Dynamics of Cerebral Tissue Injury and Perfusion After Temporary Hypoxia-Ischemia in the Rat
Evidence for Region-Specific Sensitivity and Delayed Damage

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**Background and Purpose**—Selective regional sensitivity and delayed damage in cerebral ischemia provide opportunities for directed and late therapy for stroke. Our aim was to characterize the spatial and temporal profile of ischemia-induced changes in cerebral perfusion and tissue status, with the use of noninvasive MRI techniques, to gain more insight in region-specific vulnerability and delayed damage.

**Methods**—Rats underwent 20 minutes of unilateral cerebral hypoxia-ischemia (HI). We performed combined repetitive quantitative diffusion-weighted, T2-weighted, and dynamic susceptibility contrast-enhanced MRI from before HI to 5 hours after HI. Data were correlated with parallel blood oxygenation level–dependent MRI and laser-Doppler flowmetry. Finally, MRI and histology were done 24 and 72 hours after HI.

**Results**—Severe hypoperfusion during HI caused acute reductions of the apparent diffusion coefficient (ADC) of tissue water in the ipsilateral hemisphere. Reperfusion resulted in dynamic perfusion alterations that varied spatially. The ADC recovered completely within 1 hour in the hippocampus (from 0.68±0.07 to 0.83±0.09×10^{-3} mm²/s), cortex (from 0.56±0.06 to 0.77±0.07×10^{-3} mm²/s), and caudate putamen (from 0.58±0.06 to 0.75±0.06×10^{-3} mm²/s) but only partially or not at all in the thalamus (from 0.65±0.07 to 0.68±0.12×10^{-3} mm²/s) and substantia nigra (from 0.80±0.08 to 0.76±0.10×10^{-3} mm²/s). Secondary ADC reductions, accompanied by significant T2 elevations and histological damage, were observed after 24 hours. Initial and secondary ADC decreases were observed invariably in the hippocampus, cortex, and caudate putamen and in approximately 70% of the animals in the thalamus and substantia nigra.

**Conclusions**—Region-specific responses and delayed ischemic damage after transient HI were demonstrated by MRI. Acute reperfusion-induced normalization of ADCs appeared to poorly predict ultimate tissue recovery since secondary, irreversible damage developed eventually. (Stroke. 1998;29:695-704.)

**Key Words:** cerebral ischemia, transient ■ magnetic resonance imaging ■ selective vulnerability ■ rats

A period of cerebral ischemia can lead to irreversible neuronal injury and severe neurological deficits. Early reperfusion potentially could prevent ultimate damage and result in complete recovery. However, initial injury often progresses and/or secondary damage can develop after restoration of the blood supply.1 Both primary and secondary injury do not develop uniformly in the brain; the severity and progression of damage show considerable regional differences. This region-specific response has been reported predominantly in transient global ischemia models2–4 but has also been recognized in experimental focal ischemia5,6 and after cardiorespiratory arrests in humans.7 Certain brain areas, such as the hippocampal CA1 region, are extremely susceptible to a reduction in the supply of oxygen and glucose and inevitably become damaged.8 The progression into irreversible damage in this area can nevertheless take several days.2,9,10 In other brain structures, such as the substantia nigra, injury develops much faster.11 Although these phenomena of regionally selective vulnerability and early and delayed neuronal damage have been studied extensively,1,12,13 the exact pathophysiological mechanisms underlying the heterogeneity in progression and severity of ischemic damage are still unknown. Earlier studies have suggested that local variations in CBF during and after the ischemic challenge at least partially form the basis of the heterogeneous tissue susceptibility.14–17

Characterization of the spatial and temporal patterns of ischemic injury and the origin of regional differences is of major clinical importance since specific brain areas may be...
viable for longer times and could therefore be responsive to delayed therapeutic interventions. In this view, early recognition and differentiation of these regions are crucial. The aim of our study was to evaluate the concepts of region-specific ischemic sensitivity and delayed tissue damage in association with brain perfusion dynamics in a rat model of transient HI. We were especially interested in the hyperacute region-specific tissue response in relation to long-term postischemic injury since this could provide prognostic information on the development of damage. We used noninvasive MRI techniques that allowed longitudinal and multiparametric measurements and combined these with parallel LDF measurements and histology.

