Dysexecutive Syndrome After Mild Cerebral Ischemia? Mice Learn Normally but Have Deficits in Strategy Switching
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Background and Purpose—We determined long-term functional outcome in a well-characterized mouse model of mild focal cerebral ischemia.

Methods—We subjected 129/SV mice to sham operation or 30 minutes of left middle cerebral artery occlusion (MCAo) followed by reperfusion (89% survival rate). Six weeks later, animals were tested for neurological deficits, motor coordination on an accelerating Rota-rod apparatus, and spatial navigation in a water maze task. Brain lesion size was determined on NeuN-immunostained coronal brain sections by computer-assisted volumetry.

Results—Mice had mild but distinct neurological deficits and no deficits in Rota-rod coordination or swimming speed 6 weeks after MCAo. Moreover, mice had normal spatial learning abilities in the place task. However, stroke mice had deficits in the probe trial and visible platform task, which correlated with striatal lesion size determined on NeuN-immunostained sections.

Conclusions—After mild ischemia, mice recover with mild neurological deficits and normal motor coordination. Stroke mice have no obvious deficits in spatial learning in the Morris water maze but display distinct deficits related to strategy switching and relearning. (Stroke. 2004;35:191-195.)

Key Words: animal models ■ behavior, animal ■ outcome ■ stroke, ischemic

Stroke is still the most common cause of long-term disability in adulthood.1 In addition to a wide range of motor and sensory deficits, cognitive and behavioral abnormalities are observed after stroke, including in patients with caudate infarcts.2 However, most preclinical evaluations of outcome from stroke have used morphometric assessment of infarct volume. Although it is logical to assume that the volume of infarction will be closely correlated with outcome, impairment or preservation of complex behavior may reflect changes at a subcellular level or changes in synaptic or electrophysiologic function or may reflect diffuse morphological changes that are not quantified by counting dead neurons or drawing a cursor around a contiguous infarcted region.3 Several studies have reported the occurrence of cognitive deficits in experimental models of global and focal ischemia.4–6 However, data regarding transient ischemia—especially in the mouse—are scarce.

We have recently developed a model of mild focal brain ischemia in the mouse in which neuronal cell death evolves over days in the caudato-putamen while glial cells survive the insult (Figure 1).7, 8 Neuronal death exclusively affects medium spiny projection neurons, whereas all types of interneurons remain intact.9 Moreover, mortality is high in mouse models of severe ischemia, amounting to as much as 50% to 70% after 7 days,10–12 whereas survival rates in our mild model are ≈90% at 6 weeks. This model may be ideally suited to study long-term sequelae after stroke.

Therefore, in this study, we characterized long-term functional outcome after 30 minutes of left middle cerebral artery occlusion (MCAo) and reperfusion. We evaluated sensorimotor neurological deficits using a well-established rating scale first described by Bederson et al.13 Motor coordination was tested with a standardized Rota-rod apparatus, and swimming speed was determined in a swimming pool. Spatial learning was assessed in the Morris water maze task. In addition to a standard place task over 7 days, we performed a probe trial and visible platform task to assess strategy switching and relearning.14, 15

Materials and Methods

Animals and Model of Cerebral Ischemia
All experimental procedures conformed to institutional and international guidelines. Male 129S6/SvEv wild-type mice (18 to 20 g) were housed in groups of 4 to 5 at 20°C to 21°C and standard light/dark cycle (8 AM to 8 PM). Mice were anesthetized with 1.0% isoflurane in 70% N2O and 30% O2 using a vaporizer and subjected to left filamentous 30-minute MCAo (n=9) or sham operation (n=8) with monitoring for regional cerebral blood flow and temperature as described.8 One animal died after MCA occlusion.

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Neurological Deficits
After 6 weeks, mice were tested for neurological sensorimotor deficits and were scored by a blinded observer as described by Bederson et al.,13 with the following minor modification as described: 0 = no observable deficit (normal); 1 = failure to extend the right forepaw (mild); 2 = circling to the contralateral side (moderate); and 3 = loss of walking or righting reflex (severe).

