

Case Report

## Porencephaly in a Cynomolgus Monkey (*Macaca Fascicularis*)

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**Abstract:** Porencephaly was observed in a female cynomolgus monkey (*Macaca fascicularis*) aged 5 years and 7 months. The cerebral hemisphere exhibited diffuse brownish excavation with partial defects of the full thickness of the hemispheric wall, and it constituted open channels between the lateral ventricular system and arachnoid space. In addition, the bilateral occipital lobe was slightly atrophied. Histopathologically, fibrous gliosis was spread out around the excavation area and its periphery. In the roof tissue over the cavity, small round cells were arranged in the laminae. They seemed to be neural or glial precursor cells because they were positive for Musashi 1 and negative for NeuN and GFAP. In the area of fibrous gliosis, hemosiderin or lipofuscin were deposited in the macrophages, and activated astroglia were observed extensively around the excavation area. (DOI: 10.1293/tox.25.45; J Toxicol Pathol 2012; 25: 45–49)

**Key words:** brain, cynomolgus monkey, porencephaly

The term “porencephaly” was first used by Heschl (1859) to describe a cavity in the human brain<sup>1–4</sup>. Various causes such as ischemia, hemorrhage, infection, and trauma have been considered; however, circulation disturbance is currently regarded as the most common cause of cases in humans<sup>2,4,5</sup>. Patients with porencephaly exhibit signs of delayed growth and development, spastic hemiplegia and seizures<sup>2</sup>. It is considered that differences in the pathological features are more a matter of timing and severity than fundamental differences in underlying etiology<sup>2,6</sup>. In animals, porencephaly caused by Akabane virus in cattle<sup>7</sup> and sheep<sup>8–10</sup>, Bluetongue virus in sheep<sup>6,11,12</sup>, Pestivirus in cattle<sup>13</sup> and sheep<sup>14,15</sup>, Cache Valley virus in sheep<sup>16</sup> and Col4a1 mutation in mice<sup>17</sup> has been reported. There have been only a few reports of porencephaly in monkeys: a drug-induced case<sup>18</sup>, spontaneous case<sup>19</sup> and case of Venezuelan Equine Encephalitis virus<sup>20</sup>. A histopathological description was only found in the spontaneous case<sup>19</sup>. In this paper, we report a case in an adult female cynomolgus monkey, which showed diffuse brownish excavation and a partial defect in

the full thickness of the cerebral hemispheric wall, and include its histopathological and immunohistochemical findings.

The animal was a naive female cynomolgus monkey (*Macaca fascicularis*, purpose-bred) purchased from China (Yulin Hongfeng Laboratory Animal Domesticating and Breeding Center, Guangxi, China) aged 5 years and 7 months. The animal was individually housed in a stainless steel cage (680 mm depth × 620 mm width × 770 mm height, Taiyo Stainless Co., Ltd., Kagoshima, Japan), and approximately 108 g of pellet food (HF Primate 5K91 12G 5K9J, Purina Mills, LLC, St. Louis, MO, USA) was provided daily between 14:30 and 16:00. Water conforming to the water quality standards required by the Japanese Waterworks Law was available *ad libitum* from an automatic water supply system. The animal room was maintained within a temperature range of 23°C to 29°C and a humidity range of 35% to 75%, with 15 air changes/hour and artificial illumination for 12 hrs/day (07:00 to 19:00). All procedures involving the animal were approved by the Institutional Animal Care and Use Committee of Shin Nippon Biomedical Laboratories, Ltd. and were performed in accordance with standards published by the National Research Council, USA (Guide for the Care and Use of Laboratory Animals, NIH OACU), and the U.S. National Institutes of Health Policy on Humane Care and Use of Laboratory Animals. No abnormal clinical signs including neurological symptoms were observed before necropsy, and no abnormal findings were observed in hematology, blood chemistry or urinalysis. The animal was anesthetized by an intravenous injection of sodium pento-

