



Review

Cognitive and behavioral assessment in experimental stroke research: will it prove useful?

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Abstract

Stroke in humans is associated with deficits in sensorimotor and cognitive function. Consequently, many stroke researchers recently have expanded their techniques to assess cognitive and behavioral correlates of histologically-determined stroke damage in animal models. Although the incorporation of functional outcome assessment represents an important step forward in stroke research, reports of middle cerebral artery occlusion (MCAO) induced behavioral deficits often conflict, and a significant correlation between post-stroke histology and behavior has been reported in few stroke studies. Discrepancies in behavioral outcomes among studies may be due to several factors, such as method of MCAO, duration of occlusion, strain, the timing and method of the behavioral testing and the laboratory environment. Furthermore, proper experimental and control groups, necessary to rule out potential confounding factors during cognitive testing, often are not incorporated. The goal of this review is: (1) to provide a description of the techniques most commonly employed to assess functional outcome after (MCAO) in rodents and (2) to identify potential confounding factors that may interfere with a clear interpretation of the behavioral data. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Animal models; Learning; Memory; Sensorimotor; Ischemia; MCAO

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1. Introduction

Important advances in experimental stroke research have been made during the past two decades, however little of this work has proven effective in treating stroke in humans [1]. The vast majority of this research has been aimed at preventing or ameliorating morphological and physiological damage after an ischemic insult. A common paradigm in experimental stroke research involves histological examination of the post-stroke damage in animal models that did or did not receive a pharmacological treatment. These comparisons are generally made within days of the ischemic insult. Within the last several years, however, many stroke researchers have expanded their techniques to assess the cognitive and behavioral correlates of histologically-determined stroke damage in animal models and have extended recovery periods to weeks or months. The inclusion of behavioral end-points in experimental stroke studies represents an important step forward, because in order to be of optimal clinical use, a potentially therapeutic compound should maintain or restore brain *function* after stroke. In other words, the long-term goal of these animal studies is to identify treatments that may improve the quality of life of recovering stroke survivors.

Stroke is the most common cause of permanent disability among people in the United States, and is associated with a high incidence of deficits in sensorimotor function and cognitive ability [2]. Thus, studies of functional outcome after experimental stroke have focused primarily on sensory, motor, or cognitive deficits. In some cases, the behavioral tests are easy to conduct, and the analyses and interpretation of the results are straightforward. In many cases, however, interpretation of the complex cognitive data in experimental stroke research is problematic because stroke is often associated with sensorimotor deficits that may become confounding factors in complex behavioral tests.

The goal of this paper is: (1) to provide an overview of the behavioral assays that have been used to assess stroke damage in rodents, (2) to alert investigators that behavioral studies are fraught with potential confounds and alternative explanations, and (3) to provide procedures, and analyses to avoid these potential problems with their behavioral tasks. Also the importance of taking into account strain differences, method of MCAO, experimental design differences and other nonspecific effects when assessing behavior in nonverbal animal models will be emphasized. Discussion of behavioral outcomes will be limited to studies that use middle cerebral artery occlusion (MCAO) in rodents. MCAO in rodents is considered to be a convenient, reproducible, and reliable model of cerebral ischemia in humans [3,4]. Although the extent and location of neuronal damage following MCAO varies as a function of the location and duration of MCA occlusion, proximal MCA occlusion typically causes infarction in the cerebral cortex and caudate putamen [3] while distal occlusion results in infarctions

generally limited to the neocortex [5]. The vast majority of the research into the behavioral outcomes of experimental stroke thus far has been conducted using rats; therefore, unless otherwise specified, rats were used in the studies described below.

2. Sensorimotor tasks

As stated above, MCAO typically results in extensive neuronal death in the cortex and caudate putamen [3]. However, it is often difficult to distinguish the extent to which neuronal damage in each of these two regions contributes to altered sensorimotor performance because the caudate putamen receives extensive input from the sensorimotor regions of the cortex. Neurotoxic and electrolytic lesions of the dorsomedial striatum tend to have generalized effects on locomotor activity, whereas neuronal damage that extends into the caudate putamen affects sensorimotor orientation and skilled motor control [6,7,8]. However, 'spontaneous recovery' of sensorimotor function has been reported in several models of unilateral brain damage [9,10]. Thus, the timing of sensorimotor tests following MCAO surgery is an important consideration when designing and interpreting behavioral stroke studies. The sensorimotor tasks that are the most commonly used in MCAO studies assess postural abnormalities, coordinated movements, balance, forelimb strength, locomotor activity or sensory capabilities (summarized in Tables 1–3).

2.1. Postural alterations

Several different methods have been used to examine the postural changes that occur in rodents after experimental stroke. One method involves suspending the animal by its tail and recording the position of its forelimbs and shoulders. An unmanipulated animal will typically hang straight down and extend both forelimbs toward the floor. An animal that has been subjected to MCAO may flex the forelimb contralateral to the infarct toward its abdomen or in some cases rotate the contralateral shoulder or limb medially. MCAO causes contralateral forelimb flexure that can be identified for several weeks after surgery [5,11–13] and correlates with the extent of cortical damage [11]. When the effect of MCAO on behavioral outcome was compared in several rat strains within a single study, all strains except one (Brown-Norway rat) exhibited forelimb flexure and an increase in 'c-shape bending' upon being lifted by the tail [14]. A positive correlation also is reported for the elevated body swing test (which records the direction that the animal swings upon being lifted by its tail) and striatal infarct size for several months after MCAO [15].

Another commonly used test of sensorimotor abilities involves the calculation and analysis of composite neurological scores. A variety of scoring techniques and scales have been employed across laboratories with the goal of assessing adequacy of the ischemic insult and reduced variability

