

Experimental approaches to study functional recovery following cerebral ischemia

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Abstract Valid experimental models and behavioral tests are indispensable for the development of therapies for stroke. The translational failure with neuroprotective drugs has forced us to look for alternative approaches. Restorative therapies aiming to facilitate the recovery process by pharmacotherapy or cell-based therapy have emerged as promising options. Here we describe the most common stroke models used in cell-based therapy studies with particular emphasis on their inherent complications, which may affect behavioral outcome. Loss of body weight, stress, hyperthermia, immunodepression, and infections particularly after severe transient middle cerebral artery occlusion (filament model) are recognized as possible confounders to impair performance in certain behavioral tasks and bias the treatment effects. Inherent limitations of stroke models should be carefully considered when planning experiments to ensure translation of behavioral data to the clinic.

Keywords Behavioral tests · Brain repair mechanisms · Cell-based therapy · Recovery of function · Restorative therapies · Stroke models

Introduction

Cerebral ischemia remains one of the main causes of death in western countries, which is surpassed only by heart

disease and cancer. In addition to a high rate of mortality, more than 50% of stroke patients are left with a motor disability, and stroke causes more loss of quality-adjusted life years (QALY) than any other disease in the west [1]. The aging population will see a dramatic increase in the burden of stroke if progressive improvements in health care and risk factor management are not maintained [2].

Preventive therapies and lifestyle modification are known to reduce stroke incidence and mortality [3]. Unfortunately, there is no effective drug therapy to help stroke patients during the acute phase except thrombolysis, which is available only for a small fraction of patients because of a narrow therapeutic time window [4, 5]. New endovascular devices and techniques are emerging, but again only a limited number of patients will benefit from them [6]. Neuroprotective drugs are effective in experimental settings, but despite huge effort, none have so far worked in clinical practice [7]. A number of suggested shortcomings may explain the translational failure, including the validity of experimental models and the lack of functional outcome measures. Regarding the evaluation of neuroprotective trials, readers are referred to recent reviews [7–9].

Various degrees of spontaneous recovery at weeks or months after a cerebrovascular event are observed in stroke patients [10] and have been linked to the brain's own repair mechanisms [11–13]. Early recovery following stroke is suggested to be due to the resolution of cerebral edema, the absorption of damaged tissue, improvement of local circulation, resolution of remote functional depression (i.e., diaschisis) and activation of alternate pathways [14, 15]. Later stages of recovery involve neurite growth and synaptogenesis [16], neurogenesis [17, 18] and angiogenesis [19]. Taken together, it is likely that multiple mechanisms are involved in recovery processes, and interactions between these restorative events underlie the improvement

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in functional outcome [12, 13]. In addition, emerging evidence suggests that peripheral mechanisms such as immunodepression [20] may contribute to behavioral outcome after cerebral ischemia.

Stimulating brain repair opens a new horizon that aims to facilitate neurological recovery. Cell-based therapies have emerged as one of the most promising experimental approaches to restore brain function after stroke [21–23]. The concern remains, however, whether neuronal restoration will face similar translational problems as described with neuroprotective drugs. Another issue is whether cell-based therapies enhance true recovery, compensatory strategies or just general well-being of animals after cerebral ischemia. The present review shortly describes the most common stroke models available for the assessment of cell-based therapies. A particular emphasis is placed on their inherited limitations and complications, which may affect behavioral performance of animals and bias treatment effects.

Stroke models

Animal research remains critical to the understanding of the basic mechanisms of ischemic damage and functional recovery thereafter. Preclinical models are also essential in drug development. Rat models for stroke (Table 1) are and will long be the first choice because of similar vasculature to humans, low cost, a number of well described behavioral outcome measures compared with those in mice and availability of translational imaging modalities (particularly MRI). However, there is an apparent need to confirm rat data in higher species, as suggested by the STAIR committee [24]. Excellent reviews on the technical details of different stroke models are available [25–28].

