Abstract

Stroke remains the leading cause of adult disability, with upper extremity motor impairments being the most prominent functional deficit in surviving stroke victims. The development of animal models of upper extremity dysfunction after stroke has enabled investigators to examine the neural mechanisms underlying rehabilitation-dependent motor recovery as well as the efficacy of various adjuvant therapies for enhancing recovery. Much of this research has focused on rat models of forelimb motor function after experimentally induced ischemic or hemorrhagic stroke. This article provides a review of several different methods for inducing stroke, including devascularization, photothrombosis, chemical vasoconstriction, and hemorrhagia. We also describe a battery of sensorimotor tasks for assessing forelimb motor function after stroke. The tasks range from measures of gross motor performance to fine object manipulation and kinematic movement analysis, and we offer a comparison of the sensitivity for revealing motor deficits and the amount of time required to administer each motor test. In addition, we discuss several important methodological issues, including the importance of testing on multiple tasks to characterize the nature of the impairments, establishing stable baseline prestroke motor performance measures, dissociating the effects of acute versus chronic testing, and verifying lesion location and size. Finally, we outline general considerations for conducting research using rat models of stroke and the role that these models should play in guiding clinical trials.

Key Words: forelimb; impairment; ischemia; middle cerebral artery; rat; stroke

Introduction

n the time it will take to read this article, a dozen Americans will suffer a stroke, resulting in 750,000 new stroke victims this year alone. Of the 75% who survive, most will experience upper extremity impairments, making stroke the leading cause of adult disability in the United States (Gresham et al. 1995). The need for animal models to guide the development of more effective rehabilitation therapies after stroke is obvious. Unfortunately, years of failed clinical trials of putative neuroprotective agents have cast a shadow over the use of animal models to develop clinically useful stroke treatments. However, over the last decade, there has been both a methodological and a philosophical shift in stroke research. First, researchers have begun to develop better animal models that include more comprehensive measures of both motor impairment and subsequent improvement in response to rehabilitation. Second, animal research has expanded beyond studies of neuroprotection to studies directed at identifying the fundamental neural substrates that support rehabilitation-dependent functional improvement. The purpose of this review is not to summarize these putative therapies but rather to describe several rat models of stroke currently in use in order to guide the development of novel upper extremity rehabilitation therapies.

Advantages of Using Rat Models

Modeling human neurological conditions in animals is not an easy task, primarily because the same neurological disorder may have different physical manifestations across different species. The key to successfully modeling human neurological symptoms, such as those associated with stroke, is to first identify functional rather than physical similarities in neurological impairments. No laboratory animal has been studied in more detail than the rat and this work has revealed a number of motor behaviors that can be used to study both motor impairment and recovery associated with various neurological disorders (Cenci et al. 2002). In addition to species-specific motor behaviors, detailed analysis of limb movement shows very similar motor components in human upper extremity and rat forelimb movement during reaching behavior (Whishaw et al. 2002). This information has led to the development of a battery of sen-
orsimotor tests that can measure various aspects of both motor impairment and recovery after ischemic insult. Further, investigators’ extensive knowledge of the anatomical and neurophysiological organization of the rodent motor system facilitates the identification of the neural mechanisms underlying motor recovery, information that in turn allows for the development of novel adjuvant therapies that may enhance recovery or limit impairment. In addition, rat models enable the use of more complex experimental designs to examine issues such as time course of recovery and dose-response relationships that are not feasible in non-human primate experiments.

Caveats of Translating Results from Rat Models into Clinical Studies

It is important to recognize the limitations of rat models for informing clinical research. First, animal models of stroke will never approach the complexity of the human condition and the heterogeneity of the stroke population. For example, premorbid neurological function is highly variable and frequently accompanied by diseases such as depression, cardiac ischemia, and pulmonary embolism (Cramer 2003). In addition, age, lesion location, and extent are all unpredictable in human patient populations, and rat models do not typically study the effect of this variability. Second, the nature of the damage in human patients can differ substantially from the damage experimentally induced in laboratory rats. For example, subcortical white matter infarctions are very common in human stroke victims, whereas most rat stroke models target cortical grey matter. Third, the degree of impairment between human stroke victims and rats is also different. Many acute stroke patients have little or no upper extremity movement, while rats rarely show a total loss of forelimb movement in current models of focal ischemia. Finally, the administration of rehabilitation in humans and rats is fundamentally different. In human stroke patients, rehabilitation often involves hands-on assistance or guidance from the therapist. Current rat models are entirely hands-off, requiring the rats to perform a task without assistance in a testing apparatus.

