

Behavioral Tests for Preclinical Intervention Assessment

Timothy Schallert

Departments of Psychology and Neurobiology, Institute for Neuroscience, University of Texas at Austin, Austin, Texas 78712

Summary: Select functional outcome tests commonly used for evaluating sensorimotor and cognitive capacity in rodents with focal intracerebral ischemic or hemorrhagic injury are described, along with upgrades and issues of concern for translational research. An emphasis is placed on careful quantitative and qualitative assessment of acute and long-term behavioral deficits, and on avoidance of frequent pitfalls. Methods for detecting different degrees of injury and treatment-related improvements are included. Determining the true potential of an intervention requires a set of behavioral analyses that can monitor compensatory learning. In a number of preclinical outcome tests, animals can develop remarkably effective “tricks” that are difficult to detect but frequently lead to dramatic improvements in performance, particularly with repeated practice. However, some interventions may facilitate learning without promoting

brain repair, but these may not translate into a meaningful level of benefit in the clinic. Additionally, it is important to determine whether there are any preinjury functional asymmetries in order to accurately assess damage-related changes in behavior. This is illustrated by the fact that some animals have chronic endogenous asymmetries and that others, albeit infrequently, can sustain a spontaneous cerebral stroke, without any experimental induction, that can lead to chronic deficits as reflected by behavioral, imaging, and histological analyses. Finally, a useful new modification of the water maze that involves moving the platform from trial to trial within the target quadrant is reviewed, and its advantages over the standard version are discussed. **Key Words:** Stroke, degeneration, behavior, cognitive, memory, sensorimotor.

INTRODUCTION

There is an urgent desire in patients, doctors, and neuroscientists that meaningful treatments be found for the symptoms of cerebral vessel occlusion and hemorrhage. In part because of this there is a growing interest in developing predictive preclinical functional outcome tests that can be used, along with neural pathological markers, to help in translational research. It should be stated at the outset that basic research in the field has yet to provide a set of neurological tests that one can confidently use to screen for clinically efficacious treatments. A particular problem has been the disappointing progress in finding beneficial interventions in patients, which then could be back-tested in animal models to begin to determine which animal tests would have best predicted such success. In the meantime, behavioral tests should at least be able to distinguish degrees of injury to a specific area of the brain linked to the test, and to disentangle the effects of a treatment on brain repair from the effects on

learned compensatory behaviors that masquerade as neural improvement. Interventions that primarily facilitate behavioral compensation can be very misleading, and likely would be far less helpful in the clinic than tissue protection, restorative growth, or enhanced neural function. Tests that are merely sensitive to cerebral damage typical in common stroke models can be relatively easy to devise but may or may not be sufficient to screen for treatments that will prove helpful to patients.

In brain models of focal ischemic or hemorrhagic stroke, the sensorimotor cortex or striatum in one hemisphere is frequently damaged and, with severe injury, the hippocampus and related areas may be partially affected bilaterally. To investigate adequately treatment interventions that at least show clinical promise, an extensive battery of behavioral tests is likely required. At a minimum, the tests should be able to distinguish among a range of levels of injury and also should be capable of detecting beneficial effects chronically. Whenever possible, procedures should be used that are not influenced by recurrent testing. There are a large number of behavioral tests described in the literature. The goal of this paper is not to comprehensively review all the behavioral tests described in the extant literature, nor to focus on

Address correspondence and reprint requests to: Timothy Schallert, Ph.D., Department of Psychology, University of Texas at Austin, 1 University Station, #A8000, Austin, TX 78712. E-mail: tschallert@mail.utexas.edu.

possible limitations of tests that are not discussed. A select group of tests that are reliably useful are described in some detail. Video samples can be found on the lab website (www.schallertlab.org).

BILATERAL TACTILE STIMULATION TESTS

Sensory asymmetry

A common consequence of focal cortical or striatal injury in people is contralateral sensory neglect, often followed by rapid recovery of sensory attention and a long phase of residual "tactile extinction" deficits, in which sensory stimulation applied to the ipsilateral side of the body masks detection of simultaneously applied contralateral stimulation.^{1,2} Eventually, simultaneous tactile stimulation does not produce complete extinction but instead reduces the perceptual intensity of the contralateral stimulus (obscuration).

These events have been modeled in animals.³ Rats react quickly to small sticky pieces of paper placed bilaterally on the radial aspects of the wrists, or to the bottom of the forepaws.³ The animals use their mouths to contact the stimuli and their teeth to remove them one at a time. If there is unilateral injury to the striatum or forelimb region of the sensorimotor cortex, the animals respond first to the sticky paper pieces on the good forelimb before responding to ones placed on the bad forelimb. Depending on the extent of the injury, this sensorimotor asymmetry slowly diminishes with time or can be stable and chronic.

