# New Monochromatic Synchrotron Radiation Microangiography System to Measure Intra- and Extracerebral Arteriole Change in the Rat

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**ABSTRACT.**

**BACKGROUND AND PURPOSE:** Using a newly developed angiography system, which consists of monochromatic synchrotron radiation (MSR) as an X-ray source with a high-resolution camera system, we aimed at obtaining simultaneous in vivo three-dimensional (3D) cerebral vessel images and making observations of the intra- and extracerebral arterioles in rats during reperfusion after transient forebrain ischemia.

**METHODS:** The detector features a 7 $\mu$m equivalent pixel size projected onto the input area and an input field size of 7.0 mm x 7.0 mm. The changes in the cerebral microvessels were observed continuously before and for 30 min after transient cerebral ischemia with the MSR angiography system.

**RESULTS:** Micro 3D rotational angiography provided good visibility of the rat intra- and extracerebral arterioles under basal conditions. The diameters of the middle cerebral artery and striate artery significantly increased one min after reperfusion, while the pial arteriole diameter significantly decreased. Thereafter, all of the three-type vessels significantly increased at 10 min after reperfusion.

**CONCLUSION:** We herein describe and discuss the use of an in vivo experimental model in which changes in transient cerebral ischemia in the rat cerebral microcirculation were clearly depicted with an MSR angiography system. These findings show that an assessment of the cerebral regulation system can thus be performed easily and quantitatively using this method.

**Key words** ① **synchrotron radiation** ② **transient ischemia** ③ **reperfusion** ④ **pial arteriole** ⑤ **striate artery**

Rats are useful as an experimental small animal model of intracranial diseases including cerebral vascular disorders. Cerebral angiography has been performed with an experimental rat model showing cerebral vascular obstruction and subarachnoid hemorrhage. However, cerebral angiography depicts relatively thick blood vessels, such as the internal carotid artery and middle cerebral artery, but does not show arterioles and striate arteries measuring less than 100 $\mu$m in size[1)~5)].

Therefore, the cranial window method has been used to observe microvessels[6),7)]. Pial arterioles

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distributed on the surface of the cerebrum can be observed by this method, but it is difficult to examine the blood vessels in the entire cerebrum including the striate arteries distributed in the cerebrum and the intracranial main trunk because this method allows only for local observation. To clarify cerebral circulation disorders after ischemia and reperfusion, evaluation in vivo by visualization of the blood vessels between the intracranial main trunk, the pial arterioles and striate arteries is appropriate.

The monochromatic synchrotron radiation (MSR) angiography system has a higher resolution than conventional angiography systems. Recent studies of Umetani *et al.* indicated that tumor microvessels with a diameter of 30 $\mu$m could be imaged in experimental animal models by the MSR angiography system[8),9)]. Since the intracranial structure of rats is very small, the rat model is most suitable for visualization of the entire region between the intracranial main trunk and the arterioles.

We examined in vivo 3D images and changes in the cerebral circulation in rats caused by reperfusion after ischemia in the entire brain using the MSR angiography system, and found that changes in the entire cerebral circulation of rats could be evaluated by visualization of the cerebral circulation in the entire region between the intracranial main trunk and arterioles.

## MATERIALS AND METHODS

Seven male Wistar rats (SLC Japan) weighing from 280-330 g were used in this study. The rats had access to food and water *ad libitum* and were housed in individual cages. All experimental procedures were performed in accordance with the Animal Care and Use Committee of Kawasaki Medical School (05-018, 06-023).