Spatial Navigation
Experiments were closely adapted from previously published protocols with minor modifications.14, 15 A 101-cm-diameter, 50-cm-high swimming pool with cues present at fixed positions was filled to a depth of 30 cm with 15°C opaque water. Temperature was controlled regularly, and water was exchanged once the temperature reached 16°C. A clear Plexiglas platform (11x11 cm=121 cm²) was submerged with the top located 1 cm below the surface. Swimming performance (eg, path, speed, latency, distance) was tracked with a computer-based system (TSE Systems). A full experiment consisted of a place task (learning period) with 3 trials per day for 7 consecutive days, a probe trial (spatial probe) on day 8, and a visible platform test (reverse learning with visual cues) on day 9 as described.14, 15 For the place task, which is a test for nonspatial and spatial learning, the platform was always located in the center of the same quadrant (ie, target quadrant) for an animal. Each trial consisted of maximally 90 seconds starting from 1 of the 3 remaining quadrants with the face of the animal facing the wall. If after 90 seconds an animal did not reach the platform, it was guided to the platform. After reaching the platform, animals were allowed to remain there for 30 seconds. The intertrial interval was 1 minute, and mice were quickly dried with a towel and put under a heating lamp at exactly 37°C between each trial to avoid hypothermia. In the place task, the latencies, path lengths, and swim speeds for a single day were averaged to come up with a daily mean. For the probe trial, the platform was removed, and mice were allowed to swim for 90 seconds. Trials were analyzed by preference analysis (for the target quadrant and zone) with this formula: \[ \frac{(T-A)+(T-B)+(T-C)}{3}, \] where T is the swim time in the target quadrant (or zone) and A, B, and C are swim times in the remaining 3 quadrants (or zones). In the visible platform task, a clearly visible platform was re-placed into the swimming pool but opposite the previous target quadrant. This task consisted of 3 trials starting from different quadrants with an intertrial interval of 1 minute.

Motor Coordination
Motor coordination was tested using an accelerating Rota-rod treadmill for mice (TSE Systems; 3-cm diameter) 1 day after the Morris water maze experiment was finished. After familiarization with the Rota-rod at a constant speed of 4 rpm (3 runs of 120 seconds each with an intertrial interval of 2 hours), the actual test was performed on the next day. The mice were placed on the rotating drum with an accelerating speed from 4 to 40 rpm. Maximum speed was reached after 245 seconds, and maximum testing time was 300 seconds. The time spent on the drum was registered automatically (time until drop in seconds).

Lesion Determination
At the end of the experiments, animals were deeply anesthetized and perfused transcardially with 4% paraformaldehyde in 0.1 mol/L phosphate-buffered saline. Brains were carefully removed and post-fixed in the same fixative overnight at 4°C. Coronal 40-μm sections were cut on a Vibratome (Technical Products). For NeuN immunohistochemistry, the sections were first incubated in a blocking solution containing 10% normal goat serum and 0.1% Triton X-100 in phosphate-buffered saline and then incubated overnight at 4°C with anti-NeuN antibodies (mouse monoclonal, 1:100; Chemicon) and a 3-stage avidin-biotin method with corresponding biotinylated secondary antibody. The reaction product was visualized with 3',3'-diaminobenzidine as chromogene. NeuN-negative lesion volume was quantified with a computerized image analysis system (Sigma Scan Pro 4.0, Jandel Scientific) and calculated by summing the lesion areas of each section directly.7

Statistical Analysis
Data are presented as mean±SEM. Comparisons were made by 2-way repeated-measures ANOVA, followed by Tukey’s posthoc test (water maze), the Mann-Whitney rank-sum test (neurological deficit score), and Student’s t test (Rota-rods) or Pearson’s correlation. A value of \( P<0.05 \) was considered statistically significant.