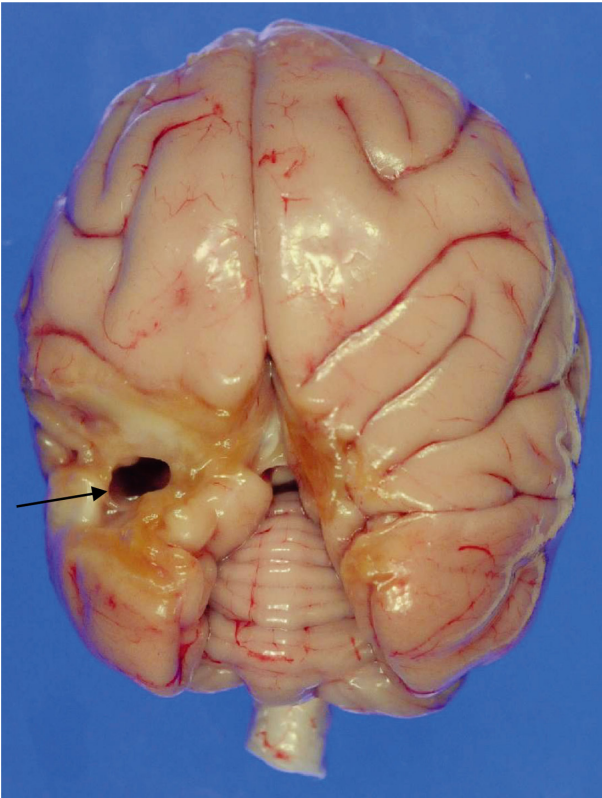
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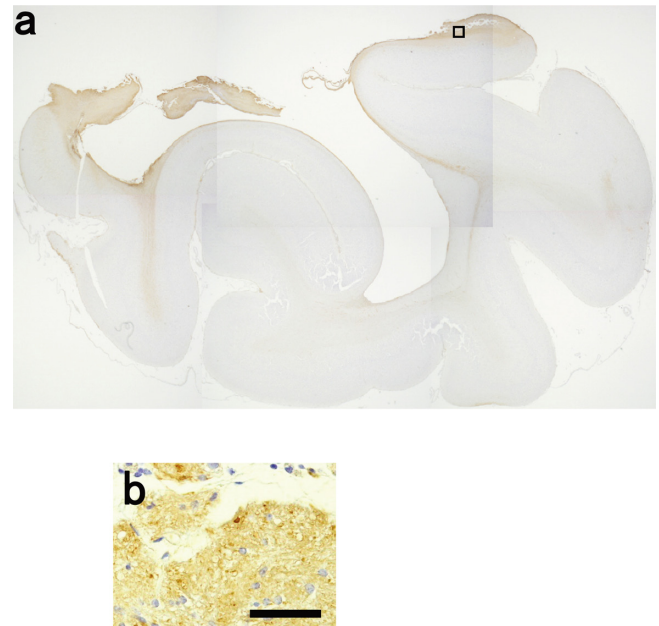
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**Fig. 1.** Gross appearance of the brain. Diffuse brownish excavation was observed. The full thickness of the cerebral hemisphere showed a partial defect and constituted open channels between the lateral ventricular system and the arachnoid space (arrow).

barbital (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) solution (64.8 mg/mL, 0.4 mL/kg) into the cephalic vein of the forearm, weighed and euthanized by exsanguination. External appearance and internal organs and tissues were examined macroscopically. After macroscopic examination, the brain was fixed in 10% neutral buffered formalin, trimmed, embedded in paraffin, sectioned and stained with HE stain. The paraffin sections were stained with Berlin blue staining and Schmorl's method as a special staining. Additionally, for immunohistochemistry, a rabbit polyclonal antibody to Musashi 1 (Musashi 1, Abcam, Tokyo, Japan), a mouse monoclonal antibody to NeuN (NeuN, Nihon Millipore K.K., Tokyo, Japan) and a rabbit polyclonal antibody to Glial Fibrillary Acidic Protein (GFAP, Dako Japan, Tokyo, Japan) were used as primary antibodies. To visualize primary antibodies, secondary antibodies, HRP-dextran complex (EnVision+, Mouse/HRP and EnVision+, Rabbit/HRP, K4000 and K4002, respectively, Dako Japan, Tokyo, Japan) and DAB+ Liquid (K3467, Dako Japan, Tokyo, Japan) were used. Expression of Musashi 1 has been observed in ependymal cells<sup>21</sup> and it was also confirmed in the section of this case. In addition, the astrocytes were positive for GFAP, and the mature neurocytes were positive for NeuN in several sections. Therefore, all of the immunohistochemistry



