Impaired Arm Function and Finger Dexterity in a Nonhuman Primate Model of Stroke
Motor and Cognitive Assessments

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Background and Purpose—Ischemic stroke is the leading cause of upper extremity motor impairments. Although several well-characterized experimental stroke models exist, modeling of upper extremity motor impairments, which are unique to primates, is not well established. Cortical representation of dexterous movements in nonhuman primates is functionally and topographically similar to that in humans. In this study, we characterize the African green monkey model of focal ischemia reperfusion with a defined syndrome, impaired dexterous movements.

Methods—Cerebral ischemia was induced by transient occlusion of the M3 segment of the left middle cerebral artery. Motor and cognitive functions after stroke were evaluated using the object retrieval task with barrier-detour. Postmortem magnetic resonance imaging and histopathology were performed to map and characterize the infarct.

Results—The middle cerebral artery occlusion consistently produced a necrotic infarct localized in the sensorimotor cortex in the middle cerebral artery territory. The infarction was reproducible and resulted in significant loss of fine motor function characterized by impaired dexterity. No significant cognitive impairment was detected. Magnetic resonance imaging and histopathology demonstrated consistent and significant loss of tissue on the left parietal cortex by the central sulcus covering the sensorimotor area. The results suggest that this species has less collateralization, which closely resembles humans.

Conclusions—The reported nonhuman primate model produces a defined and reproducible syndrome relevant to our understanding of ischemic stroke, cortical representation, and sensorimotor integration controlling dexterous movements. This model will be useful in basic and translational research addressing loss of arm function and dexterity. (Stroke. 2016;47:1109-1116. DOI: 10.1161/STROKEAHA.115.012506.)

Key Words: cognition ■ dexterity ■ focal ischemic stroke ■ nonhuman primate ■ sensorimotor cortex

The World Health Organization reports that stroke claims 6.2 million lives each year. There are ≈5.4 million stroke survivors with ≈80% requiring hand therapy.1 The estimated economic burden for stroke exceeds $56.8 billion per year in the United States alone.

Acute thrombolysis has a significant impact on the management of stroke,2,3 with a therapeutic window that may extend ≤6 hours with intra-arterial delivery.4 Yet only a minority of stroke victims benefit, and the majority experience progression of ischemia associated with neurological disabilities.

Animal models that recapitulate human disabilities and disease pathology remain an unmet need in stroke research. There has been a tremendous advance in developing rodent experimental model stroke (reviewed in Durukan and Tatlisumak5 and Carmichael6). However, the lack of success in developing neuroprotective therapies in small animal models for stroke7,8 prompted recommendations for additional research and development in large animal models with physiological, structural, and functional traits closer to those of humans before clinical trials.9,10 There has been a concerted effort in developing relevant large animal models.11,12 Interspecies comparison suggests that cerebral venous angioarchitecture in large animals is closer to that of humans13; nevertheless, such models remain relatively underdeveloped. Nonhuman primate (NHP) models offer an assessment of complex physiological, immunologic, biochemical, and behavioral outcomes most similar to those of humans.14-24 These outcome measures complement those from other animal models by improving our understanding...
Materials and Methods

Animals

The animal protocols were approved by the Institutional Animal Care and Use Committee (IACUC) for Axion Research Foundation/St Kitts Biomedical Research Foundation. The facility is accredited by Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), and all procedures prescribed by AAALAC, Office of Laboratory Animal Welfare (OLAW) standards, and National Institutes of Health guidelines were followed, including the use of anesthetics and analgesics for surgery and killing and animal welfare conditions for housing and care. Six male African green monkeys (Cercopithecus sabaeus originating from St Kitts and weighing 4.5 to 5.5 kg were used in this study. Steps taken to ameliorate suffering are included in animal handling procedures described below in this section.