Transient middle cerebral artery occlusion (filament model)

The majority of strokes are ischemic (80%), and the middle cerebral artery (MCA) is the most commonly affected vessel in stroke. Spontaneous reperfusion occurs in the majority of untreated stroke patients during the first week after the onset of symptoms [29]. Since the patients exhibit either a transient ischemia of varying duration or a permanent ischemia, the pathophysiological correlates may merge seamlessly. Thus, both reperfusion and permanent occlusion models are justified and of clinical relevance.

Transient occlusion of the MCA (MCAO) using the intraluminal thread is perhaps the most widely used reperfusion model in rats [30]. Although craniotomy is not needed, one has to realize that the model is very invasive. The operation takes 2–3 h followed by intensive

postoperative care during the first few days after operation. The basic procedure is simple. The common carotid artery is exposed through a midline ventral cervical incision under a surgical microscope and carefully separated from the adjacent nerve. Then the filament is inserted into the stump of the external carotid artery (ECA) and advanced 1.9–2.1 cm into the internal carotid artery until resistance is felt. The filament is held in that place for 30–120 min. After occlusion, the filament is removed, allowing reperfusion, and the ECA is closed. A longer occlusion time seems to produce a more consistent infarct size involving cortical areas [31]. A typical infarct includes extensive corticostriatal damage, but also variable damage to the hypothalamus outside the vascular territory of the MCA. Widespread damage is associated with complex and long-lasting behavioral impairment [32–34].

A number of modifications are described for the model, and the main adjustment is the choice of filament. The diameter of the filament varies up to 0.3 mm depending on rat strain, sex, weight and age [35]. More important is the tip of the filament, which ensures adequate blockade of the blood flow from the internal carotid artery and the back-flow from the anterior cerebral artery and posterior communicating artery. Heat blunted or rounded filament tips prevent accidental artery perforation. To further increase adhesion between the filament and the endothelial surface, a filament can be coated either with silicone or poly-L-lysine [31, 36, 37]. Both are reported to reduce interanimal variability. Commercial coated filaments are available (e.g., from Doccol Corp.), but those made from surgical suture or even from fishing line will work successfully.

Endothelin-1 model

Endothelin-1 (ET-1) is a potent vasoconstrictor that has been used to produce ischemic injury. In the method described by Sharkey et al. [38], stereotaxic injection of endothelin-1 adjacent to the MCA results in rapid, but not immediate, blood flow reduction that is followed by reperfusion over several hours. Cerebral blood flow (CBF) below 100 ml/100 g/min for at least 10 h is required to produce irreversible injury [39]. A typical injury involves the lateral cortex and dorsolateral striatum, while the forelimb motor cortex is usually spared. A major advantage compared to the filament model is that the brain regions outside the vascular territory of the MCA are spared and, for example, there is no hyperthermic response. Technically though, accurate placement of ET-1 is difficult and may explain the low success rate, which can be partially improved by increasing the ET-1 concentration and making multiple injection sites. At the behavioral level, the model is characterized by

Table 1 Advantages and disadvantages of common experimental stroke models

Stroke model	Advantages	Disadvantages
Transient MCA occlusion (filament model)	Probably the most widely used experimental stroke model Possible to assess reperfusion damage Drug penetration to the occluded area No craniectomy Permanent occlusion possible	Invasive operation Variability in lesion size and location Infarct outside MCA territory Hemorrhage High mortality Incomplete occlusion Loss of body weight Hyperthermia Immunodepression Infections
Endothelin-1 (spontaneous reperfusion)	Spontaneous reperfusion Number of variations Pure cortical or corticostriatal infarct	Craniectomy Variability in lesion size
Distal or proximal MCA occlusion (permanent occlusion)	Consistent occlusion Low mortality No loss of body weight	Craniectomy No recanalization involved Limited penetration of drugs to occluded areas
Photothrombosis	Pure cortical or corticostriatal infarct Noninvasive Exact location and size of the lesion Minimal mortality	Damage to the blood-brain barrier Severe edema Minimal penumbral region
Embolization	The best face validity Allows assessment of thrombolysis and combination therapies Number of variations	Highly variable location of lesion

MCA middle cerebral artery

long-lasting impairment in forelimb reaching and postural support [39–41].

