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Decreased Dendritic Branching in Frontal, Motor and Limbic Cortex in Rett Syndrome Compared with Trisomy 21

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Abstract. The branching of dendrites of pyramidal neurons in premotor frontal, motor and limbic cortex have been identified by us using Golgi technique to be less in Rett Syndrome (RS) brains than in non-Rett control brains. Decreased dendritic branching per se is not pathognomonic of a particular condition and has been reported in numerous disorders associated with mental retardation. This study was designed to test whether the dendritic alterations in Rett Syndrome are the same or different from the alterations present in Down Syndrome (DS), 1 specific form of mental retardation. Sections from Brodmann's areas 6, 4, 20, 43, 28, and 17 of premotor frontal, motor cortex, inferior temporal gyrus, hippocampal formation and the striate cortex from 16 Rett brains, 9 non-Rett brains and 9 Down's brains were prepared for dendrite analysis using the rapid Golgi technique. Drawings of apical and basilar dendrites of pyramidal neurons from 2 cortical layers and Ca1 were submitted to Sholl analysis. The analyses of Rett brains were compared with the analyses of the Trisomy 21 brains using the repeated measures analysis of covariance, with age as a covariate. The studies demonstrate in our sample that basal dendrites of layer III and V of frontal, layer IV of subiculum, and layer V of motor cortex and apical dendrites of layer III of frontal cortex have a significantly reduced dendritic arborization in RS compared with Trisomy 21. This study suggests that the cortical distribution of the dendritic alterations is specific for Rett Syndrome, and that the premotor frontal, motor and subicular cortex are preferentially involved in the, as yet, undefined process which affects brain growth and function in RS.

Key Words: Down Syndrome; Golgi technique; Mental retardation; Pyramidal neurons; Rett Syndrome; Sholl analysis.

INTRODUCTION

Alterations in dendrites of cortical neurons have been demonstrated in various neurologic disorders associated with mental retardation (1–5). The defective dendrites have been assumed to be the basis for the altered higher cortical function, presumably because of decreased or disordered synaptic arrangements. Most of the published studies of dendrites have been made in only 1 or 2 cortical regions so that the relevance of cortical site of the dendritic alterations in the conditions was not investigated. In our studies of Rett Syndrome (RS), which is a form of profound psychomotor retardation and nonprogressive micrencephaly (6–8), we have demonstrated site specific alterations in cortical dendrites. The basal dendrites were decreased in the limbic, motor and premotor frontal cortex, and the apical dendrites in the frontal and motor cortex (9). In order to ascertain whether these selective dendritic alterations in RS were specific to the mental handicap of this enigmatic disorder, we compared the dendrites of RS with those of Trisomy 21. We chose Trisomy 21 as a comparative example of mental retardation because it is a well defined phenotype; the brain in Trisomy 21 is decreased in weight (5, 10, 11), comparable to that in RS, and abnormal dendrites have been reported in Trisomy 21 (2, 5, 12, 13).

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MATERIAL AND METHODS

Study Subjects

Cortical regions of 16 Rett brains, 9 non-Rett brains, and 6 trisomy brains were studied. The ages of the Rett girls and women ranged from 2.9 to 35 years. The tissues and histories were provided by the Brain Tissue Resource Center of the McLean Hospital and many Rett researchers in North America, Great Britain, and Scandinavia. In some cases one half formalin fixed brains were available for study. In others only dissected brain regions were available (see comment on Table 2). The brains from 6 children with Trisomy 21 were obtained after informed consent in our institution. The ages ranged from 0.6 to 11 years. The diagnosis of Trisomy 21 had been established by chromosomal analysis during life and the phenotype was confirmed at autopsy. The brains from non-Rett children and adults were obtained after informed consent at our institution. The ages of the non-Rett children and adults were 7 months to 23 years. The cases for which brain weights are available are indicated in Table 1.

Tissue Preparation

The fixed blocks of cortex were all processed in a similar way using the modified rapid Golgi technique we have utilized in previous studies (14). The sections were then cut at 40–50 microns and the best impregnated areas were chosen to make camera lucida drawings of pyramidal neurons of layers 3 and 5 of the frontal, motor, inferior temporal, superior temporal, and occipital cortex, layers 2 and 4 of the subiculum and CA1 of the hippocampus. The 10 best impregnated neurons from each layer were drawn and analyzed.

