Mechanism of Detection of Acute Cerebral Ischemia in Rats by Diffusion-Weighted Magnetic Resonance Microscopy

Helene Benveniste, MD, PhD; Laurence W. Hedlund, PhD; and G. Allan Johnson, PhD

Background and Purpose: The aim of this study was to measure apparent diffusion coefficients in rat brain tissue exposed to ouabain, glutamate, and N-methyl-D-aspartate and to compare them with apparent diffusion coefficients found in acute cerebral ischemia.

Methods: The apparent diffusion coefficient was measured using magnetic resonance microscopy in four groups of Sprague-Dawley rats after occlusion of the right middle cerebral artery and ipsilateral common carotid artery (n=7), after ouabain exposure (n=6), during glutamate exposure (n=7), or during N-methyl-D-aspartate exposure (n=3). Ouabain, glutamate, and N-methyl-D-aspartate were applied via an intracerebrally implanted microdialysis membrane.

Results: Three hours after the induction of focal cerebral ischemia, a 33% reduction in the apparent diffusion coefficient was observed in the right dorsolateral corpus striatum and olfactory cortex. After ouabain exposure, reductions in the apparent diffusion coefficient were observed within a 1,500-μm radius of the microdialysis membrane. Quantitative analysis revealed that apparent diffusion coefficient values in ischemic and ouabain-exposed tissue fell within the same range. Glutamate and N-methyl-D-aspartate reduced the brain tissue apparent diffusion coefficient by 35% and 40%, respectively.

Conclusions: On the basis of these findings, we conclude that ischemia-induced apparent diffusion coefficient reductions are likely caused by a shift of extracellular to intracellular water.

KEY WORDS • cerebral ischemia • magnetic resonance imaging • diffusion • rats

Detection of ischemic brain tissue by conventional T2-weighted nuclear magnetic resonance (MR) imaging is delayed 3–24 hours after the interruption of cerebral blood flow (CBF).4-5 This lack of sensitivity is probably due to the dependence of T2 prolongation on the rather slow net increase in tissue water that develops several hours after the ischemic insult.6-7 Recently, MR images of incoherent microscopic proton motion, the so-called diffusion-weighted MR images, have been shown to be far superior to T2-weighted MR imaging because the ischemic brain tissue can be visualized within 1 hour.8-11 Incoherent microscopic proton motion in vivo is primarily due to diffusion, but in principle this motion reflects all transport processes within the different tissue compartments, hence the name "apparent" diffusion coefficient (ADC).12 The ADC is consistently reduced in ischemic brain areas; however, the actual pathophysiological mechanisms involved in this phenomenon are unknown.6-11 Given the potential of using ADC measurements to detect and track the evolution of tissue injury in acute clinical stroke, there is a growing need to understand this concept. One working hypothesis is that shortly after the onset of ischemia, tissue ATP reserves are depleted and the subsequent failure of the Na+, K+-ATPase pump causes water protons and ions to migrate from the faster-diffusing, extracellular space into the slower-diffusing, more restricted intracellular compartments, resulting in an ADC decrease.8 To explore this hypothesis further, we subjected brain tissue in vivo to two different insults, both of which are known to occur during the acute phases of ischemia. First, we compared brain ADC measurements in rats exposed to the glycoside ouabain, which specifically inhibits Na+, K+-ATPase,13 with the ADC measurements found in acute ischemia. Second, we tested the effect of two different excitotoxins, glutamate and N-methyl-D-aspartate (NMDA), both of which mediate acute neuronal swelling.14,15

