Neurological tests for functional outcome assessment in rodent models of ischaemic stroke

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Summary. A critical aspect in all models is the assessment of the final outcome of the modelling procedure. In the case of a focal ischaemic brain injury, apart from the determination of the size of the lesion, another valuable tool is the evaluation of the final functional deficit. Indeed, ischaemic damage leads to the appearance of different degrees of sensoriomotor and cognitive impairments, which may yield useful information on location and size of the lesion and on the efficacy of neuroprotective treatments after the acute injury. In addition, the magnitude of these impairments may also be useful to predict final outcome and to evaluate neuro-restorative therapies in a long-term scenario. To this aim, a wide range of tests has been developed which allow the quantification of all these neurological symptoms. This review intends to compile the most useful behavioural tests designed to assess neurological symptoms in studies of focal experimental cerebral ischemia in rodents induced by middle cerebral artery occlusion, the most commonly used model of ischaemic stroke.

Key words. Focal ischaemia. Memory. MCAO. Motor coordination. Neuroprotection. Neurorepair. Sensorimotor integration.

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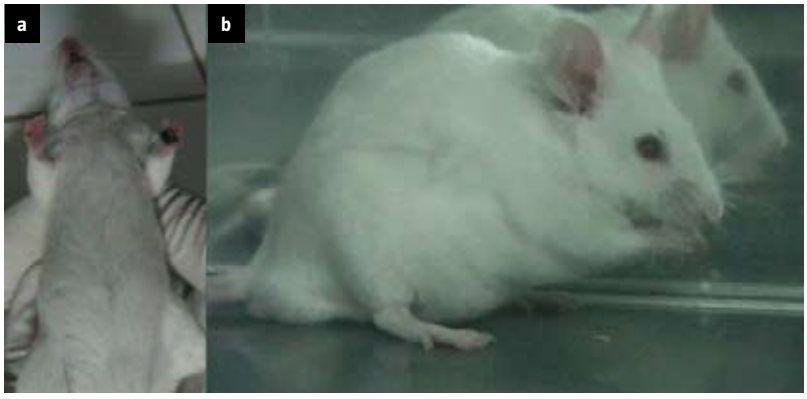
Introduction

The availability of appropriate animal disease models has served the purpose of better understanding pathology and of aid in the development of new therapies and in the improvement of diagnosis. In this context, ischaemic stroke, a major cause of death and disability worldwide, has been approached by using different experimental models, being middle cerebral artery occlusion (MCAO) in rats or mice the most extensively used (for review see [1]). A critical aspect in all models is the assessment of the final outcome of the modelling procedure. In the case of a focal ischaemic brain injury, apart from the determination of the size of the lesion, another valuable tool is the evaluation of the final functional deficit. Indeed, ischaemic damage leads to the appearance of different degrees of sensorimotor and cognitive impairments, which may yield useful information on location and size of the lesion, and on the efficacy of neuroprotective treatments after the acute injury. In addition, the magnitude of these impairments may also be useful to predict final outcome and to evaluate neuro-restorative therapies in a long-term scenario. To this aim, a wide range of tests has been developed which allow the quantification of all these neurological symptoms.

The selection of appropriate tests is therefore a critical issue in the evaluation of therapeutic strategies [2]. Apart from the above-mentioned features, tests should be informative enough as to assist in the translation to clinical practice, an objective not fully accomplished yet. This is a main concern in the scientific community, due to the failure –in clinical trials– of drugs that previously have shown efficacy at the experimental level (for review see [3]). To bridge this translation failure, the Stroke Therapy Academic Industry Round Table (STAIR) has suggested a rigorous, robust, and detailed preclinical evaluation including –among others- neurological tests [4,5]. Specifically in the area of stroke, in which 75% of the survivors develop disability due to motor impairments and also cognitive deficits, tests should also reflect the ability of different therapies to promote brain repair in long-term studies.