Indeed, animal studies may produce unrealistically favorable results as researchers typically use young, healthy rats with very specific focal damage to a particular brain structure producing comparatively mild motor impairments. Needless to say, if a treatment fails to enhance recovery under these conditions then it is very unlikely that it will work in older subjects with larger lesions and more profound impairments. It’s not that the use of older rats in stroke research is impossible—Lindner and colleagues (2003) demonstrated that many of the motor behavioral tasks that we discuss below can be effective for detecting persistent motor impairments in older rats that could be correlated with lesion volume. It is simply that younger animals tend to survive the surgeries required to induce stroke more readily than older animals. Both basic and clinical scientists must also bear in mind that animal models of stroke are not designed to mirror the human condition nor provide specific details on how therapy should be conducted in the clinic. Rather, they serve to identify fundamental neural and behavioral principles of recovery that are readily observable in the laboratory and can be used to guide the development of novel clinical therapies (Kleim and Jones 2007). They can also provide invaluable information that, with proper translation, have the potential to produce clinically useful information.

Methods for Inducing Stroke in Rat

All of the methods for inducing stroke in rats are inherently variable with respect to both the amount of damage induced and the motor impairments that result. It is not uncommon for the same procedure conducted by the same experimenters to produce vastly different levels of motor impairments. Thus it is critical to perform histological analysis of lesion size in a way that ensures the proper interpretation of observed impairments and response to putative therapies. To account for statistical variance, experimenters may also wish to sort their experimental conditions into groups as a function of impairment severity and/or lesion size. The variability in lesion size and concomitant impairments also has implications for postoperative care, as animals from the same round of surgical procedures may require different levels of care after surgery. For example, very severely impaired animals may have difficulty eating and drinking and so may need extra care during the first few hours after surgery to ensure that they can eat and drink once returned to their standard housing condition. Cases of severe impairment that include oral motor difficulties may require the administration of additional fluids (Ringer’s solution s.c.) and the placement of food mash in the cage.

Middle Cerebral Artery Occlusion

Occlusion of the middle cerebral artery (MCAo) is a widely used method for inducing stroke in rats given the high proportion of middle cerebral artery (MCA) stroke observed clinically. MCAo involves temporarily or permanently restricting MCA blood flow to the cortex and striatum. The method, location, and duration of MCA occlusion as well as the rat strain and age all affect the lesion size, location, and impairment level (e.g., Duverger and MacKenzie 1988). There are a number of ways to induce MCAo, ranging from permanent devascularization to transient occlusion, but the most widely used method involves insertion of an intraluminal suture (Kozuimi et al. 1986). The technique entails temporarily tying off the common carotid artery and insert-

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1Abbreviations used in this article: ET-1, endothelin-1; MCA, middle cerebral artery; MCAo, middle cerebral artery occlusion.
ing a suture in the internal carotid via an incision in the external carotid. The suture lodges in the junction between the anterior and middle cerebral arteries (Carmichael 2005) and remains in place either for a predetermined amount of time (1-2 hours, typically) or permanently. Depending on the duration of the occlusion, damage occurs in the frontal, parietal, temporal, and occipital cortex as well as several subcortical structures including the thalamus, striatum, and hypothalamus (Garcia et al. 1995; Kanemitsu et al. 2002).

Emboliﬁcation is also an effective method of MCAo. It involves injecting microspheres into the carotid artery that then lodge in the middle cerebral artery and produce damage comparable to that observed with permanent suture occlusion (Gerriets et al. 2003). Injections of thromboembolic clots into the MCA produce similar results by causing smaller and more variable infarctions (Wang et al. 2001; Zhang et al. 1997). Regardless of method, both temporary and permanent MCAo tend to spare forelimb motor cortex (Gharbawie et al. 2006; Windle et al. 2006).

Many MCAo models require investigators to make an incision into the external carotid artery, but this technique can damage the mastication and swallowing muscles, thus interfering with performance on some behavioral tests (Dittmar et al. 2003). A craniotomy model of MCA occlusion prevents such damage. For example, exposing the lateral cortex dorsal to the rhinal fissure and cauterizing the dorsolateral aspects of the distal MCA induces reliable sensorimotor deﬁcits (Gharbawie et al. 2005a).

