A Comparison of Long-Term Functional Outcome After 2 Middle Cerebral Artery Occlusion Models in Rats

Robin L. Roof, PhD; Gerald P. Schielke, PhD; Xiaodan Ren, MD; Edward D. Hall, PhD

Background and Purpose—Proven behavioral assessment strategies for testing potential therapeutic agents in rat stroke models are needed. Few studies include tasks that demand higher levels of sensorimotor and cognitive function. Because behavioral outcome and rate of recovery vary among ischemia models, there is a need to characterize and compare performance on specific tasks across models.

Methods—To this end, sensorimotor and cognitive deficits were assessed during a 5-week period after either permanent proximal middle cerebral artery occlusion (pMCAO) or permanent distal middle cerebral artery occlusion combined with a 90-minute occlusion of both common carotid arteries (dMCAO/tCCAO) in Sprague-Dawley rats. The EBST, hindlimb and forelimb placing, and cylinder tests were given at regular intervals postinjury to assess sensorimotor function. Cognitive function was assessed with a multitrial water navigation task.

Results—pMCAO, which caused both striatal and cortical damage, produced persistent sensorimotor and cognitive deficits. Limb placing responses and postural reflexes were impaired throughout the month of testing. A persistent bias for using the ipsilateral forelimb for wall movements in the cylinder test was observed as well as a bias for landing on the opposite forelimb. pMCAO rats were also impaired in the water navigation task. dMCAO/tCCAO, which caused only cortical damage, produced similar sensorimotor deficits, but these were greatly diminished by 2 weeks after injury. No impairment was found for water tank navigation. Correlations between forelimb placing (both models), water navigation performance (pMCAO model), and sensorimotor asymmetry (dMCAOtCCAO model) and infarct volume were observed.

Conclusions—Based on the range of functions affected and stability of observed deficits, the pMCAO model appears to be preferable to the dMCAO/tCCAO model for use in assessing therapeutic agents for stroke. (Stroke. 2001;32:2648-2657.)

Key Words: animal models ■ behavior ■ cerebral ischemia, global ■ cognition ■ stroke, experimental ■ rats

Oclusion of the rat middle cerebral artery (MCAO), as developed by Tamura et al.1 has become the model of choice for approximating the pathology and symptoms of human stroke.2,3 (STAIR stroke conference, Florida, 1999). MCAO is 1 of the most common causes of focal stroke in humans,2 and the ischemic changes associated with it closely resemble those produced in rat MCAO models.2,4 These similarities as well as the relatively constant infarct volume that can be achieved with this model make it useful for assessment of potential therapeutic treatments for human stroke.

The pathophysiology associated with MCA occlusion in rats has been well studied and described in detail elsewhere.5,6 A major limitation of this model and others like it, however, has been a lack of established, suitable behavioral test batteries with which to accurately assess patterns of neurological abnormality caused by cerebral ischemia. Until recently, the majority of studies have focused on the pathophysiology of the insult, using reduction of infarct volume as a measure of a drug’s effectiveness.7,8 Although a necessary and important measure, the amount of necrotic tissue in the primary infarct site is only 1 factor influencing functional recovery. The danger in relying on infarct volume is demonstrated by studies showing abnormal function after ischemia despite normal or near-normal histology,9,10 a phenomenon labeled “covert injury” by Squire and Zola,11 and by studies in which drugs appeared to be ineffective based on measurement of infarct volume but were found to significantly improve functional outcome (for examples, see References 10, 12, and 13). The issue of including behavioral assessment in rat stroke studies becomes even more critical with the recent interest in neurorestorative agents, the effectiveness of which will likely be reflected more by subtle changes in synapse number and dendritic structure than by changes in infarct volume.