NHP Ischemia Model

Monkeys were subjected to transient cerebral ischemia by temporarily occluding the left-side middle cerebral artery (MCA). Under general anesthesia, a curvilinear incision was made extending from anterior to the right tragus up to the midline, and the skin flap and temporal muscle were reflected inferiorly to expose the pteronial area. Using a 1 cm trephine, a craniotomy was performed on the left-side temporal bone of the skull, and the dura mater was separated from the tissue. Thereafter, using the standard microsurgical technique, the Sylvian fissure was located and the M3 in the sylvian insular cistern was exposed. Two aneurysm clips were placed with 3 mm of space between them occluding the M3 segment of the MCA. After 90 minutes of occlusion, the clips were removed and reperfusion achieved. Previous studies have reported that 90-minute occlusion of MCA produces significant stroke in NHP.25–27 The dura was closed and covered with gel foam, and the bone flap was secured with a 2.0 chromic suture. The skin was closed with 2.0 vicryl in the galea and finally sealed with dermabond glue. Post surgery, the animals were administered Flocillin (300 mg/kg) in the i.v route, the Sylvian fissure was located and the M3 in the sylvian insular cistern was exposed. Two aneurysm clips were placed with 3 mm of space between them occluding the M3 segment of the MCA. After 90 minutes of occlusion, the clips were removed and reperfusion achieved. Previous studies have reported that 90-minute occlusion of MCA produces significant stroke in NHP.25–27 The dura was closed and covered with gel foam, and the bone flap was secured with a 2.0 chromic suture. The skin was closed with 2.0 vicryl in the galea and finally sealed with dermabond glue. Post surgery, the animals were administered Flocillin (300 mg/kg) in the i.v route, the Sylvian fissure was located and the M3 in the sylvian insular cistern was exposed. Two aneurysm clips were placed with 3 mm of space between them occluding the M3 segment of the MCA. After 90 minutes of occlusion, the clips were removed and reperfusion achieved. Previous studies have reported that 90-minute occlusion of MCA produces significant stroke in NHP.25–27

Magnetic Resonance Imaging

MRI of the postmortem brains was done using the microSigna 7.0 T-MR scanner (7.0T/310/AS System) 5 weeks after the MCA occlusion surgery. The instrument comprised a 310 mm (horizontal) bore scanner, including gradient drivers, EXCITE2 electronics, 8-channel multicoil RF and multinuclear capabilities and volume RF coils, and the supporting LXi1 platform. The Resonance Research Instruments BFG-150/90-S shielded gradient insert (770 mT/m, SR-2500 T/m per second) had a bore size of 9 cm. The imaging protocol consisted of 2D multislice imaging followed by a spin-echo sequence, using the following parameters: echo time=82.5 ms, repetition time=4000 ms, number of excitations=10, 5 cm×5 cm field of view, matrix=256×256, slice thickness =0.6 mm, gap=0. Serial MRI scans from each animal were used to measure tissue loss to the ischemic lesion. The lesion was outlined and the infarct size determined as percent of healthy tissue loss in the ipsilateral hemisphere compared with the contralateral one. The results were expressed as mean±standard deviation.

Infarct Size

Brain slices throughout the rostro-caudal extent were processed for cresyl violet (CV) and hematoxylin and eosin staining. The CV-stained sections were scanned, and image J (National Institutes of Health) was used to determine the size of the infarct. For each animal (stoked [n=3] and control [n=3]), the lesion size was determined by subtracting the area of the intact tissue in the ipsilateral hemisphere from the area of the contralateral hemisphere and then expressed as a percentage of contralateral hemisphere. All the values were normalized to control animals and plotted using graph pad prism software.

Statistical Analysis

All the behavioral data were analyzed and plotted using Graph Pad Prism 5. A test was used to identify significant differences between 2 groups. For comparing multiple groups, 2-way analysis of variance with a post hoc Bonferroni analysis was performed to identify the significant differences. P<0.05 was considered statistically significant.
Results

Focal Transient Ischemic Injury
In all animals, the occlusion consistently produced apparent disability in the right hand after surgery (Figure 1A), with a focal necrotic infarct on the left parietal cortex by the central sulcus covering the sensorimotor area (Figure 1B and 1C). T2-weighted MRI scans showed the location of the lesion in the area supplied by this segment of the MCA on several serial scans (Figure 2A–2C) for each experimental case. Measurements of the infarct lesion showed 12.4±4.7% tissue loss relative to the contralateral side.

