3P5-3

Quantitative Monitoring for Cerebellar Abnormal Development of Acoustic Model Animals using Acoustic Impedance Pattern

音響インピーダンスパターンによる
自閉症モデル動物の小脳神経回路変性の定量モニタリング

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1. Introduction

Two-dimensional acoustic impedance imaging is useful for observation of biological tissue characterization. In a previous report, the methodology for microscale imaging of the cross sectional acoustic impedance of vital cerebellar slices was described[1]. The acoustic observation with 120 MHz transducers could identify three layers of rat cerebellum. Furthermore, the higher frequency 320 MHz transducers enabled more finely observation of biological cells and tissues[2, 3].

Autism, which is the mental disorder of communication, has been reported some structural abnormalities in the cerebellum. Recently, we have constructed the valproate-induced autistic animal models (VPA-rat) and reported the neuronal development in the cerebellum of the VPA-rat were different from control one[4], while the differences of neuronal networks and connections in the mature cerebellum were unclear. Optical observation of tissue is required thin-slicing and staining process for several days, whereas the acoustic imaging can be performed without staining process for a very short time.

In this study, we report the difference of the mature cerebellum in the autistic model rat from control animal using acoustic microscopy, especially, characterization of the granular layer (IGL) and the molecular layer (ML) in the cerebellar cortex. Both layers are known to relate neuronal networks.

2. Methods

Gestational day 16 (ED 16) pregnancy rat was administrated VPA, an anti-epileptic drug, to 600 mg/kg body weight per os. The offspring were employed as autism model rats.

The mature cerebellum of both VPA-rat and control rat are sampled from postnatal weeks 3 (PW3) and PW8, fixed chemically with 4% paraformaldehyde perfusion under anesthesia and rinsed in phosphate buffer solution (PBS). Each cerebellum was sliced sagittally at 400 μm thickness for the acoustic impedance observation and 50 μm thickness for optical observation using Linear slicer (DOSAKA EM CO., LTD, Japan).

To measure acoustic impedance, each slice mounted on the OptiCellTM (Thermo Scientific Nunc, USA), 75μm thickness polystyrene films, with PBS solution. PBS becomes reference material in the observation. To observe cerebellar cortex structure, the pulsed focus ultrasound (central frequency: 300 MHz) was transmitted, and the reflection from the interface between biological tissue and polystyrene film was received and interpreted into characteristic acoustic impedance. The 2D profile of acoustic impedance was acquired by mechanically scanning the transducer.

Some slices were subjected immunohistochemical staining to compared with images of acoustic impedance.

3. Results and Discussion

The layers in rat cerebellum could be observed using high-resolution acoustic impedance microscope. The IGL and the ML were differentiated as the contrast in acoustic impedance (Fig. 1a). Impedance values average has no difference between VPA-rat and control rats, whereas, the ratio of impedance (IGL/ML) in the VPA-rats was higher than it in control rats. This suggests that some abnormality would be appeared in the VPA-rat (Fig. 1b).

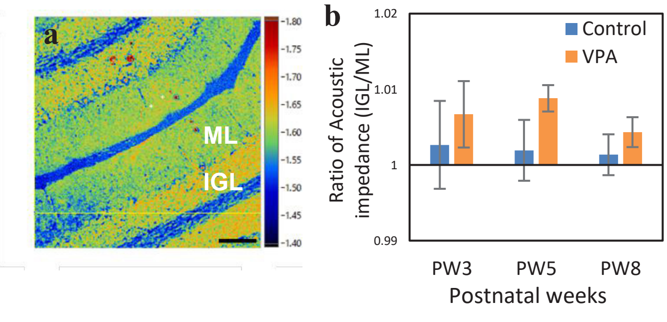

Fig. 1. The difference of acoustic impedance between IGL and ML. **a**, Acoustic impedance image. (postnatal weeks 3, control, 1.44 sq. mm., scale bar = 100 μm). **b**, Graph of acoustic impedance ratio of ML to IGL. Values are means ± S.D. (n=3-6).

In the acoustic impedance images in both VPA-rat and control rat, the dendrites of Purkinje cells could be observed as some vertical lines in the ML of PW8 (Fig. 2c, 2d). They were observed better in VPA-rats than control rats of postnatal weeks 8 (Fig. 2d), but a quantitative difference was not shown.

