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MAP2 Immunostaining in Thick Sections for Early Ischemic Stroke Infarct Volume in Non-Human Primate Brain

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Abstract

The delineation of early infarction in large gyrencephalic brain cannot be accomplished with triphenyl-tetrazolium chloride (TTC) due to its limitations in the early phase, nor can it be identified with microtubule-associated protein 2 (MAP2) immunohistochemistry, due to the fragility of large thin sections. We hypothesize that MAP2 immunostaining of thick whole-brain sections can accurately identify early ischemia in the entire monkey brain.

Using ischemic brains of one rat and three monkeys, a thick-section MAP2 immunostaining protocol was developed to outline the infarct region over the entire non-human primate brain. Comparison of adjacent thick and thin sections in a rat brain indicated complete correspondence between ischemic regions ($100.4 \text{ mm}^3 \pm 1.2\%$, $n = 7$, $p = 0.44$). Thick sections in monkey brain possessed the increased structural stability necessary for the extensive MAP2 immunostaining procedure permitting quantification of the ischemic region as a percent of total monkey brain, giving infarct volumes of 11.4, 16.3, and 19.0% of total brain. Stacked 2D images of the intact thick brain tissue sections provided a 3D representation for comparison to MRI images. The infarct volume of 16.1 cm^3 from the MAP2 sections registered with MRI images agreed well with the volume calculated directly from the stained sections of 16.6 cm^3 .

Thick brain tissue section MAP2 immunostaining provides a new method for determining infarct volume over the entire brain at early time points in a non-human primate model of ischemic stroke.

Keywords

Primate brain; stroke; immunostaining; MAP2; thick section; early ischemia; stroke; focal cerebral ischemia; infarct

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Introduction

Histological identification of the ischemic brain combined with volumetric assessment has been the gold standard for the objective assessment of drug therapy and management strategies in cerebral infarction in ischemic stroke research (Osborne et al., 1987; Brint et al., 1988; Duverger and MacKenzie, 1988). At early times after ischemic stroke the common method of histological staining with triphenyl-tetrazolium chloride (TTC) cannot clearly delineate the infarction area in either permanent (Isayama et al., 1991; Bederson et al., 1986; Hatfield et al., 1991; Bednar et al., 1994) or reperfusion models (Benedek et al., 2006). TTC stains normal tissue with bright red-colored formazan compounds produced from the mitochondrial enzymes that oxidize TTC. The lack of TTC staining (white tissue) depends on the degradation of these mitochondrial enzymes, and this does not occur until extended times after ischemia.

At early time points after ischemia the reduction in microtubule-associated protein 2 (MAP2) immunostaining has been shown to be a reliable marker of neurons that have already started down an irreversible path ultimately leading to cell death (Dawson and Hallenbeck, 1996; Pettigrew et al., 1996). Histological delineation of ischemic brain lesions can be achieved by immunohistochemical staining of thin (20-50 μ m) free-floating rat brain tissue sections with antibodies to MAP2 to measure the volume of the ischemic lesion in relation to that of the entire brain (Dawson and Hallenbeck, 1996; Kharlamov et al., 2002; Kharlamov et al., 2001). However, MAP2 immuno-staining is inadequate for the delineation of the infarct region over the entire non-human primate brain due to the fragility of the large thin sections. MAP2 immunostaining of free-floating sections from blocked non-ischemic monkey brain has been reported using commercially available (Hendry and Bhandari, 1992; Colombo et al., 1999) or custom-made (Kowall et al., 1992) antibodies, but it has never been attempted in ischemic monkey brain and never in sections from complete coronal sections. For the free-floating method, thin tissue sections of lissencephalic rat or small non-human primate (e.g., marmoset) brains (Freret et al., 2008) tolerate handling and remain intact through tissue sectioning followed by multiple transfers through different solutions in rotating tissue baths. However, for larger species of non-human primates with gyrencephalic brains (e.g., baboon, pig tailed monkeys), thin tissue brain sections have a much larger and more complicated shape, which tends to fragment during brain cutting and histochemical processing. The free-floating immunostaining procedure considerably increases the damage: thin tissue sections fold, tear, and break apart. These factors make the delineation of the infarct region over the entire brain from MAP2 immunostaining using thin tissue sections difficult if not untenable.