Thus, for a variety of reasons, there is a growing consensus that the effective evaluation of potential therapeutic interventions for human stroke in the rat MCAO model requires...
appropriate behavioral testing in addition to histological measurement.\textsuperscript{14–21} Exactly what that behavioral testing should be composed of is the topic of current discussion.\textsuperscript{2,14,15} In most cases when behavioral outcome is assessed after MCAO in rats, testing is limited to simple reflex and motor functions,\textsuperscript{20,22–24} usually during the early phase of infarction.\textsuperscript{23,24} Tests are often grouped into a basic “neurological scale” (see References 20 and 25) from which a single score can be obtained for each rat.\textsuperscript{23,24} This type of scoring system limits the information available because deficits of these basic functions represent only 1 component of the complex pattern of the sensorimotor disruption that can result from a cerebral ischemic insult. Testing of more complex sensorimotor function has tended to be neglected. There are now, however, several excellent behavioral tests that have been developed that allow evaluation of more complex sensorimotor functions, such as sensorimotor integration (eg, Schallert’s cylinder test\textsuperscript{26} and the placing test\textsuperscript{27}), unilateral sensory neglect (eg, Schallert’s sticky tape test,\textsuperscript{28}), and fine motor coordination (eg, Whishaw’s reaching task\textsuperscript{29} and the staircase test\textsuperscript{30}). These tests are sensitive enough to detect the effects of therapeutic agents and for tracking behavioral recovery over time,\textsuperscript{2,15,30} and they vary in the degree and pattern of spontaneous recovery that occurs after MCAO (for reviews, see References 2 and 14), making them useful for comparing outcome after infarcts of differing severity and for comparing the effects of different doses and/or drugs. Combining such tasks with a basic neurological scale provides a more comprehensive picture of the sensorimotor capacity of the animal.

The other important consequence of human stroke that has not been well addressed in the rat MCAO model is disruption of cognitive ability. A variety of cognitive deficits have been observed in human stroke patients, including memory dysfunction,\textsuperscript{31} topographical disorientation,\textsuperscript{32} decreased attention, and diffuse intellectual impairment.\textsuperscript{33} It is possible to test these or similar functions in rats with 1 of several already established cognitive tests, such as passive avoidance,\textsuperscript{34} the radial arm maze,\textsuperscript{35} or the Morris water maze (MWM).\textsuperscript{36}

Several laboratories have made use of the MWM to assess the behavioral effects of stroke in the rat MCAO model.\textsuperscript{18,19,37,38} This task can be modified to evaluate various aspects of cognitive functioning to provide information beyond a simple performance score. For example, the MWM is used to assess acquisition and retention, search strategies, and perseveration. The water maze task is sensitive to damage in several brain areas affected by MCAO including striatum and frontal cortex.

The selected battery was tested in 2 MCAO procedures: permanent proximal occlusion of the MCA (pMCAO) and permanent distal occlusion of the MCA combined with 90-minute bilateral common carotid artery occlusion (dMCAO/tCCAO). The pMCAO model has seen frequent use in rat stroke studies, with Tamura’s\textsuperscript{1} and modified Tamura’s\textsuperscript{20,22} methodologies being the most common. This approach involves the coagulation of the right MCA proximal to the origins of the lateral lenticulostriate arteries, producing a reliably large cortical infarction that extends to the lateral part of the striatum as well.\textsuperscript{1} The dMCAO/tCCAO model has not been well studied, although a number of studies have been published in which variations of this mixed occlusion were used.\textsuperscript{42–45} In general, occlusion of the distal portion of the MCA combined with some form of CCAO produces an infarction limited to the frontal and parietal areas of the neocortex,\textsuperscript{42,44–46} although occasionally small subcortical infarcts are reported.\textsuperscript{43}

### Materials and Methods

#### Animals and Experimental Treatments:

We used 39 male Sprague-Dawley rats (275 to 325 g at the time of stroke). Rats were housed in groups of 3 or 4 and were maintained on a 12-hour light/dark cycle with unlimited access to food and water.

All procedures performed in the experiments described here were approved by institutional committee for animal use.

#### Surgical Procedures:

Anesthesia was induced with 4% isoflurane and maintained with 2.5% isoflurane, supplemented with oxygen, during surgical procedures. Body temperature was maintained at 37.5°C using a heating blanket and a feedback temperature controller.

