Forebrain Ischemia Induces Selective Behavioral Impairments Associated With Hippocampal Injury in Rats

Thomas X. Gionet, MD; Jennifer D. Thomas, BS; David S. Warner, MD; Charles R. Goodlett, PhD; Edward A. Wasserman, PhD; and James R. West, PhD

Two groups of rats were tested on a variety of motor and cognitive tasks after either 10 minutes of two-vessel occlusion forebrain ischemia (n=8) or sham operative procedures (n=6). Histological injury was absent in the sham-operated group. In the ischemic group, hippocampal injury was restricted to field CA1, while damage in the neocortex and caudoputamen was sparse. Motor tests performed on postoperative days 18 and 28 revealed no significant differences between the ischemic and sham-operated groups. Retention performance of a radial maze discrimination task was impaired, with a significant but transient increase in both working and reference memory errors. Passive avoidance acquisition and retention were not significantly affected, although conclusions concerning the utility of this task must be reserved because of variability in the behavior of the sham-operated rats. Morris maze spatial navigation (place learning) and open-field activity were insensitive to treatment group. These functional results are consistent with the observed histological injury and what is known about hippocampal injury and behavior, and they provide further guidance for the development of neurological assays appropriate for discriminating outcome from forebrain ischemia in rats. (Stroke 1991;22:1040-1047)
Although existing investigations allow some general predictions about postischemic neurological function, no one study has evaluated the effects of ischemia on a wide range of behavioral tasks (e.g., both motor and cognitive). The goal of this project was to provide a more detailed profile of behavioral functions that are impaired and spared as a result of forebrain ischemia, using a within-subjects design.

**Materials and Methods**

With institutional Animal Care and Use Committee approval, 15 female Sprague-Dawley rats (Harlan Sprague Dawley, Inc., Indianapolis, Ind.) were trained on the radial maze (see below) for 50 consecutive days before assignment to the following surgical protocol. The rats were approximately 105 days old at the start of training.

Under 4% halothane/balance O₂ anesthesia, the rats were endotracheally intubated and mechanically ventilated with a delivered gas mixture of 1.0–1.6% halothane in 30% O₂/balance N₂. The tail artery was cannulated, and mean arterial blood pressure (MABP) was continuously recorded. Following a ventral neck incision, the common carotid arteries were isolated and incised with suture. The right internal jugular vein was cannulated, and 50 IU heparin was given intravenously. Muscle paralysis was obtained by a 0.2-mg bolus of succinylcholine, repeated as necessary. Bipolar electroencephalographic (EEG) activity was monitored continuously. Finally, a 22-gauge needle thermistor was percutaneously placed adjacent to the skull and pericranial temperature was thenceforth servo-regulated at 37.0±0.1°C by surface heating or cooling. The halothane concentration was then reduced to 0.5%, and 30 minutes were allowed for physiological stabilization.

The animals were then randomly assigned to undergo ischemia or sham procedures. Rats assigned to the ischemic group received 4 mg/kg i.v. trimethaphan followed immediately by bilateral carotid artery occlusion using temporary aneurysm clips. The MABP was maintained at 50±5 mm Hg by withdrawal and reinfusion of blood through the jugular vein catheter as necessary. After 10 minutes of ischemia (defined by an isoelectric EEG), the aneurysm clamps were released, any shed blood was rein infused, and 0.27 meq NaHCO₃ was given to minimize systemic acidosis. The catheters were removed, and the incision sites were closed with suture. Thirty minutes after ischemia, the animals were awakened. Upon recovery of spontaneous ventilation, the trachea was extubated. The rats were then placed in a chamber containing 50% O₂/50% N₂ for at least 30 minutes before being returned to their cages. The animals assigned to the sham-operated group did not undergo trimethaphan infusion, carotid occlusion, or hypotension. Otherwise, events including duration of anesthetic exposure, time to awakening, extubation, and recovery were temporally similar to those in the ischemic group.