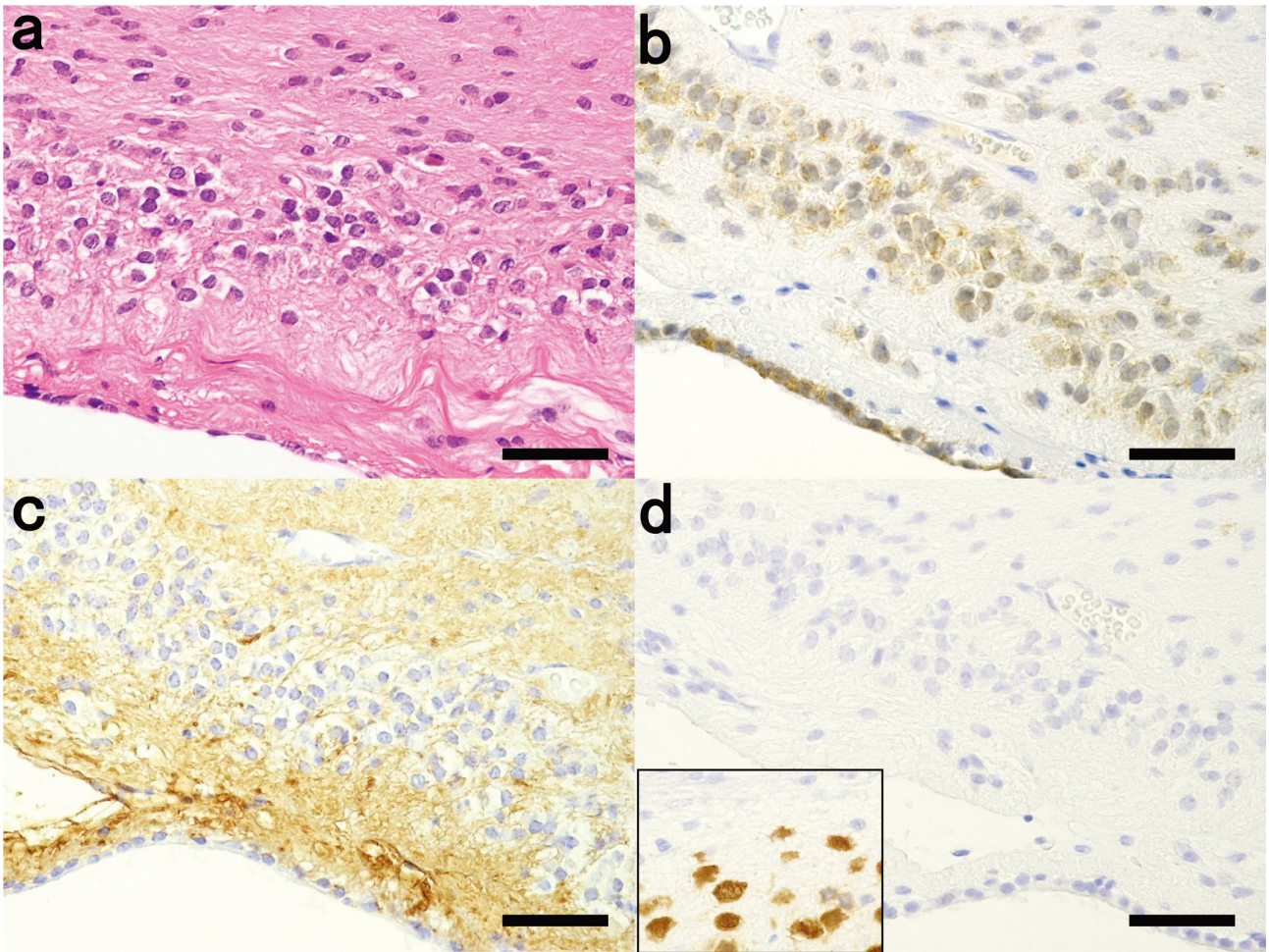
**Fig. 2.** a) The cerebral parenchyma was remarkably thin, and fibrous gliosis spread out around excavation area and its periphery. GFAP. Low magnification. b) High magnification of the fibrous gliosis (frame in a). GFAP. Bar = 50  $\mu$ m.

methods were confirmed to be appropriate.

