Recovery of Sensorimotor Function After Distal Middle Cerebral Artery Photothrombotic Occlusion in Rats

Carrie G. Markgraf, PhD; Edward J. Green, PhD; Brant Watson, PhD; Philip M. McCabe, PhD; Neil Schneiderman, PhD; W. Dalton Dietrich, PhD; Myron D. Ginsberg, MD

Background and Purpose The purpose of the present study was to delineate the behavioral correlates of focal thrombotic occlusion of the distal middle cerebral artery in rats and to compare the pattern of deficits and subsequent recovery to that following proximal middle cerebral artery occlusion.

Methods Ten Sprague-Dawley rats underwent photothrombotic occlusion of the distal middle cerebral artery with tandem occlusion of the common carotid arteries (dMCAO group); 10 animals served as operated controls. Beginning on postischemia day 2, animals were given a battery of five tests that assessed sensorimotor integration, attentional mechanisms, and muscle strength; testing continued twice weekly until day 30. Nine days of cognitive testing on the learning set of the water maze task were then given. Infarct volume and hemispheric atrophy were determined for each dMCAO animal.

Results After ischemia, the dMCAO group exhibited significant behavioral deficits in posture reflex, ability to place a forelimb to various stimuli, limb adduction during rearing, and neglect of contralateral space. These deficits showed variable recovery rates. No deficits were observed in muscle strength or cognitive performance. The deficits and patterns of recovery were related to infarct location and to degree of hemisphere atrophy.

Conclusions The present study suggests that a battery of tests is necessary to fully characterize the pattern of behavioral deficits after focal cerebral ischemia. Location of infarct damage and associated degree of hemispheric atrophy were important variables in determining behavioral outcome. The present results are compared with those of the more traditional model of electrocoagulation of the proximal middle cerebral artery. (Stroke. 1994;25:153-159.)

Key Words • cerebral arteries • photochemistry • thrombosis • rats

Observations of patients recovering from focal cerebral infarction indicate that a variety of behaviors are often affected by ischemic damage and that these behaviors can recover at different rates. Any animal model of cerebral ischemia that proposes to reflect accurately the clinical observations should demonstrate behavioral deficits and their pattern of recovery after ischemic damage, in addition to histopathological and physiological indications of cerebral ischemia. Occlusion of the proximal portion of the middle cerebral artery (MCA) in rats produces a useful model of focal cerebral ischemia, leading to consistent infarctions in the striatum and overlying neocortex. Furthermore, the pathophysiological features of this model have been well characterized (for review, see Reference 2). Previously, we examined the behavioral consequences of proximal MCA occlusion in the rat, with a battery of tests that examined various neurological and cognitive functions for a period of 6 weeks after infarction. Initial deficits were observed in sensorimo-
gain insights into the functional subdivisions of the rat neocortex by correlation of behavioral deficits with chronic histopathology; and (2) to compare the pattern of behavioral deficits and subsequent recovery of the present model to that of the more widely used proximal MCA mechanical occlusion model. A battery of tests similar to those used in the proximal MCA occlusion model was used to examine general neurological function, sensorimotor integration, muscle strength, attentional mechanisms, and cognitive abilities.

Materials and Methods

Studies were carried out using 20 male Sprague-Dawley rats, weighing 250 to 300 g, obtained from Charles River Breeders. The rats were housed individually under 12-hour light/dark conditions and allowed free access to food and water.