Materials and Methods

We used female Sprague-Dawley rats weighing 180–260 g. The rats were divided into four groups: group 1 (ouabain; n=6), 2 (glutamate; n=7), 3 (NMDA; n=3) (see Table 1 for details), and 4 (focal ischemia; n=7).
Groups 1, 2, and 3 were anesthetized with 2.5–3% halothane in a 3:1 (vol/vol) N2/O2 mixture, intubated, and ventilated with a Harvard rodent ventilator (Harvard Apparatus, South Natick, Mass.). Anesthesia was maintained with 0.5–0.75% halothane. The rat was placed in a stereotaxic frame (frame No. 9, David Kopf), and a 2.0-cm piece of a 0.35-mm (o.d.) microdialysis membrane (Diaflo Hollow Fiber H1X50; molecular weight cutoff, 50,000; Amicon, Beverly, Mass.) was stereotaxically implanted, perpendicular to the sagittal plane, into the dorsal hippocampus (coordinates: 3.6 mm behind and 3.1 mm below the bregma). To avoid disturbances of local CBF and 2-deoxyglucose metabolism, all rats in groups 1, 2, and 3 were allowed to recover for 24 hours after surgery. Technical details of the various microdialysis/MR imaging methods are summarized in Table 1. Microdialysis/MR scan time was 16 minutes for groups 1, 2, and 3, single-slice sagittal MR images were acquired in the left hemisphere 2.5 mm lateral to the midline. In group 4, single-slice frontal MR images were acquired at the level of the bregma. To verify the system calibration and stability, standards of water and dimethyl sulfoxide were included in the field of view of all studies. ADCs of standards were the same (within experimental error) for both sequences. Diffusion coefficient calculations from the four images were accomplished by a pixel-to-pixel fit to the diffusion decay given by the equation

\[ S(nTE)/S(0) = \exp[(nTE)/bD] \]

where \( S \) is the pixel intensity for a given image of the four images, TE is echo time, \( S(0) \) is the equilibrium intensity, \( D \) is the diffusion coefficient, and \( b \) is given by the integral of the pulsed gradients, \( G(t) \) (for further details, see References 12, 21, and 23). Previous efforts have demonstrated that the resulting calculated diffusion image agrees with published values of ADC for our standards within ±10%. In groups 1 and 4, we used an echo time of 40 msec, a repetition time of 2 seconds, and four excitations for each of 256 phase-encoding steps. Total scan time was 2.25 hours.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Microdialysis perfusion fluid (1)</th>
<th>MR scan Microdialysis perfusion fluid (2)</th>
<th>Microdialysis perfusion time</th>
<th>Microdialysis perfusion before MR scan</th>
<th>Microdialysis perfusion during MR scan</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n=6)</td>
<td>Mock CSF 1 mM ouabain</td>
<td>1 hour</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2 (n=4)</td>
<td>Mock CSF 10 mM glu</td>
<td>2 hours 16 minutes</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2 (n=3)</td>
<td>Mock CSF 100 mM glu</td>
<td>2 hours 16 minutes</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3 (n=3)</td>
<td>Mock CSF 1 mM NMDA</td>
<td>2 hours 16 minutes</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

The composition of the mock cerebrospinal fluid (CSF) was (in mM): 122 NaCl, 3 KCI, 1.2 CaCl2, 1.2 MgCl2, 0.4 KH2PO4, and 25 NaCO3 (pH 7.4). The osmolarity of perfusion fluids containing either ouabain (4β,20[22]-cardenolide-1β,3β,5α,11α,14,19-hexol-3-[6-deoxy-a-L-mannopyranosyl]; G-strophanthin, Sigma Chemical Co., St. Louis, Mo.), glutamate (glu; L-glutamic acid, monosodium salt, Sigma), or N-methyl-D-aspartic acid (NMDA, Sigma) was maintained at 305 mosmol/1 H2O by lowering the content of NaCl. The microdialysis membrane perfusion rate was 5 μl/min.

MR, magnetic resonance.
groups 1, 2, and 3, 40-μm-thick sagittal sections were perfused transcardially with 0.9% (wt/vol) saline for 1 minute followed by about 400 ml fixative (buffered Forma-Scent fixative, 10% [wt/vol] formalin, pH 6.9-7.1; Scientific Products, Baxter). The brain was removed and placed in fixative at 4°C for 7 days and then in fresh fixative containing 30% (wt/vol) sucrose for 3 days. In groups 1, 2, and 3, 40-μm-thick sagittal sections were cut with a freezing microtome and collected at 250-μm intervals starting 5.5 mm lateral to the midline. In group 4, 40-μm-thick frontal sections were cut starting 2.7 mm in front of and extending 3.3 mm behind the bregma. In all groups every second section was stained with cresyl violet.

The calculated MR images were analyzed for changes in the ADC using an in-house image analysis system. The mean ADC values were then obtained from selected regions of interest containing 21 pixels. Statistical evaluations used STATWORKS, and p<0.05 was considered significant. In group 1, differences in ADC values were tested among individual brain regions using a one-way analysis of variance with post hoc comparisons and a paired Student's t test. The differences between groups 1 and 4 were evaluated with a nonparametric Mann-Whitney U test, and paired data were analyzed using the Wilcoxon signed rank test. Because of limited sample sizes in groups 2 and 3, the differences were evaluated using Student's t test.