Four main criteria should be followed to select the most optimal tests for each study, namely: validity, reliability, sensitivity and utility [6]. In the field of stroke, practically all of them may be affected by multiple variables, such as the model, rodents' species, type of lesion and research protocol. Therefore, researchers have to be very careful when it comes to decide which test should be used to evaluate either sensorimotor or cognitive symptoms. There are comprehensive reports in the literature

Figure 1. Adhesive removal test [9]. A sticky tape is placed in each forepaw, and the time periods required to feel and to remove it are measured. A black sticky tape is shown in the left forepaw of a mouse (a). The mouse detects the tape and intends to remove it (b).



that review the importance of sensorimotor and cognitive function in different animal disease models in rodents [7,8]. This review summarizes scientific evidence showing some of the available behavioural tests designed to assess neurological symptoms (Table I), focusing on their application for studies of focal experimental cerebral ischemia in rodents induced by MCAO, the most commonly used model of ischaemic stroke.

Sensorimotor tests

Adhesive removal test

Described by Schallert et al [9] to highlight unilateral deficits caused by a striatal damage, this test is also known as bilateral tactile stimulation test, bilateral asymmetry test, tape removal test, or sticky label/tape test. It was originally described for rats but it has been further adapted to mice [10], and it assesses the rodent ability to feel and remove a stimulus in its forepaws.

This test consists in applying two adhesive tapes of a defined size and with the same pressure on each forepaw (Fig. 1), covering their hairless parts (pads, thenar and hipothenar). Training trials five days before surgery are needed and the animals need to become familiar with the evaluation box. The order in which the tag is placed (right or left) is alternated between each animal and training session. Then, the rodent is placed in a transparent box and two times are measured with a maximum of 120 s: 1) time to contact, is the time it takes the animal to feel each label, noticed when the animal shakes its paws or brings them to its mouth and 2) time to remove each label. Time to contact ipsilateral tag remains always unchanged after ischemia but the other times can be affected depending on the ischemia model [10]. The asymmetry between the ischaemic ipsilateral and contralateral sides is calculated. This test has been often modified changing where or how the tag is placed. For example, an adhesive around the paw can be placed to hinder its withdrawal [11].

It has been widely used in ischemia models [11, 12], being probably one of the most common sensory tests used in rats and mice in short and long term studies. In MCAO models, mainly in rats, this test have shown functional impairments correlated with the infarct size, 26 days [13] and 11 weeks after the ischaemic injury [14]. Moreover, it is able to detect sensory neglect after small cortical lesions such as those obtained with distal MCA occlusion [15], however, it loses sensitivity in models without a cortical lesion as it may occur with the intraluminal MCA occlusion during a short period of time [12]. Due to its utility, reliability and sensitivity long after an ischaemic lesion [13,14], we highly recommend this test to assess functional outcome, especially for neuro-repair and neuroprotection studies after MCAO.

Staircase test

The staircase test was developed by Montoya et al [16] to detect alterations in the use of forelimbs after unilateral or bilateral cortical damage. It was originally described for rats and further adapted on mice to assess rodents' ability to reach food pellets placed on a staircase. This complex task is evaluating not only fine motor coordination abilities, but also sensory neglect [16].

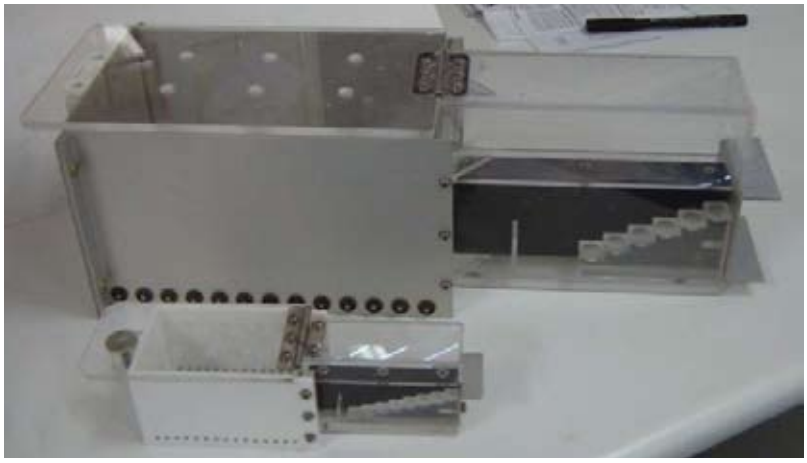
In order to stimulate the animals to reach the food pellets, they must be deprived of food until they reach 90% of their initial weight. Animals must be trained for a minimum of 15 days prior to the evaluating trials, and those animals unable to complete the training sessions have to be excluded [16]. The apparatus consists of an elevated central platform (Fig. 2), with two staircases situated on both sides of it, being each staircase baited with food pellets of sucrose (20 mg for mice and 45 mg for rats). Animals are placed on the platform for 15 minutes and required to climb it to retrieve and eat the pellets. Food pellets situated on the right or left

