Selective Dendritic Alterations in the Cortex of Rett Syndrome

DAWNA ARMSTRONG, M.D., J. KAY DUNN, Ph.D., BARBARA ANTALFPHY, A.I.M.L.T., AND RENUKA TRIVEDI, Ph.D.

Abstract. Rett syndrome, the commonest condition associated with severe mental retardation in girls, is diagnosed only by its clinical phenotype, because, to date, there is no consistent characteristic alteration in genetic, biochemical, neurotransmitter or morphologic marker. The clinical features at various ages suggest involvement of most parts of the nervous system; however, the brain in Rett syndrome is reduced in weight, without other obvious morphologic alterations. Because of the relative microcephaly, hypotheses regarding failure of development have been suggested. Supporting such hypotheses are the quantitative studies by Jellinger, Seitelberger and Kitz defining a decrease in the amount of melanin in the substantia nigra and by Bauman defining a global decrease in the size of the neurons. In this study the cerebral cortex has been examined using the rapid Golgi technique with the purpose of investigating dendrites of pyramidal neurons in six cortical regions of Rett girls from ages 2.9–35 years. Camera lucida drawings of apical and basal dendrites of two cortical layers and CA1 were prepared. These were submitted to the Sholl analysis. The Sholl analyses were tested for significance using the repeated measures analysis of covariance, with age as a covariate. The studies demonstrate that from our samples there is no evidence that the pyramidal neurons in Rett syndrome degenerate progressively with increasing age, but that the basal dendrites of layers three and five pyramidal neurons in the motor and frontal cortex, the apical dendrites of layer five of the motor cortex, and the basal dendrites of layer four of the subiculum are significantly shorter than in non-Rett brains. The dendritic trees in the visual cortex are not significantly decreased. This selective, non-progressive involvement of projection neurons of motor association and limbic cortex may have bearing on the neurologic deficits in Rett syndrome, and these areas of the brain should be investigated to search for abnormalities of trophic factors in Rett syndrome.

Key Words: Golgi; Pyramidal neurons; Rett syndrome; Sholl analysis.

INTRODUCTION

Rett syndrome was described in 1966 as a progressive disorder of females that begins in infancy as autistic behavior, gait ataxia, loss of purposeful hand movements, seizures, intermittent hyperventilation and dementia (1, 2). It has been recognized only in girls. There are familial cases, but most are sporadic with a prevalence in girls of 1:22,000 (3). Four clinical stages have been identified, and although the disease was originally described as being progressive, clinical observers now consider that the four stages possibly recognize a motor deterioration that may be a result of the initial limited motor abilities (4). It is no longer considered to be an autistic disorder. Forms fruste are recognized (5). The disease is diagnosed by its clinical phenotype after known causes of mental retardation have been excluded. Routine examinations of blood, urine and cerebrospinal fluid are normal. Special testing of the girls at various ages reveal some abnormalities in many parts of the nervous system (6–11). There is, as yet, no identified biochemical or genetic marker (12–15) for Rett syndrome, nor have routine neuropathologic studies defined a consistent pathognomonic alteration except for reports of a decrease of brain weight in Rett syndrome (16–23). Small brain size is supported by the clinical, radiological and quantitative pathological examinations which document respectively decreased head circumference (24, 25), decreased brain volume (26) and decreased size of neurons (27, 28). The decreased brain size could reflect atrophy and/or arrest of maturational processes and the Sholl study (29), in six cortical areas in the brains of Rett girls ranging in age from 2.9 to 35 years. Our goals in this study were to define whether there was a decrease in the size of the dendritic tree in the Rett brain, compared with non-Rett brains, whether the decrease was progressive with age and whether there was any specific cortical site for these changes.

MATERIALS AND METHODS

Brain Weights in Rett Syndrome

The weights of 22 brains from Rett girls were compiled from autopsy records, and from cases provided to our center by many cooperating parents, pathologists and neurologists (Fig. 1). The comparison group with normative values was obtained from Kays (30).