To rectify this inability to measure infarct volume at early times in large gyrencephalic brains used for experimental stroke studies, we hypothesize that MAP2 immunostaining on thick non-human primate brain sections would allow sectioning and processing of the tissue sections and permit the accurate, volumetric measurement of the infarct volume in comparison to the entire brain.

Materials and methods

All animal protocols were approved by the Institutional Animal Care and Use Committee of the respective institutions.

Rat Model of Brain Ischemia

Focal ischemia was induced in the male Sprague-Dawley rat using a suture model of focal brain ischemia (Koizumi et al., 1986; Longa et al., 1989). Using this model, ischemia was achieved by inserting a 4-0 monofilament suture into the right common carotid artery and advancing the suture into the internal carotid artery to the origin of the MCA from the circle of Willis. Four hours after the onset of ischemia the rat was sacrificed.

Non-Human Primate Model of Brain Ischemia

Focal brain ischemia was induced in pig-tail monkeys (*Maccaca nemestrina*, $n = 3$) using the endovascular method developed in our lab by Jungreis et al. (2003). Each animal was anesthetized with ketamine (10 mg/kg, IM), intubated and maintained under anesthesia throughout the experiment using fentanyl infusion (25 μ g/kg/h, IV). Ischemia was achieved using embolization coils to occlude the posterior cerebral artery (PCA) and a balloon catheter to occlude the middle cerebral artery (MCA) of the right hemisphere. Deflation and removal of the balloon catheter in the right MCA was performed between 3.5 and 5.4 hours after the onset of ischemia for reperfusion of that artery (Table 1). Approximately 4 hours later, the animals were sacrificed.

Brain fixing and cutting

Monkey brains were fixed with 4% paraformaldehyde solution by transcardial perfusion, followed by immersion in 4% paraformaldehyde solution for one week. The rat brain was immediately removed and fixed by immersion in 4% paraformaldehyde solution, which gives satisfactory fixation due to its smaller size (Kharlamov et al., 2001). Both rat and monkey brains were placed in 30% sucrose solution for one week until complete saturation with sucrose.

Monkey brains were pierced with a 14-gauge needle to create three holes in the left hemisphere, which were used during the assembly of brain slices to provide a marker for the left hemisphere and as fiducial marks during 3D reconstruction, as described below. Brains were frozen on dry ice, and cut using a sliding microtome (Histoslide 2000, Reichert-Jung, Nussloch, Germany). The rat brain was cut alternately into 50 μ m (“thin”) and 1 mm (“thick”) coronal sections and the monkey brain into 2.4 mm (“thick”) coronal sections. The brain sections were immediately placed in cryoprotectant solution (30% ethylene glycol, 30% glycerin, and 40% 0.1 M phosphate buffer solution).

Immunohistochemical Procedure

Both thin (50 μ m rat) free-floating, and thick (1 mm rat, 2.4 mm monkey), brain tissue sections were processed using conventional immunostaining as described fully in previous publications (Kharlamov et al., 2001; Hsu and Raine, 1981) using MAP2 antibody (HM2, Sigma, St. Louis, MO) (1:1000 non-human primate brain sections; 1:1500 rat brain sections) followed by biotinylated goat anti-mouse secondary IgG (1:500, Jackson ImmunoResearch Laboratories Inc., West Grove, PA). The tissue sections were treated using an avidin-biotinylated-peroxidase kit (ABC Elite kit, Vector Laboratories, Burlingame, CA). The specificity of MAP2 immunostaining on both thin and thick sections was confirmed by complete absence of staining in the control experiments with omission of either primary or secondary antibodies. The thin rat immunostained sections were mounted onto gelatin-coated glass slides, examined with light microscopy, and their images were digitized by a slide scanner (LS-2000, Nikon Inc., Melville, NY). Thick rat and primate immunostained sections were digitized using a high resolution flatbed scanner (HP Scanjet 4570C, Hewlett-Packard Company, Palo Alto, CA).

Infarct area and volume measurement

To compare infarct areas delineated by MAP2 in the thick and thin rat brain sections, the infarct areas in each digitized image were delineated and measured using MCID imaging software (InterFocus Imaging Ltd., Linton, England). Seven pairs of adjacent thick and thin rat brain sections were analyzed. The infarcted area on both thin and thick rat and thick monkey sections was defined as the area without MAP2 immunostaining. The areas of three regions of interest (infarction, left, and right hemispheres) in the thick sections from the monkeys were measured. The volume difference between right (lesioned) and left (non-lesioned) hemispheres was used to correct the infarction volume for the effects of edema (Swanson et al., 1990; Lin et al.,

1993). Based on infarct areas of all sections, the infarct and edema volume for each brain was calculated using the formula for the frustum of a cone and expressed both as an absolute volume and as a percent of total brain.