Table I. Neurological tests. This table summarizes the most used test in experimental studies, specifying rodents' species, ischaemia models and protocols recommended.

		Symptoms evaluated	Utility at short/ long term after ischaemia	Species	Disadvantages	Advantages
Sensorimotor tests	Adhesive removal test	Sensory neglect	+/+	Rat and mouse	Training needed Less sensitive in models without a cortical injury	High sensitivity long after ischaemia, including MCAO models with small cortical damage
	Staircase test	Fine motor coordination and sensory neglect	+/+	Rat and mouse	Long training needed Large number of excluded animals	Good sensitivity and reliability in MCAO models with small infarcts, and long after the surgery
	Beam walking test	Motor coordination	+/+	Rat and mouse	Training needed Compensatory biases in beams without a ledge	Good sensitivity in rats and mice long-after ischaemia
	Corner test	Whiskers sensitivity	+/+	Mouse	Less sensitive in models with cortical lesion Low evidence in rats	Simple and fast No training needed Evaluates long-term dysfunctions in striatal infarcts
	Rotarod test	Motor coordination and balance	+/+	Rat and mouse	Low sensitivity in mice 72 h after MCAO Confounding factors Training needed	Good sensitivity and reliability in rats
	Open field test	Locomotor activity	+/–	Rat and mouse	Low sensitivity with small infarcts Only useful in short-term protocols	Assesses anhedonia and stress
	Elevated body swing test	Muscle strength	+/+	Rat and mouse	Less experience in mice	Simple and fast No training needed Sensitive even 30 days after MCAO
	Cylinder test	Motor coordination	+/–	Rat and mouse	Decreased sensitivity in mice with small infarcts	Easy and fast to apply It is sensitive one month after ischaemia
	Foot-fault test	Motor coordination	+/–	Rat and mouse	Poor sensitivity in mild ischaemia	Very sensitive in severe ischaemia models
	Limb placement test	Sensorial limb placement	–/+	Rat	It is subjective (double blind) Sensitivity decreases due to a high spontaneous recovery	Simple and fast Detects neurological impairments in models of striatal and/or cortical lesion
	Pole test	Motor coordination	–/+	Mouse	Only used in mice It is sensitive only in models with striatal infarct	Very sensitive long after ischaemia
Mnesic tests	Morris water maze	Spatial learning and memory	+/+	Rat and mouse	Less sensitive in mice 72 h after MCAO Training needed	Very good sensitivity in rats long after ischaemia
	Passive avoidance test	Avoidance learning	+/+	Rat and mouse	Expensive equipment Training needed	Very high sensitivity long after ischaemia in rats and mice Easy to perform
Neurological scales	Modified Neurological Severity Score	Balance, muscle strength, motor coordination and reflexes	+/–	Rat and mouse	Low sensitivity at late times after ischaemia Subjective (double blind)	Gives an overall degree of the ischaemic injury Easy and fast

MCAO: middle cerebral artery occlusion.

Figure 2. Staircase test [16]. Staircases have been designed for both rat (large) and mouse (small).



side are only reachable with the right or left paw respectively. During the test the total number of pellets collected on each side are counted and this is expressed as a percentage compared with the number of pellets retrieved during the training sessions.