Golgi Studies

Portions of brains from 16 Rett girls ages 2.9–35 were studied. The subjects are listed in Table 1. Tissues and clinical 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, formalin-fixed one-half brains were available. In many cases, only dis-

From the Departments of Pathology (DA, BA), Medicine (JKD) and Pediatrics (DA, RT), Baylor College of Medicine, Houston, Texas.

Correspondence to: Dawn Armstrong, M.D., Pathology Department, MC1-2261, Texas Children's Hospital, 6621 Fannin Street, Houston, TX 77030.

TABLE I

Study Population

<table>
<thead>
<tr>
<th>Age</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.9, 4, 5, 6, 7, 10, 12, 15, 17 x 2, 20 x 2, 21, 30, 33, 35</td>
<td>Rett patients</td>
</tr>
<tr>
<td>7 months</td>
<td>Trisomy 21</td>
</tr>
<tr>
<td>8 months</td>
<td>Developmental delay, nonspecific</td>
</tr>
<tr>
<td>13 months</td>
<td>Hyperkalemia</td>
</tr>
<tr>
<td>21 months</td>
<td>Trisomy 21</td>
</tr>
<tr>
<td>8 years</td>
<td>Cystic fibrosis</td>
</tr>
<tr>
<td>17 years</td>
<td>Muscular dystrophy</td>
</tr>
<tr>
<td>19 years</td>
<td>Lupus erythematosus</td>
</tr>
<tr>
<td>23 years</td>
<td>Cystic fibrosis</td>
</tr>
<tr>
<td>30 years</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

Fig 1. (Upper) Brain weight by age for Rett syndrome versus normal. (Lower) Photograph of the camera lucida drawing of pyramidal neurons in the frontal lobe of a Rett and a non-Rett brain. The III and V cortical layers are illustrated for each brain.

The apical and basilar dendrites of each neuron were each subjected to a Sholl analysis in which the neuron is placed in the center of concentric circles, each 20 µm apart starting from the center of the neuronal soma. The analysis was extended to 220 µm from the cell soma. The numbers of dendrites which intercepted each circle were counted. The mean values of the numbers of interceptions for the apical and for the basilar dendrites of the ten neurons in each layer were then compared with mean values of apical and basilar dendrites for the non-Rett controls. The significance of differences between Rett and non-Rett Sholl analyses for apical and basilar dendrites of each layer in each cortical area were tested using the repeated measures analysis of covariance, with age as a covariate (32). Due to the number of statistical tests performed, only p values <0.01 have been considered significant.

Control Brains

Nine non-Rett brains were studied. These included neurologically normal children and adults of similar age or brain weight, normal infants, retarded children, a patient with a motor deficiency (muscular dystrophy) and a nutritionally deprived patient (cystic fibrosis).

RESULTS

Brain Weights

Weights for 22 brains from Rett patients were available (Fig. 1). The median age for the group was 10.5 years.
SELECTIVE DENDRITIC ALTERATIONS IN THE CORTEX OF RETT SYNDROME

TABLE 2
Comparison of Rett Patients Versus Non-Rett Patients with Respect to Sholl Analysis Using Repeated Measures Analysis of Covariance with Age as a Covariate