Motor and Cognitive Assessments
The object retrieval task with a barrier-detour model was used to evaluate motor and cognitive functions. During the task, the act of retrieving the reward measured the motor function, whereas the ability to learn, to detour the transparent barrier when the box orientation was modified, measured the cognitive capabilities. Three weeks after transient left MCA occlusion, the monkeys showed no significant deficit in the object retrieval task using the left (ipsilateral) healthy hand (Figure 3A1 and 3A2). However, deficits in the right hand (contralateral) fine motor skills were readily detectable. The deficit was characterized by the inability of the monkeys to grasp and retrieve the reward (fruit) using their right hand when the opening was on the respective side (Figure 3B1–3B4). There was a clear rigidity in the fingers and loss of dexterity in grasping the fruit (Figure 3B1 and 3B2), suggesting lesion in the cortical area processing the sensorimotor inputs controlling finger movement. Notably, during task performance, animals with stroke consistently used their healthy left hand against the closed side of the box for support to retrieve the fruit (Figure 3B1–3B3). These behavioral tests were video-recorded, and movie of one of the stroke animals performing the retrieval task is presented (Movie in the online-only Data Supplement). This compensatory behavior parallels the complex compensatory strategies stroke victims use to perform motor tasks.

Quantitative analysis of the fruit retrieval task revealed that the right arm motor deficit in stroke group was significantly (P<0.05) higher compared with control (Figure 4A1). The deficit was particularly severe in the animal with ID No Z288 in the stroke group. No significant differences were observed in the use of the left ipsilateral arm (Figure 4A2). Furthermore, the total motor deficit, that is, right and left combined, was significantly (P<0.05) higher in all animals with stroke compared with the controls (Figure 4A3). Overall, left MCA occlusion produced a significant (P<0.05) right arm motor deficit and total motor deficit in the stroke animals compared with the control animals (Figure 4A4).

Analysis of movement initiation time was similar among control and stroke groups (Figure 4B1). The execution of the movement was also similar between the stroke and control groups (Figure 4B2). In addition, the total number of attempts made by the animals during the tests was similar among all groups (Figure 4C1). We then determined whether animals...
with ischemic damage had a bias toward the use of the ipsilateral limb. Two out of 3 (Z267 and Z288) animals in the stroke group showed a trend in preference toward the use of ipsilateral arm; however, this difference was not significant (Figure 4C2). Interestingly, animal No Z288, which demonstrated severe contralateral motor deficit, also scored significantly \( P<0.05 \) higher in the awkward reach when compared with control (Figure 4C3).

**Figure 3.** Impaired dexterity in the contralateral arm. A1–A2, Series of photos depicting the use of normal left arm during the behavioral task. B1–B4, Sequential set of photos depicting the movement sequence of retrieval using the affected right arm. Notice the rigidity in the right arm fingers (B1 and B2), loss of dexterity in grasping the fruit and failure to grasp and retrieve the reward (B4). Red arrowhead in B1–B3 indicates the use of the left arm to support food retrieval by the right arm.

**Figure 4.** Quantitative analysis of the impaired arm function. Monkeys in the stroke group exhibited significant right motor problem (A1), total motor problem (A3), and average motor problem (A4). The monkey with ID No Z288 scored significantly higher in the awkward reach (C3). No significant differences were detected between the control and stroke groups in left motor problem (A2), initiation time (B1), execution of the task (B2), reach number (C1), hand preference (C2), awkward reach (C3), perseverative (D1), and in barrier reach (D2). **\( P<0.01 \), ***\( P<0.001 \) vs control.
The cognitive functions after stroke were evaluated by analyzing the perseverative response and number of barrier reaches made by the monkeys during each trial. Our analysis indicated that the perseverative response (Figure 4D1) and barrier reach (Figure 4D2) were similar between stroke and the control animals with no significant differences.