Six behavioral tasks were examined postoperatively in the following sequence: radial maze (postoperative [PO] days 5–16), open-field and general motor performance tasks (PO day 18), passive avoidance (PO days 20 and 21), Morris maze spatial navigation (PO days 26 and 27), and rotarod (PO day 28). The specific order of testing after the radial maze retention test was chosen to limit the potential for severe carryover effects across tasks.

The radial maze consisted of a circular platform 43 cm in diameter with 12 arms each, 89 cm long and 7.3 cm wide, extending radially from the platform. The platform was enclosed by clear Plexiglas walls 25.5 cm high with guillotine doors providing access to all arms; all doors were opened or closed simultaneously. The sides of each arm also had clear Plexiglas walls 16.3 cm high extending 33.3 cm from the center platform. A black plastic cup was present at the distal end of each arm for the placement of food reinforcers (Froot Loops, Kellogg Co., Battle Creek, Mich.). The maze was elevated 63 cm above the floor in an environment rich in visual cues.

During presurgical training, each rat was placed on a partial food-deprivation schedule designed to reduce body weight to 85% of free-feeding weight, during which approximately 15 g Teklad lab chow (Madison, Wis.) was provided each day. Water was available ad libitum. After approximately 10 days on the deprivation schedule, 8 days of radial maze preregistration were given. During this preregistration only, white sheets surrounded the maze to prevent the rats' use of distinctive visual-spatial cues in the room. Entry into all arms and unhesitating approach to the food cups were shaped over the 8 preregistration days.

At the onset of training, one of three designated patterns of six baited and six unbaited arms was randomly assigned to each animal. Rats were placed individually on the central platform and, after 5–10 seconds, the doors to all arms were opened. If the animal entered an arm and placed all four paws beyond the Plexiglas wall of the arm, an entry was recorded. Initial entries into unbaited arms within a given trial constituted reference memory errors, whereas repeated entries into a given arm (either baited or unbaited) within a trial constituted working memory errors. The trial ended after all food reinforcers were retrieved or when 10 minutes had elapsed. The rats were trained for 50 trials, one per day, before surgery, and retention was tested for 12 trials after surgery, starting on PO day 5.

Behavior in a novel open field was used as a measure of exploratory activity.13 Testing was conducted in a quiet and darkened room. The animals were placed in a square wooden box with the floor measuring 90×90 cm and walls 45 cm high. The floor was painted with a grid, each square being 15×15 cm in area (i.e., a total of 36 squares). The rats were observed over 10 minutes, and the number of peripheral squares the animal's snout entered, the number of central squares the animal's snout entered, the number of times the animal raised up on its hind limbs, and the number of defecations were recorded for each minute.
Motor function tests were performed as described by Combs and D’Alecy. Briefly, the rats were placed on a 29 × 30 cm screen (grid size 0.6 × 0.7 cm) that could be rotated from 0° (horizontal) to 90° (vertical). The animal was placed on the screen when horizontal, and the screen was then rotated into the vertical plane. The time that the rat was able to hold on to the vertical screen was recorded to a maximum of 15 seconds (allowing a total of 3 points). Next, the animal was placed at the center of a horizontal wooden rod 2.5 cm in diameter, and the time that the rat was able to remain balanced on the rod was recorded to a maximum of 30 seconds (allowing a total of 3 points). Finally, a prehensile-traction test was administered. The time that the animal was able to cling to a horizontal rope was recorded to a maximum of 5 seconds. From these three tests, a total motor score (9 possible points) was computed.

The passive avoidance apparatus consisted of two enclosed Plexiglas chambers (12.3 × 13.9 × 11.9 cm each) that differed in brightness cues; one chamber was black and unlit, the other was off-white and lit by a single 24-W lamp. Each chamber had a grid floor of stainless steel rods. On PO day 20, all rats were given acquisition training. They were placed in the bright chamber, and after 10 seconds, the guillotine door between the chambers was opened. Once the rat entered the dark chamber (defined as having all four paws in the dark chamber), the door was closed and the animal received a 0.7-mA scrambled shock for 1.0 seconds via the grid floor. Each rat was given consecutive training trials until it successfully avoided the dark chamber for 3 minutes. The intertrial interval was 30 seconds, spent in a holding cage adjacent to the apparatus. Twenty-four hours after acquisition training, the rats were returned to the room for retention testing. They were placed in the bright chamber of the apparatus, the door was opened, and latency to enter the dark chamber (to a maximum of 5 minutes) was recorded.