Analysis of Camera Lucida Drawings of Golgi Studies

The apical and basilar dendrites of each drawing of a silver impregnated neuron were subjected to a Sholl analysis (15). In this, the drawing of neuron was placed in the center of concentric

TABLE 1
Brain Weights

Rett Brains		Trisomy 21 Brains		Non-Rett Brains	
6 years	750 gm	7 months	500 gm	7 months	500 gm
7 years	1,010 gm	21 months	1,000 gm	8 months	580 gm
12 years	1,120 gm	6 years	1,050 gm	13 months	875 gm
17 years	1,090 gm	9 years	1,218 gm	8 years	1,324 gm
17 years	920 gm	10 years	1,132 gm	17 years	1,300 gm
21 years	900 gm	11 years	1,100 gm	19 years	*900 gm
21 years	900 gm			23 years	1,380 gm
21 years	900 gm				
21 years	975 gm				
33 years	915 gm				
35 years	830 gm				

* Lupus with infarcts.

circles, each was 20 microns apart, starting at the center of the neuronal soma. The analysis extended to 220 microns from the cell center. For each neuron the numbers of dendritic branches which intercepted each circle were counted. Then the mean values of the numbers of interceptions for the apical and for the basilar dendrites of the 10 neurons in each layer of the Rett brains were compared with the mean value of the numbers of interceptions for the drawings of the apical and the basilar dendrites for the Trisomy 21 brains. The significance of the differences between the Rett and Trisomy 21 Sholl analysis for apical and basilar dendrites of each layer in each cortical area was tested using the repeated measures analysis of covariance, with age as a covariate and the distance from the cell center as the repeated factor (16). Because of the numbers of statistical tests performed, only p values of <0.01 were considered significant.

RESULTS

The classic neuropathology examination of the Rett and the Trisomy brains showed no significant pathologic abnormality that could account for the decreased brain weights, which were, in most cases, less than normal weight for the age of the Rett patient. One non-Rett brain was reduced in weight. This patient had lupus erythematosus and had multiple small infarcts which were not recognized clinically. The regions submitted for Golgi studies in this case, and the Sholl analysis of this brain resembled that of the non-Rett brains.

The Dendritic Branching Patterns

The differences in dendritic branching patterns between Rett and non-Rett and between Rett and Trisomy 21 as determined by the Sholl analysis of pyramidal neurons of layers III and V in neocortical areas, and layer 2 and 4 of the subiculum and in Ca1 of the hippocampus are shown in Tables 2 and 3 respectively. In Table 2 the mean values of the Sholl analyses for the Rett brains are compared with non-Rett brains. In this analysis there is a significant difference in the basal dendrites of layer V of the *inferior temporal gyrus,* in the apical and basal dendrites of layer III and V of the *frontal cortex* and in the basal dendrites of layer III and the apical dendrites of layer V in the *motor cortex.* In Table 3 the mean values of the Sholl analysis of the Rett brains are compared with the mean values of the Sholl analyses for the Trisomy 21 brains. There is a significant difference in the basal dendrites of layer IV of the *subiculum,* of the apical and basal dendrites of layer III and of the basal dendrites of layer V of the *frontal* cortex, and of the basal dendrites of layer V of the *motor cortex.* That is, the dendritic alterations in Rett syndrome are not present in trisomy 21 brains whose dendritic branches resemble those of the other non-Rett brains.

DISCUSSION

Rett Syndrome is a profound form of mental handicap in girls of all races, with a known incidence of 1/23,000 in the USA and 1/15,000 in Scandinavia (17). It is manifest after birth as a subtle deceleration in head growth, followed by loss of hand use, loss of speech, apraxia-ataxia, hand stereotypies, frequently episodic hyperventilation, seizures, Parkinsonian-like features, and often the loss of the ability to walk. In spite of the complex clinical phenotype, the brain, except for a decrease in size, is normal in routine studies. Using Golgi studies we have identified a significant decrease in the size of the dendritic trees in the frontal, motor and temporal cortex (9). The altered regions of cortex subserve functions which are abnormal in RS, and which from MRI studies (18) have been shown to have decreased volumes. In considering the pathogenesis of the decreased dendritic fields we have postulated that there is either a failure of dendrite development or of maintenance. There is no evidence of a consistent degenerative process (19) so that failure of development is postulated. Because dendritic alterations have been observed in various forms of mental retardation (2–4, 20), we have tested the specificity of these observed dendritic alterations in selected cortical sites in

TABLE 2
Comparison of Sholl Analysis Rett vs Non-Rett Using Repeated Measures Analysis of Covariance with Age as the Covariate

	Ocipital F: DF: p:	Cal F: DF: p:	Inf Temp F: DF: p:	Subiculum* F: DF: p:	Frontal F: DF: p:	Motor F: DF: p:	Sup Temp F: DF: p:
Basal							
Basal V							
*(basal IV)	2.67	1.67	7.96	6.67	17.79	7.94	0.04
	1,25	1,21	1,22	1,20	1,25	1,16	1,9
	0.115	0.210	0.010	0.018	0.0003	0.012	0.844
Basal III	2.46		6.79	1.97	13.80	11.20	0.06
*(basal II)	1,25		1,22	1,21	1,25	1,15	1,9
	0.129		0.016	0.175	0.0010	0.004	0.816
Apical							
Apical V	6.11	0.48	6.52	0.97	19.33	11.82	0.0
*(apical IV)	1,25	1,20	1,22	1,20	1,26	1,16	1,9
	0.021	0.497	0.018	0.336	0.0002	0.0003	0.973
Apical III	3.43		2.79	1.89	12.77	6.71	0.06
*(apical II)	1,25		1,22	1,21	1,27	1,15	1,9
	0.076		0.109	0.184	0.0014	0.020	0.814

The degrees of freedom differ from area to area because for some cases only dissected brain regions were available.