### *Microangiographic system*

The present study was performed from April 2005 to July 2006 at the SPring-8 BL28B2 beamline of the Japan Synchrotron Radiation Research Institute. The experimental arrangement for X-ray imaging using MSR X-rays is shown in Figure 1. Details of the imaging system used in the present study have been described previously[8)]. In brief, the new angiography system consists of the MSR as the X-ray source and a

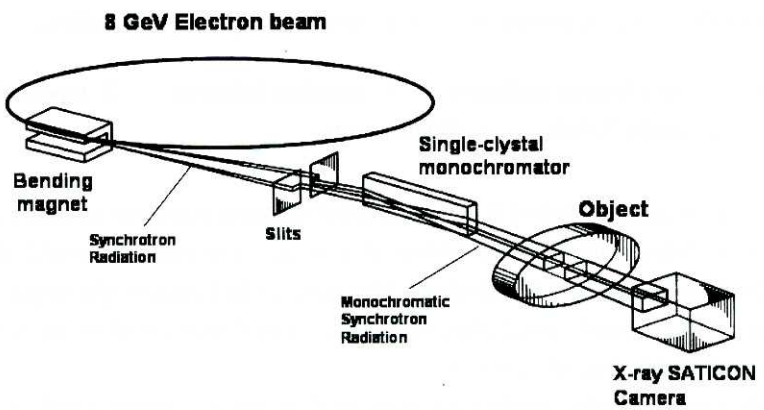


Fig. 1. Schematic presentation of the experimental setup. Synchrotron radiation is generated by bending the path of electrons at relativistic speeds using a bending magnet. The storage ring maintains the electron beam for many hours. A single silicon crystal selects a single-energy synchrotron radiation. The digital images were acquired through analog-to-digital conversion.

high-resolution camera with a video system as a detector. Monochromatic X-ray energy was adjusted to 33.2 keV just above iodine K-edge energy to produce the highest contrast image of iodine contrast material. A new SATICON camera (Hitachi Denshi Techno-System, Ltd, Japan and Hamamatsu Photonics K.K., Japan) was used as the detector. This camera has a resolution of 1050 scanning lines and can record images at a maximum speed of 30 frames/sec. Sequential images were obtained with an input field of view of 7.0 mm x 7.0 mm. High-solution images were stored in a digital frame memory system with a 1024 x 1024 pixel format and a 10-bit resolution. For images, 10 frames were integrated to obtain a higher contrast and lower noise image, and these images were obtained every 0.33 sec. 3D rotational angiography rotated by 4 degrees.

***Animal preparation***

The rats were prepared for transient global ischemia using the four-vessel occlusion model[10]. On the day before the experiment, they were anesthetized with sodium pentobarbital (50 mg/kg intraperitoneally). The skin and muscles over the first two cervical vertebrae were incised and separated from the midline. Both vertebral arteries were electrocauterized after exposition of the alar foramina under a surgical microscope.

On the day of the experiment, the rats were anesthetized with sodium pentobarbital (50 mg/kg intraperitoneally) and then underwent mechanical ventilation (model SAR-830; CWE, Inc.) using room air $O_2$ with a tidal volume of 1 ml per 100 g body weight and 40 strokes/min after tracheal intubation and muscle relaxation (vecronium bromide, 0.5 mg/kg). Anesthesia was maintained with hourly injections of sodium pentobarbital (10 mg/kg). The bilateral common carotid arteries (CCA) were isolated from surrounding tissues through a midline neck incision and silk ligatures were placed loosely around them. The pterygopalatine artery was ligated with silk ligatures. Arterial and venous catheters were placed into the femoral vessels to measure arterial blood pressure (model BSM-2301; Nihon Koden) and anesthesia. Another left external carotid artery catheter (PE-50) was connected to an automated injector (Nihon Koden) for angiography. A temperature sensor (model ATB-1100; Nihon Koden) was inserted in a temporal muscle. The duration of the whole preparation was 1 hr from the start of anesthesia to induction of global ischemia.

***Global ischemia***

The rat was placed in front of the MSR angiography system on a hand- made stereotaxic device. The arterial catheter was connected to a pressure transducer for the continuous recording of ABP. The body temperature was kept at 37℃ by a blanket and heater. Global ischemia was induced by occlusion of the bilateral CCA by tightening the silk sutures. Completion of ischemia was checked by the appearance of mydriasis. After 10 min of ischemia, reperfusion was performed by release of the silk sutures. Microangiography was performed before occluding the bilateral CCA, and 1, 10, 20 and 30 min after releasing the silk ligatures by a bolus injection of 0.2 ml of a contrast medium, iopamidol of a nonionic water-soluble contrast medium (iodine content: 370 mg/ml)(Iopamiron 370; Schering).