Rats were tested in squads of four on the place-learning task first described by Morris, using a 121-cm-diameter tank filled with 26°C water made opaque with 2% powdered milk. All rats were given 12 trials/day, with an intertrial interval of approximately 5 minutes, on 2 consecutive days. The escape platform (11 × 12 cm) was submerged 1.5 cm below the surface of the water and was in the same location during each trial. Over consecutive trials, the rats were started at each of the four compass points, randomly assigned within blocks of four trials. Time (and distance traveled) to reach the platform (to a maximum of 45 seconds) was recorded, and path lengths were obtained from video recordings of selected trials. At the end of each training day, the platform was removed and each rat was given a single 30-second probe trial. The time spent in the target quadrant and the number of crossings of the platform area were determined from video recordings of the probe trials.

The rotarod consisted of a rod constructed of knurled Plexiglas, 7.6 cm in diameter and 52 cm in length. Six aluminum disks, each 51 cm in diameter, divided the rod into segments ranging in length from 7.6 to 12.8 cm (in 1.3-cm increments) and served to minimize undesirable lateral movements. Only the 10.2-cm segment was used in this study. A Bodine motor (Kornfeld-Thorpe Electric Co., Kansas City, Kan.) with continuous variable-speed adjustment powered the rod, and the rate of rotation was monitored with a tachometer. The rod hung 107 cm above a floor of wood chip bedding 7 cm deep. A rat was placed on the stationary rod and, after 10 seconds, rotation was initiated. Four successive speeds of rotation, 3, 6, 9, and 12 rpm were used, each for 30 seconds. The direction of rotation was counter to the orientation of the rat. If the animal successfully changed orientation while the rod was rotating, the direction of rotation was also reversed. The time elapsed (in seconds) until the rat either fell from the rod or completed the task served as the performance measurement. A trial commenced when rotation began. If the animal failed to remain on the stationary bar before rotation, it was again placed on the rod, to a maximum of four consecutive placements (after which it was assigned a score of 0). Each rat received five trials, with an intertrial interval of 5–10 minutes.

Upon completion of all testing, the animals were anesthetized and the brains were perfusion-fixed in situ with 4% formalin (pH 7.35). Paraffin-embedded brain sections 5 μm thick were serially cut and stained with celestine blue and acid fuchsins.

Hippocampal CA1 and CA3 injury was graded on a 0–3 scale (0, normal brain; 1, as few as one neuron damaged; 2, many neurons damaged; and 3, most neurons damaged). Viable neurons, defined as those with a normal morphology and the absence of acidophilic staining, within the pyramidal cell layer of CA1 were counted from two sections representing bregma −3.3 mm and bregma −3.8 mm. Injury in the caudoputamen and neocortex was graded (as described for the hippocampus) at the level where the septal nuclei were at their widest. Damage was assessed by experimenters blinded to the rat’s group membership. Values are given as mean ± SEM.

**Results**

Seven rats in the sham-operated group and eight rats in the ischemic group completed the surgical protocol. One sham-operated rat was killed due to acute postoperative airway obstruction and was deleted from the analysis. Physiological values determined prior to and during the ischemic episode (or sham operation) were compared by one-way analysis of variance and are reported in Table 1.