**pMCAO**

The right MCA was occluded using the technique originally described by Tamura et al\textsuperscript{1} and modified by Bederson et al.\textsuperscript{20} Briefly, the MCA was approached through a temporal incision, and the bone overlying the vessel was removed using a dental drill. The dura was opened, and the arachnoid membrane was gently removed. The vessel was occluded by bipolar coagulation, from a point proximal to the lenticulostriate branches to the rhinal fissure. The incision was closed, and the rats were returned to their home cage after full recovery from anesthesia. The sham surgery was similar to the MCAO surgery and included opening of the dura; however, the MCA was not isolated or coagulated. Anesthesia duration was similar in all groups.

**dMCAO/tCCAO**

The right MCA was approached through a temporal incision and exposed at the level of the inferior cerebral vein. The common carotid arteries were isolated through a neck incision and occluded with aneurysm clips. The MCA was then occluded by bipolar...
coagulation just distal to the inferior cerebral vein. After 90 minutes, rats were reanesthetized, both carotid clips were removed, and the neck incision was closed. Rats were returned to their home cage after full recovery from anesthesia. The sham surgery was similar to the MCAO surgery and included opening of the dura; however, neither the MCA or carotid vessels were isolated, clipped, or coagulated. Anesthesia duration was similar in all groups.

Behavioral Testing
Each rat was subjected to a series of behavioral tests during the 30 days after stroke was induced. The tests used included (1) the limb-use asymmetry (cylinder) test, conducted preinjury and days 7, 14, 21, and 28 postinjury; (2) the forelimb and hindlimb placing tests, given on days 1, 5, 9, 13, 17, 21, 25, and 29 poststroke; (3) the EBS test, also given days 1, 5, 9, 13, 17, 21, and 29 poststroke, and (4) the MWM test, conducted days 7 and 14 after stroke. All behavioral testing was done during the animals’ light cycle. The experimenter conducting behavioral testing and scoring was blind to the experimental condition.

Description of Specific Tests
Cylinder Test
This task was designed by Schallert et al.26 For the present study, forelimb-use bias was analyzed by videotaping the rats’ movements while in a transparent Plexiglas cylinder (diameter 18 cm, height 30 cm) for 3- to 5-minute intervals. The cylinder was wide enough to allow movement but was small enough to encourage rearing and wall exploration. The height of the cylinder disallowed the rat from reaching the top edge. A mirror placed behind the cylinder allowed the experimenter to observe and record forelimb movements when the rat was facing away from the camera. Independent versus combined use of the forelimbs during vertical exploration of the walls and when landing was scored as follows. During a rear, the first forelimb placement on the wall was scored as an independent wall movement for that limb. If followed by placement of the other limb on the wall (without first removing the first paw), the movement would be scored a simultaneous limb-use movement. Each subsequent movement along the wall was scored in the same manner. Lateral exploration of the wall by alternating right and left limb placement would result in a series of “simultaneous limb-use” scores, whereas hopping along the wall using 1 limb would result in a series of independent limb-use scores for that limb. After an episode of rearing and wall exploration, the first limb to contact the ground was scored as a “landing” for that limb. If both forelimbs contacted the floor at the same time, a simultaneous limb-use score was given. Ambiguous and difficult-to-score movements were not included.

Percent-use scores were calculated for (1) movements using the nonimpaired limb relative to the total number of movements and (2) movements using the impaired limb relative to the total number of movements. The percent use of the impaired limb was then subtracted from the percent use of the unimpaired limb to create an overall limb-bias score. Limb use for wall and landing movements were analyzed separately. Average scores for each week of testing (2 testing sessions per week) were calculated for each animal.

Forelimb and Hindlimb Placing Tests
Independent testing of each forelimb for placing in response to visual, vibrissae, tactile, and proprioceptive stimulation was conducted using a test designed by De Ryck et al.17 Testing of each hindlimb for placing in response to actual and proprioceptive stimulation was also carried out. For forelimb placing, the rats were held by their torso with their forepaws hanging free and moved slowly toward the edge of a tabletop, stopping short of touching the vibrissae (for vision-induced placing), touching the vibrissae (for vibrissae-induced placing), making light contact with the front of the forepaw to the edge of the tabletop (for tactile-induced placing), and
pressing the forepaws to the edge of the table with increased pressure (for proprioceptive-induced placing). Hindlimb placing was conducted in the same manner as above but with tactile and proprioceptive stimulation applied to the front/top of each hindlimb. Each test was repeated for each paw up to 6 times or until 3 correct placements were made, whichever came first. The number of completed placing responses out of 3 for each test was recorded. Average placing scores (forelimb and hindlimb combined) for each week of testing were calculated for each animal.

