

Behavioral outcome measures used for human neural stem cell transplantation in rat stroke models

Matthew B. Jensen, Dong Y. Han,
Abdullah Al Sawaf,
Rajeev Krishnaney-Davison

Department of Neurology, University
of Wisconsin, Madison, USA

Abstract

Stroke is a leading cause of death and disability, leading to the development of various stroke models to test new treatments, most commonly in the rat. Human stroke trials focus on disability, related primarily to neurological deficits. To better model the clinical application of these treatments, many behavioral tests have been developed using the rat stroke model. We performed a systematic review of all the behavioral outcome measures used in published studies of human neural stem cell transplantation in rat stroke models. The reviewed tests include motor, sensory, cognitive, activity, and combination tests. For each test, we give a brief description, trace the origin of the test, and discuss test performance in the reviewed studies. We conclude that while many behavioral tests are available for this purpose, there does not appear to be consensus on an optimal testing strategy.

Introduction

The majority of our very limited understanding of the pathophysiology of stroke is a direct result of the use of animal models. We know too little about the complex molecular and cellular events of stroke to allow for *in vitro* or computer models, and we cannot yet access this information in human stroke victims, although rapid advances in stroke imaging holds promise.

While stroke models have been developed in many species, the rat is by far the most commonly used.^{1,2} There are a variety of models that allow for control over the size and location of focal ischemia in the rat brain causing ischemic stroke (IS), and several models of intracerebral and subarachnoid hemorrhage as well.²

The majority of preclinical testing of new stroke treatments has focused on the outcome of reducing lesion volume relative to controls. This strategy makes intuitive sense, but there has been a remarkable number of translation-

al failures in stroke, prompting great debate about the discrepancy between the results of these interventions in animals and humans.³ One explanation could be that the stroke models are invalid because the biology of humans and animals is too fundamentally different. Few authors believe this to be the case, however, because what is currently known about the pathophysiology of stroke appears to be very similar in the various species used and in non-human primates. Another explanation could be that apples are being compared to oranges, as lesion volume differences in the animals may not correlate with the neurological and functional outcomes used in human trials. This appears to be likely, as it is clear that there is only modest correlation between cerebral lesion size and severity of neurological and functional deficits in humans. For example, a very small lesion in the brainstem may cause total paralysis of one side of the body, but large lesions encompassing most of one temporal lobe may cause only subtle cognitive deficits that are not appreciable to casual observation.

There is a need, therefore, to develop neurological outcome measures for the stroke models, usually termed behavioral or functional outcome measures, which might better predict the ability of a new treatment to improve neurological deficits following human stroke. This has proved to be more difficult in rats than was perhaps initially thought, as it turns out that very large cerebral lesions in rats may cause only subtle and/or transient changes on casual observation. Most of the behavioral tests described were developed in a nonsystematic manner during the refinement of particular stroke models or with experiments of novel treatments, making it difficult to compare across published studies.

Rat stroke models

Like humans, the rat brain has two cerebral hemispheres with an exterior of grey matter cortex and an interior of white matter and grey basal ganglia, as well as a brainstem and cerebellum. The rat brain is smooth surfaced (lissencephalic), unlike the convolutions of gyri and sulci of the human brain (gyrencephalic), but there are motor and somatosensory cortices. The rat brain has much less hemispheric white matter than the human brain.

Lesions of motor cortex cause contralateral weakness, in coordination, and mobility deficits. The portion of the motor cortex injured will determine whether the forelimb, hind limb, or both are involved; the same is true for sensory loss with lesions of the sensory cortex. Injury to the striatum and deep white matter causes combinations of motor or sensory deficits. Cognitive deficits can be found with injury to most areas of the brain, but particularly with cortical injury.

Correspondence: Matthew B. Jensen, Comprehensive Stroke Program, Department of Neurology, School of Medicine and Public Health, University of Wisconsin, 1685 Highland Ave, room 7273, Madison, WI 53705-2281, USA. Tel. +1.608.263.5420 - Fax: +1.608.263-0412. E-mail: jensen@neurology.wisc.edu

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The most commonly used rat stroke model is intraluminal filament middle cerebral artery occlusion (iMCAO), which can be transient (tMCAO) of varying duration, commonly 30 to 120 minutes, or permanent (pMCAO). In this model, various types of filament, typically nylon sutures with coated or non-coated tips, are inserted and advanced up the internal carotid artery (ICA) until they lodge in the anterior cerebral artery (ACA), thereby occluding the origin of the middle cerebral artery (MCAO). Short durations of ischemia cause selective neuronal loss without disruption of tissue architecture, while longer durations of ischemia cause frank necrosis of varying volume of the MCA territory. A shorter duration of tMCAO tends to cause infarcts primarily of the striatum, as collateral circulation on the surface of the brain appears to allow the cortex to be spared. Brief duration tMCAO would, therefore, be expected to produce mild motor and sensory deficits, while longer duration of tMCAO and pMCAO would be expected to produce more severe motor, sensory, and cognitive deficits. These procedures can be combined with transient or permanent occlusion of the carotid or vertebral arteries to increase the lesion size and corresponding deficits.