Table 1
Effects of MCAO on Posture and Sensorimotor Behavior

Behavioral test	Method of MCAO	Timing of test	Behavioral effect of MCAO	Histological and behavioral correlation	Spontaneous recovery
Postural reflex during tail suspension [120]	Transient (45–120 min) left MCAO	Post-op days 1–8 and 21	Increased contralateral forelimb flexure at all durations and time points	–	No
Tail suspension [12]	Permanent rt MCAO	Post-op days 2–30	Increased contralateral forelimb flexure	–	Yes by 23 days
Tail suspension [5]	Distal rt photothrombic MCAO (cortical damage only)	Post-op days 1–30	Increased contralateral forelimb flexure	No correlation	Yes by 19 days
Tail suspension [14]	Permanent lt proximal or distal MCAO	Post-op day 2	Increased contralateral forelimb flexure	–	–
Tail suspension [14]	Permanent lt proximal or distal MCAO	Post-op day 2	'C-shape' bending of torso	–	–
Tactile stimulus localization [30]	Permanent rt MCAO	Post-op day 26	Impaired stimulus localization on contralateral side	Yes	–
Tactile stimulus localization [29]	Permanent rt MCAO	Beginning post-op day 17	Impaired stimulus localization on contralateral side	Yes	Partial behavioral recovery by 3 months
Tactile stimulus localization [31]	Permanent rt MCAO	1,2 and 3 months Post-op	Impaired stimulus localization on contralateral side	Yes at 1 and 2 months	Partial behavioral recovery by 3 months
Tactile stimulus localization [30]	Permanent lt MCAO	Post-op day 26	Impaired stimulus localization on contralateral side	No correlation	–
Schallert tape test [11]	Transient 45–120 min left MCAO	Beginning post-op day 2 through 21	Increased ratio of contra: ipsilateral tape size necessary to extinguish asymmetry	–	Full recovery within days to weeks
Schallert tape test [12]	Permanent rt MCAO	Beginning day 3 post-op to 30	Same as above and increased latency to remove tape	Predictive when used as part of composite score	Full recovery within days to weeks
Schallert tape test [10]	Permanent rt proximal MCAO	Beginning post-op day 1–30	Same as above and increased latency to remove tape	–	Minimal recovery
Schallert tape test [35]	Transient 90 min left MCAO	Post-op day 1 and 7	Increased latency to remove tape on contralateral and ipsilateral sides	–	Minimal recovery
Schallert tape test [32]	Transient 30 and 120 min rt proximal MCAO	Baseline and Post-op day 1–21	Increased latency to remove tape	Yes—30 min group only	Recovery in 1 week for 30 min group and partial recovery in 120 min group
Forelimb placement [10]	Permanent rt proximal MCAO	Beginning post-op day 1–30	Impaired forelimb placement when vibrissae stimulated	–	Partial recovery

Table 2
Effects of MCAO on quantitative measures of motoric behavior

Behavioral test	Method of MCAO	Length of test	Timing of test	Effect of MCAO on generalized locomotor activity	Histological and behavioral correlation	Report Spontaneous recovery
Open field [33]	Permanent rt MCAO (cortical damage only)	5 min	Post-op days 2–18	Increase days 5–17	–	Yes
Open field [21,33]	Permanent lt MCAO (cortical damage only)	5 min	Post-op days 2–18	No change	–	
Wheel running [33]	Permanent rt MCAO (cortical damage only)	24 h/day	Post-op days 2–18	Increase days 4–15 return to baseline by day 17	–	Yes
Wheel running [33]	Permanent lt MCAO (cortical damage only)	24 h/day	Post-op days 2–18	No change	–	
Wheel running [37]	Transient (90 and 120 min) MCAO (damage to cortex and caudate putamen)	24 h/day for 3 days	6 weeks post-op	No change	–	
Automated activity monitor [29]	Permanent rt MCAO (damage to cortex and caudate putamen)	10 h	Post-op days 17–24	No change	–	
Automated activity monitor—mice [35]	Permanent lt MCAO (damage to cortex and caudate putamen)	60 min	Post-op day 1	Decrease	No	
Automated activity monitor in RAM [34]	Transient (90 min) lt or rt MCAO (damage to cortex and caudate putamen)	3 min	Post-op days 1 and 30	No change	–	
Amphetamine-induced hyperactivity [38]	Transient (60 min) rt MCAO (damage to cortex and caudate putamen)	2 h	5 weeks post-op	Increase	–	
Amphetamine-induced rotation [31]	Permanent rt MCAO (damage to cortex and caudate putamen)	90 min	1,2,3 months Post-op	Increase ipsilateral and total number of rotations	No	No
Amphetamine-induced rotation [37]	Transient (90 and 120 min) MCAO (damage to cortex and caudate putamen)	20 min	2,3,4 weeks Post-op	Increased ipsilateral rotations for both ischemic groups	120—Yes; 90—No	No

Table 3
Effects of MCAO on qualitative measures of motoric behavior *

Behavioral test	Method of MCAO	Timing of test	Behavioral effect of MCAO	Histological and behavioral correlation	Spontaneous recovery
Initiation of walking [14]	Permanent lt. proximal and distal MCAO	Post-op day 2	Increased latency to move one body length after both types of surgery	–	
Initiation of walking—mice [39]	Transient 90 min rt MCAO	Post-op day 13	Increased latency to move one body length	No	
Elevated running beam [11]	Transient 45, 60, 90, 120 min lt MCAO	Post-op days 2–10	Increase in number of contralateral paw slips after 60, 90 or 120 min MCAO	–	
Foot fault test on elevated screen grid [12]	Permanent rt proximal MCAO	Post-op days 2–30	Increase in number of contralateral paw slips	–	Partial
Foot fault test on elevated screen grid [121]	Permanent rt distal MCAO (no subcortical damage)	Post-op days 2–60	Increase in number of contralateral paw slips	–	
Foot fault test on elevated screen grid [19]	Transient 30, 60, 90, 120 min and permanent lt MCAO	Post-op day 1	Increase in contralateral paw slips for all but 30 min group	Yes—cortex and cp	
Foot fault test on elevated screen grid [13]	Transient 90 min lt MCAO	Post-op days 2–14	No difference in number of contralateral paw slips	–	
Running wheel [11]	Transient 45, 60, 90, 120 min lt MCAO	Post-op days 2,5 and 21	Increase in number of contralateral paw slips with all durations of ischemia	–	No
Balance on rotating rod [22]	Permanent lt proximal MCAO	1,2,3,4,8,16 weeks post-op	Shorter latency to fall	–	Full recovery by 3 weeks
Balance on rotating rod [19]	Transient 30, 60, 90, 120 min and permanent lt MCAO	Post-op day 1	Shorter latency to fall for all but 30 min MCAO group	Yes—cortex and cp	
Balance on rotating rod [35]	Transient 90 lt MCAO	Post-op days 1 and 3	Shorter latency to fall on day 1	No	Recovery by 7 days
Balance on rotating rod [32]	Transient 30 and 120 min rt proximal MCAO	Post-op day 1–21	Increased latency to remove tape	No	Recovery by 7 days for 30 min group and no recovery in 120 min group
Balance on rotating rod—mice [42]	Transient 45 min rt MCAO	3 weeks post-op	Shorter latency to fall	–	
Balance on rotating rod—mice [35]	Permanent lt MCAO	Post-op day 1	Shorter latency to fall	No	
Skilled paw reaching [31]	Permanent rt MCAO	1–3 months post-op	Decreased in ipsilateral and contralateral pellet retrieval	Yes	
Skilled paw reaching [37]	Transient (90 and 120 min) MCAO (damage to cortex and caudate putamen)	13 and 22 weeks post-op	Decreased in ipsilateral and contralateral pellet	Yes	
Rearing in open field [21,33]	Permanent lt MCAO (cortical damage only)	Post-op days 2–18	Decreased frequency of rearing	No	
Rearing in open field [5]	Distal lt photothrombotic MCAO	Post-op days 1–30	Increased contralateral forelimb adduction during rearing. No effect on ipsilateral	Yes	