Sensory and motor function can be examined independently using different components of a two-part procedure. The sensory component can be assessed over time independent of the extent of practice. Total neglect, in which the animal fails to contact the stimulus on the affected side, is only very transient in rats. Tactile extinction has been modeled by making the stimulus on the good limb impossible for the animal to remove. During the tactile extinction phase, which can last for many weeks with severe injury, the animal fails to respond to the contralateral stimulus as long as there is simultaneous tactile input to the good side of the body. When the stimulus on the good side is removed by the experimenter, the animal immediately responds to the contralateral stimulus. After this phase, obscuration of sensory input replaces tactile extinction, so that only a much stronger stimulation of the contralateral side can neutralize the sensory asymmetry. In the obscuration phase, when stimuli that are impossible to remove are placed bilaterally to the forelimbs, animals spend more time attempting to remove the stimulus on the good limb but do frequently try to remove the contralateral stimulus, indicating that true extinction no longer is present. However, if the size of the ipsilateral stimulus is increased slightly, which imposes a large asymmetry, tactile ex-

tingtion re-emerges. The procedures for identifying the transition from tactile extinction to obscuration to recovery, potential explanations and the associated early literature have been detailed previously.³⁻⁶ The two-part procedure described below has been highly useful for assessing the presence and degree of somatosensory asymmetry and its recovery over time, which varies in rate depending on the extent of injury to sensorimotor regions.

Small, adhesive-backed labels (Avery Dennison, Pasadena, CA) are curled and attached to the relatively hairless distal-radial aspect of each forepaw.³⁻⁹ Very importantly, the rat must be put back into its home cage with the lid replaced, in the normal colony position, because any distraction by novelty will reduce the sensitivity of the test or cause disinterest in the adhesive stimuli, which are relatively less salient than changes to an animal's environment. Preoperatively the rats are very well handled before being given 5 unscored trials on five different days so that they reliably and rapidly remove the stickers. After this, the rats are given 2 days of scored testing, 4 trials each day separated by 10 minutes. Rats will contact and remove both stimuli within about 15 seconds. No less than 5 minutes between trials should be allowed. If a reliable left or right limb bias is found (on 75% to 100% of the scored trials), the injury should target the hemisphere opposite to the bias. Usually only about 3 rats of 10 show a reliable bias over both days, but this is variable and typically minor unless the animal has sustained a spontaneous stroke at some point in the past, which we have observed on occasion and confirmed using imaging, histological, and long-term neurological evaluation.

After a unilateral injury to the sensorimotor cortex or striatum, there is normally an immediate asymmetry in contacting and removing the stimuli (ipsilateral first). In such cases a second procedure (described below) can assess the magnitude of the sensory asymmetry.

The time it takes to remove the contralateral stimulus after contacting it is a separate motor component, which can partially improve with extended experience with sticker removal from that limb. Practice-related improvement in response time is completely independent of improvements in the magnitude of sensory asymmetry.³ Focused sensorimotor training and certain drugs can help shorten response time but the magnitude of sensory asymmetry is resistant to many of these interventions.

Magnitude of sensory asymmetry

Measuring the degree of sensory asymmetry requires a more elaborate but crucial procedure, which is much more sensitive to the extent and site of injury (e.g., striatal damage is associated with more severe and chronic deficits) and to treatment effects. Increasing or decreasing the size of the sticky piece of paper placed on

one forelimb affects its salience relative to the size of the one placed on the opposite forelimb. Thus, an intact rat, for example, will first respond to the larger of two stimuli. In animals with unilateral injury, once a sensory asymmetry is established, the size of the paper to be stuck on the bad forelimb is increased over trials while the size of the paper to be stuck on the good limb is reduced over trials until the animal no longer shows a bias. The bad to good limb ratio reflects the magnitude of asymmetry and is insensitive to practice effects, but highly sensitive to even very minor injury and to treatments that exaggerate or ameliorate the extent of brain damage.^{3,5,6,10-12} If the striatum is not too damaged, recovery can eventually occur, as reflected in the ratio defining the magnitude of asymmetry. Even total loss of cortical tissue on one side will, after several months, result in complete recovery if the striatum is not damaged, suggesting that residual tissue in the injured cortex is not necessary for recovery of sensory symmetry. However, the recovery is fragile. A sensory asymmetry returns if there is even a minor distraction, such as partially removing the cage top. In contrast to animals operated on as adults, rats with neonatal hemineodecortication fail to recover even after a year though they never display true tactile extinction.⁴

FORELIMB USE FOR VERTICAL-LATERAL EXPLORATION

Asymmetries in usage of the forelimb during spontaneous exploration of the walls of a cylindrical enclosure are common after unilateral cerebral ischemia or hemorrhage in rats^{7,13-18} and mice.¹⁹ Spontaneous unilateral strokes in rats can be detected using this test. A detailed analysis of the tapes of Derek Denny-Brown reveals that nonhuman primates with motor cortex damage show comparable forelimb-use asymmetries for weight support on the walls of a small enclosure. Recently, we found that forelimb usage asymmetries can be reliably detected in rats after a sublethal (short duration) middle cerebral artery occlusion (MCAO) that does not cause detectable tissue damage but is sufficient to produce ischemic tolerance to a longer period of MCAO.²⁰ This suggests that neural mechanisms associated with brain injury are extensively activated after mild, transient MCAO that causes no frank brain damage, but preconditions the brain against degeneration normally seen after a more severe vascular insult.