This test has been used in ischemia models including MCAO [17], giving a sensitive measure of motor dysfunction and also of sensory neglect. First described for rats [18-21], and further adapted in mice [13,15,22], it shows a high sensitivity with reliable values, mainly in long-term studies after a MCAO [18-21]. Some of the mentioned studies evaluated neuro-repair after an ischaemic lesion [18,20,21] showing a good correlation between the sensorimotor impairments and the infarct volume. Although a spontaneous recovery during the first two weeks after the injury is reported in some studies [18,19], the staircase test still has a good sensitivity even two months after ischemia [20]. One important advantage of this task is that it is useful, sensitive and reliable in ischemia models of cortical damage [21] or even of small striatal lesions [19]. Despite the long pre-training required, and the subsequent inconvenience of losing those animals unable to learn the task, according to the mentioned studies, the utility of this test evaluating the forelimb function is out of doubt even long after a MCAO. At the moment, this is not a common used test in stroke animal models, maybe due to the long training needed and the large number of animals excluded (important in studies using expensive transgenic animals) however, it is considered a very good and sensitive test to assess long-term functional outcome in neuroprotection and neuro-repair studies.

Beam walking test

Another way to assess motor coordination is the walking beam test. First described by Feeney et al [23], it consists in assessing rodents ability to traverse a graded series of narrow beams to reach an enclosed platform. The beams are wooden square strips (1 m long with 28 mm, 12 mm, or 5 mm cross sections), and/or round strips of 28 mm, 17 mm, or 11 mm diameters. Beams are wider when the experiment is with rats (3.5 cm in diameter and 200 cm long) [24] than with mice (0.7-1.2 cm in diameter and 60-120 cm long) [25]. Recently, a modification of this test, fitting a step-down ledge that prevents full slipping of the limb has been introduced (tapered/ledged beam test) [26]. The use of the ledge discourages the animals from developing compensatory strategies, normally difficult to detect, and which may influence the final results [7].

For MCAO models, a previous training phase before the ischaemic surgery is normally needed in order to have a baseline value that we may compare with the post-ischaemic trials. This test has been widely used in transient and permanent focal cerebral ischemia models in rats and mice, yielding reliable results and significant differences between the ischaemic groups and sham controls even 20 and 30 days after MCAO [27-29], and with a good correlation with infarct volume (neuroprotection) and mechanisms of neuronal migration and neurogenesis [30]. The ability of this test to evaluate hindlimb impairments after an ischaemic injury is important to discriminate the effects due to neural repair and not to the observed learning of compensatory strategies at long-term studies, what makes it a valid test for focal ischaemic protocols aimed to neuro-repair.

Corner test

Also known as Corner turn test, it was originally designed to evaluate sensorimotor deficiencies in experimental stroke models [31]. First described for mice by Zhang and later adapted for rats [32], it assesses vibrissae sensory impairments, and abnormal limb use [8].

Two boards are attached with an angle of 30º, with a small opening between them, encouraging the animal to walk and explore the corner. The turning of the animals either to the left or to the right side is evaluated over 10 trials (for a valid trial,

the rodent has to rear), and the laterality index is calculated. Some days before surgery, to normalize the data, a baseline measure is taken.

Regarding the use of this test in MCAO models, it has shown a good correlation with infarct volume in models with striatal damage (with or without a cortical lesion) as it occurs in the intraluminal suture and autologous-blood models [32-34], being able to detect sensorimotor deficits three or more months after ischemia [31]. However, in other MCAO models in which only a cortical lesion is produced, this test loses sensitivity a week after the occlusion of the MCA [35]. Although this is a good test to apply in some models of ischemia due to its simplicity, no training needed and shows reliable results, it is important to mention that a spontaneous recovery is observed during the first 10 days after the surgery [31].

Figure 3. Rotarod test [36]. The rotarod test assesses motor coordination and balance alterations when animals are placed in a rotating rod.

Rotarod test

The rotarod test, described by Dunham and Miya [36], has been used to assess motor coordination and balance alterations in several conditions including ischaemic stroke by MCAO [37].

Rodents need to be trained in either an accelerated (rod rotating from 4 to 40 rpm) or non-accelerated (constant speed) protocol for a minimum of 3-4 days prior to the evaluation day. After a habituation period (30 s), animals need to stay on the rod at 4 rpm for at least 2 minutes. After a successful training, the day previous to the surgery, a baseline trial is taken and the time until the animal falls or performs two passive rotations being cling to the rod is taken [13].

