

Orienting and Placing

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It is not difficult to document the existence of sensory or motor function asymmetries in normal rats or rats with unilateral damage to the basal ganglia, sensorimotor cortex, and related systems throughout the central nervous system, especially when the deficit on one side is near maximal. When the unilateral deficit is subtotal, quantifying the extent of asymmetry and changes over time requires unique testing methods that directly pit one hemisphere against the other.

As an illustration, if a person is slightly hard of hearing in one ear across all tones, how would you determine which ear is better and by how much? A simple test would be to put headphones on the person, play the same level of sound simultaneously in each ear, and determine which side the sound seems to be coming from. Because sound localization is influenced by relative intensity, this method could be used to confirm the existence of a sensory asymmetry. If the sound appears to come from the left, it can be concluded that the right ear (or left hemisphere) is impaired relative to the left ear. To determine the magnitude of the asymmetry, you could then raise the intensity of the sound presented to the relatively impaired ear and/or reduce the intensity of the sound presented to the better ear until the sound seemed to come from neither the left nor right side. The ratio of the sound intensity presented to the impaired ear relative to that of the sound presented to the better ear would quantify the extent of asymmetry. This two-part method is essentially the approach one can take in as-

sessing sensorimotor asymmetries in rats with partial unilateral damage to the brain, and in evaluating treatments.

Behavioral deficits in Parkinson's disease and stroke often can be traced to both sensory and movement initiation problems or an impaired ability to make appropriate motor responses to simple sensory events. In animals, unilateral damage to the sensorimotor cortex, striatum, or nigrostriatal pathway appears to have the perceptual effect of dulling somatosensory and proprioceptive sensory input on one side and, in some cases, enhancing the input from the other side. Asymmetrical sensory deficits, motor reactivity to bilateral sensory input, or predominantly motor dysfunctions can be examined with tests using a two-part method in which an asymmetry is first identified and then the extent of the asymmetry is quantified.

In animal models, it is important to select sensorimotor tests that are sensitive to the brain damage and treatment effects. This chapter describes behavioral tests that have been useful for examining the potential clinical efficacy of interventions that might be beneficial for neurological disorders. It is important to be able to distinguish whether an intervention promotes brain repair mechanisms, saves cells, enhances motor learning and retraining, or reduces the extent of secondary degeneration of tissue. We have chosen to include a subset of sensorimotor tests that we and others have found to be reliable, sensitive, quantitative, and easy to use in rat neurological models. The tests also cover the

range of cellular degeneration typical of focal ischemic injury, nigrostriatal terminal loss, and cervical spinal trauma.

ENVIRONMENTAL ENRICHMENT AND SENSORIMOTOR BEHAVIOR

Most wild rats live in a very complex environment that requires them to navigate obstacles, avoid predators, manipulate objects and circumstances to gain access to food and mates, and so forth, using a wide array of motor skills. By contrast, standard laboratory housing is severely lacking in this sort of stimulation, and laboratory "enriched" environments are still less complex than the rat's natural habitat (Greenough et al., 1976; Jones et al., 2003; Schallert et al., 2003). Even the most sedentary of people do not experience as impoverished an environment as a rat living in an isolated home cage. Therefore, to study sensorimotor behavior in the rat, it may be prudent to make some effort to house animals so that behaviors analogous to natural rat behavior are encouraged.

BILATERAL TACTILE STIMULATION TEST

Rats compulsively groom themselves and respond vigorously to any foreign substance that becomes stuck to some part of their bodies. The adaptive advantages of this behavior may include thermoregulation and maintenance against insects. Somatosensory asymmetries have been effectively determined using a test that involves reacting to, and removing, small sticky stimuli from the forelimbs. It is a two-part test; however, few investigators take advantage of both parts, which are needed to evaluate sensory function independent of the motor component. Practice effects and motor learning play a partial role in the motor aspects of this test but do not affect the sensory side, which can be investigated independently.

SENSORY ASYMMETRY

Small adhesive paper stimuli (Avery adhesive-backed labels, 113 mm²) are attached to the relatively hairless distal-radial aspect of each of the rat's forelimbs (Schallert et al., 1982, 1983, 2000; Schallert and Whishaw, 1984; Lindner et al., 2003; Fleming et al., 2003) (Fig. 12-1). The rat is placed back into its home cage so that it is not distracted by a novel environment, and it quickly uses its teeth to remove these dots one at a time. In some animals there is a small preoperative bias; in these cases, the hemisphere selected for injury can be opposite to the bias. Also, postoperative outcome can be compared against baseline values for each rat. Rats receiving unilateral lesions to brain areas subserving sensorimotor functions, especially those of the forelimbs, develop an immediate bias for removing adhesive stimuli of similar size from the unimpaired limb first. The order of contacting the ipsilateral versus contralateral stimulus reflects that there is a bias, but the magnitude of the sensory asymmetry requires further evaluation (see later). The latency to remove the stimuli can be used as a measure of motor capacity and is sensitive to practice effects, unlike the order of contact (Schallert and Whishaw, 1984).