Rats are placed in a transparent Plexiglas cylinder with no top or bottom. The use of each forelimb for weight support or weight shifting along the walls is examined using slow motion video recordings.²¹⁻²⁴ An experienced investigator can accurately score the data live, however. The test is sensitive to long-term deficits in forelimb use that might otherwise be masked by motor

learning, and the scores do not change with repeated testing.

The size of the cylinder should vary depending on the size of the animal. When the animal is standing on all four limbs in the cylinder, the distance between the wall and the snout, and the wall and the base of the tail should be about 1 cm. This encourages vertical exploration. For a typical 300-g rat, the cylinder would be 30 cm high by 20 cm in diameter. While the animal engages in vertically, oriented movements on the wall, use of the good forelimb independent of the bad forelimb, and *vice versa*, are recorded. The number of simultaneous good and bad forelimb placements on the wall and rapidly alternating wall-stepping movements also are recorded and pooled together as "both limb" movements.

To prevent habituation to the cylinder, the number of movements recorded in any one trial should be limited to about 20. Ideal testing should occur in the first few hours of the dark part of the light-dark cycle, and under very low lighting at the level of a common night light. Visibility can be boosted by red lights, under which rats behave as though there is no additional light. This encourages exploration. Some strains (e.g., Long-Evans) are more active at all times of the day. Other strains (e.g., Sprague-Dawley) tend to be highly diurnally inactive. Limb use can be calculated using the following formula: [(ipsi + 1/2 both) divided by (ipsi + contra + both)] × 100.

VIBRISSAE-EVOKED FORELIMB PLACING (UNSKILLED REACHING FOR A STABLE SURFACE)

Forelimb placing onto the corner of a table top can be elicited by unilateral vibrissae contact.^{7,25-27} The animal is held aloft with all four limbs hanging freely. With minimal training the animal relaxes and when the vibrissae on one side contact the table corner, the limb on the same side readily moves forward to gain weight support. Unilateral injury to the sensorimotor cortex or striatum, whether imposed experimentally or spontaneously by a natural stroke, impairs placing of the contralateral forelimb in response to contralateral vibrissae contact with the table. There is no effect on placing of the ipsilateral forelimb, even when only the contralateral vibrissae are contacted to elicit the response (cross-midline placing).²⁵ This suggests that the deficit on the bad side is primarily motor, not requiring sensory neglect of vibrissae stimulation.

The test requires considerable experience on the part of the examiner. The forelimb that is not being examined is lightly restrained by placing the experimenter's finger in front of that limb. Care must be taken to avoid abrupt movements, which might induce forelimb placing due to a vestibular response. The percentage of instances in

which the rat successfully places its forepaw is scored separately for each side. The only trials scored are those in which the animal does not struggle.

Extensive damage to the striatum yields a deficit that persists indefinitely.²⁵ For example, after proximal MCAO that ablates almost all anterior–lateral striatal tissue, the contralateral forelimb fails to place in response to contralateral vibrissae stimulation even after more than a year of testing. Damage limited to the cortex after distal MCAO yields comparable placing deficits but gradual recovery occurs over weeks or months. Recovery is much faster with partial injury or with treatments that reduce the extent of injury specifically to the sensorimotor cortex. Thus, after several weeks one can diagnose whether striatal damage or cortical damage has occurred in MCAO or other ischemia or hemorrhage models, and whether the damage is mild, moderate, or severe.

Even with extensive striatal damage, contralateral vibrissae stimulation can readily elicit placing of the good forelimb within the first day or two after injury. Tactile input to vibrissae-related areas of the damaged hemisphere, or perhaps input to comparable cells in the intact hemisphere that are uncrossed, can presumably activate the motor program in the intact hemisphere required for placing of the good forelimb, but not the motor program in the damaged hemisphere that corresponds to the impaired forelimb. Recovery in the ability of the bad forelimb to respond to vibrissae stimulation on the good side gradually occurs. Presumably, tactile input to the good hemisphere can gradually begin to access the motor program in the damaged hemisphere essential to moving the bad forelimb. Dendritic growth and new synapse formation in the intact hemisphere^{28–32} are well timed to reflect the progress of this cross-midline recovery. In contrast, tactile input to the damaged hemisphere apparently remains chronically unable to activate the motor program within the same damaged hemisphere, and never recovers if the damage involves extensive striatal damage.

CORNER TURN TEST

Recently, a corner turning asymmetry test was developed that is useful in stroke models.^{13,19,33} The test is well suited for testing both mice and rats. It is one of the only tests that can detect mild injury and chronically stable deficits in mice. The test takes advantage of an animal's tendency to rear and turn when reaching a sharp angled corner. The animal is placed facing a 30-degree corner formed by 2 tall walls. The walls at the corner do not make contact (a gap of about 0.5 cm remains), which encourages the animal to move very deep into the corner and rear to turn around. Ten trials on each of two test days allows for reliable preinjury assessment of turning

asymmetry. Animals with a persistent directional bias before surgery should not be used further.

