A New Model of Localized Ischemia in Rat Somatosensory Cortex Produced by Cortical Compression

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Background and Purpose—Because of its precise connectivity and functional specificity, the rat whisker-barrel system offers an excellent opportunity to study experience-dependent neuroplasticity. However, data are lacking regarding the neuroplasticity of this system after cerebral ischemia. The purpose of the present study was to develop a reproducible model for the production of ischemia/reperfusion of the posteromedial barrel subfield (PMBSF) in the rat, which is the visible representation of the large whiskers on the opposite face.

Methods—Focal cortical ischemia was induced in male Sprague-Dawley rats (n=40) by slowly compressing the intact dura (maximum 0.05 mm/s) with a 4- or 5-mm-diameter brass cylinder equipped with a laser-Doppler probe, combined with ipsilateral common carotid artery occlusion. The microvascular blood flow of PMBSF during compression ischemia was maintained at 18% to 20% of baseline flow for 1 hour. The total infarction volume was measured by 2,3,5-triphenyl-tetrazolium chloride staining at several reperfusion times, and pathological examination was performed on hematoxylin-eosin–stained sections.

Results—The infarct volumes were 36.5±9.2 (n=9), 40.7±7.7 (n=7), and 36.6±6.4 mm³ (n=5) at 24 hours, 72 hours, and 7 days after ischemia, respectively, with no significant differences among these values. There was no evidence of damage to white matter or to deep gray matter and no evidence of hemorrhage. The topographic distribution of the damaged tissue was in good agreement with that of PMBSF.

Conclusions—This stroke model produces a highly consistent cortical infarct in PMBSF and can facilitate the study of behavioral, functional, and structural consequences after cerebral ischemia/reperfusion in the rat somatosensory cortex. (Stroke. 2001;32:2615-2623.)

Key Words: cerebral blood flow ■ cerebral ischemia ■ neuronal plasticity ■ stroke, experimental ■ somatosensory cortex ■ rats

There has been recent interest in functional recovery after focal cerebral ischemia with evidence, primarily for ischemia involving primary motor cortex, for significant neuroplasticity in rats,1–4 monkeys,5 and humans.6–8 It is possible that the somatosensory cortex also has the potential for recovery of function after cerebral ischemia/reperfusion.9

The rat whisker-barrel system offers an excellent opportunity for the study of the mechanism of experience-dependent cortical plasticity.10–12 However, research into the lesion-induced plasticity of this system, particularly ischemic insult–induced functional plasticity, is limited by the lack of a reliable ischemia/reperfusion model localized to the posteromedial barrel subfield (PMBSF) in the rat.

The general models of middle cerebral artery (MCA) occlusion, such as the distal MCA occlusion model13 or the intraluminal suture model,14 usually produce relatively large infarcts extending beyond the somatosensory cortex. The suture model even includes damage to the caudoputamen. On the other hand, the ministroke model15 involves ligation of 3 to 6 branches of the MCA but does not provide an infarct large enough to include the entire PMBSF. Although a precise localized cortical infarct can be induced photochemically,16,17 controlled reperfusion is not feasible. Since the photochemically induced ischemia model is insensitive to therapies based on perfusion enhancement, it may not be appropriate for the study of functional recovery.

In the present study we investigated a simple, reproducible model to produce infarcts confined to the somatosensory cortex. Temporary ischemia is produced by compressing the intact dura over the somatosensory cortex with a cylindrical device.

By careful choice of the size of the cylinder and of the location and degree of compression, ischemia can be confined...
Materials and Methods

Animal Preparation

All procedures performed on animals were approved by the University Institutional Animal Care and Use Committee of the University of Pennsylvania. A total of 40 male Sprague-Dawley rats (weight, 306 to 438 g) obtained from Charles River Laboratory (Wilmington, Mass) were used in this study. They were anesthetized with halothane (4% for induction and 0.6% to 1.0% for surgical procedure) in a mixture of 70% nitrous oxide and 30% oxygen. Each rat was allowed to breathe spontaneously. The body temperature was monitored by a rectal probe and maintained at 37.0±0.5°C with a heating blanket regulated by a homeothermic blanket control unit (Harvard Apparatus Limited).