Each trial ends when the rat removes both stimuli, or after 2 minutes has elapsed. To avoid habituation to the stimuli, individ-



Figure 12-1. Attaching adhesive stimuli (dots) to a rat's forelimbs in preparation for the bilateral tactile stimulation test.

ual trials should occur at intervals of no less than 5 minutes. In addition, the rats used should be well handled and have received several practice trials with the test before preoperative data are collected. Experience with the test calms the rats and makes the stimuli easier to apply but does not appear to affect actual performance.

This test is generally used to examine sensorimotor integration, although, as indicated earlier, it is possible to some degree to distinguish between the sensory and motor components involved (Schallert et al., 2002). For example, a change in the latency between initial contact and subsequent removal of a dot (i.e., how much time it takes to remove the stimulus) can be an index of sensorimotor function. As in many of the tests presented here, however, it is important that such a change be represented as an asymmetry between the impaired and unimpaired limbs in unilateral lesion models to control for non-motor and nonsensory factors (e.g., motivational state, alertness) that could have a global influence on latencies to contact and remove the dot. The contralateral (impaired limb) motor component of this test is best assessed by determining the time point at which the animal makes contact with the stimulus on the impaired side and scoring how much time after that time point it takes to remove that stimulus. This difference then would be compared with a comparable score of intact control animals (i.e., how long after a control rat contacts a given stimulus before it is removed, again controlling for practice effects by equating extent of experience).

MAGNITUDE OF SENSORY ASYMMETRY

The second part of this test is used as a means of measuring the degree of sensory asymmetry. In this part, the size of the dot placed on the impaired limb is progressively increased (by overlapping two dots), while the dot on the unimpaired limb is made smaller (by cutting down one dot). The dot sizes are in-

creased or decreased by 14 mm², as illustrated in Figure 12-2, allowing for area ratios ranging from 1.3:1 to 15:1 between the impaired and unimpaired limbs, respectively. A sufficient increase in this ratio leads to a neutralization, and even a reversal (with a slightly higher ratio), in the bias for the limb that is contacted first, and the ratio at which this occurs is used as the measure of severity of the sensory asymmetry. This measure is correlated with the amount of brain damage (Schallert et al., 1983; Schallert and Whishaw, 1984; Barth et al., 1990); indeed, a small asymmetry can be detected in rats with simple burr holes in the skull. Animals are started at the 2.2:1 ratio (level 3). If the stimulus is removed from the unimpaired limb first, animals then are tested at *two* levels higher. If the stimulus is removed from the impaired limb first, the animal is tested at *one* level lower. This process is continued until the experimenter has determined between which two levels the bias exists and assigned the rat a score that reflects this ratio (e.g., a score of 2.5 is given if the animal's bias reverses between levels 2 and 3).

Acute and chronic asymmetries on this test have been demonstrated in models of cortical injury and ischemia, parkinsonism, and spinal cord injury (Schallert et al., 2000). As recovery occurs, the ratio defining the magnitude of asymmetry becomes smaller inde-

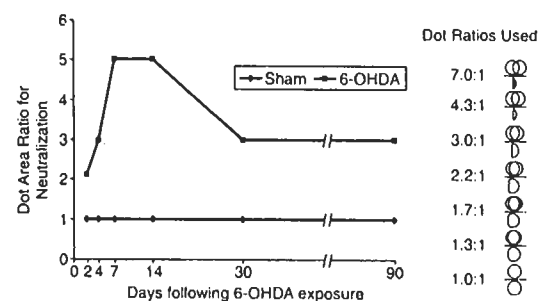


Figure 12-2. (Left) Data from an animal model of 6-hydroxydopamine-induced parkinsonism, in which sensory asymmetries measured with the bilateral tactile stimulation test improved over time but did not return to sham-operated control levels. (Right) Schematic of the different area ratios of the "dots" used in the test.

pendent of how much or how little practice occurs. Depending on the degree of striatal or nigrostriatal damage, full recovery can occur, even in hemidecorticate rats (Schallert and Whishaw, 1984). However, small changes in the testing environment (e.g., partially opening the home cage while testing) can partially reverse recovery so that the ratio becomes larger, possibly because the striatum is being taxed. This is important because it suggests that the testing environment can have a major influence on measures of functional outcome.

LIMB-USE ASYMMETRY ("CYLINDER") TEST

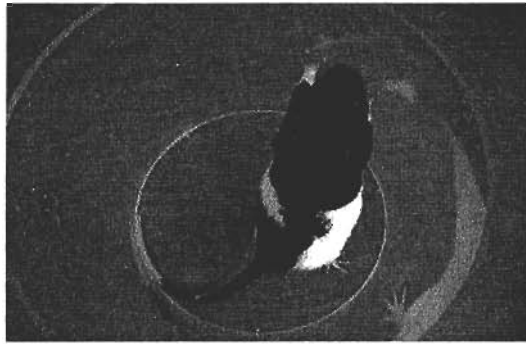
The limb-use asymmetry test evaluates the forelimb use of rats placed in a transparent Plexiglas cylinder. It has been used in a wide variety of motor system injury models, including middle cerebral artery occlusion, spinal cord injury, traumatic brain injury, parkinsonian models, cortical ablation, and focal cortical ischemia (Schallert et al., 2000; Schallert and Tillerson, 2000; Tillerson et al., 2001, 2002; Lindner et al., 2003). A notable feature is a high degree of sensitivity to chronic deficits not noticeably masked by postlesion compensatory behaviors, as well as to chronic sensorimotor deficits that many tests fail to detect. The test is also easy to use and score, has a high inter-rater reliability, is well correlated with the extent of lesions, including a wide range of dopamine depletion (even 50% or less) (Tillerson et al., 2001), and is relatively unaffected by practice effects or, it seems, the compensatory strategies often adopted by animals after motor system insults (Schallert et al., 2002).