**Elevated Body Swing Test**

The EBST, described in detail by Borlongan et al., was used to test asymmetric motor behavior. Rats were held by the base of the tail and elevated ~1 inch from the tabletop. The direction of the first body swing, defined as an upper body turn of >10 degrees to either side, was recorded for each of 30 trials (3 sets of 10 trials over 5 minutes). The numbers of left and right turns were recorded, and the percent of turns made to the side contralateral to the stroked side bias on this task, whereas rats with unilateral stroke show a strong tendency to turn toward the side contralateral to the stroked hemisphere was determined. Intact animals do not typically exhibit a side bias on this task, whereas rats with unilateral stroke show an overall asymmetry score was obtained for each animal by combining percent asymmetry in wall movements for the cylinder test with percent turn bias for the EBST.

**Morris Water Tank Test**

Cognitive testing was conducted using the water tank task originally designed by Morris et al. On day 7 after stroke, the rats were given a series of 6 trials, 1 hour apart in a large dark-colored tank (160 cm in diameter) filled with clear water at a temperature of 22.0±1.0°C. A 13×13-cm submerged platform (2 mm below water surface) was placed in the northwest quadrant of the pool. The release point was always the southern end of the pool. The rats were lowered into the pool facing the wall and were released. Each rat was given a maximum of 90 seconds to find the submerged platform. If it did not find the platform in that time, the rat was physically guided to it. After remaining on the platform for 20 seconds, the rat was removed and placed in a dry cage. One hour later, each rat was given a second trial, using the same release position and platform position, to measure retention of platform location. This process was repeated a total of 6 times for each rat, each trial 1 hour apart. These 6 trials were then followed by a probe trial for which the platform was removed from the pool. Each rat was allowed to swim for 30 seconds before being removed. The percent time and portion of the swim path that each rat spent in the area previously containing the platform were determined as measures of platform location retention by each rat. The entire procedure was repeated 1 week later (day 14 after stroke).

The swim paths of the rats were recorded with a computer-interfaced camera tracking system (Noldus Ethovision). For each trial, the following parameters were analyzed: length of path to platform and latency to platform (measure animals’ ability to learn and remember the location of the platform), swim velocity (to assess the effects of physical ability to swim on results), percent of swim path limited to outer annulus (swimming in the outermost 15 cm of the pool rather than searching for platform), as well as percent of path in platform annulus (36-cm-diameter circular area surrounding platform) and initial heading error (disparity between actual initial heading and correct direction of platform during the first 3 seconds of swim time), both indicative of memory for general location of platform. For the first of the 6 regular trials and for the probe trial, each rat’s swim path was also analyzed for degree of sinuosity and tortuosity, as well as for relative turn angle and relative turn bias. These measures were used to assess the influence of any motor impairment (eg, persistent turning in 1 direction) on performance.

**Statistical Analyses**

Results were analyzed using 2-way ANOVA with independent variables of treatment group and day of testing (time). Planned comparisons were made between each MCAO group and its sham equivalent, as well as between the 2 MCAO groups at each time point. Four planned comparisons per time point were made, and the modified Bonferroni method was used to correct for multiple comparisons.

**Histology**

After the final behavior test, rats were killed and brains removed and frozen for histological analysis of the infarct volume. Brains were sectioned at −17°C on a cryomicrotome. Twenty-micron coronal sections were collected at 600-μm intervals and stained with hematoxylin and eosin. Lesion volume was measured using the stereological software package GRID (Olympus). A grid of points was randomly superimposed over a video image of each tissue section, and the number of points overlaying an area of interest was counted. Total lesion volume was estimated using the equation \( V = 1 \times A(p) \times \Sigma P \), where \( V \) is lesion volume, \( t \) is the distance between sections analyzed (1200 μm), \( A(p) \) is the surface area associated with 1 grid point, and \( \Sigma P \) is the total number of grid points associated with an area of interest in all of the sections examined.

