Morphometric Evidences for Regional Variation in Potential of Neural Plasticity

Evidencias Morfométricas de Variación Regional en Potencial de Plasticidad Neural

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SUMMARY: The neural plasticity showing the ability of nervous system to change its structure and function is a well-documented fact. However regional variation within a CNS structure to undergo plastic changes has been shown by limited studies.

Along medial-lateral sequences of parasagittal sections, the molecular layer thickness of primary fissure borderlands in rat cerebellar left hemisphere was studied to assess the regional difference in plasticibility.

Despite the homogeneity of cerebellar histology, this study showed that there is a significant interlobular difference between ML thicknesses of Prf borderlands. In addition, it revealed that the thickness alters in a significant trend within each borderland.

The quantitative heterogeneity of cerebellar architecture such as variation of cortical thickness may provide some evidences to show that different regions of a homogenous cortex, even two adjacent borderlands and areas within them, can have different potentials for plasticity.

KEY WORDS: Plasticibility; Molecular layer; Primary fissure; Cerebellum; Rat.

INTRODUCTION

Neural plasticity showing the ability of nervous system to change its structure and function is a well-documented fact. (Diamond, 2001) Indeed, this is the basis of normal learning and memory. (Diamond; Crepel *et al.,* 1996).

Alterations of cortical thickness as well as other structural changes were studied by some researchers to show the neural plasticity in response to different exposures (Diamond). Despite these abundant attempts showing dynamism of neural tissues, regional variation within a CNS structure to undergo plastic changes which may be called as "regional plasticibility" was shown by limited studies (Anderson *et al.*, 1994, 2002).

The logic way to show the difference between the plasticibility of two regions is to compare their plastic changes following an exposure. However, the results of such a comparison may be exposure-dependent and can be differ with each exposure (Anderson *et al.*, 2002).Therefore, to have a constant scope of regional difference in plasticibility, the tissue characteristics such as cortical thickness which may reflect the ability for plastic changes can be compared.

In this study, the two lobular borderlands of primary fissure (Prf) in rat cerebellar left hemisphere were the subjects of the comparison. The thickness of molecular layer (ML) which is a synaptic zone and abundant of neural processes was chosen as comparing characteristic between the borderlands (Kiernan, 1998). Therefore, along a medial-lateral range of study, the lack or presence of difference between two borderlands (Interlobular variation) and within them (Intralobular variation) were assessed. (Fig.1).

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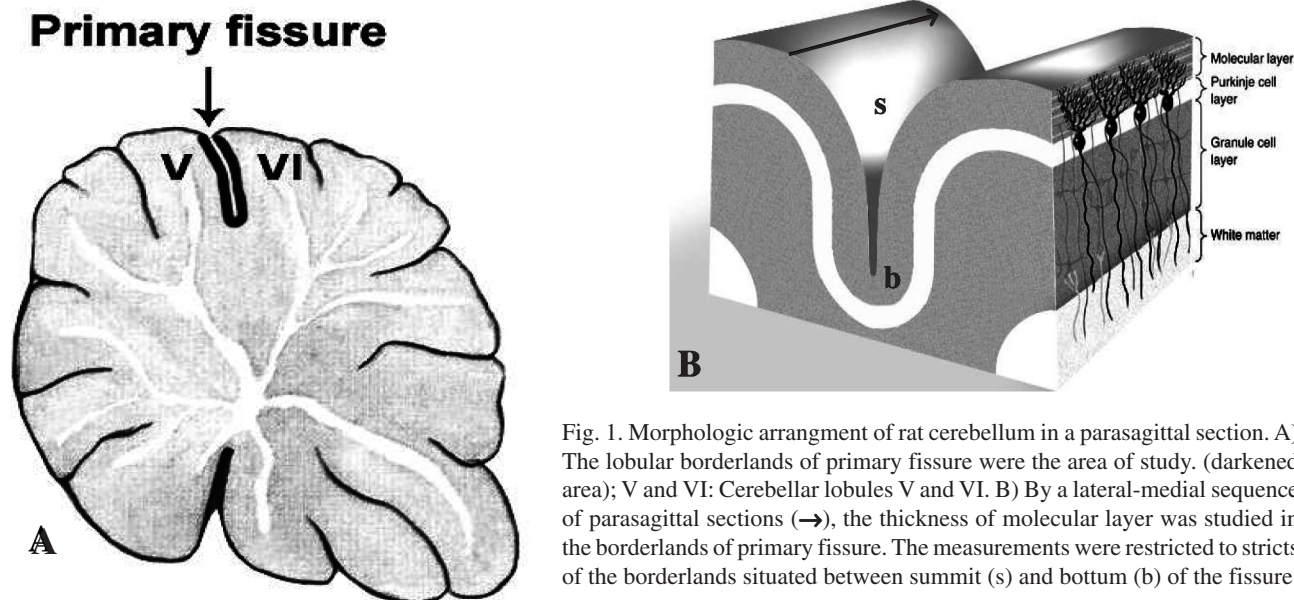
Fig. 1. Morphologic arrangment of rat cerebellum in a parasagittal section. A) The lobular borderlands of primary fissure were the area of study. (darkened area); V and VI: Cerebellar lobules V and VI. B) By a lateral-medial sequence of parasagittal sections (→), the thickness of molecular layer was studied in the borderlands of primary fissure. The measurements were restricted to stricts of the borderlands situated between summit (s) and bottum (b) of the fissure.

