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Long-Term Functional End Points Following Middle Cerebral Artery Occlusion in the Rat

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MCAO Rats Staircase test Rotation Operant behavior

THE middle cerebral artery occlusion (MCAO) model of stroke is considered to be among the most valid representations of the pathology seen in human stroke populations, and is often used as such in the preclinical evaluation of putative neuroprotective agents. Damage following MCAO is demonstrated in striatum and overlying cortex, much as would be seen in human thrombotic/embolic occlusion of the MCA (21,22). One issue surrounding preclinical use of this and related models for evaluation of neuroprotective activity is the duration of protection. It is now clear that neuroprotection can be temporary, in that neuronal death may only be delayed rather than permanently conferred (3–5), depending upon a number of factors that include magnitude and duration of the neuroprotective treatment, as well as magnitude of insult. However, the majority of neuroprotection studies have studied behavioral and histologic endpoints over a limited time frame (e.g., 1–2 weeks). This is largely due to practical time constraints, and to the fact that many functional deficits appear to recover rapidly in rats (unlike in humans) in the absence of treatment, the time course of which depends upon the behavioral assessment used. For example, short-term neurologic deficits, such as spontaneous rotation, lack of orientation to tactile stimulation, and postural torsion are readily demonstrated in the rat MCAO model (1,14,15), but appear to recover within days to weeks of the surgery. Other tasks such as the tactile adhesion test (tape test), in which latency to contact or to remove a piece of adhesive tape placed on the paw (20), or beam balance tests, assess functional integrity of tactile inputs as well as motor dexterity, but deficits in these tasks also appear to recover typically within several weeks of the occlusion (1,15). Given that functional end points recover over the short term while histologic end points can deteriorate over the long term, the need for better long-term functional end points is very clear. In addition, given the fact that protection or recovery is measured in the clinic primarily by assessment of function, it is critical that functional protection can be preclinically demonstrated with any candidate treatment.

Another issue involved in the current use of behavioral end points is the fact that much of the volume of damage (and demonstrable protection) is noted in cortex, but primarily only motor or sensory endpoints are used. There have, however, been a number of studies addressing cognitive deficits

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following MCAO in the rat employing techniques such as Morris Water maze (13,15), radial arm mazes (19,23) and passive avoidance (2,16), each of which assesses aspects of short-term memory function, as well as learning ability. These tests appear to demonstrate good sensitivity to deficits that result from MCA occlusion, yet also appear to recover within several weeks of the surgery, especially under repeated testing paradigms.

Only a few tests have been shown to possess sensitivity on the order of months beyond the surgery. The staircase test (9,10,17), which assesses fine motor coordination, and amphetamine-induced rotation, have been previously shown to be sensitive to MCA occlusion for up to 3 months. However, both of these are primarily sensorimotor tests. In human stroke, hallmarks of the typical cortical damage beyond somatosensory deficits can include deficits in higher order processing (e.g., new learning ability, behavioral flexibility/adaptation), as well as behavioral restraint [i.e., appropriate withholding of responses; (8,12)]. In an attempt to address some of these cortical deficits, an operant differential reinforcement of low rates task (DRL) was employed, and both acquisition (learning) of the task, as well as subsequent performance was studied. The test measures ability to withhold responding for a fixed period of time (behavioral restraint), and as employed in the present study, also assesses behavioral adaptation. Only one group has previously used schedule-controlled behaviors to assess function following experimental cerebral ischemia (6,7). In those studies, animals were trained to respond under a multiple fixed-ratio fixed-interval schedule prior to global ischemia, and large decreases in response rates were observed, which recovered over the course of several weeks. We chose to use the DRL schedule largely because we felt that it might be more sensitive to cortical damage than other types of operant tasks, given the requirements of the task, which involves aspects of temporal perception, behavioral restraint, and perhaps sustained attention.

Hence, in the present study, both sensorimotor and cognitive testing was conducted to study the potential utility of these tests as a baseline from which to assess long-term functional neuroprotection as well as assessment of promotion of recovery of function.

METHOD

Subjects

The studies were carried out using male Wistar (Kyoto) rats obtained from Harlan Labs, weighing 280–310 g. All rats were housed individually with free access to food and water on a 12 L:12 D cycle. All animal care and use was carried out according to U.S.D.A. and A.A.L.A.C. guidelines.

Surgical Procedure

Middle cerebral artery (MCA) monofilament occlusion model. All animals were fasted overnight, with free access to water, prior to surgery. Rats were subjected to the following set of procedures to induce an infarct via MCAO model. During the procedure rectal temperature was maintained at 37.5 ± 1° C by a heating pad and lamp. The animal was anesthetized with 2–3% halothane anesthesia in a 30% oxygen/70% nitrous oxide mixture, and an incision was made just below the mandibles, extending approximately 1–2 cm caudally. The bifurcation of the external common carotid artery (ECA) and the internal common carotid artery (ICA) were exposed, and the right ECA was permanently tied off and bisected, with the remaining ECA stump used to isolate the ICA. A microaneurysm clip was placed across both the CCA and ICA to prevent bleeding during the insertion of the suture. A small hole was then made above the temporary tie and just below the permanent tie on the external carotid artery stump, and a monofilament suture inserted into the ECA past the temporary tie and into the lumen of the ICA. The temporary clip on the CCA/ICA bifurcation was then removed and the monofilament advanced approximately 20 mm into the ICA until proper resistance was felt. At this point MCA occlusion was achieved and the duration of the ischemia was limited to either 90 or 120 min. The monofilament was held in place during the ischemic period by tightening a suture around the ECA. The study also included a sham group of rats that underwent an identical surgical procedures except for the insertion of the monofilament suture into the ICA.

The rats were allowed to recover from anesthesia during the period of ischemia, and the animals were observed for behavioral asymmetries and other appropriate behaviors during the period of ischemia. No analgesic was administered during this time due to possible interaction with the behaviors measured or with lesion development. Three characteristic asymmetries (forepaw extension, spontaneous rotation, and salivation) were scored on a three-point rating scale: (1 = mild, 2 = moderate, and 3 = severe). Following the appropriate duration, the rats were reanesthetized, the incision opened, the monofilament removed, and the suture on the ECA was ligated to prevent bleeding. Reflow was established back to the ICA and ultimately the middle cerebral artery. The incision was closed, the rat was again removed from anesthesia, and placed on a heating pad until fully recovered. Ultimately, six animals given sham lesions, five given a 90-min ischemia, and seven given a 120-min ischemia were entered into the study.

