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MR Detection of Secondary Changes Remote from Ischemia: Preliminary Observations after Occlusion of the Middle Cerebral Artery in Rats

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PURPOSE: To determine whether secondary MR changes occur in the thalamus or the substantia nigra after middle cerebral artery (MCA) occlusion in rats. **METHODS:** Sprague-Dawley rats were subjected to MCA occlusion. At varying intervals, proton density–, T1-, and T2-weighted images were obtained with a 4.7-T superconductive MR unit. **RESULTS:** T2-weighted images revealed an area of high signal intensity in the ipsilateral substantia nigra 4 days after occlusion. A lesion of low signal intensity appeared in the ipsilateral thalamus 7 days after surgery on proton density– and/or T2-weighted images. **CONCLUSION:** MR showed secondary changes in the thalamus and the substantia nigra after MCA occlusion in rats. MR imaging should provide more information on the neuropathology of the delayed neuronal degeneration after cerebral ischemia.

Index terms: Animal studies; Arteries, cerebral, middle; Brain, ischemia; Brain, magnetic resonance

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The mechanism by which neuronal degeneration occurs in an area remote from a primary ischemic lesion has been studied in laboratory animals. In rats, neuronal loss and atrophy have been observed in the ipsilateral thalamus (1–4) and the ipsilateral substantia nigra (5, 6) 1 or 2 weeks after middle cerebral artery (MCA) occlusion. Since the thalamus and the substantia nigra are located outside the ischemic region in the rat MCA occlusion model, degeneration of these areas might represent secondary neuronal damage that develops gradually.

These neuropathologic changes can be interpreted as being the result of either retrograde or anterograde degeneration or transneuronal cell death. However, the mechanism giving rise to these neuropathologic changes is still not clear, necessitating more detailed study for clarification.

In clinical studies, we have detected secondary changes in the thalamus using X-ray computed tomography (CT) (7) and in the substantia nigra using magnetic resonance (MR) imaging (8). In animals anterograde (wallerian) degeneration has been reported in studies using MR imaging (9, 10). Recently, MR equipment has been developed that allows high-resolution imaging in animals. Thus, we tried to detect secondary changes after MCA occlusion in rats using this more sensitive MR equipment.

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Materials and Methods

Animal Model and Experimental Design

We anesthetized 25 male Sprague-Dawley rats (ages, 9 to 10 weeks; weight, 320 to 350 g) with 2% halothane. The proximal part of the left MCA was exposed via a transretroorbital approach and permanently occluded using a microsurgical technique (11, 12). For the control group, we prepared sham-operated rats, the MCAs of which were exposed but not occluded. After surgery, the animals were transferred to observation cages and permitted free access

MR findings in the ipsilateral thalamus and the substantia nigra after middle cerebral artery occlusion in rats

Animal	Days after Surgery	Proton Density–Weighted	T2-Weighted	
		Thalamus*	Substantia Nigra*	Thalamus*
1	1	–	–	–
2	1	–	–	–
3	1	–	–	–
4	4	–	High	–
5	4	–	High	–
6	4	–	High	–
7	7	Low	–	Low
8	7	Low	–	Low
9	7	Low	–	–
10	14	Low	–	Low
11	14	Low	–	Low
12	14	Low	–	Low
13	28	Low	–	Low
14	28	Low	–	Low
15	28	Low	–	–

* – indicates no significant abnormality.

to food and water until the time of the imaging experiments.

The rats were reanesthetized with 1% isoflurane via a face mask, and their body temperature was maintained between 36°C and 37°C. Proton density-, T2-, and T1-weighted images were obtained at 1, 4, 7, 14, and 28 days after MCA occlusion (n = 3 at each time point). The sham-operated rats were also imaged as outlined above (n = 2 at each time point). MR imaging was performed in each animal only once.