MRI

Using a 3 Tesla whole body scanner (General Electric Medical Systems, Milwaukee, WI), an anatomical proton 3D spoiled gradient recalled echo (SPGR) data set was acquired (50 axial slices, slice thickness = 1.0 mm, field-of-view = 16 cm, voxel size = 0.6 mm × 0.6 mm × 1.0 mm, echo time = 15 ms, repetition time = 35 ms, flip angle = 20°, scan time = 5.8 min), as previously described (LaVerde et al., 2007) within two hours of ischemic onset.

3D brain reconstruction

Using the fiducial marks, every other thick monkey brain MAP2 image was stacked and registered using ImageJ (Abramoff et al., 2004) (available from: Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, <http://rsb.info.nih.gov/ij/>, 1997-2008) into a 3D volume file and analyzed using the medical image analysis program AMIDE (Loening and Gambhir, 2003). Infarct volumes of the 3D MAP2 data were available from the region-of-interest output from AMIDE.

Statistics

A paired t-test was used to compare infarct areas in the rat thin and thick sections. A p value <0.05 was assumed to infer statistical significance.

Results

Physiological parameters (arterial blood pressure and heart rate) in all three monkeys were within normal levels (Table 1). However, for the purposes of the MAP2 immunohistochemical response to ischemia, these values are relatively unimportant.

Fig. 1 shows MAP2 immunostained thin 50 μ m (top) and the adjacent thick 1 mm (bottom) rat brain sections after 4 hours of focal brain ischemia. Decreased MAP2 immunostaining indicating the infarct region can be seen in both the thick and thin sections on the right side of the brain. Qualitatively, the location and shape of the infarct region were similar for both the thick and the thin sections. The infarct area of the thick (1 mm) sections was $100.4\% \pm 1.24\%$ ($n = 7$, $p = 0.44$) of the infarct area of the thin (50 μ m) sections, demonstrating good quantitative agreement between the thick and thin sections. The established thick rat brain tissue section MAP2 immunostaining protocol was then implemented on non-human primate stroke brains ($n = 3$). The resulting thick (2.4 mm) MAP2 immunostained sections for one non-human primate stroke brain are shown in Fig. 2. The decreased MAP2 immunostaining indicating the infarct region varied in the extent and anatomic location over the 15 sections shown in Fig. 2. These thick MAP2 brain sections kept their structural and anatomical integrity thereby allowing the expression of the infarct volume as a percent of total brain or in cubic centimeters (Table 2) and the 3D reconstruction of the ischemic injury of the entire brain for alignment and comparison with the MRI images (Fig. 3). The volume of the MAP2-determined infarct region (16.1 cm^3), shown as the yellow 3D region-of-interest (Fig. 3), compares well with the infarct volume calculated directly from the individual sections (16.6 cm^3 , Table 2).

Discussion

Our major result is that despite the early phase (~8 h) after MCA occlusion and reperfusion the infarct volume in the entire monkey brain was identified using MAP2 immunostaining of thick coronal sections. By evaluating the infarct and the whole brain volumetrically, infarct

volume was expressed as a percentage of total brain, in addition to the absolute volume in cubic centimeters, permitting comparison between animals with different brain weights. In addition, the anatomical relation of the infarct was compared to MRI images after 3D stacking, registration, and alignment of the MAP2 immunohistochemical images.

In several studies, paraffin embedding of blocked portions of baboon brain was performed, permitting thin coronal sectioning that maintained the anatomic integrity (Young et al., 1997; Giffard et al., 2005) and definition of the infarcted area by hematoxylin and eosin histological staining four weeks after onset. MAP2 immunostaining of thick paraformaldehyde-fixed monkey brain sections used in the present study is easier compared to large object paraffin embedding and large format thin sectioning, both of which require extremely careful handling. Thick (2-5 mm) brain sections have been used for triphenyl-tetrazolium chloride (TTC) analysis of infarct area and volume in baboon brain (Huang et al., 2000; Del Zoppo et al., 1986) at 3 and 10 days, respectively, and in rhesus macaques at 2 days (West et al., 2009), but never for immunostaining.