Behavioral procedures. The sequence of testing is outlined in Table 1, and the details of each procedure described below. The sequence was chosen to ensure that subjects were well recovered (as assessed by weight gain) prior to initiation of behavioral testing. Methamphetamine rotation was tested first to give additional confidence that lesions were present prior to the many weeks of testing.

Methamphetamine rotation. Animals were individually transported to the laboratory and allowed to acclimate for 5 min. (+)-Methamphetamine HC1 (Sigma Chemical), prepared as a 1 mg/ml solution in saline, was then administered IP at 1 mg/kg (as salt) in a dose volume of 0.1 ml per 100 g body weight. The animal was returned to the holding cage for 5 more min. The animal was then placed in a round opaque

TABLE 1

SEQUENCE OF BEHAVIORAL TESTING FOLLOWING SURGERY

Week Postsurgery	2–4	5	6	13–14	15–21	22
Tests	methamphet rotation	operant acquisition	wheel	staircase	DRL	staircase
Duration	once per week (three determinations)	single day	3 days	daily (11 days)	daily (27 days)	daily (7 days)

plastic open top container (30 cm diameter, 24 cm high) and observed for the direction and number of 360 degree turns per 5 min interval over a 20-min period. Partial turns, delays or climbing activity were not factored.

Staircase test. The training and testing procedures and apparatus have been thoroughly described previously (9,17). Briefly, the apparatus consists of an elevated platform on which the rat rests, with staircases on either side of the animal. A staircase consists of seven steps each containing a small food well, that were baited with three 45-mg Noyes pellets per well. There were also two "free" pellets placed on the platform at the beginning of each session to assist in initiation of the session. The apparatus is designed such that the rat can only use the respective arm and forepaw (left or right) to pick up pellets on each side of the platform (left or right). The size of the interior area of the staircase could be adjusted to the size of animal being tested.

All animals were food restricted to approximately 75–90% of ad lib weight prior to the initiation of testing. Weight restriction was adapted to the individual subject, and in some animals, up to a 25% decrease was required to train the task. The animal was placed in the staircase for a 20-min test session once daily with the number of pellets eaten and dropped/not eaten on each side recorded after the conclusion of the session. The "free" pellets consumed were not included with the data collected during the session. All rats were fed the remainder of their chow ration upon the completion of daily testing.

Operant acquisition with delayed reinforcement. Animals were fasted for 24-h, then placed into standard two-lever operant chambers for a single 14-h, overnight session. The procedure used was a modification of one previously described (11). Operant session were scheduled such that delivery of a food pellet was initially contingent upon a single lever press on either of the levers in the chamber (FR1). Following delivery of each 20 reinforcers, the response requirement was increased in the following series: FR2 (achieved following 20 reinforcers) FR3 (40 reinforcers), FR5 (60 reinforcers), FR7 (80 reinforcers), and FR10 (100 reinforcers). Delivery of the food pellet was always delayed by 4 s following completion of any FR requirement. The delay of reinforcement was instituted because previous studies have shown a marked increase in the sensitivity of the paradigm to acute drug effects following initiation of the delay (Hudzik, unpublished observations), and it was anticipated that a similar sensitivity increase would be evident following surgery. The dependent variables included the time required to achieve FR3 (emit the first 40 responses), time required to achieve FR 10 (emit the first 100 responses), and the total reinforcers earned.

Running-wheel chambers. Following acquisition of operant responding, animals individually resided for 3 days in clear Plexiglass shoebox cages (50 cm L $\times$ 27 cm W $\times$ 36 cm H), which were fitted with running wheels with magnetic switches for counting wheel turns (Mini-Mitter), water bottles, and a grid floor. These cages resided in a room dedicated to the study, which was on a 12 L:12 D cycle. An operant manipulanda (MED Associates, St. Albans, VT) was attached to the rear wall of the cages, 4 cm above the floor, and a food cup to the perpendicular wall close to the lever. Food pellet dispensers were mounted outside the cages, as was a stimulus lamp. Events in the chamber were controlled and recorded via interface (MED Associates) to a microprocessor. Food was continuously available under an FR1 schedule of reinforcement (each lever press resulted in delivery of a 45-mg food pellet). Because a running wheel turn was the primary dependent variable, standard rodent chow was made freely available on the tops of the cages. The operant manipulanda were activated to assess whether generalization of responding from the prior operant acquisition would occur. Wheel turns during the dark and light cycles were recorded, as were total lever presses.

Operant DRL. Animals were trained to respond under a DRL schedule of reinforcement in the standard, Gerbrands operant chambers used in the initial operant response acquisition. Animals were initially trained under a 7-s DRL schedule, in which only responses that were preceded by at least 7 s of no responding were reinforced. Animals were trained under the DRL-7 schedule for 17 days. Following this initial training, the schedule was changed to a DRL-10 s schedule for 5 days, then to a DRL 15-s schedule for an additional 6 days. The total lever presses, reinforcers earned, the percentage of reinforced responses, as well as the individual interresponse times (the time elapsed between each response; IRT) were recorded.

Histological procedures. To estimate the volume of the infarcted tissue the animals were anesthetized and perfused with 10% formalin solution buffered to pH 7.4 and the brains were stored in situ for 24 h. The brains were subsequently embedded in paraffin, and a series of 5 μm-thick sections (up to eight section per animal) were cut at 2-mm intervals through the forebrain beginning at about 2 mm anterior to bregma (18) and stained with hematoxylin and eosin (H&E). These H&E slides were assessed for histological damage using a microscope and a C-Imaging system (Compix, Cranberry Township, PA) in the cortical and subcortical regions as evidenced by loss of tissue, damaged neurons (as evidenced by pyknosis and karyorhexis), and a proliferation of phagocytic cells. In addition, the amount of tissue atrophy (the difference between the infarcted and non infarcted hemispheres) was measured as well. A volumetric measure of these areas was calculated by summing the product of areas and the interval distance. Eight sections were used (between 2 mm anterior to bregma, and ending 10 mm posterior to bregma). The amount of damage is expressed as the percent area of the contralateral hemisphere to control for differences between animal brain sizes as well as fixation and other artifacts. None of the subjects had any indication of hemorrhage as a result of the surgical procedure.