Surgical Preparation

Rats were deprived of food 12 hours before surgery. Using sterile surgical procedures, the right dMCA was occluded photochemically in 10 rats (dMCA group), following previously described surgical procedures. This procedure involved anesthetizing the rat with 2% halothane in a mixture of 70% nitrous oxide and 30% oxygen, paralysis with pancuronium bromide, intubation, and maintenance on 1% halothane on a Harvard small animal respirator. The right dMCA segment was exposed above the rhinal fissure through a small burr hole drilled above the junction of the zygoma and squamosal bones, according to the procedure of Chen et al. Care was taken not to injure the dura during exposure of the vessel. An argon laser-activated dye laser (Coherent, Inc) tuned to 562 nm was used to irradiate the dMCA at a power of 20 mW. The beam was focused on the vessel at three points simultaneously: (1) distally at the Y-shaped juncture of the frontal and parietal branches, (2) proximally above the rhinal fissure, and (3) at a point midway between the distal and proximal irradiation points. Rose bengal (15 mg/mL, 0.67 mL/kg body wt) was injected intravenously via a femoral vein catheter before irradiation, and the dMCA was irradiated until completely occluded as evidenced by lack of blood flow through the vessel and by the presence of thrombotic material in the vessel. Immediately after irradiation, both carotid arteries (CCA) were occluded for 60 minutes by tightening previously placed ligatures around the vessels. The contralateral CCA was occluded as evidenced by lack of blood flow through the vessel and by the presence of thrombotic material in the vessel. Arterial blood pressure was constantly measured during the irradiation period, the dMCA was inspected for occlusion, and normal, 1 if the placing response was slow or delayed, and each instance of these bouts in which the contralateral forepaw or ipsilateral forepaw was retracted from the surface or walls of the cage was noted (limb adduction). A percent score was thus derived for forelimb adduction for rearing and for standing for each limb.

Postural reflex test. Rats were tested for degree of abnormal posture when suspended by their tails 1 cm above the floor. Intact rats extended both forelimbs toward the floor. Rats displaying this behavior were given a score of 0. Abnormal posture included flexing the contralateral limb toward the body and/or rotating the contralateral shoulder and limb medially. If abnormal posture was noted, the animal was placed on a sheet of soft plastic-backed paper that could be gripped by its claws. Lateral pressure was applied from behind the shoulders so that the rat's forelimbs slid gently to the left and then to the right. Rats that resisted sliding in both directions were given a score of 1, reflecting abnormal limb flexion; severely affected rats demonstrated a decreased resistance to the lateral push and were given a score of 2.5

Elicited forelimb placing. Elicited forelimb placing reactions to each of three stimuli (visual, tactile, proprioceptive) were measured for each forelimb to examine sensorimotor integration. For visual placing, animals were cupped in the experimenter's hands with the forelimbs hanging free and were slowly tilted and held 10 cm above the tabletop. Intact rats reached toward the table with both limbs; by moving the animals laterally toward the table edge visual placing could be assessed sideways as well. Tactile placing was assessed by lightly contacting the dorsal and then the lateral surface of the paw to the table edge. Intact rats placed the paw on the table top immediately; impaired rats were either slow to place or did not place at all. Proprioceptive placing was assessed by pushing the paw against the table edge to stimulate limb muscles. For each test, scoring was 0 if the placing response was immediate and normal, 1 if the placing response was slow or delayed, and 2 if the placing response did not occur within 2 seconds. The total scores for the contralateral and ipsilateral limbs were calculated.

Vertical screen test. To assess muscle strength, rats were placed on a screen (540.2 x 22.5 cm; grid openings, 15 mm2) in the horizontal position. The screen was rotated over the course of 2 seconds to the vertical position, and the rat was observed for 5 seconds; a score of 0 was given if the rat gripped the screen for the full 5 seconds; a score of 1 was given if the rat slipped but did not fall; a 1 was recorded if the animal fell during the 5-second period; and a 2 was assigned if the rat fell immediately.
Bilateral asymmetry test. Sensitivity to simultaneous forelimb stimulation was measured by means of a test developed by Schallert and colleagues that is similar to contralateral neglect testing in humans. Adhesive-backed labels were attached to the distal radial region of both forelimbs. The rat contacted and removed them with its mouth. The test was conducted in the dark under red light and consisted of two parts. The first part indicated the presence or absence of an asymmetry in limb choice to remove the first patch; the second measured the magnitude of the asymmetry. On return to the testing cage, the latency and order of contact/removal of the patch was recorded for five trials. All rats with an ipsilateral bias (ie, the ipsilateral stimulus was contacted first or greater than 70% of trials) were given an additional test to assess the magnitude of asymmetry. In the latter test, the size of the contralateral stimulus was progressively increased, and the size of the ipsilateral stimulus was decreased by an equal amount. With sufficient increases in the contralateral to ipsilateral ratio, an ipsilateral bias can be overcome so the rat will not respond preferentially to the ipsilateral side. The ratio at which this bias was overcome was recorded as the asymmetry magnitude for each animal.

