NXY-059, a Free Radical–Trapping Agent, Substantially Lessens the Functional Disability Resulting From Cerebral Ischemia in a Primate Species

Jonathan W.B. Marshall, PhD; Katharine J. Duffin, BA; A. Richard Green, DSc; Rosalind M. Ridley, ScD

Background and Purpose—NXY-059 is a novel nitrone with free radical–trapping properties that has a considerable neuroprotective effect in rats. We have now examined the efficacy of this drug at reducing long-term functional disability in a primate model of stroke.

Methods—Twelve monkeys were trained and tested on a variety of behavioral tasks used to dissociate and quantify motor and spatial deficits. Five minutes after permanent occlusion of the right middle cerebral artery, monkeys received a 1-mL intravenous infusion of either saline or NXY-059 (28 mg·kg⁻¹), and osmotic minipumps, model 2001D, were implanted subcutaneously to provide continuous drug or saline infusion for 48 hours. Drug-filled pumps released NXY-059 at 16 mg·kg⁻¹·h⁻¹. The monkeys were retested 3 and 10 weeks after surgery to assess functional disability. Surgery, behavioral testing, and histology were all done blinded to treatment condition.

Results—NXY-059–treated monkeys were significantly better at reaching with their hemiparetic arm than were saline-treated monkeys when retested 3 weeks (P<0.01) and 10 weeks (P<0.01) after surgery. Drug treatment also significantly lessened the degree of spatial perceptual neglect (P<0.01), a debilitating though ameliorating consequence of this infarct. NXY-059 treatment reduced the overall amount of brain damage by >50% of saline-treatment values, with similar levels of protection afforded to both white and gray matter.

Conclusions—This novel drug has a substantial protective effect, lessening the disability caused by an experimentally induced stroke in a primate species. These findings provide considerable encouragement for the clinical development of NXY-059. (Stroke. 2001;32:190-198.)

Key Words: behavior, animal ■ hemineglect ■ hemiparesis ■ monkeys ■ neuroprotection

Free radicals play a major role in the damage caused by hypoxia and reperfusion during cerebral ischemia.¹ Studies in rats have shown that the spin-trapping agent α-phenyl-N-tert-butyl nitro-trap (PBN) is neuroprotective when administered up to 3 hours after transient middle cerebral artery (MCA) occlusion.² The novel nitrone-based compound NXY-059 (disodium 4-[(tert-butylimino)-methyl]benzene-1,3-disulphonate N-oxide) also has free radical–trapping properties and has been shown to have better neuroprotective effects than PBN at equimolar doses in rats.³ NXY-059 reduced infarct size when given up to 5 hours after the onset of ischemia. This is an acceptable therapeutic time window for stroke treatment in humans. These data suggest that NXY-059 has considerable potential as a neuroprotective drug treatment for stroke.

Extrapolating findings from rodents to humans is, however, fraught with difficulties, and potential neuroprotective treatments must be tested in other species for greater assurance of efficacy before large multicenter clinical trials are undertaken. Although histological measurement has been the main criterion for estimating neuroprotective drug efficacy in rodents, long-term behavioral assays are also necessary. Functional recovery is what matters in the clinic. It is also important to test potential compounds with clinically realistic dosing regimens, for example, administered as sustained infusions at plasma levels that are well tolerated in humans.⁴ For these reasons, we have developed a model of stroke with a New World species of monkey, the marmoset, in which we can measure the functional outcome of neuroprotective drug treatment.⁵ An advantage of the use of marmosets is that as primates, they are considerably closer in the phylogenetic tree to humans than are rodents. Marmosets also have a brain weight–to–body weight ratio in excess of the human and a larger white matter–to–gray matter ratio than have rodents.

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The measurement of white matter damage and its protection may be important; there has been little evidence for the drug-induced protection of white matter in animal models of stroke that may account for the lack of effect of such drugs in the clinic.8

Permanent occlusion of the right M1 segment of the MCA in marmosets produces a large infarct to the lateral frontal, temporal, and parietal cortex that extends to the caudate, putamen, and underlying white matter.9 We use permanent occlusion because, although many strokes spontaneously reperfuse, a substantial number do not do so within a short time, if at all.10 After this occlusion, monkeys have a persistent hemiparesis of their contralesional arm, a motor neglect of this arm, and a transitory contralesional spatial neglect. These disabilities are analogous to the symptoms seen in humans after a stroke of the same arterial territory.11 We have used and further developed various tests that dissociate and quantify these motor and cognitive disabilities. The monkeys are tested shortly after and at a later time point after the induced stroke.