<table>
<thead>
<tr>
<th>Site</th>
<th>Occipital</th>
<th>CA1*</th>
<th>Inferior temporal</th>
<th>Subiculum†</th>
<th>Frontal</th>
<th>Motor</th>
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<tbody>
<tr>
<td>Apical layer III</td>
<td>F = 1.19‡</td>
<td>F = 1.73</td>
<td>F = 2.63</td>
<td>F = 3.27</td>
<td>F = 6.19</td>
<td>F = 8.65</td>
</tr>
<tr>
<td>DF = 1.20</td>
<td>DF = 1.14</td>
<td>DF = 1.15</td>
<td>DF = 1.15</td>
<td>DF = 1.20</td>
<td>DF = 1.9</td>
<td></td>
</tr>
<tr>
<td>p = 0.2876</td>
<td>p = 0.2096</td>
<td>p = 0.1256</td>
<td>p = 0.0908</td>
<td>p = 0.0218</td>
<td>p = 0.0164</td>
<td></td>
</tr>
<tr>
<td>Basal layer III</td>
<td>F = 2.85</td>
<td>F = 4.16</td>
<td>F = 8.53</td>
<td>F = 4.83</td>
<td>F = 11.16</td>
<td>F = 14.02</td>
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<td>DF = 1.15</td>
<td>DF = 1.15</td>
<td>DF = 1.19</td>
<td>DF = 1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p = 0.1071</td>
<td>p = 0.0593</td>
<td>p = 0.0106</td>
<td>p = 0.042</td>
<td>p = 0.0034</td>
<td>p = 0.0046</td>
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</tr>
<tr>
<td>Apical layer V</td>
<td>F = 1.91</td>
<td>F = 4.27</td>
<td>F = 1.62</td>
<td>F = 6.52</td>
<td>F = 21.89</td>
<td></td>
</tr>
<tr>
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<td>DF = 1.14</td>
<td>DF = 1.19</td>
<td>DF = 1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p = 0.1824</td>
<td>p = 0.0565</td>
<td>p = 0.2238</td>
<td>p = 0.0194</td>
<td>p = 0.0012</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal layer V</td>
<td>F = 2.63</td>
<td>F = 6.06</td>
<td>F = 10.56</td>
<td>F = 11.12</td>
<td>F = 11.36</td>
<td></td>
</tr>
<tr>
<td>DF = 1.20</td>
<td>DF = 1.15</td>
<td>DF = 1.14</td>
<td>DF = 1.19</td>
<td>DF = 1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p = 0.1207</td>
<td>p = 0.0264</td>
<td>p = 0.0058</td>
<td>p = 0.0035</td>
<td>p = 0.0074</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* CA1 is reported for the pyramidal layer.
† Subiculum is reported for layer II instead of III and layer IV instead of V.
‡ F = the test statistic from the repeated measures analysis of covariance, DF = degrees of freedom, p = p value.

(range 4–35 years). Normative mean values and their 95% confidence limits for the comparison group (Fig. 1) define brain weights in grams for Rett girls and the mean for each age of the non-Rett comparison group, together with the 95% confidence interval. The brain weights of all Rett girls are below the lower 95% confidence limits. We observed no trend to suggest that there is a larger difference for the older Rett women.

Golgi Studies

In most cortical areas the pyramidal neurons in Rett syndrome were simplified with a decrease of dendritic length and branching patterns. The terminations of the apical dendrites were not always apparent in the 50 μm sections. However, because the branching of the dendrites is concentrated near the cell soma (33), the analysis was carried out to 220 μm, a distance that included all but the termination of some of the apical dendrites. An occasional neuron had unusual clusters of dendrites, but for the most part the abnormality was a decrease in numbers or length of primary and second order dendrites. This decrease was not always apparent without Sholl analysis (Fig. 1 upper panel).

An example of the Sholl analysis (Fig. 2a, b, c, d) is presented for all Rett cases and non-Rett cases in the layers of frontal cortex. These values represent means of all of the Rett and non-Rett analyses at these sites and are plotted in two graphs for each area of analysis. In these examples there is a decrease of dendritic branching in the Rett cases at each site, layer three apical (a) and basilar (b), and layer five apical (c) and basilar (d). The basal dendrites of layers three and five of the Rett cases are significantly less than the non-Rett cases. The comparison analysis for each of the six cortical layers is presented in Table 2.