**Stroke Location and Size**

To analyze ischemic zone and quantify the size of the infarct, CV and hematoxylin and eosin staining were performed on serial sets of brain sections (Figure 5). The histological analysis demonstrated that the stroke animals had a focal necrotic infarct on the left sensorimotor cortex (Figure 5A). The infarct was evident by the high infiltration of monocytes, proliferation of connective tissue, and the large number of darkly stained and necrotic cells on CV-stained sections (Figure 5B1 and 5B2). The ischemic damaged tissues showed reactive astrocytes with vesicular nuclei, figures of shrunk neuronal perikarya with condensed pyknotic nuclei, and perivascular lymphocytic cuffing indicative of inflammatory reaction (Figure 5B1–5B3). In contrast, CV- and hematoxylin and eosin–stained control brain tissue showed prominent neurons with cytoplasmic extensions, clear nucleus, and nucleolus (Figure 1 in the online-only Data Supplement). The CV-stained sections were used to outline the infarct and quantitatively measure the infarct size (see Method Section). Stroke animals had significant tissue loss (11%) in the ipsilateral hemisphere ($P<0.05$) compared with control animals (Figure 5C).

**Discussion**

We describe an NHP model of focal ischemia reperfusion with a defined syndrome, loss of arm function, and impaired dexterity. We used the object retrieval task with a barrier-detour behavioral paradigm to quantify the fine motor skills and cognitive deficits. MRI and histopathologic analysis demonstrated the location of the infarct in the sensorimotor region of the cortex and a significant loss of the tissue in the lesioned hemisphere.

Ischemic stroke is the leading cause of upper extremity motor impairments and in severe cases causes hemiparesis. It is estimated that each year ≈80% of stroke survivors require hand therapy. The death of the neurons with highly specific functions in the infarct zone causes severe sensorimotor impairments and paralysis in patients. Gross motor dysfunction recovery occurs early on, whereas the recovery of fine motor functions, such as grip strength, fine manipulation, and writing, is difficult and sometimes requires extended periods of physiotherapy.

Developing NHP animal models with consistent and a reproducible ischemic lesion and syndrome is desirable for studying the multifaceted brain response to stroke and for developing therapeutic approaches. Various NHP models of stroke have been reported using different surgical techniques in the marmosets, African green monkey, baboon, macaques, and squirrel monkeys. However, to date, there has been less emphasis on outcome measures and disabilities relevant to daily living activities, such as dexterous movements. We report an African green monkey model of transient focal ischemia induced with open surgery to access the MCA through a pterional craniotomy. The location of the lesion is critical to successfully demonstrate the clinical ischemic stroke. MRI and histopathologic analysis confirmed that the necrotic infarct produced was localized in the sensorimotor cortex in the MCA territory. This parallels...
the embolic obstruction of the MCA that is a major cause of ischemic stroke in humans. These data suggest that the African green monkey has less collateralization, which closely resembles humans. Another species with less collateralization is Macaca fascicularis, while the baboon (Papio anubis) and rhesus (Macaca mulatta) exhibit collateralization that requires occluding other segments of the MCA.

Studies have previously reported the correlation between functional outcome and size of the infarct. In the squirrel monkey, focal stroke induced by vascular coagulation using a bipolar electrocoagulator in M1 hand area of the cortex produced motor deficits according to the extent of the injury. In the present study, the analysis of infarct size indicated that all the monkeys with stroke had significant loss of tissue and functional impairments. The evidence suggests that the craniotomy approach to access and occlude MCA works for the African green monkey and provides consistent well-tolerated focal ischemia. The advantages of this approach complement features offered by other NHP models of stroke, such as the transorbital approach, and photothrombotic stroke. These data are in agreement with previous reports and suggests that the open surgical model provides less variability in volume and outcome measures and rare mortality in comparison to the endovascular approach, including the autologous blood clots model. The restoration of blood flow and reperfusion post occlusion in the ischemic area are readily achieved and provide a transient model to study reperfusion injury in NHP. Post-surgical complications were minimum with no mortality.

