Observation of Autism Model Rat Brain by Phase-Contrast X-Ray CT

Prenatal exposure to the antiepileptic drug valproic acid increases the risk of having offspring with an autism spectrum disorder. The phase-contrast X-ray imaging technique based on crystal X-ray interferometry enables the observation of fine differences in density within biological soft tissues like the brain without contrast agents. We report here the advantage of this technique in visualizing detailed morphological changes in the brains of a valproic acid-induced rat model of autism. Our results suggest that phase-contrast X-ray CT could reveal important information about the neurobiological basis of autism spectrum disorder.

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder characterized by difficulties with social communication and social interaction and repetitive patterns of behavior. Prenatal exposure to the antiepileptic drug valproic acid (VPA) is one of the risk factors for the development of ASD [1]. Recently, many researchers have studied ASD using a rodent model of autism, created by exposing rat fetuses to VPA on day 12.5 of gestation [2]. The noninvasive imaging technique plays an important role in gaining insights into changes in the brain morphology of ASD. The conventional absorption X-ray imaging technique cannot depict the internal structure of the brain without using contrast agents due to small differences in absorption within the soft tissues of the brain. Phase-contrast X-ray imaging is a new imaging method that offers approximately 1000 times higher sensitivity than the conventional imaging technique. The method can clearly depict morphological changes of soft biological samples such as the brain [3-6]. In this work, we investigated the advantage of the phase-contrast X-ray imaging technique for detailed visualization of morphological changes in the brains of a VPA-induced rat model of autism.

Autism rat offspring were created by exposing pregnant Sprague Dawley rats to VPA (600 mg/kg i.p.) on the 12.5th day of gestation (VPA group). Normal control rats were given normal saline under the same conditions (control group). Brains from 6 VPA and 3 control rats at 13 weeks old were used in this study. Brains were extracted under anesthesia and fixed with 10% formalin

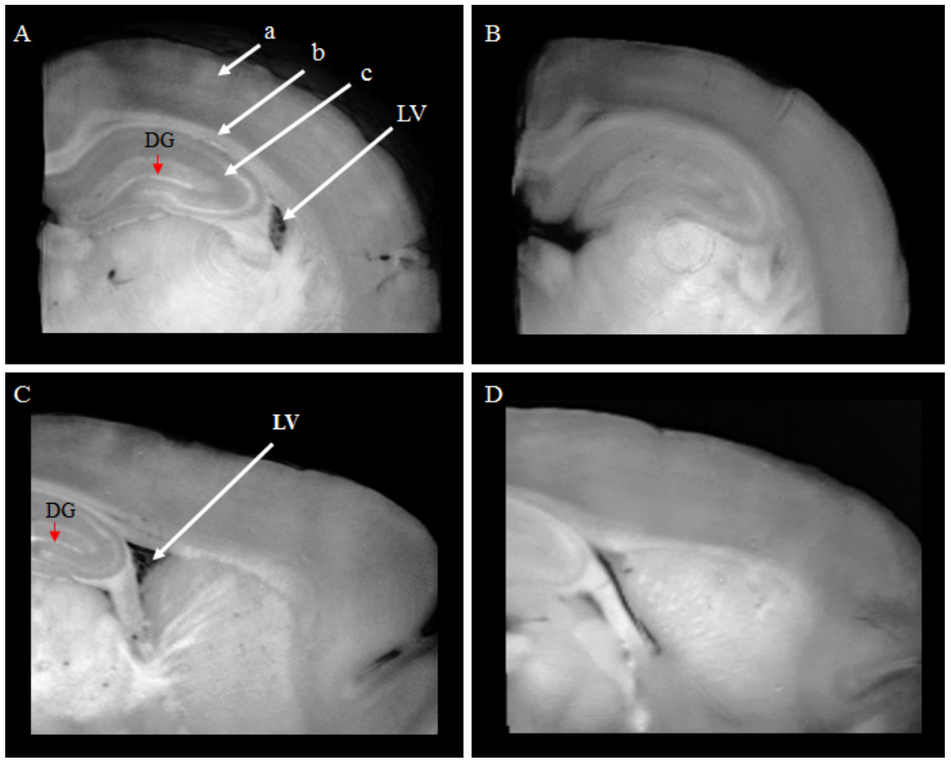

Figure 1: Phase-contrast X-ray CT image of rat brains; Axial image (**A:** VPA, **B:** Control), Sagittal image (**C:** VPA, **D:** Control); a: cortex, b: corpus callosum, c: hippocampus, LV: lateral ventricle, DG: dentate gyrus