Water maze. The water maze apparatus consisted of a round pool (4 ft in diameter; 23.5 in. deep) filled with 21°C water rendered opaque by the addition of white, nontoxic powdered paint. The maze was situated in a well-lighted room containing a variety of cues outside of the maze. Four points around the rim of the pool were designated north (N), south (S), east (E), and west (W), thus dividing the pool into four quadrants. A round platform (11 cm in diameter) was located 2 cm beneath the surface of the water. The rat's swimming behavior was recorded and analyzed using a microcomputer-based tracking system (Prototype Systems Ltd, Boulder, Colo). The system automatically recorded the position of the animal throughout each trial by tracking a small piece of black tape on the rat's head, using a digital camera suspended above the pool. Latency to reach the platform (in seconds) and path length (in centimeters) were recorded for each trial. Animals received 16 trials per day, grouped into eight pairs of trials with an intertrial interval of 2 minutes, for 8 consecutive days. On each trial, the rat was released facing the wall of the pool from one of the four starting locations (N, S, E, or W) according to a pseudorandom sequence that varied daily. On each day, each rat was exposed to two start locations twice. The rat was allowed to swim until the platform was reached or until 60 seconds had elapsed, at which time the rat was placed by hand onto the platform. In either case, the rat was allowed to remain on the platform for 10 seconds. After the first trial of the pair, the second trial was given immediately, from the same start location. The platform location was changed daily to one of eight locations around the pool.

Chronic Histopathology

After the completion of water maze testing (during the seventh postischemic week), rats were anesthetized with 2% halothane and perfused transcardially with a mixture of 40% formaldehyde, glacial acetic acid, and methanol (1:1:8 vol/vol/vol). The brains were removed and processed for paraffin histopathology by methods previously described. Coronal sections were prepared at 10 μm through the extent of the infarction and stained using hematoxylin-eosin. Twelve coronal sections were examined using a Nikon microscope. Each drawing was then traced onto a digitizing tablet (Summagraphics) interfaced to a VAX mini-computer system, which computed areas at each coronal level. Total infarct volume and total hemispheric volume were derived by means of numerical integration of sequential infarct areas.

Statistical Analyses

Physiological variables were compared between groups using a t test. Split-plot ANOVAs were performed on the behavioral data, followed by simple effects comparisons where appropriate. Intertest correlations were performed using a Spearman's rank correlation test, comparing each of the tests to each other on the test day when deficits were maximal. Each test was also correlated with volume of damage using the Spearman's rank correlation test.

### Results

#### Physiological Variables

Measurements of blood pressure, PCO₂, PO₂, and pH taken during surgery did not differ between groups, as depicted in the Table. Blood pressure rose significantly in the dMCAO group during CCA occlusion.

#### Histopathology

Eight weeks after surgery, a well-demarcated infarct was detected in all experimental rats, which consisted of a zone of pallor with extensive panneerosis and gliosis. Representative sections depicting the anterior-posterior extent of the ischemic damage are shown in Fig 1. The ipsilateral hemisphere was consistently smaller than the uninfarcted contralateral hemisphere in all experimental animals in accordance with observations of Persson et al in the chronic phase of proximal MCA occlusion. Therefore, to measure accurately the area of cerebral tissue destroyed, the area of overt infarction was measured as well as the area of the ipsilateral hemisphere (IPH) and the area of the contralateral hemisphere (CLH). Total tissue loss was defined as the area of overt infarction + (CLH - IPH), thus yielding a measure that reflected combined infarct and tissue atrophy. The mean volume of infarcted tissue (±SEM) was 105 ± 37 mm³; the mean volume of infarct plus atrophy (±SEM) was 340 ± 95 mm³ for the same group. The mean volume of atrophy alone was 235 ± 75 mm³. These two latter values were significantly correlated (r = 0.88, P < 0.05), indicating that the larger the volume of infarction, the greater the degree of atrophy. By means of Zilles’ atlas of the cortex, areas of damage were identified on the stained tissue for the dMCAO group. As is evident in Fig 1, the infarct caused consistent damage to the primary somatosensory cortex and the forelimb region. Three animals with the largest infarcts had ischemic