It is important to mention that there are several misleading issues in this test [6]. First, is the tendency of animals to cling to the beam and rotate with it when they lose balance. The second is related to some animals that refuse the test, falling as soon as they are placed on the beam, and it is related with learning that the consequences of falling are innocuous. A third source or error is related to mouse weight: heavy mice perform worse than light ones, that is important to take into account for genetic or lesion-induced weight loss. Finally, in the accelerated protocols, motor coordination impairments can be confounded with fatigue, being fixed speed tests more sensitive to ensure that a rapid fall is attributable to failure in motor coordination rather than to fatigue [38].

Multiple studies using the intraluminal suture model of MCAO (transient and permanent MCAO) have shown the efficacy of this test on neuroprotection and neuro-repair studies in rats [39-41]. However, a focal transient ischemia study in rats (intraluminal suture model) did not find any significant differences between sham and MCAO groups 15 days after the ischaemic insult [27]. On the other hand, motor coordination in mice assessed with the rotarod has been used only in neuroprotection studies (24 and 48 hours after MCAO) [42]. One study, in which multiple sensorimotor tests were used to evaluate mice for 30 days after a transient intraluminal MCAO, failed to observe significant differences in the rotarod between ischaemic and sham operated mice three days after MCAO [13].

Despite the mentioned sources of error, the evidence shows that this test could be used for evaluation of motor coordination and skill learning [43] with a good sensitivity and reliability in rodents [39,40].

Open field test

The open field test was described by DeFries et al [44] and was designed to evaluate locomotor activity. It has been widely used to evaluate the anxiety of animals in response to stress or some drugs [45].

Animals are placed in a box (80 cm^2 chamber, 20 cm high walls) and the floor is divided in equal squares (5 × 5 cm) by 1 cm wide lines. Animals are positioned somewhere in the box and video-recorded for a specified time. Spontaneous activity is

Figure 4. Cylinder test [53]. Exploratory activity of a mouse in the cylinder test is video-recorded to assess limb use asymmetries.

thus recorded (the number of times the rodent crosses the floor squares with both hind paws) and latency (time lapse for crossing the first square), rest, or rearing can also be assessed. The test has to be performed in environmental controlled conditions, in a dark room with red light, controlled ventilation system and a temperature of 21 ± 3 °C.

This is not a common used test to assess the functional outcome after focal cerebral ischemia. In some models of focal brain ischemia this test shows hyperactivity in animals with mild damage, as a consequence of stress, and hypoactivity with large infarcts [46], but being only sensitive 72 hours after MCAO [46,47]. However, some studies have shown activity deficits beyond two weeks after stroke, and in these cases the impairments, were associated with anhedonia and post-stroke depression [48], two common symptoms of stroke. Therefore, this trial does not seem to be a sensitive test to assess locomotor activity long after MCAO, but it shows reliable results during the first 72 hours after the ischaemic insult. In addition, it could be useful to assess anhedonia long after ischemia.

Elevated body swing test

The elevated body swing test was described by Borlongan and Sanberg [49] in order to evaluate Parkinson's disease motor symptoms. In this test, the animal is placed in a plexiglas box (40 × 40 × 35.5 cm), and allowed to habituate for 2 min and attain a neutral position (defined as having all four paws on the ground). Then, the animal is elevated 2 cm above the ground and a swing is counted whenever the animal moves its head out of the vertical axis more than 10° to either the left or the right side. Three or more trials are recommended for each testing day, and the number of swings to each side is expressed as a percentage [50].

Although this test is not commonly used in ischemia, there are studies reporting significant differences between the treated groups after MCAO, correlating their results with a reduced infarct volume and increased neuronal plasticity even 28 days after the ischaemic insult [18,51,52].

Considering that it is a simple and quick test to do, it could be taken into account in MCAO studies on neuroprotection and neuro-repair studies.

Cylinder test

It was described by Schallert et al [53], and it is also known as limb-use asymmetry test. It has been used in rodents to evaluate the limb use and asymmetries during exploratory activity caused by unilateral cerebral damage, mainly in Parkinson's disease.