**Endothelin-1**

An alternative method to physically blocking vessels is to induce them to constrict through administration of endothelin-1 (ET-1), a potent vasoconstrictor (Yanagisawa et al. 1988) that reduces local blood ﬂow to produce ischemic injury when injected directly into brain tissue (Fuxe et al. 1997). Blood ﬂow reduction is rapid but not immediate (Macrae et al. 1993) and reperfusion occurs over several hours (Biernaskie et al. 2001; Macrae et al. 1993). This proﬁle may be more representative of human stroke than the immediate reduction and reperfusion that occur with the intraluminal suture or clip models of MCAo. Intracerebral injection or topical application produces a localized and dose-dependent ischemic lesion with minimal edema (Windle et al. 2006), and thus makes it possible to target infarction to speciﬁc brain regions, particularly subcortical areas that are more diﬃcult to achieve using physical occlusion methods. For example, Frost and colleagues (2006) have shown that injection of ET-1 into the internal capsule can cause white matter damage thought to mimic lacunar inﬁrctions observed in many stroke patients. However, ET-1 receptors are not restricted to endothelial cells and are found on both neurons and glial cells (Nakagomi et al. 2000). Although the speciﬁc action of ET-1 receptor binding is unknown, there is the potential for confounding natural brain responses to stroke and rehabilitation.

**Photothrombosis**

This model involves injecting photosensitive dyes (e.g., Rose-Bengal) into the bloodstream and inducing photocoagulation by irradiating speciﬁc areas of tissue. The photocoagulation causes endothelial damage, platelet activation, and vascular occlusion in the vascular bed of the irradiated area (Watson et al. 1985). The advantage to this model is the creation of very focal ischemic inﬁrctions with minimal surgical invasion. The disadvantage is that there is very little reperfusion or ischemic penumbra and the nature of the occlusion causes signiﬁcant vasogenic and cytotoxic edema that more often follows traumatic brain injury than ischemic strokes in humans (Carmichael 2005).

**Devascularization**

Devascularization of the cortical surface, either via electrocoagulation of surface vessels (Kleim et al. 2003) or by pial stripping (Gonzalez and Kolb 2003), can induce cortical inﬁrctions. Both techniques produce focal cortical damage that extends from pia to white matter beneath the devascularized area. The limitations of this method are that it can produce mechanical damage to the underlying tissue and hemorrhagia and it does not permit reperfusion (Del Bigio et al. 1996). The degree of impairment depends on the volume of cortex that has been affected.

**Methods for Inducing Focal Hemorrhagic Damage in Rats**

Although the majority of human strokes are ischemic, approximately 15% are hemorrhagic and many ischemic strokes are associated with secondary bleeding or hemorrhagic conversion or transformation in the inﬁrcted areas (Lyden and Zivin 1993). One method of experimentally inducing intracortical hemorrhagic strokes is by infusing bacterial collagenase (DeBow et al. 2003b; Del Bigio et al. 1996; MacLellan et al. 2004, 2006; Rosenberg et al. 1990) or whole blood (Xue and Del Bigio 2000) into cortical and/or subcortical areas. These compounds disrupt vessel integrity, inducing cerebral bleeding; variations in the volume of infused compound can modulate the lesion volume (MacLellan et al. 2006).

**Methods for Measuring Forelimb Impairment in Rats After Stroke**

Numerous tasks are effective for assessing forelimb impairment in rats after stroke (Table 1). The tests range from those that measure acquired (skilled) sensorimotor behaviors to those that measure preexisting (unskilled) sensorimotor behaviors. Acquired behaviors, which require a signiﬁcant amount of training in order to reach behavioral...
Table 1 Various methods used for inducing stroke in rats (model), the area of the brain damaged (infarction), and the tasks on which the animals were impaired (impairments)\(^a\)