MATERIAL AND METHOD

This study was performed on two 6-week male Albino Wistar rats (E1 and E2) bought from Iranian Pasteur Institute. Both experiments were kept in same animal house with a non-enriched environment, 12 hours light/12 hours dark cycle, standard food regimen and water *ad libitum*.

After 18 weeks of keeping, the 24-week experiments were anesthetized by chloroform inhalation and killed with cervical dislocation. The skulls were dissected and cerebella were taken. To fix them, they were emerged in Clark solution for 48 hours. After fixation, the left hemispheres of each of two cerebella were separated from reminder at their junction with relevant vermises. The hemispheres were embedded in paraffin and 6μm parasagittal serial sections were prepared from each of them.

For a simple random selection, every 3-5 sections of each hemisphere were mounted on a glass slide. Consequently, a lateral-medial series of slides contained the parasagittal serial sections were provided for each hemisphere. As the count of sections mounted on the slides was variable between 3 to 5 sections, no equal interval was put in the lateral-medial position of the slides which is necessary in a simple random selection. Finally, the slides with numbers of 50, 60, 70, 80, 90, 100 and 110 for E1 and 50, 60, 70, 80 and 90 for E2 were selected.

The picture of Prf and the borderlands in each selected sections, stained with H&E, was captured by means of Olympus digital light microscopy and an ACC camera. On the spot, it was transferred to the computer. The ML thicknesses of Prf borderlands were measured by Olysia Autobioreport software, Olympus corporation. The area of study in each section was a strict of borderlands situated between the summit to bottom of the fissure. (Fig. 1).

Cumulatively, 710 and 642 data were collected for ML thicknesses of borderlands V and VI of E1, respectively. In E2, the counts of measurements were 281 for borderland V and 303 for borderland VI.

Considering 6μm thickness of each sections and numbers of selected sections, we studied the left cerebellar hemisphere of E1 in the range of 1113μm to 2421μm from lateral extreme toward the vermis (with a length of 1308μm). However, this range was 1161μm to 2145μm for E2 in the same direction. (With a length of 984μm).

Because of quantitative character of the data, the normality of their distributions in each borderland of the experiments was assessed by one-sample Kolmogrov-Smirnow test. After confirmation of the normality, the mean ML thicknesses of the borderlands were compared with each

other using independent sample T-test. The significance was set at p≤0.05.

To determine the relationship between ML thickness and lateral-medial position within the borderlands by the regression methods, in addition to normality of data distribution, the constancy of their variances was confirmed. Consequently, the different regression models such as linear, exponential, logarithmic, inverse, power and etc showing ML thickness in a lateral-medial transition within the borderlands were fitted.

RESULTS

The mean thicknesses of Prf borderlands in each of experiments were summarized in Table I. As shown in this table, in both experiments, the Molecular layer of borderland VI was significantly thicker than the corresponding layer of borderland V (p≤0.0009). Also, the same finding was found with combined values of the experiments. (p≤0.0009).

Table I. Mean thicknesses of Molecular layer in Prf borderlands of the experiments.