* Strain specific effect and more pronounced after distal than proximal ligation

of outcome within a treatment cohort. Such scales typically involve assigning each animal a score from zero to three (no deficit to severe deficit, respectively) based on performance in a series of tasks that assess posture, resistance to lateral push, and circling behavior [4]. Other laboratories incorporate additional measures and use a wider scale which presumably increases the sensitivity of the neurological assessment and allows for an increased level of outcome assessment if extended for days after ischemia [16]. Intra-ischemic neurological scoring can be essential in neuroprotection studies, allowing a-priori exclusion of animals with unsuccessful occlusion prior to the period of behavioral testing [16,17]. Pre-selecting animals based on intra-ischemic neurological score decreases the histological and behavioral variability often associated with MCAO [18]. Some studies report a significant correlation between infarction volume and neurological score [4,19,20], whereas others report a MCAO-induced deficit but no correlation with the histological outcome [21]. When animals survive for several weeks after surgery, there is often a 'spontaneous' partial or complete recovery of MCAO-induced neurological deficits [20,22–24–26,27]. The advantages of using composite neurological scores to assess sensorimotor deficits following MCAO include: (1) the technique is not particularly time consuming, and (2) it does not require special equipment or extensive personnel training. However, composite scores typically provide a fairly crude and subjective assessment of sensorimotor behavior and do not provide information about which specific behaviors are improved or exacerbated following a treatment. Within the last several years, it has become common to use neurological scores in conjunction with more sensitive measures of sensorimotor behavior to identify what are often subtle sensory and motor deficits caused by MCAO.

2.2. *Hemi-neglect and sensorimotor integration*

There are many reports that rats subjected to MCAO fail to orient toward contralateral sensory stimuli. The lack of a response to contralateral sensory stimuli is often referred to as hemi-neglect. However, this failure to respond following stroke can result from sensory inattention (hemi-neglect), an inability to initiate the motor responses that signal sensory awareness or a combination of deficits in these two components (sensorimotor integration) [7]. However, it is possible through proper behavioral testing to dissociate the sensory, motor, and motivational components of stimulus neglect [7]. For example, using a 'nose poke board' rats can be trained to respond to visual stimuli presented to one eye by withdrawing their nose from a central hole and poking it into a hole to their left or right [7]. By recording response accuracy to stimuli presented to the ipsilateral versus contralateral eye, latency to initiate response and latency to complete response, the investigator can differentiate between sensory inattention (no response to stimuli presented to contralateral eye only) and a motor deficit

(inability to initiate or complete contralateral motor responses regardless of which eye was presented with the stimulus). In this behavioral paradigm, rats with unilateral striatal dopamine lesions exhibit normal sensory attention, but fail to orient toward contralateral stimuli because of a motor deficit [7]. Using a similar protocol, it was determined that endothelin-1 induced MCAO does not result in dysfunctional sensory attention [28]. Most experimental stroke studies report hemi-neglect or sensorimotor integration deficits without attempting to dissociate the sensory, motor, and motivational components. Presumably this reflects the complex nature and expense of assessing sensory attention in tasks that are not confounded by deficits in motor control.

One method used to assess sensorimotor integration involves placing an animal on a pedestal and using a thin pointer to lightly touch several different regions of the animal's body. The animal is then assigned a score based on its response to the stimulus. MCAO is associated with an impaired ability to localize the stimulus on the side of the body that is contralateral to the infarct [29–31]. There is a strong correlation between sensorimotor deficits in this task and infarction volume when animals are tested within two months of right MCAO [30,31]; however, by three months the animals display some behavioral recovery and the correlation is no longer significant [31]. Using a similar protocol, animals were subjected to permanent left MCAO, then tested for sensorimotor integration three weeks later; these rats showed performance deficits compared to sham animals, but there was no significant correlation between the extent of sensorimotor deficits and lesion size [30].

Contra-lateral sensory neglect following MCAO also can be demonstrated by placing adhesive labels on the fore paws of the animal and recording the order in which the labels are removed. Animals that have been subjected to MCAO tend to remove the tape on the paw that is ipsilateral to the infarct before they remove the tape that is on the contralateral paw [10–12,28,32]. By systematically increasing the size of the tape attached to the contralateral paw and decreasing the size of the tape attached to the ipsilateral paw, until the animal no longer exhibits a bilateral removal bias, the magnitude of sensory asymmetry can be determined [11,12]. At early time points, there is a correlation between sensorimotor deficit as measured by the tape test and the duration of ischemia [32], but within several weeks of surgery, rats subjected to either transient [11,32] or permanent MCAO [12] exhibit a return to control levels of task performance.

2.3. *Locomotor activity*

A variety of different methods have been used to assess generalized locomotor activity in rodents subjected to MCAO (Table 3). A fairly inexpensive and technically simple way to measure locomotor activity is via the open field test. In the open field test, the animal is placed in an

open arena (typically 1 m²) that is divided into 16 squares. The number of squares that the animal crosses during the testing period is recorded and used as an index of locomotor activity. Permanent occlusion of the right [33], but not left [21,33], middle cerebral artery of rats increases the number of squares crossed in the open field. Locomotor activity as measured by wheel running also is elevated during the first seventeen days after permanent occlusion of the right MCAO, then returns to baseline [33]. However, the open field and wheel running data are not corroborated by locomotor data collected from animals with permanent occlusion of the right MCAO using automated photocell activity monitors [29]. Using a similar photocell apparatus, there also is no significant difference in locomotor activity reported between the control group and animals subjected to either transient [34] or permanent [30], right or left MCAO. In addition to the obvious difference in the method of locomotor activity assessment, there are several factors that could account for the discrepancy between the open field, running wheel and activity monitor data, including strain differences and timing of the locomotor measurement. Importantly, the rats in the open field and running wheel study [33] returned to baseline activity levels after seventeen days, while the rats in the study that used the photocell activity monitors were not tested until the seventeenth day after surgery [29]. Thus, it is possible that the animals in the latter study would have exhibited increased locomotor activity if measured at an earlier time point. In contrast to rats, mice subjected to permanent MCAO exhibited a significant decrease in locomotor activity 24 h after surgery [35].