Materials and Methods

Animal Model

Experimental protocols were approved by the Groningen Medical School Animal Experiment Committee. We used a modified Levine model (global hemispheric ischemia induced by unilateral carotid artery ligation with hypoxic ventilation) in the rat. Male Wistar rats (weight, 305 to 370 g) were anesthetized with subcutaneous injections of a mixture of 0.315 mg/mL fentanyl citrate and 10 mg/mL fluanisone (0.55 mL/kg), 0.315 mg/mL fentanyl citrate and 10 mg/mL fluanisone (0.55 mL/kg), midazolam (0.55 mL/kg), and atropine (0.05 mL/kg). Body temperature was kept at 37°C with a heating pad. Animals were endotracheally intubated and mechanically ventilated with O2/N2O (30%/70%). After placement around it. The deflated balloon (20-mm length; 3-mm diameter) was isolated, and a polyethylene tube (10-mm length; 3-mm diameter) was placed around it. The deflated balloon (20-mm length; 3-mm diameter) was inserted into the polyethylene tube. Inflation of the balloon inevitably accomplished unilateral occlusion of the CCA.

Cerebral HI was induced by reducing the O2 fraction of the breathing mixture to approximately 10% (keeping the breathing volume constant by adjusting the N2O supply), in combination with occlusion of the left CCA. The O2 fraction was carefully adjusted between 9% and 12% to induce an MABP drop of at least 50% (but never less than 30 mm Hg). After 20 minutes the breathing gas composition was normalized, and the balloon of the occluding device was deflated.

MRI Experiments

After surgery the animals were immobilized in a specially designed stereotaxic device and placed in an animal cradle. MRI was done on a 4.7-T SISCO/Varian Instruments NMR spectrometer (horizontal bore) with a 120-mm gradient insert (gradients up to 220 mT/m). A Helmholtz volume coil (85-mm diameter) and an inductively coupled surface coil (20-mm diameter), placed on the rat’s head, were used for signal transmission and detection, respectively. Brain coordinates were determined from a sagittal scout image, acquired with the use of a FLASH sequence. Dynamic susceptibility contrast-enhanced (bolus track) MRI (FLASH, TR, 11 milliseconds; TE, 7 milliseconds; two acquisitions; 15° flip angle; FOV, 30×30 mm; 64×64 data matrix; slice thickness, 1.7 mm; 120 consecutive images) was performed in combination with an intravenous bolus injection of Gd-DTPA (0.5 mmol/kg) given during the acquisition of the 13th MR image (time resolution, 0.7 second per image). Damage in our modified Levine model develops predominantly in areas served by the middle cerebral artery. Therefore, we performed bolus track MRI of a brain slice encompassing a relatively large middle cerebral artery territory, which was at the position 1 mm posterior to bregma according to Paxinos and Watson. The selected slice matched with the (single) slice in the BOLD MRI experiments and with slice number five in the multislice MRI experiments (see below).

Multiphase DW MRI was performed with a combination of the following images at 37°C with a heating pad. Animals were endotracheally intubated and mechanically ventilated with O2/N2O (30%/70%). After approximately 1 hour, halothane (0.8%) was added to the breathing mixture to maintain long-term anesthesia. The tail vein was catheterized to allow administration of the NMR contrast agent dimeglumine-dextran-pentetate (Gd-DTPA) (Schering) (0.5 mol/L). The left femoral artery was cannulated for continuous recording of the blood pressure (Datascope 3000 Monitor, Datascope Corp) and frequent analysis of blood gases (ABL 505/Osm4, Radiometer). After a neck incision the left CCA was isolated, and a polyethylene tube (10-mm length; 3-mm diameter) was placed around it. The deflated balloon (20-mm length; 3-mm diameter) of a remotely inflatable coronary balloon dilatation catheter (Datascope; shaft length, 1.35 m) was inserted into the polyethylene tube. Inflation of the balloon inevitably accomplished unilateral occlusion of the CCA.

Cerebral HI was induced by reducing the O2 fraction of the breathing mixture to approximately 10% (keeping the breathing volume constant by adjusting the N2O supply), in combination with occlusion of the left CCA. The O2 fraction was carefully adjusted between 9% and 12% to induce an MABP drop of at least 50% (but never less than 30 mm Hg). After 20 minutes the breathing gas composition was normalized, and the balloon of the occluding device was deflated.

MRI Protocol

A scheme explaining the timing of the experiments is given in Fig 1. MRI was done from approximately 1 hour before to 2 hours after HI (n = 10). In four of the animals MRI experiments were continued up to 5 hours after induction of HI. DW and T2-weighted MRI were performed repeatedly. Bolus track MRI was done on an hourly basis. It requires approximately 30 minutes to ensure essentially complete clearance of Gd-DTPA. Therefore, around the period of HI, bolus track MRI experiments were performed just before the end of HI (19 minutes after HI) in one group (n = 4) and just after the end of HI (25 minutes after HI) in another group of animals (n = 4). Since circulation of the contrast agent Gd-DTPA influences the tissue T2* (MNR, echo time). The selected slice matched with the (single) slice in the BOLD MRI experiments and with slice number five in the multislice MRI experiments (see below).