TABLE 3
Comparison of Sholl Analysis Rett vs Trisomy 21

	Occipital F: D: p:	Cal F: D: p:	Inf. Temp F: D: p:	Subiculum* F: D: p:	Frontal F: D: p:	Motor F: D: p:	Superior Temp F: D: p:
Basal							
Basal V	1.49		7.13	9.94	10.63	16.73	0.03
*(basal IV)	1,17	0.10	1,15	1,13	1,17	1,8	1,6
	0.239	1.14	0.0178	0.008	0.005	0.004	0.869
		0.758					
Basal III	3.22		8.15	0.62	15.57	7.27	0.83
*(basal II)	1,17		1,15	1,14	1,17	1,8	1,6
	0.090		0.012	0.445	0.001	0.027	0.398
Apical							
Apical V	3.09		3.72	0.13	8.38	8.83	0.09
*(apical V)	1,17	0.37	1,15	1,13	1,17	1,8	1,6
	0.097	1,14	0.073	0.719	0.010	0.018	0.773
		0.550					
Apical III	3.32		2.73	0.09	9.77	2.40	0.03
*(apical II)	1,17		1,15	1,14	1,18	1,8	1,6
	0.086		0.119	0.764	0.006	0.160	0.870

The degrees of freedom differ from area to area because for some cases only dissected brain regions were available.

RS, by comparing them with dendrites in the Trisomy 21 brain.

We chose the brain in Down Syndrome (DS) as a comparison study for several reasons. Trisomy 21 has a well-characterized phenotype and the brain is small, like the Rett brain, without any specific pathologic alteration in routine studies. MRI studies in DS (21), as in RS (18), have defined a decreased brain volume (9). In DS altered dendrites and spines have been observed in the occipital (13, 22), motor (5), hippocampal and cingulate cortices (23) but this is the first study in which multiple regions have been compared. We have found that this examination of multiple regions in each brain is a useful application of the Golgi technique that is notoriously capricious.

In a multiple area analysis the more normal areas in each brain serves as an internal control. When 7 cortical regions in RS and Trisomy 21 were compared (using age as a covariate), we observed no significant difference between the dendritic branch lengths in the *occipital cortex (area 17), Ca1,* and the *superior temporal cortex (area 22).* In RS the basal dendrites in the *inferior temporal cortex,*(area 20) and the apical dendrites of layer V in *frontal (area 6)* and *motor cortex (area 4)* approached a significant difference (0.01) from those dendrites in Trisomy 21. However, there was a significant difference between RS and Trisomy 21 in the basal dendrites of layer IV in the *subiculum,* layer III and V of the *frontal cortex (area 6)* and layer V of the *motor cortex (area 4).* The apical dendrites of layer III of the *frontal cortex* (area 6) in RS were also greatly and significantly reduced compared with those in Trisomy 21.

When the Sholl analysis of dendritic branching patterns in RS is compared with all non-Rett brains in the study (Trisomy 21 and "non-retarded brains"), the same selected cortical areas are significantly reduced in RS. However, when the basal dendrites of layer V of the motor cortex in RS are compared with those in Trisomy 21 alone, there is an even greater difference (Tables 2, 3). This correlates with the profound motor handicap in RS (24–26). The other areas of involvement, or of sparing, also correlate generally with the observed clinical features of RS. For example, the relatively normal occipital dendrites reflect the grossly intact vision of the Rett patient. The marked involvement of the frontal and limbic regions corresponds to the profound deficits in mental functioning and the emotional lability in RS. The implication that altered function is associated with decreased dendritic branching is speculative but it seems reasonable to assume that a decreased dendritic tree would be indicative of decreased numbers of spines with a decreased potential for synaptic modulation. Belichenko (27–29) has reported alterations in dendritic spines in some regions of the Rett cortex. This lends credence to the idea that in RS the altered dendrites in these regions are defective in their postsynaptic apparatus.

Although the investigation of dendritic spines in RS needs to be expanded, it can be concluded that the decreased brain weight in RS as in Trisomy 21 correlates with alterations in the dendrites. When the dendritic arborizations in brains of these 2 conditions are compared with those of non-Rett brains, they are reduced in all regions studied. However, in RS there are selected regions which are significantly more reduced than in Trisomy 21.

In addition to the possible functional implications of the selective involvement of the cortical dendrites in RS, there is the possibility that these cortical regions have a common etiology for their reduced dendritic branching pattern. The identification of this common deficiency may suggest to us the pathoetiology of the brain lesion resulting in RS. For example, the development of the frontal, motor and limbic cortex depends upon similar trophic factors (e.g. the brain stem derived catecholamines) (30–36). Our study confirms the selective nature of the cortical involvement in RS and suggests that continued investigation of factors involved in development of these specific cortical regions is important in understanding RS.

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