***Vessel diameter measurement***

The images were stored digitally. A temporal subtraction operation was performed for flat-field correction. The summation image taken before injection was subtracted from raw images taken after injection to eliminate the superimposed background structure (Image Pro Plus version 3.0; Media Cybernetics, Inc). The microvessel diameter was measured using a 7 $\mu$m equivalent pixel size as a standard

(NIH Image; National Institute of Health). Measurement of the vascular inner diameter was performed in the middle cerebral artery (MCA) immediately before first branching, and in the pial and striate arteries immediately after each branching. The diameters of the pial and striate arteries that were clearly observed on all images were measured (Fig. 3A, B, C, D).

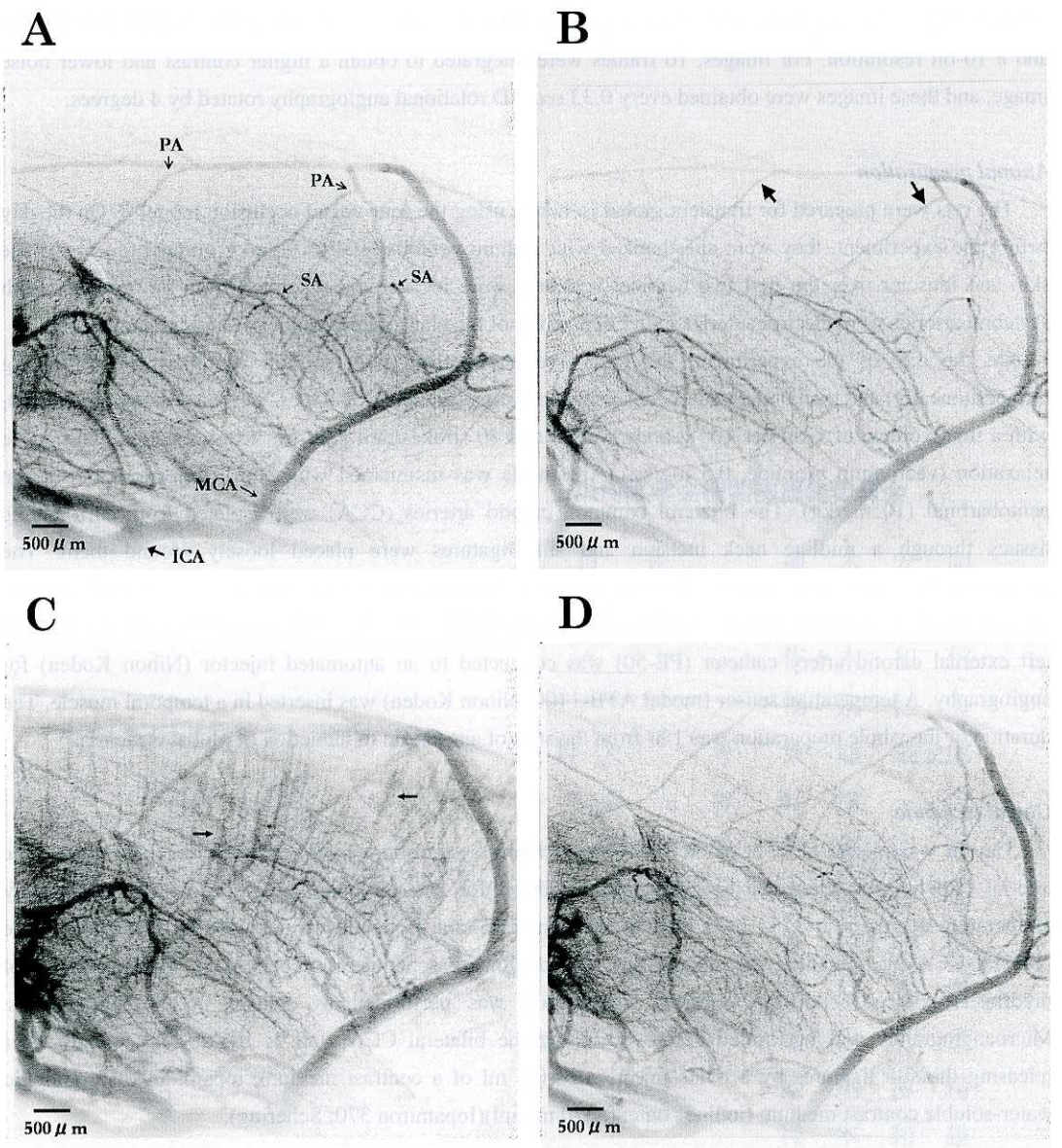


Fig. 3A. Basal conditions

Fig. 3B. Images obtained at 1 min after reperfusion. The pial arterioles were significantly constricted (large arrow). On the contrary, the MCA and striate artery were significantly dilated at 1 min after reperfusion.

Fig. 3C. Images obtained at 10 min after reperfusion. The pial arteriole, MCA and striate artery remained dilated. Early venous filling was observed (small arrow).

Fig. 3D. Images obtained at 30 min after reperfusion. All vessels returned to basal values.

***Statistical Analysis***

The changes with time are expressed as the means ± SD. The mean basal value was calculated in all vessels. A statistical analysis was performed using the paired t-test. The first value was taken as the control value. Values of $P < 0.05$ were considered to be significant.

## RESULTS

The mean arterial blood pressure significantly increased by 122 ± 6% during ischemia and then returned to its basal level immediately after reperfusion. There was no significant difference in the mean arterial blood pressure when microangiography was performed.