In the present study, we have examined the ability of NXY-059, administered after arterial occlusion at a drug level that is tolerated in humans, to ameliorate both the motor and cognitive deficits produced in this primate model of stroke. Behavioral assessment was made both shortly after arterial occlusion and many weeks later.

Materials and Methods

Animals
Fourteen laboratory-bred, adult common marmosets (Callithrix jacchus), ~12 months of age at the start of the experiment, were used. Two of these monkeys were used for pharmacokinetic analysis and the remaining 12 monkeys were divided between drug (n=6) and saline (n=6) treatments for the main study. All monkeys were kept within a large colony and had good visual and auditory interaction with other monkeys. All procedures were carried out in accordance with United Kingdom Home Office regulations. Surgery, behavioral testing, and histological analysis were all done blinded to the drug-treatment condition. There were no deaths as a consequence of the surgery or drug treatments.

Pharmacokinetic Analysis
A pharmacokinetic study was performed on 2 marmosets by an intravenous bolus of NXY-059 (104.9 μmol·kg⁻¹) and a mean constant-rate subcutaneous infusion (48.6 μmol·h⁻¹·kg⁻¹) through osmotic minipumps (Alzet model 2001D). Data were fitted to a 1-compartment model with intravenous bolus and maintenance infusion, which showed a good fit. These data were applied to dose calculations for the main study. NXY-059 was assayed by coupled infusion, which showed a good fit. These data were applied to dose calculations for the main study. All monkeys were kept 682 mol/L, with accuracy of 92% to 104%, and the intra-assay coefficient of variation was 4.5%.

Surgery
Twelve monkeys were anesthetized with Saffan (alphaxalone [9 mg/mL]/alphadolone acetate [3 mg/mL], 0.15 mL/100 g, Glaxo Vet Ltd) administered intramuscularly. After turning a large cranial flap and opening the dura over the right lateral frontal cortex, the right M1 segment of the MCA, 2 mm medial to the olfactory tract, was permanently occluded and bisected by electrocoagulation at this site. After surgery, the monkeys were placed in incubators to maintain body temperature during recovery from anesthesia; they remained there for 3 to 5 days and were nursed until they were capable of self-care. Sham-operated control monkeys were not used in this study because a previous study had shown no evidence of any behavioral deficits or brain damage in sham-operated monkeys,6 and we wished to avoid unnecessary surgical procedures. The mortality rate from this procedure across all studies is <10%, with most deaths having occurred early in the development of this model.

NXY-059 Treatment
Five minutes after permanent MCA occlusion (pMCAO), the monkeys received a 1-mL intravenous infusion of either saline (n=6) or NXY-059 (73.5 μmol·kg⁻¹) (n=6), and 2 primed osmotic minipumps (Alzet model 2001D) were implanted subcutaneously to provide prolonged drug or saline infusion. Drug-filled pumps contained 682 μmol/mL of NXY-059, which was released at 8 μL·h⁻¹ from each pump. Twenty-four hours after pMCAO, the minipumps were replaced with 2 further primed minipumps, under Saffan anesthesia, to provide continuous drug administration for a total of 48 hours, at which time the pumps were removed. A blood sample (0.5 mL) was taken 24 hours after pMCAO, and plasma was stored at −20°C until analyzed at AstraZeneca R&D Södertälje.

Physiological Variables
During surgery, blood pressure was monitored by an ultrasonic Doppler flow detector (model 811-B, Perimed UK Ltd), with the probe attached to the tail below an inflatable cuff and pressure gauge. Heart rate and percentage blood oxygen saturation (PO₂) levels were monitored with a Tiger pulse oximeter (Thames Medical). Body temperature was measured with a rectal thermometer. Recordings were taken at 10-minute intervals during surgery. After surgery, rectal temperature was monitored 2, 4, 24, and 48 hours after pMCAO.

