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Alterations in the Postnatal Development of the Cerebellar Cortex due to Zinc Deficiency

III. Impaired Dendritic Differentiation of Basket and Stellate Cells

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The effects of zinc deficiency and undernutrition on the dendritic differentiation of basket and stellate cells were studied in 21-day-old rats. A morphometric analysis of the dendritic branching of basket and stellate neurons was used that took into account the cell's position in the molecular layer. Zinc deficiency and undernutrition during the suckling period impaired the dendritic differentiation of cerebellar basket and stellate cells. The effects of zinc deficiency were not due totally to the reduced food intake of lactating dams. In the lower 65–75% of the molecular layer of zinc-deficient (ZD) pups, the dendritic field area, the total dendritic length and the number of branches per interneuron were reduced by 45–61%. In the lower 50–60% of the molecular layer, undernutrition reduced the dendritic field area, the total dendritic length and the number of branches per neuron by 32–44%. A comparison of ZD and undernourished (pair-fed) pups indicated that the dendritic field area and total dendritic length of neurons of ZD animals were 43% and 30% smaller in the lower half of the molecular layer. The number of branches per neuron was not significantly different between ZD and undernourished animals. The area of the soma was unaffected by dietary treatment. A delay in the onset of dendritic differentiation and a retarded rate of dendritic growth were considered possible mechanisms for the impaired dendritic differentiation.

INTRODUCTION

Zinc deficiency occurs in animals and humans[12,19,24]. Although the effects of zinc deficiency on the developing brain of humans are unknown; it has become increasingly evident in experimental animals that zinc deficiency impairs the growth of the brain (reviewed in ref. 20). Impaired growth of the cerebellar cortex, a delayed disappearance of the external granule cell layer, impaired aquisition of granule cells and retarded differentiation of Purkinje cells were described in our previous reports[5,6].

Basket and stellate cells are interneurons of the cerebellar molecular layer[14]. In the rat these interneurons are formed and differentiate during the first three postnatal weeks[1,2]. Like the Purkinje cells, the normal differentiation of the dendrites of basket and stellate cells depends on interactions with appropriate numbers of parallel fibers[3,13,16–18,21]. The impaired maturation of the molecular layer[5,6] and the decreased numbers of granule cells[5] of animals subjected to early postnatal zinc deficiency suggests that the dendritic growth of these neurons might be impaired by zinc deficiency.