Animals with unilateral damage to any brain region linked to sensory or motor functions of the forelimb or hindlimb turn preferentially ipsilaterally. Weight shifting movements in the forelimb and hindlimb on the side opposite to which the animals turn are impaired. Even animals with sciatic nerve damage in one hindlimb will turn away from the impaired side. The sensitivity of the test to hindlimb impairment is important because stroke models often damage hindlimb-related brain areas. In instances in which the animal does not readily enter the corner and rear, the tail can be gently tapped or the corner can be slowly moved toward the animal, which encourages rearing and turning around. The percentage of trials in which the animal turns away from the affected side is assessed. After each trial, the animal is left alone for at least 30 seconds before another trial is run. If instead the animal is picked up and put back facing the corner it may begin to stop turning around after several trials. Only turns involving full rearing are included. Typically, 10 to 20 trials are carried out for each animal. The correlation between corner turn asymmetry and the extent of tissue injury (in sensorimotor cortex and striatum) is very high.

LEDGED BEAM TEST

Another test useful for hindlimb function involves limb use while walking down a narrow (1.5 cm wide) elevated beam. The beam can be fitted with a step-down ledge that serves as a "crutch" to prevent full slipping of the limb.^{34,35} Rats can be easily encouraged to walk toward a home–cage goal at the end of the beam, or to a dark familiar compartment. During training, the animal is placed on the beam near the goal, and over trials the distance between the goal and the starting place is progressively increased. The distinguishing feature of the beam is that it has an underhanging ledge (2 cm wide, positioned 2 cm below the upper beam surface). Normal animals keep all limbs on the upper beam surface as they traverse, whereas injured rats chronically use the ledge for support of the bad hindlimb. This use of the ledge for support discourages the animals from developing compensatory strategies that are difficult to detect and vary from animal to animal. For scoring, foot faults per step are scored for each hindlimb. Depending on the site and extent of damage, there can be some recovery over the first few weeks which may be attributable to neural restoration or retreat from diaschisis.

If the ledge is removed, animals with MCAO injury at first show slipping with the bad hindlimb but quickly learn to traverse the beam without foot faults by, for example, extending the tail contralaterally for balance while shifting weight support primarily to the good

limbs. This strategy masks the deficit, but if the ledge is put back along the beam the animals almost immediately begin to use it for bad limb stepping, making the impairment readily detectable once again. This test may be useful for determining the extent to which a therapeutic intervention promotes brain repair and not simply motor learning. A treatment that is more likely to be related to brain repair is one that leads to a reduction in the use of the ledge as a crutch. Treatments that only reduce limb slippage when the ledge is not available are unlikely to be more than minimally promising, although they may enhance motivation or compensatory motor adjustments, particularly during physical therapy, which could be quite beneficial. Note that forelimb function can be examined using a ledged beam, but the beam itself should be proportionally narrower and the hindlimbs suspended slightly off the beam surface by the experimenter so that the animal can walk with the forelimbs without foot faults by the hindlimb.

COGNITIVE FUNCTION: MOVING PLATFORM AND STRATEGY SWITCHING VERSIONS OF THE WATER MAZE

Deficits in learning, memory, mental flexibility, and inhibitory control are common in people who sustain focal cerebral injury. Even if an intervention shows enormous promise in a battery of sensorimotor tests in animals, cognitive function should be assessed as well. Interventions such as glutamate antagonists that can enhance sensorimotor function after brain injury³⁶ might have an adverse effect on cognitive functions.

The Morris water maze³⁷ has been used extensively to examine learning and memory deficits in stroke and many other neurological models. Damage to many different areas in the forebrain can be detected using various versions of this task. Both qualitative and quantitative changes in function can be assessed. In most learning tasks performance decrements are typically documented without understanding the nature of the deficits. If a treatment improves outcome, it is essential to know what aspect of cognitive function has been affected and its magnitude in order to design assessment measures for clinical trials. Recent modifications of existing procedures, including moving the platform from trial to trial within the target quadrant of the water tank,³⁸ provide several advantages that are discussed at the end of this section.

In 1982, two labs showed that damage to the hippocampus caused impairment in spatial memory function in the water maze.^{39,40} Rats are natural swimmers. Indeed, in the wild they will voluntarily swim long distances⁴¹ and they even have a diving bradycardia reflex, which is an oxygen and fuel conservation mechanism characteristic of animals such as whales, seals, and ducks

that hunt for food underwater.⁴² When placed in a tank of cool water with a submerged platform located in one of four quadrants, they immediately search for a way to escape.⁴³ Most animals naturally swim toward any visible structure to climb onto, so the first strategy that most normal rats adopt is to search along the wall of the tank rather than in the target quadrant. They quickly learn the futility of this strategy and begin to search the interior of the tank. Over trials the animal may go through several search strategies that progressively decrease the time required to escape. Intermediate strategies include swimming a certain distance away from the wall or crisscrossing the tank without retracing paths. After many trials the animal usually begins to use visual cues in the room to locate the hidden platform (visual acuity can vary among strains, so the cues should be very striking and visible from the water surface). Animals with damage to the hippocampus or related areas of the brain are slower to inhibit searching along the wall, and once they do, they are much slower to progress from one strategy to another. Eventually they may learn to escape rapidly by using 1 or 2 single landmarks to adjust their trajectory, but this is not the optimal strategy achieved by control animals.