Histological sections were matched with the MR images by using the shape of the corpus callosum, striatum, lateral ventricles, cerebellum, and hippocampus as guides. Silver-impregnated and cresyl violet-stained sections corresponding to MR imaging sections were analyzed for brain tissue damage using a light microscope (Leitz, Wetzlar, FRG).

Results

All animals survived implantation of the microdialysis membrane. Postoperatively, rats showed no signs of seizure activity, paresis, or behavioral alterations.

Figure 1 demonstrates diffusion-weighted MR images from a typical group 1 rat after microdialysis membrane perfusion with mock cerebrospinal fluid (top) and ouabain (bottom). The microdialysis membrane appears as a circular, low-signal-intensity area (arrow in Figure 1, top). On diffusion-weighted MR images (see Figure 1), a more intense signal reflects a more reduced ADC, and low-signal-intensity areas, such as white matter areas in the cerebellum, and the corpus callosum represent regions with high ADCs. (Regional brain tissue ADC variability probably reflects differences in myelin bundle density and direction because the ADC magnitude within heavily myelinated brain regions depends on the direction of the diffusion gradient. For example, if the diffusion gradient direction is parallel to the orientation of the myelin bundles, the ADC increases.) Table 2 presents ADCs from rats in group 1. As shown, only the hippocampal ADC was affected by ouabain. Figure 2 again illustrates measurements obtained 40 minutes after ouabain exposure, demonstrating significantly reduced ADC values at different distances from the microdialysis membrane. These reductions in the ADC were observed within a 1,500-μm radius of the microdialysis membrane (see also Figure 1, bottom).

All the rats in group 4 survived surgery. On diffusion-weighted MR images obtained 40 minutes after the onset of focal ischemia, high-signal-intensity areas were observed in the dorsolateral portion of the right caudate putamen and frontoparietal (somatosensory area) cortex. The ADC measurements from the right and left dorsolateral caudate putamen demonstrated that right-sided ADCs were significantly lower than those of the contralateral left (Table 3). Figure 2 demonstrates that ADC measurements from the right dorsolateral caudate putamen and ouabain-treated tissue fall within the same range.

In group 2, four rats were exposed to 10 mM glutamate and three to 100 mM glutamate (see Table 1). The hippocampal ADC was unaffected by microdialysis membrane perfusion with 10 mM glutamate but was decreased during perfusion with 100 mM glutamate (Figure 3, top). However, ADC reductions in the hippocampus induced by 100 mM glutamate were observed only within a 500-μm radius from the microdialysis membrane (compare Figure 1, bottom, with Figure 3, top). By contrast, perfusion with NMDA induced changes in the hippocampal ADC extending approximately 1,100 μm from the microdialysis membrane (Figure 3, bottom). Quantitatively, the ADC reductions induced by either 100 mM glutamate or NMDA seemed to be similar to those induced by ouabain (Table 3).

The histological examination of brains from groups 1, 2, and 3 revealed scattered accumulations of extravasated erythrocytes and polymorphonuclear leukocytes in the hippocampal tissue close to the microdialysis membrane track. Degenerating neurons were always observed in the immediate vicinity of the membrane track. On cresyl violet-stained and silver-impregnated brain sections from ouabain-exposed rats, we found 1) areas of pallor, indicating cellular edema, within an approximate 1,500-μm radius from the microdialysis membrane track and 2) degenerating neurons with shrunken somata and dendrites in the periphery of this radius. The tissue within the area of pallor was not necrotic. No histological abnormalities were seen in brain regions remote from the implantation site. On silver-impregnated brain sections from rats exposed to 100 mM glutamate and 1 mM NMDA, we observed areas of pallor extending approximately 500 and 1,000 μm from the membrane track, respectively (compare Figure 4, top). The tissue within the area of pallor was not necrotic. No histological abnormalities were observed in remote brain regions in these groups.