MATERIALS AND METHODS

The methods of producing zinc deficiency in suck-

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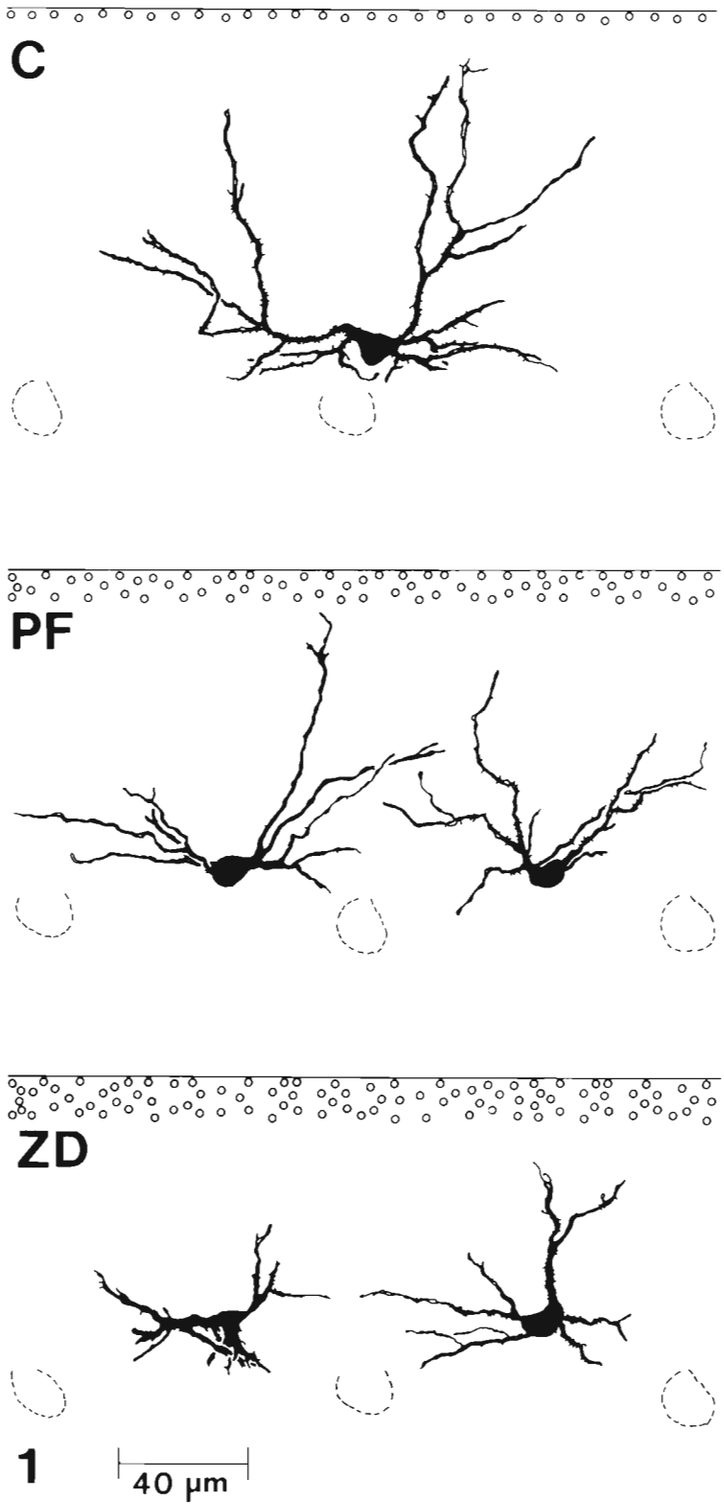

Fig. 1. Basket cells of ZD, PF and normal (C) 21-day-old rats.