A number of modifications are described for the use of ET-1, which are relevant to stroke recovery studies. These include topical application of ET-1 onto the surface of the cortex [42, 43], which results in localized, reproducible focal cortical ischemic damage. Another modification is intracerebral injection of ET-1 to the cortex or in combination with the cortex and striatum [39].

Permanent proximal middle cerebral artery occlusion (Tamura model)

Direct occlusion of the MCA is possible through craniotomy followed by electrocoagulation or suturation of the MCA. However, a filament model where the filament is tied in permanently has more or less replaced the models that require craniotomy. Tamura et al. [44] described a permanent MCAO model, where using a subtemporal craniotomy, the proximal regions of the MCA are exposed below the olfactory tract and the MCA is permanently electrocoagulated. The zygomatic arch can be left intact.

As a result, an extensive neocortical and striatal infarct is produced, which is associated with persistent sensorimotor and cognitive deficits [45–47]. Disadvantages of the model include the demanding surgery. The collateral blood flow plays important roles in the evolution of the infarct and the way the treatment has access to regions at risk.

Permanent distal middle cerebral artery occlusion

A distal MCAO model was introduced by Chen et al. [48]. In brief, an incision is made between the eye and external auditory canal. The distal portion of the MCA is exposed through a small burr hole, and the artery is occluded just above the rhinal fissure by means of clip, suture or electrocoagulation. In normotensive rats, this has to be combined with ipsilateral permanent or bilateral transient (45–60 min) common carotid artery occlusion to produce consistent lesions. A typical lesion is demonstrated in the frontoparietal cortex, while subcortical structures are spared. In contrast to proximal MCA occlusion, sensorimotor deficits recover over time [45, 46, 49–51]. Weight loss or feeding difficulties are not as severe as in the

filament model, although there is damage to the facial muscles. Again, variations in vascular patterns complicate the operation.

Photothrombosis

Stroke models based on photochemical reactions require photosensitive dyes such as Rose Bengal and erythrosine B [52]. Light activation of the dye causes formation of free radicals and endothelial cell damage, aggregation of platelets and eventually occlusion of the vessel.

For the operation, the rat is placed in a stereotaxic frame, and the skull is exposed. An argon laser (514.5 nm) is used to activate photochemical dye in the original method [53]. Now less expensive lasers are available. A cold white light produces a similar lesion if the skull surface temperature is kept constant by cool air flow. When using cold light, an aperture ($\varnothing$ 1–4 mm) placed between the light source and the exposed skull determines the size of the lesion. The photochemical dye is administered intravenously via a microinjection pump, and the light is then turned on. The typical dose for Rose Bengal is 20–40 mg/kg. Higher doses are reported to be toxic to the liver. An elegant modification of this is the ring model, in which a ring-shaped laser beam produces an annular cortical lesion [54]. The region inside is suggested to undergo similar pathological processes as the penumbral region. The photothrombotic distal MCAO model has been described in spontaneously hypertensive rats [55].

The main advantage of the photothrombosis models is their noninvasive operation, which produces a consistent infarct with a precise location and size. In addition, mortality is low, and the success rate is high. The unique feature in the photothrombotic models is occlusion of small cortical vessels, so major arteries or branches are not affected. Thus, there is not much penumbral area or collateral flow, which is deemed important for neuroprotection and recovery processes. Another disadvantage of photothrombosis is damage to the blood-brain barrier, resulting in severe edema [56]. Delayed hemorrhage in the infarct border zone has also been reported [57]. Thus, the clinical relevance and application of this model remains to be seen.