***Basal conditions***

The MSR angiography system provided good three-dimensional visibility of the MCA, pial arterioles and striate arteries under basal conditions (Fig. 2A, 2B). In addition, this system could detect microvessels with a diameter of 30 $\mu$m. The mean MCA diameter was 198 ± 30 $\mu$m, the mean pial arteriole diameter was 56 ± 14 $\mu$m, and the mean striate artery diameter was 42 ± 8 $\mu$m (Fig. 3A).

***Reperfusion***

The MCA diameter significantly increased at 1 and 10 min after reperfusion as shown in Figure 3B and 3C. The diameters of the striate artery significantly increased at 1 and 10 min after reperfusion as shown in Figure 3B and 3C. The extent of vasodilatation after 10 min reperfusion was 140 % for MCA. On the contrary, the pial arteriole diameter significantly decreased by 25 % at 1 min after reperfusion, and thereafter significantly increased at 10 min after reperfusion as shown in Figure 3B and 3C. Changes in pial arteriole diameter are shown in Figure 4. Diameters of these vessels tended to return to base line values at 30 min after reperfusion as shown in Figure 3D. As shown in Figure 3C, early venous filling was observed at 10 min after reperfusion.

## DISCUSSION

We developed a method to visualize the changes in the cerebral blood vessels after ischemia and reperfusion in the entire brain of rats using the MSR angiography system, by which in vivo arterioles with a diameter of less than 100 $\mu$m could be examined on high-quality images. Evaluation is difficult with two-dimensional images, because the cerebral microvessels are complex. It is necessary to see them in three-dimensional images. With the MSR angiography system, it was possible to observe blood vessels of different diameters showing various changes after cerebral ischemia and reperfusion in a single visual field.