**Correlations Between Histology and Behavioral Outcome**

Correlation analyses were carried out between measures of infarct volume and those behavioral measures on which significant impairments were observed. Because cortical and subcortical structures may play different roles in the behaviors tested, separate analyses were conducted for cortical and subcortical infarct volume.

**Results**

**Permanent Proximal MCAO**

**Sensorimotor Tests**

Both forelimb \( (F_{1,136}=201.01, P<0.0001) \) and hindlimb \( (F_{1,136}=412.89, P<0.0001) \) placing were impaired in rats with...
pMCAO compared with sham-operated controls. While pMCAO rats showed no significant improvement in hindlimb placing (as indicated by lack of significant interaction with time), some improvement in forelimb placing did occur ($F_{7,136}=2.9, P=0.01$). However, pMCAO rats were still significantly impaired at forelimb placing (as well as in hindlimb placing) compared with sham-operated controls at the end of the 30 days of testing. These data are shown in Figures 1A and 1B.

Turn bias, as measured by the EBS test, was significantly higher in pMCAO rats compared with sham-operated controls ($F_{1,136}=299.29, P=0.0001$). pMCAO rats showed a strong and persisting tendency to turn their upper bodies to the side contralateral to the stroked hemisphere, whereas sham-operated rats showed no bias. These data are shown in Figure 2A.

pMCAO also produced a persistent bias toward using the ipsilateral forelimb for wall exploration ($F_{1,85}=25.2, P<0.0001$) and using the contralateral forelimb for landings ($F_{1,85}=35.84, P<0.0001$) in the cylinder test. This forelimb use bias did not recover over the 30-day test period. Sham-operated rats showed no significant bias in forelimb use. These data are shown in Figures 3A and 3B.

**Morris Water Tank Test**

Permanent MCAO resulted in significant impairments in the Morris water tank test. pMCAO rats took significantly longer ($F_{1,85}=14.29, P<0.001$) to reach the platform during the 5 regular trials. There also was a difference in swim velocity between groups ($F_{1,85}=13.37, P<0.001$), with pMCAO rats swimming at a slightly higher average velocity compared with sham-operated controls. This difference in velocity would influence the latency comparison but not the length of path comparison. The data for length of swim path are shown in Figure 4A. Performance during the probe test also suggests impaired retention of the platform location after pMCAO. Sham-operated rats spent a greater percentage of their total time ($t_{17}=2.96, P<0.001$) and of their total swim path ($t_{17}=4.176, P<0.001$) in the annulus that previously contained the platform than did pMCAO rats, as shown in Figure 4B. The opposite pattern was seen for movement in the outer annulus (outer ring of the pool), as shown in Figure 5A. pMCAO rats spent a significantly greater percent of their total time ($t_{17}=4.1, P<0.001$) and of their total swim path ($t_{17}=3.126, P<0.01$) circling the outside of the pool than did sham-operated controls. In addition, pMCAO rats had a greater average error in their initial heading than did shams (47.2 versus 13.5 degrees off from correct heading; $t_{17}=8.4, P<0.0001$), shown in Figure 5B.

There were no differences between the 2 groups in their extent of sinuosity ($P=0.49$, NS), meandering ($P=0.55$, NS), relative angular velocity ($P=0.37$, NS) or relative turn angle ($P=0.42$, NS) in swim paths. The lack of group differences in these measures suggest that the performance disparity be-
between pMCAO and sham-operated rats was not due to a simple motor impairment causing problems with turning. Impaired performance by the pMCAO rats was also seen in the MWM retest 1 week later. Sham rats showed significant retention of the platform location from the previous week as exhibited by short latencies and swim paths beginning with the first trial of the retest session. In contrast, the performance of pMCAO rats on trial 1 of the retest did not differ significantly from their performance on trial 1 of the first session. Over the remaining retest session trials, pMCAO rats took longer (F_{1,85}=86.44, P<0.0001) and used longer swim paths (F_{1,85}=80.22, P<0.0001) to reach the platform compared with sham-operated rats (latter shown in Figure 4C). During the probe trial, pMCAO rats spent less time in the quadrant that previously contained the platform compared with sham-operated controls (t_{17}=2.23, P<0.05, shown in Figure 4D).

