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Original article

Neurobiology of Rett syndrome: a genetic disorder of synapse development☆

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Abstract

Rett syndrome is a developmental disorder that restricts brain growth beginning in the first year of life and evidence from neuropathology and neuroimaging indicates that axonodendritic connections are especially vulnerable. In a study of amino acid neurotransmitter receptors using receptor autoradiography in tissue slices of frontal cortex and the basal ganglia, we found a biphasic age-related pattern with relatively high receptor densities in young RS girls and lower densities at later time. Using microarray analysis of gene expression in frontal cortex, we found that some of the most prominent alterations occurred in gene products related to synapses, including the NMDA receptor NR1 subunit, the cytoskeletal protein MAP-2 and synaptic vesicle proteins. Using a new antibody that recognizes MeCP2, the transcription factor mutated in RS, we established that most neurons in the rodent brain express this transcription factor. We hypothesize that a major effect of mutations in the MeCP2 protein is to cause age-related disruption of synaptic proliferation and pruning in the first decade of life.

Keywords: Rett syndrome; MeCP2; Glutamate; Gene microarrays; Gene expression

1. Introduction

Gaining a better understanding of the neurobiology and pathogenesis of Rett syndrome (RS) is important for designing therapeutic strategies [1,2]. A feature of RS neuropathology that sets it apart from many other pediatric disorders is its impact on developing neurons and axonodendritic connections [1]. During the decade before the discovery that mutations in *MECP2* are responsible for the disorder [3], we focused on the clinical and pathologic evidence that RS disrupts synaptic development [4]. We hypothesized that RS disrupts genetic programs that control elaboration and pruning of axonal and dendritic processes that form synapses during early postnatal life [5]. Analysis of postmortem brain tissue with neurotransmitter receptor autoradiography and brain imaging by our group and others continues to support this hypothesis [6–8]. In addition, our recent studies of differential gene expression using microarray analysis of cDNA prepared from postmortem RS brain tissue suggest that some of the largest and most consistent changes in gene expression involve axons, dendrites and neurotransmitter receptors. It seems likely that some of these changes result from a defect in gene silencing caused by mutations in *MECP2* [9]. In this brief review, we present an updated hypothesis that incorporates our recent data on gene expression in RS along with data using other approaches.

2. RS disrupts brain growth, especially of axonodendritic connections

Clinical and neuropathological evidence suggests that Rett syndrome (RS) involves a disorder of brain growth, especially the growth and development of gray matter and of axonodendritic connections [1]. Clinically, girls display deceleration in head growth along with psychomotor regression, loss of social and speech skills and autistic-like behavior. Brain MR imaging suggests preferential involvement of gray matter, especially in prefrontal, posterior-frontal and anterior temporal regions [1,2,10]. Prominent neuropathological features include reductions in cortical thickness and markedly reduced neuronal size and dendritic arborization with relative preservation of neuronal number [11–14]. The neuronal cell packing density has been reported to be increased, consistent with a reduction in the development of axons and dendrites to connect neurons [11,14]. In a small number of cases, synaptic density has been reported

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to be decreased markedly [1]. Levels of the important cytoskeletal microtubule associated protein (MAP) associated with dendrites, MAP-2, are also severely reduced in RS [15]. During development, interactions between MAP-2 and tubulin are important for extension of neuronal growth cones and dendritic growth [16]. This evidence suggests that brain growth decelerates in the postnatal period in RS because the growth of axonodendritic connections that form the neuropil is restricted. It is unclear if the decrement in MAP-2 is directly related to the MeCP-2 mutation.

3. Relationship of RS to other transcriptional disorders that disrupt brain development

It is noteworthy that at least two other developmental disorders associated with mental retardation, Rubinstein–Taybi (R–T) syndrome and cretinism, involve stunted axonodendritic development that is a consequence of disrupting gene transcription during critical periods of brain development [17–20]. In R–T syndrome, the genetic defect is in the gene encoding CREB binding protein (CBP), a transcriptional co-activator that regulates the expression of numerous other genes [19]. In cretinism, lack of thyroid hormone during early brain development allows the nuclear thyroid receptor to silence transcription of many genes [20]. Neuropathologic studies of both conditions have also demonstrated an increased cell packing density in the brain that resembles the neuropathology of RS [17,18]. Although the disorders are quite different in some of their manifestations, the shared features of severe mental retardation and stunted axonodendritic growth suggests some similarity with respect to disrupted gene expression in the developing brain.