A polyethylene catheter (PE-50) was placed into the tail artery, and arterial pressure was continuously monitored and recorded on a Grass polygraph recorder (model 7D; Grass Instruments). Samples for blood gas analysis (arterial pH, PaO2, and PaCO2) were taken from the tail artery catheter during the ischemic period and measured with a blood gas analyzer (BG 3; Instrumentation Laboratory). The right common carotid artery (CCA) was isolated, and loops made from a polyethylene catheter (PE-10) were carefully passed around the CCA for later remote occlusion.

Laser-Doppler Flowmetry

The microvascular blood flow in the right primary somatosensory cortex was continuously monitored with a laser-Doppler flowmeter (LDF) (PeriFlux 4001; Perimed). The animal’s head was positioned in a stereotaxic frame, and a right parietal incision between the right lateral canthus and the external auditory meatus was made. With the use of a dental drill constantly cooled with saline, a 6-mm burr hole, the center of which was located at 6 mm lateral to the sagittal suture and 2 mm caudal to the coronal suture, was made over the primary somatosensory cortex. The bone at the burr hole was completely removed, and the dura was kept intact. A needle-type thermistor electrode (HH23; Omega) was placed at the edge of the burr hole, which was continuously perfused with saline. The brain temperature was maintained at 36.5±0.5°C by an infrared heating lamp.18,19 The LDF probe (tip diameter of 1 mm, fiber separation of 0.25 mm) was fixed in a brass cylinder (4 or 5 mm in diameter and 18 mm in length). The cylinder, held by a micromanipulator, was placed perpendicular to the exposed dura just touching the dura (Figure 1).

Care was taken to position the laser-Doppler fibers of the flow probe over a tissue area devoid of large (＞75 μm) blood vessels.20 One technique for avoiding large vessels was to rotate the cylinder; since the flow probe was slowly raised to the baseline position (just touching the dura), this had the effect of moving the fibers of the probe without displacing the cylinder with respect to the barrel cortex. If this did not permit large vessels from being avoided（≈10% of the animals）, the entire cylinder was moved. Movement was rarely >0.3 mm, but in a few animals it was up to 0.5 mm. If greater movement was necessary, the animal was not used in the study.

Focal Cerebral Ischemia

Focal cerebral ischemia was produced by compression of the right primary somatosensory cortex combined with ipsilateral temporary CCA occlusion. After right CCA occlusion with the remote occluder, the cylinder was gradually advanced onto the brain (2 to 3 mm) at a speed of 0.05 mm/s until the cerebral blood flow (CBF) was reduced to 18% to 20% (groups A and B; see below) or 30% to 35% (group C; see below) of the value before CCA occlusion. CBF was maintained at these values for 60 minutes by adjusting the micromanipulator. After the period of compression ischemia, the cylinder was slowly raised to the baseline position (just touching the dura), and the cataroid loop was released. CBF was monitored for 10 minutes of reperfusion. The tail arterial catheter was removed, and all of the wounds were sutured. Gentamicin sulfate (10 mg/mL; SoloPak Laboratories) was topically applied to the wounds to prevent infection. The animals were returned to their cages after awakening, and water and food were allowed ad libitum.

Protocol

The rats subjected to compression ischemia were divided into 4 groups according to the size of brass cylinder used (groups A and B) and according to the degree of cerebral ischemia (groups C and D).

To examine the effect of reperfusion time on the infarct volume, group B rats were further subdivided into 3 groups (groups B-1, B-2, and B-3). Furthermore, we added group D, in which ischemia time was limited to <2 minutes and the rate of brain compression was the same as that in the other groups. Groups were as follows: group A (n=7): 4-mm cylinder, CBF=18% to 20% of baseline, 24 hours of reperfusion; group B-1 (n=11): 5-mm cylinder, CBF=18% to 20% of baseline, 24 hours of reperfusion; group B-2 (n=7): 5-mm cylinder, CBF=18% to 20% of baseline, 72 hours of reperfusion; group B-3 (n=5): 5-mm cylinder, CBF=18% to 20% of baseline, 7 days of reperfusion; group C (n=5): 5-mm cylinder, CBF=30% to 35% of baseline, 24 hours of reperfusion; and group D (n=5): 5-mm cylinder advanced 3.0 mm into the brain at a rate comparable to the other groups, ischemia limited to 2 minutes, 24 hours (n=2) and 7 days (n=3) of reperfusion.