Measuring amphetamine-induced alterations in locomotor behavior is a means of assessing hemispheric asymmetry in dopamine sensitivity following MCAO. In general, animals that have sustained chronic damage to dopaminergic neurons in the striatum will turn toward the hemisphere that is receiving less dopamine receptor stimulation [36]. The imbalance in dopamine receptor stimulation between the damaged and undamaged hemispheres can be further exaggerated through the use of dopamine releasing drugs (i.e. amphetamine). Thus, because there is extensive death of dopaminergic neurons in the striatum following MCAO, animals that are treated with 1 mg/kg of d-amphetamine exhibit a significant increase in the number of ipsilateral rotations and the total number of rotations [29,31,37]. However, a significant correlation between lesion size and rotation behavior was reported in one ischemic group in one study [37]. When animals are tested within one month of surgery, those subjected to either right or left MCAO exhibit an increase in both asymmetrical and total amphetamine-induced rotation compared to control animals [30]. However, the only significant correlation between rotation behavior and lesion size occurs at one rostrocaudal level in the right MCAO group [30]. Animals subjected to one hour of MCAO also exhibit increased amphetamine-induced general locomotor activity compared to sham-operated

animals [38]. It appears that the age of the animals at the time of MCAO may also be an important factor to consider; the effect of amphetamine on locomotor activity lasts longer in young than old ischemic animals [38].

The behavioral tests described above are primarily used as quantitative measures of locomotor behavior. There are also several qualitative measures of motoric behavior that have been used in experimental stroke studies including: initiation of walking, number of foot faults during challenged walking, performance on a rotating rod and skilled paw reaching. Latency to initiate walking is measured by placing the animal in the center of a circle that has a radius of approximately one body length, then recording the amount of time that elapses before the animal has moved entirely out of the circle. Latency to move one body length is increased in both rats and mice after MCAO [14,39]. However, MCAO-induced impairment in latency to move one body length appears to be strain-specific and is more severe after distal than proximal MCA ligation in rats [14]. In mice, 90 min, but not 60 min, of transient MCAO results in an increase in latency to move compared to sham operated animals [39].

MCAO is associated with an increase in the number of contralateral foot faults (also called 'paw slips') that occur when animals are subjected to a task that requires good motor coordination. Several challenging motor tasks have been shown to be sufficiently sensitive to discriminate between the performance of MCAO and sham-operated animals including an elevated running beam [11], an elevated screen grid [12,19,40], and a running wheel [11]. Although the apparatuses that have been used to challenge the animals differ between studies, the dependent variables (number of steps taken versus the number of times each paw slip from the apparatus) remain essentially the same. In a related task, latency to fall from a rotating rod is significantly decreased in animals that have been subjected to MCAO versus the sham operation [19,32,35,41,42], but MCAO treated animals appear to recover function by three weeks and are no longer different than shams [22].

Paw reaching is another qualitative measure of motoric behavior that has been used successfully in experimental stroke studies to identify deficits in skilled motor control [28,31,43,44]. The apparatus consists of an elevated center platform that has a narrow descending stair case on both sides of the platform. Each step is baited with a food pellet. The animals are food-restricted for two days, then placed in the apparatus. The number of successful versus unsuccessful pellet retrievals is recorded for both the ipsilateral and contralateral paws. Permanent MCAO results in a long term (>3 months) deficit in successful contralateral and ipsilateral pellet retrieval compared to sham-operated animals [31]. Although paw reaching appears to be a sensitive measure of MCAO-induced deficits in skilled motor control it requires fairly extensive animal training and utilizes an apparatus that is not yet commercially available.

3. Studying sensorimotor function as a behavioral end point versus potential confound

The same techniques are used to assess sensorimotor function regardless of whether the primary purpose of doing so is: (1) to identify MCAO-induced alterations in sensorimotor behavior or (2) to rule out MCAO-induced alterations in sensorimotor behavior as potential confounding factors in additional behavioral tests. Experimental design, however, should reflect the primary purpose for studying sensorimotor behavior. For example, because there is often spontaneous recovery of sensorimotor function after focal ischemia (reviewed above), investigators who are interested primarily in studying the effects of MCAO on sensorimotor behavior should choose a method of MCAO method that results in a large infarction volume and begin behavioral testing within days of the surgery. In contrast, investigators who are interested primarily in assessing the effects of MCAO on cognitive function or another complex behavior should choose a shorter duration of MCAO and a post-surgical recovery period that is long enough to allow sensorimotor recovery to occur prior to commencing additional testing [39]. Waiting until MCAO-induced sensorimotor deficits are no longer detectable will minimize the possibility of sensorimotor deficits becoming a confounding factor in additional behavioral tests [39]. It also is advisable to conduct a complete battery of sensorimotor tasks both pre- and post-operatively in order to rule out preexisting or nonspecific group differences that might interfere with the interpretation of the behavioral data [45–47].

4. Using behavioral tests to study hypothetical constructs

Many behavioral processes represent hypothetical psychological constructs. Hypothetical constructs are inferred processes that cannot be measured directly; only performance on a test designed to assess one of these constructs can be measured. Examples of hypothetical constructs include attention, motivation, perception, arousal, anxiety, learning, and memory. For instance, unlike the number of apoptotic cells in a particular brain region, learning cannot be assessed directly. Only *performance* on a test that has been designed to measure learning and memory can be examined. Thus, a poorly designed test or an inappropriate test will not effectively measure what has been learned. If the test is not reliable (repeatable results are obtained) or valid (measures what it purports to measure), then unreliable or invalid behavioral conclusions will be drawn. Because animal models are nonverbal, all cognitive tests require performance in a nonverbal test; therefore, test performance, in most cases, is dependent upon some sort of motor output (e.g., depressing a lever or running a maze). If an animal repeatedly fails to run a maze correctly after MCAO, but this failure is due to motor impairments, then

performance in the maze is not a valid measure of cognitive ability in that model. As described above, impairment of sensorimotor abilities is often reported in rodent models of stroke and any alterations in motor function, sensory capabilities, or motivation can become confounding factors when measuring cognitive performance [48,49]. Consequently, not all behavioral tests are equally valid for all animal models, and when assessing MCAO-induced alterations in cognitive function, it is important to rule out sensorimotor or motivational deficits as possible confounds in the cognitive testing.

5. Behavioral tests that assess MCAO-induced deficits learning and memory

The two most common types of mazes used to assess spatial learning and memory are the radial arm maze (RAM) and the Morris water maze (MWM) [50]. Although RAM and MWM have traditionally been used to assess spatial deficits associated with hippocampal damage [51–53], performance in these mazes also can be altered by damage to the caudate-putamen [54–57] or cortex [58,59]. Consequently, several laboratories have studied MCAO-induced alterations in spatial memory and learning as measured by the RAM and MWM. Associative learning and memory also has been assessed in animals subjected to experimental stroke through the use of active and passive avoidance paradigms. During avoidance training the animal are taught to actively or passively avoid a part of the apparatus that they have learned to associate with receiving an electric shock.