For ischemic rats, injury ratings for CA1 ranged between 1 and 3, whereas ratings for the CA3 and dentate gyrus were either 0 or 1. Four ischemic rats exhibited only moderate injury (ratings of < 3 on at least one side) whereas the other four exhibited...
TABLE 1. Physiological Values Obtained Immediately Before or After Ischemic Insult or Sham Operation in Rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sham-operated</th>
<th>Ischemic</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=6)</td>
<td>(n=8)</td>
<td></td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>236±6</td>
<td>241±5</td>
<td></td>
</tr>
<tr>
<td>Plasma glucose (mg/dl)</td>
<td>93±15</td>
<td>97±20</td>
<td></td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>44±1</td>
<td>43±1</td>
<td></td>
</tr>
<tr>
<td>PaO2 (mm Hg)</td>
<td>138±9</td>
<td>129±15</td>
<td></td>
</tr>
<tr>
<td>PaCO2 (mm Hg)</td>
<td>39.3±0.6</td>
<td>38.9±0.3</td>
<td></td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.37±0.01</td>
<td>7.35±0.01</td>
<td></td>
</tr>
<tr>
<td>Mean arterial blood pressure (mm Hg)</td>
<td>112±5</td>
<td>110±4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>111±5</td>
<td>92±4*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>115±5</td>
<td>118±4</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SEM. *p<0.05 different from sham-operated group by one-way analysis of variance.

severe, bilateral damage (ratings of 3 on both sides). In the neocortex, two rats had a rating of 2, five had a rating of 1, and one had a rating of 0. In the caudoputamen, one rat had a rating of 2, six had a rating of 1, and one rat had no acidophilic neurons. All sham-operated rats were rated 0 in all regions.

A high correlation was found between the CA1 cell counts of the two independent experimenters (r=0.96) as well as between the two sections (r=0.97). The mean of the two independent counts for each section was obtained, and the sum of the two hemispheres for each section were used for statistical analysis. Rats exposed to forebrain ischemia had significantly fewer viable hippocampal CA1 cells than those in the sham-operated group (Figure 1). At bregma -3.3 mm, 540±60 versus 272±80 nonacidophilic CA1 neurons were present in the sham-operated versus ischemic groups, respectively (F(1,12)=6.32, p<0.03). Similarly, at bregma -3.8 mm, 602±50 viable CA1 neurons were identified in the sham-operated group versus 270±82 in the ischemic group (F(1,12)=10.09, p<0.01).

FIGURE 1. Photomicrographs of coronal sections of CA1 field of rat hippocampus (bregma -3.3 mm) evaluated 29 days after either 10 minutes of sham operative procedure (A) or forebrain ischemia (B). Note dense acidophilic tissue and absence of intact neurons in pyramidal cell layer of ischemic example. Pyr, pyramidal cell layer; SO, stratum oriens; SR, stratum radiatum. Bar=100 μm.
Forebrain ischemia induced a severe but temporary, deficit in retention performance on the radial maze task, as indicated by increases in both working and reference memory errors (Figure 2). Errors were averaged over blocks of six trials and analyzed with repeated-measures analysis of variance, with treatment as a between-subjects variable and type of error (reference or working memory) and trial block (last preoperative block, first postoperative block, second postoperative block) as within-subjects variables. This analysis confirmed a significant group x trial block interaction ($F(2,24)=4.47$, $p<0.05$), as well as significant main effects of error type ($F(1,12)=10.63$, $p<0.05$) and trial block ($F(2,24)=8.27$, $p<0.01$). Error type did not interact with group or trial block, indicating that the increase in errors induced by ischemia was not a function of the type of error. Newman-Keuls tests on total (working+reference memory) errors confirmed that the ischemic rats were significantly different from the controls on the first postoperative block ($p<0.05$) but not on the other two blocks. Analysis of change scores (first postoperative block minus last preoperative block) confirmed that the change in total errors across the two trial blocks spanning the surgery was significantly greater in the ischemic group ($F(1,12)=4.85$, $p<0.04$).

Initial inspection of open-field performance as a function of time (1-minute blocks) indicated that the two groups did not differ in their activity level over time, so statistical analyses were performed on the measures summed over the 10-minute test. Table 2 presents group means for the number of central plus peripheral squares entered, the number of hind leg rearings, and the number of defecations. One-factor analysis of variance for each measure indicated that the ischemic group did not differ significantly from the sham-operated group on any measure.

The total motor score (maximum of 9) obtained on the three general motor performance tasks for the ischemic and sham-operated groups is presented in Figure 3. A Mann-Whitney $U$ test revealed no significant difference between the groups.