	Borderland V	Borderland VI
Experiment 1	40.44 (6.95, 0.26) 1	49.43 (10.50, 0.41)
Experiment 2	41.53 (8.25, 0.49)	45.57 (13.57, 0.78)
Total	40.75 (7.35, 0.23)	48.19 (11.71, 0.38)

1. μm (SD, SEM)

The scatter diagrams showing the lateral-medial transition of ML thickness in Prf borderlands were drawn for each experiment where the horizontal axis is representative of the distance that each section had from the lateral extreme of left hemisphere and vertical axis shows the relevant thicknesses measured in each sections. (Figs. 2, 3, 4, 5) Although all the fitted regression models were significantly appropriate (p≤0.05); but as the linear model is easier to understand, it was chosen to associate the diagrams.

In Fig. 2, it is shown that in a lateral-medial trial, the closer to vermis, the thicker were the layers. The slope of the line is positive and the model was significantly appropriate. (p≤0.0009; R^2= 0.035)
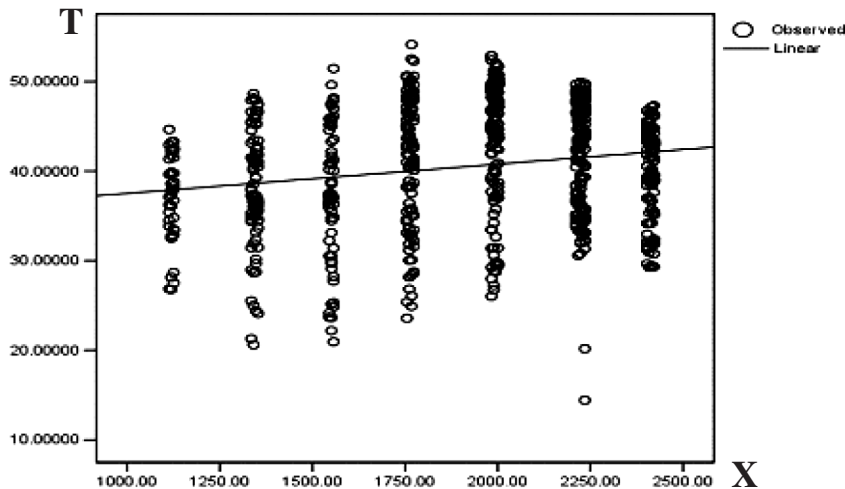

Fig. 2. Lateral-medial transition of ML thickness in Prf borderland V of E1. The equation of fitted line was T=34.249+0.003x with p≤0.0009 and R^2=0.035.
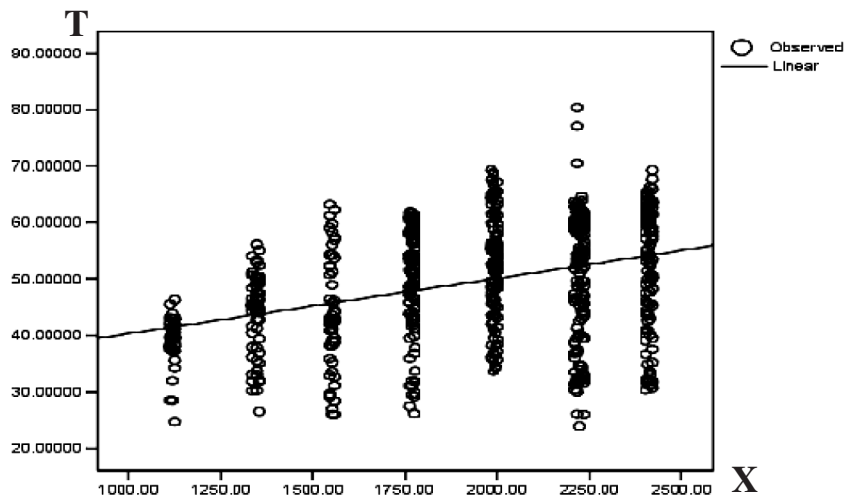T: ML thickness (μm); X: lateral-medial position (μm).

Fig. 3. Lateral-medial transition of ML thickness in Prf borderland VI of E1. The equation of fitted line was T=30.507+0.010x with p≤0.0009 and R^2=0.129.
T: ML thickness (μm); X: lateral-medial position (μm).