4. Excitatory neurotransmission is altered in RS

In addition to neuropathologic and imaging evidence that neuronal connections form abnormally in RS, clinical evidence suggests that excitatory synaptic activity may be particularly affected [21–24]. Seizures and abnormalities in the electroencephalogram are common in girls with RS during the encephalopathic stage of the disorder during the first decade of life [21,22]. Using electromagnetic stimulation of the motor cortex, Eyre et al. [23] found that motor cortex stimulation evoked action potentials in arm and hand muscles at low threshold with short latency and longer duration, findings consistent with enhanced excitatory activity in motor cortex. Yamanouchi et al. [24] also found giant visual evoked potentials in girls with RS. Heightened excitatory neuronal activity would also be consistent with the hyperkinetic hand-wringing movements and breathing abnormalities seen in RS [25–29].

Synapses that use the excitatory amino acid neurotransmitter glutamate play an important role in circuits in the cerebral cortex, basal ganglia and brainstem responsible for movement and breathing [30,31] and several neurochemical studies also suggest that glutamate mediated neurotransmission is disrupted in RS. Two studies of cerebrospinal fluid in RS girls found elevations in glutamate, suggesting that synaptic levels of glutamate may be elevated [32,33]. A study using MR spectroscopy in living patients also found elevations in the glutamate/glutamine peak [34]. We examined the distribution and density of four subtypes of glutamate receptors in postmortem tissue from frontal cortex in RS girls and controls and found a trend for them to be elevated in younger girls less than eight years and lower than control in older girls (Fig. 1). The elevation in younger girls and decrease in older girls was especially marked and was statistically significant for the density of NMDA (*N*-methyl-D-aspartate) type glutamate receptors [6]. We found a similar trend for an age-related bimodal distribution of glutamate receptors in the basal ganglia from girls with RS, especially for AMPA receptors [7]. Studies of NMDA receptor development in postmortem temporal lobe from non-RS infants indicate that densities remain considerably higher than adult levels during the first several months of postnatal life [35]. This suggests that the high levels of excitatory receptors in RS brain during the first decade could represent a persistence of the immature state. These neurochemical results indicate that both presynaptic and receptor-mediated components of excitatory neurotransmission are altered in RS. They are compatible with the hypothesis that disrupted development of glutamate synapses is responsible for the cortical hyperexcitability, seizures, and possibly other signs seen in young girls with RS. They could also be related to the dendritic pathology and abnormalities in MAP-2 reported in RS since MAP-2 changes following glutamate receptor activation are an integral part of the excitatory synaptic response [15].

It is interesting that receptors for the inhibitory amino acid receptor GABA showed a similar trend to be higher in younger patients and lower in older ones, but to a lesser degree. GABA receptor density was elevated in the caudate of younger girls with RS but reduced in older girls [6]. Another study utilizing SPECT scanning for the closely related benzodiazepine receptor also found reduced levels in frontal cortex in adults with RS [36]. Both excitatory and inhibitory amino acid receptors tend to be expressed in higher than normal numbers in younger children during the period of active synaptogenesis but in lower numbers later in childhood when synaptic pruning is beginning. These results for amino acid neurotransmitters provide the first age-related neurochemical data that is consistent with the age-related clinical stages previously described for RS [1,2].

5. Microarray analysis of gene expression in RS

Analysis of a much larger number of genes expressed in brain tissue has become available using the technique of