Rats are useful as a small animal model for studies of various intracranial diseases including cerebrovascular disorders. However, because of their small size, examination of their cerebrovascular circulation by cerebral angiography is limited to large vessels such as the ICA and MCA[1)~5)]. Recently, magnetic resonance angiography has been used for examinations using rats, but this, as with cerebral angiography, is only effective for large blood vessels[11)]. The cranial window method has been used in various animal models including the rat for visual examinations of small blood vessels in vivo. However,

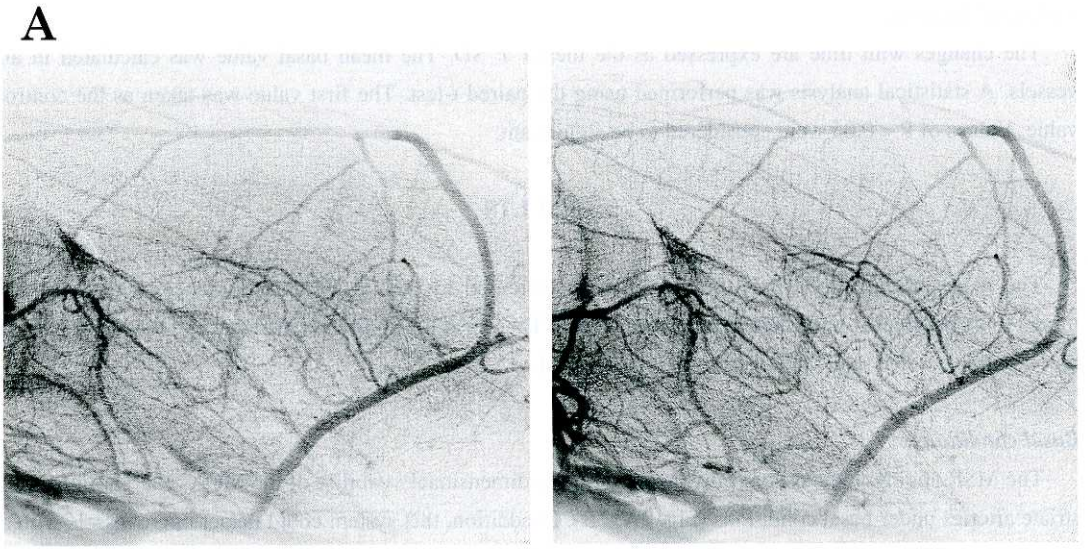


Fig. 2A. Digitally subtracted stereo-images of monochromatic synchrotron radiation microangiographs of normal rat cerebral arteries. Good demonstration and separation of arterioles with a diameter of less than 100 $\mu$m.

Fig. 2B. Diagram showing the principal cranial arteries in the rat. MCA: middle cerebral artery, PA: pial arteriole, and SA: striate artery

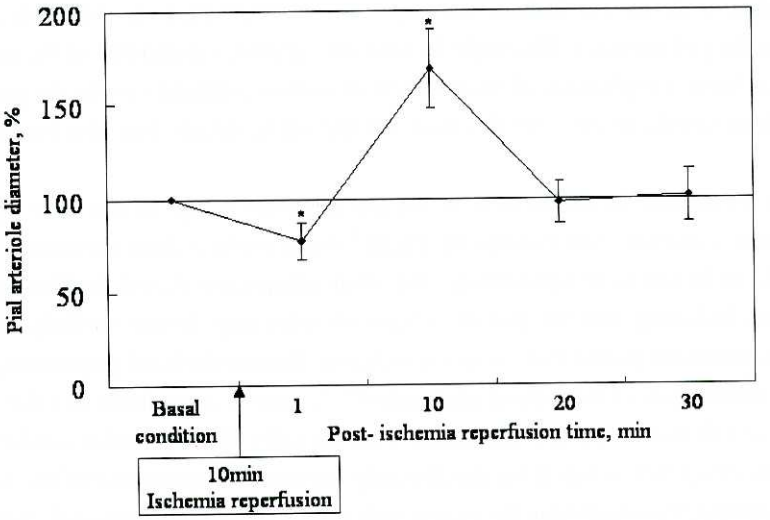


Fig. 4. Changes in pial arteriole diameter expressed as a percent of basal values. Each asterisk (*) shows $p<0.05$ as compared with the basal condition.