Multislice DW MRI was performed with the use of a spin-echo sequence (TR, 2 seconds; TE, 33 milliseconds; two acquisitions; FOV, 30×30 mm; 64×64 data matrix; eight contiguous 1.7-mm slices) with four different b values (0 to 1848 s/mm2), which allowed calculation of the tissue-water ADC. The measured ADC values in the brain are dependent on the direction of the diffusion-encoding gradient because of diffusion anisotropy, particularly in white matter. Nevertheless, the degree of ADC reduction in ischemic areas (arising predominantly in gray matter) is not influenced by changes in anisotropy. Besides, areas displaying a significant ADC decrease have been shown to be highly correlative with histological damage when the diffusion-sensitive gradient is applied along the z axis (ie, parallel to the long axis of the brain), which was done in the present study. Multislice T2-weighted MRI was also done with a spin-echo sequence, with three different echo times (30, 80, and 130 milliseconds).

BOLD MRI was done with a FLASH sequence (TR, 35 milliseconds; TE, 30 milliseconds; flip angle, 15°; FOV, 30×30 mm; 64×64 data matrix; slice thickness, 1.7 mm). Images of a slice 1 mm posterior from bregma were collected during the onset and completion of HI (120 consecutive images; time resolution, 2.2 seconds per image).

Analysis of MR Data

Bolus track MRI data were processed as described previously. Briefly, contrast agent–induced SI changes were converted to changes in BOLD MRI. A scheme explaining the timing of the experiments is given in Fig 1. MRI was done from approximately 1 hour before to 2 hours after HI (n = 10). In four of the animals MRI experiments were continued up to 5 hours after induction of HI. DW and T2-weighted MRI were performed repeatedly. Bolus track MRI was done on an hourly basis. It requires approximately 30 minutes to ensure essentially complete clearance of Gd-DTPA. Therefore, around the period of HI, bolus track MRI experiments were performed just before the end of HI (19 minutes after HI) in one group (n = 4) and just after the end of HI (25 minutes after HI) in another group of animals (n = 4). Since circulation of the contrast agent Gd-DTPA influences the tissue T2*, T2*-sensitive BOLD MRI during onset and completion of HI was performed in four separate animals that were not subjected to bolus track MRI experiments. DW, T2-weighted, and bolus track MRI were also performed 24 hours (n = 6) and 72 hours (n = 5) after HI in separate groups of animals. Control experiments were performed in animals that underwent either 20 minutes of hypoxia without CCA occlusion (n = 4) or CCA occlusion under normoxic conditions (n = 4). The imaging protocol in these experiments was as described above, except that BOLD MRI was omitted.

Selected Abbreviations and Acronyms

- ADC = apparent diffusion coefficient
- BOLD = blood oxygenation level–dependent
- CBF = cerebral blood flow
- CCA = common carotid artery
- DW = diffusion-weighted
- FLASH = fast low-angle shot
- FOV = field of view
- HI = hypoxia-ischemia
- LDF = laser-Doppler flow, flowmetry
- MABP = mean arterial blood pressure
- NMR = nuclear magnetic resonance
- rCBV = relative cerebral blood volume
- ROI = region of interest
- SI = signal intensity
- tpeak = bolus peak time
- TR = repetition time

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were fitted to a curve, which is proportional to the rCBV. Pixel-by-pixel fitting of the \( \Delta R_2^* \) time plots allowed generation of two-dimensional maps of the hemodynamic parameters. Brain maps of the ADC and T2 were generated from the DW and T2-weighted MRI measurements, respectively, by monoeponential fitting on a pixel-by-pixel basis.

Parametric images were analyzed in anatomic ROIs with use of the image analysis software package ImageBrowser (SISCO/Varian). The time course of the tissue water ADC and T2 was assessed in specific areas known to be differentially vulnerable to an HI insult. These were the substantia nigra, dorsal hippocampus, thalamus, parietal cortex, and caudate putamen (ROI positioning is exemplified in Figs 5, 6, and 7). ROIs were positioned in both ipsilateral and contralateral areas. Ipsilateral \( t_{\text{peak}} \), \( \Delta R_2^* \), and rCBV were measured in the parietal cortex and caudate putamen. Since variations in bolus injections prevent a direct longitudinal comparison of hemodynamic parameters from consecutive perfusion measurements, these parameters were expressed relative to the value in corresponding contralateral brain areas measured at the same time.

### Laser-Doppler Flowmetry

In six rats, regional blood flow in the parietal cortex of both hemispheres was measured by means of LDF, essentially as described by De Wildt et al. from 2 minutes before (baseline) up to 2 hours after induction of HI (see also Fig 1). The parietal cortex was exposed on both the left and right hemispheres by a trepanation (approximately 6 mm²); the dura was left intact. Two LDF probes (PF 302, Perimed) were stereotaxically placed on the exposed dura at 1 mm posterior and 5 mm lateral to bregma. Regional LDF was expressed as a percentage of baseline.