MR Imaging

All examinations were performed using a 4.7-T imager/spectrometer system equipped with a 33-cm horizontal bore magnet with a gradient strength of 50 mT/m. For radio-frequency transmission and detection, we used a 5-cm-diameter quadrature coil. The coil was positioned close to the animal's head to gain a high signal-to-noise ratio in the central regions, including the thalamus and the substantia nigra. To achieve reproducible images, a T1-weighted midsagittal scout view was taken, and nine consecutive coronal sections were selected. The section thickness was 2 mm with a 128 × 128 matrix, interpolated to 512 × 512 over a field of view of 4 × 4 cm^2. We obtained T1-weighted (600/20/2 [repetition time/echo time/excitations]), T2-weighted (2500/100/2), and proton density–weighted (1800/25/1) spin-echo images.

Results

MR findings in this study are presented in the Table. Ischemia developed in all rats and was seen on T2-weighted images as cerebral infarction with high signal intensity in the left caudate putamen and cortex (Fig 1). The area of infarction was manifested as slightly high signal intensity on proton density–weighted images and as isointense signal on T1-weighted images 1 day after occlusion (Fig 1). Small cortical high signal intensity, observed on T2-weighted images in some sham-operated rats, was considered to be due to bleeding and/or metal dust caused by drilling at the site of craniectomy or dural incision. A slight asymmetry of the signal intensity was observed in peripheral areas, including the cortex, especially on T1-weighted images. This may have been due to inhomogeneity of the B_1 field caused by the position of the coil.

T2-weighted images obtained 4 days after MCA occlusion revealed high signal intensity in the ipsilateral substantia nigra in all animals (Fig 2). However, the lesion was not observed at 1, 7, 14, and 28 days after MCA occlusion. The lesion in the substantia nigra was manifested as a slight high-intensity signal on proton density–weighted images and as an isointense signal on T1-weighted images (Fig 2).

The signal intensity on proton density–weighted images and/or T2-weighted images decreased in the ipsilateral thalamus as early as 7 days after MCA occlusion (Fig 3). The low signal intensity was located in the ventral thalamus, and was observed even at 28 days after MCA occlusion. T1-weighted images revealed no signal abnormality in the thalamus (Fig 3).

In sham-operated rats, no significant abnormality was observed in the substantia nigra or in the thalamus at 1, 4, 7, 14, and 28 days after MCA occlusion.

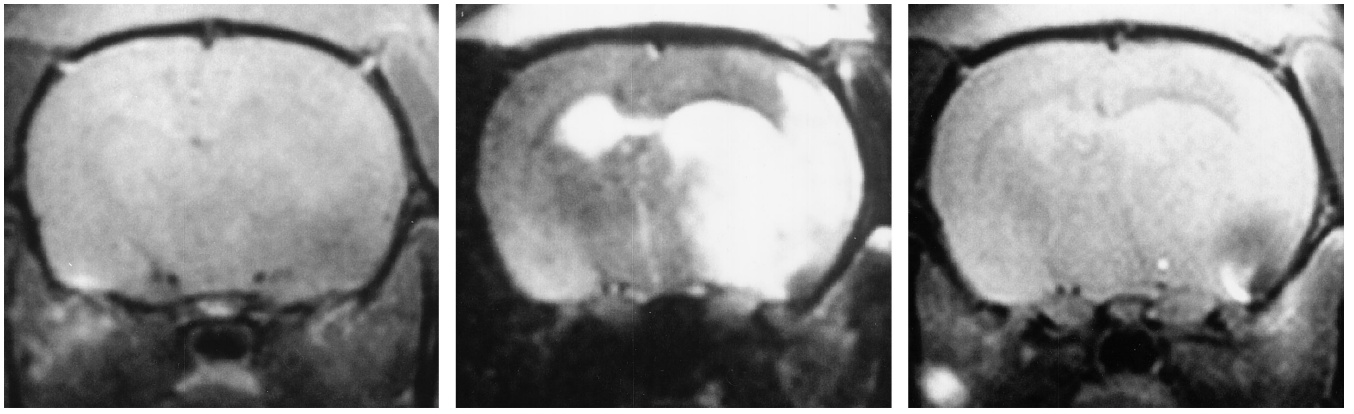

Fig 1. Coronal images of a rat brain 1 day after left MCA occlusion (animal 1).