Rats are tireless explorers, in both their natural environments and laboratory home cages. They often explore vertical surfaces by rearing up on their hindlimbs and exploring the surface with their front paws and vibrissae (Gharbawie et al., 2003). The cylinder test takes advantage of this tendency and of the common

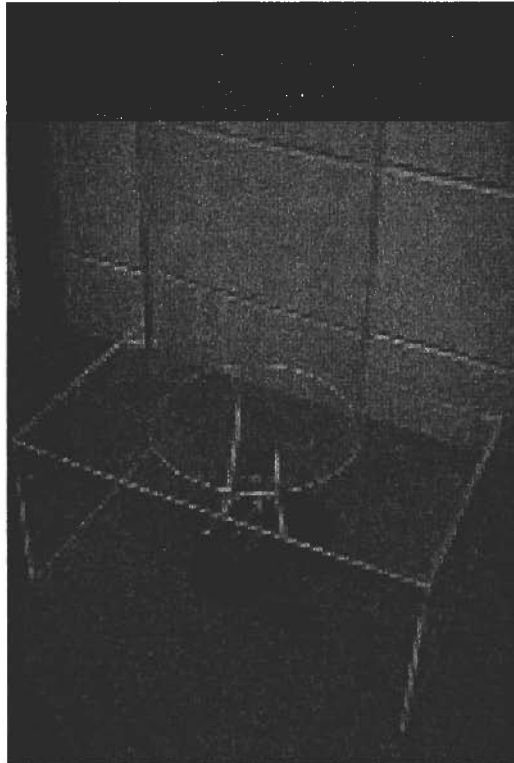
impairment in the initiation of movement and control of static stable equilibrium, especially center of gravity (Schallert et al., 1979, 1992). A rat is placed in an upright Plexiglas cylinder, open at both ends and measuring 30 cm high by 20 cm in diameter, that rests on a tabletop. The number of independent placements observed for either the right or left forelimb, as well as the number of "both limb" (i.e., simultaneous or near-simultaneous) placements, made onto the inner wall of the cylinder during rears is recorded. These limb placements occur when the rat shifts its weight, touches the cylinder wall, or steps to regain center of gravity during lateral movements along the cylinder wall ("wall stepping").

The data can be recorded over a set period of time in the cylinder or until a certain number of placements has been made. (We prefer the latter technique because different rats, and especially different strains, can vary widely in their activity levels in the cylinder.) To film the rat's behavior for later rating, (1) a camera is placed over the cylinder (Fig. 12-3A), (2) a camera is positioned to the side of the cylinder with a mirror angled behind and to the side to enable the experimenter to see the rat from all angles during live rating so that no limb movement is missed, or (3) the cylinder is placed atop a raised, transparent surface with a mirror positioned beneath at a 45° angle, with the camera aimed at the mirror to film the limb placements from below (Fig. 12-3B). Care should be taken so that the rats do not habituate to the cylinder lest they become inactive. This can be avoided by testing during the dark cycle and by dividing long trials into shorter segments separated by several minutes, during which the rat is placed back in the home cage.

Limb use is scored as the percentage of left, right, or both-limb wall placements relative to the total number of placements observed. One can also obtain a single limb-use asymmetry score by subtracting the percent independent use of the impaired limb from the percent independent use of the



A

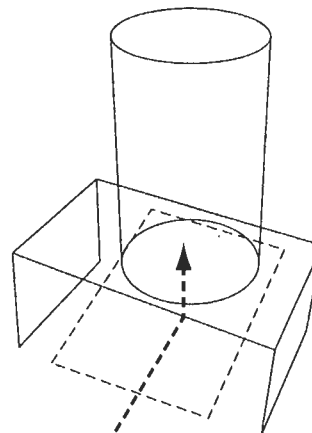


Setup 2:

Place a mirror at 45° angle
beneath cylinder and film at 45°
angle to mirror.

Setup 1:

Place camera directly beneath
cylinder.