Postoperative Records
In our laboratory, we routinely keep postoperative records of all monkeys, detailing the food and water intake, drug administration, and notes on their well-being and behavior. With the knowledge of the adverse effects seen in the clinic with other neuroprotective drugs such as the NMDA antagonists, we were particularly diligent in noting any unusual behavior that might follow drug treatment. The number of days after surgery when the monkeys first started eating without assistance was also noted.

Behavioral Tasks
All behavioral testing was performed in a modified home cage with an internal Plexiglas enclosure to prevent the monkeys from hanging upside down from the bars while they performed the tasks. The enclosure, entered from the rear, contained a small central perch on which the monkeys stood while performing the tasks. Before surgery, all monkeys were familiarized with all the tasks with the exception of rotation, which is a spontaneous behavior. When the monkeys were well practiced with the tasks, a formal preoperative test was done. The monkeys were retested 3 and 10 weeks after surgery.

Hill-and-Valley Staircase Tasks
In these tasks, the monkeys were required to reach through vertical slots in a Plexiglas screen attached to the front of the cage to retrieve food rewards from the steps of 2 staircases outside the cage (see Figure 1). A small piece of marshmallow was placed on each step of the staircases to give a total of 5 pieces on each side. The monkeys were allowed 5 minutes to retrieve all the food bits and were scored by an observer who stood 1 to 2 m away from the cage front. Only successful reaches, defined as food taken securely through the slot, were counted. The score for each piece depended on the distance from the relevant slot (score 1 for the nearest piece, 5 for the furthest piece). The total score was summed to give a maximum score of 15 for each side. In the hill task, there were two laterally positioned slots and the staircases rose toward
the center of the apparatus. The monkeys therefore used their right hand to reach to the right staircase and their left hand to the left staircase. In the valley task, there was one centrally positioned slot and the staircases rose toward the outside of the apparatus. The right arm was therefore used to reach to the left staircase and vice versa. Each monkey was tested with both designs of staircase in a random order such that they received 3 trials with each design. Examining the use of each arm into either hemispace allows the effects of a unilateral motor impairment, confined to one arm in either hemispace, to be dissociated from a unilateral perceptual spatial impairment, confined to one hemispace with either arm.

Two-Tube Choice Test
In this test, 2 black plastic tubes (diameter, 3 cm; depth, 5 cm) fixed to small Plexiglas strip 2 cm apart from one another, with a food reward in each tube, were presented to the monkey. The task is illustrated in Figure 2. Once the monkey had reached into one tube and retrieved a reward, the choice of side was noted and the tubes were removed and rebaited. The tubes were randomly presented in front and to the left or right sides of the monkey, in a random order such that there were 10 trials at each position and a total of 30 trials. After right-hand–sided pMCAO, monkeys reached almost exclusively to the right of the two rewarded tubes, even when both tubes were presented on the monkey’s ipsilesional side, such that this tube was less accessible than the left tube.7 This is a test of “extinction,” that is, the tendency for attention to items in ipsilesional hemispace to overshadow attention to items in contralesional hemispace.12

Six-Tube Search Task
In this task, the monkeys were required to search for one marshmallow piece hidden in any one of six locations (see Figure 3). Six black plastic tubes (diameter, 3 cm; depth, 5 cm), separated by 0.2 cm, were fixed to a Plexiglas strip, which was presented horizontally at the front of the home cage. Monkeys could only reach into the tubes if they entered the enclosure in the cage and stood on the small central perch. Only one tube was baited with a marshmallow reward, and the monkey was required to search for the reward. The time taken to reach into the rewarded tube, starting from when the tubes were first presented, or from when the monkey first jumped on the central perch, was recorded. After right-hand–sided pMCAO, this task was carried out 30 times with the tubes baited in a pseudo–random order such that each of the 6 tubes was baited 5 times. Because there was only one reward available on each trial, this task is a test of spatial neglect rather than extinction.

Rotation
The number and direction of spontaneous 360-degree rotations of the monkeys while housed singly in the home cage, with nest box and angled perches removed, were recorded for a 30-minute period by an experimenter who sat ~3 m away from the cage in the home room. Although rotation is rarely seen in the clinic, a bias to rotate in one direction can be a sensitive marker of unilateral brain dysfunction in animal models of disease.