Results
All mice subjected to MCAo (“stroke”) exhibited neurological deficit scores of ≥2 30 minutes after reperfusion, whereas all sham-operated mice had a score of 0. At 6 weeks, stroke animals...
still had distinct deficits (typically a mild forepaw palsy but no circling behavior) with a median score of 1.0 (0.50 and 1.0 for 25% and 75% confidence intervals) versus 0.0 (0.0 for 25% and 75% confidence intervals) in sham animals ($P_{<0.01}$, Mann-Whitney rank-sum test). Motor coordination on Rota-rod was not different between groups at 6 weeks (time until drop, 200±27 versus 189±23 seconds for stroke versus sham animals, respectively; $P_{>0.05}$).

For assessment of spatial learning, we exposed animals to a Morris water maze task. In the place task (Figure 2), we found that animals in both the sham and stroke groups showed a rapid decrease in latency and path length to find the platform, so that by day 5 they were performing near an asymptotic level of accuracy. Two-way repeated-measures ANOVA showed a significant effect of trial day on latency ($F_{6,84}=11.357; P_{<0.05}$) but no differences between groups at 6 weeks (time until drop, 200±27 versus 189±23 seconds for stroke versus sham animals, respectively; $P_{>0.05}$).

Analysis for path length yielded similar results (see Figure 2B). Swimming speed varied slightly over time during the place task; however, there were no differences in swimming speed between sham and stroke mice (Figure 2C). Together, these data indicate that stroke mice have no deficit in spatial learning compared with sham controls.

On day 8, animals were tested in a probe trial in which the platform is removed and animals are allowed to swim for 90 seconds (Figure 3). As in the place task, swimming speed was not different between sham and stroke animals (18.4±2.7 versus 17.9±1.3 cm/s). We noticed that sham animals spent less time in the target zone in the last minute of the trial (ie, from 31 to 90 seconds). In contrast, stroke animals had a higher preference for the target zone over time (see selected swim paths in Figure 3A). Two-way repeated-measures ANOVA revealed a statistically significant interaction between time (ie, intervals of 0 to 30, 31 to 60, and 61 to 90 seconds) and group (stroke or sham) for the number of target crossings ($F_{2,24}=3.874; P_{<0.05}$) and similarly time spent in the target zone ($F_{2,24}=3.937; P_{<0.05}$). Posthoc analysis (Tukey’s test) revealed that sham mice but not stroke mice had significant differences over time ($P_{<0.05}$ for 61 to 90 seconds versus 0 to 30 seconds; Figure 3). Moreover, there was a significant effect of time ($F_{2,24}=3.987; P_{<0.05}$) and a time-by-group interaction for target zone preference ($F_{2,24}=4.372; P_{<0.05}$). Tukey’s posthoc testing revealed significant differences between stroke and sham animals during the 31- to 60-second interval ($P_{<0.05}$) and the 61- to 90-second interval ($P_{<0.05}$; Figure 3B).

In the last part of the water maze (ie, day 9), we performed a visible platform task in which a visible platform is placed in the quadrant opposite the former target quadrant (Figure 4). In this trial, animals typically revisit the target zone learned in
the first trial, but they quickly find the visible platform in the second and third trials (see swim patterns in Figure 4A). Two-way repeated-measures ANOVA revealed a significant effect of trial on latency ($F_{2,24}=4.581; P<0.05$; Figure 4B). Posthoc testing, however, demonstrated a significant effect of trial only in the sham group ($P<0.05$ for trial 1 versus 3). Indeed, in the stroke group, brain lesion volume significantly correlated with latencies in trials 2 and 3 (Figure 4B). Average brain lesion volume on NeuN-immunostained sections was $9.7\pm1.1\ mm^3$ and confined to the caudato-putamen in the stroke group (Figure 1).