Embolization

A number of animal models have been developed to mimic thromboembolic stroke. These involve injection of microspheres, macrospheres, a thrombotic clot or purified thrombin into the internal carotid artery. With microspheres ($<50\ \mu\text{m}$), placement and ultimate lodgment is not controlled, which results in multiple small lesions in variable locations [58]. Macrospheres such as TiO_2 spheres (300–400 μm) produce a more consistent permanent

occlusion of the MCA with a purely cortical infarct [59]. Autologous or heterologous blood clots have the best face validity to assess safety and efficacy of antithrombotic therapies [60]. Both spontaneously developing clots and thrombin-induced clots of variable number and size have been used. The generation of thrombus in the MCA is also possible by local injection of purified thrombin [61]. Although embolic models have their own advantages, less experience of their use in neuroprotective and restorative studies is available.

Peripheral complications in the filament model

The main inherent complications in all the stroke models are variability in lesion size and location, high mortality and hemorrhage [26, 62]. Anatomical variations of vascular collaterals in different rat strains explain part of the variability in neuronal damage [63, 64], and use of different rat strains may also contribute to the observed discrepancies in treatment efficacy [65]. A particular limitation of the filament model is the difficulty in filament insertion and the high mortality in aged rats [66, 67]. The lack of experience of the operator is another reason for variability, and only practice will improve this. Laser Doppler monitoring or behavioral testing during occlusion or after surgery can help to exclude rats with incomplete or partial occlusion. The peripheral complications varying from loss of body weight to infections are only well described for the filament model and are next discussed in detail.

The filament model is compromised by the poor health of the animal due to loss of body weight [68–70], postoperative stress, hyperthermia [71, 72] and immunodepression and peripheral infections [20, 73], which all affect behavioral outcome. Interestingly, stroke patients are also susceptible to many of these complications, and they have a substantial effect on outcome and often impede functional recovery [74]. Since the treatment may affect complications rather than true brain repair and plasticity, it is of importance to recognize and monitor complications whenever possible.

Dittmar et al. [68] provided evidence that transection of the ECA, which is necessary for filament insertion, produced ischemic tissue damage of the lingual and pharyngeal musculature, leading to impaired mastication and swallowing functions, and eventually loss of body weight. Although other factors such as the extensive corticostriatal damage per se are likely to contribute to the body weight loss [69], special care is needed to overcome this complication and to improve the overall condition of rats. Importantly, feeding difficulties and loss of body weight complicate all food-rewarded behavioral tasks. Poor

Table 2 Common behavioral tests for assessment of sensorimotor recovery after stroke

Behavioral test	Function	Advantages	Disadvantages
mNSS	Motor and sensory functions, balance and reflexes	Fast analysis, testing possible at acute phase	Subjective rating, spontaneous recovery, compensation
Rotarod	Coordination, balance and motor functions	Fast analysis, objective rating	Spontaneous recovery, compensation
Limb-placing	Limb responses to tactile and proprioceptive stimulation	Fast analysis, testing possible at acute phase	Subjective rating
Adhesive tape removal	Forelimb sensory asymmetry	Easy to score, possible to differentiate sensory and motor components	Affected by motivational state and alertness, motor aspects by practice effects and motor learning
Cylinder test	Spontaneous forelimb use	No pretraining	Complicated by immobility, habituation to the test
Ledged tapered beam-walking	Hindlimb functions	Not affected by compensation, both forelimb and hindlimb functions can be measured, long-lasting impairment	Complicated by immobility and overactivity
Reaching tasks	Skilled forelimb use	Sensitive to lesion size, differentiate true recovery vs. compensation, long-lasting impairment	Usually laborious, food-deprivation, larger infarcts do not allow recovery

mNSS modified neurological severity score

health of ischemic rats and postoperative care also cause additional stress, which are likely to affect behavioral outcome. Kirkland et al. [75] showed that post-lesion stress delays motor recovery and, in addition, leads to an enlarged infarct size.