Statistics. Comparisons of means were made by ANOVA, where three or more comparisons were made, followed by Dunnett's post hoc tests to compare sham-lesioned subjects' performance to that of the experimental groups. Two-factor ANOVA was used to compare operant acquisition curves (factor A = lesion type, factor B = hour in chamber, data on cumulative reinforcers). Spearman rank correlations between histologic and behavioral end points were made.

RESULTS

Outcome and Neurologic Assessment

Surgeries continued until an n of at least 6 was obtained for each group. All stroked rats which were entered into the study scored 3 in neurological assessment following occlusion of the MCA, with one exception (one rat in the 120-min ischemia group scored 2). One rat initially assigned to the 90-min group scored 0 in the neurological assessment was excluded from the study. There was one rat from the 120-min group that died during surgery, and one that died 24 h after surgery. One animal in the 90-min group died 5 weeks following surgery, and its data were excluded from all analyses. MCAO oc-

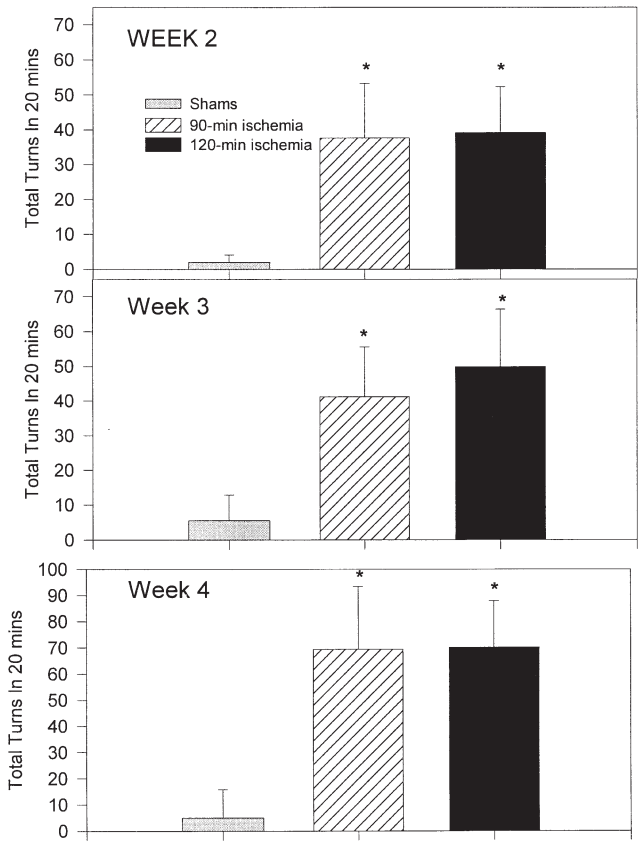

FIG. 1. Methamphetamine (1 mg/kg) induced turning behavior 2–4 weeks following ischemia. *Significantly different from control (ANOVA, Dunnett's post hoc comparison to control).

cluded rats maintained an average body weight of 30–40 g lower than starting weight for the first week of the study, then began to gain weight over the successive week. Ischemic 120-min rats lost on average 10 g more than 90-min ischemic rats. By the end of the second week postischemia, ischemic rats had returned to preischemic weight levels. Sham-lesioned rats, in contrast, lost on average 15 g body weight on the first day after surgery, but regained presurgical weight within 2–3 days of surgery.

Methamphetamine-Induced Turning

Two weeks following ischemia, both ischemic groups showed a comparable magnitude of modest ipsilateral turning (Fig. 1, upper panel), which remained unchanged by the third week. By the fourth week following ischemia, the amount of turning increased similarly (~1.7-fold) in both ischemic groups (lower panel).

Learning Ability

Control rats acquired lever pressing (achieved FR-3 by emitting more than 40 responses) within 5 ± 1.5 h. Neither ischemic group differed from this, achieving FR3 within 6 ± 2 and 5.1 ± 2 h for the 90-min and 120-min groups, respectively. Control rats earned 109 ± 12 reinforcers during the course of the 14-h session (Fig. 2), which did not differ significantly from the two ischemic groups, suggesting that no deficit in ability to learn to emit and perform the response was present.

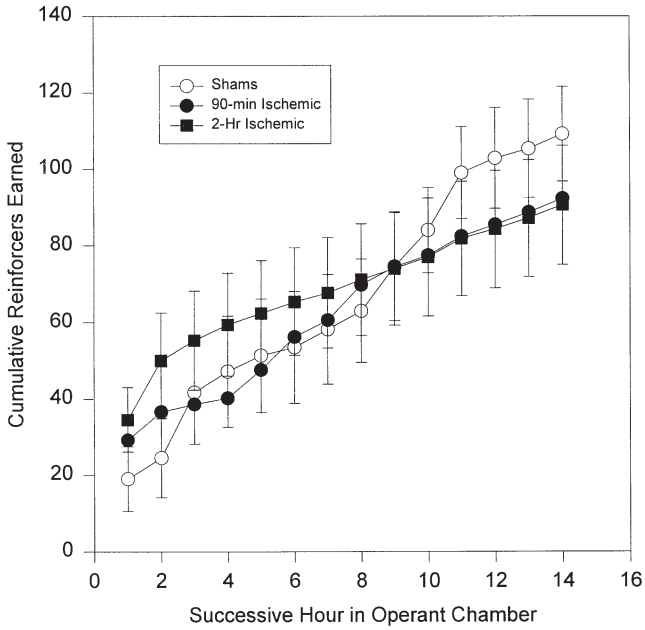

FIG. 2. Operant acquisition curves, shown as cumulative reinforcers (food pellets) earned during successive hours of the 14-h test session. Two-factor ANOVA revealed no significant differences between groups, (F(2) = 0.64, $p = 0.52$).