Histology
Twenty weeks after surgery, the monkeys were deeply anesthetized and perfused transcardially with 200 to 300 mL saline followed by 250 to 300 mL of 10% formal saline (ie, 10% formalin in saline). The brains were removed and immersed in 10% formal saline. Before blocking and paraffin wax embedding, the brains were examined to ensure that the MCA had been bisected at the site adjacent to the olfactory tract. Coronal sections (8 μm) were taken at regular levels through the brains within the areas of visible infarct. Sections were stained with solochrome cyanine and cresyl violet counterstaining. Images of stained sections at ~1 mm intervals through the brain from AP 14.5 to AP 2.5 of the stereotaxic atlas13 were videocaptured with a digital camera connected to a Leica M420 Wild microscope. With the use of a computerized image analysis system (Global Laboratory Image, Data Translation Ltd), the area of the contral-
sional hemisphere and the area of intact ipsilesional hemisphere, that is, excluding areas of infarct damage and neuronal loss delineated by microscopic analysis of the sections, were measured. To adjust for size differences between monkeys, the average area of the contralateral hemisphere of all the monkeys at each stereotaxic level was used to transform all the measurements to a standard size of brain. The area of tissue damage for each monkey was then calculated by subtracting the area of the ipsilateral hemisphere from the area of the contralateral hemisphere. Further analyses measured the amount of damage to the cortex, white matter, caudate, and putamen.

Correlation and multiple regression compared the amount of damage to the cortex, white matter, caudate, and putamen and the total amount of damage to the ipsilesional hemisphere, with the behavioral deficits measured 3 and 10 weeks after pMCAO. The motor deficit was determined from the monkey’s ability to reach with the contralesional arm on the valley staircase task, that is, when the reach was not contaminated by spatial deficits. Spatial neglect was scored according to the latency to find a reward hidden in the most contralesional tube of the 6-tube search task. Total functional disability was determined from the combined score of their motor and spatial deficits, with an equivalent weighting given to each deficit.

Statistics
The data were analyzed with multifactorial ANOVA with 2-tailed post hoc Newman-Keuls t tests to compare specific differences between the groups. ANOVA was performed with the software package GB-Stat version 6.5 PPC, (Dynamic Microsystems, Inc).

Results
One monkey was excluded from the analysis, before its treatment was known, because visual inspection indicated that its right MCA had not been occluded. This left 5 saline-treated monkeys and 6 NXY-059–treated monkeys.

NXY-059 Plasma Level
Analysis of blood samples taken 24 hours after minipump implantation showed that the plasma unbound drug concentra-

Figure 2. Two-tube choice task, illustrated in a, with results shown in b as number of reaches (±SEM) to left tube by saline-treated (n=5, white bars) and NXY-059–treated (n=6, gray bars) monkeys before pMCAO and 3 and 10 weeks after surgery, when tubes were presented solely on right-hand side of monkey. Results were analyzed by ANOVA followed by post hoc Newman-Keuls analysis. *P<0.05, significant difference between groups; †P<0.05, significant difference from before pMCAO.

Figure 3. Six-tube search task, illustrated in a, with results shown as latency (±SEM) to find food reward hidden in 1 of 6 tubes positioned from monkey’s far right (tube 1) to their far left (tube 6) by saline-treated (n=5, white bars) and NXY-059–treated (n=6, gray bars) monkeys before pMCAO (b); 3 weeks after pMCAO (c); and (d) 10 weeks after pMCAO. Results were analyzed by ANOVA followed by post hoc Newman-Keuls analysis. *P<0.05, **P<0.01, significant difference between groups.

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After surgery, there were no behavioral abnormalities or other side effects to identify drug-treated monkeys. There was no significant difference in the length of time before the monkeys could eat unassisted, although NXY-059–treated monkeys started 3.3±0.4 days after surgery, whereas saline-treated monkeys did not start until 4.6±0.8 days.

**Postoperative Records**

After surgery, there were no behavioral abnormalities or other side effects to identify drug-treated monkeys. There was no significant difference in the length of time before the monkeys could eat unassisted, although NXY-059–treated monkeys started 3.3±0.4 days after surgery, whereas saline-treated monkeys did not start until 4.6±0.8 days.