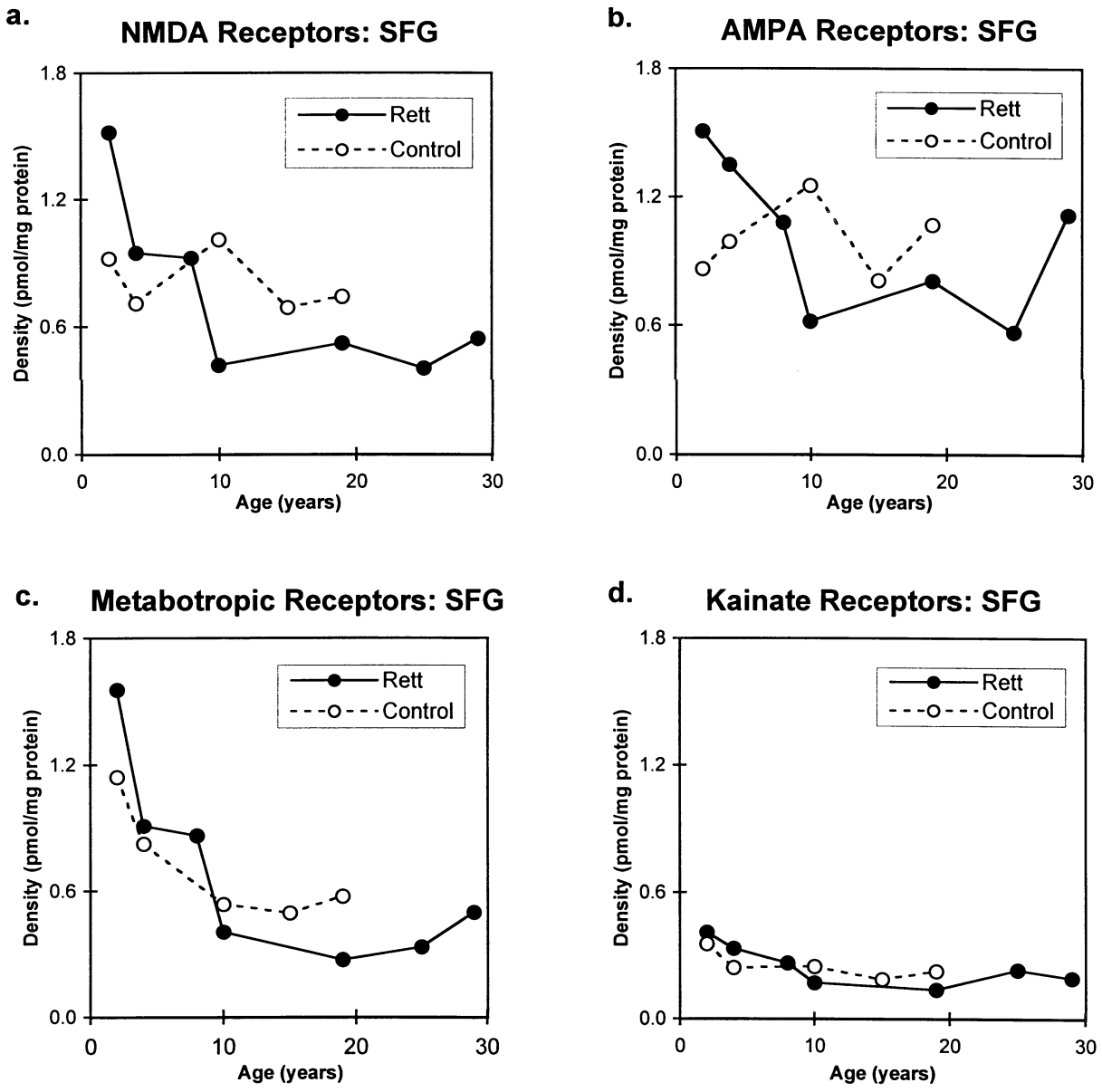

Fig. 1. Density versus age plots for NMDA, AMPA, metabotropic and kainate subtypes of glutamate receptors in frontal lobe cortex from RS patients and controls obtained from neurotransmitter receptor autoradiography in tissue slices (from Ref. [6]). Values plotted were averaged over 2-year bins for each group. In younger RS patients, densities for NMDA, AMPA and metabotropic, but not kainate receptors, tended to be higher than controls but in older patients they were lower than control values.

cDNA microarray analysis [37,38]. Through this technique, messenger RNA (mRNA) prepared from brain tissue is allowed to hybridize with hundreds or thousands of cDNA sequences immobilized on nylon or other solid support filters. Use of several commercially available arrays, which contain some similar genes in the same experiment, allows the results to be confirmed. Further confirmation of individual findings can be obtained by performing reverse transcription followed by the polymerase chain reaction (RT-PCR) to measure mRNA levels and Western blotting to measure protein expression. Table 1 shows some of the synaptically-related genes that are increased or decreased by over 1.5-fold in postmortem samples of frontal cortex from