5.1. Radial arm maze (RAM)

In the RAM, the arms of the maze project out from a central platform like spokes of a wheel. Typically, a cup at the end of several of the arms contains some small food reward for the animal. Because the animals are usually food-deprived to motivate the foraging behavior, they will work to find the food in the most efficient manner. The optimal strategy is to visit each arm once before returning to any previously visited arm. Thus, to perform the task successfully, animals must remember where it has been and where food has been and/or remains available [50]. In other words, animals require both their reference and working memories. Reference memory contains the stable memories about the task; e.g., that food is located at the end of each arm. Working memory contains short-term information; e.g., which arms have or have not been visited. Unmanipulated rats perform this task nearly perfectly on an 8-arm RAM [60], and alternative strategies for solving the maze, such as following algorithms (e.g., always turn right upon leaving each arm and visiting the next arm encountered) or using odor cues (e.g., to determine if food remains at the end of a particular arm) have been generally ruled out [59,61]. Temporal measurements and error scoring (revisiting arms

that do not contain food) are the parameters that are typically used as indices of cognitive function in the RAM.

Permanent occlusion of the right MCAO is associated with a long-term deficit in acquisition of the radial task [62], whereas 90 min of right MCA occlusion is associated with a transient deficit in the acquisition and retention of the radial arm task during the first two weeks following surgery [34]. Even though the right and left MCA occlusions resulted in infarcts of similar size, temporary occlusion of the left MCA had no effect on acquisition of the radial arm task under the same behavioral protocol used following right MCAO [34]. The underlying cause of the difference in RAM performance following right versus left MCAO is unknown, but requires further study because a lack of functional hemispheric asymmetry has been reported in lesion studies that use the RAM [63].

The radial arm task requires extensive motor coordination, training, and food-restriction for motivation; therefore, it is not an ideal method for the assessment of cognitive abilities in animal models of stroke. Animals that require longer to navigate the maze because of locomotor deficits, or those that are not motivated to seek out the food, will appear to possess deficits in learning. Importantly, there have been several reports that MCAO results in significantly lower body weight at several time points following surgery [13,19,20,22,27,62,64,65]. Thus, a significantly lower body weight suggests that the MCAO animals may not be as motivated to seek out food as the sham-operated animals, which could become a confounding factor when attempting to assess cognitive function via the radial arm maze. For these reasons and others, most investigators have chosen to use another tool in assessing cognitive ability, viz., the Morris water maze.

5.2. Morris water maze (MWM)

The Morris water maze is not actually a ‘maze’ per se; rather it typically consists of a round tank filled with opaque water [53]. The animal is trained to swim to a hidden platform that is located just beneath the surface of the water. Because the water temperature is usually maintained at room temperature, it is aversive, and presumably the animals are highly motivated to find the platform to get out of the water. Because the platform is not visible, the animals must depend on their spatial memory and use extra-maze visual cues to locate the platform. The animals are usually introduced into the water at different locations around the tank. Data collection in the water maze can be easily automated and it does not require food-restriction, but in common with the RAM, the water maze task does require complex and coordinated movement, which is often impaired after stroke.

The Morris water maze has been used to study impairments in spatial learning and memory in several experimental stroke studies [5,12,13,20,25,26,40,66–72,66–72]. However, the experimental protocols and indices of perfor-

mance used in these studies vary considerably and consequently, the conclusions are not always consistent (Table 4). Most of the studies report an MCAO-induced increase in latency to locate the platform or an increase in path length, but often a significant difference between the ischemic and control animals is reported at only one of several time points (suggesting the possibility of a Type I statistical error). Differences among the outcomes in these studies are not likely to be due to whether the left or right MCA was occluded, because there is no functional asymmetry in water maze performance after unilateral hemi-decortication or hippocampal lesioning of the left versus right hemisphere [63]. Additional research also is necessary to determine if there is a species difference in the effects of MCAO on performance in the water maze. In mice that were pre-trained in a working memory version of the Morris water maze, permanent left MCAO did not affect performance as measured by path length [66]. Alternatively, training the mice in the water maze *prior* to inducing MCAO may have provided the animals with non-spatial strategies that allowed them to successfully navigate the maze despite ischemia-induced neuronal death. This MWM pre-training phenomenon has been documented in other models of impaired spatial learning and memory [73,74]. All of the MCAO studies report no difference in mean swim speed between sham and MCAO groups, but one study indicated that there was a transient disruption in maze performance that was attributed to a nonspecific surgical effect in both the MCAO and sham groups [66].

Traditionally, latency to locate the platform and path length have been used as indices of learning and memory in the water maze, however, the relatively imprecise nature of these measurements has led to the development of additional methods for assessing learning strategy in this task [48]. For example, during probe trials when the platform is removed, the percent time spent, or distance traveled, in the goal quadrant (which contained the platform during the training trials) versus the quadrant opposite the goal quadrant is a useful measure for analyzing search pattern [48]. However, in the two experimental stroke studies in which a probe trial was conducted, the outcomes differed. In the first study, there was no difference between the MCAO and sham groups in the percent distance traveled in the goal quadrant or the number of times the experimental animals crossed overtop of where the platform had previously been located [72]. It should be noted that the overall effects of MCAO on MWM performance in this study were slight. In contrast, the percent time spent in the goal quadrant was significantly greater for the sham operated animals than the MCAO animals in another study [24]. In addition, the MCAO animals spent more time swimming near the wall of the tank (thigmotaxis) during the training sessions than the sham-operated animals [24], which is consistent with the swim patterns observed in animals with lesions of the caudate putamen [54] or hippocampus [75]. The effects of caudate putamen lesions on thigmotaxis in the MWM are of