Running Wheel

None of the ischemic groups differed from control in terms of activity in the running wheels over the 3-day exposure period (Fig. 3), nor on any individual day (data not shown), although the 90-min ischemic rats ran significantly less than the 120-min ischemic rats [ANOVA, $F(2, 51) = 4.04$, $p < 0.05$]. Generalization of operant responding to the running wheel cages occurred in all groups, because all responded on the levers during the 3-day period. However, despite the fact that food was freely available in the cages, both ischemic groups, emitted more operant responses than control rats [ANOVA, $F(2, 51) = 3.4$, $p < 0.05$].

Staircase Test

Both ischemic groups performed significantly more poorly than sham rats (Fig. 4, Table 2) over the course of training and performance [ANOVA, $F(2, 17) = 4.7$ $p = 0.03$]. Although all groups improved their performance over the course of training, in general, the 90-min ischemic animals retrieved fewer pellets than the 120-min ischemic animals. When studied as a function of completed trials (pellets eaten) by laterality (Table 2), both groups exhibited bilateral deficits with respect to control (fewer pellets were eaten on both the left and right sides). However, only the 120-min ischemia group animals exhibited a significant preference for the right paw (significantly more pellets were retrieved from the right side than the left).

Operant DRL

All rats emitted similar numbers of responses during the course of DRL training, confirming that there was no deficit

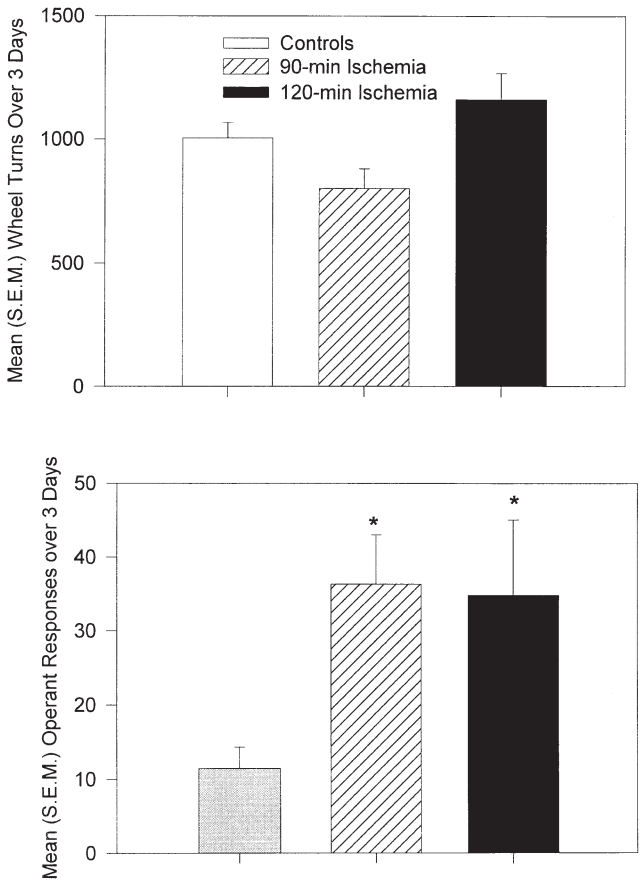

FIG. 3. Behavioral performance in the running-wheel operant chambers. Upper panel: total wheel turns. Lower panel: operant responses. *Significantly different from control (ANOVA, Dunnett's post hoc comparison to control).

in ability to emit operant responses, or in motivation to respond. Under the DRL-7, each group earned similar numbers of reinforcers (Table 3), although a higher proportion of longer IRTs was noted in MCAO animals in general (Fig. 5).

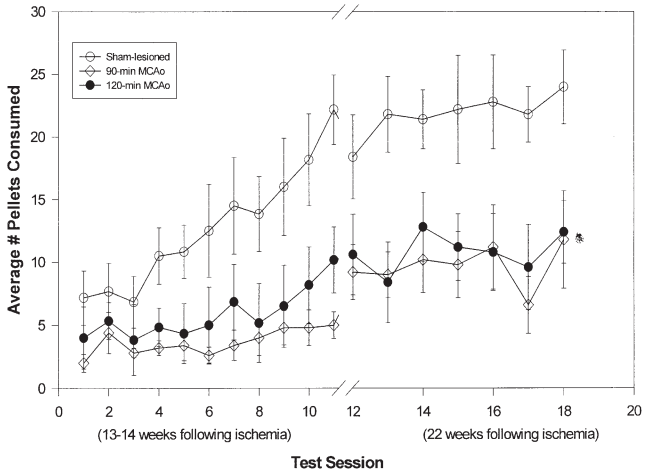

FIG. 4. Group averages for total pellets consumed in the staircase test on successive daily sessions beginning 3 months and 5 months following ischemia.

The percentage of reinforced responses for each group declined, as expected, with each change in the DRL value imposed, but declined significantly, with respect to shams, only in the 120-min ischemia group (Table 3). Control rats maintained average IRT values that were proximal to the DRL value imposed, (e.g., IRTs increased as a function of increases in DRL value). The 90-min ischemia rats, however, maintained IRTs within a restricted range (9.5–11.5 s), regardless of the DRL value in effect (Fig. 6). The 120-min ischemic group decreased mean IRTs in response to an increase in DRL from 7 to 10 s, then returned IRTs to their initial value (9 s) in response to increasing the DRL value from 10 to 15 s.

Histology

The infarct volumes, as determined by H&E stained sections, for the 90-min and 120-min groups were similar in size and the largest portion was composed of atrophied tissue (Table 4). The atrophy is the area of complete necrosis with loss of tissue, which presented as lack of tissue and a shrunken hemisphere. Although the infarcted area of striatum were not

TABLE 2

EFFECTS OF MCAO ON PELLETS EATEN, BY SIDE (MEAN AND SEM)

	Grand Mean Left Side	Grand Mean Right Side	Week 13 Left Side	Week 13 Right Side	Week 22 Left Side	Week 22 Right Side
Sham	7.73	8.14	6.03	6.58	10.88	10.71
	0.48	0.47	0.56	0.55	0.66	0.69
90 min	2.83*	3.18*	1.67*	2.00*	4.66*	5.03*
	0.333	0.28	0.21	0.26	0.68	0.42
120 min	3.86*†	5.92*†	3.22*†	4.87*†	5.02*†	7.86*†
	0.38	0.41	0.44	0.45	0.71	0.75

*Significantly different from control (ANOVA, Dunnett's post hoc comparison to control).
†Significantly different from other side (left vs. right) within group (*t*-test).