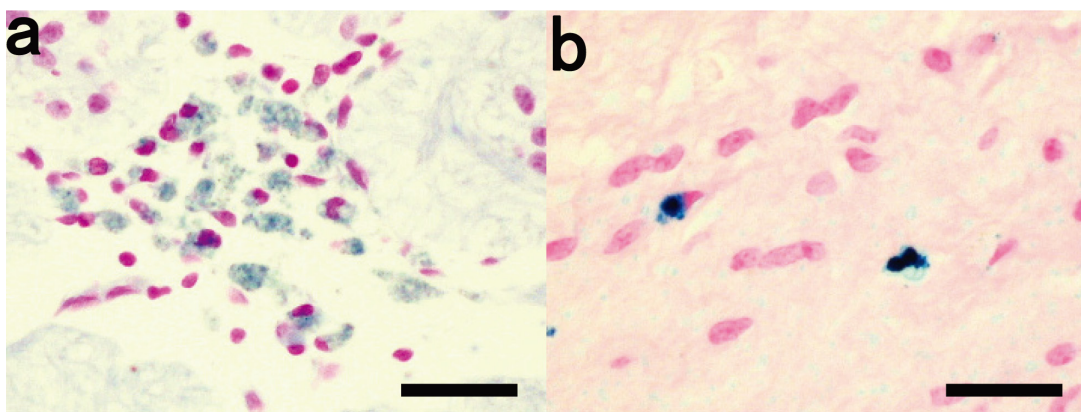
Macroscopically, brownish excavation (30  $\times$  20 mm) was observed in the left parietal lobe and its periphery. There was a partial defect of the full thickness of the hemispheric wall (3  $\times$  5 mm) in the excavation area, which constituted open channels between the lateral ventricular system and the arachnoid space. Judging from these findings, this case was diagnosed as porencephaly. Additionally, the area of the bilateral occipital lobe extending from the excavation area was slightly atrophied (Fig. 1). No other abnormal findings were observed in any organ or tissue.

Histopathologically, there was an increased amount of GFAP-positive fibers. They were expressed fibrous gliosis and the cerebral parenchyma was remarkably thin in the excavation area and its periphery (Fig. 2). In the roof tissue over the cavity, small round cells with round nuclei and bright and slight cytoplasm were arranged in the laminae. The cells were positive for Musashi 1 and negative for NeuN and GFAP (Fig. 3). In the area of fibrous gliosis, a small number of macrophages containing brown pigments was observed. The pigments were revealed to be hemosiderin or lipofuscin deposits by Berlin blue staining or Schmorl's method (Fig. 4). Additionally, activated astroglia were observed extensively around the excavation area (Fig. 5).

Heschl described three types of porencephaly<sup>2,3</sup>: 1. partial full thickness hemisphere defects separated by membranes from both the ventricular system and the arachnoid space, 2. partial full thickness hemisphere defects in open communication with the ventricles but separated from the arachnoid space by a membrane and 3. partial full thickness



**Fig. 3.** Small round cells arranged in the laminae were observed in the roof tissue over the cavity, and they were positive for Musashi 1 and negative for NeuN and GFAP. a) HE stain. Bar = 50  $\mu\text{m}$ . b) Musashi 1. Bar = 50  $\mu\text{m}$ . c) GFAP. Bar = 50  $\mu\text{m}$ . d) NeuN. Bar = 50  $\mu\text{m}$ . Inset: Mature neurocytes that were positive for NeuN.

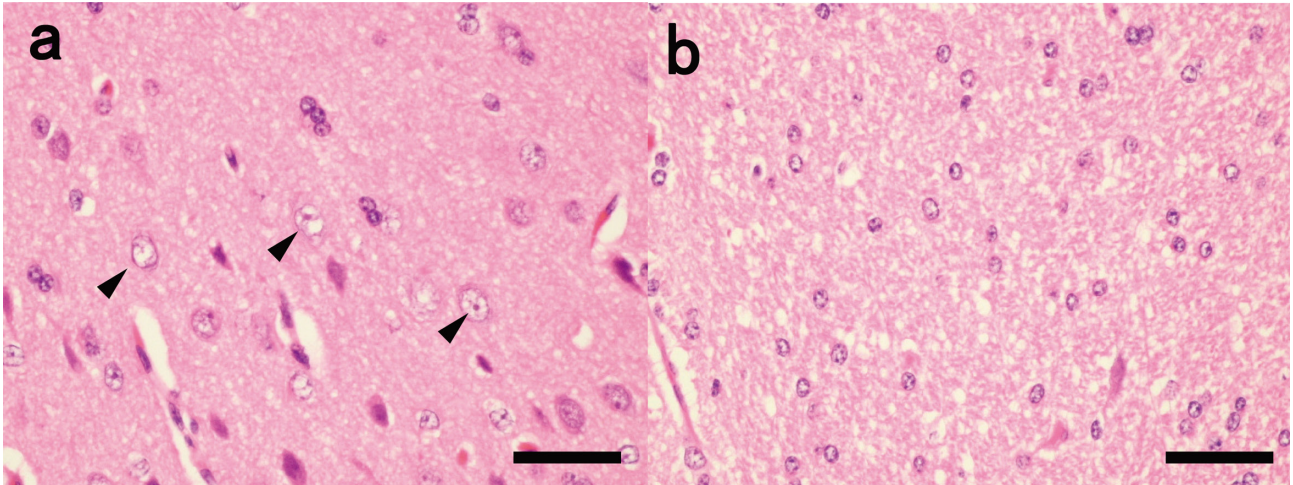


**Fig. 4.** Macrophages containing brown pigments were observed. The pigments were positive for Berlin blue staining or Schmorl's method. a) Schmorl's method. Bar = 50  $\mu\text{m}$ . b) Berlin blue staining. Bar = 50  $\mu\text{m}$ .