The passive avoidance task failed to distinguish the ischemic from the sham-operated group, for either the number of training trials to acquisition criterion or for crossover latency during retention testing (Table 2). The groups did not differ significantly on either measure on the basis of Mann-Whitney $U$ tests. The lack of group differences on the retention test was a function, in part, of the relatively poor performance of two control rats (latencies of <20 seconds). However, data from the ischemic group suggested that the retention test may be sensitive to the extent of hippocampal injury in that retention crossover latencies of animals with severe damage (range of 2-33 seconds) was lower than (and did not

<table>
<thead>
<tr>
<th>Behavioral test</th>
<th>Group</th>
</tr>
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<tbody>
<tr>
<td>Open-field test (cumulative events over 10-min trial)</td>
<td></td>
</tr>
<tr>
<td>Squares entered</td>
<td>Sham-operated</td>
</tr>
<tr>
<td></td>
<td>Ischemic</td>
</tr>
<tr>
<td></td>
<td>($n=6$)</td>
</tr>
<tr>
<td></td>
<td>($n=8$)</td>
</tr>
<tr>
<td>Hind leg rearings</td>
<td>$49\pm7$</td>
</tr>
<tr>
<td></td>
<td>$48\pm7$</td>
</tr>
<tr>
<td>Defecations</td>
<td>$2\pm1$</td>
</tr>
<tr>
<td></td>
<td>$3\pm1$</td>
</tr>
<tr>
<td>Passive avoidance</td>
<td></td>
</tr>
<tr>
<td>Acquisition training trials (median no.)</td>
<td>$4$</td>
</tr>
<tr>
<td>Retention crossover latency (median sec)</td>
<td>$37.5$</td>
</tr>
<tr>
<td></td>
<td>$34.0$</td>
</tr>
<tr>
<td>Morris maze target search time (sec)</td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>$7\pm2$</td>
</tr>
<tr>
<td></td>
<td>$9\pm1$</td>
</tr>
<tr>
<td>Day 2</td>
<td>$10\pm1$</td>
</tr>
<tr>
<td></td>
<td>$11\pm2$</td>
</tr>
<tr>
<td>Rotarod</td>
<td></td>
</tr>
<tr>
<td>Time on rod per trial (sec)</td>
<td>$47\pm9$</td>
</tr>
<tr>
<td></td>
<td>$45\pm6$</td>
</tr>
</tbody>
</table>

Values are mean±SEM unless noted.
such as the one employed in this experiment are 
within-subjects variable, indicated a significant effect 
was not sensitive to the damage produced by the 
ischemia on a variety of motor and cognitive tasks,
Models 
were sensitive to ischemic injury. Models 
the results indicate that only relatively complex cog-
nies declined as a function of day (F(1,12)=56.9,
nor the search time for the probe trials (Table 2)
Thus, comparable 
overlap with) the latencies of rats with partial dam-
the two groups also did not differ on acquisition of the 
Morris maze spatial navigation place-learning task. Escape latencies and path lengths were highly 
correlated so only latencies are reported. Neither the 
escape latencies over the two training days (Figure 4) 
nor the search time for the probe trials (Table 2) 
revealed significant treatment effects. Escape laten-
cities declined as a function of day (F(1,12)=56.9,
confirming that the performance of both groups 
improved over training. Repeated-measures analysis 
of variance of target quadrant search time, with 
treatment as a between-subjects variable and day as a 
within-subjects variable, indicated a significant effect 
of only day (F(1,12)=5.18, p<0.05). Thus, comparable 
 improvement in performance over the 2 days was 
present for the two groups, indicating that this task 
was not sensitive to the damage produced by the 
ischemic insult. Furthermore, when the partial injury 
cases (n=4) were omitted from the analysis, no 
significant group differences emerged, so even severe 
CA1 injury failed to produce deficits in simple place 
navigation.
Total time spent on the rotarod, averaged across 
the five trials, is presented in Table 2. Repeated-
measures analysis of variance indicated no significant 
main or interactive effects of treatment.

