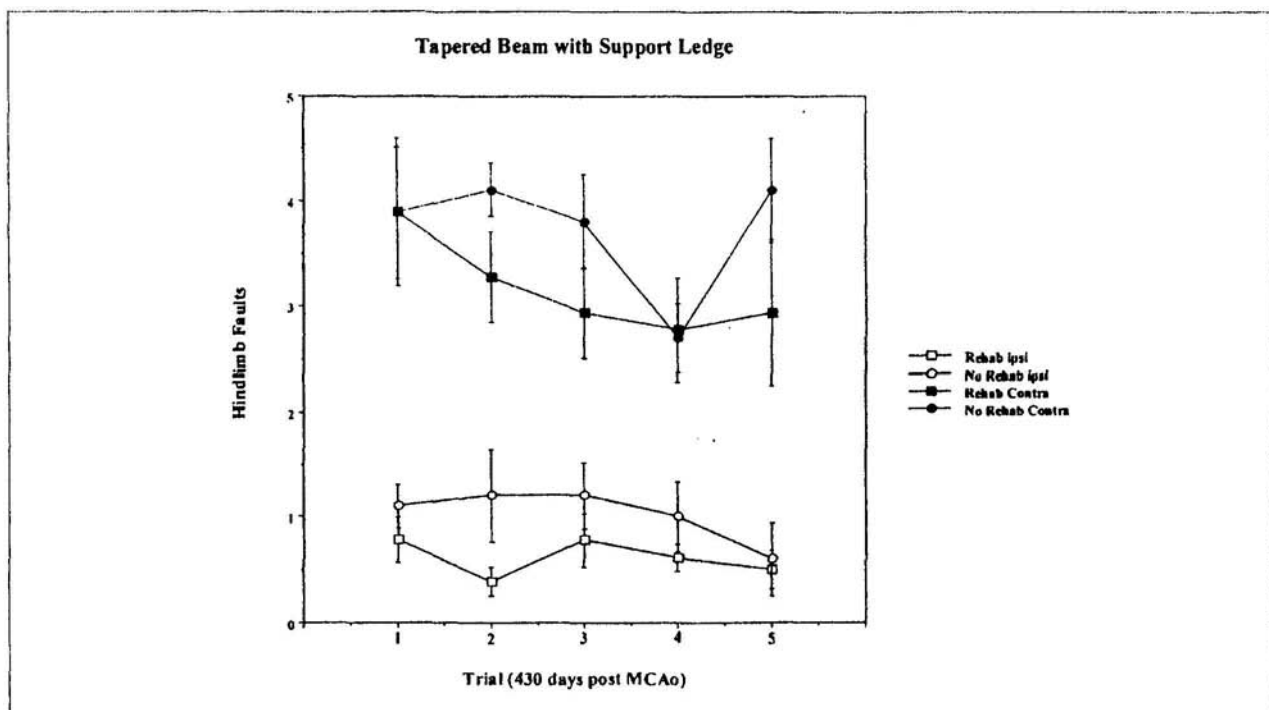


Fig. 2. Hindlimb faulting on the ledged tapered beam in rats 430 days after MCAo, with or without rehabilitative training on a random grid floor.

et al., 2000b). However, we trained specifically to the grid test but did not assess how general the rehabilitative therapy might have been. As can be seen in Fig. 2, all grid-trained animals with MCAo faulted with their impaired (contralateral) hindlimbs much more frequently than with their non-impaired hindlimbs, and the rehabilitative grid training had at best only a minor effect at this late period. It is possible that training earlier after the injury and/or cellular intervention might have improved outcome, but these data together suggest that motor training to the grid did not generalize to the ledged tapered beam. This finding may have implications for rehabilitative therapy trials in which the patients are trained to the tests being used to evaluate the effects of therapy.

The advantage of the ledged tapered beam test is that the deficit displayed is chronic, easy to score and highly sensitive. The ledge provides a place to step with the impaired limbs so that the animals are not induced to compensate using alternative motor strategies (Jones and Schallert, 1992, 1994; Schallert et al., 1997; Schallert et al., 2000b). This permits more accurate evaluation of the deficit.