Preventing less optimal strategies

Successive transitions in spatial learning in animals can be facilitated, including those with hippocampal dysfunction, by introducing certain bridging manipulations. For example, on the initial trials a large circular hidden platform that occupies nearly the entire tank can be provided, which is detected almost immediately and redirects their search away from the wall.^{38,44,45} Over trials the platform is gradually reduced in size and incrementally shifted toward the middle of the target quadrant until its size and location are typical of the standard water maze setup. This procedure eliminates ineffective strategies such as searching along the wall or at a certain distance from the wall, so that hippocampal animals rapidly acquire a spatial strategy comparable with control animals, as indicated by probe trials in which the platform is removed. However, although they spend as much time as normal animals swimming in the target quadrant during the probe trial, the trajectories of the hippocampal animals are often not as precise as that of controls. Most importantly, as they arrive at the location of the now-withdrawn platform, the hippocampal animals swim straight through the target area to the wall without stopping or even reducing speed, and must begin their search again. In contrast, control animals slow down as they arrive at the learned location⁴⁶ and begin to paddle specifically in that general area using their forelimbs to “feel” for the platform.⁴⁵ The hippocampal animals do not display these normal reactions to expectancy violation. It can be argued that damage to the

hippocampus retards or prevents them from strategy switching and perhaps from having a “declarative” memory-like understanding of platform location,^{45–48} but does not abolish the capacity to use distal cues to find a hidden platform located in a fixed position.

Once spatial learning plateaus, if the platform is then relocated to random locations along the perimeter of the pool, control animals, but not hippocampal animals, display remarkable pliancy.⁴⁵ Normal animals readily abandon their search within the target quadrant and adopt the newly optimal nonspatial strategy of swimming only along the perimeter of the tank without changing directions. Ironically, in rats with ischemic injury or damage to the hippocampus, a wall-swimming strategy, which they have so much trouble giving up in the early trials of the standard water maze procedure, now comes with extreme difficulty.^{38,45} Instead, they continue to search in the middle of the former target quadrant for many more trials. Animals with mild focal cerebral ischemia behave similarly to rats with partial hippocampal damage.³⁸

Moving the platform within the target quadrant

A simple, but in our lab, a very useful modification of the standard water maze procedure is to change the location of the hidden platform randomly within a fixed target quadrant from trial to trial.³⁸ On one trial, for example, the platform would be positioned equidistant from the center and edge of the pool, but on other trials it is moved to different locations against the wall, close to the center of the pool, and near the other quadrants but at varying distances from the wall, all within the confines of the target quadrant. This method allows one to probe spatial memory early and continuously as the animals learn. In addition, the animals are unlikely to adopt an intermediate strategy of looping around the tank at a specific distance away from the wall, a response that gets them to a *fixed* platform quite rapidly without the use of distal visual cues, and which does not require the hippocampus.

In the moving platform version, if the platform is removed (as in a standard probe trial, which is a consensus best measure of spatial memory presence)^{40,49} the animals do not panic because they do not expect to find the platform in a given location. Moving the platform from trial to trial also improves the utility of standard probe trials, and allows one to run multiple probe trials even early in acquisition without a negative influence because the animals will have had extensive experience with searching in the target quadrant without finding the platform. Even animals with mild distal occlusion of the MCAO, which show no more than a transient deficit in the standard water maze, display deficits in probe trials after learning under the moving platform procedure.

One could argue that a probe trial at the end of acquisition may not be needed as much in the moving platform

version because the longer searches provide daily assessment comparable to a short probe trial in a fixed platform procedure. Note, however, that in the moving platform version of the water task, removing the platform from the tank to probe the level of memory retention greatly enhances the time and distance spent in the target quadrant, at least in intact animals. In contrast, there are two major concerns, widely discussed, associated with running probe trials when the platform is always in a fixed location:

- 1) The animals that have the best retention and awareness know instantly that the platform is no longer in its usual place and may more quickly leave the area and begin to search in other quadrants, or they may panic. In contrast, dysfunctional animals may persevere in their search within the target quadrant, which may increase the time spent and distance traveled within the target quadrant, thus giving the false appearance of better retention.^{50,51}
- 2) A probe trial introduces confounding information with the potential for adversely influencing the performance of an animal in subsequent trials when the platform is put back into the tank at its former target location, perhaps more so in animals that have greater cognitive capacity and better memory. Memory impairment for such disinformation in brain-injured animals could be, paradoxically, an asset for them in future probe trials. If so, this certainly would have important implications for estimating the potential of some preclinical treatment candidates.

Neither of these two concerns would be problematic in the moving platform version of the task. Others previously have developed procedures that required the animal to wait within the correct quadrant for the platform to be available.^{52,53} Moving the platform within the target quadrant across trials is less expensive and achieves much the same benefit, but without requiring the animal to interrupt its search for a set period of time.

CONCLUSION

The sensorimotor and cognitive tests described above are not the only ones that should be considered useful for assessing functional outcome after unilateral focal ischemic or hemorrhagic injury. Others, for example, include forelimb reaching ability,^{32,54–57} tongue extension capacity,⁵⁸ forelimb inhibition during swimming,^{59,60} postural asymmetry when suspended upside down,^{6,61} and grid walking.¹⁰ Meaningful beneficial treatments for stroke may require large functional improvements across a broad range of behavioral tests.