<table>
<thead>
<tr>
<th>Measurement</th>
<th>dMCAO Group</th>
<th>Sham Group</th>
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<tbody>
<tr>
<td>PO2, mm Hg</td>
<td>152.2±34.2</td>
<td>134.8±42.1</td>
</tr>
<tr>
<td>PCO2, mm Hg</td>
<td>34.9±0.7</td>
<td>35.2±0.8</td>
</tr>
<tr>
<td>pH</td>
<td>7.47±0.03</td>
<td>7.46±0.02</td>
</tr>
<tr>
<td>BP, before MCAO, mm Hg</td>
<td>104±9</td>
<td>103±9</td>
</tr>
<tr>
<td>BP, during CCAO, mm Hg</td>
<td>125±33*</td>
<td>100±10</td>
</tr>
</tbody>
</table>

Values are mean±SD. dMCAO indicates distal middle cerebral artery occlusion; BP, blood pressure; MCAO, middle cerebral artery occlusion; and CCAO, common carotid artery occlusion. *P<.05 compared with the previous measure for that group.
damage in the hind-limb region and supplementary somatosensory cortex as well. The hippocampus and thalamus were unaffected. The animals in the SHAM group did not show any ischemic damage.

Behavioral Testing

The pretest scores for each test of the sensorimotor battery were averaged; t tests performed on these data showed no significant differences between groups on any measure before infarction.

Elicited limb placing. The dMCAO animals showed greater impairment than did the SHAM animals in elicited limb placing responses for the contralateral limb. An ANOVA revealed a significant group×days interaction (F_{9,162}=3.68, P<.01). As can be seen in Fig 2A, in which the higher scores indicate greater impairment, the scores decreased significantly over days (F_{9,162}=3.96, P<.01), demonstrating that recovery was occurring, but simple effects tests performed comparing the dMCAO group with the SHAM group on each day revealed that the infarcted animals never reached the level of the SHAM group’s performance during the 30-day recovery period (F_{9,162}=3.66, P>.05). As is evident in Fig 2B, the dMCAO and SHAM groups did not differ in elicited limb placing for the ipsilateral limb. An ANOVA revealed no significant differences between groups or over days (F_{9,162}=4.1, P=NS; F_{9,162}=1.39, P=NS).

Posture reflex test. As is evident in Fig 3, animals in the dMCAO group exhibited greater impairment than the animals in the SHAM group in abnormal postural reflexes, and their scores decreased over days, as the significant interaction indicates (F_{9,162}=3.45, P<.01). Simple effects tests revealed that the dMCAO group had recovered to SHAM levels by day 19.

Spontaneous limb use. Asymmetries in spontaneous limb use were noted during rearing, with the contralateral limb adducted significantly more often for the dMCAO group than for the SHAM group (F_{9,162}=8.12, P<.01). These differences decreased over days (F_{9,162}=6.48, P<.01), as Fig 4A shows, indicating that recovery was occurring. There were no differences in
day of testing.

This deficit did not change over days in either group (F\(_8,144=1.32, \text{P}=\text{NS})

FIG 4. A. Line graph shows mean (±SEM) percentage of rearing bouts in which the contralateral limb

adduction of the ipsilateral limb between groups

(F\(_8,144=1.80, \text{P}=\text{NS})

adducted, which also showed partial recovery. Distal middle cerebral artery occlusion (dMCAO)

Bilateral asymmetry test. The dMCAO group exhibited a significant neglect of the contralateral stimulus

(F\(_{1,14}=13.18, \text{P}<.01)

adducted for the two groups, distal middle cerebral artery occlusion (dMCAO)

and sham, over the 30 postischemic testing days. B. Mean (±SEM) percentage of rearing in which the ipsilateral limb was

was not impaired in learning the maze. Initially the infarcted hemisphere swells, then atrophies dramati-

some intriguing possible interpretations of behavioral deficit after focal cerebral ischemia. While others have found that a general measure of neurologic function such as the posture reflex test correlated well with infarct area,\cite{17} those studies were conducted within the first 24 hours after infarction. Initially the infarcted hemisphere swells, then atrophies dramati-