Morphometric Measurement of Damaged Volume

The rats were killed with an intraperitoneal injection of pentobarbital sodium (150 mg/kg) after reperfusion. Either 2,3,5-triphenyltetrazoli-um chloride (TTC) staining or hematoxylin-eosin (H&E) staining was performed to calculate the infarct volume. For reperfusion times of 0.5 and 7 days, groups B-1 and B-2 and group B-3. TTC staining was used for the measurement of infarct volume, while for the 7-day reperfusion time (group B-3), H&E staining was used. Two of the rats in group B-1 were stained with H&E for pathological examination.

The brains from animals to be stained with TTC were carefully removed from the skull and cooled in ice-cold PBS for 15 minutes to facilitate cutting. They were sectioned in the coronal plane at 1-mm intervals with the use of a rodent brain matrix (RB-4000C; ASI Instruments). The brain slices were incubated in PBS containing 2% TTC (Sigma) at 37°C for 10 minutes. The TTC-stained sections were photographed from the posterior surface of the slices with a digital camera (C-900 ZOOM Olympus) equipped with a macro lens (Raynox). The damaged area was determined at each cross-sectional level selected by reference to the corresponding level in an atlas of the rat brain with the use of a computer-based image analyzer (NIH Image, version 1.59). To avoid artifacts due to edema, the damaged area was calculated by subtracting the area of the normal tissue in the hemisphere ipsilateral to the stroke from the area of the hemisphere contralateral to the stroke.21 The total volume of infarct was calculated by summation of the damaged volume of the brain slices.

In animals to be stained with H&E, the brains were removed similarly and stored in 10% buffered formalin acetate for at least 2 days. After fixation, brains were embedded in paraffin, and 5-μm
sections were obtained at 250-μm intervals and stained with H&E. The sections were imaged under a light microscope at 31.25-fold magnification and digitized with a charge-coupled device camera (SPOT version 3.0.4, Diagnostic Instrument Inc). Areas of damaged brain tissue along with left and right hemispheric areas were delineated with the image analyzer. Criteria used to determine brain cell damage were similar to those used by Dereski et al. Briefly, if the area consisted of a majority of either eosinophilic neurons or ghost neurons with no nucleus and loss of all cellular detail (coagulative necrosis) or cavitation with neuronal loss, these areas were defined as damaged tissue. The infarction areas were summed and multiplied by the distance between slices to give the infarct volumes. Correction for shrinkage due to processing was made by comparing undamaged hemispheric areas on H&E-stained sections with those on TTC-stained sections at 3 different levels separately (−1.3, −2.3, and −3.3 mm from bregma) as follows. The mean areas of the undamaged (left) hemisphere stained with TTC in groups A, B-1, and B-2 at these 3 levels were calculated. These areas were divided into the corresponding left hemispheric area in the H&E-stained animals (group B-3), resulting in a ratio at 3 levels for each rat. The values obtained were averaged, and a shrinkage coefficient for volume correction was individually calculated from the following equation:

\[
SC_i = \left( \frac{\sum_{j=1}^{i} A_{H&E,j} A_{TTC,j}}{3} \right)^{1/2}
\]