We found that, in contrast to sham animals, several stroke animals revisited the formerly learned target in trials 2 and 3 of the task (see swim pattern video records in Figure 4A). The time that mice spent in the quadrant that had previously contained the platform varied as a function of trial ($F_{2,24}=5.133; P<0.05$; Figure 4C); however, posthoc testing revealed significant differences between trials in the sham group ($P<0.05$ compared with trial 1; Figure 4C) but not the stroke group. In the stroke mice, brain lesion volume significantly correlated with time spent in the former target quadrant (trial 2; Figure 4C).

Discussion

Here, we characterized long-term functional outcome after mild focal brain ischemia in the 129/SV mouse. Interestingly, motor coordination on a Rota-rod was not affected by cerebral ischemia, and stroke mice displayed only mild sensorimotor neurological deficits at 6 weeks. Moreover, we demonstrate that, at 6 weeks after mild brain ischemia, mice have no apparent deficits in spatial learning in the Morris water maze: stroke mice had similar latencies and path lengths to find the platform in a standard place task over 7 days compared with sham controls (Figure 2).

However, stroke mice displayed distinct deficits in the probe trial and visible platform task. Although in the first 30 seconds of the probe trial stroke mice spent as much time in the presumed target as sham controls, they did not switch strategies afterward and explored the remaining quadrants of the swimming pool in the last minute of the trial (Figure 3). In the visible platform task (relearning with visible cues), stroke mice in contrast to sham mice tended to revisit the former target zone in trials 2 and 3 (Figure 4). Brain lesion areas determined on NeuN-immunostained sections reflecting neuronal loss were confined to the caudato-putamen of the left hemisphere as predicted from previous studies (Figure 1). $8^{-10}$ Importantly, lesion size of individual animals significantly correlated with latencies in trials 2 and 3 and with time spent in the former target quadrant in the visible platform task (Figure 4). This finding indicates that animals with larger lesions tend to revisit the formerly learned target and to have longer latencies to find the new platform. We identified distinct deficits in strategy switching and relearning after mild cerebral ischemia in the mouse. Although learning and memory seem to be unaffected, the observed impairment in behavioral flexibility partially resembles the dysexecutive syndrome observed in patients with basal ganglia disorders (eg, deficits in mental and motor switching).$16$

There are some caveats with regard to our data. Neurological sensorimotor deficits and changes in visual acuity may affect performance during spatial navigation. However, we performed the water maze experiments 6 weeks after surgery, when residual deficits were mild and when MCAo had no effects on motor coordination as determined on a Rota-rod. Notably, in contrast to rats, mice use their tails for swimming, and swimming speeds in the stroke mice were not different from those in controls (Figure 2). The fact that stroke mice had equal latencies and path lengths in the place task (which depends on visual cues) implies that any changes in visual acuity in the stroke mice would not have an impact on performance in the water maze task.

It has previously been reported for rats that caudato-putamen lesions may affect acquisition, retention, and selection of spatial navigation strategies.$17$ In addition, several authors have detected disturbances of memory acquisition and learning after MCAo in rats using avoidance tasks and maze tasks.$18^{-22}$ Of interest, using passive avoidance test and transfer latency in the elevated-plus maze, Gupta and coworkers$6$ detected normal learning and memory in rats subjected to transient focal ischemia.

So far, evidence is lacking that insight gained from animal models of cerebral ischemia is of any relevance to the
pathophysiology of stroke in humans. None of the neuroprotective strategies developed in rodent models proved effective in clinical trials. Of note, long-term stroke survival is woeful in the mouse stroke models, with mortality rates often >50% after 24 to 72 hours and functional outcome rarely tested at late time points. In contrast, in our model, survival rates are 90% at 6 weeks. Therefore, our model may be better suited for the study of long-term functional outcome as suggested by the Stroke Academic Industry Roundtable as the gold standard for preclinical neuroprotective and restorative drug development. It remains to be determined, however, whether the distinct dysexecutive deficits reported in this study are amenable to treatment.

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