Histology
Permanent MCAO resulted in unilateral tissue loss and necrosis in parietal, temporal, and insular cortex and in the dorsal-lateral caudate putamen (Figure 6). The lesion often extended into the frontal, perirhinal, and occipital cortex as well. All animals had both cortical and caudate putamen tissues affected. The mean total infarct volume was 205±59 mm³. The area of damage extended from 5.5 to −7.5 mm relative to bregma and was contained wholly in the right hemisphere. The average infarct volumes were 144±62 and 62±15 mm³ in the cortex and caudate putamen, respectively.

Correlation Between pMCAO Infarct Size and Behavioral Measures
Correlation analyses were conducted for infarct volume (mm³) and performance on those tasks or task groups for which significant differences were seen between injured and sham-operated rats. For the pMCAO model, these included (1) average forelimb and (2) hindlimb placing scores, (3) asymmetric limb use for wall movements and for (4) landings, (5) average distance to platform over water tank test trials 2 to 5, and (6) probe trial performance.

Significant correlations with infarct size were found for 2 of the behavioral measures. Forelimb placing performance correlated with subcortical infarct volume (r=0.67, P<0.05), as shown in Figure 7A. Performance on the water maze retest also correlated with subcortical infarct volume (r=−0.67, P<0.05), as shown in Figure 7B.

Distal MCAO Occlusion Model
Sensorimotor Tests
Both forelimb (F_{1,144}=67.55, P<0.0001) and hindlimb (F_{1,144}=55.42, P<0.0001) placing was impaired in rats with dMCAO/tCCAO compared with sham-operated controls. Unlike in the pMCAO model, improvement over time by dMCAO/tCCAO rats was observed in both forelimb (F_{15,144}=5.15, P<0.0001) and hindlimb (F_{15,144}=2.84, P<0.01) placing. Nevertheless, dMCAO/tCCAO rats were
significantly impaired compared with sham-operated controls at hindlimb placing on all days of testing and at forelimb placing on all except day 21. These data are shown in Figures 1C and 1D.

Turn bias, as measured by the EBS test, was significantly higher in dMCAO/tCCAO rats compared with sham-operated controls ($F_{1,144} = 48.14, P < 0.0001$). As with the pMCAO rats, dMCAO/tCCAO rats showed a strong and persisting tendency to turn their upper bodies to the side contralateral to the stroked hemisphere, whereas sham-operated rats showed no bias. These data are shown in Figure 2B.

Morris Water Tank Test

No significant impairments in the water tank test were seen after dMCAO/tCCAO. Rats with dMCAO/tCCAO did not differ from sham-operated controls in distance, latency to platform, or initial heading error.

Histology

Distal MCAO with temporary CCAO resulted in tissue loss and necrosis in the parietal and temporal cortex. The mean infarct volume was $113 \pm 43 \text{ mm}^3$, and the damage extended from 5.5 to $-7.5 \text{ mm}$ relative to bregma. Only cortical tissue was affected.

Correlation Between Infarct Size and Behavioral Measures

Correlation analyses were conducted for infarct volume ($\text{mm}^3$) and performance on those tasks or task groups for which significant differences were seen between injured and sham-operated rats. For the dMCAO/tCCAO model, these included (1) average forelimb and hindlimb placing scores and (2) asymmetric limb use for wall movements. Forelimb placing performance correlated with total infarct volume ($r = 0.68, P < 0.05$), as shown in Figure 7C. In addition, overall sensorimotor asymmetry score correlated with total infarct volume ($r = 0.665, P < 0.05$), as shown in Figure 7D.

Discussion

The present study examined the types and patterns of behavioral deficits resulting from 2 versions of the rat MCAO model with the purpose of developing an appropriate functional test battery with which potential treatments for stroke might be assessed. Several specific behavioral tasks were assessed for potential inclusion in this battery. In addition, the 2 MCAO models were compared for their usefulness as experimental stroke models in which behavioral outcome could be assessed.