Motor enrichment therapies, which are typical for stroke patients, should be examined as possible counter-

ventions to promote brain repair and reorganization more reliably; however, the safety of some early intense motor rehabilitation interventions may be an issue of potential concern.^{15,62–66}

Considerable progress is being made in designing useful, evidence-based outcome measures for human stroke diagnosis and research. It is not easy to match deficits in rats and people, but test selection and interpretation of the nature of the deficit is critical in both if translational research is to be successful.

Treatment effects on learned behavioral compensation should not be mistaken for improvements in the integrity of the brain. If a treatment helps a patient switch from using the affected hand (or both hands) for a set of tasks (such as keyboard and mouse use) to using mainly the unaffected hand in a better-than-normal way, this is a compensatory strategy that will be readily noticed and the treatment would not be regarded as revolutionary with exceptional prescriptive potential. On the other hand, if a treatment helps a patient learn to use the unaffected limb in a subtle way, such as weight support during a bimanual task, or to use a trunk flexion response to facilitate reaching for an object, this might not be so readily detected. It is important to understand and document the contribution of learned motor compensation, which may be most prominent when eloquent regions of the brain are extensively injured.

Careful monitoring of the nature of the behavioral changes is required to evaluate treatment effects. The assessment should be long term because delayed secondary neural degeneration may cancel early beneficial effects, or the treatment itself might exaggerate late cell loss. Note, however, that histological evidence that a treatment can protect against delayed secondary degeneration does not ensure that it will be beneficial functionally. For example, many neurological deficits are caused by pathological release of neural activity due to a loss of inhibitory control. Saving the uncontrolled cells in such cases may be detrimental.⁶⁷

Acknowledgments: Thanks to Cole Husbands for editorial comments. Funded by NS19608, NS042345, HD39386, HD-02-023.

REFERENCES

- Benton AL. Jacque Loeb and the method of double stimulation. *J Hist Med Allied Sci* 1952;11:47–53.
- Benton AL, Levin HS. An experimental study of 'obscuration'. *Neurology* 1972;22:1176–1181.
- Schallert T, Whishaw IQ. Bilateral cutaneous stimulation of the somatosensory system in hemidecorticate rats. *Behav Neurosci* 1984;98:518–540.
- Schallert T, Whishaw IQ. Neonatal hemidecortication and bilateral cutaneous stimulation in rats. *Devel Psychobiol* 1985;18:501–514.
- Schallert T, Upchurch M, Lobaugh N, et al. Tactile extinction: distinguishing between sensorimotor and motor asymmetries in rats with unilateral nigrostriatal damage. *Pharmacol Biochem Behav* 1982;16:455–462.
- Schallert T, Upchurch M, Wilcox RE, Vaughn DM. Posture-independent sensorimotor analysis of inter-hemispheric receptor asymmetries in neostriatum. *Pharmacol Biochem Behav* 1983;18:753–759.
- Schallert T, Fleming SM, Leasure JL, Tillerson JL, Bland ST. CNS plasticity and assessment of forelimb sensorimotor outcome in unilateral rat models of stroke, cortical ablation, Parkinsonism, and spinal cord injury. *Neuropharmacology* 2000;39:777–787.
- Fleming SM, Delville Y, Schallert T. An intermittent, controlled-rate slow progressive degeneration model of Parkinson's disease: antiparkinson effects of Sinemet and protective effects of methylphenidate. *Behav Brain Res* 2005;156:201–213.
- Markgraf CG, Green EJ, Hurwitz BE, et al. Sensorimotor and cognitive consequences of middle cerebral artery occlusion in rats. *Brain Res* 1992;575:238–246.
- Barth TM, Jones TA, Schallert T. Functional subdivisions of the rat somatic sensorimotor cortex. *Behav Brain Res* 1990;39:73–95.
- Schallert T, Hernandez TD, Barth TM. Recovery of function after brain damage: severe and chronic disruption by diazepam. *Brain Res* 1986;379:104–111.
- Hernandez TD, Schallert T. Seizures and recovery from experimental brain damage. *Exp Neurol* 1988;102:318–324.
- Hua Y, Schallert T, Keep RF, Wu J, Hoff JT, Xi G. Behavioral tests after intracerebral hemorrhage in the rat. *Stroke* 2002;33:2478–2484.
- Karhunen H, Virtanen T, Schallert T, Sivenius J, Jolkkonen J. Forelimb use after focal cerebral ischemia in rats treated with an alpha 2-adrenoceptor antagonist. *Pharmacol Biochem Behav* 2003;74:663–669.
- MacLellan CL, Grams J, Adams K, Colbourne F. Combined use of a cytoprotectant and rehabilitation therapy after severe intracerebral hemorrhage in rats. *Brain Res* 2005;1063:40–47.
- Roof RL, Schielke GP, Ren X, Hall ED. A comparison of long-term functional outcome after 2 middle cerebral artery occlusion models in rats. *Stroke* 2001;32:2648–2657.
- Nakamura T, Xi G, Hua Y, Schallert T, Hoff JT, Keep RF. Intracerebral hemorrhage in mice: model characterization and application for genetically modified mice. *J Cereb Blood Flow Metab* 2004;24:487–494.
- Wu J, Hua Y, Keep RF, Schallert T, Hoff JT, Xi G. Oxidative brain injury from extravasated erythrocytes after intracerebral hemorrhage. *Brain Res* 2002;953:45–52.
- Li X, Blizzard KK, Zeng Z, DeVries AC, Hurn PD, McCullough LD. Chronic behavioral testing after focal ischemia in the mouse: functional recovery and the effects of gender. *Exp Neurol* 2004;187:94–104.
- Hua Y, Wu JM, Pecina S, et al. Ischemic preconditioning procedure induces behavioral deficits in the absence of brain injury? *Neurolog Res* 2005;27:261–267.
- Schallert T, Kozlowski DA, Humm JL, Cocke RR. Use-dependent structural events in recovery of function. *Adv Neurol* 1997;73:229–238.
- Tillerson JL, Cohen AD, Philhower J, Miller GW, Zigmond MJ, Schallert T. Forced limb-use effects on the behavioral and neurochemical effects of 6-hydroxydopamine. *J Neurosci* 2001;21:4427–4435.
- Tillerson JL, Cohen AD, Caudle WM, Zigmond MJ, Schallert T, Miller GW. Forced nonuse in unilateral parkinsonian rats exacerbates injury. *J Neurosci* 2002;22:6790–6799.
- Schallert T, Tillerson JL. Intervention strategies for degeneration of dopamine neurons in parkinsonism: optimizing behavioral assessment of outcome. In: Emerich DF, Dean RLI, Sanberg PR, eds. *CNS diseases: innovate models of CNS diseases from molecule to therapy*. Totowa, New Jersey: Humana Press; 2000:131–151.
- Woodlee MT, Asseo-Garcia AM, Zhao X, Liu SJ, Jones TA, Schallert T. Testing forelimb placing "across the midline" reveals distinct, lesion-dependent patterns of recovery in rats. *Exp Neurol* 2005;191:310–317.
- Schallert T, Woodlee MT. Brain-dependent movements and cerebral-spinal connections: Key targets of cellular and behavioral enrichment in CNS injury models. *J Rehabil Res Dev* 2003;40:S9–S17.
- Wolgin DL, Kehoe P. Cortical KCl reinstates forelimb placing