**TABLE 1. Systolic Blood Pressure, Heart Rate, PO2, and Rectal Temperature of Saline-Treated and NXY-059–Treated Monkeys for the 30 Minutes Before and After pMCAO**

<table>
<thead>
<tr>
<th>Physiological Variable</th>
<th>Treatment</th>
<th>Before pMCAO</th>
<th>After pMCAO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>–30 min</td>
<td>–20 min</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>Saline</td>
<td>72.0±4.1</td>
<td>69.0±5.2</td>
</tr>
<tr>
<td></td>
<td>NXY-059</td>
<td>76.3±5.0</td>
<td>72.8±8.2</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>Saline</td>
<td>226.2±16.6</td>
<td>206.0±20.9</td>
</tr>
<tr>
<td></td>
<td>NXY-059</td>
<td>256.2±17.7</td>
<td>239.3±19.3</td>
</tr>
<tr>
<td>PO2, %</td>
<td>Saline</td>
<td>94.4±1.7</td>
<td>95.6±0.9</td>
</tr>
<tr>
<td></td>
<td>NXY-059</td>
<td>95.6±0.4</td>
<td>97.0±0.3</td>
</tr>
<tr>
<td>Rectal temperature, °C</td>
<td>Saline</td>
<td>34.9±0.2</td>
<td>34.9±0.2</td>
</tr>
<tr>
<td></td>
<td>NXY-059</td>
<td>34.7±0.2</td>
<td>34.7±0.2</td>
</tr>
</tbody>
</table>

NXY-059 treatment had no significant effects on any of the physiological parameters measured.

**Hill-and-Valley Staircase Tasks**

Three-way ANOVA with group (saline-treated versus NXY-059–treated), arm (ipsilesional versus contralesional), and time (before surgery versus 3 weeks after surgery versus 10 weeks after surgery) as factors revealed a significant difference between the groups on both tasks (hill: $F_{2,18}=9.18$, $P<0.01$; valley: $F_{2,18}=17.67$, $P<0.01$). Post hoc Newman-Keuls $t$ tests showed that at 3 weeks, NXY-059–treated monkeys significantly better with their affected left arm than were saline-treated monkeys on both tasks, that is, reaching into both contralesional and ipsilesional spaces (hill: $t=16.68$, $P<0.01$; valley: $t=13.26$, $P<0.01$). Furthermore, on the valley task, NXY-059–treated monkeys were significantly better with their unaffected right arm than were saline-treated monkeys, in which the reach was into the contralesional neglected side of the apparatus ($t=13.74$, $P<0.01$).

At 10 weeks, NXY-059–treated monkeys were still significantly better with their affected left arm than were saline-treated monkeys at reaching into both contralesional and ipsilesional spaces (hill: $t=19.70$, $P<0.01$; valley: $t=17.53$, $P<0.01$). By this time, both groups could reach into the contralesional neglected space with their unaffected right arm without any significant deficit. The results are shown in Figure 1.

**Two-Tube Choice Task**

Two-factorial ANOVA, with group (saline-treated versus NXY-059–treated) and time (before surgery versus 3 weeks after surgery versus 10 weeks after surgery) as factors, compared the tendency of the monkeys to reach to the left of the two tubes when the pair of tubes was presented on the monkey’s ipsilesional side. There was an overall significant group effect ($F_{1,10}=6.58$, $P<0.05$); the results are shown in Figure 2. Before surgery, when the tubes were presented to the monkey’s right side, the monkeys had a natural bias to reach to the left tube because this tube was nearer and more easily reached. Three weeks after surgery, all the monkeys reached significantly less to the left tube than they had before surgery (saline: $t=12.06$, $P<0.05$; NXY-059: $t=7.47$, $P<0.05$). There was no significant difference between the 2 groups. Ten weeks after pMCAO, the NXY-059–treated monkeys had fully recovered, and they now reached significantly more to the previously ignored left tube than did the saline-treated monkeys ($t=11.83$, $P<0.05$).

**Six-Tube Search Task**

ANOVA with group (saline-treated versus NXY-059–treated), tube (1 to 6), and time (before surgery versus 3 weeks after surgery versus 10 weeks after surgery) as factors showed a group difference ($F_{1,32}=5.56$, $P<0.05$). Post hoc Newman-Keuls $t$ tests showed that at 3 weeks, NXY-059–treated monkeys were significantly better at finding rewards hidden in the 3 leftmost tubes than were saline-treated monkeys (tube 4: $t=8.79$, $P<0.05$; tube 5: $t=20.51$, $P<0.01$; tube 6: $t=37.74$, $P<0.01$). Ten weeks after surgery, saline-treated monkeys still had a small residual impairment at finding a reward hidden in the leftmost tube. NXY-059–treated monkeys were significantly better than the saline-treated monkeys at finding rewards in this tube (tube 6: $t=20.98$, $P<0.01$). The results are shown in Figure 3.