When the Sholl analyses for all of the Rett girls is compared with the Sholl analyses for all of the comparison patients at apical or basilar dendrites, in each layer and at six brain sites, the following sites in Rett syndrome have significantly fewer branches (p < 0.001) (Fig. 3): the basal dendrites of the pyramidal neurons of layers three and five in the motor and frontal lobes, and the basal dendrites of layer four of the subiculum. In the motor cortex the apical dendrites of layer five pyramidal
neurons have significantly fewer branches in Rett syndrome. There is no significant difference in dendrites of the occipital lobe, in CA1 of the hippocampus or in the inferior temporal gyrus. There is a trend (using p < 0.05) for the apical dendrites of layers three and five frontal, and of layer three motor and the basal dendrites of layers three and five inferior temporal lobe and of layer two of the subiculum to be less than in the corresponding regions from the non-Rett brains. The Sholl analyses were tested by an analysis of covariance, with age as a covariant.

**DISCUSSION**

This study has demonstrated in our sample that there is no progressive decline in brain weight or of dendritic branching in Rett syndrome during the ages of 2.9-35 years, providing additional evidence that the pathology of Rett syndrome is a manifestation of maturation arrest. Belichenko et al (34) studied Rett cortex with confocal microscopy and came to the same conclusion. This interpretation of the cortical morphology supports the clinical impressions that Rett syndrome is not a progressive disease, but that the changing clinical features possibly represent a motor deterioration that may be the consequence of limited initial motor abilities.

Polialkov (35) in his studies of the ontogenesis of the human brain has identified three periods of cortical development and organization. During the intratuteral period the cortex develops neurons which establish two-way cortico-subcortical projectional connections involving the fifth cortical layer and subcortical regions (basal ganglia, brainstem and cord). At birth, the second period of development begins with a rearrangement of connections of the lower levels of the cortex with those of layer three. He refers to this period of brain maturation as a period of "intensive development of the system of cortical projectional-associative connections which is coupled at the level of the lower part of layer three" (35). The growth of the bodies and dendries, and the development of interneurons is the morphologic expression of this rearrangement. This period proceeds into the second year of life. The final stage of cortical ontogeny, which goes on for an extended time, involves the associative cortical connections in the uppermost cortical layers.

Our demonstration of decreased dendritic territories in both layer five and layer three of selected areas of the cortex could suggest that the rearrangement of connections which occurs during the second stage of cortical development is incomplete in Rett syndrome. Belichenko's study endorses this conclusion (34). Is it justified to suggest that alterations in ten neurons per layer in each brain is representative of the whole cortical layer? It will be important to examine additional cases; but our study of the pyramidal neurons in six different cortical areas in each case has provided controls within each brain so that the consistent alteration of dendritic trees in the frontal, motor and subicular cortex may indeed reflect regional alterations in these neurons and that they do significantly influence function. In support of our interpretation that the dendritic alterations at these sites do correlate with function is our demonstration of comparatively normal dendrites in the visual cortex of Rett girls, whose visual system seems to be intact. Also, collaborating our observations, are the CT and MRI studies which have defined a selected decrease in the volume of the frontal and temporal lobes in Rett syndrome (6, 26). Our identification of abnormalities of the pyramidal neurons of layers three and five of the cortex would correlate with a defective coupling of projectional and associative capabilities within selected areas of the cortex, and such a phenomenon would, for example, explain Eyre and Kerr's observations (7) of delayed cortical responses in the motor system of Rett girls. From our identification of selective regional involvement we may suggest other functional correlations. Alterations of dendritic trees in the frontal lobe may compromise this association area which is essential for the integration of information from the sensory and limbic cortex. Lesions in this area have been correlated with changes in mood and personality, absence of a delayed response, and increased distractibility and motor hyperactivity (36). These features can be recognized in some stages of Rett syndrome.