TABLE 3
DRL PERFORMANCE (MEAN AND SEM)

DRL-7	Reinfs	% Reinf	DRL-10	Reinfs	% Reinf	DRL-15	Reinfs	% Reinf
Sham	84.61	40.63	Sham	59.78	38.37	Sham	36.27	28.16
	13.96	7.75		10.73	7.83		7.93	7.20
90-min	90.47	56.59	90-min	82.27	52.15	90-min	39.13	27.65
	13.96	12.42		13.15	9.55		9.69	7.63
120-min	89.57	53.58	120-min	51.00	26.30*	120-min	20.72	12.78
	13.72	10.97		9.42	5.08		9.39	6.55

Group mean (SEM) of last 3 days for each DRL level.
Reinfs = reinforcers earned. % Reinf = % of responses that were reinforced.
*Significantly different from sham group (ANOVA, Dunnett's post hoc).

significantly different between the 90- and 120-min groups, there was a greater amount of cortical damage in the 120-min compared to the 90-min group.

In addition, there were qualitative differences in the appearance of the infarcted regions of the tissues in both the striatum (Fig. 7) and the cortex (Fig. 8). In all cases the region contralateral to the infarct appeared normal (Fig. 7A and 8A), although there may be some selective cell loss or changes in other histological parameters or function that are not observable in H&E staining. In the striatum, animals receiving a 90-min occlusion displayed striasomes, although they appeared abnormally small and stained lighter than the contralateral side (see Fig. 7A and B). However, there were few of the characteristic large neurons in the infarcted striatum, while there were numerous darkly staining cells, some of which may be microglia (Fig. 7B). Similar changes were noted in the cortex, where there was minimal damage in the 90-min group, although the neuropil in the outermost cortical layer appeared abnormal (Fig. 8B). In contrast, the cortical region of the 120-min infarct group has numerous darkly staining cells and long streaks of glial scars (Fig. 8B), which were also seen in striatal tissue in the group receiving 120 min of ischemia but not in the 90-min group (data not shown). Therefore, although the striatal infarct sizes were similar for the 90- and 120-min MCA occlusion groups, the qualitative damage appeared to be more severe in the group with the longer oc-

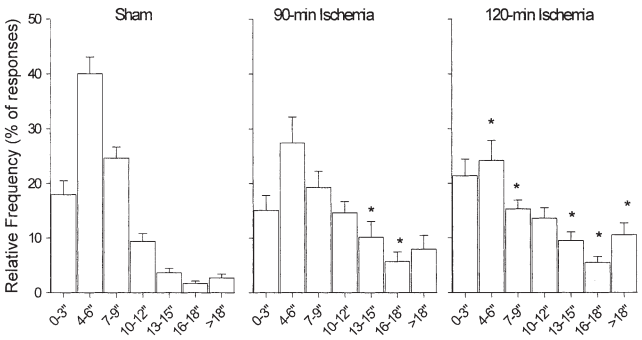

FIG. 5. Relative frequency distributions of IRTs over the final 3 days of the DRL-7 condition. Indicates significant difference from distribution representing control (sham-lesioned) rat's responding at a given interval (ANOVA, Dunnett's post hoc).

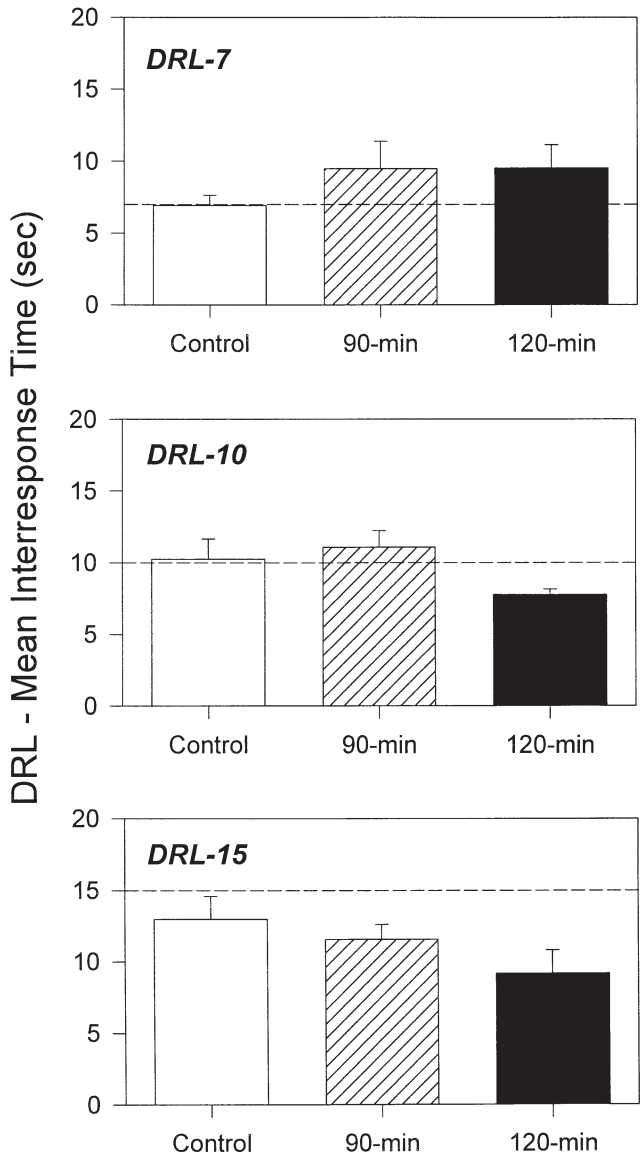

FIG. 6. Average (±SEM) IRT over the final 3 days of each DRL condition. The broken line indicates the DRL value.