With craniotomy, it is possible to directly occlude the distal MCA (dMCAO) with suture, clips, or cautery. This can also be transient or permanent (tdMCAO or pdMCAO), and can be combined with concomitant transient common carotid artery occlusion (tCCA) to cause more severe ischemia. This occlusion occurs more distally along the MCA than iMCAO, which tends to cause cortical infarcts sparing the striatum, producing variable motor, sensory, and cognitive deficits.

Certain types of lasers and light-activated compounds in the blood may produce focal brain injuries via micro- or macro-vascular thromboses, with or without craniotomy. These photochemical stroke models cause discrete injuries of various size and location, leading to variable motor, sensory, and cognitive deficits.

Emboli may be introduced into any of the cervical arteries to occlude cerebral arteries of various sizes depending on the size of the embolic materials. These embolic ischemic stroke models (EISM) can use either clot or non-clot materials such as metal or plastic spheres. Clot can be made from various sources and processed in different ways to encourage or discourage spontaneous thrombolysis, while non-clot EISM are permanent. Attempts have been made to create EISM that selectively cause MCAO, but in general, most of these models cause single or multiple infarcts of variable location and size, therefore causing variable motor, sensory, and cognitive deficits.

Intracerebral hemorrhage models can be targeted (such as by injection of blood or proteolytic enzymes) or untargeted (such as thrombolytic administration and blood pressure elevation), causing variable deficits. Subarachnoid models may produce focal deficits, but more commonly produce stupor or death due to the rapid increase in intracranial pressure.

Materials and Methods

We performed a systematic review with the objective of identifying all the behavioral outcome measures used for human neural stem cell transplantation in animal stroke models. We searched PubMed, Science Citation Index, and Biological Abstracts for articles that matched our inclusion criteria (Appendix 1). We included any behavioral test used in controlled experiments transplanting cultured human cells expressing neural stem cell markers *in vitro* prior to transplantation into a rat stroke model of focal cerebral ischemia, intracerebral hemorrhage, or subarachnoid hemorrhage. Our search was performed in April of 2010, and limited to full articles in the English language published prior to January 1, 2010. No other limits were used. This searched produced 510 results. We reviewed titles, abstracts, or full articles to determine if our inclusion criteria were met. We found 21 articles that matched our inclusion criteria.⁴⁻²⁵ The references included in these articles were reviewed for more matching studies, but none were found. An ischemic stroke model was used in 19 studies while two studies used an intracerebral hemorrhage model; none of the

included studies used a model of subarachnoid hemorrhage.

For each test identified, we give a brief description, the origin of the test as could be determined by tracing references back from the reviewed article, and a discussion of the test performance in the reviewed studies. The tests are categorized as motor, sensory, cognitive, activity, and combination tests, although the true neurological function assessed by any of the tests is a matter of debate.

Review

Motor Tests

Elevated body swing

Description

The elevated body swing test (EBST) consists of lifting the rat by the base of the tail, whereby it will laterally swing its body to one side or the other; repeated trials are performed to see if it has a bias toward one side. A swing bias toward one side has been interpreted as a motor deficit.

Origin

In 1995 Borlongan *et al.* reported the development of the EBST and its successful use to distinguish a unilateral Parkinson's disease rat model from sham.²⁶ In a separate 1995 article, Borlongan *et al.* reported that the EBST successfully distinguished sham from 60 min tifMCAO rats up to two months after stroke.²⁷

Use in human neural stem cell transplantation studies

Several studies reported significant improvement of grafted rats relative to control on the EBST up to six months after transplantation, using 30, 60, and 90 min tifMCAO rat models.^{5-8, 16, 25}

Rotarod

Description

The rotarod is a horizontal cylinder large enough to stand on, which is then rotated at a variable speed or acceleration until the rat falls off. Diminished speed tolerance has been interpreted as a motor deficit.

Origin

In 1957 Dunham *et al.* reported the development of the rotarod test to assess neuroactive drugs on rodents, and in 1994, Hamm *et al.* found it useful to distinguish rats with traumatic brain injury from sham.^{28,29} In 2000 Chen *et al.* reported that the rotarod test successfully distinguished pre-stroke rats from the same rats up to one week after 120 min tifMCAO.³⁰

Use in human neural stem cell transplantation studies

Several studies reported significant improvement of grafted rats relative to control on the rotarod test up to two months after transplantation, using 60, 90, and 120 min tifMCAO, and intrastriatal collagenase rat models.^{9,11,12,19-21}

Turning in alley

Description

The turning-in-alley test (TIAT) consists of the rat being placed facing the back wall of an alley, and the time for turning around recorded over repeated trials. A longer time to turn around has been interpreted as a motor deficit.