### Histology

After the MRI experiments at 24 and 72 hours after HI (see Fig 1), rats were killed by means of an overdose of anesthetic and directly transcardially perfused with saline (50 mL) followed by approximately 500 mL 4% paraformaldehyde in 0.1 mol/L phosphate buffer (pH 7.4). Brains were removed and stored in the same fixative solution. Sections of 30 \( \mu \)m were cut with a cryostat microtome and stored in 4% phosphate-buffered paraformaldehyde. Every fifth section was stained by means of an improved Gallyas silver impregnation procedure, which labels damaged cell bodies and their processes. The silver-impregnated sections were stored in 0.1 mol/L phosphate buffer (pH 7.4) and thereafter mounted on gelatin-coated slides. Silver-impregnated cells, indicative of neuronal degeneration, are displayed as bright white on dark-field photomicrographs.

### Statistical Analysis

All values are expressed as mean±SD. Data were analyzed with the use of one-way ANOVA with repeated measures. Values measured after HI were compared with pre-HI values by a paired or unpaired Student’s \( t \) test analysis. Differences were considered significant if \( P<.05 \).

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**Results**

### Physiological Variables

During the period of HI, MABP and \( \text{PaO}_2 \) dropped significantly (Table 1) which, in combination with occlusion of the left CCA, established global hemispheric ischemia. Although the \( \text{PaCO}_2 \) was also lowered during HI, its value always remained in the physiological range. Reperfusion together with normoxic ventilation normalized all systemic variables.

### BOLD MRI

Continuous rapid acquisition of BOLD MR images during the onset and cessation of HI was done to obtain information on the acute cerebral hemodynamic responses. The BOLD MR SI is affected by changes in the oxygenation level of blood as deoxy-hemoglobin acts as an intrinsic (T2* shortening) contrast agent.

As a result of the induction of hypoxia, the SI on the BOLD images significantly (\( P<.05 \), relative to pre-HI value) dropped to 80% to 85% of baseline in the ipsilateral and contralateral ROIs in the parietal cortex and caudate putamen in approximately 1 minute (Fig 2A and 2B). Subsequently, we detected an elevation of the ipsilateral BOLD SI starting approximately 2 minutes after induction of HI, which was, however, not statistically significant. At the end of the HI period, the BOLD SI had returned to 90% to 95% of pre-HI values in ipsilateral and contralateral ROIs; the overshoot of the SI reasonably reflected luxury perfusion (see also LDF results). However, in the ipsilateral cortex and caudate putamen the BOLD SI showed small changes within the examined time period, pointing toward a state of sustained hypoperfusion during the first minutes of reperfusion.

### Bolus Track MRI

Repetitive T2*-sensitized MRI experiments performed in combination with intravenous bolus injections of the NMR

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**TABLE 1. Physiological Parameters in Rats Subjected to a 20-Minute-Period of Cerebral HI**

<table>
<thead>
<tr>
<th>Time After HI</th>
<th>MABP, mm Hg</th>
<th>( \text{PaCO}_2 ), mm Hg</th>
<th>( \text{PaO}_2 ), mm Hg</th>
<th>( \text{pH} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>88±12</td>
<td>43±7</td>
<td>140±27</td>
<td>7.42±0.05</td>
</tr>
<tr>
<td>15 min</td>
<td>39±10*</td>
<td>35±6*</td>
<td>41±6*</td>
<td>7.39±0.06</td>
</tr>
<tr>
<td>90 min</td>
<td>88±17</td>
<td>40±4</td>
<td>148±18</td>
<td>7.40±0.08</td>
</tr>
</tbody>
</table>

Values are mean±SD. *\( P<.05 \) vs pre-HI.
contrast agent Gd-DTPA enabled the estimation of several hemodynamic parameters. Two-dimensional brain maps of hemodynamic parameters are shown in Fig 3. More specific analysis of regional perfusion differences was done by quantification of the hemodynamic parameters in ROIs in the parietal cortex and caudate putamen shown in Table 2, which presents the ipsilateral values relative to contralateral. A critically affected brain circulation was revealed by a significantly altered ipsilateral $t_{\text{peak}}$ and $\Delta R2^{*}_{\text{max}}$ during HI (Table 2). Fig 3 demonstrates that 1 hour after the insult the perfusion status had strongly improved as a consequence of successful reperfusion. However, perfusion in the ipsilateral hemisphere was still impaired at this stage. The delayed ipsilateral $t_{\text{peak}}$ in particular reflected a diminished blood supply. Signs of hyperemia (short $t_{\text{peak}}$, elevated $rCBV$) were displayed in the ipsilateral caudate putamen. In addition, the shorter ipsilateral $t_{\text{peak}}$ compared with contralateral indicates that the maximal concentration of the contrast agent during bolus passage is reached later in the ipsilateral than in the contralateral area.