- following damage to the internal capsule. *Physiol Behav* 1983;31:197–202.
28. Jones TA, Schallert T. Overgrowth and pruning of dendrites in adult rats recovering from neocortical damage. *Brain Res* 1992; 581:156–160.
 29. Jones TA, Schallert T. Use-dependent growth of pyramidal neurons after neocortex damage. *J Neurosci* 1994;14:2140–2152.
 30. Schallert T, Leasure JL, Kolb B. Experience-associated structural events, subependymal proliferation activity, and functional recovery after injury to the central nervous system: a review. *J Cereb Blood Flow Metab* 2000;20:1513–1528.
 31. Chu CJ, Jones TA. Experience-dependent structural plasticity in cortex heterotopic to focal cortical damage. *Exp Neurol* 2002; 166:403–414.
 32. Luke LM, Allred RP, Jones TA. Unilateral ischemic sensorimotor cortical damage induces contralesional synaptogenesis and enhances skilled forelimb reaching with the ipsilateral forelimb in adult male rats. *Synapse* 2004;54:187–199.
 33. Zhang L, Schallert T, Zhang ZG, et al. A test for detecting long-term sensorimotor dysfunction in the mouse after focal cerebral ischemia. *J Neurosci Methods* 2002;117:207–214.
 34. Schallert T, Woodlee MT, Fleming SM. Disentangling multiple types of recovery from brain injury. In: Kriegstein J, Klumpp S, eds. *Pharmacology of cerebral ischemia*. Stuttgart: Medpharm Scientific Publishers; 2002:201–216.
 35. Bliss T, Kelly S, Ankur S, et al. Transplantation of hNT neurons into the ischemic cortex: cell survival and effect on sensorimotor behavior. *J Neurosci Res* 2006;83:1004–1014.
 36. Barth TM, Grant ML, Schallert T. Effects of MK-801 on recovery from sensorimotor cortex lesions. *Stroke* 1990;21:153–157.
 37. Morris RGM. Spatial localization does not require the presence of local cues. *Learn Motiv* 1981;12:239–260.
 38. Choi SH, Woodlee MT, Hong JJ, Schallert T. A simple modification of the water maze test to enhance daily detection of spatial memory in rats and mice. *J Neurosci Methods* 2006;156:182–193.
 39. Morris RGM, Garrud P, Rawlins HN. Place navigation impaired in rats with hippocampal lesions. *Nature* 1982;297:681–683.
 40. Sutherland RJ, Kolb B, Whishaw IQ. Spatial mapping: definitive disruption by hippocampal or medial frontal cortical damage in the rat. *Neurosci Lett* 1982;31:271–276.
 41. Russell JC, Towns DR, Anderson SH, Clout MN. Intercepting the first rat ashore. *Nature* 2005;437:1107.
 42. Whishaw IQ, Schallert T. Hippocampal RSA (theta), apnea, bradycardia and effects of atropine during underwater swimming in the rat. *Electroencephalogr Clin Neurophysiol* 1977;42:389–396.
 43. D'Hooge R, De Deyn PP. Applications of the Morris water maze in the study of learning and memory. *Brain Res Rev* 2001;36: 60–90.
 44. Day LB, Schallert T. Anticholinergic effects on acquisition of place learning in the Morris water task: spatial mapping deficit or inability to inhibit nonplace strategies? *Behav Neurosci* 1996;110: 998–1005.
 45. Day LB, Weisand M, Sutherland RJ, Schallert T. The hippocampus is not necessary for a place response but may be necessary for pliancy. *Behav Neurosci* 1999;113:914–924.
 46. Whishaw IQ, Cassel JC, Jarrad LE. Rats with fimbria-fornix lesions display a place response in a swimming pool: a dissociation between getting there and knowing where. *J Neurosci* 1995;15: 5779–5788.
 47. Kimble DP. Hippocampus and internal inhibition. *Psychol Bull* 1968;70:285–295.
 48. Karhunen H, Pitkanen A, Virtanen T, et al. Long-term functional consequences of transient occlusion of the middle cerebral artery in rats: a 1-year follow-up of the development of epileptogenesis and memory impairment in relation to sensorimotor deficits. *Epilepsy Res* 2003;54:1–10.