Origin

In 2004 Chu *et al.* reported the development of the TIAT and stated that it successfully distinguished pre-stroke rats from the same rats up to one month after 90 min tifMCAO.¹¹

Use in human neural stem cell transplantation studies

In the same 2004 report, Chu *et al.* stated there was significant improvement of grafted rats relative to control on the TIAT test up to one month after transplantation.

Rotameter

Description

The rotameter test consists of the rat moving in a bowl-shaped device that counts turns and direction, usually after the administration of amphetamine to increase rotations. A tendency to rotation primarily in one direction has been interpreted as a motor deficit.

Origin

In 1970 Ungerstedt *et al.* reported the development of the rotameter test, and stated that it successfully distinguished a unilateral Parkinson's disease rat model from control.³¹ In 2001 Veizovic *et al.* reported that the rotameter test successfully distinguished sham and 60 min tifMCAO rats up to seven weeks after stroke.³²

Use in human neural stem cell transplantation studies

Two studies reported significant improvement of grafted rats relative to control on the rotameter test up to three months after transplantation in 60 and 70 min tifMCAO rats.^{13, 23}

Ledged beam

Description

The ledged beam test (LBT) consists of the rat walking on a narrow beam with ledges on the sides and counting each time a hind limb touches the ledge. Asymmetry has been interpreted as a motor deficit.

Origin

In 2002 Schallert *et al.* reported the development of the LBT and stated that it successfully distinguished MCAO rats from sham.³³

Use in human neural stem cell transplantation studies

One study reported significant improvement of grafted rats relative to control on the LBT up to one month after transplantation in rats with pdMCAO plus 60 min tCCA0.14

Cylinder

Description

The cylinder test consists of the rat being placed in a cylinder that is wide enough to allow movement and to encourage wall exploration. The rat explores the space by rearing and placing one or both forelimbs on the cylinder wall, and then using one or both forelimbs to land back on the floor. All of these movements may be counted for a certain length of time. Decreased use of the impaired forelimb has been interpreted as a motor deficit.

Origin

In 2000 Schallert *et al.* reported the development of the cylinder test and its successful use to distinguish a unilateral Parkinson's disease rat model from sham.³⁴ In a separate 2000 article, Schallert *et al.* reported that the cylinder test successfully distinguished sham and pdMCAO rats up to one month after stroke.³⁵

Use in human neural stem cell transplantation studies

Two studies reported significant improvement of grafted rats relative to control on the cylinder test up to two months after transplantation, using rats with 90 min tifMCAO and pdMCAO plus 60 min tCCA0.^{17,24}

Staircase

Description

The staircase test consists of the rat being placed in a box with narrow staircases descending on both sides with a food pellet on each step. Each step is progressively further away and harder to reach with the forelimb. Limitations of the number of pellets retrieved have been interpreted as a motor deficit.

Origin

In 1991 Montoya *et al.* reported the development of the staircase test and its successful use to distinguish rats with unilateral cortical suction lesions from sham.³⁶ In 2009 Hicks *et al.* reported that the staircase test successfully distinguished sham and rats with pdMCAO plus 60 min of bilateral tCCA0 up to two months after stroke.²⁴

Use in human neural stem cell

Transplantation studies

In the same 2009 report, Hicks *et al.* stated that they did not see significant improvement of grafted rats relative to control on the staircase test through two months after transplantation.

Sensory tests

Limb placing

Description

The limb placing test (LPT) scores limb placing reactions to visual, tactile, and proprioceptive stimuli, usually by using the edge of a table to produce the stimulus. Slow, incomplete, or absent placing has been interpreted as a sensory deficit of the tested modality.

Origin

In 1989 De Ryck *et al.* reported the development of the LPT and its successful use to distinguish photochemical stroke model rats from sham up to three weeks after stroke.³⁷

Use in human neural stem cell transplantation studies

Several studies reported significant improvement of grafted rats relative to control on the LPT up to three months after transplantation, using 60 and 90 min tifMCAO, and intrastriatal collagenase rat models.^{9,11,12,18,23}

Adhesive removal

Description

The adhesive removal test (ART) consists of placing a small adhesive-backed tape circle or strip to both forelimbs. The times to contact and/or removal are recorded. A variant test involves using progressively larger adhesive pieces on the contralateral side with progressively smaller dots on the ipsilateral side. A longer time for contact and removal of the adhesive material has been interpreted as a sensory deficit of that limb.