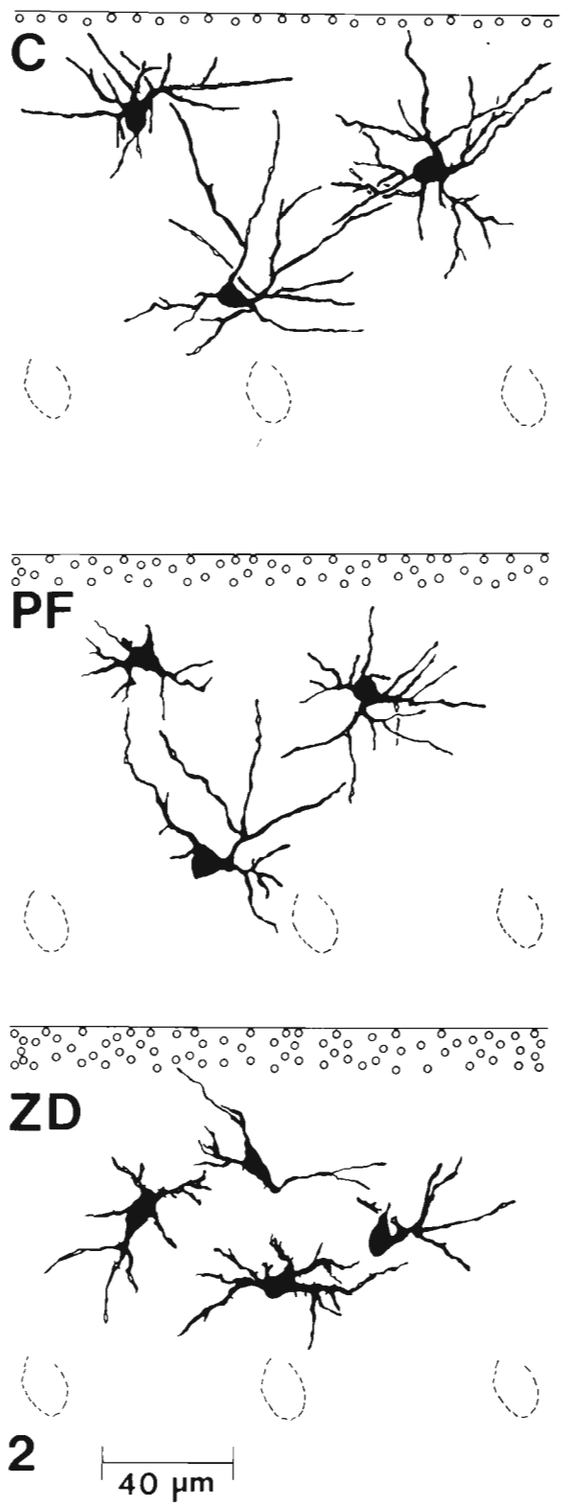

Fig. 2. Stellate cells of ZD, PF and normal (C) 21-day-old rats.

ling rats and the procedures for controlling the effects of undernutrition in the ZD dams were described previously[5,6]. The processing of cerebellar tissue for the Golgi method were described in the preceding paper[6]. All animals examined were 21-day-old rats.

Quantitative methods

Only neurons with dendrites that did not appear truncated by sectioning were considered in this study. Basket and stellate cells along the posterior superior fissure[9] were chosen randomly from a pool of well-impregnated neurons. All neurons were

drawn at the same magnification with a ×100 objective. A total of 17 ZD, 12 pair-fed (PF, undernourished) and 15 normal control pups were studied. With a computer and digitizing tablet the distance from the center of the soma to the upper border of the Purkinje cell layer, the dendritic field area, the total dendritic length and the area of the soma were measured. The area of the dendritic field was determined by connecting, in a clockwise direction, all the ends of the terminal dendritic branches and computing the area within this circumscribed field. A centrifugal ordering method was used to order the dendritic branches[22]. A total of 41 cells from ZD animals, 24 cells from PF animals and 29 cells from normal animals were analyzed. Basket and stellate cells could not always be distinguished from each other. Therefore, in the quantitative analysis no attempt was made to differentiate between basket and stellate cells.

For each measured parameter, a simple linear regression analysis was performed. Regressions were computed using the cell's location in the molecular layer as the independent variable and the measured parameters as the dependent variables. Significant regressions were compared by a multiple regression analysis. The Johnson–Neyman test of significance[8] was used to determine at what depth of the molecular layer two regression lines differed at $P < 0.05$.

The distance from the center of the soma to the Purkinje cell layer or the relative position of a cell in the molecular layer was used to indicate the location of a cell in the molecular layer. The cell's relative position in the molecular layer was a standardized measurement (the molecular layer was of unequal thickness between dietary groups[6]). The cell's relative position in the molecular layer was computed as the distance from the center of the soma to the external granule cell layer divided by the thickness of the molecular layer (expressed as a percent). A cell at the external granule cell–molecular layer interface had a relative position in the molecular layer of zero. A cell located at the border of the molecular layer and the Purkinje cell layer had a relative position of 100. Analyses based on the distance of the soma from the Purkinje cell layer or the cell's relative position in the molecular layer yielded similar results and therefore, only the results from the cell's relative position in the molecular layer were reported.

RESULTS

Effects of zinc deficiency

Basket and stellate cells were tentatively identified on the bases of the morphology of their dendritic and axonal arborizations[14]. Basket cells of ZD animals were found in the lower third of the molecular layer and stellate cells were found predominantly at higher levels of the molecular layer (Figs. 1 and 2). In ZD animals the dendritic arbors of some of the basket and stellate cells were obviously immature and reduced in size (Figs. 1 and 2). However, the variations in the size and shape of the dendritic trees at varying depths of the molecular layer made it difficult to qualitatively assess the impaired dendritic growth of basket and stellate cells. For this reason, the dendrites of stellate and basket cells were analyzed quantitatively.