The rodent is placed in a transparent cylinder, and the exploratory activity is video-recorded. Two behaviours are noted: a) simultaneous or independent contact of the left or right forelimb with the wall during a rear or when a lateral movement is initiated; b) simultaneous or independent contact of the left or right forelimb with the floor after rear (land movements). Each behaviour is expressed as a percentage [53].

Although it has been recently described, this test is easy to perform and it has been commonly used to assess functional outcome after ischaemic stroke. In rats, this test has shown a good sensitivity in models with [41,54] or without [55] striatal damage only for the first month after the ischaemic insult, maybe due to a strong spontaneous recovery. Much less use in mice, it has shown similar results [56], but with a minor sensitivity with small infarcts [35]. Therefore, this test detects limb-use asymmetry

during exploratory activity for the first weeks after the ischaemic injury but not beyond this time. It could be a reliable test for neuroprotection studies in which the neurological sequels are evaluated for a couple of weeks, but it does not seem a proper trial for neuro-repair studies where the neurological outcome is measured 30 or more days after the ischaemic lesion.

Foot-fault test

This test was described by Hernández and Schallert [57] to evaluate motor coordination of the forelimbs in mice and rats. The animal is placed in a grid and is video recorded, counting how many steps needs the animal to cross the wire and the percentage or the total number of foot-faults (when the foot falls or slips) is reckoned [58].

It is a quite frequent test used in rats and mice, detecting deficiencies in motor coordination beyond a month in models in which a cortical and striatal ischaemic lesion is produced, like the intraluminal suture and autologous-blood clot models [31,59]. Furthermore, although a clear spontaneous recovery is observed, one study showed a clear correlation between motor coordination impairments and the brain lesion even beyond 17 weeks after the surgery [60]. Conversely, negative or no differences have been seen in MCAO models with small cortical lesions as in the endothelin-1 model [58,61]. Therefore, due to the spontaneous recovery observed, and its limited used only in MCAO models with large infarcts, we do not recommend using this test for neuro-repair studies. However, its simplicity and utility makes it a proper test for neuroprotection studies (24 and 48 hours after MCAO) using the intraluminal suture and autologous blood-clot models.

Other sensorimotor tests

There are other tests that assess limb function and asymmetry after ischemia. In general, these tests are more specific for some ischemia models, being in these cases quite reliable. The most common are:

- *Limb-placement test* [62]: a scale that estimates the sensorimotor disabilities of the limbs, focusing on the forelimbs.
- *Pole test* [63]: a test which evaluates motor coordination.

Some studies that have used the limb-placement test were able to detect long-term disabilities after cortical [60] and striatal lesions [64]. On the other hand, the pole test has been mainly performed in mice, detecting long-term alterations only in models with a striatal injury [13,65]. Both tests show a spontaneous recovery in the first weeks after ischemia, but they are sensitive enough to assess long-term motor dysfunctions in neuro-repair or neuroprotection especially when the striatum is infarcted.

Table II. Neurological scale (adapted from [78]).

Symptom	Score	Type of brain damage
Normal	0	
Forelimb hemiparesis while held by the tail	1	Cortex damage
Circling toward the paretic side	2	Striatal damage
No spontaneous movements	3	Large brain damage
Animal death	4	Massive damage

Cognitive tests

Morris water maze

This test is used to evaluate learning and memory deficits. Described by Morris [66], it consists of a circular pool filled with water, and virtually divided into four arbitrary, equally spaced quadrants, in which the animal (rat or mice) swims searching for a way to escape (submerged platform). A traditional Morris water maze paradigm, where the platform is placed in the same position of the target quadrant during the training and probe trials is the one that has been widely used. However, this protocol has the disadvantage that control and experimental groups diminish their differences with repeated testing [67]. Conversely, a recent modification has been introduced, where the location of the platform is randomly changed within a fixed target quadrant from trial to trial [68]. This method allows to probe spatial memory in early trials continuously as animals learn, avoids animals to adopt a strategy of looping around the tank (response that gets them to a fixed platform rapidly without using the visual cues, behaviour that does not require the hippocampus), and finally reduces animal's anxiety as they do not expect to find the platform in a fixed location [7].

