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
appropriate behavioral testing in addition to histological measurement.14–21 Exactly what that behavioral testing should be composed of is the topic of current discussion.2,14,15 In most cases when behavioral outcome is assessed after MCAO in rats, testing is limited to simple reflex and motor functions,20,22–24 usually during the early phase of infarction.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.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 test26 and the placing test27), unilateral sensory neglect (eg, Schallert’s sticky tape test,28), and fine motor coordination (eg, Whishaw’s reaching task29 and the staircase test30). These tests are sensitive enough to detect the effects of therapeutic agents and for tracking behavioral recovery over time,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 tests 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,31 topographical disorientation,32 decreased attention, and diffuse intellectual impairment.33 It is possible to test these or similar functions in rats with 1 of several already established cognitive tests, such as passive avoidance,34 the radial arm maze,35 or the Morris water maze (MWM).36

Several laboratories have made use of the MWM to assess the behavioral effects of stroke in the rat MCAO model.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 has been used in rat MCAO studies to test acquisition,18,19,38,39 long-term retention,39 swim patterns and search strategy,19,38,40 and simple cued versus cognitive learning.18 Such cognitive tests can supply important information about the functional state of the animal after MCAO in addition to its sensorimotor capacity. There is also evidence that cognitive impairments may be longer lasting than general sensorimotor deficits,18,19,39,41 which allows for chronic behavioral testing.

The goals of the present study were to design and test a behavioral testing battery for use with the rat MCAO model. Two major issues were addressed in this study: the selection of specific tasks and a comparison of 2 versions of the MCAO procedure. We sought tasks that would assess the range of deficits associated with MCAO, including both basic and complex sensorimotor as well as cognitive function. For sensorimotor testing, we included the elevated body swing task (EBST) for basic postural reflexes and asymmetrical trunk function, the limb placing tests to assess integration of sensory input with motor responses of the forelimbs and hindlimbs, and the cylinder test to detect asymmetry in forelimb use and sensorimotor integration. Using a water tank task, we assessed several aspects of cognitive functioning, including task 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’s1 and modified Tamura’s20,22 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.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.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,42,44–46 although occasionally small subcortical infarcts are reported.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 5 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 al1 and modified by Bederson et al.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.47 Testing of each hindlimb for placing in response to tactile 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.,46 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 hemisphere was determined. Intact animals do not typically exhibit a side bias on this task, whereas rats with unilateral stroke show a strong tendency to turn toward the side contralateral to the stroked hemisphere.41,49 Average scores for each week of testing (2 testing sessions in each case) were calculated for each animal. In addition, 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.36 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 within 1 cm of the wall (measure the tendency to swim in the innermost 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 $V = \frac{1}{2} \times A(p) \times \Sigma \rho$, where $V$ is lesion volume, $A(p)$ is the surface area associated with 1 grid point, and $\Sigma \rho$ 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 ($F_{7,136}/H11005=2.9$, $P=0.01$). 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 ($F_{1,136}/H11005=299.29$, $P=0.0001$). 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 ($F_{1,85}/H11005=25.2$, $P<0.0001$) and using the contralateral forelimb for landings ($F_{1,85}/H11005=35.84$, $P<0.0001$) in the cylinder test. These data are also shown in Figure 2A. 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-
tween 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 $205 \pm 59 \text{ mm}^3$. The area of damage extended from 5.5 to $-7.5 \text{ mm}$ relative to bregma and was contained wholly in the right hemisphere. The average infarct volumes were $144 \pm 62$ and $62 \pm 15 \text{ mm}^3$ in the cortex and caudate putamen, respectively.

**Correlation Between pMCAO 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 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 ($r = 0.704$, $P < 0.05$), 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/tCCАО 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 \((F_{1,144}/H11005/48.14, P/H11021/0.0001)\). As with the pMCAO rats,
dMCAO/tCCAO rats showed a strong and persisting ten-
ddency 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±43 mm\(^3\), and the damage extended
from 5.5 to −7.5 mm relative to bregma. Only cortical tissue
was affected.

Correlation Between Infarct Size and
Behavioral Measures
Correlation analyses were conducted for infarct volume
\((mm^3)\) 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 behav-
ioral deficits resulting from 2 versions of the rat MCAO
model with the purpose of developing an appropriate func-
tional 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 substanc-
tial recovery does not occur after dMCAO/tCCAO. A task
such as the staircase test developed by Montoya et al\(^{27}\) 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. Probe trial: percent of total swim path in annulus pre-
viously 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.

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 cor-
tical and subcortical damage resulted from pMCAO, whereas
only cortical damage resulted from dMCAO/tCCAO.
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, 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. 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, and 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.
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 neuropahtology 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


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