A, T1-weighted image (600/20/2) reveals only slight signal asymmetry due to artifact (see text).

B, T2-weighted image (2500/100/2) reveals a high-intensity lesion in left caudate putamen and cortex. Lateral ventricles are also shown as high-intensity areas.

C, Proton density–weighted image (1800/25/1) reveals slightly high intensity in the area of infarction.

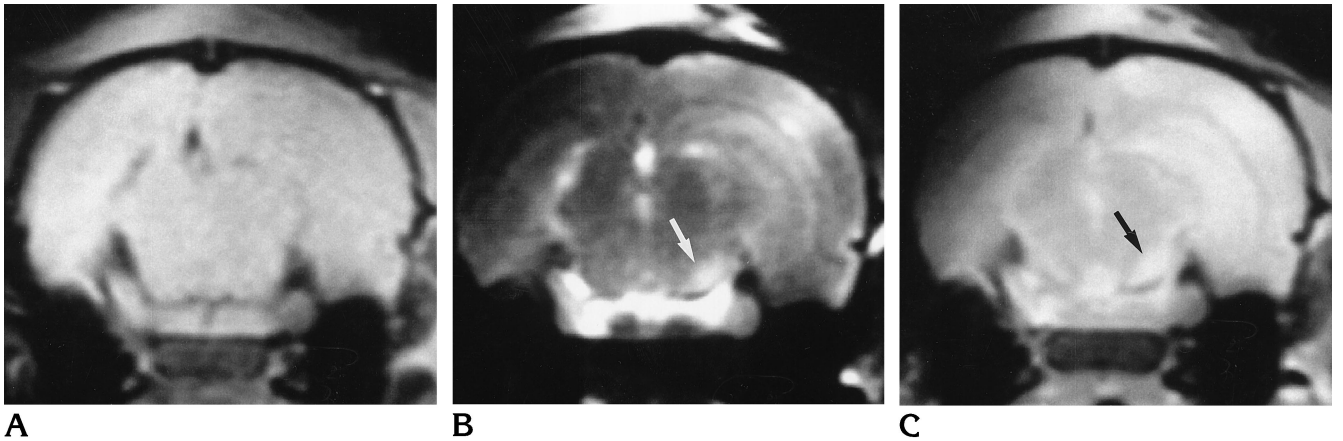

Fig 2. Coronal images of a rat brain 4 days after left MCA occlusion (animal 5).

A, T1-weighted image (600/20/2) shows no signal abnormality in the thalamus and the substantia nigra.

B, T2-weighted image (2500/100/2) reveals an area of high signal intensity in the left substantia nigra (*arrow*). Periinfarct edema is manifested as high signal intensity in the left cortical and subcortical regions.

C, Proton density–weighted image (1800/25/1) reveals slightly high signal intensity in the left substantia nigra (*arrow*). The left cerebral cortex and subcortical regions exhibit high signal intensity.

Discussion

We have shown that MR imaging can be used to detect secondary changes in the thalamus and the substantia nigra after MCA occlusion in rats. It has been reported that focal cerebral ischemia can cause neuropathologic changes not only in the area of infarction but also in certain distant, nonischemic areas remote from the original infarction (1–5, 7). The results of neuropathologic studies of the ipsilateral thalamus (1, 2) and substantia nigra (5) after MCA occlusion in rats have also been reported. In the ventral thalamus, small vacuoles, degenerated presynaptic terminals, and axons were found in the neuropil 2 days after surgery, and cell swelling and peripheral chromatolysis of the thalamic neurons were observed 4 days after surgery. Neuronal necrosis in the ipsilateral thalamus was observed 2 weeks after MCA occlusion. The ipsilateral thalamus then progressively shrank during the months after MCA occlusion (1, 2). In the substantia nigra, some degenerated dark neurons were observed 7 days after surgery. Neuronal loss, gliosis, and atrophy of the ipsilateral substantia nigra also occurred approximately 2 weeks after MCA occlusion (5). Yamada et al (6) reported that many neurons showing swollen chromatolytic