The simple linear regression analysis indicated that the size of all somatic and dendritic parameters were dependent upon the location of the soma in the molecular layer (Table I). The positive slopes of the lines indicated the largest cells were in the lower molecular layer.

A comparison of regression lines indicated zinc deficiency significantly impaired the dendritic growth of basket and stellate cells (Table II). In the lower 75% of the molecular layer, the area of the dendritic field and total dendritic length were reduced by approximately 61% and 55%, respectively (Fig. 3). The total number of branches per neuron was reduced by 45% in the lower 65% of the molecular layer (Fig. 4). For example, for the cells of normal rats located directly above the Purkinje cell layer (i.e. cells with a relative depth of 100), the dendritic field area, the total dendritic length and the number of branches per cell were computed to be 6570 μm, 760 μm and 50 branches, respectively. In ZD pups the size of these parameters were 2630 μm, 340 μm and 27 branches, respectively. The area of the soma was not altered by zinc deficiency (Table II; Fig. 4).

Effects of undernutrition

Undernutrition significantly impaired the growth of the dendrites of basket and stellate cells (Figs. 1 and 2; Table II). In the lower half of the molecular layer the dendritic field area was reduced by 32% (Fig. 3). In the lower 56–60% of the molecular layer

TABLE I

Simple linear regression analysis

Equations for the dendritic parameters are in the form, y = mx, where y = dendritic parameter, m = slope, x = cell's relative position in the molecular layer. Somatic area is based on the equation, y = mx+b, where b = y intercept. R^2 is the coefficient of determination[8]. All regressions are significant at $P < 0.008$.

	Equation	R^2
Dendritic field area (A)		
Control	A = 67.5×	0.93
Pair-fed	A = 45.9×	0.86
Zinc-deficient	A = 26.3×	0.83
Total dendritic length (L)		
Control	L = 7.6×	0.96
Pair-fed	L = 4.8×	0.94
Zinc-deficient	L = 3.4×	0.88
Number of branches (B)		
Control	B = 0.50×	0.82
Pair-fed	B = 0.28×	0.85
Zinc-deficient	B = 0.27×	0.80
Somatic area (S)		
Control	S = 0.36× + 42.7	0.43
Pair-fed	S = 0.48× + 34.8	0.46
Zinc-deficient	S = 0.25× + 47.0	0.17

the total dendritic length and the number of branches per neuron were reduced by approximately 44% (Figs. 3 and 4). The size of the soma was not affected by undernutrition (Fig. 4 and Table II).

The dendrites of the basket and stellate cells of PF pups were significantly larger than the interneurons of ZD animals. The dendritic field area and the total dendritic length of the interneurons of the lower half of the molecular layer, were 43% and 30% smaller in the ZD animals (Fig. 3 and Table II). The number of branches per neuron and the area of the soma were not significantly different between these two groups (Fig. 4 and Table II).

TABLE II

Multiple regression analysis

Numbers are *P* values for specific comparisons. Control (C), pair-fed (PF), zinc-deficient (ZD), non-significant (NS).

	C vs ZD	*C vs PF*	*ZD vs PF*
Dendritic field area	0.001	0.0001	0.001
Total dendritic length	0.0001	0.0001	0.001
Number of branches	0.001	0.001	NS
Somatic area	NS	NS	NS

DISCUSSION

Previous work showed that in normal animals the interneurons near the Purkinje cell layer differentiated prior to the interneurons of the upper molecular layer[1,2,16,17]. Thus, cells in the lower molecular layer were developmentally more advanced than cells near the external granule cell layer[1,2,16,17]. In this study the significant linear regressions of the dendritic and somatic measurements with depth probably reflected the aforementioned differences in the time of origin and the extent of dendritic differentiation of neurons at varying depths of the molecular layer.