Scoring. Originally we counted only the number of foot faults and half faults for each limb over 5 trials across the entire beam. Fig. 2 uses this older scoring system, whereas Table 1, which assesses MCAo rats on the beam at 450 days after injury, reflects the more advanced scoring system now used for the ledged beam, as follows. The total number of steps along the tapered portion of the beam is counted, and then divided by three. Then, for each of the three bins of difficulty, the number of contralateral limb faults and the number of ipsilateral limb faults

is counted; each of these is divided by the number obtained above to give the percentage faults per step. Percent ipsi faults per step is then subtracted from percent contra faults per step to give a percent asymmetry for each of the three bins of difficulty, with higher percentages indicating a greater relative degree of faulting with the contralesional limb.

It is worth noting that even on the ledged beam, we have observed a small degree of early recovery before the deficit stabilizes. This could be due to early brain repair mechanisms, or learned motor compensation or practice effects; more investigation into what occurs during this early phase could prove interesting. Nevertheless, as can be seen in the data presented here, the deficit quickly stabilizes and significant differences in beam performance are seen chronically.

Impairment of procedural learning and its dissipation over time can be studied using this test. Once an animal's deficit stabilizes on the ledged beam, the ledge can be removed at different time points to determine the rate at which the animal learns to compensate for the lack of the ledge. If there is an early suppression of the capacity to learn but this capacity recovers later, then the animal should be slower to perform maximally when the ledge is removed early after the injury but much faster later. We have some evidence that when the ledge is removed late after the injury, the recovery is almost immediate, i.e., over a few trials within a few hours, indicating that motor learning capacity has recovered.

In summary, the novel tapered beam with ledges situated on both edges of the beam and below the upper surface encourages the animal

Table 1. Ledged tapered beam hindlimb faults in MCAo vs. control animals.

	Beam Section (bins of difficulty)		
	Wide	Medium	Narrow
Control	-3%	2%	2%
MCAo	15%	47%	68%

Values shown are percent contralateral limb faults per step minus percent ipsilateral limb faults per step. Data obtained 450 days after MCAo.

to display its deficit and allows the experimenter to detect improvement related to reduced injury or enhanced brain integrity that might be associated with cellular or pharmacological intervention strategies. More importantly, this test ensures that treatment-induced enhancement of motor outcome is not improperly attributed to brain repair *per se*.

Limb-use asymmetry (cylinder) test

The limb use asymmetry test was developed by Schallert et al. to measure forelimb use during vertical exploration, and has been used by a number of labs to evaluate impairment after MCAo, TBI, nigrostriatal dopamine depletion, and focal cortical injury (see Schallert et al., 2000, for review; Schallert and Tillerson, 2000; Tillerson et al., 2001, 2002). This section describes updated methods of analysis, and includes helpful hints that make the test easier to use.

The rats are placed in a clear Plexiglas cylinder, 20 cm in diameter and 30 cm high. The testing sessions are videotaped and forelimb usage scored blindly at a later date. A mirror is placed at an angle behind the cylinder to allow detection of limb use when the animal faces away from the camera. The cylindrical shape of the chamber encourages vertical exploration of the walls with the forelimbs, as well as landing activity. The cylinder is high enough that the animal cannot reach the top edge by rearing and wide enough to permit a 2 cm space between the tip of the snout and the base of the tail when the animal is not rearing. An advantage of this test is that inter-rater reliability is very high ($r > .95$), even with relatively inexperienced raters.