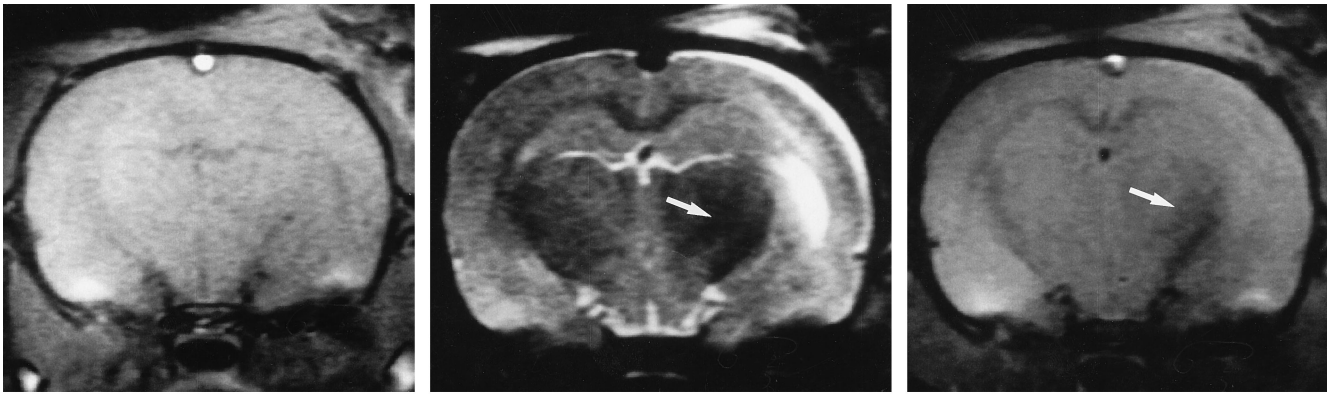

Fig 3. Coronal images of a rat brain 7 days after left MCA occlusion (animal 7).

A, T1-weighted image (600/20/2) shows no signal abnormality in the thalamus and the substantia nigra.

B, T2-weighted image (2500/100/2) reveals an area of slightly low signal intensity in the left thalamus (*arrow*). The left internal capsule also exhibits low signal intensity due to wallerian degeneration. The area of infarction in the left subcortical region is manifested as high signal intensity.

C, Proton density–weighted image (1800/25/1) reveals slightly low signal intensity in the ventral thalamus (*arrow*) and the internal capsule.

features and vacuoles were noted 3 days after 2-hour MCA occlusion. Neuronal changes were not observed in these remote areas within 2 days after focal cerebral ischemia. Since the thalamus and the substantia nigra are located outside the ischemic area, shrinkage of the ipsilateral thalamus and substantia nigra after MCA occlusion reflects secondary ischemic neuronal damage that develops gradually. Although both regional changes were similar in their sequential morphological appearance, MR findings were different. We observed low signal intensity in the thalamus and high signal intensity in the substantia nigra.

Two types of neuronal degeneration have been found to be the cause of secondary degeneration in remote areas after focal ischemic damage (13). First, after impairing a neuron at the cell body or proximal axon, the distal axon and its surrounding myelin sheath undergo degeneration. These changes are referred to as anterograde (wallerian) degeneration. Severing an axon also causes the cell body of the affected neurons to degenerate. This is retrograde degeneration. In addition, both types of degeneration may have an effect across a synapse; namely, transneuronal anterograde and retrograde degeneration (5, 13).