where \(SC_i\) is volumetric shrinkage coefficient for animal \(i\), \(A_{H&E,j}\) is area of the undamaged hemisphere determined from H&E-stained sections of animal \(i\) at level \(j\), and \(A_{TTC,j}\) is the mean area at level \(j\) of the undamaged hemisphere in the TTC-stained section. The corrected infarct volume of each animal was obtained by dividing the measured infarct volume by the shrinkage coefficient of that animal. The precise location of the damage with respect to the location of PMBSF was made from the H&E-stained sections (group B-3). Although individual barrels could not be discerned from these 5-μm-thick sections, the PMBSF boundaries could be determined. The general position of the PMBSF in the H&E-stained coronal sections was determined from coronal section figures taken from the atlas of Paxinos and Watson (labeled SIBF). Determination of the PMBSF boundaries was facilitated by the significant contrast in layer IV cell density between the PMBSF (dense accumulation of granule cells; granular neocortex) and the neocortical areas bordering the PMBSF (no granule cells; agranular cortex or much less dense granule cells; dysgranular cortex).24,25

### Topographic Distribution of Infarct Tissue

To examine the distribution of infarcted tissue with respect to PMBSF, we reconstructed the areas of PMBSF and infarct tissue in each section. This was accomplished by measuring the distance from the superior aspect of the brain at the midline to the 2 edges of the infarct as determined from the stained section. These measurements were made at each level, permitting the reconstruction of the infarct area and comparison of the extent of PMBSF from the rat brain atlas.23

### Statistical Analysis

The data are expressed as mean±SD. The significance of differences between groups was determined with Student’s \(t\) test for comparison of pushing distance. ANOVA followed by post hoc comparison tests to probe for the source of the differences was used for the remaining data, with Bonferroni correction made for multiple comparisons.

### Results

#### Physiological Variables

Data on animal body weight, blood gas analyses, and mean arterial blood pressure (MABP) are summarized in Table 1. No significant differences were detected in animal body weight among the groups. The values of arterial blood gases were within physiological range, with no significant difference among the groups. MABP remained fairly constant throughout the experiment, and there were no significant differences in MABP before and during compression ischemia in each group. There were also no significant differences in MABP among the groups.

### Pushing Distance and Size of Compression Cylinder

The original studies were done with the 4-mm-diameter cylinder chosen because it was close to the size of PMBSF. Since by the end of the ischemia the distance that the cylinder was advanced into the brain to produce a CBF of 18% to 20% of baseline was 3.43±0.19 mm, it was decided to switch to a 5-mm cylinder, with which the compression distance was only 2.84±0.41 mm by the end of ischemia \(P<0.01\); Figure 2). There was no difference in pushing distance between groups in the group B animals (5-mm-diameter cylinder). The pushing distance for the animals in which CBF was reduced to 30% to 35% of control (group C) was 2.74±0.74, which was not significantly different than for the animals in which CBF was reduced to 20% of control.

#### LDF Measurement

Figure 3 demonstrates the relative blood flow before, during, and after 60 minutes of compression ischemia in groups B-1 and B-2 (\(n=18\)). After right CCA occlusion, CBF immediately decreased to 70% to 80% of the baseline value. During the first 4 to 5 minutes of compression, CBF tended to dip

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### TABLE 1. Physiological Parameters

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight, g</th>
<th>pH</th>
<th>(P_{aO_2}), mm Hg</th>
<th>(P_{aCO_2}), mm Hg</th>
<th>MABP, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>A ((n=7))</td>
<td>350±36</td>
<td>7.409±0.033</td>
<td>103±15</td>
<td>40.5±2.8</td>
<td>91±8</td>
</tr>
<tr>
<td>B-1 ((n=11))</td>
<td>371±37</td>
<td>7.397±0.027</td>
<td>96±12</td>
<td>40.6±6.0</td>
<td>98±9</td>
</tr>
<tr>
<td>B-2 ((n=7))</td>
<td>366±29</td>
<td>7.389±0.015</td>
<td>93±16</td>
<td>42.9±5.5</td>
<td>93±9</td>
</tr>
<tr>
<td>B-3 ((n=5))</td>
<td>341±33</td>
<td>7.383±0.032</td>
<td>109±13</td>
<td>42.9±6.9</td>
<td>96±8</td>
</tr>
<tr>
<td>C ((n=5))</td>
<td>358±24</td>
<td>7.419±0.047</td>
<td>111±14</td>
<td>43.1±7.1</td>
<td>95±7</td>
</tr>
<tr>
<td>D ((n=5))</td>
<td>359±31</td>
<td>7.408±0.023</td>
<td>101±4</td>
<td>41.6±4.4</td>
<td>98±7</td>
</tr>
</tbody>
</table>