<table>
<thead>
<tr>
<th>Model</th>
<th>Infarction</th>
<th>Task impairment</th>
<th>References (see text)</th>
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<tr>
<td>Topical ET-1</td>
<td>FL-Mctx</td>
<td>Single pellet(^1,12)</td>
<td>O’Bryant et al. (2007)(^1)</td>
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<td></td>
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<td>Cylinder(^2,3)</td>
<td>Windle et al. (2006)(^2)</td>
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<td>Staircase(^1)</td>
<td>Hsu and Jones (2006)(^3)</td>
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<td>Forelimb placing(^3)</td>
<td>Adkins and Jones (2005)(^12)</td>
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<td>Reaching quality(^3)</td>
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<tr>
<td>Intracortical ET-1</td>
<td>FL-Mctx</td>
<td>Staircase(^2)</td>
<td>Windle et al. (2006)(^2)</td>
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<td>Cylinder(^2)</td>
<td>Gilmour et al. (2005)(^10)</td>
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<td>Foot fault(^10)</td>
<td>Gilmour et al. (2004)(^19)</td>
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<td>Tray reaching(^10,19)</td>
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<td>MCA ET-1</td>
<td>Lat-Ctx</td>
<td>Staircase(^2,21)</td>
<td>Windle et al. (2006)(^2)</td>
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<td>Cylinder(^2,21)</td>
<td>Biernaskie et al. (2005)(^14)</td>
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<td>Single pellet(^14,21)</td>
<td>Riek-Burchardt et al. (2004)(^18)</td>
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<td>Reaching quality(^14)</td>
<td>Biernaskie et al. (2004)(^21)</td>
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<td>Foot fault(^18,21)</td>
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<td>Beam walking(^21)</td>
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<td>Intracortical + intrastratal ET-1</td>
<td>FL-Mctx striatum</td>
<td>Staircase(^2,4,13)</td>
<td>Windle et al. (2006)(^2)</td>
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<td></td>
<td></td>
<td>Cylinder(^2,4,13)</td>
<td>Hewlett and Corbett (2006)(^4)</td>
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<td>Foot fault(^13)</td>
<td>Winde et al. (2005)(^13)</td>
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<tr>
<td>Photothrombosis</td>
<td>FL-Mctx</td>
<td>Foot fault(^5)</td>
<td>Shanina et al. (2006)(^5)</td>
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<td>Cylinder(^5)</td>
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<td>Intracapsular ET-1</td>
<td>Internal capsule</td>
<td>Forelimb placing(^6)</td>
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<td>Cylinder(^6)</td>
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<tr>
<td>Electrocoagulation</td>
<td>FL-Mctx</td>
<td>Single pellet(^7)</td>
<td>Kleim et al. (2003)(^7)</td>
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<td>Pial strip</td>
<td>FL-Mctx</td>
<td>Cylinder(^8,24,35)</td>
<td>Gonzalez et al. (2006)(^8)</td>
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<td>Forepaw inhibition(^8,35)</td>
<td>Gharbawie et al. (2005a)(^18)</td>
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<td>Tray reaching(^8,18,24,36)</td>
<td>Teskey et al. (2003)(^22)</td>
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<td>Single pellet(^8,18)</td>
<td>Gonzalez and Kolb (2003)(^24)</td>
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<td>Reaching quality(^8,18)</td>
<td>Kolb et al. (2007)(^35)</td>
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<td>Pasta matrix(^22)</td>
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<td>Sunflower seed(^24)</td>
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<td>MCAo</td>
<td>Lat-Ctx striatum</td>
<td>Rotor rod(^9,23,36)</td>
<td>Erdo et al. (2006)(^9)</td>
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<td>Beam walking(^9)</td>
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<td>Grip strength(^9)</td>
<td>Woodlee et al. (2005)(^15)</td>
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<td></td>
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<td>Single pellet(^1,11,16,18,25,34)</td>
<td>Gharbawie et al. (2005a)(^16)</td>
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<td>Staircase(^23,26,29)</td>
<td>Gharbawie et al. (2005b)(^18)</td>
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<td>Forelimb placing(^15,23,26-28,31,32)</td>
<td>Lindner et al. (2003)(^23)</td>
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<td>Cylinder(^15,23,24,27,28,31)</td>
<td>Gonzalez and Kolb (2003)(^24)</td>
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<td>Adhesive removal(^23,31,36)</td>
<td>Bland et al. (2001)(^27)</td>
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<td>Forepaw inhibition(^24)</td>
<td>Roof et al. (2001)(^28)</td>
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<td>Sunflower seed(^24)</td>
<td>Palmer et al. (2001)(^29)</td>
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<td>Foot fault(^27,28,30,36)</td>
<td>Pitsikas et al. (2001)(^30)</td>
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\(^a\)ET-1, endothelin-1; MCA, middle cerebral artery; MCAo, middle cerebral artery occlusion; FL-Mctx, forelimb motor cortex; Lat-Ctx, lateral cortex.
asymptote, include primarily tests of skilled forelimb reaching such as single pellet reaching, tray reaching, pasta matrix reaching, and Montoya staircase tasks. They also require food restrictions for the rats, usually to reduce the body weight of an adult to 90% of its prestudy weight. (We caution, however, that food restriction may interfere with other behavioral tasks, especially if the lesion technique itself—e.g., proximal MCAo—reduces body weight.) Unskilled tasks that test preexisting motor behaviors include the cylinder task, forelimb placing, swimming (forepaw inhibition), sunflower seed opening, foot fault, and adhesive dot removal. These unskilled tasks typically require only a few training sessions to familiarize the animals with the demands of the task in order to produce reliable measures.