**Rotation**

ANOVA with group (saline-treated versus NXY-059–treated) and time (before surgery versus 3 weeks after surgery versus 10 weeks after surgery) as factors was used to analyze the results. The group effect approached significance ($F_{1,10}=3.69$, $P=0.087$) but not the group×time interaction ($F_{1,18}=1.32$, $P=0.29$). Before surgery, both groups had a slight preference to rotate to the right side (saline-treated group: 69±6% of rotations to the right, that is, toward the lesion; NXY-059–treated group: 61±5% of rotations to the right). Three weeks after surgery, the saline-treated group
rotated almost solely to the right and significantly more than the
NYX-059–treated group (saline-treated group, 95±5% of
rotations to the right; NYX-059–treated group, 68±12%
rotations to the right, \(t=10.81, P<0.05\)). Ten weeks after
surgery, saline-treated monkeys continued to rotate primarily
to the right (92±8% rotations to the right), whereas NYX-
059–treated monkeys rotated less but not significantly to this
side (75±6% rotations to the right).

**Quantitative Histological Analysis**

The saline-treated group had large infarcts in the right
hemisphere that extended to the subcortical structures, with
almost total loss of the caudate and putamen. There was
visibly less damage in the NYX-059–treated group (saline-treated group, 95±5% of
rotations to the right; NYX-059–treated group, 68±12%
rotations to the right, \(t=10.81, P<0.05\)). Ten weeks after
surgery, saline-treated monkeys continued to rotate primarily
to the right (92±8% rotations to the right), whereas NYX-
059–treated monkeys rotated less but not significantly to this
side (75±6% rotations to the right).

Discussion

We have shown that NYX-059, a novel drug with free
radical–trapping properties, has substantial protective effects,
not only at reducing the amount of brain damage as measured
histologically but, more importantly, at reducing the func-
tional disability that follows an experimentally induced
stroke. These protective effects of NYX-059 probably relate,

**Figure 4.** Photomicrographs of coronal sections stained with
solochrome cyanine and cresyl violet. Sections from the stereo-
taxic level A 9.5 are from pair of twins; twin in a received saline;
twin in b received NYX-059. Scale bar=5 mm. cd indicates cau-
date; put, putamen.

![Figure 4](image_url)

**Figure 5.** Area (mm²±SEM) of tissue damage to ipsilateral hemi-
sphere compared with contralateral hemisphere between stereo-
taxic levels A 14.5 and A 2.5 of saline-treated (n=5, □) and
NYX-059–treated (n=6, ◆) monkeys. Results were analyzed by
ANOVA followed by post hoc Newman-Keuls analysis. *\(P<0.05\),
**\(P<0.01\), difference between groups.
TABLE 2. Percentage Protection Afforded to Cortex, White Matter, Caudate, and Putamen Across Stereotaxic Levels in NXY-059–Treated Monkeys Compared With Saline-Treated Monkeys

<table>
<thead>
<tr>
<th>Stereotaxic Level</th>
<th>A 14.5</th>
<th>A 12.5</th>
<th>A 10.5</th>
<th>A 8.5</th>
<th>A 6.5</th>
<th>A 4.5</th>
<th>A 2.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortex</td>
<td>18.8%</td>
<td>27.1%</td>
<td>57.0%</td>
<td>69.2%</td>
<td>65.8%</td>
<td>55.7%</td>
<td>46.5%</td>
</tr>
<tr>
<td>White matter</td>
<td>57.4%</td>
<td>53.0%</td>
<td>33.7%</td>
<td>33.1%</td>
<td>66.1%</td>
<td>56.7%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Caudate</td>
<td>...</td>
<td>56.7%</td>
<td>36.8%</td>
<td>46.8%</td>
<td>69.5%</td>
<td>62.3%</td>
<td>...</td>
</tr>
<tr>
<td>Putamen</td>
<td>...</td>
<td>...</td>
<td>18.9%</td>
<td>38.6%</td>
<td>35.6%</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