Group 4 rats were killed 3–5 hours after the middle cerebral artery occlusion. On cresyl violet-stained brain sections, pallor was observed in the right dorsolateral part of the corpus striatum and olfactory cortex. In three rats, pallor was also observed in the right frontoparietal cortex. Examination of corresponding silver-impregnated brain sections revealed degenerating neurons with shrunken somata and dendrites at the edges of the described pallor area (Figure 4, bottom). Tissue in the right dorsolateral part of the corpus striatum was not yet necrotic because this area appeared dark brown.
FIGURE 1. Top panel: Diffusion-weighted magnetic resonance (MR) image of a group 1 rat after microdialysis membrane perfusion with mock cerebrospinal fluid (CSF). Location of microdialysis membrane is indicated by arrow. On diffusion-weighted images, a more intense signal reflects a more reduced apparent diffusion coefficient (ADC); e.g., low-signal-intensity areas such as white matter areas in cerebellum and corpus callosum represent regions with high ADCs. Perfusion with mock CSF did not cause changes in ADC (see Table 2 for absolute ADC values). Bottom panel: Diffusion-weighted MR image of same group 1 rat after microdialysis membrane perfusion with ouabain. Ouabain increased hippocampal signal intensity within a 1,500-μm radius of membrane (see Figure 2 for absolute ADC values). On corresponding diffusion-calculated images, ADCs of water and DMSO were measured, at 28°C, to be $2.53 \times 10^{-3}$ and $0.92 \times 10^{-3}$ mm²/sec, respectively, values within 10–20% of published literature values.

Discussion

We found that intracerebral application of ouabain induced reductions in the ADC similar to those observed in acute cerebral ischemia. It is questionable whether ADC reductions in the right ischemic dorsolateral caudate putamen can be compared with those measured in the hippocampus after ouabain exposure. However, we find this justifiable because ADC reductions within ischemic gray matter areas demonstrated similar values. Changes in the ADC occur so rapidly after the cessation of blood flow that histologically, there is no sign of tissue necrosis but only evidence of tissue edema. We detected a 32% reduction in the ADC in the right dorsolateral caudate putamen approximately 3 hours after the interruption of blood flow. (MR images were initiated and concluded 40 minutes and 2 hours, 56 minutes, respectively, after occlusion.) Despite the lack of CBF measurements, it seems reasonable to conclude that the right dorsolateral caudate putamen was ischemic throughout the imaging period because our previous experience with the middle cerebral artery occlusion model always demonstrated necrosis in this particular area.

Recent studies suggest that ischemia, i.e., interruption of CBF per se and the resultant brain tissue hypothermia, may cause part of the ADC reduction. Although the lack of vascular perfusion resulting in a loss of motion from intravascular protons in ischemic tissue might contribute to the decrease in the ADC, it seems unlikely that...

rather than black on silver-impregnated brain sections (Figure 4, bottom).
The significance of differences in apparent diffusion coefficient (ADC) values among individual brain regions in group 1 rats after exposure to mock cerebrospinal fluid (CSF) was assessed by one-way analysis of variance with post hoc comparisons using a paired t test. The following list of region pairs had significantly different ADC values: Frontal cortex vs. hippocampus, \( p=0.031 \); caudate putamen vs. thalamus, \( p=0.015 \); caudate putamen vs. hippocampus, \( p=0.013 \); parietal cortex vs. thalamus, \( p=0.011 \); parietal cortex vs. hippocampus, \( p=0.003 \); thalamus vs. cerebellum, \( p=0.025 \); and hippocampus vs. cerebellum, \( p=0.018 \).

The significance of differences in apparent diffusion coefficient values in brain regions before and after perfusion with ouabain was assessed by the Wilcoxon signed rank test. 

Values are mean±SD.

<table>
<thead>
<tr>
<th>Region</th>
<th>ADC ( \times 10^{-3} ) with mock CSF (mm²/sec)</th>
<th>ADC ( \times 10^{-3} ) with ouabain (mm²/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olfactory bulb</td>
<td>0.67±0.05</td>
<td>0.73±0.10</td>
</tr>
<tr>
<td>Frontal cortex</td>
<td>0.72±0.08</td>
<td>0.74±0.10</td>
</tr>
<tr>
<td>Caudate putamen</td>
<td>0.68±0.05</td>
<td>0.68±0.05</td>
</tr>
<tr>
<td>Frontoparietal cortex</td>
<td>0.66±0.04</td>
<td>0.62±0.11</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.79±0.05</td>
<td>0.47±0.16</td>
</tr>
<tr>
<td>Thalamus</td>
<td>0.75±0.07</td>
<td>0.77±0.06</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.65±0.07</td>
<td>0.73±0.05</td>
</tr>
</tbody>
</table>

Values are mean±SD.