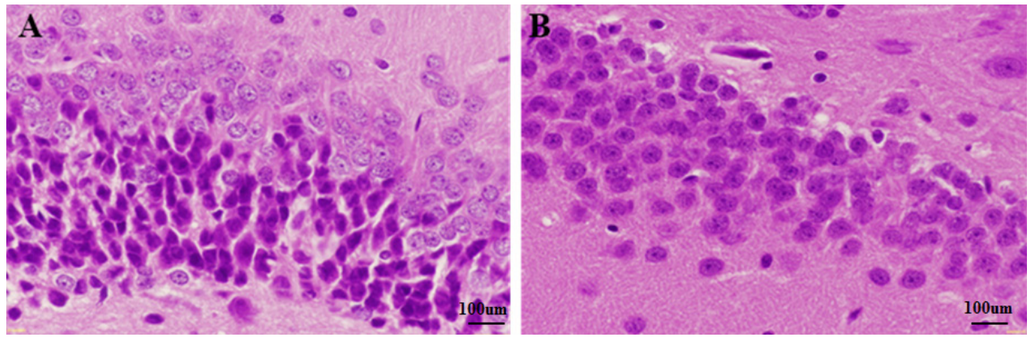

Figure 2: Dentate gyrus region of hippocampus (×40) (H&E staining); **A:** VPA, **B:** Control. Cell number and density are increased in VPA rats.

for imaging. A two-crystal X-ray interferometer-based phase-contrast X-ray imaging system [7] was used. An X-ray camera with a 2560×2100-pixel sensor of pixel size 6.5×6.5 μm^2 was used to detect interference patterns. The data were acquired with monochromatic X-rays of photon energy (17.8 keV). The CT image was reconstructed by a filtered back-projection method. The density of the hippocampal dentate gyrus on phase-contrast X-ray images was calculated by reflective index, which was determined directly from phase shift information [8].

After the phase-contrast CT imaging, the brains were embedded in paraffin. Then, 3 mm-thick sections were cut in the coronal plane. Hematoxylin-eosin (H&E) staining was carried out to examine the abnormal histopathological structures. The stained sections were imaged using an optical microscope (Olympus FSX100; Olympus, Tokyo, Japan). The animal experimental protocol was approved by the Ethics Committee of the Animal Care and Experimentation Council of the National Institute for Environmental Studies, Japan.

Phase-contrast X-ray CT images of VPA rat brain and control rat brain are shown in **Fig. 1**. Phase-contrast X-ray CT demonstrated excellent tissue conspicuity and was able to reveal the detailed anatomical structures of the rat brain, including the cortex, corpus callosum, hippocampus, and lateral ventricle depending on the different densities. Especially, a high density was observed in the hippocampal dentate gyrus of VPA rat brain compared to that of the control rat brain. The results of quantitative analyses showed that the absolute density values of the hippocampal dentate gyrus in the VPA and control rat brain were 1.0478 ± 0.0004 g/cm^3 and 1.0384 ± 0.0004 g/cm^3, respectively. Thus, the absolute density of the hippocampal dentate gyrus of VPA rats was higher than that of the control rats by 9.4 mg/cm^3. Increased density might be caused by various neurodegenerative processes in the hippocampal dentate gyrus. This is considered to be associated with the etiology of ASD in prenatal exposure of VPA because the hippocampus is an important region for memory function. In addition, mild to moderate expansion of the lateral ventricle, an associated finding of ASD, was found in VPA rats. H&E staining also revealed increased cell proliferation in the hippocampal dentate gyrus of VPA rats compared to that of the control rats (**Fig. 2**). This finding was consistent with increased density in the hippocampal dentate gyrus of VPA rats on phase-contrast CT imaging.

Our findings indicate that phase-contrast X-ray CT clearly depicted minute histopathological changes in the hippocampal dentate gyrus. Thus, phase-contrast X-ray CT can depict the autistic neuronal morphology in detail, and will be a useful tool for various developmental neurotoxicity research.

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