unusual behaviors and the monkey’s well-being. There was nothing in the records to identify drug-treated monkeys nor to indicate any behavioral side effect of drug administration. Specific behavioral tasks were used to measure and disassociate motor and spatial deficits. Motor disability, uncontrolled by the effects of visual spatial deficits, was assessed by examining performance with the affected contralesional arm when used in ipsilesional space. Measurement of spatial deficits was made with tasks in which marmosets were free to use their ipsilesional nonaffected arm to avoid the influence of contralesional motor disability on their performance. Before surgery, the monkeys readily performed and were adept at all the tasks. After surgery, all the monkeys were keen to do the tasks, but the saline-treated monkeys were constrained by their disability with regard to their success.

The main finding of this study was that NXY-059 has a substantial ability to lessen the functional disability that follows pMCAO in this species of monkey. The most impressive effect was the reduction in motor disability of the contralesional arm. After pMCAO of the M1 segment, monkeys usually do not attempt to use their contralesional arm in formal tests, a form of motor neglect.9 NXY-059–treated monkeys were considerably better at using their affected arm than were saline-treated monkeys when tested both 3 and 10 weeks after surgery. Only one of the saline-treated monkeys retrieved any marshmallow, and then only a couple of pieces that were nearest to the slots. Remarkably, 4 of the 6 NXY-059–treated monkeys used their affected arm at near-normal preoperative levels, even when the reach required full extension of the arm to retrieve the food rewards furthest from the slots. The results from the 10-week time point demonstrate that NXY-059 had not just enhanced the natural rate of recovery but had reduced the long-term disability in these monkeys. This is important; large strokes are associated with permanent disability and poor recovery in humans.

A second important drug effect was its influence on a cognitive deficit, spatial neglect. After pMCAO, marmosets have a profound neglect of contralesional space seen in the valley staircase task and the 6-tube search task. On these tasks, saline-treated monkeys had a severe spatial neglect 3 weeks after surgery, but NXY-059–treated monkeys had substantially less spatial neglect. The reduction of spatial neglect in these monkeys is of considerable functional benefit. In humans, stroke-induced neglect is debilitating and can greatly hinder a patient’s rehabilitation.18 It is unlikely that these animals had a hemianopia because the infarct did not intrude into primary visual system. However, even if they had such a defect, it is unlikely to have produced such results; hemianopia caused by optic tract section does not result in behavioral impairment in freely moving monkeys who readily accommodate to this disability.19

Although patients tend to recover from the more florid symptoms of spatial neglect, as do marmosets, more subtle symptoms may persist and can be irksome to the patient. In this study, saline-treated monkeys still had a small residual impairment on the 6-tube search task at 10 weeks, whereas NXY-059–treated monkeys had fully recovered and showed no impairment. NXY-059–treated monkeys were also significantly better than saline-treated monkeys on the 2-tube choice task at 10 weeks, although they were not initially any better when first tested at 3 weeks. The 2-tube choice task is a sensitive indicator of a spatial deficit; the monkeys often show significant deficits on this task when they have recovered from the more profound spatial neglect seen in the other tasks.7 We think that this task is a measure of extinction, that is, the tendency for one stimulus to inhibit response to more contralesional stimuli, where detection of one stimulus is unaffected,12 and indicates a longer-lasting, more subtle impairment. NXY-059 treatment, therefore, not only reduced the initial obvious spatial neglect but also improved recovery from a more subtle spatial deficit.

Histological analysis showed that the volume of brain damage was reduced by >50% with NXY-059 treatment. The protection seen with NXY-059 was not restricted to the cortex but also included considerable protection of the white matter, caudate, and putamen. This is important, as there has been little published evidence for the drug-induced protection of white matter or subcortical neurons in animal models of stroke.8 The studies that have examined the effects of NXY-059 in rat models of stroke have as yet only reported on the histopathological analysis of overall infarct volumes and not on specific damage to the cortex, white matter, or striatum.4,14 Although correlation and multiple regression analysis were performed, it was not possible to isolate any areas or structures that related specifically to a particular behavioral disability. The large amount of damage to the cortex was itself related to similar levels of damage to the white matter, caudate, and putamen, which may have obscured the results of these analyses. The limitation of spatial neglect may therefore relate to protection of the parietal cortex, damage to which has long been associated with neglect in humans30 or to protection of the underlying white