The following behaviors are scored: (a) independent use of the left or right forelimb to contact the wall of the cylinder, to initiate a weight-shifting movement, or to regain center of gravity while moving laterally in a vertical posture along the wall during a rear, and (b) simultaneous, or near-simultaneous, use of both the left and right forelimb to contact the wall of the cylinder during a rear and for lateral movements along the wall. Behavior is expressed in

terms of (1) percent use of the contralateral (impaired) forelimb, (2) percent use of the ipsilateral (non-impaired) forelimb, and (3) percent simultaneous (both) limb use, relative to the total number of ipsilateral, contralateral and simultaneous (both) limb use observations. A single score can be obtained by subtracting the percent use of the contralateral forelimb from the percent use of the ipsilateral forelimb. Using this method, a higher score indicates a greater asymmetry, with zero being the baseline for an animal that uses its limbs symmetrically (negative scores, though uncommon, are certainly possible and would indicate a preference for use of the impaired limb). Note that when calculated in this manner this asymmetry score, with no change in use of the impaired forelimb independent of the non-impaired forelimb, would be smaller if the percent use of both limbs simultaneously is increased, and larger if it is not. Thus, if the impaired forelimb is not used independent of the intact forelimb in each of two different conditions, but in one condition the impaired limb is nevertheless used frequently together with the intact forelimb, then the limb use asymmetry score in this latter case would be lower (i.e., better) than if the impaired limb were not used at all. However, a treatment that improves the percentage of instances in which both limbs are used together but does not increase the percent independent use of the impaired forelimb may be less promising as a therapeutic option in the clinic.

Preoperative asymmetries can be calculated as baseline, which may improve reliability somewhat. Consistent preoperative biases are rare and when they occur, they are small and not consistently in the left or right direction of bias.

For a given day of testing, about 20 movements involving weight-bearing contact with the walls of the cylinder should be scored, though not necessarily consecutively in one session. To reduce habituation to exploration of the walls of the cylinder, the animal can be tested in the dark part of the light/dark cycle, home cage shavings can be placed on the floor of the cylinder, the animal can be picked up briefly, put into its home cage for one second, and returned to the cylinder, odors can be waft-

ed above the cylinder opening, the lights can be turned off briefly, and different cylinders can be used in 1-2 minute intervals. Use of red light in a dark room also helps, as long as the image is sharp enough to detect use of each forelimb. Finally, testing the animals in the colony room improves exploration.

Animals with focal ischemic or other damage to the sensorimotor cortex or striatum, and rats with unilateral MCAo show acute and chronic biases toward using the intact forelimb for vertical exploration and weight shifting. This asymmetry can be reduced detectably by neurotrophic factor interventions, but use of the impaired forelimb independent of the non-impaired forelimb has not been demonstrably improved by non-neuroprotective agents that improve performance in standard motor tests (see Schallert et al., 2000 and Schallert et al., 2000b, for review).

As a final note, unpublished evidence from our lab suggests that asymmetries in vibrissae length can affect performance on the cylinder test, even in unlesioned control animals. Therefore, it is advisable to ensure that vibrissae are the same length on both sides during surgery. If one side is inadvertently clipped during surgery, the other side should be trimmed to match to ensure that the test can remain reliably sensitive to small deficits.

Sensory asymmetry tests

This test has been called the sticky tape test, the dot test, and the patch removal test. Originally we used the term "bilateral simultaneous extinction test" because it seemed analogous to human neurological tests used clinically (reviewed in Schallert et al., 1982, 1983; Schallert and Whishaw, 1984, 1985; Schallert et al., 1986; Jones and Schallert, 1992; Schallert et al., 2000). In stroke patients, simultaneous extinction is one of the most reliable chronic residual deficits that has been documented (Rose et al., 1994).