Table 4
Effects of MCAO on Spatial Learning and Memory

Behavioral test	Method of MCAO	Timing of training	Beginning of test	Behavioral effect of MCAO (compared to sham)	Anatomic specificity	Spontaneous recovery
Radial arm maze [62]	Permanent proximal rt MCAO	Post-op	1 month post-op	Required more training sessions to reach criterion	–	–
Radial arm maze [34]	Transient (90 min) rt MCAO	Pre-op	Post-op day 3	Number of maze errors increased at one of nine time points and time required to successfully run maze increased at three of nine time points	–	Yes
Radial arm maze [34]	Transient (90 min) rt MCAO	Post-op	Post-op day 3	Number of maze errors increased at one of 28 time points and time required to successfully run maze increased at three of 28 time points	–	–
Radial arm maze [34]	Transient (90 min) lt MCAO	Post-op	Post-op days 1–14	No effect	–	–
Morris water maze [20]	Permanent lt proximal MCAO	Post-op	8 weeks post-op	Increased latency to locate platform and increased path length and time in goal quadrant at several time points	All three behavioral measures correlate with cortical infarction	–
Morris water maze [68]	Permanent lt proximal MCAO	Post-op	2 weeks post-op	Increased latency to locate platform at all time points	–	–
Morris water maze [69]	Permanent lt proximal MCAO	Post-op	1 week post-op	Increased latency to locate platform at all time points	–	–
Morris water maze [67]	Permanent lt proximal MCAO	Post-op	4 weeks post-op	No difference in percentage of animals locating the platform	–	–
Morris water maze [13]	Transient (90 min) lt MCAO	Post-op	Post-op days 17 and 18	Non-significant decrease in latency to locate platform on the second trial day	–	–
Morris water maze [25]	Permanent lt proximal MCAO	Post-op	5 weeks post-op	Increased latency to locate platform and increased in path length	Correlation between latency to locate platform and total infarct volume	–
Morris water maze [71]	Permanent lt distal MCAO	Post-op	2 weeks post-op	Increased latency to locate platform at seven of eight time points	–	–
Morris water maze [12]	Permanent rt proximal MCAO	Post-op	5 weeks post-op	Increased latency to locate platform; no change in path length	–	–
Morris water maze [40]	Permanent rt distal MCAO (cortical damage only)	Post-op	Post-op day 3	Increased latency to locate platform at one of five time points	–	–
Morris water maze [72]	Transient (60 min) rt MCAO	Post-op	13 months post-op	Increased latency to locate platform at one of nine time points	–	–
Morris water maze (mice) [96]	Permanent lt MCAO (no subcortical damage)	Pre-op	Post-op day 4	No difference	–	–

particular interest because MCAO often results in damage to this brain region.

An additional analysis that may be useful in differentiating between the performance of MCAO and sham-operated animals in the Morris water maze is proximity score [48]. One shortcoming of using the traditional measures of path length and escape latency in the water maze is that they do not provide any indication of the type of search strategy that is being used by the animals to locate the hidden platform. Consequently, animals from two different treatment groups may utilize very different search strategies and still have similar path lengths and escape latencies [48]. The advantage of using the proximity score is that it is more representative of the spatial distribution of the search than the traditional measures [48]. The proximity score is calculated by determining the distance of the animal from the goal during each second of the trial and is used as a measure of deviation from the ideal path to the platform once an animal is placed in the pool. Although, calculating proximity scores does not require additional animal training or probe trials, it does require software that may not be available with all video tracking systems.

It is important to include in all water maze studies, a series of trials in which a visible platform is used, to rule out group differences in sensorimotor deficits and motivation [48,53] which may affect performance in the MWM. For example, if MCAO induces forelimb weakness that is severe enough to result in decreased swim speed compared to controls, then latency to escape will be increased whether the platform is hidden or visible. Thus, when there is a strong correlation between performance on the hidden and visible platform versions of the water maze, it is not possible to clearly attribute altered performance in the hidden platform version of the task to a cognitive rather than sensorimotor or motivational deficit [76]. Given the high incidence of sensorimotor deficits after MCAO, it is particularly important to include the visible platform sessions in any experimental stroke study incorporating the water maze [12], but few researchers do so.

5.3. *Passive and active avoidance*

Passive and active avoidance tasks are the most commonly used measures of cognitive function (specifically associative learning) in experimental stroke studies. Although several different apparatuses and experimental protocols have been used, the underlying principal of passive and active avoidance remains the same: the animals are taught to avoid the part of the apparatus in which they have previously received electric shocks. One example of a passive avoidance task would be to place an animal into a cage that is partitioned into a light and dark chamber. Rodents prefer dark, enclosed spaces, so when placed into the light chamber, the animal will typically venture into the dark chamber within several seconds. When the rat enters the preferred dark chamber, it receives a mild electric shock

(the learning trial). On subsequent tests, the animal must remember not to go into the dark chamber, otherwise it will receive additional electric shocks. Thus, in a passive avoidance task, an animal must learn to suppress a behavior that it would normally exhibit in order to avoid receiving additional electric shocks. Requiring fewer training sessions prior to acquiring the task and displaying increased latencies to enter the dark ‘shock’ chamber on subsequent tests indicate successful learning and memory retention.

In an active avoidance task, an animal must learn to exhibit a behavior that it normally would not exhibit in order to avoid receiving an electric shock. An example of an active avoidance task would be to place an animal into an apparatus like the one described above, except that the animal is placed into the dark chamber where it subsequently receives a series of electric shocks until it crosses into the light chamber (learning trial). On subsequent trials, the animal must remember to actively walk to the light chamber within seconds of being placed in the apparatus in order to avoid receiving additional electric shocks. For active avoidance tests, requiring fewer training sessions prior to acquiring the task and displaying decreased latencies to enter the light chamber on subsequent tests indicate successful learning and memory retention.

Training the animals in the passive or active avoidance task prior to surgery allows one to assess the effects of MCAO on task retention, whereas training the animals post-surgery allows one to assess both task acquisition and retention, but not independently. Despite the high degree of diversity in the type of apparatus and experimental protocol used in passive avoidance testing, all of the studies using MCAO in rats and mice report significant impairments in the acquisition [27,39,77] or retention [20–23,25,27,39,69,77–82] of the passive avoidance task compared to sham-operated animals. However, performance in the passive avoidance task is rarely correlated with histological damage [20,21,23,39]. MCAO also results in a significant impairment in the retention of an active avoidance task [82], which has been correlated to cortical infarction volume.

Although performance in both active and passive avoidance tasks can be altered by MCAO-induced sensorimotor deficits, the more conservative approach would be to use the passive avoidance paradigm in experimental stroke research. If the MCAO animals are not able to move freely around the apparatus, then there is the increased chance of reporting a ‘false positive’ effect when using an active avoidance paradigm and a ‘false negative’ effect when using a passive avoidance paradigm. However, given the robust effects of MCAO on passive avoidance in the studies listed above, it appears that several different passive avoidance protocols are sufficiently sensitive to detect differences in performance even when the MCAO animal’s movement may be hindered by sensorimotor deficits. It is still important to assess and report group differences in sensorimotor behavior on each test day of the passive avoidance protocol.

Overall, associative learning paradigms appear to be the most sensitive and consistent indicators of MCAO-induced cognitive impairment. As described above, deficits in active and passive avoidance have been reported consistently across laboratories despite the use of different methods of MCAO, behavioral protocols and testing apparatuses. In contrast, MCAO-associated alterations in RAM and MWM performance have been highly variable (Table 4). Whether the variability in RAM and MWM results represents imperfect behavioral testing conditions or a marginal effect of MCAO on spatial learning memory remains to be specified. Thus, for researchers who are interested in assessing MCAO-induced alterations in cognitive function but have minimal behavioral testing experience, the passive avoidance paradigm is recommended because it is 'user-friendly' and the effects of MCAO in this paradigm are robust.