Acute hyperthermia is another well-known inherent complication in the filament model. Hyperthermia is due to variable damage to the hypothalamus by the blockade of blood flow to the hypothalamic artery by the filament [72, 76, 77]. Even mild hyperthermia aggravates the histopathological outcome in MCAO rats [71]. After severe intracerebral hemorrhage, however, it was shown that mild to moderate hyperthermia does not worsen behavioral outcome [78]. On the other hand, some of the ischemic animals are hypothermic, possibly because of weight loss and immobility.

In addition, severe ischemic damage produced by the filament model induces a profound immunodepression [20]. This is mediated by injury-induced release of immune modulators such as interleukins, which activate the hypothalamo-pituitary-adrenal axis and sympathetic nervous system, leading to depression of peripheral immunity, followed by infections [20, 73]. Infection prevention improves survival and neurological outcome in ischemic mice [79]. Delayed and prolonged peripheral inflammation in the absence of a febrile response is also known to impair functional and histological outcome in MCAO rats [80]. Recently Engel et al. [81] suggested that prophylactic antibiotic therapies should be included in experimental stroke studies to prevent infectious complications.

Behavioral tests for the assessment of sensorimotor impairment and recovery

Numerous tests are available to assess behavioral impairment following cerebral ischemia [82–87]. The tests vary from simple tasks measuring general severity of neurological impairment to more demanding reach tasks that measure upper extremity function (Table 2). To evaluate the effect of cell-based therapies, a battery of behavioral endpoints should be selected that are sensitive to the degree of injury, sites of damage and severity of impairment [88]. In clinical settings, recovery of function after stroke is most often measured as improvement of fine motor skills, such as the dexterity of the hand, or gross motor skills, such as walking or maintenance of gait.

Some complications of the stroke models may have a direct impact on behavioral outcome (Fig. 1). Particularly poor health status due to the loss of body weight, stress, hypothermia and infections may impair behavioral performance. Thus, one could assume that quick resolution of impairment (spontaneous recovery) or treatment induced behavioral improvement may arise from improved recovery from the complications, rather than stroke recovery. This is especially likely with simple tests assessing gross neurological impairment following stroke.

Simple tests for assessing gross behavioral impairment

Perhaps the most common test in stroke recovery studies is the modified neurological severity score (mNSS), which

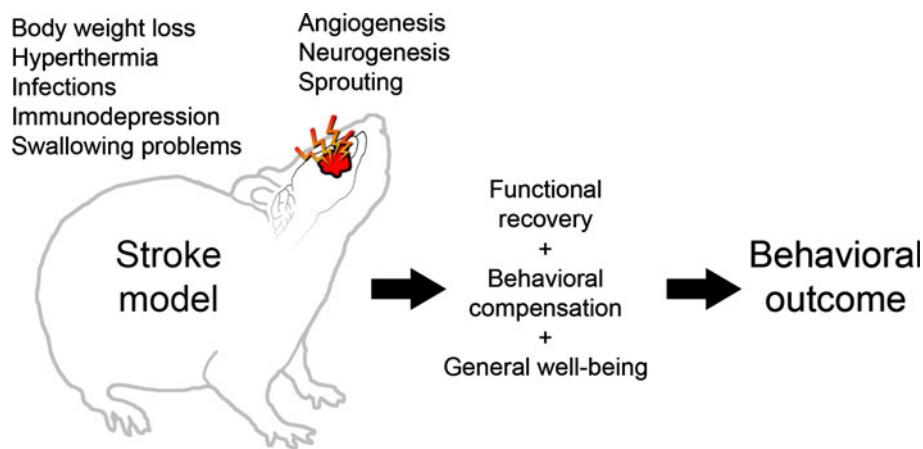


Fig. 1 Focal cerebral ischemia activates the brain's own repair mechanisms, such as angiogenesis, neurogenesis and sprouting. However, at the acute phase behavioral performance is compromised by poor health of the animal due to loss of body weight, postoperative

stress, hyperthermia, immunodepression and peripheral infections. These complications and poor general well-being may impair functional recovery and increase compensatory strategies, leading eventually to biased interpretation of behavioral outcome data

consists of several subtests measuring reflexes, balance, sensory responses and simple motor functions [37, 89, 90]. One point is given for the inability to perform each task. Scoring is subjective, and summing up scores from different subtests may mask specific deficits. Spontaneous recovery or compensation is evident, and the test is less useful for long-term studies.