Table 1
Changes in mRNAs from postmortem cerebral cortex of girls with RS[a]

Increased > 1.5X Control	Decreased > 1.5X Control
NMDA Receptor NR1 subunit	MAP-2
Metabotropic mGluR1 receptor	Synapsin II
Glial EAAT1 Transporter	Synaptogyrin 3
Type 3 GABA transporter	Synaptotagmins 1, 5
	Syntaxin 1A
	Annexin VI

[a] From Colantuoni et al., submitted.

six girls with RS as compared with six age- and gender-matched controls. A number of genes related to synapses were among the most dramatically differentially expressed genes. Several genes encoding proteins associated with synaptic function were consistently down regulated in RS, included syntaxin 1A, the synapsin binding protein annexin VI, and other synaptic vesicle-associated proteins. On the other hand, mRNA for the NR1 subunit of the NMDA receptor as well as the metabotropic mGluR1 receptor subunit was elevated in RS consistent with data from our receptor autoradiographic studies. Messenger RNA for the glial excitatory amino acid transporter type 1 (EAAT1) and the type 3 GABA transporter were also elevated. The microarray results provide further support for the hypothesis that abnormalities in synaptic development are a fundamental component of pathology in RS and demonstrate that presynaptic abnormalities in nerve terminals play a prominent role. These results are consistent with earlier reports of abnormalities in cholinergic and dopaminergic neurons that project long axons into the cerebral cortex and basal ganglia, respectively [39–43].

6. Which abnormalities are a direct result of mutations in MECP2?

The results reviewed here seem quite compatible with the type of pathology expected with mutations in a transcription factor like MeCP2, which is likely to control expression of multiple other genes. However, little is actually known about which genes are under direct control of MeCP2. Although it might be expected that mutations in a transcriptional silencer like MeCP2 would cause inappropriate up-regulation of other genes, the opposite could also occur through up-regulation of genes that suppress others. These preliminary results indicate that a number of genes related to synapses are in fact down regulated in brain tissue. This raises the question of which changes in brain are directly related to mutations in MeCP2 and which reflect secondary effects. For example, several studies have documented the involvement of the cholinergic nucleus of Meynert in the pathology of RS [41–43]. In rodents, neonatal lesions that deprive the cerebral cortex of its cholinergic innervation in the postnatal period produce dendritic pathology, reductions in MAP-2 protein and glutamate receptor abnormalities that resemble RS [15,44–47]. This suggests that a defect in the cholinergic projection to cerebral cortex could cause secondary pathologic changes in neurons that are independent of the direct effect of mutations in *MECP2*. Another example relates to reduced levels of nerve growth factor (NGF) that have been reported in cerebrospinal fluid from RS patients [48]. If these results reflect levels of NGF in RS brain, they could result from abnormalities in neuronal activity rather than the gene defect because NGF production is heavily influenced by excitatory amino acid receptors [49]. Results of cDNA microarray studies in brain need to be interpreted cautiously because gene expression in the nervous system is regulated by activity-dependent neuronal interactions as well as by intrinsic genetic programs.

A related question is whether the *MECP2* mutation affects certain neuronal systems because MeCP2 is differentially expressed in specific neuronal sub-populations in