Tabla III. Modified Neurologic Severity Score [79].

	Score
Motor tests (muscle status-hemiplegia) (normal = 0; maximum = 6)	0-6 [a]
Raising the rat/mouse by the tail: (normal = 0; maximum = 3)	
Flexion of forelimb	1
Flexion of hindlimb	1
Head moving more than 10° to the vertical axis within 30 s	1
Placing the rat/mouse on the floor: (normal = 0; maximum = 3)	
Normal walk	0
Inability to walk straight	1
Circling toward the paretic side	2
Falling down to the paretic side	3
Sensory tests (not for mice)	0-2 [a]
Placing test (visual and tactile test)	1
Proprioceptive test (deep sensation, pushing the paw against the table edge to stimulate limb muscles)	1
Beam balance tests (normal = 0; maximum = 6)	0-6 [a]
Balances with steady posture	0
Grasps side of beam	1
Hugs the beam and one limb falls down from the beam	2
Hugs the beam and two limbs fall down, or spins on beam (> 60 s; 30 s for mice)	3
Attempts to balance on the beam but falls off (> 40 s; 20 s for mice)	4
Attempts to balance on the beam but falls off (> 20 s; 10 s for mice)	5
Falls off: no attempt to balance or hang on to the beam (< 20 s; 10 s for mice)	6
Reflexes absent and abnormal movements (normal = 0; maximum = 4)	0-4 [a]
Pinna reflex (a head shake when touching the auditory meatus)	1
Corneal reflex (an eye blink when touching the cornea with cotton)	1
Startle reflex (motor response to a brief noise from snapping; not for mice)	1
Seizures, myoclonus, myodystony (not for mice)	1

One point is awarded for inability to perform the tasks or for lack of a tested reflex. For rats: 13 to 18 indicates severe injury; 7 to 12, moderate injury; 1 to 6, mild injury. For mice: 10 to 14 indicates severe injury; 5 to 9, moderate injury; 1 to 4, mild injury. [a] An accumulative score is given.

Concerning MCAO, this test seems to be sensitive detecting spatial memory impairments in rats. Several studies have shown significant differences between MCAO and sham operated rats evaluated 7 and 14 days as well as 8 weeks after the ischaemic insult [69,70]. On the other hand, Bouet et al failed to report spatial learning and memory impairments in the Morris water maze between ischaemic and sham operated mice 72 hours after the surgery [13]. In accordance with this, other studies have not found any differences in spatial memory but a difference in strategy switching and relearning in mice after MCAO [71]. The different results between species could be explained by the variability in the protocols used (number of trials per day, inter-trial interval, duration of the acquisition period and delay for the retention probe test, pre- or postsurgical training). The mentioned studies show that the Morris water maze is a recommendable test to use in rats. However, due to the less experience with this test in mice after MCAO, it should be used with caution and it will depend on the type of lesion.

Passive avoidance test

The passive avoidance is a generic name for learning how to avoid aversive stimulation by not taking a certain action. This kind of test was introduced by Pearl [72] and, since then, it has been used in numerous studies [13,73,74]. As it was previously described [72,75], the animal is placed on a brightly lighted platform and, 30s later; a door to a dark, enclosed chamber with a stainless grid floor is opened (Fig. 3). The latency time to cross to the dark room is measured. When the animal crosses to the dark room, the door is closed and a constant current (0.4-0.7 mA) is delivered for 2 s. After the shock, the animal is immediately removed from the box. To evaluate retention, 24 hours later the animal is again placed onto the brightly lighted platform and the time to cross to the dark chamber is measured. A total of 300s is taken as the limit time for the animal to go into the darkened chamber. During the retention trial no current shock is delivered to avoid reacquisition.

This test has been used in animal models of focal ischaemic stroke (intraluminal suture and ligature models) obtaining clear differences in the retention phase between the ischaemic and sham groups [49,76]. Bouet et al demonstrated the utility of this test, finding a significant correlation between an increased retention latency period and the ischaemic lesion [13]. This is in agreement with other studies in rat [69,70] and mice [77], showing that retention latency period is impaired even 7 weeks after a focal brain ischemia. Given its sensitivity detecting early and delayed acquisition or retention impairments after focal ischemia, this test seems to be a quite reliable and sensitive test to use in MCAO models.