 49. Hodges H. Maze procedures: the radial-arm and water maze compared. *Brain Res Cogn Brain Res* 1996;3-4:167–181.
 50. Gerlai R, Roder J. Spatial and nonspatial learning in mice: effects of S100 beta overexpression and age. *Neurobiol Learn Mem* 1996; 66:143–154.
 51. Lindner MD, Schallert T. Aging and atropine effects on spatial navigation in the Morris water task. *Behav Neurosci* 1988;102: 621–634.
 52. Buresova O, Krekule I, Zahalka A, Bures J. On-demand platform improves accuracy of the Morris water maze procedure. *J Neurosci Methods* 1985;15:63–72.
 53. Spooner RI, Thomson A, Hall J, Morris RG, Salter SH. The Atlantis platform: a new design and further developments of Buresova's on-demand platform for the water maze. *Learn Mem* 1994;1:203–211.
 54. Whishaw IQ. Lateralization and reaching skill related: results and implications from a large sample of Long-Evans rats. *Behav Brain Res* 1992;52:45–48.
 55. Montoya CP, Campbell-Hope LJ, Pemberton KD, Dunnett SB. The "staircase test": a measure of independent forelimb reaching and grasping abilities in rats. *J Neurosci Methods* 1991;36:219–228.
 56. Adkins-Muir DL, Jones TA. Cortical electrical stimulation combined with rehabilitative training: enhanced functional recovery and dendritic plasticity following focal cortical ischemia in rats. *Neurol Res* 2003;25:780–788.
 57. Cenci MA, Whishaw IQ, Schallert T. Animal models of neurological deficits: how relevant is the rat? *Nat Rev Neurosci* 2002;3: 574–579.
 58. Whishaw IQ, Schallert T, Kolb B. An analysis of feeding and sensorimotor abilities of rats after decortication. *J Comp Physiol Psychol* 1981;95:85–103.
 59. Stoltz S, Humm JL, Schallert T. Cortical injury impairs contralateral forelimb immobility during swimming: a simple test for loss of inhibitory motor control. *Behav Brain Res* 1999;106:127–132.
 60. Kolb B, Tomie JA. Recovery from early cortical damage in rats. IV. Effects of hemidecortication at 1, 5, or 10 days of age on cerebral anatomy and behavior. *Behav Brain Res* 1988;28:259–274.
 61. Felt BT, Schallert T, Shao J, Liu Y, Li X, Barks JD. Early appearance of functional deficits after neonatal excitotoxic and hypoxic-ischemic injury: fragile recovery after development and role of the NMDA receptor. *Dev Neurosci* 2002;24:418–425.
 62. Kleim, JA, Jones TA, Schallert T. Motor enrichment and the induction of plasticity before and after brain injury. *Neurochem Res* 2003;28:1757–1769.
 63. Risedal A, Zeng J, Johansson BB. Early training may exacerbate brain damage after focal brain ischemia in the rat. *J Cereb Blood Flow Metab* 1999;19:997–1003.
 64. DeBow SB, McKenna JE, Kolb B, et al. Immediate constraint-induced movement therapy causes local hyperthermia and exacerbates cerebral injury in rats. *Can J Physiol Pharmacol* 2004;82: 231–237.
 65. Bland ST, Schallert T, Strong R, Aronowski J, Grotta JC, Feeney DM. Early exclusive use of the affected forelimb after moderate transient focal ischemia in rats: functional and anatomic outcome. *Stroke* 2000;31:1144–1152.
 66. Schallert T, Fleming SM, Woodlee MT. Should the injured and intact hemispheres be treated differently during the early phases of physical restorative therapy in experimental stroke or parkinsonism? *Phys Med Rehab Clin* 2003;14:1–20.
 67. Schallert T, Lindner MD. Rescuing neurons from trans-synaptic degeneration after brain damage: helpful, harmful, or neutral in recovery of function. *Can J Psychol* 1990;44:276–292.