B

Figure 12-3. (A) Top-down view of a rat making placements in the cylinder, filmed from a camera placed over the cylinder. (B) Alternate setup that can be used to film the rat from below the cylinder.

unimpaired limb. Higher numbers indicate a greater bias for use of the unimpaired limb. The former scoring method is advantageous in that it provides more information about both- versus independent-limb use events. It should be noted, however, that even with the latter scoring method, a large number of both-limb use events lower the asymmetry score, albeit not as much as would an equal number of independent impaired-limb placements. An alternative formula is one that we recently adopted because it reduces variability even further and sets a nonbias at 50%:

$$\frac{[(\text{ipsi} + \frac{1}{2} \text{both})]}{(\text{ipsi} + \text{contra} + \text{both})} \times 100$$

An additional measure can be obtained from this test. The use of a single limb to make lateral weight-shifting movements, independent of the other limb, is reduced in the impaired limb and enhanced in the nonimpaired limb and reflects a very high degree of functional integrity. That is, when a rat rears and places one forelimb on the wall of the cylinder and then makes a lateral movement to another location on the wall during the same rear sequence, this is considered an independent lateral weight-shifting movement, as opposed to a simple limb placement on the wall (which for the contralateral limb may show some recovery). The number of independent-limb weight-shifting movements along the wall for the ipsilateral forelimb can be compared with that of the contralateral forelimb. After unilateral injury to the sensorimotor cortex, striatum, or other motor areas, such movements are rarely observed in the affected forelimb but are commonly chronic in the unaffected forelimb (where they are even more frequently observed than in either limb of control animals, suggesting a reorganization in the intact hemisphere).

Preoperative baseline values should be obtained before animals undergo surgery or other experimental manipulations. Although there is no consistent population bias in limb

preference in the cylinder, some rats do display a predilection for independent use of one limb. When this occurs, experimental lesions can be applied contralateral to this preferred limb so that experimental effects are not confounded by the preexisting limb-use bias. Animals without a preoperative bias can be randomly assigned the lesion side.

Motivation differs between strains of rats. Long-Evans hooded rats, for example, are more active and thus might be considered preferable as animal models, all other considerations being equal. Some rats, especially Sprague-Dawley rats (in our experience), may not initially engage in an adequate amount of wall exploratory behavior in the cylinder. By and large, however, the behavior can be encouraged in any rat with the use of any number of "tricks" that do not affect the limb-use asymmetry score itself, including the following:

- Momentarily turning out the lights in the testing room and testing during red light
- Blowing into or tapping the top of the cylinder
- Placing a dark cage cover (especially the rat's own) over the cylinder
- Placing shavings from the rat's home cage into the cylinder
- Scooting the cylinder (with the rat inside) gently a few cm along the tabletop
- Lightly touching a pencil eraser or cotton tipped applicator to the rat's nose
- Dangling another rat into the cylinder momentarily
- Presenting novel scents or treats at the top of the cylinder
- Picking up the rat and replacing it into the cylinder
- Placing the rat in a new cylinder
- Picking up the rat, flipping over the cylinder, and putting the rat back in

TESTS OF FORELIMB PLACING

Researchers have developed a variety of forelimb-placing tests. Limb placing is usually triggered by visual or vestibular cues or by

contacting the limb being tested with a surface (Wolgin and Kehoe, 1983; Marshall, 1982). Rats use their vibrissae to gain bilateral information about the proximal environment, and this information is integrated between the hemispheres. When the bottoms of all four feet indicate that there is no stable surface for support, the rat is motivated to respond to the first object that one set of vibrissae contacts. In exploring its natural world, the rat frequently encounters surfaces that are unstable or, in the case of a cliff, unsuitable for locomotion. All four limbs must be able to respond to information from either set of vibrissae.

The test we describe next is the vibrissae-elicited forelimb placing test, which uses stimulation of the rat's vibrissae to trigger a placing response (Barth et al., 1990; Schallert et al., 2000; Lindner et al., 2003). This is a nice feature in light of the very important role that the vibrissae play in the rat's sensory environment—indeed, they are thought to be one of the primary tools rats use to explore their world. In addition, the test can be adapted to investigate neural events in the sensorimotor system that occur across the midline (as described next), a feature that is more difficult to implement using other placing triggers.

In this test, the rat's torso is supported by the investigator and suspended such that all four legs hang freely in the air. The experimenter then brings the rat toward the edge of

a tabletop or another flat surface, taking care to avoid abrupt movements that might trigger placing due to a vestibular response. If such responses are noted, they should be extinguished by taking the rat through the testing motions in open space (i.e., away from the tabletop) a few times. In the traditional, same-side version of this test, the rat's vibrissae are brushed against the table edge on the same side of the body in which forelimb placing is being evaluated. The percentage of trials in which the rat successfully places its forepaw onto the tabletop is recorded for each side. In addition, the triggering stimulus can be provided by moving the rat head on toward the table edge, thus providing chin-based and/or bilateral vibrissae stimulation, or by holding the rat on its side and stimulating the whiskers *opposite* the limb being evaluated (Fig. 12-4 demonstrates these different types of vibrissae stimulation). In all of these testing scenarios, the experimenter should gently restrain the limb not being tested. Naturally, this requires a tame rat that has been well handled for some time before testing, and which ideally has had a chance to acclimate to the test and the experimenter before being introduced to the experimental manipulation. Trials should be counted only when the rat is relaxed and does not struggle, and achieving this can require a great deal of practice on the part of the experimenter. Intact rats will place with 100% success in all variants of this test.