since this method has a drawback in that observation of the striate arteries, which are distributed in the cerebrum, is impossible, the parenchymal circulation of the brain must be speculated on from the pial circulation. Confocal laser microscopy, which has been used in recent studies, provides a method for the evaluation of blood vessels in the brain, but the visual depth is limited to the partial brain surface even when using this method[6),7),12)]. Therefore, it is impossible to simultaneously visualize the difference in regulation in blood vessels of various diameters distributed in different regions.

The spatial resolution of conventional medical x-ray imaging is affected by the size of the x-ray source and the source-to-detector distance. In contrast, the MSR angiography system has a spatial resolution of more than 30 $\mu$m, which is due to the nearly parallel X-rays emitted from its source. MSR angiography has been suggested as a possible tool for diagnosing cardiovascular disease in microvessels and malignant tumors in various organs[8),9)]. The microvessels are of arteriole size.

One problem is exposure to synchrotron radiation. To minimize this effect, irradiation was blocked during the intervals between the angiography recordings. Six consecutive angiography recordings were conducted every 10 min with normal rats, yielding clear images without any significant changes in the intracranial vascular diameters. Another problem is that vessel diameter is affected by blood pressure. However, in this study, there were no significant changes in blood pressure at the time of angiography.

In the four-vessel occlusion rat model of 15-min ischemia, Pinard *et al*[7)] reported that the diameter change in arterioles of the cortical surface was not significant for 5 min after reperfusion and, thereafter, a significant increase was observed from 5 to 15 min after reperfusion. Their results were not consistent with our findings of pial arteriole diameter change at 1 min after reperfusion. On the contrary, the diameter of the striate arteries in the present study showed a significant increase at 1 min after reperfusions. The striatum nourished by the striate arteries is reported to be more vulnerable than the neocortex perfused by the pial arterioles[13)]. Liachenko *et al* reported that transient hyperperfusion after 12 min of circulatory arrest in rats started from the thalamus and hypothalamus and later shifted to the cortex[14)]. Judging from Liachenko's

report, in addition to the present findings, the striate arteries might be more vulnerable to ischemia and reperfusion than the pial arterioles. This might be caused by hypoxic vulnerability of the striatum following global ischemia. Another explanation might exist with or without collateral vessels: the pial arterioles have abundant collateral vessels on the cortical surface but the striate arteries have few because they are end arteries.

The significant increase in the diameter of the pial arterioles during 10 min after reperfusion in the present study was consistent with findings by Pinard[7] using confocal laser microscopy. Therefore, our ischemia model can be said to be reproducible. The striate arteries also dilated significantly during 10 min after reperfusion, indicating that the pial and striate arterioles may behave similarly except during an immediate post-reperfusion period after 10 min of ischemia. Because the basal ganglia may have 54 - 73% the number of microvessels of the cortical gray matter[15),16)], there is a possibility that the more prolonged ischemia is, the more behavior between the pial and striate arterioles after reperfusion will differ.

Early venous filling, which was defined as the early angiographic appearance of the venous structures from the arterial phase, was observed in the present study during reperfusion. Ohta *et al*[17] reported that there was a significant correlation between the appearance of angiographic early venous filling during intra-arterial reperfusion therapy and post-therapeutic hemorrhagic complications. Although the appearance of early venous filling seems to be a good predictor of hemorrhagic complications after reperfusion therapy, its pathophysiologic basis is incompletely understood. Clear detection of early venous filling in rats with the MRS angiography system could help us clarify its pathophysiologic basis.

## CONCLUSION

We herein described an *in vivo* experimental model in which rat 3D cerebral vessels and microcirculation changes in transient global cerebral ischemia were clearly depicted using the MSR angiography system. Our results indicate that an assessment of the cerebral regulation system can be performed both easily and quantitatively using this method.

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