Discussion
In characterizing the effects of transient forebrain ischemia on a variety of motor and cognitive tasks, 
the results indicate that only relatively complex cogni-
tive tasks were sensitive to ischemic injury. Models 
such as the one employed in this experiment are 
frequently used to screen potential neuroprotective 
pharmacological and physiological therapies that 
may eventually be extrapolated to the human sce-
nario of global cerebral ischemia. In the laboratory, 
assays of histological outcome are valuable in deter-
mining the efficacy of such therapies; however, clin-
ically, the critical outcome determinant is neurolog-
ical function and quality of life. Definition of 
appropriate postischemic neurological assays may 
thus improve the utility of rodent experimental mod-
els for studying this disease process.

The histological damage observed in the ischemic 
rats is characteristic of the model employed (i.e., 
dense damage in hippocampal field CA1 and sparing 
of injury in the neighboring hippocampal field CA3 
and dentate gyrus). Neocortical and caudoputaminal 
damage was sparse. The fact that histological analysis 
was performed 30 days after reperfusion might raise 
some concern. Neuronal necrosis, while requiring 
various region-specific intervals for maturation, is 
clearly evident by 96 hours after ischemia (although 
note Mudrick and Baimbridge (19,20). The extent to 
which phagocytosis of necrotic neurons was com-
pleted by PO day 30 (and thus eliminated acidophilic 
evidence of cellular injury) has not been determined. 
Nevertheless, there are reasons to believe that the pattern of histological injury observed in this study 
was valid. First, artifacts resulting from the delay of 
histological analysis of the hippocampus were cir-
cumvented by confirming the histological ratings with 
direct counts of viable cells. Second, other investiga-
tors have observed remnants of acidophilic neurons 
as late as 13–15 weeks after ischemia, suggesting that 
the course of phagocytosis is prolonged.21 Finally, the 
anatomic distribution of ischemic cell damage was 
similar to that observed in numerous previous appli-
cations of this model in which recovery intervals were 
restricted to 5–7 days.422–24 Such studies presumably 
would have encompassed the intervals when the 
number of acidophilic neurons was maximal.
Postischemic performance on individual behav-
ioral tasks in this study was similar to that observed 
in previous investigations. The only task that reliably 
segregated the ischemic from the sham-operated rats 
was the retention of radial maze discrimination. 
Volpe et al14 demonstrated that performance in a 
similar task was dependent on the duration of pre-
ischemic training. A training interval of 36 trials was 
associated with a transient postischemic impairment 
of reference memory, whereas deficits in working 
memory were more persistent. When the preischemic 
training was extended to 80 trials, both working and 
reference memory function recovered to control lev-
els within 12 trials. Our experiment is perhaps more 
similar to the latter case in that we used a 50-day preischemic training interval and observed transient 
deficits in both working and reference memory, and 
our results are consistent with the recent report of 
Kiyota et al.13 In addition, the impaired reference 
and working memory performance with subsequent 
recovery found in our studies is consistent with the 
behavioral profile observed in rats with hippocampal
neurotoxic lesions. Thus, radial maze discrimination is sensitive to forebrain ischemic insults, and behavioral effects are consistent with histological changes in the hippocampus. Because our results (with retention testing begun on PO day 5) are similar to those of Volpe et al (retention testing was initiated 30 days after ischemia), it can be concluded that deficits in radial maze performance can be observed at a variety of postischemic intervals. This flexibility suggests value for the radial maze task in a number of different experimental designs. In contrast, the drawback to this analysis is the intense labor involved in both preischemic training and post-ischemic trials required to produce such an effect, and the effect is relatively transient. Such a labor-intensive task is not ideal for routine studies screening particular pharmacological effects.

The remaining task we assessed failed to discriminate the ischemic from the sham-operated rats, even when considering only the four rats with severe histological damage. In the case of the Morris maze, Auer et al and Kiyota et al, using somewhat different training schedules, also found the place-learning task to be insensitive to forebrain ischemia. It can be concluded that substantial loss of dorsal CA1 neurons does not impair the acquisition of simple place learning. In contrast, Morris et al and Olds et al found that near-complete ablation of the rat hippocampus impaired the acquisition of simple place learning. In the latter study, unlesioned rats successfully performing the same task were demonstrated to exhibit changes in protein kinase C concentrations in CA3, but not in other regions of the hippocampal formation. Thus, the lack of deficits observed for simple place learning following ischemic injury may be attributed in part to the sparing of CA3 neurons and perhaps to the residual neurons of CA1. In contrast, when the place-learning task was made more difficult by altering the position of the escape platform each day (learning-set task), ischemic rats consistently performed more poorly than controls. Unfortunately, the specificity of ischemic effects to the learning-set task outlined by Auer et al was not available to us until after the present study was begun. Given the labor-intensive characteristics of the radial maze and the potential sensitivity of the Morris maze learning-set task, it could be that the latter may be superior for routine screening studies.