![Figure 2](image)

**Figure 2.** Relative SI on BOLD images (Rel. BOLD SI) (mean±SD) as a function of time after induction of HI. A, Relative BOLD SI vs time in the ipsilateral (●) and contralateral (○) parietal cortex. B, Relative BOLD SI vs time in the ipsilateral (●) and contralateral (○) caudate putamen. Relative BOLD SIs are expressed as percentages of pre-HI values. The large error bars during reperfusion in B are due to the high individual variation.

![Figure 3](image)

**Figure 3.** Typical examples of two-dimensional maps of a rat brain slice representing the bolus peak time ($t_{\text{peak}}$), the inverse of the maximal $\Delta R2^{*}$ ($1/\Delta R2^{*}_{\text{max}}$), and the $rCBV$ as calculated from bolus track MRI experiments at 80 minutes and 72 hours after HI. High signal intensities on the $t_{\text{peak}}$, $1/\Delta R2^{*}_{\text{max}}$, and $rCBV$ maps represent a longer bolus peak time, reduced maximal $\Delta R2^{*}$, and elevated $rCBV$, respectively. All these parameters were increased at 80 minutes after HI on the ipsilateral side (right on the images). Ipsilateral hyperemia is demonstrated after 72 hours (shortened $t_{\text{peak}}$, increased maximal $\Delta R2^{*}$, enlarged $rCBV$), especially in the caudate putamen. A longer ipsilateral $t_{\text{peak}}$ compared with contralateral indicates that the maximal concentration of the contrast agent during bolus passage is reached later in the ipsilateral than in the contralateral area. A lower ipsilateral $\Delta R2^{*}_{\text{max}}$ compared with contralateral indicates that the maximal concentration of the contrast agent during bolus passage is lower in the ipsilateral than in the contralateral area.)

to 7.5 seconds, whereas during HI ipsilateral and contralateral $t_{\text{peak}}$ values were $38.6±9.0$ and $12.6±3.9$ seconds in the parietal cortex and $27.4±8.3$ and $13.1±4.1$ seconds in the caudate putamen, respectively. Strong vasodilatation was delineated by the major elevation of the $rCBV$ to 300% to 500% of pre-HI values in both the parietal cortex and caudate putamen. This is not evident from Table 2 because it occurred in both the ipsilateral and contralateral hemispheres. A few minutes after reperfusion, significant decreases of the individual $t_{\text{peak}}$ and increases of the $\Delta R2^{*}_{\text{max}}$ values were found in all contralateral areas (mean $t_{\text{peak}}$ decreases [relative to pre-HI value]: $1.2±1.6$ and $1.7±1.8$ seconds in contralateral parietal cortex and caudate putamen, respectively; mean $\Delta R2^{*}_{\text{max}}$ increase [relative to pre-HI value]: $40.2±18.5$ and $37.3±8.8$ s$^{-1}$ in contralateral parietal cortex and caudate putamen, respectively), suggestive of an increase in intravascular flow velocity. In four of six rats hemodynamic parameter values also pointed toward postischemic hyperperfusion in the ipsilateral hemisphere (shorter $t_{\text{peak}}$ increased $\Delta R2^{*}_{\text{max}}$), whereas in two rats ipsilateral perfusion was still compromised 5 minutes after HI. At this stage, comparison between the two hemispheres revealed that the mean ipsilateral blood supply was lower than contralateral (Table 2). After a few hours the hemodynamic parameters had largely returned to pre-HI values in the cortex, while the perfusion remained diminished in the ipsilateral caudate putamen. Interestingly, after 24 and 72 hours the ipsilateral $rCBV$ and $\Delta R2^{*}_{\text{max}}$ had clearly increased, particularly in the caudate putamen. In addition, the shorter ipsilateral $t_{\text{peak}}$ compared with contralateral denoted pronounced hyperperfusion in the caudate putamen. No significant changes in any of the above hemodynamic parameters were found in rats subjected to
hypoxia without CCA occlusion or to CCA occlusion without hypoxia (data not shown).

**Laser-Doppler Flowmetry**

Although the bolus track MRI provided detailed information on ischemia-induced perfusion changes, it does not allow calculation of the absolute or relative CBF. Furthermore, bolus track MRI experiments were only performed at hourly intervals because of the relatively slow washout of the contrast agent. To compare the hemodynamic parameters deduced from the MRI experiments with continuous relative flow measurements, we performed parallel LDF studies. During HI, LDF dropped to 12-13% of baseline values in the ipsilateral and contralateral parietal cortex, respectively (Fig 4). On induction of reperfusion the contralateral LDF improved almost immediately, whereas it took a few minutes longer for the ipsilateral LDF to recover. In both hemispheres postischemic hyperperfusion occurred, peaking approximately 5 minutes after HI completion on the contralateral side and approximately 6 minutes later on the ipsilateral side. Approximately 1 hour after HI, cortical LDF had reached values of 70% to 80% of baseline in both hemispheres.