Discussion

The present study demonstrates that photothrombotic occlusion of the dMCA produces deficits on various sensorimotor tasks and that these deficits show variable recovery rates. There was a significant posture reflex abnormality that recovered by day 23 after ischemia; there was a large deficit in the ability to place a limb to tactile stimuli that showed partial recovery, and an initial deficit in rearing with the contralateral limb adducted, which also showed partial recovery. Distal MCA photothermbotic occlusion led to deficits in sensory neglect as measured by the bilateral asymmetry test, but as a group the dMCAO animals showed no recovery. There were no deficits in either muscle strength or in complex spatial mapping ability, as reflected by the water maze test. The finding that only one pair of tests correlated well with one another indicates that the tests that compose the sensorimotor battery do not all measure the same aspects of sensorimotor integration. Thus, it is important to examine the various aspects of these behaviors with a battery of tests.

While it may seem initially surprising that the overall volume of tissue injury was not correlated with behavioral deficits on four of the five tests, this result actually suggests some intriguing possible interpretations of behavioral deficit after focal cerebral ischemia. While others have found that a general measure of neurologic function such as the posture reflex test correlated well with infarct area,\cite{17} those studies were conducted within the first 24 hours after infarction. Initially the infarcted hemisphere swells, then atrophies dramati-
cally over 42 days. Therefore, one might not expect in the present study to find a correlation between behavioral measures taken on day 3 after occlusion and the volume of infarct plus atrophy measured after 56 days. Alternatively, the rather modest variability in the sizes of the infarcts of the 10 dMCAO animals may have precluded a statistically significant correlation.

Variation in infarct plus atrophy size and a lack of correlation between extent of injury and behavioral outcome suggest that functional outcome depends as much on the location of the ischemic damage and concomitant hemispheric atrophy as its extent. It is evident, comparing individual animals' patterns of infarction with their respective deficits, that the three animals with the largest areas of damage (infarct volume >200 mm$^3$ and infarct plus atrophy volume >600 mm$^3$) exhibited the greatest behavioral deficits on all tests and showed little to no recovery on any measure. Conversely, the three animals with the smallest damage (infarct volume <40 mm$^3$ and infarct plus atrophy volume <200 mm$^3$) exhibited few behavioral deficits, and these recovered quickly. The four animals with intermediate-sized infarcts (80 to 88 mm$^3$) were of greatest interest. They exhibited remarkably different patterns of deficit and recovery from one another on the battery of tests. The predictive variable seemed to be degree of hemispheric atrophy: animals with greater atrophy in general had larger deficits and slower recovery than those with less hemispheric atrophy. In this regard, these results support the conclusions of Tominaga and Ohnishi, who found, after similar dMCAO surgery, that motor deficits were significantly correlated with water content of the brain (ie, edema) in the acute phase of the infarction, and edema is characteristically followed by atrophy in focal ischemia.

In addition, in animals with intermediate-sized infarcts, location was critical in determining behavioral outcome after focal cerebral ischemia, an observation that points to the importance of knowing various subdivisions of the rat neocortex. In this regard, these results agree with those of DeRyck and colleagues, who examined forelimb placing responses after photochemical lesions in various regions of the neocortex. They found that recovery varied as a function of the anterior-posterior coordinate measured from bregma. Barth et al also point to the significance of functional subdivisions of the rat cortex by examining small lesions in various cortical regions and observing the effects on sensorimotor behaviors that were unique to the placement of the lesions. For example, in their study, animals with rostral forelimb area damage were significantly more impaired on the bilateral asymmetry test than either animals with caudal forelimb area or anterior motor cortex lesions. Ischemia in the present study typically included the rostral forelimb and caudal forelimb areas but spared the anterior motor cortex, and deficits were seen on the bilateral asymmetry test with little recovery. This pattern of sensory neglect with prolonged recovery is similar to that seen in a few selected human cases of pure sensory stroke after cortical infarctions. The lack of deficit in the water maze task was not surprising because the striatum and hippocampus, both structures known to play a role in cognitive mapping ability, were unaffected. Furthermore, although previous studies involving the effects of bilateral damage to the frontal and parietal cortex indicate that these two structures participate in spatial mapping, the damage in the present study was unilateral, thus reducing the possibility of uncovering a deficit.