defects constituting open channels between the ventricular system and the arachnoid space (Fig. 6). In this case, the defect constituted open channels between the ventricular system and the arachnoid space, and it falls into the category

of type 3.

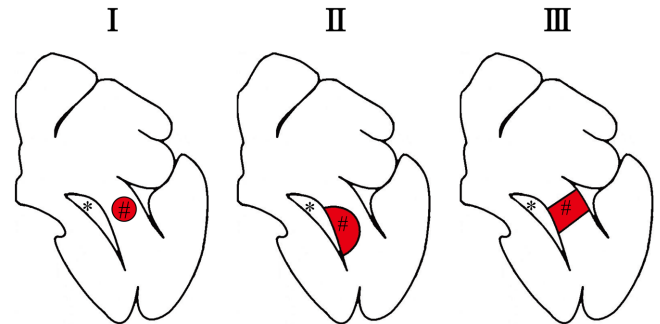
Friede<sup>1</sup>, Barth<sup>3</sup> and Norman<sup>22</sup> defined porencephaly as circumscribed hemispheric necrosis that occurs in utero or during brain development. Friede<sup>1</sup> stated that macroscopi-



**Fig. 5.** a) Activated astroglia (arrowheads) were observed extensively around the excavation. HE stain. Bar = 50 µm. b) Control HE stain. Bar = 50 µm.

cally revealed disturbances in the development of the adjoining cortex are proof of porencephaly and defined two types: 1. polymicrogyria bordering the defect or extending over much of the convexity of the hemisphere or patches of polymicrogyria found at a distance and 2. no polymicrogyria, but the arrangement of adjacent gyri is abnormal in that some or all radiate toward the defect, often descending into its depth. A previously reported spontaneous cynomolgus monkey case showed similar macroscopic changes to the present case, but it was more serious and widespread and was accompanied by severe neurological signs<sup>19</sup>. Additionally, a hobnail or polymicrogyric appearance was observed, but the layered small round cells were not described in the reported case. In the present case, although there was no polymicrogyria or abnormal arrangement of adjacent gyri indicating a disturbance in the development in macroscopic examination, diffuse atrophy of the cerebral hemisphere around the defect and layered small round cells in the roof tissue over the cavity were observed, and the roof tissue was suspected to be immature brain tissue. To investigate whether or not the layered small round cells were immature neurons or glia, an immunohistochemical examination was conducted, and the small round cells were positive for Musashi 1 and negative for NeuN and GFAP. Musashi 1 was reported to exist in the proliferating ventricular zone in fetuses<sup>23</sup> and in neural stem cells under the ependyma in the postnatal CNS<sup>21</sup>. Additionally, expression of Musashi 1 was observed in astrocytes ranging from GFAP-negative glial precursors in the subventricular zone to GFAP-positive differentiated astrocytes, which had already finished migration<sup>24</sup>. The layered small round cells in the present case were thought to be neural or glial precursor cells, and although there was no disarrangement of gyri, persisting immature cells suggested that the first damage occurred during development of the brain.

Although fibrous gliosis and reactive astrocytes are a common response to almost any type of injury to the adult



**Fig. 6.** Three categories of porencephaly (illustrated according to Heschl's definition)<sup>2,3</sup>. \*: Lateral ventricle #: defect area

central nervous system, lesions in fetal and neonatal animals have been reported to induce little or no fibrous gliosis or hypertrophic glial forms<sup>1</sup>. However, even though damage is thought to have occurred at an early stage of gestation, the present case has extensive fibrous gliosis with activated astroglia, which suggests the possibility that the destruction of the thin fragile tissue around the partial defect of the cerebral hemisphere caused by the mechanical power of the cerebrospinal flow persisted until just before the necropsy.

This case was very rare because it developed and existed without any neurologic symptoms despite the wide range of brain lesions. Additionally, the small round cells arranged in the laminae in the roof tissue over the cavity were the characteristic feature of this case because there has been no report describing similar histopathology in the monkey, and immunohistochemistry was helpful in understanding that the first damage occurred during development of the brain.

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