Multiple testing sessions are of critical importance to assess the impact of any rehabilitation treatment. It is also important to include tasks that are not part of the rehabilitation therapy, to assess the generalizability of motor improvement and to better distinguish between lesion size and therapeutic interventions (MacLellan et al. 2006). For example, single pellet or tray reaching can be effective as a rehabilitative intervention as well as a measure of motor improvement. Furthermore, most upper extremity impairments in stroke patients are so severe that they affect even the simplest of movements and therefore simple motor tests, such as the Fugl-Meyer, are sufficient to assess recovery (Duncan et al. 2000).

Preclinical tests fall into two categories, based on whether they involve endpoint measures (“how well are the animals doing the task?”) or qualitative measures (“how are they doing the task?”). Examples of endpoint measures are accuracy in reaching, number of strands of pasta obtained, or number of foot faults, whereas qualitative measures examine the normality of motor elements in a single reaching movement. The use of these two different measures makes it possible to differentiate between true recovery of motor function as opposed to the development of compensatory motor strategies. For example, increases in accuracy on a skilled reaching task may result from restoring the movement sequence used before the insult, developing a completely new movement sequence, or some combination of the two. The neural mechanisms that underlie restoration of the original movement sequence are likely very different from those involved in development of a completely new motor pattern. Indeed, analysis shows that improvements in reaching accuracy after stroke are due, at least in part, to adaptations in the original movement sequence (Gharbawie and Whishaw 2006). The strategy adopted in response to a given therapy may be influenced by any number of factors, including the size and location of the damage, the nature/efficacy of the rehabilitation treatment, or the timing of the treatment after insult. It is essential to understand the contribution of these factors in order to optimize the kind of rehabilitation administered to each patient.

Cylinder Task

The cylinder task is effective in examinations of rodent forelimb use for postural support. The test encourages the rodent, inside a specially designed cylinder, to use the walls for upright support and vertical exploration. The cylinder walls reveal forelimb asymmetries that have resulted from different forms of brain injury, including cortical damage (Jones and Schallert 1992) and nigrostriatal neurodegeneration (Schallert et al. 2000). A video camera with slow-motion playback capability records the number of times the animal uses the ipsilateral (affected) or contralateral (less affected) forelimb alone or uses both simultaneously for upright support. Intact animals typically use both limbs equally for upright support. But after damage to the motor system, animals show an asymmetric reliance on the less-affected (ipsilateral) limb, although they may return to a more symmetrical use of the forelimbs over time depending on the type and extent of injury.

Forelimb Placing

The vibrissae-stimulated forelimb placing and extinction placing tests are methods for revealing sensorimotor/proprioception deficits. To determine whether an animal has asymmetrical sensorimotor perception, the test is designed to hold the animal by the torso with its forelimbs hanging freely, and then slowly move the animal laterally toward the edge of the table or countertop until the vibrissae of one side make contact with the edge. The apparatus records data about the percentage of time in which vibrissae stimulation elicits a “placing response” for each side: intact animals typically quickly place the ipsilateral forelimb on the edge of the surface when the ipsilateral vibrissae brush the table edge. In contrast, rats with damage to the motor system often do not respond to vibrissae stimulation on the affected side (e.g., Schallert et al. 2000). This deficit is even more pronounced with the extinction placing test, in which, as the animal’s vibrissae on one side brush the edge of the countertop, the experimenter gently stimulates the contralateral vibrissae, thus providing competing vibrissal stimulation (Schallert et al. 2000).

Swim Task (Forepaw Inhibition)

Kolb and Whishaw (1983) first noted that normal rats hold their forelimbs immobile under their chin while swimming and use their hindlimbs to propel them through the water. However, after brain injury, the animals make swim strokes with the forepaw contralateral to the damage. This task entails training the rats for 2 days to swim to a visible platform at the end of a rectangular aquarium. Once trained, the rats swim uninterrupted from one end to the other, facilitating the observation of forepaw inhibition behavior. On the third day, investigators videotape three trials for each
animal (a trial is defined as the animal’s swim from one end of the aquarium to a platform at the other end). These three trials establish a prelesion baseline measure. Most rats make fewer than one forepaw stroke per trial, and the number of strokes by each forelimb on each trial is the basis for calculating the swim score, which determines the postlesion assessment of forepaw inhibition behavior. The swim score of forelimb inhibition for each group is the number of contralateral forelimb strokes minus the number of ipsilateral forelimb strokes. After revascularization or MCA occlusion the scores increase from just above 0 to between 2 and 3. Even with several weeks of testing the scores do not return to prestroke levels in the absence of rehabilitative training. The test is very sensitive to brain injury and resistant to recovery (Gonzalez and Kolb 2003).