In the rat brain, infarct analysis in the first few hours after ischemia can be done by MAP2 immunostaining of thin (20-50 μ m) brain tissue sections (Dawson and Hallenbeck, 1996; Kharlamov et al., 2002; Kharlamov et al., 2001); however, in the non-human primate, thin (20-50 μ m) brain tissue section MAP2 immunostaining using free-floating sections is not functionally possible using whole brain sections. Test experiments on thick (1 mm) vs thin (50 μ m) rat brain sections (Fig. 1) demonstrated that the MAP2 immunostaining of thick brain sections is equivalent to that of thin sections, suggesting that MAP2 immunostaining of thick non-human primate brain tissue section was accurate. Specifically, tissue sections were stained appropriately and the successful delineation of the infarct region was achieved in each slice through the brain (Fig. 2, Table 2). The thick brain tissue sections better maintain their structural and anatomical integrity and shape throughout the extensive MAP2 immunostaining procedure. These 2D images of the thick brain sections can be stacked into a 3D volume of the entire MAP2 immunostained brain for comparison with other imaging modalities such as MRI, as shown in Fig. 3 for SPGR images (LaVerde et al., 2007), and positron emission tomography.

Considering the more comparable degree of microvascular collaterals and ratio between white and grey matter between primate and human brain and the fact that monkey readily adapts to standard clinical, surgical, and radiographic procedures, the nonhuman primate model of stroke provides a high degree of translational potential to the clinical stroke. The possibility of infarct volume evaluation at early times after ischemia in the primate gives an additional advantage to the non-human primate ischemic model.

In conclusion, the thick brain tissue section MAP2 immunostaining protocol successfully allows the histological delineation of the infarct region over the entire brain at early time points in a non-human primate model of ischemic stroke.

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References

- Abramoff MD, Magelhaes PJ, Ram SJ. Image processing with ImageJ. *Biophotonics International* 2004;11:36-42.