unusual behaviors and the monkey’s well-being. There was nothing in the records to identify drug-treated monkeys nor to indicate any behavioral side effect of drug administration. Specific behavioral tasks were used to measure and disassociate motor and spatial deficits. Motor disability, uncontaminated by the effects of visual spatial deficits, was assessed by examining performance with the affected contralesional arm when used in ipsilesional space. Measurement of spatial deficits was made with tasks in which marmosets were free to use their ipsilesional nonaffected arm to avoid the influence of contralesional motor disability on their performance. Before surgery, the monkeys readily performed and were adept at all the tasks. After surgery, all the monkeys were keen to do the tasks, but the saline-treated monkeys were constrained by their disability with regard to their success.

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matter, lesions of which produce a marked neglect in monkeys.19

The marmoset, like the squirrel monkey, is a nongyrencephalic species, but whether this limits the extrapolation of these findings to humans is not known. Some drugs are neuroprotective in nongyrencephalic rats21 and in gyrencephalic cats.22 An advantage of the use of marmosets is that as primates, they are considerably closer in the phylogenetic tree to humans than are rodents and cats. Marmosets also have many practical advantages over Old World monkeys for modeling human central nervous system disease.23 In particular, their small size makes them relatively easy to handle for both behavioral testing and for providing the necessary postoperative care that is needed for animals with such large infarcts.

It is difficult to compare NXY-059 quantitatively with other drugs we have tested against the cerebral ischemia produced by pMCAO of the M1 segment24-26 in the absence of dose-response data. However, at the dose chosen, the results obtained with NXY-059 were striking. NXY-059 not only protected against the development of spatial neglect and improved recovery from more subtle spatial deficits, it also substantially attenuated the contralesional hemiparesis. Most remarkably, considering that motor deficits are the main long-term disability from stroke in monkeys (and in humans), 4 of 6 monkeys had near-normal motor performance. This drug thus appears to fulfill the criteria for a potentially successful neuroprotective stroke treatment. It has a long therapeutic window, at least in rats; it does not produce overt adverse effects in monkeys; it has excellent protective effects measured histologically in rats and in monkeys on both gray and white matter; it substantially lessens the level of functional disability in a primate species; and in monkeys, neuroprotection has been demonstrated at a plasma level safely tolerated by stroke patients.15 This is extremely encouraging for advancing NXY-059 to further clinical trials.

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References

The accompanying article by Marshall et al shows that treatment with the free radical trapping agent NXY-059 reduces infarct volume and improves behavioral outcome in a model of focal cerebral infarction in the marmoset. There are several interesting features in this study. First, the study was performed in the New World monkey, the marmoset. While the marmoset has a smooth, nonconvoluted brain unlike the convoluted brain of humans, this species is nonetheless much closer to man than are rats, mice, or cats, standard species used in preclinical stroke studies. Second, the study demonstrates clearly that a treatment which reduces infarct volume can also be expected to improve behavioral outcome after strokes. Because behavioral outcome, not infarct volume, is the relevant issue on which stroke drugs are evaluated in humans, this is an important and reassuring association.

Third, this study in marmosets makes an important point that is highly relevant to human stroke drug clinical trials. Specifically, this study makes the point that different classes of neurological deficits may recover spontaneously to different degrees following stroke and consequently may be differentially treatable by various stroke therapies. Figure 1 in the article shows that sensorimotor disability of the affected arm did not recover spontaneously in vehicle-treated animals during the 10 week-observation period, which allowed observation of a robust drug treatment effect over this entire interval. In contrast, hemispatial neglect recovered considerably in vehicle-treated animals over this same time period, ultimately blunting any drug treatment effect after 10 weeks of observation. This observation mimics a commonly observed phenomenon in human stroke patients, namely, that a patient with hemispatial neglect often recovers spontaneously after a middle cerebral artery territory stroke, whereas one with dense hemiparesis may not. This point underscores the value of this primate model: hemisensory neglect cannot be easily measured in rodents. Moreover, these data remind us that sensorimotor and cognitive deficits and their recovery are fundamentally different in human stroke patients, and that clinical stroke trials may not be well served by the “lumping” together of such fundamentally different classes of disabilities in “global” stroke outcome scales.

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