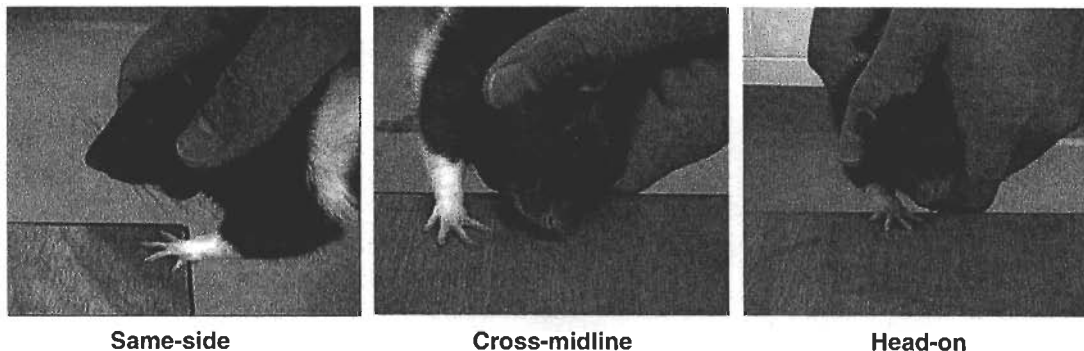


Figure 12-4. Forms of vibrissae-elicited forelimb placing, demonstrating the proper grip and orientation of the rat for this test.

The same-side version of the test has been used in the evaluation of many central nervous system injury models (Schallert et al., 2000). At our laboratory, we have begun to investigate the recovery of the cross-midline type of placing response in rats receiving cortical (via middle cerebral artery occlusion or focal ischemia to the forelimb area of sensorimotor cortex) or nigrostriatal (via 6-hydroxydopamine infusions to the nigrostriatal bundle) injury. One striking feature noted here is that vibrissae stimulation applied to the "good" (i.e., ipsilesional) side of the body is able to trigger a placing reaction in the impaired forelimb long before stimulation of the contralesional vibrissae can. In contrast, lesions to the nigrostriatal system lead to a complete failure of placing in the contralesional limb in this test, consistent with parkinsonian akinesia. Also, the placing deficit recovers over a period of weeks in the cortical injury models (the rate of recovery depending on the extent of damage to the forelimb area of the sensorimotor cortex and especially to the extent of striatal damage) but persists chronically in parkinsonian models (Felt et al., 2002; Woodlee et al., 2003).

For example, after middle cerebral artery occlusion that damages the striatum, the contralateral forelimb no longer responds to information from the vibrissae about the location of stable surfaces, although the ipsilateral forelimb can respond appropriately to information from the contralateral vibrissae (suggesting that the deficit is not due to a pure sensory impairment associated with the contralateral vibrissae). Moreover, except for severe damage to nigrostriatal dopamine terminals, in which the contralateral forelimb is akinetic, the contralateral forelimb recovers placing in response to ipsilateral vibrissae stimulation. That is, sensory information sent to the intact hemisphere can eventually control motor function associated with the damaged hemisphere, which is typical of normal rats.

TESTS OF HINDLIMB FUNCTION

Rats do not normally use their hindlimbs to initiate or execute complex movement. In this regard, we like to think of rats as being "front-wheel drive," a phenomenon that is illustrated in Figure 12-5, wherein rats supported solely

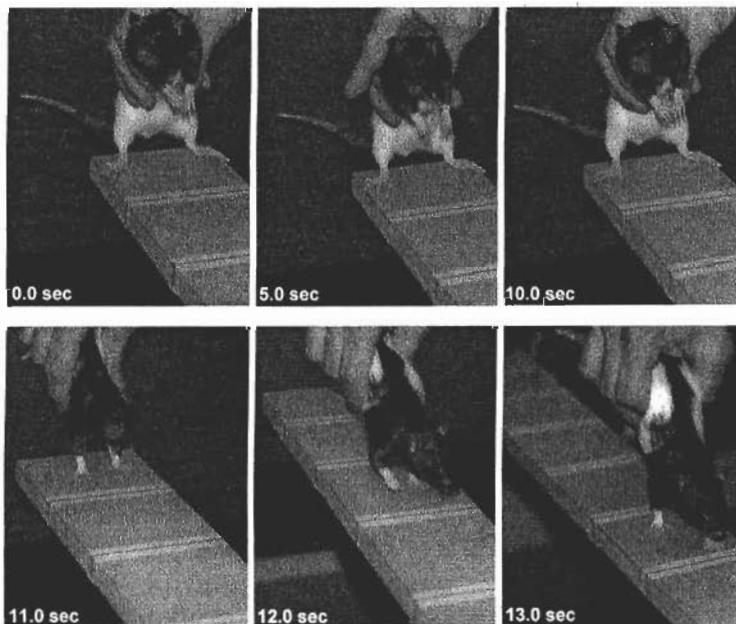


Figure 12-5. Rats operate primarily via "front-wheel drive." This series depicts a rat remaining stationary for 10 seconds when supported only on its hindlimbs (rats do not walk on their hindlimbs even if given more time) but proceeding to move briskly along a beam when flipped over so that it can support its weight on its forelimbs.

on the forelimbs or hindlimbs initiate movement only when on the forelimbs (Schallert and Woodlee, 2003). In part, this is because the vibrissae are not in contact with the ground and thus there is essentially a “stop” signal. This makes testing of hindlimb function rather difficult, but some tests have been developed that provide reliable measures of hindlimb function. This is good news for researchers of spinal cord injury, because experimental animal models of these conditions often involve lesioning caudal to the thoracic cord so that animals can maintain forelimb function and thus continue to care for themselves postoperatively. Also, research into sciatic nerve injury, a widely used model of peripheral nerve damage, can benefit from well-developed tests of hindlimb function.