**TABLE 2. Relative Ipsilateral ΔR2*max, rCBV, and Differences in tpeak Between Ipsilateral and Contralateral Parietal Cortex and Caudate Putamen Before, During, and After HI Onset**

<table>
<thead>
<tr>
<th>Time</th>
<th>Cx</th>
<th>CP</th>
<th>Cx</th>
<th>CP</th>
<th>Cx</th>
<th>CP</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔR2*max, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36±8 min</td>
<td>0.46±0.43</td>
<td>0.66±0.46</td>
<td>98.2±17.3</td>
<td>101.1±10.7</td>
<td>109.7±22.9</td>
<td>107.2±22.4</td>
</tr>
<tr>
<td>19±0 min</td>
<td>26.82±13.02*</td>
<td>15.45±5.66*</td>
<td>15.7±5.4*</td>
<td>37.3±13.8*</td>
<td>103.7±101.0</td>
<td>149.7±87.9</td>
</tr>
<tr>
<td>25±1 min</td>
<td>3.83±7.47</td>
<td>1.41±2.03</td>
<td>60.1±40.8*</td>
<td>74.1±34.3*</td>
<td>118.4±51.0</td>
<td>106.7±26.4</td>
</tr>
<tr>
<td>86±6 min</td>
<td>1.00±0.92</td>
<td>1.40±0.46*</td>
<td>83.9±18.4*</td>
<td>74.0±14.0*</td>
<td>114.8±29.2</td>
<td>103.1±23.6</td>
</tr>
<tr>
<td>151±10 min</td>
<td>0.42±0.53</td>
<td>1.29±0.31*</td>
<td>87.6±6.7</td>
<td>70.1±14.8</td>
<td>91.8±8.6</td>
<td>85.9±9.7*</td>
</tr>
<tr>
<td>245±10 min</td>
<td>0.64±0.49</td>
<td>1.22±0.60</td>
<td>80.9±3.1</td>
<td>76.0±18.1</td>
<td>93.4±22.6</td>
<td>93.6±12.7</td>
</tr>
<tr>
<td>24±3 h</td>
<td>0.04±0.37</td>
<td>-0.66±0.46*</td>
<td>123.8±9.2*</td>
<td>189.0±33.4*</td>
<td>143.1±25.9*</td>
<td>160.4±26.4*</td>
</tr>
<tr>
<td>70±3 h</td>
<td>0.29±0.85</td>
<td>-0.86±0.81*</td>
<td>108.2±33.1</td>
<td>167.9±39.0*</td>
<td>130.1±40.3*</td>
<td>148.5±21.3*</td>
</tr>
</tbody>
</table>

Cx indicates parietal cortex; CP, caudate putamen. Percentages are percentages of contralateral value. Values are mean±SD. A positive ΔR2*max indicates that the maximal concentration of the contrast agent during bolus passage is reached later in the ipsilateral than in the contralateral ROI. A relative ΔR2*max below 100% indicates that the maximal concentration of the contrast agent during bolus passage is lower in the ipsilateral than in the contralateral ROI.

*P<.05 vs pre-HI.

**DW and T2-Weighted MRI**

Multislice quantitative DW and T2-weighted MRI were performed at regular intervals during the entire experimental protocol. DW MRI can monitor the development of acute cytotoxic edema from the associated reduction of tissue water ADC. T2 prolongation of brain tissue water, measured by T2-weighted MRI, is known to be due to vasogenic edema and is indicative of irreversible tissue damage. Fig 5 shows typical ADC maps of coronal brain slices obtained before, during, and acutely after HI. The results of region-specific quantitative analysis of the ADC maps are given in Fig 6. During HI, the ADC was significantly reduced in major parts of the ipsilateral hemisphere. ADC declines relative to pre-HI values were found in all animals in the ipsilateral dorsal hippocampus, parietal cortex, and caudate putamen and in...
eight and seven of 10 rats in the ipsilateral thalamus and substantia nigra, respectively. Within 1 hour after reperfusion in combination with normoxia, the ADC normalized completely in the hippocampus, parietal cortex, and caudate putamen followed by a small temporary overshoot (see Fig 6). However, in some regions, typically the ipsilateral substantia nigra and thalamus, the mean ADC continued to progressively decline directly after HI and only partially recovered at later time points. There were no significant T2 changes in the brain during the first hours after HI; nevertheless, the T2 in the substantia nigra and thalamus showed a tendency to increase during these acute stages (Fig 7). ADC (and T2) maps revealed no significant alterations in these parameters in the brains of sham-operated rats (data not shown).