The regression analysis provided quantitative estimates of the impaired dendritic growth of basket and stellate cells by zinc deficiency. The decreased slope of the regressions of ZD animals indicated that zinc deficiency and to a lesser extent undernutrition impaired the dendritic differentiation of basket and stellate cells. This was consistent with previous reports[5,6] that indicated the impaired dendritic growth of cerebellar neurons can not be attributed totally to the inanition in the ZD dams. Dietary effects were minimal in neurons near the external granule cell layer since the interneurons in this region of the molecular layer had just begun to differentiate.

Similar studies have not been reported. However, West and Kemper[23] have reported that after pre- and postnatal protein malnutrition there was a 16% decrease in the length of the oblique dendrites of basket cells. Our findings are consistent with the impaired growth of the cerebellum[4,10], cerebellar cortex[5,6,10] and Purkinje cell dendrites[6,10,15] in ZD and undernourished rat pups.

A combination of factors may contribute to the impaired growth of the basket and stellate cells of ZD pups. Possible mechanisms include a delay in the onset of dendritic differentiation and a retardation in the rate of dendritic growth.

The persistence of the external granule cell layer[4,5] and decreased numbers of granule cells[5] of 21-day-old ZD rats suggest that the production of neurons in the cerebellar cortex may be delayed. Since zinc deficiency was begun prior to the major period of the formation and differentiation of basket and stellate cells[1,2], it was possible that the reduced size of the dendritic arbor may be related to a delay in the for-

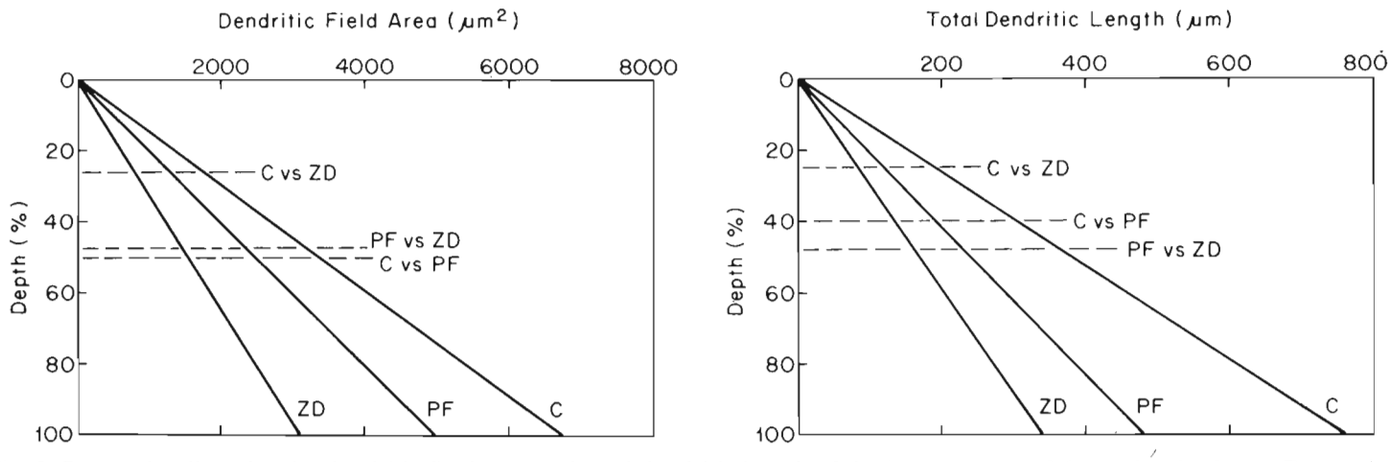

Fig. 3. Regression lines for the dendritic field area and total dendritic length of the interneurons of the molecular layer. Regressions were graphed with the independent variable (depth) on the y-axis. The border between the external granule cell layer and the molecular layer is represented by zero. The bottom of the molecular layer is 100. Specific comparisons of regression lines by the Johnson–Neyman test of significance are marked by the dashed lines. For specific comparisons, neurons located below the level of the molecular layer indicated by the dashed lines differ at $P < 0.05$.

mation and thus a delay in the onset of the dendritic differentiation of neurons at equivalent depths of the molecular layer.

