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Involvement of endoplasmic reticulum stress and neurite outgrowth in the model mice of autism spectrum disorder

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ABSTRACT

Neurodevelopmental disorders are congenital impairments, impeding the growth and development of the central nervous system. These disorders include autism spectrum disorder (ASD) and attention-deficit/hyperactivity disorder in Diagnostic and Statistical Manual of Mental Disorders-5. ASD is caused by a gene defect and chromosomal duplication. Despite numerous reports on ASD, the pathogenic mechanisms are not clear. The optimal methods to prevent ASD and to treat it are also not clear. Other studies have reported that endoplasmic reticulum (ER) stress contributes to the pathogenesis of neurodegenerative diseases. In this study, we have investigated ER stress condition and neuronal maturation in an ASD mice model employing male ICR mice.

An ASD mice model was established by injecting with valproic acid (VPA) into pregnant mice. The offspring born from VPA-treated mothers were subjected to the experiments as the ASD model mice. The cerebral cortex and hippocampus of ASD model mice were found to be under high ER stress. The mRNA levels of *Hes1* and *Pax6* were decreased in the cerebral cortex of the ASD model mice, but not in the hippocampus. In addition, the mRNA level in *Math1* was increased in the cerebral cortex. ER stress inhibited dendrite and axon extension in primary culture derived from the cerebral cortex of E14.5 mice. Furthermore, dendrite outgrowth was suppressed in primary culture derived from the cerebral cortex of ASD model mice by the same method.

These results indicated the possibility that ER stress induces abnormal neuronal maturation in the embryonal cerebral cortex of ASD model mice employing male ICR mice. Therefore, ER stress may contribute to the pathogenesis of ASD.

1. Findings

Neurodevelopmental disorders are defined as congenital developmental impairments of the central nervous system. Autism spectrum disorder (ASD), which is characterized by abnormal behavior such as a deficit in social communication ability and ritual behaviors, is included in the list of neurodevelopmental disorders. It has been reported that ASD is caused by genes defects such as neurexin-neuroligin-shank complex (Gjørlund et al., 2012), chromosomal duplication on 15q11-q13 encoding $GABA_A$ receptor subunits and ubiquitin ligase (Nakatani et al., 2009; Pizzarelli and Cherubini 2011; Greer et al., 2010), and antenatal environmental factors such as medication, alcohol, and cigarette smoking (Landgren et al., 2010; Christensen et al., 2013). In addition, it is known that neuronal differentiation and neuronal maturation may be involved in the pathogenesis of ASD. However, the pathogenic mechanism of ASD is not clear, and information regarding prevention and treatment of ASD is rare. In this study, we investigated whether endoplasmic reticulum (ER) stress is one of the pathogenic mechanisms of ASD.

The ER is a multifunctional organelle required for protein folding and processing. When the unfolded proteins accumulate in the ER lumen, the ER is under stressful condition. It is well-known that ER stress causes neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease (Kaneko et al., 2010; Omura et al., 2013), but its function in neurodevelopmental disorders such ASD is not known. On the other hands, it has been

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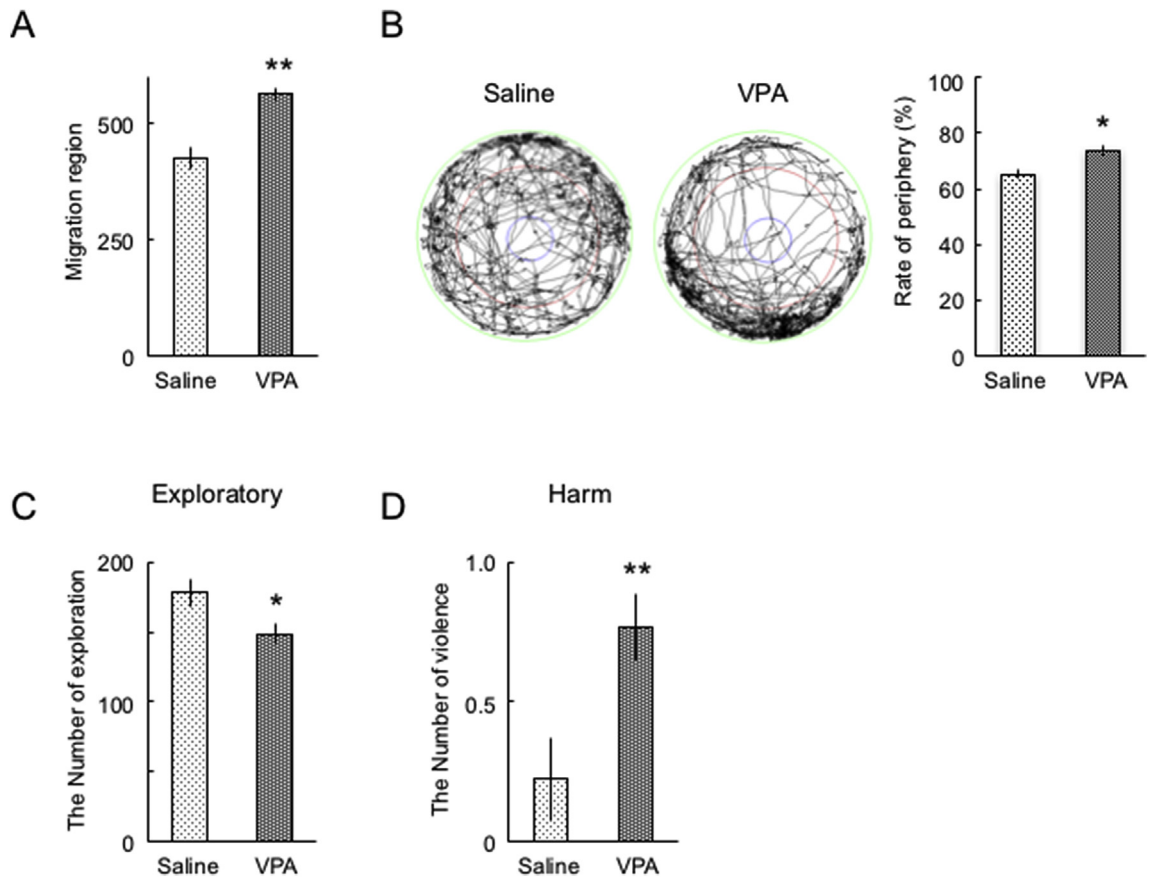