Fig 8 demonstrates that 24 and 72 hours after the HI period all examined ipsilateral areas exhibited ADC and T2 changes (see also Figs 6 and 7). The brain water ADC was significantly reduced in the ipsilateral dorsal hippocampus, parietal cortex, and caudate putamen 24 hours after HI. In addition, the T2 was significantly prolonged in these areas (see also Fig 7). In the substantia nigra and thalamus these alterations were less evident; in 27% of the animals at 24 and 72 hours after HI explicit changes were even absent in these areas. After 72 hours the tissue changes on the ADC (Figs 6 and 8) and T2 maps (Fig 7 and 8) were more pronounced, and the T2 values in particular had further increased. Interestingly, at this stage aberrations were also seen in white matter areas (typically the corpus callosum and external capsule). These areas were characterized by very high ADC and long T2 values, presumably as a result of vasogenic edema. Ventricular dilatation and small but significant ADC reductions and T2 elevations were also detected in the contralateral hemisphere during these stages (Figs 6, 7, and 8).

Histology
Histological analysis was done after the MRI experiments at 24 and 72 hours after HI and clearly revealed ipsilateral tissue damage. Silver-stained areas, indicative of neuronal degeneration, matched with areas with ADC and T2 abnormalities (Fig 9). Silver staining was most pronounced in the areas with highly increased T2 values, typically the dorsal hippocampus, parietal cortex, and caudate putamen. The silver staining was less intense in the substantia nigra and thalamus.

Discussion
In this study we assessed the ongoing brain perfusion and tissue response after 20 minutes of HI in rats to gain a better understanding of region-specific tissue susceptibility and delayed damage after a transient ischemic episode. Multiparametric MRI, LDF, and histological data revealed a sequence of alterations that dynamically varied with time and between different brain structures.
Hemodynamics

Critical flow alterations in the ipsilateral hemisphere were clearly demonstrated by the bolus track MRI data and LDF measurements. Unilateral occlusion of the CCA in combination with hypoxic ventilation gave rise to a severe ipsilateral perfusion reduction. In the contralateral hemisphere we observed moderate hypoperfusion. Immediate tissue deoxygenation was evident from the rapid BOLD MRI experiments. Interestingly, we detected a rapid partial recovery of the ischemia-induced BOLD SI reductions, which has also been found during acute focal ischemia in the rat.36,37 Although the exact mechanism responsible for this is unknown, factors involved could be secondary changes in the microcirculation, a decline in the oxygen extraction fraction, or vasodilatation, which all decrease the local amount of deoxygenated hemoglobin. In addition, the regional decrease in paramagnetic tissue oxygen could counterbalance the increase in paramagnetic deoxyhemoglobin, as suggested by Hossmann and Hoehn-Berlage.38 After induction of reperfusion, recovery of the BOLD MR SI reflected reoxygenation. Luxury perfusion emerged readily in the first 10 to 20 minutes of reperfusion in both hemispheres. Thereafter, a state of mild hypoperfusion was evident in cortical and striatal areas, which persisted for several hours and was most pronounced in the caudate putamen. These findings are in agreement with those from other studies that also described posts ischemic early reactive hyperemia and delayed hypoperfusion and that were associated with changes in metabolic activity.15,16,39 Finally, at 24 and 72 hours after HI we detected hyperemia in the ipsilateral caudate putamen that could be due to reported increased levels of lactate, acting as a local vasodilator, and autoregulatory dysfunction in this area.40

Tissue Parameter Changes

The drastic HI-induced perfusion deficit was accompanied by a drop of the tissue water ADC in all investigated ipsilateral brain regions. Acute ADC reductions have been described in several experimental ischemia models as well as in human brain ischemia and are typically associated with the development of cytotoxic edema.35–37,41 Cytotoxic edema or cellular swelling is a direct result of the loss of ionic gradients due to ischemia-induced energy failure that develops below a CBF threshold level of approximately 20% to 25% of the baseline value in acute ischemia.42 Accordingly, similar CBF threshold levels have been reported for ADC reductions in early ischemia.25,43–45 Perfusion reduction in the contralateral hemisphere was insufficient to result in significant ADC changes. Reperfusion of the brain completely normalized the ADC in the ipsilateral dorsal hippocampus, parietal cortex, and caudate putamen within 1 hour. In the substantia nigra and thalamus, however, the mean ADC remained below baseline values, suggesting irreversible tissue impairment. This was confirmed by significant ADC reductions, T2 elevations, and silver staining in these areas after 24 and 72 hours. Still, also in...
regions where the ADC initially normalized, severe tissue injury was demonstrated after 24 and 72 hours. At these time points, small but significant ADC decreases and T2 elevations and ventricular dilatation occurred in the contralateral hemisphere. Whether this was directly caused by the moderate contralateral hemodynamic changes or was an indirect result of the comprehensive ipsilateral changes, such as hemispheric swelling, remains unclear.

