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The epigenetic change of granule cell precursors causes excess folding of cerebellar lobules to induce ASD-like malformation
自閉症モデル動物における神経細胞の変化とエピジェネティクス

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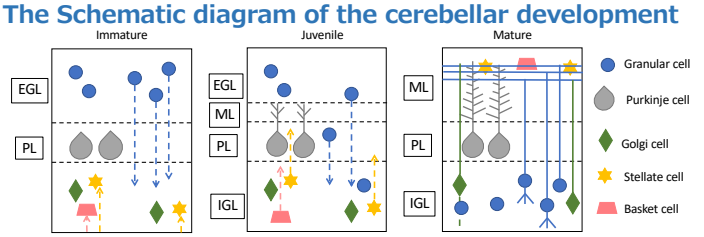
Introduction

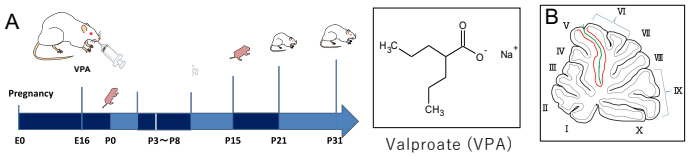
Valproate (VPA), the popular anticonvulsant and mood stabilizer, is known as an inducer of autism. It has many kinds of physiological properties, including the inhibition of histone deacetylase (HDAC). Recently we reported VPA administration to rat fetus caused developmental changes and malformation in the cerebellar cortex, correlated with autism. In the VPA-administrated cerebellum, the dendrites of Purkinje cells were elongated earlier than in vehicles and immature granule cells were left in the external germinal layer even in P16. Additionally, VPA-administrated rat showed the excess folding between the V to VI lobules of cerebellar vermis within two weeks after birth with dose-dependent and administrated-period dependent manner and maintained in adult. This alteration would be deeply related with epigenetic change of neuronal development due to VPA.

The Schematic diagram of the cerebellar development

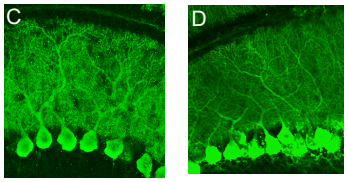


After birth, the two layers, the External Granular Layer (EGL) and the Purkinje Layer (PL) are in the cerebellar cortex. With maturing, many granule cells migrate down to the Internal Granular Layer (IGL) and extend their axons towards the Molecular Layer (ML), and Purkinje cells become developed with elongation of their dendrites. Other interneurons migrate up to the ML to develop the complex neuronal circuits with each other.

Materials & Methods



200-600mg/kg VPA was dosed P.O. to pregnant rat at E16, sometimes at E14 or E18. Offspring was fixed at P3 to P21, and observed its cerebellum (Cb) stained with anti-Calbindin D-28k, anti-glast, anti-GFAP, anti-reelin and anti-H3K27 antibodies.
In Hematoxylin-Eosin image, we calculated the ratio of the length of Purkinje layer (red line) to the depth of gyrus (green line).



PCs in P14 control Cb　PCs in P14 VPA-ad Cb

In VPA-ad Cb, Purkinje cells (PCs) showed the abnormal form of dendrites and broken lines of soma (C: control Cb, D: VPA-ad Cb both in P14).

Result 1. Excess folding of cerebellar lobules

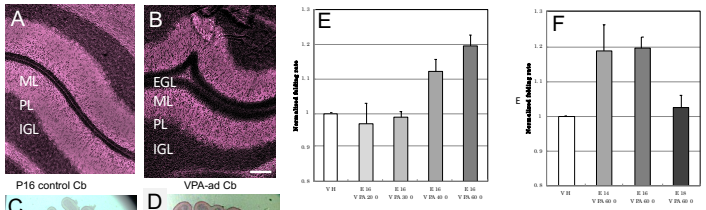
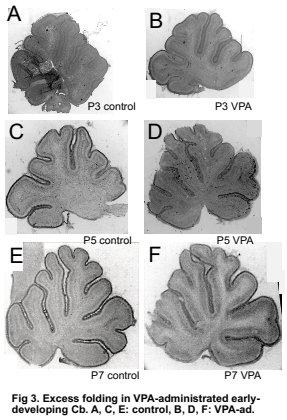


Fig 2. Excess folding in VPA-administrated Cb and dose- or timing-dependencies.

Excess folding was observed at the primary fissure between the V and VI lobules in VPA-ad Cb (B and D) and appeared in the Cb administrated the VPA 400 mg/kg above with dose-dependent manner (E).

The E14 VPA 600 mg/kg administrated rat pups showed similar abnormal development of the cerebellar cortex which was observed in the VPA 300 and 400 mg/kg administrated rat, however, the E18 VPA administrated pups did not (F).

Result 2. Epigenetic changes in Cb early development



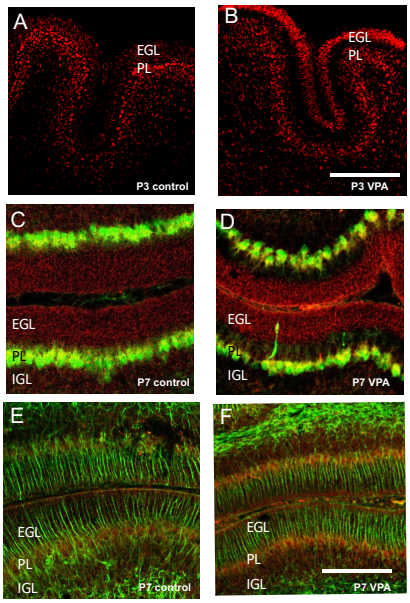


Fig 3. Excess folding in VPA-administrated early-developing Cb. A, C, E: control, B, D, F: VPA-ad.

Fig 4 Distribution of acetylated H3K27 positive cells (A, B), Calbindin D28k (green) and reelin (red) (C, D) and GFAP (green) and GLAST (red) (E, F).

Even in P3 VPA-ad Cb, acetylated H3K27 positive cells were observed in the EGL(Fig.4B),whereas excess folding was observed after P5 (Fig.3). As VPA is known as non-specific HDAC inhibitor, it would make granular neuron precursors deacetylate and develop. Reelin expressed higher in the EGL in P7 VPA-Cb animals (Fig.4D) than control animals (Fig.4C), and GLAST, glutamate transporter on astrocytes was developed earlier in P7 VPA-ad Cb (Fig.4F) than control (Fig.4E).

Conclusion

VPA is a famous antiepileptic agent and a well-known HDAC inhibitor which causes wide-spread epigenetic change. Even in P3 cerebellum of VPA-administrated rat, H3K27-acetylated granule cells appeared in the EGL and the irregular folding appeared in P5. Epigenetic-changed granule cells expressed reelin earlier than control and migrated down to the Internal granular layer making the line for the excess folding. The expression of the astrocyte-specific glutamate transporter, GLAST was increased in P7 cerebellar cortex of VPA-admin.
In the developing cerebellum, the granule cells in the IGL secrete Reelin to regulate the position of Purkinje neurons. We suggest the epigenetic alteration of granule cells with VPA would induce excess reelin expression and early development of granule cells.