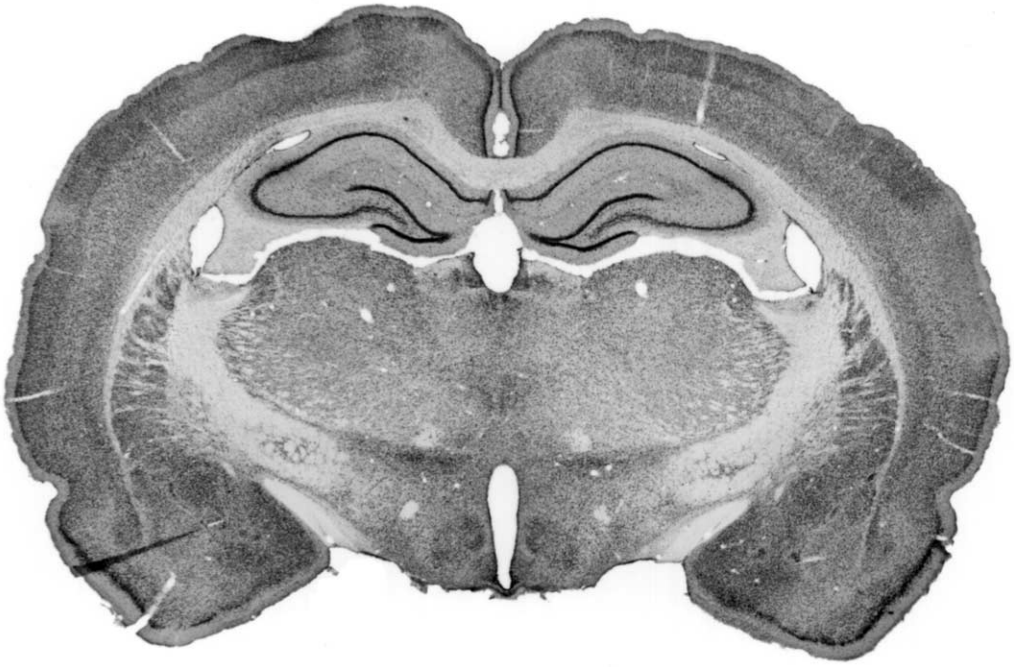

Fig. 2. Low magnification photomicrograph of MeCP2 immunostaining in adult rat brain. The overall pattern of MeCP2 immunoreactivity resembles a Nissl stain since it is ubiquitously distributed in cell nuclei throughout the brain.

the brain (e.g. nucleus basalis cholinergic projection to cortex). Our recent studies in rodent brain using a newly generated antibody to the C-terminal portion of MeCP2 suggest that this is not the case. As shown in Figs. 2 and 3, immunostaining for the transcription factor is found in most neurons throughout the brain and does not appear to be restricted to any particular system.

7. Neurobiology of RS: how does the MECP2 mutation disrupt brain development

The discovery of *MECP2* mutations in RS along with information about the protein's expression and that of other genes in RS brain provide a basis for imagining how RS disrupts the developing brain. All of the new information is consistent with the clinical and pathological data suggesting that it is primarily a disorder of neuronal development and especially both pre- and post-synaptic components of synapses. The clinical picture tells us that the brain is developing fairly normally during fetal life, or at least far better than during later childhood [1]. The emergence of head deceleration and psychomotor regression during infancy, coincides with the timing of synapse proliferation in human cerebral cortex [1,2,5] (Fig. 4). Pathological evidence of synaptic pathology and reduced synaptic numbers in RS suggests that mutated *MECP2* has inhibited this proliferative phase. This suggests that the physiologic function of non-mutated *MECP2* may be to facilitate synapse proliferation by silencing genes that have been inhibiting the process during fetal life. If this is true, then many of the clinical manifestations and changes in gene expression found in RS could be reactions to inhibitory genes that are inappropriately 'left on'. For example, one explanation for the higher densities of glutamate receptors in brains of younger RS girls may reflect attempts of normal developmental programs for receptor development to 'break through' the block presented by inhibitory genes left on by mutated *MECP2*. Since the phase of pruning of extra synapses is underway in cerebral cortex at age 10 years, the genetic programs promoting synaptic proliferation are probably down regulated at this time. Down-regulation of programs that direct synapse proliferation could explain the trend for reduction of glutamate receptors below control in older girls with RS, at a time when some of the most troublesome signs, such as seizures and hand-wringing behavior, are remitting.