The limb-placing test is another test to assess forelimb and hindlimb responses to tactile and proprioceptive stimulation. Anatomical correlates of the behavioral impairment are nicely described in the Rose Bengal model [91]. The modified test consists of seven subtasks where tactile stimulation is elicited by contacting the limb being tested with a table surface, and proprioceptive stimulation is elicited by pulling down the limb being tested [47, 92, 93]. The test is also performed to measure sensorimotor perception by stimulating the vibrissae that elicit the limb placement on the edge of the table. The impairment is scored as no response, delayed and/or incomplete response, and normal response. The test is easy to perform, but scoring is subjective and variable between examiners. The animals can be tested immediately after operation to confirm completeness of MCAO and to assign rats into experimental groups based on the severity of impairment.

The rotarod is used to assess sensorimotor coordination and balance [70, 89, 90, 94]. In this test a rat is placed on a rotating rod and the speed of rotation is gradually increased so the rat's ability to remain on the rotating rod is measured. The test is easy to perform and is sensitive to brain injury; however, spontaneous recovery occurs, which may partly be explained by compensatory strategies such as postural adjustment and tail deviation. The clinical relevance of the test is unclear.

Somatosensory asymmetry

The adhesive tape removal test measures sensory asymmetries produced by stroke [46, 51, 95]. Briefly, the adhesive tape is placed on the dorsal side of forelimbs. The order and the time to contact the tape and time to remove the tape are recorded to measure sensory and motor deficits, respectively [96]. The latency to remove the tape is sensitive to practice effects, motivational state and alertness. Handling and pretraining of rats by an experienced person helps to carry out the test. The degree of sensory asymmetry can be further studied by increasing the size of the tape to the impaired forelimb and decreasing it for the unimpaired forelimb. The ratio of these is related to the severity of brain damage [83, 96].

Tests measuring forelimb impairment

The cylinder test measures spontaneous forelimb use during vertical exploration [96]. An impairment of forelimb function after stroke causes asymmetrical use of upper extremities for postural support [40, 41, 97]. For the test, the rat is placed in a transparent cylinder (Ø 20 cm) for video recording. Mirrors are placed behind the cylinder so that behavior can be clearly viewed when the animal is turned away from the camera. Alternatively, a mirror is placed at a 45° angle beneath the cylinder so that behavior can be filmed from below the cylinder. To increase explorative activity, the test can be carried out in the dark using red light. The number of contacts by both forelimbs and by either the impaired or unimpaired forelimb is counted to calculate the score for the impaired forelimb. No pretraining is needed, impairment is easy to score, and the test is sensitive enough to pick up treatment effects [40, 41].

Montoya's staircase test measures skilled forelimb use [98]. The animals are pretrained to retrieve pellets from a descending staircase and exhibit deficits in reaching and grasping after stroke. For the test, food-deprived rats are placed in the testing box (e.g., from Campden Instruments). The removable staircase with two or three food pellets on each of the steps is inserted into the box. After the 10-min test session, pellets retrieved (and eaten) and dropped by the forelimb for each side are counted separately. The task requirements involve natural responses for a rat, and consequently training is relatively rapid and simple. The test provides objective measurement of reaching performance rather than being dependent upon experimenter ratings. Impairment is permanent in the filament model [45].