Phase 1: Detection of sensory and motor asymmetries. In this test a small adhesive patch (Avery adhesive backed labels, 1.2 cm diame-

ter or 113 m²), is placed on the radial aspect of each wrist and the time and order (L versus R) to touch the patch and then to remove it during 4 trials are recorded. Animals are first tested to indicate the presence of an asymmetry. This is done by removing the animal from the home cage and attaching adhesive stimuli to the relatively hairless distal-radial aspect of both forelimbs. The labels are attached first to either the left or the right forelimb in a random fashion. After being returned to the home cage, rats contact and remove the adhesive stimuli one at a time using their teeth. The patches are just sticky enough to stay on when the animal moves, but when the animal purposefully removes them no hair is detectable on the patch. The order and latency of stimulus contact and removal are recorded for each of 4 trials. We use the order of contact to determine the presence of a bias. Differences in latencies for contact and removal following patch application to the impaired versus non-impaired forepaws indicate the extent of the deficit. This test measures both cutaneous sensitivity and sensorimotor integration. Improvement shown on this test with different interventions will therefore indicate the efficacy of that intervention in terms of improved sensorimotor integration. Note that the animals should be well handled, experienced with adhesive attachment and removal before surgery, and tested in the home cage within the colony or in a familiar room.

As noted above, the percentage of trials for which the ipsilesional patch is contacted before the contralesional patch is the primary measure of sensory asymmetry and should be presented without regard to latencies. However, the latency to remove the patch from the impaired forelimb can be used as a secondary measure of motor function. Because the animal must pull the tape upward and hold the limb or move it downward at the same time (a double movement that typically fails to recover; see Schallert and Whishaw, 1984), the latency to remove the tape from the contralateral forelimb can be longer than the latency simply to contact the tape. One can compare the difference in latencies to contact versus remove the tape on the good limb with the latencies to contact versus

remove the tape from the bad limb. This difference, if significant, could indicate a motor impairment and is the only aspect of this test that can change with repeated testing. In contrast to the *order* of tape removal (i.e., ipsi versus contra), latencies between animals are highly variable, particularly in the latency to first contact any patch, in part because some animals immediately settle down after being placed back into the home cage whereas other animals explore for a period of time. This variability makes it difficult to detect treatment effects statistically and has little or nothing to do with sensorimotor function.

Placing very small patches on the bottom of the feet or on the ulnar aspect of the wrists rather than the radial aspect may increase the difference between the latency to contact the patch and the latency to remove the patch. The animal must rotate the limb to pull off the patch efficiently, which is a movement that is often very impaired after striatal or extensive cortical injury to forelimb regions (Whishaw and Pellis, 1990). Note that the animal may learn tricks to rotate the limbs, such as using the good limb to rotate the bad limb. Again, analysis of latencies to remove patches, rather than the order of contact, can lead to misinterpretations of sensorimotor effects if not accompanied by careful behavioral observations.

Phase 2: Determining magnitude of sensory asymmetry. The degree of somatosensory asymmetry can be assessed using an extension of the above test (Schallert et al., 1982, 1983; Schallert et al., 1986). If the animal shows either a 25% or 75% preference for removing the stimulus from the unaffected forelimb first, a fifth trial is given. All animals with a bias (removal of the stimulus from the unaffected limb first on more than 70% of the trials) are then tested to determine the magnitude of the somatosensory asymmetry.

To do this, the size of the affected limb (A) stimulus is progressively increased and the size of the unaffected limb (U) stimulus is simultaneously decreased by an equal amount (14.1 mm²). A sufficient increase in the A/U ratio causes a reversal of the original bias wherein the rat begins to respond to the stimulus placed

on the affected limb first. The A/U ratio necessary to reverse the initial bias is proportional to the degree of brain damage (Barth et al., 1990b; Schallert and Whishaw, 1984, 1985; Aronowski et al., 1996; Schallert et al., 1983). Seven levels of stimulus pairs can be used. Each animal begins at level 3. Every time the animal removes the U stimulus first, it is tested again **two** levels up (higher A/U ratio), and if the animal removes the A stimulus first, it is tested **one** level down (smaller A/U ratio). A score is given to reflect the two levels between which the animal reverses; e.g., a score of 3.5 if the animal removes the A stimulus first at level 4 and the U stimulus first at level 3.

Animals with focal damage to the forelimb area of the sensorimotor cortex or to the striatum show acute asymmetries in this task, as do animals with unilateral MCAo's (Schallert et al., 2000, for review) and rats with other types of unilateral cortical or striatal injury.