The passive avoidance task failed to discriminate between the ischemic and the sham-operated animals, a finding consistent with that of other investigators. This task is also dependent on learning and memory, presumably involving some of the same neural substrates necessary for the other cognitive tasks. However, avoidance tasks using electric shock are particularly prone to the intrusion of nonassociative factors on behavioral performance. The inability of the passive avoidance task used in the present study to separate the treatment groups was related in part to unexplained variation in retention performance of the sham-operated rats. Thus, sample size or specific procedural parameters may be critical determinants for the usefulness of passive avoidance in rat models of ischemia. Although further analysis with larger sample sizes should resolve this question, the limitation identified here is relevant. Most investigations assessing drug or physiological effects on histological outcome following ischemia have been successful in identifying intergroup differences with group sizes of 5–10 rats, comparable to the group sizes employed in our study. Thus, use of the passive avoidance task would likely require revisions of the procedures used in this study to enhance its sensitivity to group differences.

Finally, motor function was found to be undisturbed in both the general motor and rotarod tasks. These findings are logical given the sparse histological damage observed in the neocortex and basal ganglia and the preserved cerebellar perfusion (via the open vertebrobasilar system) common to this model. Thus, although transient motor impairment (at 24 hours after ischemia) has previously been reported, tests of motor function are not reliable indicators of long-term neurological function. Whether recovery from these transient motor deficits represents functional plasticity or is attributable to other factors (e.g., recovery from residual anesthetic/surgical effects) is not known. It is unlikely that decrements in motor performance are accountable for differences observed in the radial maze task. In a previous experiment, no difference in motor function was observed between two-vessel occlusion ischemic rats and sham-operated animals 7 days after ischemia. Furthermore, Volpe et al concluded that the observed similarity in maze exploration speed between ischemic and sham-operated rats was indicative of the absence of motor impairment from transient forebrain ischemia.

Two methodological features of our report warrant critical consideration. First, the order of administration of the functional assays was not randomized. All rats underwent the same sequence of tests after the same respective postischemic intervals. We chose this design primarily because of our desire to standardize the postischemic retention testing interval on the radial maze. The second limitation, alluded to above, was sample size, which was restricted by the number of rats originally pretrained on the radial maze. However, most investigations seeking to distinguish drug and physiological effects on outcome from ischemia use sample sizes similar to those reported here. Comparison groups (i.e., drug-treated and untreated groups) are typically all rendered ischemic, and the decrease/increase in damage is subsequently assessed. Such studies may require behavioral assays that are even more sensitive than those used here since histological differences between groups would be expected to be more subtle than those reported in this investigation (i.e., ischemia versus no ischemia). One behavioral assay that may be sensitive to such variations in outcome is conditional spatial learning in a T-maze, a task that is heavily dependent on
working memory function and is sensitive to damage to the hippocampal system.\textsuperscript{32–35}

In conclusion, rodent models of transient forebrain ischemia produce profiles of regional histological injury that are similar to those observed in some human survivors of cardiac arrest. Similarly, neurological deficits associated with forebrain ischemia are consistent with the histological injury observed (i.e., preserved motor function with impaired performance in complex learning and memory tasks). Many typical laboratory behavioral tasks are either not affected or are impaired only during the first few days after ischemia. An accumulating body of information indicates that tasks that require more complex cognitive functions will be of greatest value in identifying both correlates of histological injury as well as screening modalities for therapeutic interventions when rodent models of forebrain ischemia are employed.

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References


\textbf{Key Words} • behavior, animal • cerebral ischemia • rats
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