Adhesive Dot Removal

The adhesive dot removal test indicates that rats with brain injury show forelimb sensory asymmetries similar to those in human hemineglect patients. Small adhesive stimuli (labels) are randomly placed on the distal-radial aspect of both forelimbs. After their return to the home cage, the rats contact and remove the stimuli one at a time using their teeth. The order and latency of stimulus contact and removal is recorded for each of four trials. The order of contact determines whether animals show a bias for the stimulus on the forelimb that is either unaffected or affected by the injury. The test includes a fifth trial for animals that show either a 25% or 75% preference for removing the stimulus from the unaffected forelimb first. All animals with a bias (removal of the stimulus from the unaffected limb first on more than 70% of the trials) are tested to determine the magnitude of the somatosensory asymmetry. Assessment of the magnitude of asymmetry involves progressively increasing the size of the stimulus to the affected limb (A) and decreasing it for the unaffected limb (U). The A/U ratio necessary to reverse the initial bias is proportional to the degree of brain damage (Barth et al. 1990; Schallert and Whishaw 1984).

Montoya Staircase Test

The Montoya staircase task is a reach-to-grasp test for assessing skilled forelimb use. The task requires rats to learn to reach from a central platform with their forelimbs to retrieve food pellets at variable distances on a descending “staircase.” The test is a challenging skilled motor task even for intact animals and is highly sensitive to the impairing effects of motor system damage (e.g., Montoya et al. 1991). The rats ascend to an elevated shelf above which a low ceiling enforces a relatively fixed supine position. On either side of the animal is a shelf with six descending steps with shallow wells that contain food pellets. The pellets in lower wells are more difficult to grasp than those in wells higher on the staircase. The percentage of pellets that the animal retrieves determines the assessment of changes in skilled forelimb use. Use of a distinct color (e.g., with food coloring) for the pellets in each stair step reveals the percentage of pellets retrieved from a particular staircase level (see Adkins and Jones 2005).

Foot Fault

Various forms of foot fault tasks are effective for assessing forelimb and hindlimb function. The general approach is to place the animals on an elevated grid or ladder that requires them to carefully place their paws on the wire between openings (Hernandez and Schallert 1988). A video camera positioned below the grid records the rats’ stepping pattern. With each weight-bearing step, the paw may fall or slip between the wires, and when it does it counts as a foot fault. The contrast between the number of faults for the forepaw contralateral to the infarction and the number of successful steps gives the percentage of contralateral forelimb foot faults per forelimb steps. Fault calculations can also take into account different sizes of grids (Stroemer et al. 1995) or ladder rung spacing (Metz and Whishaw 2002). Performance in all cases is sensitive to cerebral infarction.

Sunflower Seed Opening

Rats are inherently adept at opening shelled seeds to obtain food, and sunflower seed opening is an effective measure of bilateral object manipulation (Whishaw et al. 1998) as well as of motor impairments after stroke (Gonzalez and Kolb 2003). The rats first manipulate the seed into a preferred position and then chew away a portion to facilitate splitting open the shell to obtain the seed (Whishaw et al. 1998). In this task, the animal receives a preset number of seeds and the investigator records the total amount of time the animal spends manipulating, opening, and consuming the seeds as well as the number of pieces of shell the animal broke in its efforts to open the seed. The animals perform the task in a clear plastic arena with five sunflower seeds placed in one of the corners. A mirror under the box is angled to allow the experimenter to videotape the animal’s activity from a ventral view. The experimenter starts timing the moment the animal touches the first seed and stops the timer every time the animal is distracted, with a limit of 300 seconds and 30 pieces of shell. Intact animals typically open the five seeds in 35 seconds and produce 11 pieces of shell (Gonzalez and Kolb 2003). After either MCA occlusion or cortical devascularization, rats show a marked increase in both the time to open the seeds and the number of pieces produced, although both of these measures decline over several weeks of training (Gonzalez and Kolb 2003).

Pasta Matrix Reaching Task

In the pasta matrix reaching task, rats reach through a high vertical slot for pasta pieces on a shelf attached to the out-
side of the front wall (Ballermann et al. 2000, 2001). The shelf contains an array of 13 × 20 holes for holding uncooked pieces of pasta, which are inserted so that they extend above the top of the shelf. The measure of performance is the number of pieces the rat obtains. In the half-matrix configuration, the pasta pieces fill only half the matrix holes. This configuration forces the animals to use only the paw contralateral to the pasta array, prevents them from using the unimpaired paw, and allows for comparisons between ipsi- and contralesional paws. Animals with motor cortex infarctions show a reduction in the number of pieces of pasta they can obtain with the contralesional paw. Further, with motor rehabilitation the rats progressively increase the number of pieces of pasta obtained (Teskey et al. 2003). The task therefore provides a sensitive measure of both motor impairment and improvement after stroke.