Neurological scales

There are different types of scales that measure the functional impairment after ischemia. They usually evaluate diverse symptoms of the ischaemic injury,

mainly motor deficits; some also evaluate the sensory neglect and more rarely, the absence of reflexes. These scales can be modified depending on the species (mice or rats), giving a total score for each parameter of ischaemic damage.

A frequently used scale is one designed by Bederson et al [78]. It quickly assesses the severity of the brain infarct, giving a score that reflects the extension of the lesion (Table II). Although it differentiates between cortical, striatal and massive damage, it is only sensitive in a short period of time after ischemia.

Probably, one the most recommended scales is the mNSS (Modified Neurological Severity Score) [79]. It is fast, easy to perform, and assesses motor deficits, sensory neglect, balance and reflexes. Table III shows the conventional scale, in which 1 point is given for the inability to perform the task properly or the absence of the sign. For rats, the mNSS gives a maximum of 18 points and of 14 for mice.

The mNSS has been used in almost all ischemia models, observing neurological deficits even 1h after ischemia, and contrary to other scales, is still useful 35 days after surgery [79], although a spontaneous recovery can be observed during time [80].

These scales are subjective, its sensitivity is low and are only useful in short-term studies due to the spontaneous recovery. However, they are easy to perform and give a rapid general evaluation about the severity of the ischaemic injury. Therefore, their use is highly recommended but always accompanied with other tests sensitive enough depending on the ischaemic model and the study objectives.

Conclusions

Ways to assess extent of brain damage and degree of recovery are important issues in ischemia models, being motor and cognitive testing a relevant procedure in terms of translational science. Numerous behavioural tests have being designed to accomplish this goal, but not all of them provide equal, efficient and valid results, a fact that may be explained by the experimental approach (ischemia model, training, drug used, evaluation times, etc.) of each study.

Regarding models of MCA occlusion, a high variability in the results depending on the test and type of brain lesion (cortical, striatal or both) is observed; in addition, not all the tests described are useful for neuro-repair studies. Several issues have to be taken into account in order to select the most efficient, reliable and valid test. First is the consideration of pre-stroke values, which need to be similar in all animals to avoid possible biases between the compared groups; the second one is size and location of the brain infarct, as they could affect the sensitivity of some tests; and finally, the phase after stroke (acute or chronic) to be studied. With these points in mind, and with the evidence reviewed above, we highly recommend to select a battery of sensorimotor and cognitive tests which, in combination, are able to discriminate chronic recovery and compensatory mechanisms learned, two main characteristics that could influence the final results.

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Tests neurológicos para la evaluación del pronóstico funcional en modelos de ictus isquémico en roedores

Resumen. Un aspecto crítico en todos los modelos experimentales de patologías del sistema nervioso es la evaluación del pronóstico neurológico final. En el caso de una lesión cerebral isquémica focal, además de la determinación del tamaño de la lesión, una valiosa herramienta es la evaluación del déficit funcional final. Ello se debe al hecho de que el daño isquémico produce diferentes grados de deterioro sensoriomotor y cognitivo, que pueden proporcionar información sobre la ubicación y el tamaño de la lesión y sobre la eficacia de los tratamientos neuroprotectores después del daño agudo. Además, la magnitud de estas alteraciones también puede ser útil para predecir el resultado final y para evaluar terapias reparadoras a largo plazo. Con este fin se ha desarrollado una amplia gama de tests que permite la cuantificación de todos estos síntomas neurológicos. Esta revisión tiene como intención recopilar los tests de comportamiento más útiles diseñados para evaluar los síntomas neurológicos en los estudios de isquemia cerebral focal experimental en roedores inducida por oclusión de la arteria cerebral media, el modelo más utilizado para el estudio del ictus isquémico.

Palabras clave. Coordinación motora. Integración sensoriomotora. Isquemia focal. Memoria. Neuroprotección. Neurorreparación. Oclusión.