A relatively new hindlimb test that we developed is the ledged tapered beam-walking test (Schallert et al., 2002). In this test, rats are trained to traverse an elevated beam that is tapered along its extent and has an underhanging ledge (2 cm wide, dropped 2 cm below the upper beam surface) that the rat can use as a crutch if it slips (see Figure 12–6 for dimensions and setup). Footfaults (slips) made with the hindlimbs can be measured as an index of hindlimb function. A footfault can be rated as a half-fault if the paw slips off the upper sur-

face of the beam without falling all the way to the ledge or as a full fault if the paw is placed fully on the ledge. The difficulty of the rat’s traverse increases as it moves along the narrowing beam, thus leading to more footfaults. For this reason the beam can be divided into three “bins” of difficulty along its extent, and these can be scored separately or weighted relative to each other to develop a single score. We generally run rats for five trials on a given day of testing, with several minutes between each trial (during which the rat’s cagemate, for example, can be tested) to avoid habituation to the test.

An important feature of this test is that the presence of the ledge allows the rat to display a deficit that it might normally make compensatory adjustments to hide. Rats are well known for compensating to overcome lesion-induced deficits, and indeed this can make the development of good behavioral tests difficult. Some compensatory motor adjustments appear to be more automatic in that they appear immediately in response to an impairment, whereas other adjustments require new learning. If one wishes to test the direct effect of a therapeutic intervention on the system in question, it is important to have tests that will target the deficit directly and be minimally affected by these compensatory be-

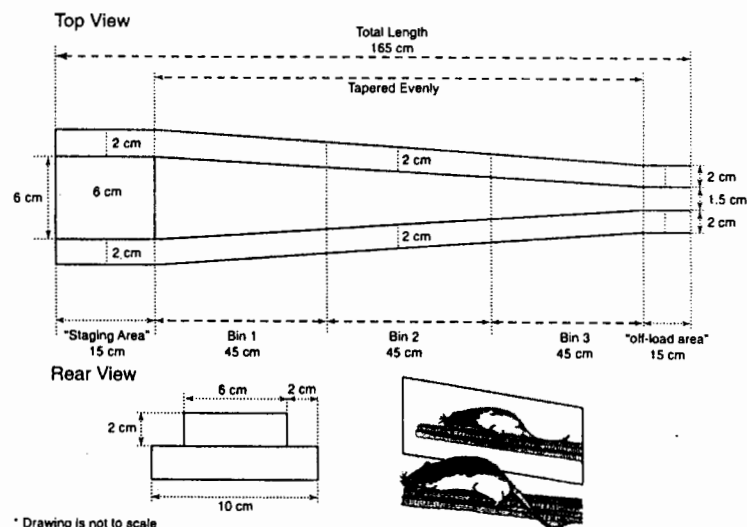


Figure 12–6. The ledged tapered beam, showing dimensions for construction. (Lower right) Rat running the beam, making a full left hindlimb footfault (slip) onto the ledge.

haviors. If the test is influenced by compensation, it may not be clear if the therapy is actually ameliorating the deficit *per se* rather than enhancing motor learning mechanisms that allow for development of the compensatory behavior. Beam-walking tasks that do not use a ledge are plagued by this problem, because rats very quickly learn to make compensatory postural adjustments to keep themselves from falling off the beam. Limb dysfunction may still exist, but the shift in body weight can hide it. With the ledged beam, there is less threat of falling and therefore compensation is less of a problem. In fact, a beam with a detachable ledge can be used to measure the ability of the rat to learn compensatory skills. For example, even several weeks after the insult, rats sustaining brain damage due to middle cerebral artery occlusion (a commonly used stroke model) continue to show a stable deficit on the ledged beam test (Schallert et al., 2002). If the ledge is removed, however, the rats learn to compensate over the course of just a few trials until they are running the beam successfully with no footfaults. This does not necessarily indicate recovery of limb function, though, because rats will begin to make foot faults again if the ledge is subsequently replaced. The speed with which rats are able to shift between displaying a deficit on the ledged beam and learning to compensate in the ledge's absence may be reflective of the level of impairment and the capacity of motor learning circuits that may or may not have been affected by the lesion.

Some hints make the use of the beam more successful. Preoperatively, rats must be trained to run the beam without fault, and preferably without stopping to explore the beam or its surroundings during the run. There is no prescribed number of training trials needed to achieve this result; each rat can simply be trained until this criterion is reached. Good training eases the testing phase, because stopping to encourage the rat to traverse the beam becomes less necessary.