Tray Reaching

Several different versions of skilled reaching are suitable for testing forelimb motor performance, and rats can be readily trained to reach outside their home cage to obtain food. The tray reach task involves placing the rats in clear plastic training boxes that are open at the front with small metal bars. Outside the bars is a long tray containing food fragments weighing ≈ 30 mg each (chick feed is a frequent choice for this task). Animals learn to reach between the bars, grasp the food, and retract it to eat it. Subjects typically train for a total of 15 days before the lesion to establish a baseline measure of motor performance. Success is calculated as the percentage of successful reaches.

Single Pellet Reaching

Whishaw and Pellis (1990) trained rats to reach for and retrieve a single food pellet located on a shelf outside their cage. This task is considerably more difficult than the tray reaching because the animals must target a single food pellet rather than simply reaching into a tray filled with food. The exercise begins with a brief period of pretraining to familiarize the rats with the reaching task. This component involves placing them in pretraining cages that have trays filled with food pellets (45 mg; Bioserv) mounted on the front. The rats have to reach outside the cage and retrieve pellets from the tray; they remain in pretraining until they make 10 successful reaches. After pretraining, the rats transfer to a clear plastic cage with a 1-cm slot at the front. In this cage, they train each day to reach through the slot and retrieve individual food pellets from a table outside the cage. Training parameters may include time limits, number of pellets, or number of reaches. The rats may use either limb, and the preferred limb is noted for each animal. Videotaping of the sessions makes it possible afterward to assess reaching performance. Each time the animal grasps the food pellet and brings it into the cage and to its mouth without dropping it counts as a successful reach. The experimenters then calculate the percentage of successful reaches ([# successful retrievals/total # of reaches] × 100).

Skilled Reaching Movement Analysis

In addition to analyzing success rates in skilled reaching tasks, it is possible to analyze changes in the performance of the reaching movements. One method of analysis for 10 successful reaches is an adaptation of the qualitative reaching movement rating scale that Whishaw and colleagues (e.g., Whishaw et al. 1993; Metz and Whishaw 2000) developed, based on Eshkol-Wachman movement notation (Eshkol and Wachman 1958). This movement analysis is sensitive to compensatory forelimb movements that reveal enduring impairments or compensatory strategies in reaching and grasping motor action patterns after brain injury (e.g., Garabawie et al. 2005a; Metz and Whishaw 2000; Whishaw et al. 1993). The analysis has eight components:

1. Aim: The elbow is adducted while the digits retain their alignment with the midline of the reaching window and are oriented toward the food pellet;
2. Digits semiflexed: Digits are semiflexed before reaching through the window;
3. Digits open: The digits are open and extend toward the pellet as the limb advances;
4. Advance: The limb advances directly through the reaching window and extends to the food pellet;
5. Grasp: The pads of the palm or the digits touch the food and grasp it by closing the digits around the pellet;
6. Supination 1: The paw is dorsiflexed and supinated 90° as the limb withdraws through the reaching window;
7. Supination 2: The paw is supinated again by approximately 45° to bring the pellet to the mouth;
8. Release: The digits open and release the pellet into mouth.

During video slow-motion replay, each movement is rated with a score of 0 (normal), 0.5 (slightly abnormal), or 1 (absent or highly abnormal).
Important Considerations for Study Design

Stroke Model

There is no general agreement on the “best” model for inducing ischemic damage, but middle cerebral artery occlusion (MCAo) may be the most clinically relevant. There are also several methods for permanently or transiently occluding the MCA. A number of studies have used intraluminal occlusion, but it has several drawbacks in studies of sensorimotor deficits. First, the infarct size is variable and typically does not affect motor cortex. Second, blocking much of the length of the MCA with a suture may occlude other arteries (Dittmar et al. 2003; McColl et al. 2004) and thus cause damage to other brain regions not typically affected in human MCA stroke. Finally, and most importantly, the surgeries can be very invasive, requiring manipulation of multiple arteries that may result in a high mortality rate and health problems in those animals that do survive (Dittmar et al. 2003; Sharkey and Butcher 1995).