TABLE 4

PERCENT REDUCTION IN VOLUME (INFARCT) RELATIVE TO THE CONTRALATERAL HEMISPHERE

	% Infarct CPU	% Infarct CTX	% Atrophy	% Total Volume of Ischemic Damage	Total Volume of Ischemic Damage mm^3
90-min	1.89	0.11	17.5	19.48	104.7
SEM	0.63	0.11	8.75	9.1	45.3
120-min	1.14	2.54	15.4	19.1	109.8
SEM	0.61	1.19	4.5	5.45	27.9

(Mean, and SEM), and volume of damage in mm^3.
CPU = caudate/putamen; CTX = cortex.

clusion. Also, the large amount of glial scarring seen in the 120-min group was absent in the 90-min group. There was little difference among groups in terms of average volume of damage as a function of anterior/posterior plane (Fig. 9).

Spearman rank correlations between individual infarct values and performance across each of the behavioral endpoints (Table 5) revealed that only in the 120-min ischemic group was there a statistically significant correlation between striatal infarct size and number of rotations ($r = 0.93$, $p < 0.02$). Pooling all ischemic rats for correlational analysis revealed two additional relationships (Fig. 10). When all MCAO rats were entered into a comparison between pellets eaten in the staircase apparatus and volumetric assessment of damage, there was a significant negative relationship (greater damage was associated with fewer pellets retrieved from the left side). Under these conditions, IRTs were also related to amount of damage (greater damage was associated with longer IRTs under the DRL-7 schedule).

DISCUSSION

Of the behavioral test employed in the present study, methamphetamine-induced rotation, the staircase test, and the DRL schedule appeared to exhibit longer term sensitivity to behavioral deficits consequent to MCA occlusion. Ipsilateral rotation, indicative of striatal damage, was measured for up to 4 weeks following MCA occlusion, the amount of which tended to increase over time. Assessment of rotational behavior has been previously shown to be sensitive for up to 3 months following MCA occlusion (9), although the absolute duration of sensitivity has not yet been established. This end point was used in the present study as a means of confirming

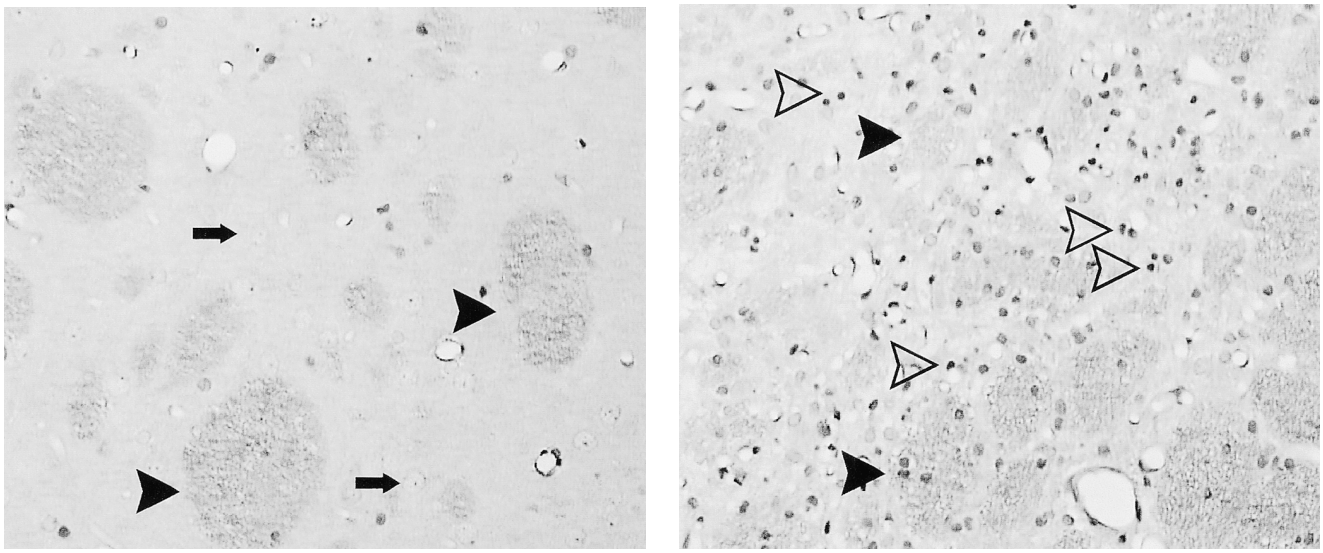

FIG. 7. Long-term histological outcome of striatal tissue using H&E-stained sections from an MCA-occluded rat 23 weeks after the surgical procedure in both the unlesioned (left panel) and lesioned hemispheres (right panel). Sham-lesioned animals and lesioned animals demonstrate a normal neuropil and numerous neurons and several striasomes (e.g., large arrowheads) in both the contralateral and sham surgical side. In the group receiving a 90-min occlusion, the contralateral striatum (A) showed normal striasomes (arrowheads) and neurons (arrows), while the infarcted striatum (B) had few normal appearing neurons, smaller, fainter staining striasomes (dark arrowheads), and numerous small darkly staining cells (open arrowheads), some of which may be microglia. The group of animals that received an occlusion that lasted for 120 min also displayed a normal contralateral striatum with apparently normal neurons; however, the infarcted striatum had few normal large neurons numerous small dark staining cells and large, radiating glial scars, similar to the scars in cortical tissue (see Fig. 8B).