On the other hand, the graininess of the IGL in the VPA-rats was different from control rat. Therefore, the skewness of acoustic impedance values in the region of interesting (ROI) in the IGL was calculated. In the VPA-rat, deviation of particle distribution from acoustic impedance has showed compared to control (Table 1, Fig. 3b, 3d). Especially, it was indicated in the PW8 rat (Table 1). These results suggest that the condition of the granule cell-clusters in the IGL would be different between the VPA-rat and control rat. In the VPA-rat cerebellum, the granule cell clusters would be ununiform.

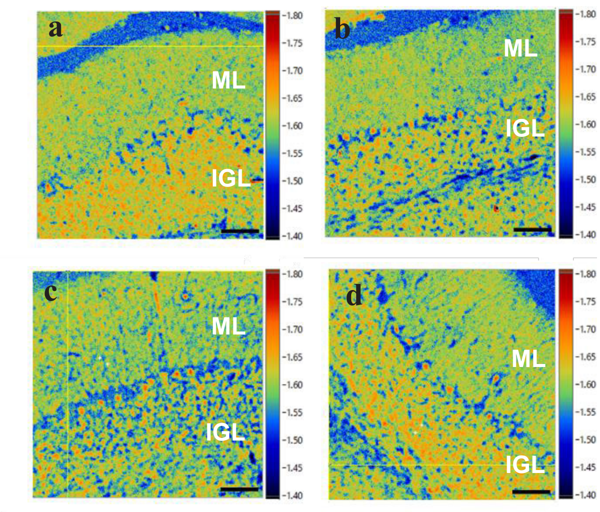

Fig. 2. Particle distribution from Acoustic impedance images. **a**, control of PW 3, **b**, VPA-administrated rat of PW 3, **c**, control of PW 8, **d**, VPA-administrated rat of PW 8. All images are scanned 3600 sq. µm. (scale bar=100 µm).

Table 1 Averaged acoustic impedance values of skewness from cerebellum cotex of VPA-administrated rats and control (1.44 sq. mm.)

Postnatal weeks	layar of cerebellum cortex	Group	n	Mean ± SD
PW3	IGL	control	3	-0.458 ± 0.194
		VPA	4	-0.555 ± 0.103
	ML	control	3	-0.283 ± 0.155
		VPA	4	-0.382 ± 0.203
PW8	IGL	control	5	-0.364 ± 0.240***
		VPA	5	-0.583 ± 0.298
	ML	control	5	-0.355 ± 0.175
		VPA	5	-0.449 ± 0.318

Comparison between the group of VPA and control in IGL in PW8 using *t* test revealed significant difference(***p-0.003)

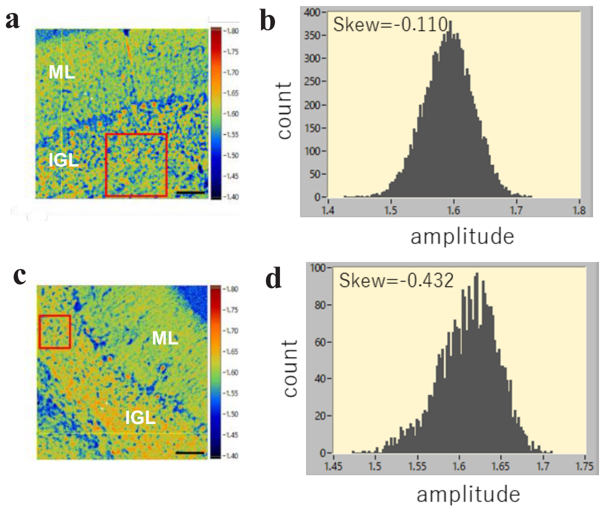

Fig. 3. Histogram of acoustic impedance amplitude. **a**, **c**, Red frame showed the region of interesting (ROI), and **b**, **d**, the histogram of acoustic impedance amplitude was shown in the ROI. **a**, **b**, control of PW 8, and scanned 3600 sq. µm. (scale bar=100 µm). **c**, **d**, VPA-administrated rat of PW 8, and scanned 3600 sq. µm. (scale bar=100 µm).

4. Conclusion

We suggest that the high-resolution acoustic impedance microscopy could finely analyze of cerebellar structure difference between the autistic model rat and control. In the future, quantitative observation of maturation-dependent alternation in cerebellum using the acoustic impedance microscopy would be useful to recovery some diseases and diagnosis of disease severity.

5. References

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