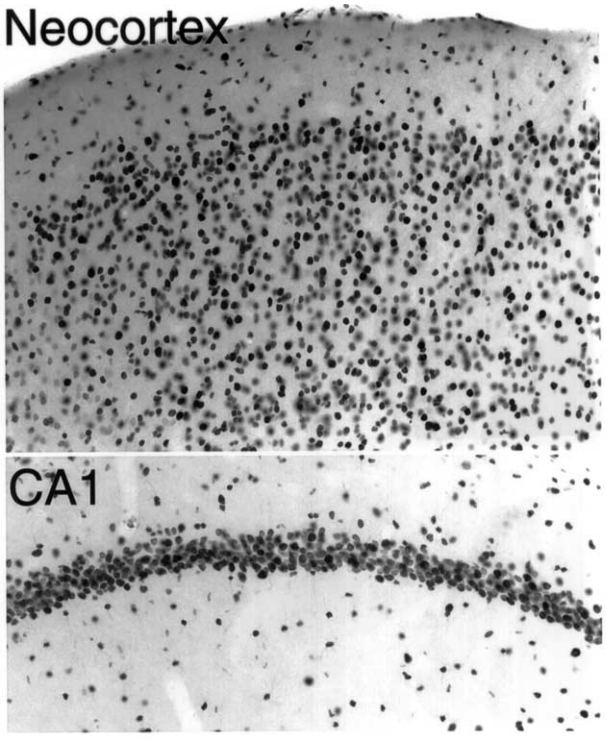

Fig. 3. Higher magnification photomicrographs of MeCP2 immunostaining in neocortex and CA1 hippocampus of an adult rat. The distribution of this C-terminal antibody is nuclear and it ubiquitously stains neurons.

8. An updated neurobiologic hypothesis for RS

Based on the exciting developments in RS research we can modify our working hypothesis that RS disrupts genetic programs that control elaboration and pruning of axonal and dendritic processes to add that it does this by *failing to silence genes that antagonize the synaptic elaboration process* (Fig. 4). This antagonism may produce dramatic clinical signs because it opposes powerful programs directing neuronal synapses, including excitatory and other transmitter receptors, to proliferate. An everyday analogy for this process might be driving a car with the emergency brake accidentally engaged. If the car is accelerated enough to move it, signs of the brake left on soon appear: a burning odor, a screeching sound and maybe smoke. In the brain, an analogy may be drawn between these signs of friction and the signs of excessive brain excitement that appear during the encephalopathic phase of RS. Just as when the car stops, the severe clinical signs in the human brain may abate after age 10 as the brain's genetic programs for synapse proliferation are down regulated.

9. Conclusion

Considerable evidence suggests that RS is a disorder of brain development that disrupts genetic programs controlling the elaboration and pruning of neuronal connections during the early postnatal period. Clinical, neuropathological, neurochemical and gene expression data from microarrays indicate that synaptic development is disrupted in RS, especially development of excitatory amino receptors that use glutamate as a neurotransmitter. In most cases, RS is caused by mutations in the *MECP2* transcription factor, and preliminary data on the expression of this protein in rodent brain suggests that it is ubiquitously expressed in neurons throughout the brain. MeCP2 has been reported to act as a