Modeling hand dexterity impairments seen in humans during the chronic phase of ischemic injury is lacking. NHP offer unique opportunities to address this gap and further our understanding of cortical control of arm function. The object-retrieval task with a barrier-detour described in this study has been used to evaluate the motor and cognitive functions in various neurological disorders. By carefully monitoring the subject’s activity during the task, several motor and cognitive functions can be quantified. The behavioral changes observed in the current model are clinically relevant because recently it has been suggested that initial severity of upper limb weakness can be an important predictor of recovery from upper limb motor dysfunction in stroke patients. It is important to acknowledge that although this animal model is promising, from a translational perspective, its effectiveness has yet to be validated in humans. Although NHP are close to humans on many aspects, some differences remain to be considered and studied, such as the expression and sequence homologies of a therapeutic target and validity for drug screening. In addition, ethical, infrastructural, and financial issues are inherent to NHP models and must be taken into consideration. Therefore, NHP models are valuable for studies leading to first-in-human clinical trials and ideally extending on Stroke Treatment Academic Industry Roundtable (STAIR)-compliant studies with compelling evidence-based efficacy and safety results in rodents, sheep, pig or other animal models.

We report no significant cognitive deficits, which is not surprising given that the focal ischemic infarct was mainly located in the sensorimotor cortex. However, we cannot exclude cognitive disabilities in early time points during the first or second week post stroke. Future studies will be required to investigate both motor and cognitive deficits in various early time points. Cognition may play a role in sensorimotor performance in NHP and in patients with brain injuries. Our data showed no significant difference in the ipsilateral arm performance between stroke and control animals (data not shown). These findings support previous observations in humans that impairment in dexterity in the ipsilateral hand correlates with cognitive deficits affecting perception and control of action.

This study represents a different approach to developing NHP models by addressing a single aspect of neurological dysfunction, that is, impaired dexterity, and may lead to better understanding of the plasticity in the integration of sensorimotor inputs during the recovery period. Better understanding of fundamental cellular and molecular brain-coping mechanisms may translate to novel hand neuro-restorative strategies.

Acknowledgments
We thank the staff of the St Kitts Biomedical Research Foundation for technical support.

Sources of Funding
This work was supported by the Kleberg Foundation (Dr Daadi), Axion Research Foundation (Dr Redmond), and Bernard and Ronni Lacroute, the William Randolph Hearst Foundation, Russell and Elizabeth Siegelman (Dr Steinberg).

Disclosures
None.

References


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Stroke. 2016;47:1109-1116; originally published online March 8, 2016; doi: 10.1161/STROKEAHA.115.012506

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Cover title: nonhuman primate stroke and dexterity

Number of pages: 19
Number of figures: 5
Total number of words: 5,715

Online Supplements: Supplemental Figure I; Video recording of the object retrieval task with a barrier-detour.

Keywords: Focal ischemic stroke, nonhuman primate, sensorimotor cortex, dexterity, cognition.
SUPPLEMENTAL MATERIAL

Figure and Video Legends

Supplemental Figure I. **A.** Representative low power image of the cresyl violet (CV) stained brain section from a control animal. **A1 & A2.** Inserts depict high power images of the section. Cortical neurons (arrowheads) appear healthy with dendritic and axonal projections. **B.** Representative low power image of the haematoxylin and eosin (H&E) stained brain section from a control animal. **B1 & B2.** High power inserts of the H&E staining. Arrowheads indicate the morphology of healthy cortical neurons with visible nucleus and nucleolus. In contrast to stroke animals, notice the lack of inflammatory reaction around the small blood vessels. Bars: (A, B) 2.5 mm, (A1, A2, B1, B2) 15 µm.

**Video recording of the object retrieval task with a barrier-detour.** Video recording showing African green monkey with stroke performing the retrieval task with barrier-detour. The first part of the video shows the ipsilateral left arm performing the fruit retrieval. The orientation of the box opening was then changed. The second part of the video shows motor problems experienced during the use of the right contralateral arm in retrieving the fruit.