- Bederson JB, Pitts LH, Germano SM, Nishimura MC, Davis RL, Bartkowski HM. Evaluation of 2,3,5-triphenyltetrazolium chloride as a stain for detection and quantification of experimental cerebral infarction in rats. *Stroke* 1986;17:1304–1308. [PubMed: 2433817]
- Bednar MM, Fanburg JC, Anderson ML, Raymond SJ, Dooley RH, Gross CE. Comparison of triphenyltetrazolium dye with light microscopic evaluation in a rabbit model of acute cerebral ischaemia. *Neurol Res* 1994;16:129–132. [PubMed: 7519330]
- Benedek A, Moricz K, Juranyi Z, Gigler G, Levay G, Harsing LG Jr, Matyus P, Szenasi G, Albert M. Use of TTC staining for the evaluation of tissue injury in the early phases of reperfusion after focal cerebral ischemia in rats. *Brain Res* 2006;1116:159–165. [PubMed: 16952339]
- Brint S, Jacewicz M, Kiessling M, Tanabe J, Pulsinelli WA. Focal brain ischemia in the rat: Methods for reproducible neocortical infarction using tandem occlusion of the distal middle cerebral and ipsilateral common carotid arteries. *J Cereb Blood Flow Metab* 1988;8:474–485. [PubMed: 3392112]
- Colombo JA, Schleicher A, Zilles K. Patterned distribution of immunoreactive astroglial processes in the striate (V1) cortex of New World monkeys. *Glia* 1999;25:85–92. [PubMed: 9888300]
- Dawson DA, Hallenbeck JM. Acute focal ischemia-induced alterations in MAP2 immunostaining: description of temporal changes and utilization as a marker for volumetric assessment of acute brain injury. *J Cereb Blood Flow Metab* 1996;16:170–174. [PubMed: 8530550]
- Del Zoppo GJ, Copeland BR, Harker LA, Waltz TA, Zyroff JM, Hanson SR, Battenberg E. Experimental acute thrombotic stroke in baboons. *Stroke* 1986;17:1254–1265. [PubMed: 3810730]
- Duverger D, MacKenzie ET. The quantification of cerebral infarction following focal ischemia in the rat: influence of strain, arterial pressure, blood glucose concentration, and age. *J Cereb Blood Flow Metab* 1988;8:449–461. [PubMed: 2968987]
- Freret T, Bouet V, Toutain J, Saulnier R, Pro-Sistiaga P, Bihel E, MacKenzie ET, Roussel S, Schumann-Bard P, Touzani O. Intraluminal thread model of focal stroke in the non-human primate. *J Cereb Blood Flow Metab* 2008;28:786–796. [PubMed: 18000514]
- Giffard C, Young AR, Mezenge F, Derlon JM, Baron JC. Histopathological effects of delayed reperfusion after middle cerebral artery occlusion in the anesthetized baboon. *Brain Res Bull* 2005;67:335–340. [PubMed: 16182942]
- Hatfield RH, Mendelow AD, Perry RH, Alvarez LM, Modha P. Triphenyltetrazolium chloride (TTC) as a marker for ischaemic changes in rat brain following permanent middle cerebral artery occlusion. *Neuropathol Appl Neurobiol* 1991;17:61–67. [PubMed: 2057051]
- Hendry SH, Bhandari MA. Neuronal organization and plasticity in adult monkey visual cortex: immunoreactivity for microtubule-associated protein 2. *Vis Neurosci* 1992;9:445–459. [PubMed: 1333277]
- Hsu SM, Raine L. Protein A, avidin, and biotin in immunohistochemistry. *J Histochem Cytochem* 1981;29:1349–1353. [PubMed: 6172466]
- Huang J, Mocco J, Choudhri TF, Poisik A, Popilskis SJ, Emerson R, DeLaPaz RL, Khandji AG, Pinsky DJ, Connolly ES Jr. A modified transorbital baboon model of reperfused stroke. *Stroke* 2000;31:3054–3063. [PubMed: 11108772]
- Isayama K, Pitts LH, Nishimura MC. Evaluation of 2,3,5-triphenyltetrazolium chloride staining to delineate rat brain infarcts. *Stroke* 1991;22:1394–1398. [PubMed: 1721247]
- Jungreis CA, Nemoto E, Boada F, Horowitz MB. Model of reversible cerebral ischemia in a monkey model. *AJNR Am J Neuroradiol* 2003;24:1834–1836. [PubMed: 14561612]
- Kharlamov A, Jones SC, Kim DK. Suramin reduces infarct volume in a model of focal brain ischemia in rats. *Exp Brain Res* 2002;147:353–359. [PubMed: 12428143]
- Kharlamov A, Kim DK, Jones SC. Early visual changes in reflected light on non-stained brain sections after focal ischemia mirror the area of ischemic damage. *J Neurosci Methods* 2001;111:67–73. [PubMed: 11574121]
- Koizumi J, Yoshida Y, Nakazawa T, Ooneda G. Experimental studies of ischemic brain edema: 1. A new experimental model of cerebral embolism in rats in which recirculation can be introduced in the ischemic area. *Jpn J Stroke* 1986;8:1–8.
- Kowall NW, McKee AC, Yankner BA, Beal MF. In vivo neurotoxicity of beta-amyloid [beta(1-40)] and the beta(25-35) fragment. *Neurobiol Aging* 1992;13:537–542. [PubMed: 1461341]

- LaVerde G, Nemoto E, Jungreis CA, Tanase C, Boada FE. Serial triple quantum sodium MRI during non-human primate focal brain ischemia. *Magn Reson Med* 2007;57:201–205. [PubMed: 17191243]
- Lin TN, He YY, Wu G, Khan M, Hsu CY. Effect of brain edema on infarct volume in a focal cerebral ischemia model in rats. *Stroke* 1993;24:117–121. [PubMed: 8418534]
- Loening AM, Gambhir SS. AMIDE: a free software tool for multimodality medical image analysis. *Mol Imaging* 2003;2:131–137. [PubMed: 14649056]
- Longa EZ, Weinstein PR, Carlson S, Cummins R. Reversible middle cerebral artery occlusion without craniectomy in rats. *Stroke* 1989;20:84–91. [PubMed: 2643202]
- Osborne KA, Shigeno T, Balarsky AM, Ford I, McCulloch J, Teasdale GM, Graham DI. Quantitative assessment of early brain damage in a rat model of focal cerebral ischaemia. *J Neurol Neurosurg Psychiatry* 1987;50:402–410. [PubMed: 3585350]
- Pettigrew LC, Holtz ML, Craddock SD, Minger SL, Hall N, Geddes JW. Microtubular proteolysis in focal cerebral ischemia. *J Cereb Blood Flow Metab* 1996;16:1189–1202. [PubMed: 8898691]
- Swanson RA, Morton MT, Tsao-Wu G, Savalos RA, Davidson C, Sharp FR. A semiautomated method for measuring brain infarct volume. *J Cereb Blood Flow Metab* 1990;10:290–293. [PubMed: 1689322]
- West GA, Golshani KJ, Doyle KP, Lessov NS, Hobbs TR, Kohama SG, Pike MM, Kroenke CD, Grafe MR, Spector MD, Tobar ET, Simon RP, Stenzel-Poore MP. A new model of cortical stroke in the rhesus macaque. *J Cereb Blood Flow Metab* 2009;29:1175–1186. [PubMed: 19384334]
- Young AR, Touzani O, Derlon JM, Sette G, MacKenzie ET, Baron JC. Early reperfusion in the anesthetized baboon reduces brain damage following middle cerebral artery occlusion: a quantitative analysis of infarction volume. *Stroke* 1997;28:632–637. [PubMed: 9056623]