Vibrissae-evoked placing test

Forelimb placing asymmetry is scored using the vibrissae-elicited forelimb placing test. Animals are held by their torsos, allowing the forelimbs to hang free. While holding the animal, the experimenter makes gentle up and down movements in space prior to place testing, which facilitates muscle relaxation and eliminates any struggling movements. Independent testing of each forelimb is performed by brushing the respective vibrissae on the edge of a table top once per trial for 10 trials. If tension in the muscles or struggling movements are evident, a placing trial will not be attempted or counted. No more than 3 trials at a time should be given without allowing the animal to stand on the tabletop for a moment.

Intact animals place the forelimb of both sides quickly onto the countertop. Animals with unilateral damage, depending on the site of injury, will show varying degrees of impairment in limb placing ability, while still placing the unimpaired limb reliably. A score of one is given each time the rat places its forelimb on the tabletop in response to the vibrissae stimula-

tion. Percent unsuccessful placing responses are determined (number missed \times 10) for the impaired and unimpaired forelimbs. Typically, animals place successfully on 100% of trials with the unimpaired limb (Barth et al., 2000a; Schallert et al., 2000, for review; Tillerson et al., 2001,2002).

Cross-midline vibrissae -evoked placing tests

In a variation of the aforementioned test, rats can also be tested for the ability of the vibrissae on one side to elicit placing movements in the forelimb on the opposite side. When performing this test, the animal is again held by the torso and habituated to the testing situation by gentle up and down motions in space. The limb not being tested should be held gently in place against the torso by the experimenter. The animal is then turned on its side, and the downward-pointing vibrissae brushed against the counter edge. The opposing limb (which is on top, if the rat is being held properly) is then rated for percent unsuccessful placing as described above. So, for example, a rat would be held sideways with the left vibrissae pointing down, while the experimenter moved the rat towards the tabletop, keeping the left forelimb gently restrained. When held correctly, intact rats will always place the opposing (in this example, right) forelimb in this cross-midline test.

This test takes more experimenter skill to master than the "traditional" placing test, but recent unpublished evidence from our lab suggests it may be of great use in investigating cross-hemispheric recovery-related events (such as those described in Carmichael and Chesselet, 2002) or synaptogenesis in the intact cortex (Jones and Schallert, 1992, 1994; Jones et al., 1996). For example, following unilateral ischemic injury to the forelimb area of the sensorimotor cortex, the "good" (ipsilesional) vibrissae are able to elicit placing in the impaired forelimb significantly earlier than can the "bad" (contralesional) vibrissae. Too, the "bad" vibrissae can elicit placing in the unimpaired forelimb almost immediately following such injury, suggesting that vibrissae impair-

ment is not the root cause of placing deficits in this type of injury (Schallert, Woodlee, and Adkins, 2002, unpublished data). Finally, our investigations with parkinsonian rats lesioned with 6-OHDA indicate that neither form of placing challenge (direct or cross-midline) is able to make the impaired forelimb place, in contrast to the cortically injured rats.

Corner or alley turn tests

The corner test was recently developed by Schallert using mice and rats from the Chopp lab. This test is a variation on a narrow alley test which has previously been used in the assessment of frontal cortex and striatal injury (Schallert et al., 1982, 1983; Teitelbaum et al., 1983; Barth et al., 1990b). In the test, a mouse is placed between two boards which taper together to form a corner of 30 degrees; a small opening can be left between the two boards at the corner to encourage exploration into the corner. Upon entry into the corner, vibrissae on both sides are stimulated. The number of turns made in each direction to exit the corner is counted over ten trials (with appropriate refractory time between trials, usually about 30 seconds), and the percentage of ipsilesional versus contralesional turns are compared as a measure of the deficit. Turns are counted only if they include a full rear along either wall. Normal mice turn in either direction at random, while mice with ischemic damage to the SMC will preferentially turn toward the non-impaired side. This test has been shown to correlate well with infarct volume in chronically examined mice following middle cerebral artery occlusion, with an r value of 0.93 (Zhang et al., 2002). This correlation may have been high because the infarct involved proportionally more striatal and sensorimotor regions rather than non-motor regions. The test is also highly sensitive to relatively minor chronic deficits that may not be picked up by other tests, possibly because it is influenced near simultaneously by multiple sensory and motor functions; e.g., vibrissae sensitivity and limb use (fore- and hind-limb) and postural biases.