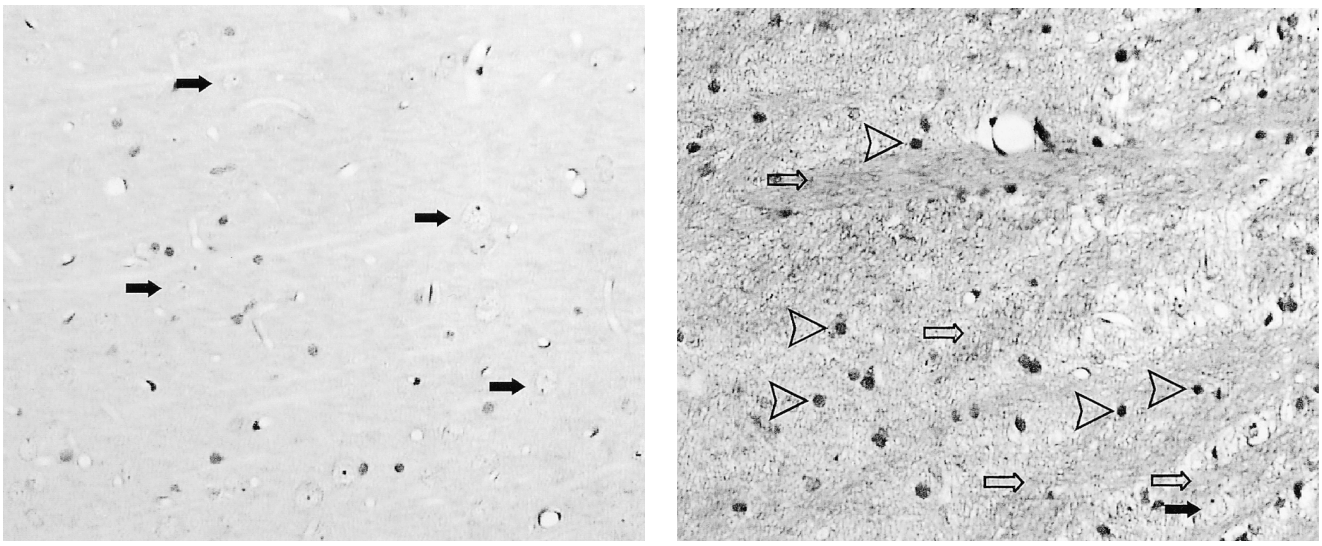

FIG. 8. Long-term histological outcome of cortical tissue using H&E-stained sections from an MCA-occluded rat 23 weeks after the surgical procedure in both the unlesioned (left panel) and lesioned (right panel) hemispheres. In both the 90- and 120-min occlusion groups the contralateral hemisphere appeared normal (A). The 90-min occlusion group demonstrated few normal-appearing neurons in the infarcted region, while the group receiving an occlusion lasting for 120 min demonstrated very few neurons (B, dark arrow) but numerous darkly staining cells (open arrowheads), some of which may be microglia, and numerous glial scars (open arrows). Sham-lesioned animals demonstrated normal tissue staining and numerous, normal-appearing neurons in both the contralateral and the surgery hemisphere.

striatal damage, but did not appear to be related to the duration of the ischemia, inasmuch as both groups of ischemic rats produced equivalent numbers of turns on the average. Unlike previous studies (9), caudate-putamen lesion size was correlated with amount of rotation for the longer duration ischemic rats.

The staircase test showed perhaps the greatest degree of disruption of the tests employed. Ischemic rats successfully consumed only about 50% of the pellets consumed by the control rats, and the test remained sensitive for up to 22 weeks following ischemia, despite some improvement in performance over time for all groups. Both groups showed bilateral deficits with respect to control, although the contralateral paw tended to show relatively greater deficits. Oddly, animals subjected to 90-min of ischemia appeared to perform more poorly in the staircase test than those subjected to 120-min of ischemia. This is difficult to reconcile with the fact that the groups did not differ from each other in terms of the magnitude of striatal damage or by the functional deficit as probed by methamphetamine rotation. The groups did, however, differ in terms of the magnitude of cortical damage. It is possible that cortical damage can either benefit recovery of function (e.g., by reducing factors associated with inhibition of axonal sprouting/growth, such as myelin-associated glycoproteins) or protect in some manner (e.g., by reducing potentially neurotoxic excitatory input). This observation will require replication before these speculations can be tested.

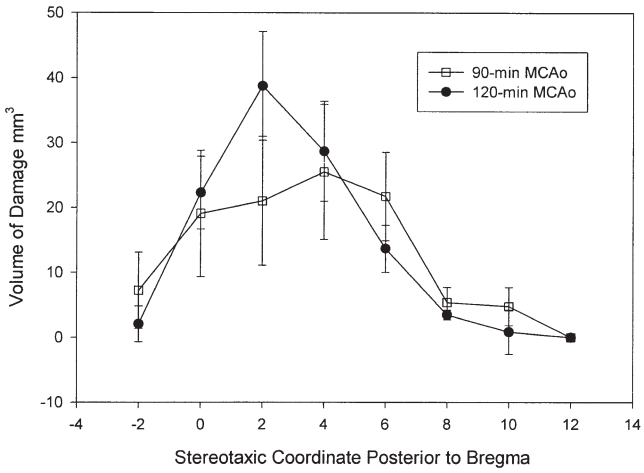

FIG. 9. Mean (±SEM) lesion volume (mm^3) as a function of successive, 2-mm section.

The majority of tests conducted to assess deficits following MCA occlusion are related to sensorimotor performance, although the damage incurred in the model is not restricted to motor regions of brain. Two tests were employed in the present study to address potential cortically mediated deficits. The first test, operant acquisition, assesses the ability to explore a novel environment, make contact with, depress a lever for food pellet delivery, and establish the contingency between lever pressing and food pellet delivery. Additionally, to potentially increase the sensitivity of the test, delivery of the reinforcer was delayed following lever presses by 4 s. This test, which has been previously shown to be very sensitive to the disruptive effects of acute drug administration (11) was, however, insensitive to ischemic damage at the time period during which it was measured. This observation has recently been replicated in a different set of rats with a permanently occluded MCA, as well as in rats that have been infarcted by occlusion of the jugular and submitted to hypoxia (Hudzik, unpublished observations), which would tend to argue that unilateral striatal and cortical lesions of this type (ischemic) do not impair operant learning.