However, the use of the water maze in the present study in addition to the battery of sensorimotor and muscle strength tasks allows direct comparison of the behavioral consequences after dMCAO to those after proximal MCAO. The pattern of behavioral deficit/recovery after dMCAO was less homogeneous than after proximal MCAO. In the present study, elicited limb placing and bilateral asymmetry behaviors did not show recovery by the end of the 30-day testing period, whereas after proximal MCAO, all sensorimotor integration behaviors initially affected recovered by post-ischemia day 30. A cognitive deficit, measured by water maze performance, was observed after proximal MCAO but was not seen after dMCAO. Muscle strength was not affected in either group. The differences in deficit/recovery patterns between dMCAO and proximal MCAO may be due to several factors. First, after proximal MCAO, the striatum is consistently and severely damaged, and this damage may contribute to the behavioral deficits observed and hence to their consistency. Furthermore, differences in cortical location exist following proximal MCAO and dMCAO; with dMCAO the infarct is located in more superior cortex (Fig 1), whereas with proximal MCAO the infarct is typically concentrated in more lateral and inferior cortex away from primary forelimb areas. Thus, with proximal MCAO, forelimb cortex might represent an area in which infarction is variable, increasing the possibility of a correlation between histopathology and behavior. In contrast, after dMCAO, the forelimb region is always damaged; thus, a significant correlation is difficult to attain. Second, the present dMCAO was produced by photochemical occlusion, as opposed to electrocoagulation of the proximal MCA. It is known that photochemical occlusion produces blood-borne factors that acutely affect the blood-brain barrier and cerebral blood flow, unlike during mechanical occlusion of a vessel. Therefore, the damage after photochemical dMCAO may have produced a wider variety of acute physiological changes that could have influenced behavioral outcome, although the long-term expression of these factors has not been studied.

In summary, this article describes the pattern of deficit and recovery on a variety of tasks after photochemical dMCAO. Cortical infarction produces a heterogeneous pattern of deficit/recovery that is related to not only infarct location but to degree of associated hemispheric atrophy. These results provide the basis on which further studies can be expanded, examining, for example, the therapeutic benefits of various treatments.

References

The behavioral manifestations of cortical ischemia represent important clinical problems that are often poorly analyzed or addressed with focal cerebral ischemia models in animals. Understanding the behavioral correlates of brain injury is an important part of expanding our understanding of the morbidity associated with stroke. The article by Markgraf et al, from the Cerebral Vascular Disease Research Center in Miami, Fl, provides an important advance in the ability to use animal models to characterize behavioral correlates of focal thrombotic occlusion of the distal middle cerebral artery in rats. This study initiates a scientific approach to developing specific measures for evaluating the often difficult aspects of behavioral changes after brain injury. An interesting result from this study is the conclusion that a battery of tests may be more useful than single evaluations in fully characterizing the pattern of behavioral deficits after focal cerebral ischemia. In addition, this article provides a standard by which further studies can expand by using a battery of tests to evaluate behavioral changes in ischemia and, more specifically, to evaluate the therapeutic benefits of various treatments using these animal models.

Although animal models have limitations in their application to human behavioral dysfunction, the careful evaluation of recovery of sensorimotor function in this rodent model has obvious clinical implications. Specific patterns of sensory neglect with prolonged recovery were seen in this animal model and could be considered similar in some aspects to human cases of pure sensory stroke after cortical infarctions. As in the human condition, this animal model takes into account not only the location and size of the infarct but the associated degree of hemispheric atrophy. These were all shown to be important in determining behavioral outcome and have obvious clinical implications in our understanding of the role of brain injury on behavioral aspects in man. The development of well-characterized animal models to study morbidity, especially as it relates to behavior, is an important advance. This type of quantitative evaluation in an animal model is ideally suited for drug development and testing of potential therapies to minimize or help alleviate some of the behavioral correlates associated with brain injury.

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