Inhibitory control of forelimb movement during swimming

The tests presented thus far in this chapter quantify an absence of motion or disuse in the impaired forelimb. However, another possible consequence of motor cortical damage can be a loss of appropriate motor inhibition in the contralesional limb. The swim test was developed to display and quantify this phenomenon (Stoltz et al., 1999; Kolb and Tomie, 1988). Normal rats, when swimming in a straight line, swim with their forelimbs held stationary beneath their chin while the hindlimbs do the paddling (Schapiro et al., 1970). Rats with lesions to the forelimb region of the sensorimotor cortex, though, tend to use the impaired forelimb for stroking movements, while holding the non-impaired forelimb motionless as do normal rats. Data suggest that this "disinhibition" impairment may be chronic, which is a useful feature for preclinical treatment research (Stoltz et al., 1999).

In the test, rats are trained before surgery to swim in a straight line from one end of a 120 x 43 x 50 cm tank, filled with water deep enough to prevent the rat from touching bottom, to the other end of the tank where a wire mesh platform is positioned onto which the rat can climb. During training, another rat can be placed on the platform to make the "escape" point salient to the swimming rat. Early in training, the rat may be released close to the platform, and then moved progressively further away until the rat is adept in traversing the length of the tank in a straight line towards the platform. This direct "bee-line" must be the end goal of pre-training because rats will paddle with their forelimbs during turning (which is generally observed early in the pre-training phase; Whishaw and Schallert, 1977). The water is maintained at 18 °C, as studies have shown that swimming behaviors may be disrupted at higher temperatures (Bruner and Vargas, 1994; Whishaw and Vanderwolf, 1971). After the trials, the rat can be placed in a shallow pool of 37 °C water to warm them back up before being dried and returned to the home cage.

After surgery, a well-trained rat will continue to swim straight to the escape platform when

released from the opposite end, but will generally display some paddling with the contralesional forelimb, with the amount varying depending on the location and extent of the lesion. On testing days, rats can be given five trials each, and scoring is calculated as follows:

$$\sum \left[\frac{(\text{strokes made with impaired limb}) - (\text{strokes made with non-impaired limb})}{\# \text{ of trials}} \right]$$

Trials in which the rat does not swim directly to the platform are not counted when scoring. This scoring method is advantageous in that it indicates degree of asymmetry without being affected by individual differences between rats who may spontaneously paddle with both forelimbs more often than others.

This test is an excellent indicator of chronic forelimb impairment, as it has been demonstrated that asymmetries after unilateral electrolytic lesions to the FL-SMC persist for up to eight weeks following the lesion, without significant abatement (Stoltz et al., 1999). The usefulness of this test in assessing hindlimb dysfunction has not yet been investigated. It is, however, entirely possible that some measure of time spent stroking with each hindlimb could be a good quantifier of limb use asymmetry following lesions to the hindlimb areas of the motor cortex.

Spatial navigation water task

This section describes a variant of the Morris water maze (MWM) test. The procedures are adapted from Day et al., 1999 and Day and Schallert, 1996, which is modified from Morris et al., 1982; Sutherland et al., 1982; and Whishaw et al., 1995.

Animals are handled for 10-15 minutes daily for 4 days prior to the MWM test. The water maze consists of a 7-foot diameter, 3-foot deep pool that is temperature controlled and maintained at 30 °C. Small Styrofoam beads can be used to cover the water surface, which is important for the later hidden platform tests (Lindner and Schallert, 1988). Various visual cues are

located and maintained in place around the room. A number of automated systems can be used to allow for easier systematic video recording and data collection and organization.