When setting up the beam, the experimenter may want to place the rat's home cage at the end to serve as a reinforcer. The cage may also be covered with a dark cloth to make it more enticing. During the initial training, the experimenter can encourage the rat to run by tapping on the beam in front of the rat, picking up the rat's tail from behind to encourage it to move away, or "tucking" the rat's hindquarters with the experimenter's hands. On early trials, the rat frequently stops to sniff the beam or have a look around the testing room, but this generally ceases during the course of pretraining. Objects should not be placed to the side or below the beam because these tend to distract the animal. A comprehensive review of the setup, use, and scoring of the beam can be found in Schallert et al. (2002).

Other opportunities exist for testing hindlimb function. Although rats rely primarily on their forelimbs for most movement, the hindlimbs are used in behaviors such as jumping, swimming (in which the forelimbs usually stay stationary as the hindlimbs paddle [Whishaw et al., 1981; Kolb and Tomie, 1988; Stoltz et al., 1999]), and backing out of tunnels or other tight areas in which the rat cannot turn around. They can also use the hindlimbs to maintain balance during a rear; one can also quantify hindlimb stepping in the cylinder test (see earlier) as an index of hindlimb function, if the cylinder is set up to be filmed from below (Fleming et al., 2002). In the cylinder, rats with unilateral nigrostriatal system damage mimicking a hemiparkinsonian state tend to leave the impaired hindlimb planted in one place and pivot around this akinetic limb by stepping with the unimpaired limb.

CONCLUSION

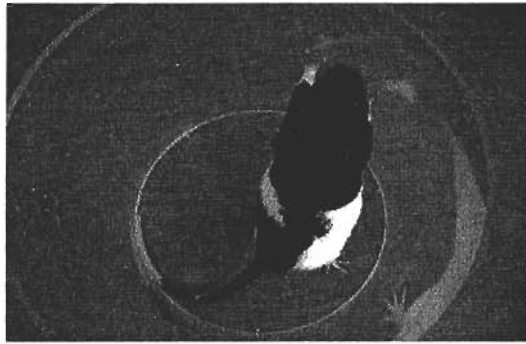
The sensorimotor tests described earlier are certainly not the only ones that should be considered useful, but in our experience these

qualify as among the best for assessing functional outcome after unilateral focal ischemic injury, nigrostriatal degeneration, traumatic head injury, damage to the intrinsic neurons of striatum, and cervical spinal hemisection. It is possible to use aspects of these tests along with others to determine the location and extent of injury and the degree of improvement over time. With practice, investigators can reliably and rapidly evaluate treatment effects. Our Web site (<http://www.schallertlab.org>) has downloadable videos and information that can help new researchers adopt these and related tests of sensory and motor function.

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A

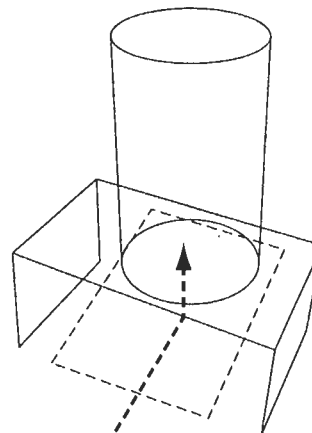


Setup 2:

Place a mirror at 45° angle
beneath cylinder and film at 45°
angle to mirror.

Setup 1:

Place camera directly beneath
cylinder.



B

Figure 12-3. (A) Top-down view of a rat making placements in the cylinder, filmed from a camera placed over the cylinder. (B) Alternate setup that can be used to film the rat from below the cylinder.

unimpaired limb. Higher numbers indicate a greater bias for use of the unimpaired limb. The former scoring method is advantageous in that it provides more information about both- versus independent-limb use events. It should be noted, however, that even with the latter scoring method, a large number of both-limb use events lower the asymmetry score, albeit not as much as would an equal number of independent impaired-limb placements. An alternative formula is one that we recently adopted because it reduces variability even further and sets a nonbias at 50%:

$$\frac{[(\text{ipsi} + \frac{1}{2} \text{both})]}{(\text{ipsi} + \text{contra} + \text{both})} \times 100$$

An additional measure can be obtained from this test. The use of a single limb to make lateral weight-shifting movements, independent of the other limb, is reduced in the impaired limb and enhanced in the nonimpaired limb and reflects a very high degree of functional integrity. That is, when a rat rears and places one forelimb on the wall of the cylinder and then makes a lateral movement to another location on the wall during the same rear sequence, this is considered an independent lateral weight-shifting movement, as opposed to a simple limb placement on the wall (which for the contralateral limb may show some recovery). The number of independent-limb weight-shifting movements along the wall for the ipsilateral forelimb can be compared with that of the contralateral forelimb. After unilateral injury to the sensorimotor cortex, striatum, or other motor areas, such movements are rarely observed in the affected forelimb but are commonly chronic in the unaffected forelimb (where they are even more frequently observed than in either limb of control animals, suggesting a reorganization in the intact hemisphere).

Preoperative baseline values should be obtained before animals undergo surgery or other experimental manipulations. Although there is no consistent population bias in limb

preference in the cylinder, some rats do display a predilection for independent use of one limb. When this occurs, experimental lesions can be applied contralateral to this preferred limb so that experimental effects are not confounded by the preexisting limb-use bias. Animals without a preoperative bias can be randomly assigned the lesion side.