The significance of differences in apparent diffusion coefficient values in brain regions before and after perfusion with ouabain was assessed by the Wilcoxon signed rank test. 

Values are mean±SD.

In groups 1, 2, and 3, "control" refers to microdialysis membrane perfusion with mock cerebrospinal fluid; in group 4, "control" refers to the left, nonischemic, dorsolateral caudate putamen. In groups 1, 2, and 3, "treated" refers to microdialysis membrane perfusion with 1 mM ouabain, 100 mM glutamate, and 1 mM N-methyl-D-aspartate (NMDA), respectively; in group 4, "treated" refers to the right, ischemic, dorsolateral caudate putamen. It is clear that the apparent diffusion coefficient (ADC) reductions induced with ouabain, glutamate, and NMDA are identical to those induced by ischemia. There is a small discrepancy in the absolute values of ADC for groups 1 and 4 and 2 and 3. Although these subtle differences may be due to differences in diffusional restriction, they are most likely due to systematic errors introduced by the different echo times used (40 msec for groups 1 and 4 and 20 msec for groups 2 and 3).

Values are for comparisons of treated and control groups that were evaluated using the Wilcoxon signed rank test. 

Values are mean±SD.

In groups 1, 2, and 3, "control" refers to microdialysis membrane perfusion with mock cerebrospinal fluid; in group 4, "control" refers to the left, nonischemic, dorsolateral caudate putamen. In groups 1, 2, and 3, "treated" refers to microdialysis membrane perfusion with 1 mM ouabain, 100 mM glutamate, and 1 mM N-methyl-D-aspartate (NMDA), respectively; in group 4, "treated" refers to the right, ischemic, dorsolateral caudate putamen. It is clear that the apparent diffusion coefficient (ADC) reductions induced with ouabain, glutamate, and NMDA are identical to those induced by ischemia. There is a small discrepancy in the absolute values of ADC for groups 1 and 4 and 2 and 3. Although these subtle differences may be due to differences in diffusional restriction, they are most likely due to systematic errors introduced by the different echo times used (40 msec for groups 1 and 4 and 20 msec for groups 2 and 3).

Values are for comparisons of treated and control groups that were evaluated using the Wilcoxon signed rank test. 

Values are mean±SD.
(0.1×3.25×10^{-2} \text{ mm}^2/\text{sec}) + (0.9×1.43×10^{-4} \text{ mm}^2/\text{sec}), or 0.45×10^{-2} \text{ mm}^2/\text{sec}, again in excellent agreement with the values we measured.

A previous autoradiographic study of [3H]ouabain binding to sections of rat brain demonstrated a relatively homogeneous distribution of Na⁺, K⁺-ATPase binding sites in gray matter areas, thereby explaining the observed uniform effect of ouabain on the ADC in the hippocampus (the ADC reductions occurred within a radius of approximately 1,500 \mu m, independent of membrane location within the hippocampus).³⁸ Ouabain did not affect more remote brain regions because it was administered locally, and its distribution was limited by diffusion.³⁸ The theoretical path length (\bar{x}, in a direction perpendicular to the dialysis membrane) of brain ouabain diffusion was calculated using the Einstein equation:

\[ \bar{x} = \sqrt{2 D^* \tau} \]

where \( \tau \) is diffusion time, i.e., the time from the start of ouabain perfusion through the microdialysis membrane to the conclusion of the MR experiment (=3.8 hrs), and \( D^* \) is the diffusion coefficient of ouabain in brain tissue. Although \( D^* \) is unknown, an estimate of \( \bar{x} \) can be obtained by inserting the brain tissue diffusion coefficient for the neurotransmitter dopamine (molecular weight of 156)³⁹:

\[ \bar{x} = \sqrt{2 \times (3.8 \text{ hr} \times 3,600 \text{ sec/hr}) \times 0.68 \times 10^{-10} \text{ m}^2/\text{sec}} \]

= 1,360 \mu m
FIGURE 4. Top panel: Dorsal left hippocampal formation of rat 2 hours and 16 minutes after microdialysis membrane perfusion with 100 mM glutamate (×112, silver-impregnation stain). Microdialysis membrane track in CA1 hippocampal area is indicated by arrow. Perfusion with 100 mM glutamate caused tissue edema, represented by area of pallor, extending ≈500 μm away from membrane track. Argyrophilic CA1 neurons can be observed at edges of pallor area.