Values are mean±SD. Blood gas data at 30 minutes after start of compression ischemia.
slightly below 20% of baseline value and to fluctuate between values of 15% and 30%. Accordingly, a finer adjustment of the micromanipulator was required. After approximately 10 minutes of ischemia, CBF was easily maintained at 18% to 20% of baseline and fluctuated by 2.6% to 5.1% over the ischemic period (Table 2). To maintain CBF at this level throughout 60 minutes, however, it was necessary to increase the amount of compression by 1.15/H110060.36 mm (Figure 3).

Reversal of compression and right CCA occlusion after 60 minutes of ischemia led to hyperemia (179/H1100642% of preischemic baseline), which lasted for the period of observation. In some animals (n=7) followed for 30 minutes of reperfusion, CBF remained significantly above the preischemic level, at a level very similar to the CBF after 10 minutes of reperfusion. All experimental groups showed comparable degrees of ischemia (Table 2.).

Pathological Findings of Barrel Field in H&E Staining

H&E-stained sections 1 day after compression ischemia showed complete coagulative necrosis and cavitation with severe edema involving the whole cortical layer (Figure 4). The majority of neurons appeared shrunken with eosinophilic cytoplasm and pyknotic nuclei. However, no change in vascular morphology was detected including hemorrhage or hematoma, and no significant histological change was detected in the piriform cortex contralateral to the site of compression. H&E-stained sections 7 days after compression ischemia showed a well-demarcated necrosis with massive infiltration by inflammatory cells. Light microscopic examination of both 7-day and 1-day H&E-stained sections indicated no obvious damage to subcortical white matter. At both intervals, the demarcation between normal and abnormal tissues was sharp, without the interpolated zone of neuronal ischemia lacking tissue necrosis that we have customarily observed in other cerebral infarction models.26,27 The animals subjected to a brief period of ischemia (group D) demonstrated no significant damage throughout the whole cortical thickness except for the presence of a small number of nonspecific dark neurons in cortical layer I-II. Only in 1 animal (from group B-3), in which the infarct volume was small compared with the other animals (25.4 mm3 ), was there a suggestion of a small area of densely aggregated layer IV cells that were spared by the infarct. When compared with the size and density of layer IV cells in the same neocortical region on the contralateral (control) side, it strongly suggested that the spared area contained stellate cells associated with the PMBSF barrels. Because of the small size of the spared area, this suggests that it may represent the sparing of a single solid barrel or, at most, 2 such barrels. In a few cases, a small area of dense aggregated layer IV cells was spared at the rostral portion of the PMBSF and may have spared the rostral-most solid barrels or the caudal-most hollow barrels associated with the rostral sinus hairs on the face. The section thickness (5 um) and the staining method used (H&E) would not permit resolution of the type of barrel(s) spared in that rostral border zone.
Infarct Volume

To compare infarct volumes in the animals stained with H&E and TTC, it was necessary to calculate shrinkage due to processing of the H&E sections; this was accomplished by examining the control (left) hemisphere in both groups. Since no pathology was found in the opposite hemispheres (see above), these served as controls in this study. The values of hemispheric area in the undamaged side were relatively constant among the TTC-stained rats. The mean areas of this hemisphere in the TTC-stained sections were 67.0±2.1, 73.9±3.9, and 79.5±3.1 mm² (n=16) at 1.3, 2.3, and −3.3 mm from bregma, respectively. The shrinkage coefficient in the 5 rats of group B-3 ranged from 0.60 to 0.88, and the shrinkage coefficient–corrected infarct volume ranged from 25.4 to 41.1 mm³ (mean, 36.6±6.4 mm³). The infarct areas in the TTC-stained sections were mainly distributed from the level of −0.3 mm to −4.3 mm from bregma (Figure 5). Figure 6A demonstrates the effect of reperfusion time on infarct volume in the group B rats. No significant differences in infarct volume were detected when we compared days 1, 3, and 7. At 24 hours of reperfusion the infarct volume measured by TTC staining in the studies using the 5-mm-diameter cylinder (36.5±9.2 mm³; group B-1) and the 4-mm-diameter cylinder (37.0±11.4 mm³; group A) are almost identical (Figure 6B). In contrast, the infarct volumes in group C (CBF ≈30% to 35% of baseline) rats were significantly smaller and fairly variable (6.3±6.2 mm³), and no infarct was seen in the animals that were compressed in a manner similar to groups A and B, but in which the ischemia was limited to <2 minutes (group D).