Tray reaching [99] and single pellet reaching [100] are more demanding tests to be used to assess specific forelimb functions, such as extending the forelimb, aiming and grasping, and retrieving a pellet. Single pellet reaching can be combined with a detailed movement analysis [101]. This reveals any compensatory strategies in reaching and grasping motor action patterns after brain injury. The reaching tasks are usually laborious and pretraining alone may take weeks. Although true recovery and/or compensation can be differentiated, the problem is that extensive damage produced by MCAO may not allow any recovery.

Tests measuring hindlimb impairment

Testing of hindlimb functions is difficult because rats do not normally use their hindlimbs for complex movements [96]. One option is a ledged tapered beam-walking test [51, 93, 96]. The beam-walking apparatus consists of a tapered beam with underhanging ledges on each side to permit foot faults without falling, and thus rats are less prone to learn compensatory postural motor strategies. The end of the beam can be connected to a black box or home cage to motivate the rats to traverse the beam. A rat's performance is videotaped and later analyzed by calculating the slip ratio for the impaired hindlimb. Steps onto the ledge are scored as a full slip, and a half slip is given if the limb touches the side of the beam. The ratio between number of slips and total steps is related to the severity of impairment. The impairment seems to be permanent after transient MCAO [102]. Forelimb impairment can be also tested, although the foot fault task may be more sensitive for this.

Does the behavioral test or stroke model affect treatment outcome after cell-based therapy?

Cell transplantation has emerged as an experimental approach to restore brain function after stroke [21–23].

Both stereotaxic transplantation of cells into the brain and systemic delivery have been applied. The former approach expects that grafted cells migrate to the ischemic boundary to replace the lost neurons [103]. Given that stroke often produces large ischemic damage, it is not known whether a targeted approach can provide efficient and wide cell engraftment, even with the aid of anatomical and functional imaging to explore the location of cell transplantation. Another concern is the invasive nature of intracerebral transplantation. The second approach is intravascular administration [104, 105], which does not necessarily rely on the cellular replacement, but rather on the activation of the brain's own repair mechanisms [106]. Intra-arterial injection seems to represent an effective and safe route to deliver cells to the brain [107]. Surprisingly, it was also shown that intravenously injected cells did not have to enter the central nervous system in order for therapeutic effects to emerge [108]. The stem cells may actually exert some of their effects on peripheral organs [109]. In particular, the spleen seems to play an important role by contributing to ongoing inflammation and brain injury after stroke [110]. On the other hand, complications related to cell therapy itself are several (e.g., immunological rejection, tumor formation), which may attenuate functional benefit [111–113].

The mNSS, the adhesive-removal test, and the rotarod test are the most frequently used behavioral tests to assess the efficacy of cell-based therapy following focal cerebral ischemia [114]. Less frequently used tests include the cylinder test, ledged tapered beam-walking test and Montoya's staircase test. Interestingly, the more frequently used behavioral tests are more likely associated with a positive treatment effect. There is no apparent difference in positive treatment effects between transient and permanent MCAO models. Thus, it is the behavioral test used rather than the stroke model that biases the treatment outcome after cell-based therapy. The positive effects observed through specific behavioral tests could be partly due to treatment-enhanced neuroplasticity associated with experience-related compensatory strategies [84].

The open question is, whether stroke model-related complications contribute to treatment effects. It could be expected that in the testing of ischemic animals, which suffer from infections and severe loss of body weight, behavioral tests such as mNSS do not measure sensorimotor impairment, but merely general animal well-being. In turn, behavioral improvement by cell-based therapies may be due to recovery of complications rather than promotion of brain repair per se [115]. More importantly, this may be true not only for cell therapy, but for neuroprotective [7–9] and restorative pharmacotherapies [13] as well.

Conclusions

Animal research remains critical to the understanding of basic mechanisms of recovery processes after stroke, which information is needed for the development of restorative therapies. A number of experimental stroke models are available, and some have been used for 20–30 years. The inherent complications of the models, such as recently shown immunodepression, should be appreciated because these may affect behavioral outcome. In some cases the cell-based therapy may improve complications and not the recovery process or functional outcome per se.

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