In the rat MCA occlusion model, it has been suggested that thalamic degeneration might be predominantly retrograde degeneration of the thalamocortical pathway (2–4). The ventroposteromedial nucleus neurons are most frequently affected (4). Other studies have suggested that neuronal degeneration in the ipsilateral substantia nigra might be produced by a transsynaptic, neurotransmitter-mediated disinhibition as a result of loss of inhibitory γ-aminobutyric acid (GABA)ergic input (5, 14–16). These may explain the differences in the MR imaging findings.

Secondary thalamic change was manifested by low signal intensity on proton density– and/or T2-weighted images in the ventral nuclei in our study. The low signal intensity may have resulted from reduction of mobile protons or loss of uniformity of local magnetization. It is well known that calcification of tissues and hemoglobin degradation products, such as deoxyhemoglobin and hemosiderin, shorten the T2 relaxation time. However, there is no evidence of calcium deposition or hemorrhage in the thalamus. Hill et al (17) reported that the ventral thalamus in rats was relatively rich in transferrin receptors and iron as compared with other parts of the thalamus. This area might be one of the neural structures in which uptake of iron occurs. Dietrich and Bradley (18) concluded in their study that low-signal-intensity lesions in the ventral thalamus might be caused by the accumulation of iron in the neurons due to impairment of axonal transport. It has been shown at autopsy (19) and by means of MR imaging (18, 20) that ferruginous neurons have appeared in the thalamus in association with cortical lesions

in children. The results of these studies coincide quite well with our MR observations.

On the other hand, secondary change in the substantia nigra was manifested by high signal intensity on T2-weighted images. We have observed coupled increases in local cerebral blood flow and glucose use, and a subsequent long-lasting decrease in the concentration of GABA, an inhibitory neurotransmitter, in the ipsilateral substantia nigra after MCA occlusion in rats (15, 16). Our studies, therefore, suggest that the mechanism of neuronal degeneration in the ipsilateral substantia nigra may be a transsynaptic, neurotransmitter-mediated disinhibition occurring as a result of destruction of the striatum (5, 15, 16). Increased intensity on T2-weighted images may be due to edema, infarction, gliosis, or a plaque of demyelination, which prolongs the T2 relaxation time (21). However, we cannot fully explain the cause of the high-signal-intensity lesion in the substantia nigra in our study. Since the signal abnormality appeared transiently, only at 4 days after MCA occlusion, it is likely to represent reversible edema of the nigral neuron or the axonal terminal of the striatonigral pathway. In clinical cases, T2-weighted images revealed an area of high signal intensity in the ipsilateral substantia nigra after striatal infarction (8). Changes in the ipsilateral substantia nigra appeared approximately 2 weeks after the stroke and then became less intense and smaller a few months later. Findings on MR images were quite the same for humans and rats; however, changes in rats could be detected earlier than those in humans.

Grossman et al (9) reported that areas of wallerian degeneration produced high signal intensity on the long-repetition-time/long-echo-time images from days 208 to 285 after radiation injury. Lexa et al (10) observed low signal intensity in the thalamocortical pathways on proton density– and T2-weighted images at days 25 and 44 after ablation of the visual cortex. We observed low signal intensity in the internal capsule on proton density– and/or T2-weighted images 7 days after MCA occlusion, which might have been due to wallerian degeneration of the corticospinal tract. At this time, since the thalamic changes were manifested as low signal intensity on proton density– and T2-weighted images, we must distinguish wallerian degeneration from thalamic changes by anatomic location. In the chronic stage of MCA occlusion, wallerian degeneration is manifested as high signal intensity. On the other hand, thalamic degeneration may remain as low signal intensity due to iron accumulation. Thus, we can recognize the difference between wallerian and thalamic degeneration in the chronic stage.

MR imaging may prove to be a sensitive detector of early changes in secondary degeneration. In this study, we detected two types of secondary degeneration with MR imaging. MR is very useful in following neuropathologic changes after cerebral infarction, and provides much important information on brain networks. A more detailed study involving histopathologic examination is necessary to interpret the signal changes on MR images.

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