Topographic Distribution of Infarct Tissue

The reconstructed figures derived from tissues of 16 infarcted rats demonstrated a good agreement between the area of PMBSF and the individual infarct areas (Figure 7). In 6 rats (37.5%) the center of infarct was shifted slightly posteriorly,
resulting in enlargement of the infarct posterior to PMBSF. The overlap between PMBSF and the area of infarct was 76.1±9.6%, and the percentage of the barrel field that was infarcted was 92.0±9.2%.

**Discussion**

We have developed a new reproducible, localized ischemia/reperfusion model to produce focal damage to the S1 barrel field of the rat. During the development of this model it was necessary to examine whether traumatic, as opposed to ischemic, injury was a component of this model. In addition, it was important to investigate a number of factors, including the size of the compression cylinder and the magnitude of the compression necessary for reproducible damage, and to characterize the damage produced in this model.

To achieve the desired CBF reduction (18% to 20% of control) throughout the ischemic period, it was necessary to depress the cerebral cortex by approximately 3 mm. The possibility of traumatic cortex by approximately 3 mm. The possibility of traumatic brain injury should be examined as the dominant mechanism for tissue injury, since many models of traumatic brain injury involve compression of brain tissue. Traumatic brain injury models compress cortical brain tissue from 0.2 to 4.8 mm at a rate of 15 to 1700 mm/s, whereas in our model the brain is compressed at a rate of only 0.05 mm/s. We believed that traumatic damage would not occur if the brain were compressed slowly. Histological examination by H&E-stained sections obtained 24 hours and 7 days after compression ischemia indicates that the tissue damage is consistent with ischemia/reperfusion injury rather than a traumatic brain injury. There were no subarachnoid hemorrhages or subdural hematomas observed by macroscopic examination, and no evidence of hematoma in either the cortex or the white matter was detected in the 1-day H&E-stained sections, which are frequently seen in general experimental models of traumatic brain injury. Additional evidence of an inflammatory process, such as the presence of neutrophils, without the initial breakdown of the vasculature suggests that local reperfusion occurred after compression ischemia. The observation that the animals in group D did not exhibit any significant damage throughout
the cortex except for the presence of a few nonspecific dark neurons in cortical layer I-II in some animals indicated that the damage is likely due to ischemia and not trauma. Furthermore, the small infarcts observed in group C (CBF reduced to only 30% to 35% of control) support the conclusion that focal ischemia/reperfusion is the dominant pathophysiological mechanism in this model since the tissue compression was comparable in both amplitude (2.7 to 2.8 mm) and time of compression (60 minutes). No significant histological damage was detected in the contralateral piriform cortex, suggesting that there was no obvious “contra- trencoup” mechanism as is frequently seen in cerebral trauma.