Fig. 3 shows that the ML thickness of Prf borderland VI of E1 changes in the same manner as the borderland V. The fitted equation for this diagram has a positive coefficient showing the increase in mean ML thicknesses of borderland VI from lateral extreme toward vermis. (p≤0.0009; R^2= 0.129).

Comparison between the fitted equations showed that the coefficient for borderland VI equation was more than the same one for borderland V. (0.010 Vs 0.003).

In E2, as shown in Figs. 4 and 5, the ML thicknesses of both borderlands clarify a decreasing lateral-vermial transition.

A significant linear equation was fitted for each of diagrams. (p≤0.0009; R^2= 0.106 for borderland V and p≤0.0009; R^2= 0.075 for borderland VI)

Comparison between the fitted equations showed that the coefficient for borderland VI was more than the same for borderland V.
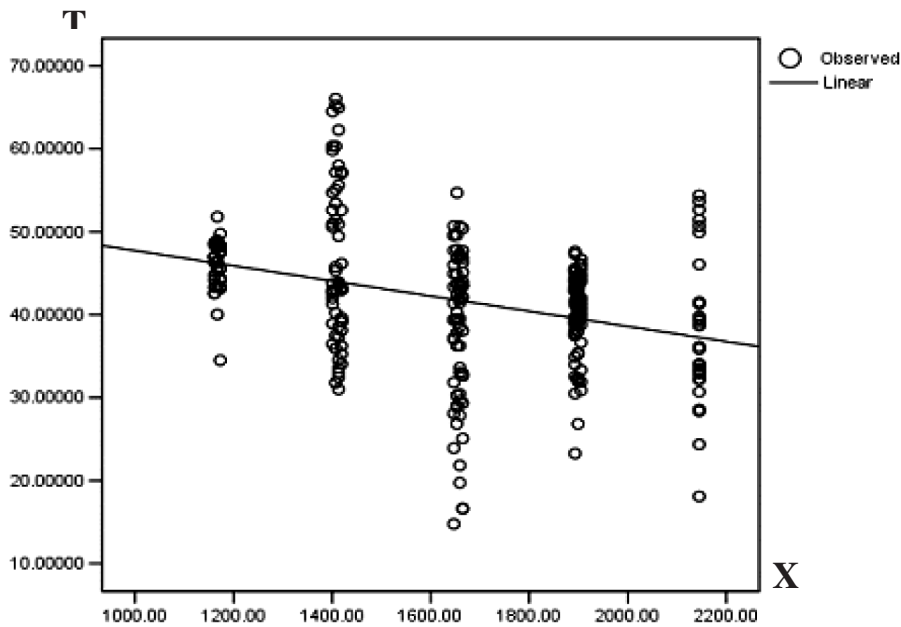

Fig. 4. Lateral-medial transition of ML thickness in Prf borderland V of E2. The equation of fitted line was T= 56.868-0.009x with p≤0.0009 and R^2=0.106.
T: ML thickness (μm);
X: lateral-medial position (μm).
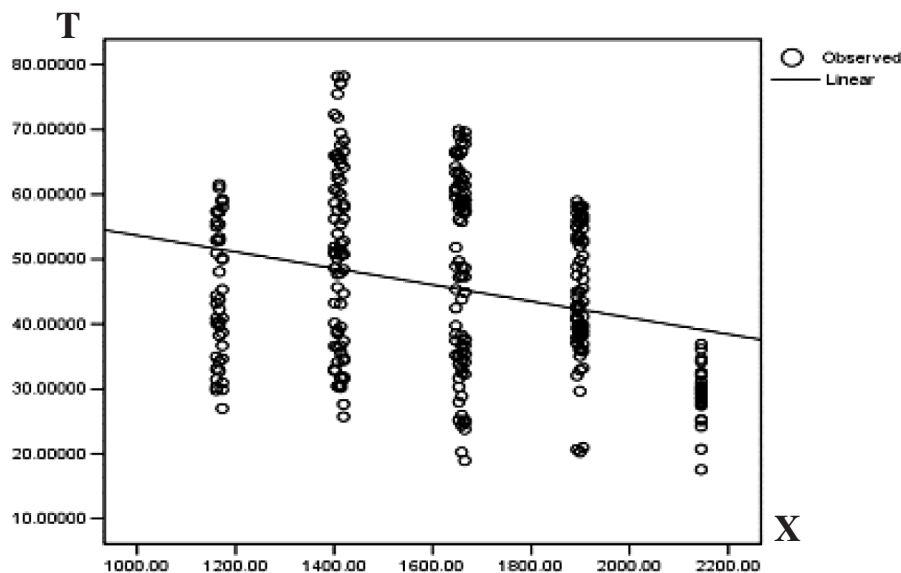

Fig.5: Lateral-medial transition of ML thickness in Prf borderland VI of E2. The equation of fitted line was T = 66.371-0.013x with p≤0.0009 and R^2 =0.075.
T: ML thickness (μm);
X: lateral-medial position(μm).