6. Measuring anxiety following experimental stroke

In humans, stroke is associated with an increased incidence of anxiety although the etiology remains unknown [83]. Damage to the cortex and caudate putamen is not typically associated with increases in anxiety, however, it is necessary to rule out the possibility that experimental stroke causes increased anxiety in rodents which could then affect performance in additional testing. Anxiety is commonly assessed using an open field test or the elevated plus maze. In the open field test, the animal is placed in an open arena (1 m²), and its movement is recorded during the testing period. The floor is marked off by 16 squares and the number of squares crossed is typically counted. Also, the amount of time that the animal spent in the open field (inner squares) is compared to the time the animal was moving along the walls of the arena. Animals normally prefer to avoid the open center and spend most of their time near the walls, presumably for protection. Experimental manipulations that alter this avoidance of open spaces subsequently can affect performance in a learning task. Although the open-field has been used as a measure of locomotor activity in experimental stroke studies, it has not been used to assess anxiety.

The elevated plus maze also can be used to determine anxiety levels. Animals are placed in the center of an elevated plus maze with two open arms and two closed arms (~67 × 5.5 cm for mice). The closed arms have 15 cm high, black tinted Plexiglas and 65 cm long detachable roof. The maze is mounted 75 cm above the floor on a tripod. Choice behavior, the number of visits to each arm, and the time spent in each arm, as well as in the central area are recorded. Incidents of grooming, rearing, and number of fecal boli produced are also typically recorded. The elevated plus-maze exploits the natural tendency of rodents to suppress exploration of the open arms when they are anxious [84,85]. Measurement of the amount of time that

the experimental animal spends exploring the open arms of the maze provides a sensitive index of anxiogenic-like behavior. In rats, transient right MCAO decreases the number of arms visited within the first week after surgery, but does not alter other measures of anxiety in the elevated plus-maze [86].

7. Correlation between histology and behavior

Through reviewing the behavioral stroke literature described above, it quickly becomes apparent that it is the exception rather than the rule to find a close correlation between histological and behavioral outcomes. In fact, there are several examples of studies in which behavioral stroke outcomes are improved by experimental manipulation in the absence of any corresponding change in histology [23,78,79,87,88]. Among the factors that could account for the lack of a close histological-behavioral association are functional compensation or recovery, as well as, histological limitations. As described below in more detail, there are many redundancies in the mechanisms underlying behavior, so behavioral changes may be measurable only after the severity of the infarction on has reached a critical threshold [6]. For some tasks, even when a behavioral deficit has been identified soon after stroke, there is partial to full functional recovery over time (reviewed above). Therefore, the timing of the behavioral tests and tissue collection may influence the degree of correlation between these two end points. Recovery of sensorimotor function also has been reported in several models of unilateral brain damage and the mechanisms responsible for this functional recovery are a topic of intense investigation [9,10]. Kinematic (frame by frame) analysis of motor behaviors has been used successfully to study apparent behavioral recovery following cortical lesions [89] and may be useful in understanding the mechanisms responsible for functional recovery following MCAO also.

The two most commonly used methods for quantifying the amount of histological damage following MCAO are calculating the volume of infarcted tissue via image analysis or counting the number of surviving neurons with normal morphology. These methods allow one to differentiate live versus dead tissue, but are not designed to identify diffuse morphologic changes within non-MCA territory [11]. Also, classifying neurons merely as dead or alive does not provide information on the effectiveness of intracellular or intercellular communication in the remaining live neurons. In other words, a neuron may have survived the ischemic insult, but it is not functioning at full capacity. For example, infarction quantification may not reveal MCAO-induced damage to the hippocampus, but changes in long-term potentiation in that region have been reported following MCAO and may negatively alter performance in cognitive tests [90,91]. In addition, distribution of cell death within a region may be a more important determinant of behavioral function than the

size of the infarction [92]. Thus, the lack of a close correlation between the histological and functional outcomes of MCAO emphasizes the need to include both outcome measures in experimental stroke studies.

8. Variability of behavioral results in experimental stroke studies

Even under the best testing conditions behavior is highly variable. It is a process that results from numerous complex subprocesses. There are many redundancies in the mechanisms underlying behavior, and to make matters more difficult, there are large individual differences in both the physiological mechanisms of behavior and the behavioral output [46]. Consequently, behavioral research often requires large sample sizes, consistent testing conditions, and sophisticated statistical analyses to detect the usually subtle changes within the wide range of normal behaviors that are caused by any experimental treatment [47]. This enormous inter-individual variation in behavior continues to be a problem for many investigators who have recently added behavioral tests to their studies—whole animals do not behave like the biochemical processes that underlie their behaviors. The ‘whole’ does not necessarily equal the sum of its parts in the study of behavior. It is rare to see three to five-fold changes in behavior after an experimental manipulation.

Maintaining a consistent testing environment is paramount when studying behavior and will help reduce intra-experiment variability. Among the many subtle environmental variables that can influence behavior are ambient temperature, time of testing during circadian cycle [93], lighting intensity [94], and housing conditions [95]. In addition, the detailed and accurate reporting of testing conditions in publications is likely to improve replicability between laboratories.

The effects of MCAO on avoidance and sensorimotor behavior (Table 1) are remarkably consistent across experimental stroke studies. In contrast, the greatest discrepancy in results is associated with the quantitative measurement of motoric behavior (Table 2) and performance in the Morris Water Maze (Table 4) following MCAO. For example, when locomotor activity is measured via open field activity [21,33] and wheel running [33], right MCAO causes hyperlocomotor activity while left MCAO has no effect on activity levels. However, when automated activity chambers are used to measure locomotion there is no increase in activity in animals subjected to right MCAO. Although a running wheel and automated locomotor chamber should yield similar results when animals are subjected to MCAO, there are several discrepancies between the two studies that may account for the inconsistency in results including: (1) extent of neuronal damage, (2) circadian differences, (3) length of post-operative recovery period prior to initiating testing and (4) strain differences. Without further testing it is not possible to determine which of these factors contributes to the

opposing behavioral results in the wheel running and locomotor chamber studies.