The basic mechanism of CBF reduction seems to be compression of the vascular lumen under the brass cylinder. However, the possibility remains that the initial compression insult increases vulnerability to ischemia, and damage occurs at levels of local CBF not conventionally defined as sub-threshold.33 Possible mechanisms of increased vulnerability include energy failure in cells damaged by trauma and the effects of excitotoxins released by neurons damaged at the time of the initial insult.34 Although there is ample evidence that trauma to the brain and spinal cord produced by the standard trauma models produces profound changes in intracellular calcium, cytokines, gene expression, intracellular proteases, and neurotrophic factors, there is limited evidence in the literature about the effect of tissue compression or stretching at a rate and magnitude comparable to that used in this study. In a study examining the mechanical and electric responses of the squid giant axon to elongation, rapid elongation (5 mm/s or 31% length per second) produced a transient loss of action potentials, whereas slow elongation (0.05 mm/s, similar to the rate of compression in the present study) resulted in only negligible changes in membrane potential.35 If NTera2-neurons (NT2-N) cells are subjected to slowly applied fluid shear stress, no change in cytosolic calcium is elicited, whereas high strain rates produce rate-dependent changes in acute cytosolic calcium and lactate dehydrogenase release.36 Evidence that a slow rate of tissue deformation does not produce damage and does not increase the susceptibility of the tissue to damage by concomitant factors such as hypoxia comes from the studies of Cargill and Thibault.37 They found that when NG108-15 cells are plated onto elastic membranes that can be deformed in a prescribed fashion, no changes in intracellular calcium are found at low rates of deformation, whereas significant changes in intracellular calcium occur when the strain rate is high. If the tissue is made hypoxic during low rates of deformation, changes in intracellular calcium are comparable to those seen in un-stressed cells. These data indicate the relative importance of the dynamics of tissue deformation in traumatic injury. Further detailed studies must be undertaken, however, to determine what factors, independent of the ischemia, may contribute to tissue susceptibility and damage in this model.

TTC staining is widely used in animals euthanatized up to 3 days after an ischemic insult.38–40 There is some controversy about the use of TTC staining in sections obtained 7 days after an ischemic insult. Some investigators report that infarct volumes estimated by TTC staining did not change between 1 day and 7 days after MCA occlusion41 and that the extent of striatal and cortical infarction in TTC-stained sections at 7 days after MCA occlusion correlates well with corresponding H&E-stained brain sections.42 However, TTC staining may underestimate infarct volume as a result of the presence of active glial cells and macrophages 7 days after ischemic insults.32 Glial cells surrounding the damaged tissue and macrophages with intact mitochondria that infiltrate the infarcted region both contain enzymes that can reduce TTC and thus cause the tissue to falsely stain red.43 Even in the H&E-stained sections, loss of tissue through macrophage phagocytosis and cellular shrinkage or crenation may lead to underestimation of infarct volume in the chronic phase.44 However, the effect of tissue loss in the H&E sections was not prominent 7 days after MCA occlusion.45 Accordingly, we used H&E staining to examine infarct volume for the rats killed 7 days after compression ischemia. Infarct volumes in TTC-stained and H&E-stained sections were measured by the indirect method to minimize the effect of edema that must be taken into consideration after an ischemic insult.21,32 Edema reaches a maximum at approximately 3 days after occlusion and then decreases gradually over the next 1 to 2 weeks.46

In general, there is a good correlation between infarct volume measured with TTC and that observed from H&E-stained sections from 1 to 2 days after an ischemic insult.32,43,47,48 Quantitative comparison of TTC and H&E sections must be done with care because of possibility of shrinkage of the section during processing. It was therefore necessary to correct the H&E data with the use of a shrinkage coefficient derived from the hemisphere contralateral to the insult, which was identified as normal by both techniques. Although the shrinkage coefficient ranged widely from 0.65 to 0.88 in the group A animals, the corrected infarct volume in H&E sections 7 days after compression ischemia was almost identical to that in TTC-stained sections obtained 3 days after compression ischemia. This suggests that the infarct volume remained constant over the first week after compression ischemia.

We wanted the infarct volume to be slightly larger than the volume of PMBSF to ensure that the entire barrel field was made ischemic. The areas surrounding the PMBSF involved by the infarct are the less cell-dense agranular and dysgranular (area rostral to PMBSF)49 neocortices. It is very unlikely that their damage would effect any behavioral responses associated with this PMBSF ischemic model, and thus they would not compromise the present results. Chapin and Lin50 have shown that the neurons contained within the area rostral to PMBSF possess relatively high-threshold cutaneous response characteristics with large receptive fields and low-threshold responses associated with activation of individual PMBSF vibrissae. Although the infarct volumes (mean, 36.5 mm³) were generally larger than the estimated volume of PMBSF (∼30 mm³), the topographic distribution of damage is of greater importance in this model.