There is a major problem in defining and obtaining the appropriate control cases for this study. The age-matched
control brains are heavier and larger than the Rett brains, and for this reason brains of children having the same body weight as some of the Rett girls were included. A brain from a patient with cystic fibrosis was included as a control for the effects of altered nutrition on dendrite growth. Three brains from retarded children were analyzed in order to identify whether the Rett brain differed from the brain of other forms of mental handicap. Our analysis does suggest that the Rett brain shows a regional arrest of dendritic development which differs from these various controls. A study which includes additional Trisomy 21 brains is in progress.

Dendritic alterations are not unique to Rett syndrome. A limited number of quantitative analyses have been published for the normal human brain and have shown, for example, that the basal dendrites of supragranular pyramidal neurons in Wernicke's area in adults exhibit the following normal variations: dendritic length and segments are greater in women than in men, they are greater in the left than the right hemisphere, and they are greater in more highly educated subjects (37). In normal aging, the basal dendrites of pyramidal neurons of the motor cortex decrease, layer five showing a greater decrease than layer three (38).

In human disease, Huttenlocher (39) has studied pyramidal neurons in layers three and five of the middle frontal gyrus in 11 patients with severe mental defect. He found decreased numbers of branches and decreased length of apical and basal dendrites in the five youngest patients. Buell and Coleman (40) examined the pyramidal neurons of layer two of the parahippocampal gyrus in aged and demented adults and demonstrated that in normal aging there was continuation of growth of dendrites that did not occur in dementia. Takashima et al (41) showed that the basal dendrites of pyramidal neurons in the visual cortex were shorter in Trisomy 21 than in control brains. Harper and Corbett (42) demonstrated decreased terminal dendritic branches in the basal dendrites of layer three pyramidal neurons in the superior frontal and motor cortex in alcoholic patients. There have been qualitative differences defined in pyramidal neurons of the prefrontal cortex (43) and the occipital cortex in phenylketonuria (44).

This Golgi study of the human cortex in severe mental retardation differs from previous ones by its inclusion of six different cortical areas, allowing us to suggest, as above, that the selective dendritic alteration may contribute significantly to the pathophysiology of Rett syndrome. We have considered, too, that the selective dendritic alterations may reflect important aspects pertaining to the pathophysiology of Rett syndrome. The growth and the preservation of dendrites depends upon the presence of axons (45–48), their synapses, the presence of appropriate input through the axons, the presence of trophic factors and the presence of neurotransmitters. Considering these factors within the context of what is known about Rett syndrome, it may be important to examine again the role of the neurotransmitters in Rett syndrome. The variable observations about alterations of neurotransmitters and brain peptides in Rett syndrome have been considered chiefly in relation to their role as traditional transmitters (4). If we re-examine them as trophic agents in the development and maintenance of neural structure, the transient alterations of some of these factors may be more meaningful. We know, for example, that neurotransmitters are essential in several aspects of neural growth. Endogenous opioids regulate dendritic growth in rat brain (49). Postnatal rats deprived of noradrenaline from the locus ceruleus develop elongated apical dendrites without branches in layer six of the neocortex (50). In neonatal rats exposed to the neurotoxin 6-hydroxydopamine, of the five cortical areas which were studied, the basal dendrites of layers three and five of the frontal and cingulate cortex showed decreased lengths and branching (51). In neonatal mice deprived of cholinergic input, dendritic development of layer five pyramidal neurons in the somatomotor cortex was altered (52). Experimental work has illustrated the necessity of other kinds of trophic factors. For example, deviant development of neurons in layers five of the visual and motor cortex have been produced by undernutrition (53), and hypothyroidism in neonatal rats is associated with deficient dendritic arborization (54).

A consideration of a deficiency of neurotransmitters as growth factors in Rett syndrome has been previously suggested by Nomura and Segawa (6). Certainly the investigation of trophic factor deficiency in Rett syndrome opens up many possible avenues for investigation, including all of the biologic requirements of growth factors such as receptors and their genes, and the second and third intracellular messenger systems. It is hoped that these kinds of studies will reveal more about the pathogenesis of Rett syndrome.

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REFERENCES


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