The behavioral effects of MCAO on performance in the Morris Water Maze also are highly variable. Animals subjected to left MCAO exhibit an increase in latency to escape and in some cases a concomitant increase in path length [20,25,68,69]. Animals subjected to right MCAO typically exhibit an increase in latency to escape at some time points [12,40,71,72] but no difference in path length [12]. Also, the only study conducted using mice reports no effect of MCAO on water maze performance [96]. Again, among the possible sources of variation are extent and distribution of neuronal damage, length of post-operative recovery period prior to initiating testing, experimental protocol (training pre- versus post-ischemia), and strain differences. In addition, the majority of the studies that report differences in the MWM have relied on latency to locate the platform as the primary index of learning and memory. As stated above, latency to escape is a relatively imprecise measure [48] that could be confounded easily by MCAO-induced sensorimotor deficits. Inclusion of the visible platform trials in future studies could rule out sensorimotor deficits as a potential source of variation between studies using the MWM.

9. Strain and species differences in behavior

There have been several reports that strain influences the extent of histological damage that results from MCAO in rats [97–99] and mice [100,101]. Ischemic outcome can even be influenced by the breeding conditions of different vendors [98,99]. It is therefore reasonable to expect that the pattern of sensorimotor deficits that occur following MCAO in rats may be partially strain dependent [14]. In addition, strain differences in performance on many of the cognitive and sensorimotor tasks that are favored by stroke researchers have been identified in rats [64,102] and mice [49,96,103–106]. Thus, a combination of strain differences in task-specific performance and the distribution or extent of neuronal death following MCAO may be partially responsible for variability in some of the functional stroke outcomes described above.

10. Assessing functional outcomes in mice

Several studies have examined the histological consequences of MCAO in mice but few describe the effect of MCAO on functional outcome in this species [18,35,39,42,66]. As in rats, MCAO is reported to alter neurological score [18,35], visual paw placement [18], proprioceptive paw placement [18], balance on a rotarod [35,42], latency to move one body length [39] and passive avoidance [39] in mice. MCAO did not alter performance in the only mouse study that used the MWM as a measure of cognitive function, however, the non-traditional scoring

method used may not have been sufficiently sensitive to detect group differences [66]. Thus, it appears that the effects of MCAO on sensorimotor function and at least one measure of cognitive function in rats and mice may be similar. However, all of the MCAO behavioral studies have been conducted in male C57/BL6 mice, so additional studies are required before making assumptions regarding the generality of the findings described above.

Increased availability of knockout and transgenic mice is likely to result in increased use of mice as animal models of behavioral stroke research in the near future. The use of mice with targeted disruption of specific genes (i.e., knockouts) is an extremely valuable tool in stroke research because: (1) disabling a gene is often a very precise and ‘clean’ ablation, (2) the effects of the gene product can be abolished without the behavioral side-effects of drugs, and (3) genetic manipulations may be the only way to determine the precise role of many endogenous factors on behavior. However, several potential problems with using transgenic and knockout animals in behavioral studies have been identified [45–47]. For example, the products of many genes are essential to normal function, and inactivating the gene may induce gross morphological or physiological abnormalities that can complicate interpretation of discrete behavioral effects. Also, unexpected compensatory or redundant mechanisms might be activated when a gene is missing, and cloud interpretation of the normal contribution of the gene to behavioral outcome. An additional caveat of using knock-out mice is that typically the gene of interest is missing not only at the time of the experiment, but throughout ontogeny. However, as ‘inducible knockouts’ become available, and researchers are able to inactivate genes in precise regions of the nervous system and during specific time periods, the issue of ontogenetic influences will be resolved.

Strain differences also are an important consideration in assessing behavioral data in knockout mice. The stem cells used in producing knockout mice are often from 129 Sv mice against a C57/BL6 background. If the animals have not been backcrossed over several generations, using the appropriate control animals becomes problematic because the knockout and wild type mice differ not only in the presence or absence of the gene of interest, but in their genetic background. Thus, behavioral differences that are not specific to the presence or absence of the gene of interest may occur. It is especially important to conduct a series of preliminary studies on knockout (or transgenic) mice and wild-type mice to identify any genetically mediated alterations in the sensorimotor or complex behavioral tasks of interest prior to subjecting the animals to experimental stroke. If there are behavioral differences between genotypes prior to stroke, then it becomes very difficult to interpret any group differences in behavior that are identified post-ischemia. Knowing in advance which behaviors differ in the knockout (transgenic) and wild type mice, will allow researchers to choose the appropriate tests to measure post-ischemic functional outcome. Finally, it is important to

remember that in knockout mice behavioral tests study the effects of the *missing* gene (and gene product), not the effects of the gene directly [45,107].

11. Validity of behavioral ischemia models

Stroke in humans is commonly associated with impaired sensorimotor ability, reduced cognitive function and a change in affect. Approximately 70–85% of all patients experience hemi-paresis immediately following stroke [108]. A substantial proportion of stroke survivors will experience recovery of impaired motor function, but timing and extent of recovery varies greatly among individuals [109–112]. The mechanisms underlying functional recovery in humans are not well understood [113] but appear to involve compensatory changes in uninjured sensory and motor structures [114,115]. Following ischemia, rodents also exhibit sensorimotor deficits that often dissipate over time (reviewed above). Although several rodent studies have evaluated therapies that improve sensorimotor function following ischemia [40], large clinical trials will need to be conducted before it can be determined how well rodent behavioral models predict human functional recovery following stroke. Historically, preclinical testing of neuroprotective drugs in rodent models of acute ischemic stroke (incorporating primarily histological outcomes) has not been highly predictive of outcome in clinical stroke trials [1]. However, the inclusion of behavioral endpoints and longer survival periods may improve the predictive value of rodent ischemia models.

Changes in affect also are reported in many stroke victims. In some clinical studies, as many as 65% of stroke survivors are diagnosed with depression within the first year following an ischemic event. Post-stroke depression is associated with increased cognitive deficits [116], decreased general social interaction [117], and decreased sexual motivation and function [118]. Despite the prevalence of post-stroke depression, its etiology remains unknown and currently there are no rodent behavioral models that have been developed to study post-stroke changes in affective behaviors.

12. Summary

The simultaneous presentation of histological and behavioral end-points in experimental stroke studies is advantageous when assessing potentially therapies because, as reviewed above, it is uncommon to find a significant correlation between these two measures of ischemic damage. Nor should researchers be frustrated by the lack of correlation between some functional tests and infarction size; complex behaviors are typically influenced by input from several brain regions rather than one or two structures. In addition, when comparing the apparently conflicting results from several studies, it is important to remember that studies of

the functional outcomes of experimental stroke can be influenced by many variables including method of MCAO, experimental protocol, and genetic factors. Even when these variables are held constant, environmental factors, such as room temperature, light intensity, and time of testing during the circadian cycle can alter outcome [119]. A heightened awareness of how all of these factors can collectively alter behavioral outcomes will allow a better understanding of the circumstances under which MCAO alters functional outcome and may lead to an increase in the reliability and replicability of behavioral results across laboratories.

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