DISCUSSION

Despite the homogeneity of cerebellar histology, this study showed that there is a significant interlobular difference between ML thicknesses of Prf borderlands. In addition, by a lateral-medial study of parasagittal sections, it revealed that the thickness alters in a significant trend within each borderland showed the intralobular variation.

The cytoarchitecture of molecular layer is designed in a way made it as a modifier structure. Indeed, by inhibitory interneurons like basket and satellite cells associated by dendrites of Golgi cells, it can modify any exciting volley entering through parallel and climbing fibers to Purkinje cells. This fine tuning task of molecular layer between the inputs of Purkinje cells and Purkinje output to deep nuclei play an important role in cerebellar function (Kiernan).

Long-term depression (LTD) of synaptic transmission at parallel fiber-Purkinje cell synapses is thought to be a cellular substrate of synaptic plasticity in the cerebellum. This use-dependent change in synaptic efficacy is induced by conjunctive stimulation of parallel and climbing fibers (Crepel *et al.*,). In concert with LTD at excitatory synapses of Pukinje fiber, a robust and long-lasting potentiation of inhibitory synaptic transmission is induced following stimulation of excitatory climbing fibers, that was termed "rebound potentiation" (RP) (Kano, 1996).

As the thickness can reflect the neuronal content, the homogenous areas with different thicknesses may have difference in quantity of histologic facilities. Therefore, it can be reasonably postulated that, in mentioned areas such as Prf borderlands, neurons like Purkinje cells can be trapped by different compositions of presynaptic fibers and,

consequently, show synaptic heterogeneity even have no difference in postsynaptic receptors (Lambolez *et al.*, 1992).

Excitatory and inhibitory populations (parallel/climbing fibers Vs basket/satellite and Golgi cells) play differently in LTD and RP (Crepel *et al.*; Kiernan and Kano). Hence, as areas with different thicknesses may have difference in neuronal populations, it can be theorized that these mechanisms share differently in plasticity of various cerebellar regions.

Found trends and equations can help guide to develop a computer model integrating the morphometric characteristics of rat cerebellar hemisphere. Such model can simulate an artificial tissue displaying the plastic changes of different cerebellar regions in response to different exposures as well as pathologic processes and therapeutics.

CONCLUSION

Based on these postulated differences in mechanisms of neural plasticity, the quantitative heterogeneity of cerebellar architecture such as variation of cortical thickness may provide some evidences to show that different regions of a homogenous cortex, even two adjacent borderlands and areas within them, can have different potentials for plasticity.

RESUMEN: Aunque la plasticidad neural muestra la capacidad del sistema nervioso para cambiar su estructura y función, lo cual es un hecho bien documentado, pocos estudios han mostrado la variación regional, dentro de la estructura del sistema nervioso central para sufrir cambios plásticos.

A través de disecciones parasagitales, secuenciadas de medial a lateral, se estudió el grosor de la capa molecular, en el límite de la cisura primaria de hemisferio izquierdo del cerebelo, de ratas, con el propósito de evaluar las diferencias regionales en plasticidad.

A pesar de la homogeneidad de la histología cerebelar, el estudio mostró que hay una diferencia interlobular significativa entre el grosor de la capa molecular en el límite de la fisura prima. Agregado a ello, fue revelado que los cambios de grosor tienen una tendencia significativa dentro de cada límite.

La heterogeneidad cuantitativa de la arquitectura cerebelar, tal como la variación en el grosor cortical, puede proveer algunas evidencias que muestran que regiones diferentes de un corte homogéneo, aún de límites y áreas adyacentes dentro del mismo, pueden tener diferentes potenciales para plasticidad.

PALABRAS CLAVE: Plasticidad; Capa molecular; Fisura prima; Cerebelo; Rata.

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