Our results suggest the tasks selected were appropriate for use in assessing functional recovery in a rat MCAO model. Measurable impairments were observed for all of the tasks after pMCAO and for several tasks after dMCAO/tCCAO. In addition, the lack of correlation between performances on the various tasks suggests that each measured some unique aspect of functioning. This is not to suggest that these are the only tasks appropriate in this regard. For example, the battery lacks a measure of sensorimotor function on which substantial recovery does not occur after dMCAO/tCCAO. A task such as the staircase test developed by Montoya et al. might serve this purpose.

The second issue addressed in the present study was the relative value of the 2 types of MCAO for use in behavioral

![Figure 5](http://stroke.ahajournals.org/)

**Figure 5.** Probe trial: percent of total swim path in annulus previously containing platform (A) or in outer annulus (B) after pMCAO or sham surgery. *Significant difference between groups at that time point at the 0.05 level.

A persistent bias toward using the ipsilateral forelimb for wall exploration was seen for dMCAO/tCCAO rats ($F_{1,90} = 60.11, P < 0.0001$). This forelimb use bias for wall movements lessened somewhat over time ($F_{4,91} = 5.23, P < 0.001$) but did not recover completely. No such limb bias was seen for landings in the cylinder test. Sham-operated rats showed no significant bias in forelimb use for either wall movements or landings. These data are shown in Figures 3C and 3D.

**Morris Water Tank Test**

No significant impairments in the water tank test were seen after dMCAO/tCCAO. Rats with dMCAO/tCCAO did not differ from sham-operated controls in distance, latency to platform, or initial heading error.

**Histology**

Distal MCAO with temporary CCAO resulted in tissue loss and necrosis in the parietal and temporal cortex. The mean infarct volume was $113 \pm 43 \text{ mm}^3$, and the damage extended from 5.5 to $-7.5 \text{ mm}$ relative to bregma. Only cortical tissue was affected.

**Correlation Between Infarct Size and Behavioral Measures**

Correlation analyses were conducted for infarct volume ($\text{mm}^3$) and performance on those tasks or task groups for which significant differences were seen between injured and sham-operated rats. For the dMCAO/tCCAO model, these included (1) average forelimb and hindlimb placing scores and (2) asymmetric limb use for wall movements. Forelimb placing performance correlated with total infarct volume ($r = 0.68, P < 0.05$), as shown in Figure 7C. In addition, overall sensorimotor asymmetry score correlated with total infarct volume ($r = 0.665, P < 0.05$), as shown in Figure 7D.

**Discussion**

The present study examined the types and patterns of behavioral deficits resulting from 2 versions of the rat MCAO model with the purpose of developing an appropriate functional test battery with which potential treatments for stroke might be assessed. Several specific behavioral tasks were assessed for potential inclusion in this battery. In addition, the 2 MCAO models were compared for their usefulness as experimental stroke models in which behavioral outcome could be assessed.

Our results suggest the tasks selected were appropriate for use in assessing functional recovery in a rat MCAO model. Measurable impairments were observed for all of the tasks after pMCAO and for several tasks after dMCAO/tCCAO. In addition, the lack of correlation between performances on the various tasks suggests that each measured some unique aspect of functioning. This is not to suggest that these are the only tasks appropriate in this regard. For example, the battery lacks a measure of sensorimotor function on which substantial recovery does not occur after dMCAO/tCCAO. A task such as the staircase test developed by Montoya et al might serve this purpose.

The second issue addressed in the present study was the relative value of the 2 types of MCAO for use in behavioral

**Figure 6.** Photographic display of representative hematoxylin and eosin–stained brain sections taken from sham-operated, pMCAO, and dMCAO/tCCAO rats 40 days postinjury. Both cortical and subcortical damage resulted from pMCAO, whereas only cortical damage resulted from dMCAO/tCCAO.
assessment after stroke. In general, deficits after pMCAO were more severe and more persistent than those after dMCAO/tCCAO, and affected both cognitive and sensorimotor functions. Behavioral deficits observed after dMCAO/tCCAO, on the other hand, were milder (consistent with Reference 50), were limited to sensorimotor function, and subsided substantially during the month of testing. Both factors can be important when assessing the effects of therapeutic compounds. Because both sensorimotor and cognitive impairments are often seen after stroke in humans,2,32 the ability to test both types of function increases the relevancy of the test battery. A test battery that encompasses a wider variety of functions would also have a greater likelihood of detecting beneficial effects of therapeutic agents. The persistence of behavioral deficits after pMCAO is advantageous in that it allows testing of functional recovery at extended time-points after the ischemic event. Chronic or late-stage behavioral testing is especially critical when assessing neurorestorative agents. Not only may benefits of some agents appear only after a period of time, but extended testing can determine whether the beneficial effects of a compound are sustained or simply delay the appearance of a deficit.13,51,52 Based on these 2 factors, range of functions affected and stability of observed deficits, the pMCAO model appears to be preferable to the dMCAO/tCCAO model for use in assessing therapeutic agents for stroke. However, because information can be gleaned from by comparing the effects of a drug on behaviors with different rates of recovery and because some therapeutic agents may be more effective in cases of mild impairment, the use of both models in some cases may prove beneficial.