The points of pool entry should be randomized across days and held consistent across the rats for a given test day. Learning and memory measures should be gathered in cued and non-cued levels of the MWM. Before testing, the rats should be acclimated to the pool, swimming, and getting to and onto a cued platform.

Cued phase: The rats begin daily sessions of four 1-minute swim trials with the point of pool entry randomized as described above. At the cued level, the platform is positioned just above water and is cued visually, using a cylindrical cue with black and white stripes of the optimally detectable spatial frequency for rats as established by Prusky et al. (2000) for the strain of rats used, visible from all parts of the pool. Test sessions continue daily until the rat reaches criterion (reaching the platform within 20 seconds, for 3 or more of the 4 swim trials on each of two consecutive days), or it has completed 8 days of testing. The rats that reach criterion are given a single 30 sec probe trial (visual cue presented but no platform) on day 9 to assess memory for cued platform location. These rats then proceed to the un-cued level (see below). The rats that are not successful at the cued level (though no failures are expected) would continue testing at the cued level. Any lack of success at the cued level could be due to factors such as visual discrimination deficits, interference from pre-potent strategies (e.g., thigmotaxis-the preference to search the pool periphery), or affect-related behaviors (e.g., fear to traverse the open pool).

Un-cued phase: The rats that reach criterion at the cued level proceed to the un-cued level. This level assesses the rat's ability to shift from 1) a visual stimulus to no visual cue; and 2) the expectation of platform location in the quadrant established at the cued level. The platform location is changed to a different quadrant from the cued level described above and is presented as submerged and un-cued, and therefore hidden. The rats receive four 30-second swim trials

as described above with the point of entry randomized for each day. The rats continue until they reach criterion (as described above) or until 6 days are completed. If they reach criterion, they are presented with a 30 second probe trial on the next day.

A 30-second probe trial should be given to assess memory for the platform location (measured as percent time spent in each of 4 quadrants with the platform removed). It is important to limit the duration of the probe trial because normal rats that know the location of the platform well will often search for it only briefly in the old location, and finding it no longer there, will begin searching elsewhere in the pool, whereas impaired animals that have only marginally learned the specific location of the platform may spend a longer period of time persisting in a search area near the old location (displaying a failure to disinhibit).

Matching to place challenge: The location of the platform can also be changed every day, starting each day by examining how well the rats remember from the previous day. Each day will thus have a learning curve over four trials. This variation is more difficult for the rats to learn but is more sensitive to minor deficits in hippocampal function, for example (Whishaw et al., 1995). Distance swum should be used as the primary measure, and direct swims along an imaginary corridor can also be used.

Alternatively, a probe trial can be run at the beginning of each day, for 30 seconds, after which the platform can be placed at a new random location. To increase the rigor and sensitivity of the test for minor injury, the random locations of the platform should include the center and areas near the walls.

Summary

Ideally, functional outcome tests for stroke models are sensitive to the injury, detect chronic sparing of tissue, are not overly influenced by repeated testing, and target the impairment. We have suggested seven distinct but interactive types of recovery-influencing processes that have the potential to affect outcome. Designing

tests to measure these processes independent of one another will continue to be a major challenge, particularly if convenience is desired in order to expedite research. Long-term observations are essential because residual deficits are not easily detected or are not obvious in the acute stages of recovery. Immediately after the injury the eventual residual abnormalities may be so subtle they are overwhelmed by more extreme impairments.

We have selected for review a few tests that have some of the major features needed for preclinical utility. This battery of tests includes methods for at least partially determining whether a preclinical treatment restores the original function or simply enhances performance by means of improving learning of alternative strategies. Most investigators have concentrated on brain repair mechanisms and degenerative events that may counteract such mechanisms. An important but under-investigated type of recovery is the learning of novel compensatory strategies that masquerade as brain repair. New tests such as the ledged tapered beam test may begin to isolate and assess this type of recovery.

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