Overall, the topographic distribution of damaged tissue appears to correspond well with the distribution of PMBSF. However, the overlap is not precise in some animals for a number of reasons. One reason is the difference in shape between PMBSF and the compression cylinder. The shape of PMBSF is relatively irregular, being closer to a rectangle,
whereas the cylinder was circular. Even if the cylinder was successfully placed over the center of the barrel field, the corners of the PMBSF may be spared from damage. The use of an oval compression probe may have been advantageous.

Another reason for the mismatch between the ischemic area and the barrel field is the technique for probe/cylinder placement. Although attempts were made to place the cylinder at 6 mm lateral to the sagittal suture and 2 mm caudal to the coronal suture, this was sometimes difficult. The depression of the cylinder onto the brain surface depended on the blood flow data obtained from the laser-Doppler probe that was situated in the center of the cylinder. In an attempt not to place the laser-Doppler probe over a large vessel, it was sometimes necessary to move the cylinder as much as 0.5 mm away from the desired location, thus shifting the ischemic area. Additionally, because of the vascular pattern under the probe, the cylinder frequently compressed the parietal branch of the MCA. This may have yielded a slight enlargement of the infarct, particularly dorsal to PMBSF.

Initially we assumed that a 4-mm brass cylinder would be appropriate because the extent of PMBSF is approximately 4 mm from the most rostral portion (bregma −0.26 mm) to the most caudal portion (bregma −4.16 mm). Although the infarct volume in group A (4-mm cylinder) was comparable to PMBSF, the distance that the cylinder was advanced into the brain to obtain the desired CBF reduction was larger. To reduce the risk of traumatic injury, a 5-mm brass cylinder was therefore used, leading to significantly less tissue compression. There was no significant difference in infarct volume between group A (4-mm cylinder) and group B (5-mm cylinder). Moreover, the infarct volume data in group B were less variable, and it was technically easier to maintain the decreased CBF reduction with the use of a 5-mm cylinder.

Difficulty in maintaining CBF at the desired level was only encountered in the first few minutes of compression ischemia. There were a few rats (5 in groups A and B) for which it was fairly difficult to maintain CBF throughout the compression period, probably because of the presence of a large vessel under the laser-Doppler probe. Use of a cylinder with 2 laser-Doppler probes, each centered 1 mm from the center of the cylinder, would facilitate a LDF measurement free from large-vessel interference. The abrupt recovery of CBF immediately after the end of compression was a common finding in this model. In contrast, CBF generally remains significantly depressed up to 2 hours after injury in the area circumscribing the trauma following experimental traumatic brain injury in rats. In some animals LDF increased significantly during the period of reperfusion up to a level approximately 2 times the baseline value. The apparent hyperemia may have been in part an artifact due to shifting of the tissue under the probe during release of compression.

Conclusions

We developed a reproducible ischemia/reperfusion model that produces focal damage to the SI barrel field of the rat using a cylinder to compress the surface of the somatosensory cortex. The model produces an infarct volume of 36.5 ± 9.2 mm³ (n = 9), 40.7 ± 7.7 mm³ (n = 7), and 36.6 ± 6.4 mm³ (n = 5) at 24 hours, 72 hours, and 7 days after ischemia, respectively, with the infarction confined to the cortex only. Infarct volume remains constant throughout the 7-day observation period, and the histopathology of the damaged tissue demonstrates a consistent pattern of cortical infarction. There was no evidence of damage to white matter or deep gray matter and no evidence of hemorrhage. The topographic distribution of the damaged tissue is in good agreement with that of the primary somatosensory cortex barrel field. This technique is promising for the production of ischemia/reperfusion in which the severity, size, and distribution can be controlled in a variety of anatomic locations.

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References


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