An alternative to physical occlusion is the stereotaxic injection of ET-1 adjacent to the MCA. This method is less invasive, does not require a large craniectomy, and produces a pattern of ischemic damage similar to the more traditional MCAo models (Sharkey et al. 1993). An advantage of the ET-1 MCAo model is that blood flow restriction is not immediate (Macrae et al. 1993) and reperfusion occurs over several hours (Biernaskie and Corbett 2001; Macrae et al. 1993). These effects are more representative of human stroke than the immediate restriction and reperfusion seen with mechanical restriction. Windle and colleagues (2006) have recently compared the effects of topical, intracortical, and intracortical + intrastralial ET-1 administration on lesion size, motor function, and study success rate, with success defined as the percentage of animals that survived the manipulation and exhibited significant motor impairment. They report the combined administration of intracortical + intrastralial ET-1 as the most successful approach as it yielded the highest number of animals with significant motor impairments.

Using a Battery of Sensorimotor Tests

The second consideration when determining study design is the choice of an appropriate battery of sensorimotor tests that will be sensitive enough to detect a range of motor impairments across a significant time span. A summary of the tasks that reliably reveal changes in motor performance after injury is provided in Table 1. We recommend the use of tasks that enable measures of both preexisting and acquired motor abilities and that allow for both endpoint and qualitative assessments. For example, the single pellet reaching, forepaw inhibition, forepaw placement, and cylinder tasks facilitate a more comprehensive examination of the impact of a given therapy on recovery versus compensation and the generalizability of any observed improvements. A second important consideration is the time required to administer the tests and analyze performance as well as the sensitivity of the tasks for detecting impairments/recovery. A summary of the relative amounts of time required to perform and analyze the tests, as well as their sensitivity to detecting motor impairments, is shown in Table 2. Experimenters should base their choice of tasks on the nature of the experimental questions they are studying.

Establishing Stable Prestroke Measures of Motor Performance

The establishment of prestroke measures of motor performance requires the averaging over time of multiple testing sessions prior to insult. Measures from a single testing session can be highly variable and unreliable. It is also important to counterbalance prestroke performance across treatment conditions, assigning animals such that the mean prestroke performance on all tests is equivalent. Such advance measures can prevent the potential problem of having animals in any one treatment condition with higher or lower prestroke levels of performance, which would make relative levels of improvement difficult to interpret.

Motor Testing Is a Form of Rehabilitation

If the purpose of the experiment is to assess the impact of rehabilitation on some measure of brain structure (dendritic

<table>
<thead>
<tr>
<th>Task</th>
<th>Time to administer</th>
<th>Time to analyze</th>
<th>Sensitivity to lesion size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cylinder task</td>
<td>Low</td>
<td>Medium</td>
<td>Medium</td>
</tr>
<tr>
<td>Forelimb placing</td>
<td>Low</td>
<td>Very low</td>
<td>Medium</td>
</tr>
<tr>
<td>Swim task</td>
<td>Low</td>
<td>Very low</td>
<td>Medium</td>
</tr>
<tr>
<td>Adhesive dot removal</td>
<td>Medium</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Montoya staircase</td>
<td>Low</td>
<td>Medium</td>
<td>Low</td>
</tr>
<tr>
<td>Foot fault</td>
<td>Low</td>
<td>Medium</td>
<td>Low</td>
</tr>
<tr>
<td>Sunflower seed opening</td>
<td>Medium</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Pasta matrix</td>
<td>Medium</td>
<td>Very high</td>
<td>Medium</td>
</tr>
<tr>
<td>Tray reaching</td>
<td>Medium</td>
<td>Low to high</td>
<td>Medium</td>
</tr>
<tr>
<td>Single pellet</td>
<td>High</td>
<td>Very high</td>
<td>High</td>
</tr>
<tr>
<td>Movement analysis</td>
<td>Very high</td>
<td>Very high</td>
<td>Very high</td>
</tr>
</tbody>
</table>
models have included the creation of an exhaustive battery of sensorimotor tests to examine the neural substrates underlying functional impairments and recovery as well as the efficacy of adjuvant therapies, and they therefore have the potential to help guide the development of novel rehabilitation therapies for human stroke patients. However, it is important to keep in mind that animal models will never duplicate the complexities of the human condition. The success of translational rehabilitation research will depend on both clinical and basic scientists working to adapt the inherent limitations of animal models. Basic scientists must identify preclinical measures of motor performance that are homologous to the clinical measures. Translation of the efficacy of various treatments in animal models will depend on bringing the preclinical and clinical measures closer together. Finally, we caution that animal research will not provide information on specific methods of delivery for motor rehabilitation in the clinic. Animal research will, however, reveal neurobiological phenomena related to motor recovery and identify fundamental principles that may help to guide the optimization of motor rehabilitation.

References


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Oppositions