Motivation differs between strains of rats. Long-Evans hooded rats, for example, are more active and thus might be considered preferable as animal models, all other considerations being equal. Some rats, especially Sprague-Dawley rats (in our experience), may not initially engage in an adequate amount of wall exploratory behavior in the cylinder. By and large, however, the behavior can be encouraged in any rat with the use of any number of "tricks" that do not affect the limb-use asymmetry score itself, including the following:

- Momentarily turning out the lights in the testing room and testing during red light
- Blowing into or tapping the top of the cylinder
- Placing a dark cage cover (especially the rat's own) over the cylinder
- Placing shavings from the rat's home cage into the cylinder
- Scooting the cylinder (with the rat inside) gently a few cm along the tabletop
- Lightly touching a pencil eraser or cotton tipped applicator to the rat's nose
- Dangling another rat into the cylinder momentarily
- Presenting novel scents or treats at the top of the cylinder
- Picking up the rat and replacing it into the cylinder
- Placing the rat in a new cylinder
- Picking up the rat, flipping over the cylinder, and putting the rat back in

TESTS OF FORELIMB PLACING

Researchers have developed a variety of forelimb-placing tests. Limb placing is usually triggered by visual or vestibular cues or by

contacting the limb being tested with a surface (Wolgin and Kehoe, 1983; Marshall, 1982). Rats use their vibrissae to gain bilateral information about the proximal environment, and this information is integrated between the hemispheres. When the bottoms of all four feet indicate that there is no stable surface for support, the rat is motivated to respond to the first object that one set of vibrissae contacts. In exploring its natural world, the rat frequently encounters surfaces that are unstable or, in the case of a cliff, unsuitable for locomotion. All four limbs must be able to respond to information from either set of vibrissae.

The test we describe next is the vibrissae-elicited forelimb placing test, which uses stimulation of the rat's vibrissae to trigger a placing response (Barth et al., 1990; Schallert et al., 2000; Lindner et al., 2003). This is a nice feature in light of the very important role that the vibrissae play in the rat's sensory environment—indeed, they are thought to be one of the primary tools rats use to explore their world. In addition, the test can be adapted to investigate neural events in the sensorimotor system that occur across the midline (as described next), a feature that is more difficult to implement using other placing triggers.

In this test, the rat's torso is supported by the investigator and suspended such that all four legs hang freely in the air. The experimenter then brings the rat toward the edge of

a tabletop or another flat surface, taking care to avoid abrupt movements that might trigger placing due to a vestibular response. If such responses are noted, they should be extinguished by taking the rat through the testing motions in open space (i.e., away from the tabletop) a few times. In the traditional, same-side version of this test, the rat's vibrissae are brushed against the table edge on the same side of the body in which forelimb placing is being evaluated. The percentage of trials in which the rat successfully places its forepaw onto the tabletop is recorded for each side. In addition, the triggering stimulus can be provided by moving the rat head on toward the table edge, thus providing chin-based and/or bilateral vibrissae stimulation, or by holding the rat on its side and stimulating the whiskers *opposite* the limb being evaluated (Fig. 12-4 demonstrates these different types of vibrissae stimulation). In all of these testing scenarios, the experimenter should gently restrain the limb not being tested. Naturally, this requires a tame rat that has been well handled for some time before testing, and which ideally has had a chance to acclimate to the test and the experimenter before being introduced to the experimental manipulation. Trials should be counted only when the rat is relaxed and does not struggle, and achieving this can require a great deal of practice on the part of the experimenter. Intact rats will place with 100% success in all variants of this test.

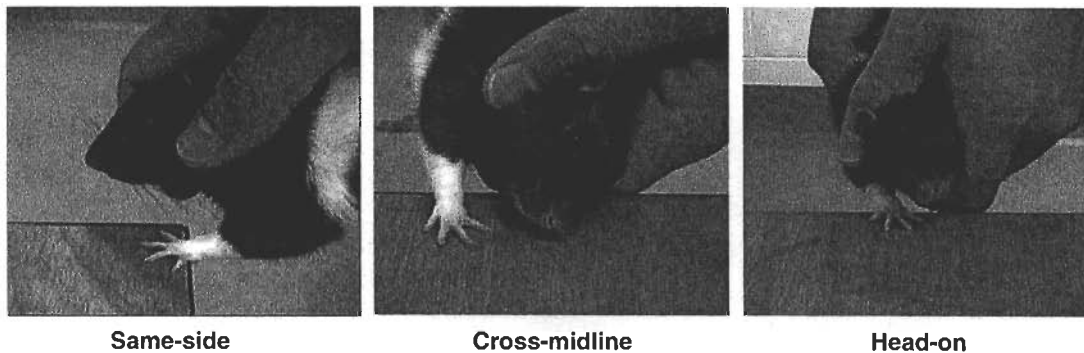


Figure 12-4. Forms of vibrissae-elicited forelimb placing, demonstrating the proper grip and orientation of the rat for this test.