Origin

In 1982 Schallert *et al.* reported the development of the ART and its successful use to distinguish a unilateral Parkinson's disease rat model from sham.³⁸ In 1992 Markgraf *et al.* reported that the ART successfully distinguished sham from pdMCAO rats up to 20 days after stroke.³⁹

Use in human neural stem cell transplantation studies

Several studies reported significant improvement of grafted rats relative to control on the ART up to three months after transplantation, using 60, 70, and 90 min tifMCAO, and pifMCAO rat models.^{10,13,19,21,23}

Cognitive tests

Passive avoidance

Description

The passive avoidance test (PAT) uses a mild electric shock to teach the animal to move and stay away from certain areas of the cage. Diminished ability to do so has been interpreted as a deficit of learning.⁴⁰

Origin

In 1978 Sanberg *et al.* reported the development of the PAT and its successful use to distinguish a unilateral Huntington's disease rat model from sham.³⁸ In 1993 Nishino *et al.* reported that the PAT successfully distinguished sham from 60 min tifMCAO rats up to 3 months after stroke.⁴¹

Use in human neural stem cell transplantation studies

Several studies reported significant improvement of grafted rats relative to control on the PAT up to six months after transplantation, using 60min tifMCAO rat models.^{5, 6, 8}

Activity tests

Locomotor activity

Description

The locomotor activity test (LAT) consists of one of several systems to quantify open field locomotor activity, such as specific movements around the cage during a period of time. Most of these systems use direct observation or beams of light arranged in a grid, with a computer recording each time the animal moves through a beam. Changes in activity patterns with stroke, usually hyperactivity, have been interpreted to be either motor or cognitive deficits. Motor deficits could cause decreased movement in general or a different pattern of movements to compensate for the deficits. Cognitive deficits could conceivably lead the animal to explore the environment more because of a deficit of spatial perception or memory.

Origin

In 1987 Sanberg *et al.* reported the development of a LAT and its successful use to distinguish rats that received neuroactive drugs from control.⁴² In 1995 Borlongan *et al.* reported that the LAT successfully distinguished sham from 60 min tifMCAO rats up to 2 months after stroke.²⁷

Use in human neural stem cell transplantation studies

One study reported significant improvement of grafted rats relative to control on the LAT up to two months after transplantation, using a 30min tifMCAO rat model.¹⁶

Combination tests

Neurological severity score

Description

The neurological severity score (NSS) is a composite of motor (muscle status, abnormal movement), sensory (visual, tactile, proprioceptive), reflex, and balance tests.

Origin

In 2002 Li *et al.* reported the development of the NSS and its successful use to distinguish 120 min tiffMCAO rats from sham up to two weeks after stroke.⁴³

Use in human neural stem cell transplantation studies

Several studies reported significant improvement of grafted rats relative to control on the NSS up to two months after transplantation, using 90 and 120min tiffMCAO, and pifMCAO rat models.^{10,19,20,22}

Motor behavior index

Description

The motor behavior index (MBI) is a composite of spontaneous activity, symmetry of movement of the limbs when suspended by the tail, climbing a wire cage, and reaction to touch of the body and vibrissae.

Origin

In 1995 Garcia *et al.* reported the development of the MBI and its successful use to distinguish 30 and 60 min tiffMCAO, and pifMCAO rats from sham up to one week after stroke.⁴⁴

Use in human neural stem cell transplantation studies

One study reported significant improvement of grafted rats relative to control on the MBI up to three weeks after transplantation, using a 120 min tiffMCAO rat model.¹⁵

Bederson

Description

The Bederson test was a composite of forelimb flexion with tail suspension, response to lateral push, and circling with walking.

Origin

In 1986 Bederson *et al.* reported the development of the Bederson test and its successful use to distinguish proximal versus distal pifMCAO rats at one day after stroke.⁴⁵

Use in human neural stem cell transplantation studies

One study reported significant improvement of grafted rats relative to control on the Bederson test up to two months after transplantation, using a 30 min tiffMCAO rat model.¹⁶

Conclusions

Animal stroke models, primarily using the rat, are currently necessary to further our understanding of the disease and to test new treatments in the hopes of improving outcomes for our patients. The ability of a new treatment to reduce infarct volume in an animal model of stroke has not translated to improvement on neurological and functional outcome measures in human trials. There are many behavioral outcome measures that have been developed for rat stroke models assessing multiple domains of neurological function. Despite this there does not appear to be consensus on an optimal behavioral testing strategy in any particular model, or for any particular category of treatment under evaluation. Hopefully, in the coming years, we will see multiple new treatments successfully translate promising new interventions from preclinical studies into positive clinical trials, which would then allow us to go back and re-assess the most useful behavioral outcome measures in the various rat stroke models. Until such information is available, we feel that a reasonable testing strategy would include an assessment of multiple domains of neurological function including motor, sensory, and cognitive tests, which can be tailored to the stroke model being used, and refined through iterative use in the lab of each investigator, based on their experimental aims and available resources.

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