Fig. 1. ASD model mice display abnormal behaviors. (A) By measuring the total migratory behavior around the area of the cage, we revealed that ASD model mice had an increased migration area compared with controls. (B) Pictures display the migrated trajectory of mice. By measuring the percentage time spent in the periphery compared to the total zone, we revealed that ASD model mice spent more time in the periphery, indicating increased anxiety compared with controls. (C) This graph displays the exploratory behavior of mice regarding a novel object. ASD model mice showed less interest and less exploratory behaviors than the controls. (D) This graph displays the harming behaviors for the social communication test. ASD model mice showed a higher number of violent behaviors. Values are presented as the means ± SE from 9 to 10 independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, significantly different to controls.

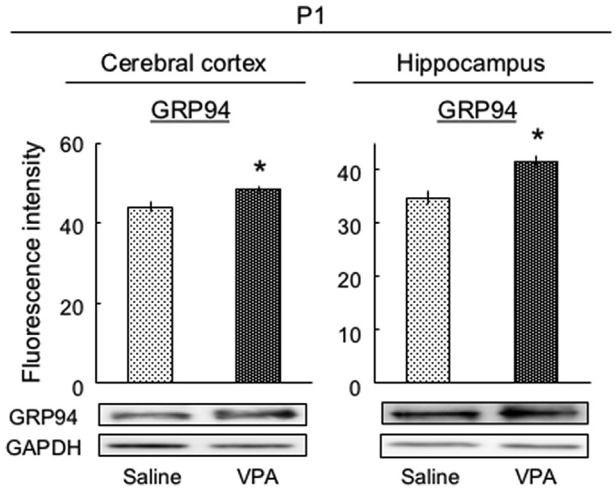

Fig. 2. The brain of the ASD model mice is under the ER stress. Pregnant mice were given VPA (500 mg/kg) at E9.5. After delivery, offspring animals were decapitated at P1 for preparation of lysates of the cerebral cortex and hippocampus. Tissue lysates were subjected to immunoblot analysis for determination of GRP94. These experiments were carried out at least eight times with similar results under the same experimental conditions. The expression of GAPDH, the housekeeping protein, did not change in these experiments. Values are presented as the means ± SE from eight independent experiments. $^{*}p < 0.05$, significantly different to controls.

reported that ER stress is increased by alcohol consumption, cigarette smoking, and other environmental factors such as a high-fat diet during pregnancy. We have previously reported that mild ER stress in the differential process induces abnormal neuronal differentiation and maturation (Kawada et al., 2014). Here, we investigated whether ER stress in the developmental brain of ASD mice induces abnormal neuronal differentiation and maturation using a ASD mice model.

In this study, we employed male ICR mice (n = 7–9), which were housed with room temperature at 21 °C, and artificial 12: 12 h light: dark cycle before the experiments. Food and water were available ad libitum. The reporting of the animal experiments conforms to the animal experiment guidelines of *Chiba Institute of Science*. Before the study launch, ethical approval was obtained by the ethics committee of *Chiba Institute of Science*. To establish ASD model mice, we injected valproic acid (500 mg/kg, i. p. VPA) into pregnant mice at 9.5 days gestation. The offspring born from VPA-treated mothers were subjected to the experiment as ASD model mice. We observed a few deformed pups in VPA-induced offspring at E14.5. We used the VPA-induced offspring group except for the deformed pups. Primary cultured neurons were prepared from the cerebral cortex of the offspring mice at embryonic day 14.5 (E14.5). Locomotor activities in open-field and social communication activities were measured using SMART V3.0 (Bioresearch Center, Japan). The mice were acclimatized for 30 min before the trial in the

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