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. 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. 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.

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 6 in a temperature-controlled room (18°C to 22°C) with 12-hour 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. One animal died after MCA occlusion.
C. A clear Plexiglas platform (11 cm x 11 cm x 121 cm²) was sub-
regularly, and water was exchanged once the temperature reached
16°C. Temperature was controlled to a depth of 30 cm with 15°C
swimming pool with cues present at fixed positions was filled to a
loss of walking or righting reflex (severe); 2 = circling to the con-tra-
lateralse side (moderate); and 3 = loss of walking or righting reflex (severe).

Spatial Navigation
Experiments were closely adapted from previously published proto-
cols 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 (11 x 11 cm x 121 cm³) was sub-
merged 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: \[(T - A) + (T - B) + (T - C)/3,\]
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 in 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 immuno-
histochemistry, 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.8 The reaction product was visualized with 3,3-
diaminobenzidine as chromogen. 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-rod) or Pearson’s correla-
tion. 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

Figure 1. 129/SV mice were exposed to 30 minutes of MCAo
followed by reperfusion and killed 6 weeks later. NeuN immuno-
histochemistry (40-μm coronal brain section) demonstrates neu-
ronal sparing confined to the caudato-putamen.

Figure 2. Latencies (A), path lengths (B), and swimming speed
(C) in stroke and sham animals on a place task in a swimming
pool. Mean±SEM (average of tree trials per day); n=8 per
group. For statistical analysis, see text.
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 sham and stroke mice (group, $F_{(1,14)}=0.0543; P_{0.80}$) and no significant group-by-trial interaction ($F_{(6,84)}=1.137; P_{0.35}$). 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
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. Furthermore, 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 coworkers6 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

Discussion

Here, we characterized long-term functional outcome after mild focal brain ischemia in the 129/SvJ 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

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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.\textsuperscript{10–23} 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.\textsuperscript{23} It remains to be determined, however, whether the distinct dysexecutive deficits reported in this study are amenable to treatment.

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References
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