Bottom panel: Coronal section at level of caudate-putamen from group 4 rat 3 hours and 45 minutes after occlusion of right middle cerebral artery and ipsilateral common carotid artery (×58, silver-impregnation stain). Necrosis (i.e., black tissue) is not yet present within occluded right hemisphere at this early postocclusion time. However, degenerating, argyrophilic neurons in caudate-putamen are observed at the edges outlining the pending infarct (arrows).
This estimate approximates the observed ADC reductions within a 1,500-μm radius from the microdialysis membrane.

The relation between ADC reductions and acute cell swelling was further supported by the glutamate and NMDA experiments. When glutamate activates the NMDA- and non-NMDA-type receptors that are linked to Na⁺ and K⁺ channels, it mediates transmembrane ion and water shifts with subsequent neuronal swelling.⁴⁰ Our results further suggest that ischemia-induced glutamate release at the time of anoxic depolarization⁴¹ may contribute to the rapid ADC reductions observed during ischemia.

The effectiveness of the sodium-dependent, high-affinity glutamate uptake mechanisms located on both neurons and astrocytes⁴² was demonstrated indirectly on diffusion-weighted MR images from group 2 because 1) high glutamate concentrations were required to affect the hippocampal ADC, 2) the glutamate-induced ADC changes were observed only in close proximity to the microdialysis membrane, and 3) relatively low NMDA concentrations affected the hippocampal ADC far from the microdialysis membrane. In contrast to NMDA, when glutamate crosses the microdialysis membrane and enters the brain interstitial space, uptake mechanisms⁴⁰ will continuously remove it. Thus, our experiments agree with previous reports demonstrating that in vivo, the interstitial threshold concentration for glutamate cytotoxicity is reached only when it is delivered in sufficiently large amounts to counteract the uptake mechanisms.⁴³,⁴⁴ Unfortunately, at this time it is not possible from our microdialysis/MR imaging experiments to estimate the actual interstitial glutamate concentration range for cytotoxicity.

Ischemia-, ouabain-, glutamate-, and NMDA-exposed brain tissue was nonnecrotic but edematous at the time of histological examination. Our histological results agree with previous studies in which brain tissue was exposed to ouabain.⁴³,⁴⁵,⁴⁶ Cornog et al⁴⁵ demonstrated that ouabain does not change blood–brain barrier permeability in rats. Therefore, it is reasonable to assume that ouabain-induced edema is of the cytotoxic type. The same reasoning may be applied to the ischemia data because Schuerer and Hossmann⁴⁷ showed that the permeability of the blood–brain barrier to serum proteins does not change during the initial 4 hours after middle cerebral artery occlusion.

In conclusion, our data indirectly support the hypothesis that ischemia-induced ADC reductions are caused by a shift of extracellular to intracellular water resulting from a rapid breakdown of the Na⁺, K⁺-ATPase pump. To characterize this relation in more detail and to provide more direct evidence, MR time resolution needs further improvement because ischemia-induced extracellular to intracellular water shifts in the brain are known to occur within 2 minutes. Finally, ongoing experiments employing glutamate/NMDA antagonists may help define ischemic brain injury thresholds in greater detail.

Acknowledgments
We are particularly grateful to G.P. Cofer for his expert help and absolute mastery of the CSI system. We also wish to thank neuropathologist B. Crain and statistician C. Beam for helpful suggestions in reviewing this work.

References
1. Sauter A, Rudin M: Calcium antagonists reduce the extent of infarction in rat middle cerebral artery occlusion model as determined by quantitative magnetic resonance imaging. Stroke 1986; 17:1228-1234
44. Rosenberg PA, Aizenman E: Hundred-fold increase in neuronal vulnerability to glutamate toxicity in astrocyte-poor cultures of rat cerebral cortex. Neurosci Lett 1989;103:162–168
Mechanism of detection of acute cerebral ischemia in rats by diffusion-weighted magnetic resonance microscopy.
H Benveniste, L W Hedlund and G A Johnson

Stroke. 1992;23:746-754
doi: 10.1161/01.STR.23.5.746

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1992 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/23/5/746

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org/subscriptions/