Figure 1.

MAP2 immunostaining of thin, 50 μm (A) and adjacent thick, 1 mm (B) rat brain sections after four hours of focal brain ischemia. Decreased staining (shown by the dotted red lines) can be seen in the infarct region of the right hemisphere of the brain in both thick and thin sections. The location and shape of the infarct region on both sections are virtually identical. Quantitative analysis confirmed the similarity of the infarcted area of these two adjacent sections: the infarct area of this thick 1 mm section was 101.7 % of the infarct area of the thin 50 μm section. The thick brain section maintains its structure and shape throughout the MAP2 immunostaining procedure.

Figure 2.

Fifteen thick, 2.4 mm coronal MAP2-immunostained sections through ischemic non-human primate (monkey # 3) brain represent every other section starting with section “a” as the most rostral section and ending with section “o” as the most caudal section. Ischemic grey matter of the right hemisphere revealed by loss of specific MAP2 immunostaining is delineated by the red lines. Cortical infarction was observed in all 15 sections. In sections “c”- “g”, ischemic subcortical areas were observed as well. Pronounced ischemia was observed in the caudal part of the brain (sections “j”- “o”) comprising most of the right side. In more rostral brain regions (sections “f” – “i”), the infarct encompasses the inferior and medial portions of the sections.

Figure 3.

Fifteen stacked color-scale images of thick sections stained with MAP2 antibody (shown on Fig. 2) co-registered with the gray-scale SPGR proton MR data set of monkey #3. 3D views represent transverse (A, D), coronal (B, E), and sagittal (C, F) views of monkey brain. In panels A, B, and C, MAP2 sections overlap the proton images, which can be partly seen as the light grey area outside the MAP2 sections. The infarct area can be clearly seen on the right hemisphere of the coronal view (B, red arrow) as light staining compared to the more intense staining of corresponding areas in the contralateral hemisphere. In panels D, E, and F, the infarct area identified from the MAP2-stained sections is displayed in solid yellow and is most clearly visualized in panel E. The infarct volume of 16.1 cm^3 from the MAP2 sections registered

with the MRI images compared well with the infarct volume calculated directly from the individual sections 16.6 cm^3 (Table 2). The crossed red lines indicate the relative location of the other two orthogonal sections.

Table 1

Experimental parameters and characteristics

Animal #	Weight (kg)	Gender	MCA occlusion (hr)	MCA reperfusion (hr)	Total time from onset (hr)	mean BP (mmHg)	Heart rate (beats per minute)
Monkey #1	6.5	male	5.2	3.7	8.9	85 ± 2.9	83 ± 4.4
Monkey #2	7.4	female	4.1	3.8	7.9	63 ± 2.1	78 ± 2.5
Monkey #3	8.5	male	3.5	3.8	7.3	105 ± 4.6	92 ± 9.5

Table 2

Infarct volumes

Animal #	Total brain volume	Infarct volume	Infarct volume corrected for edema	Infarct volume
	(cm ³)	(cm ³)	(cm ³)	(% of total volume)
Monkey #1	60.5	7.3	6.8	11.4
Monkey #2	57.2	11.8	10.2	18.3
Monkey #3	79.1	16.6	14.7	19.0