Parallel fibers have been suggested to influence the dendritic differentiation of basket and stellate cells[16,17]. During development, a deficit of parallel fibers causes the dendritic arbors of basket and stellate cells to be distorted[7], reduced in size[3,13,18,21] and abnormally oriented[3,13,18]. In view of these findings it is possible that the impaired acquisition of granule cells[5] in ZD animals may retard the rate of dendritic growth by reducing the number of parallel fibers interacting with the interneurons.

Alterations in the metabolism of neurons also have been postulated to impair dendritic growth[10,11]. Impaired RNA and protein synthesis in the brain[20] and other tissues[12,24] of ZD animals have been described. As was previously suggested for the Purkinje cells of ZD animals[6], reduced levels of RNA and protein synthesis may directly impair the growth of the dendrites.

Similar mechanisms may be responsible for the impaired dendritic growth of the interneurons of undernourished rat pups. The smaller magnitude of the effects of undernutrition, as compared to zinc deficiency, were probably related to the lesser effects of undernutrition, on neurogenesis, RNA and protein synthesis.

The growth of the cell bodies of basket and stellate cells was not altered by dietary treatment. This might indicate that relative to dendritic growth, the growth of the cell bodies of the basket and stellate cells is less sensitive to the effects of undernutrition and zinc deficiency. Another possibility is that the technique

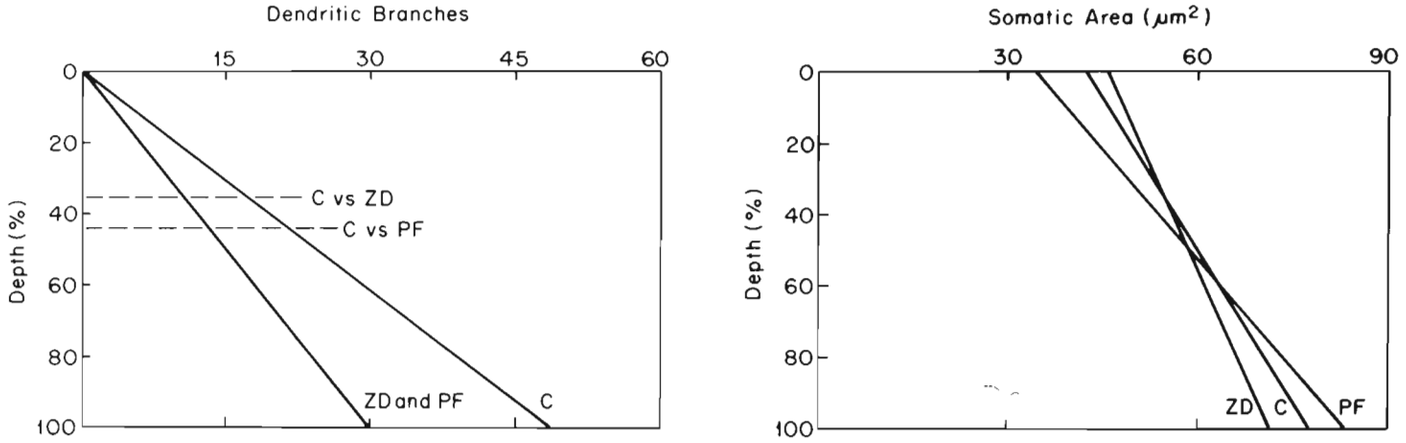

Fig. 4. Regression lines for the number of dendritic branches per neuron and the area of the soma of the interneurons of the molecular layer. See Fig. 3 for explanation.

used to evaluate somatic growth is less sensitive in evaluating alterations of somatic differentiation than of dendritic differentiation. Difficulties in defining the somatic-dendritic boundaries of immature neurons may produce errors in estimating somatic area which could affect the regression analysis.

It is evident from this and the preceding reports[4-6], that the acquisition and the differentiation of some cerebellar neurons is impaired when zinc deficiency is imposed during the major period of cerebellar cortical development. The long-term consequences of the retarded synaptic and dendritic maturation in the molecular layer of ZD rats is unknown.

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