Running in a wheel was insensitive to ischemic damage. This was somewhat surprising in light of motor deficits ob-

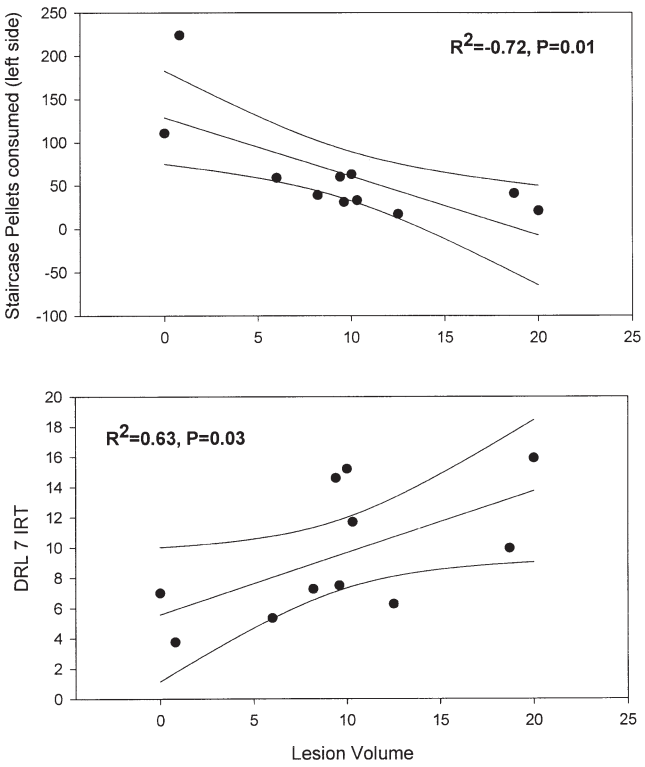

FIG. 10. Regressions. 95% confidence intervals, and Spearman rank correlation coefficients of volumetric damage measurements (% infarct) against pellets eaten from the left (affected) side of the staircase apparatus (upper panel) and IRT averaged over the last 3 days under the DRL-7 schedule (lower panel). Data are for all MCAO rats combined.

served in the staircase test. However, it may be the case that the relatively gross motor movements in wheel running are less vulnerable than the very fine motor output required by the staircase test. Although all animals were able to generalize responding from their initial overnight session in the operant chambers to responding on the operant manipulanda in the running wheel chambers, animals subjected to ischemia responded more than the sham-lesioned rats over the 3-day observation period. It is unlikely that the increased responding was due to the relative preference for eating the 45-mg food pellets over the biscuits on the top of the cage, because, on average, only 1–2 g of the food pellets was consumed per day. The responding may, however, have been due to an overgeneralization or perservation on the previously learned operant response. However, given the unanticipated nature of this finding, it is difficult to speculate on the cause based upon the data gathered in the present study.

Rats learned to respond under and to perform a DRL-7 schedule at similar rates between groups, although both ischemic groups had longer IRTs than controls. However, a clear deficit was uncovered when the schedule requirements were changed such that longer pauses between responses were required (10 and 15 s). Both ischemic groups failed to appropriately alter their behavior as the schedule requirements were changed. The 90-min ischemic rats maintained IRTs at similar level, regardless of the DRL schedule in effect, and the 120-min ischemic rats initially reduced IRTs in response to an increase in the DRL schedule, and maintained that IRT level when the schedule was further increased, suggesting perservation on the previously learned response. Perseverative errors are commonly found during neuropsychological assessment in human patients that have sustained frontal cortical injury (8,12). Future experiments, perhaps employing simpler behavioral end points, could be designed to study this specific deficit. It is also possible that the relative impact of changing the DRL value differed between groups, thus explaining the differential response to the change in DRL. For example, because the ischemic groups generally had longer IRTs, increasing the DRL value would have resulted in a relatively lower reduction in reinforcement density, thus resulting in lesser behavioral adaptation to the change. This possible explanation merits further study. Given the fact that IRTs were longer in the ischemic groups (thus, the relationship observed between number of reinforcers earned and duration of ischemia, and the correlation between damage and IRT), it will be of inter-

TABLE 5

SPEARMAN RANK CORRELATION COEFFICIENTS AND *p*-VALUE

90-min Ischemia	MA Turns	DRL Reinforcers Earned	Staircase Total Pells Consumed	Pellets consumed Left Side	Pellets consumed Right Side
CTX	−0.35	0.71	0.35	0.35	0.18
	0.52	0.13	0.52	0.52	0.78
CPU	0.2	0.2	−0.20	−0.6	0.46
	0.78	0.78	0.78	0.35	0.45
Volume	0.30	−0.50	−0.70	−0.88	0.05
	0.68	0.45	0.23	0.08	0.95
120-min Ischemia					
CTX	0.17	0.32	0.29	−0.75	0.23
	0.71	0.49	0.56	0.10	0.66
CPU	0.84*	0.69	−0.3	−0.08	−0.38
	0.03	0.14	0.49	0.80	0.92
Volume	0.26	0.49	−0.26	−0.48	−0.08
	0.66	0.36	0.66	0.35	0.92

CPU = caudate/putamen; CTX = cortex.

est in future studies to design the schedule parameters to take advantage of this observation.

The present study showed that deficits following MCAO can be present for periods of time in excess of 22 weeks in rats, which extends earlier observations (9). However, the relationship between duration of ischemia, damage, and deficit measured critically depends upon the time at which behavior is assessed, and the range of damage initially induced, which was, in the present study, perhaps more restricted in magnitude than would have been necessary for such comparisons. It is not clear why the lesion sizes reported in the present study are lower than those traditionally reported for infarcts of this nature (including in our own hands in other studies). The possibility that smaller lesions were simply induced in the particular batch of rats used in the present study cannot be ruled out. It is also possible that over the 5-month period during which animals were kept alive following surgery, there was shifting of cerebral mass and other compensatory changes that may have contributed to lower apparent lesion size as well as to variability. Further, the repeated testing of the subjects in itself could potentially promote recovery of function, thus contributing to the variability seen. Additionally, the general lack of significant correlation of lesion size with deficits seen in the present study may also reflect the difficulties in application of quantitative histologic techniques under conditions temporally far removed from the initial insult. Therefore, long-term analyses of neuroprotection cannot rely solely on histologic endpoints, but may better be conducted in conjunction with behavioral/functional end points.

In summary, the present study demonstrated that methamphetamine-induced rotation and the staircase test, perhaps both reflective of striatal damage, appear to be sensitive to MCAO ischemia for at least 4 and 22 weeks following ischemia, respectively. Additionally, although acquisition and initial performance of operant DRL responding was insensitive to ischemia beginning at 15 weeks following stroke, animals had longer IRTs and a possible suggestion of perservative deficits when challenged with changes in the schedule parameters. These studies provide an initial framework with which to assess long-term neuroprotection and functional recovery.

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