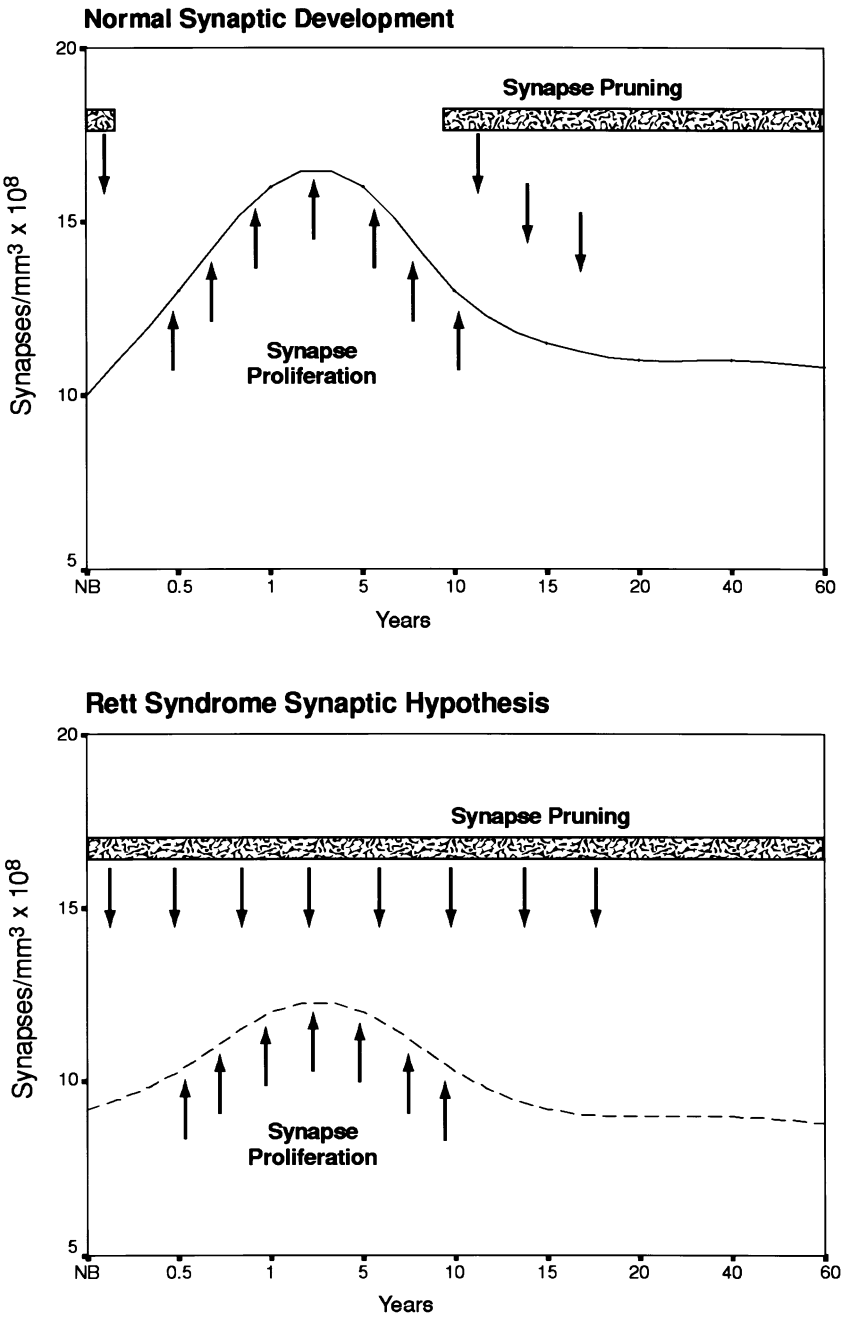

Fig. 4. Diagram illustrating a hypothesis for the pathogenesis of the disorder of neuronal development in RS. Data from neuropathology, neurochemistry, neuroimaging, and gene microarrays indicate that developing synaptic connections are a major target for the disorder. Clinically, the period of developmental stagnation after relatively normal progress to 7–18 months [2] coincides with the time when synapses are proliferating in human cerebral cortex [5]. MeCP2 has been reported to act as a transcriptional silencer for genes that are regulated by methylated CpG islands. This suggests that in RS, mutated *MECP2* may be unable to suppress expression of genes that should be 'turned off' during the first few months of postnatal life. We hypothesize that some of these MeCP2-regulated genes act to inhibit synapse proliferation and/or stimulate synapse pruning. In the upper panel, these genes are shown being turned off by normal MeCP2 during the period of maximal synapse proliferation during normal development (values taken from Ref. [5]). The situation in RS is shown in the lower panel, where mutated *MECP2* is unable to turn off these hypothetical regressive genes. In this case, they remain on, antagonizing the actions of genes that are stimulating synapse proliferation (values are hypothetical). The early 'encephalopathic' or 'destructive' phase of RS during the first decade [2] may result from conflict between proliferative and regressive programs on at the same time. The later phase of relative stability seen in many girls with RS may occur because genetic programs for synapse proliferation are eventually down regulated and do not act to overcome the block in synapse development.

transcriptional silencer for other genes, suggesting that mutations in RS may allow genes that inhibit synaptic proliferation to remain 'turned on'. Many of the clinical signs of RS during the first decade of postnatal life could result from antagonism between powerful genetic programs that stimulate synapse proliferation and conflicting

programs for synaptic regression that are accidentally 'left on' by mutated *MECP2*. It seems likely that the neurobiological and clinical signs of RS reflect a combination of primary effects of the mutated gene (e.g. over-expression of specific genes) and secondary compensatory effects related to attempts to overcome blocks in expression of other programs.

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