Delayed Brain Damage

Delayed neuronal damage and/or reperfusion-induced secondary injury have been frequently observed in both experimental and clinical studies of cerebral ischemia. The exact mechanisms underlying delayed/secondary damage, however, are still unclarified. Possible factors involved include sustained impairment of calcium homeostasis, long-term glutamate accumulation, reflow-induced formation of free radicals, secondary hypoperfusion, secondary impairment of mitochondrial function, and apoptosis. The reappearance of reduced tissue water ADCs after 24 and 72 hours in our study suggested cytotoxic edema due to secondary energy depletion and dissipation of ion gradients. Accordingly, Ordidge et al recently found a correlation between delayed ADC reductions and secondary loss of high-energy phosphates in a neonatal model of HI. Secondary energy failure is a well-known phenomenon in perinatal HI encephalopathy, and the extent of the delayed impairment of energy metabolism has been shown to correlate with the magnitude of the cerebral infarction. Despite the initial recovery of mitochondrial function and high-energy phosphate levels on early reperfusion, secondary energy failure could definitely also be a major factor responsible for delayed damage in adult brain. In our study, postischemic hypoperfusion did not reach threshold levels known to cause energy failure in normal, nondisturbed brain tissue. However, a mismatch between CBF and metabolic rate could arise in hypermetabolic postischemic brain. Secondary processes, such as the protective mechanisms against the massive loss of calcium homeostasis, activation of free radical scavenging enzymes, and apoptosis-related protein synthesis, possibly lead to high glucose and oxygen demands that may not be met by the compromised perfusion status.

Region-Specific Sensitivity

Certain brain regions appeared more susceptible to an ischemic insult than other areas. In the hippocampus, cortex, and caudate putamen, the frequency of the occurrence of primary ischemia-induced ADC changes was higher than in the substantia nigra and thalamus. The manifestation of delayed damage was also more prominent in the former areas. Since ADC reductions in ischemia only develop below a certain CBF threshold, the above suggests that in the substantia nigra and thalamus perfusion levels occasionally would not be reduced to below threshold levels for ischemic damage. Selective vulnerability has been associated with heterogeneities in local CBF. In fact, our results demonstrated differences in ischemic and postischemic perfusion between cortical and striatal areas. Unfortunately, in our study we could not compare the perfusion status in these areas to those in the hippocampus, thalamus, and substantia nigra. However, previous studies have shown that CBF reductions during global ischemia are less drastic in the diencephalic thalamus and substantia nigra than in forebrain regions (dorsal hippocampus, cortex, and caudate putamen). Nevertheless, if an early ADC drop occurred in the substantia nigra or thalamus, the reduction was profound and irreversible, in contrast to the reversible changes in the other investigated regions. This may imply a higher intrinsic susceptibility to ischemia for the thalamus and substantia nigra. We believe that the region-specific response to ischemia is associated with heterogeneities in local ischemic and postischemic hemodynamics. However, other factors such as differences in postsynaptic organization, differences in time needed for exposure to cumulative threshold levels of glutamate, and variations in mitochondrial capacity and in the activity of free radical defense systems.
may also be involved. Such factors could be the cause of the differences in intrinsic susceptibility between brain regions apart from the hemodynamic component.

In conclusion, in this study heterogeneous tissue susceptibility and delayed damage after transient HI in rat brain were demonstrated by means of in vivo MRI. Areas exhibiting ADC reductions during the ischemic episode were invariably injured in the chronic phase, despite early postschismic recovery on perfusion. For the clinical situation, this may imply that early restoration of the blood supply in acute stroke (eg, by thrombolysis) should be combined with cytoprotective therapy to reduce postschismic secondary damage. Clinical studies evaluating our findings, in which (DW) MRI could play an essential role, are eagerly awaited.

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References

It is well recognized that brief periods of cerebral ischemia resulting from either a transient disruption of the blood supply or a period of hypoxia can lead to delayed tissue damage. This delayed infarction may occur days or even weeks after the initial insult, depending upon the nature and the site of the insult. With the advent of therapies that are efficacious in acute stroke (especially thrombolitics), there has been a resurgence of interest in secondary or delayed damage as a potential therapeutic target. Indeed it seems likely that protection against the acute ischemia-induced damage may serve to unmask secondary mechanisms that lead to eventual cell death.

In this article the authors exploit a number of MRI approaches to assess changes in tissue status and perfusion that result from 20 minutes of hypoxia-ischemia in the rat brain. Using diffusion-weighted MRI, they show that a decline in ADC of the brain water occurs in all brain regions exposed to the insult; however, within 1 hour after reperfusion, the ADC had returned to normal in the hippocampus, cortex, and caudate putamen, but not in the thalamus or substantia nigra. But the seminal observation of this study is that secondary mechanisms that lead to eventual cell death.

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