Efforts in the present study to correlate the severity of behavioral deficits with infarct volume were only marginally successful. For both MCAO models, infarct volume correlated with forelimb placing. Additionally, water maze retest performance correlated with subcortical infarct volume after pMCAO, and overall sensorimotor asymmetry correlated with infarct volume after dMCAO/tCCAO. Thus, correlation exists between infarct size and measures of both cognitive and sensorimotor behavior for pMCAO and with measures of sensorimotor behavior for dMCAO/tCCAO, which follows the pattern of deficits produced by the 2 models. However, we did not find any correlation with infarct volume among the remainder of our behavioral measures. Attempts to correlate behavioral measures with infarct volume by other researchers have also met with mixed results.17,19,20,30,31,38,39,53

Figure 7. Correlations between measures of infarct volume and behavior. A, Correlation between infarct volume and forelimb placing after pMCAO ($r = -0.67$, $P < 0.05$). B, Correlation between subcortical infarct volume and performance on the final water maze after pMCAO ($r = 0.79$, $P = 0.006$). Dotted lines show 95% confidence level based on linear regression analysis. C, Correlation between infarct volume and overall sensorimotor asymmetry score after dMCAO/tCCAO ($r = 0.71$, $P = 0.022$). Dotted lines show 95% confidence level based on linear regression analysis.
versus noncortical tissue, which were assessed separately in this study. Even so, use of even the measures of cortical and subcortical infarct volume in a correlation analysis may mask or dilute relationships that do exist. In support of this, Yonemori et al. examined different sections containing the infarct and found a stronger correlation with behavior for those sections that contained parietal cortex and caudate-putamen than with total infarct volume. A similar strategy was attempted in the present study on a post-hoc basis. Although the number of correlation analyses needed to assess each behavioral measure with multiple brain sections becomes prohibitive if corrections for multiple comparisons are made, some interesting patterns appeared. For example, several measures of water maze performance (heading error, probe trial performance) correlated significantly with extent of cortical infarct in the anterior most sections, corresponding to prefrontal cortex areas, despite the fact that no correlation between those measures and overall infarct volume was found.

An additional reason for the lack of correlation between behavioral measures and infarct volume in the present and other studies is that behavioral deficits resulting from an ischemic event may also be due to diffuse damage outside the primary infarct site. In addition to the primary infarct, diffuse neuronal death in distal areas, diaschisis, and biochemical changes would be expected to contribute to behavioral outcome. The development and use of methods for detecting and quantifying pathological and biochemical changes distal from the main site of damage, although time consuming, would help to remedy this problem (for discussion, see References 14 and 17). In any event, the lack of correlation between infarct size and behavioral outcome reinforces the notion that parallel examination of neuropathology and behavioral outcome should be performed to evaluate potential neuroprotective or neurorestorative compounds. This is especially important in light of the lack of success clinical trials have seen when trying to replicate findings from animal studies. Inclusion of behavioral assessment in animal studies may identify before going to the clinic those cases in which a potential treatment alters infarct but does not affect the organism on a functional level.

References


A Comparison of Long-Term Functional Outcome After 2 Middle Cerebral Artery Occlusion Models in Rats
Robin L. Roof, Gerald P. Schielke, Xiaodan Ren and Edward D. Hall

Stroke. 2001;32:2648-2657
doi: 10.1161/hs1101.097397

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/32/11/2648

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/