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A Review of Gene Expression Patterns in the Malformed Brain

Mercedes F. Paredes and Scott C. Baraban*

Epilepsy Research Laboratory, Box 0520, Department of Neurological Surgery & The Graduate Program in Neuroscience, 513 Parnassus Ave, University of California, San Francisco CA 94143

Abstract

Major advances in the identification of genes expressed in malformation-associated epileptic disorders have been made. Some of these changes reflect the complex gene interactions necessary for proper neurodevelopment, whereas others suggest specific synaptic aberrations that could result in a hyperexcitable, and ultimately, epileptic condition. Here we review reported changes in gene expression associated with a malformed brain, with particular emphasis on how these changes provide clues to seizure genesis.

Index Entries: Animal model; dysplasia; epilepsy; human; malformation; pediatric.

Introduction

Epilepsy is a fairly common neurological disorder characterized by recurrent seizure activity. Without full knowledge of the underlying cause of seizures, appropriate diagnosis and treatment for epileptic patients has proven difficult. A growing body of recent literature suggests that abnormal cortical architecture underlies a variety of early-onset epilepsy disorders *(1–3).* As a group, cortical malformations exhibit a broad spectrum of anomalies, often reflecting a disturbance of neuronal migration or some type of proliferative disorder. Brain malformations that fall under the term "malformations of cortical development" (MCD) include, but are not limited to, lissencephaly, polymicrogyria, focal cortical dysplasia, and periventricular nodular heterotopia *(4).* Early-onset epileptic disorders associated with MCD are often resistant to conventional antiepileptic treatments and regions of disorganized cortex can act as seizure foci *(5,6).* As such, a great deal of experimental investigation is currently directed toward a better understanding of the anatomical and functional properties of dysplastic cells.

* Author to whom all correspondence and reprint requests should be addressed. E-mail: baraban@itsa.ucsf.edu

Access to surgically resected tissue from epileptic patients with a cortical malformation has helped in identifying abnormalities that are associated with, and are potentially the cause of, seizure genesis in the disorganized brain. Additional details about disorganized brain tissue have emerged from studies using animal models of malformation-associated epilepsy. Although histological and physiological studies provide important characterization of disorganized tissue, there is a paucity of work examining the molecular differences between normal and dysplastic neurons. Furthermore, most of the avaialble information is descriptive in nature and offers little information on the mechanistic significance of the reported findings. Here we will discuss the types of molecular abnormalities that can be assocaited with an abnormally formed brain and specualte on how these findings may provide insight into the development of epilepsy in a malformed brain.

Cytoskeletal Abnormalities in the Malformed Brain

Brains obtained from patients with cortical dysplasia (CD), a fairly common form of MCD, often have large hypertrophic neurons, characterized by enlarged nuclei and a prominent nucleolus, dispersed throughout the cortex *(7).* In a subset of CD cases, referred to as focal cortical dysplasia type II, there is another class of dysplastic cells, referred to as "balloon" cells, which have an abundant, glassy eosinophilic cytoplasm. Balloon cells express mature and immature markers of both glial and neuronal origin *(8,9)* and are believed to arise from proliferation of abnormal cells in the germinal zone *(4).* The function of these cells is currently unknown, but it is tempting to speculate that they play some role in the initiation of abnormal electrical discharges. An early indication that dysplastic cells are different from normal cortical neurons came from the observation that these cells have intracytoplasmic fibrillar inclusions; this pathological finding is similar to the neurofibrillary tangles seen in the brains of patients with Alzheimer's disease. Although tangles in CD cases were immunoreactive against phosphorylated neurofilaments, tau, and ubiquitin, they were not reactive against paired helical filaments *(8).*

Intracytoplasmic inclusions may interfere with normal neuronal function and create a condition where dysplastic neurons exhibit abnormal, potentially hyperexcitable activity. Another reported difference between normal and dysplastic cells concerns microtubule-associated protein 2 (MAP2). For example, Yamanouchi et al. *(7)* described higher protein and mRNA levels of the low-weight form, MAP2c, within large neurons in specimens from patients with focal CD and hemimegalencephaly. This smaller form of MAP is normally expressed during embryonic and postnatal periods *(7)* and its expression is developmentally regulated. Animal model findings have substantiated the existence of MAP2 abnormalities in clusters (heterotopia) of displaced hippocampal neurons. For example, immunocytochemical analysis of MAP2 expression in rats exposed to methylazomymethanol (MAM) *in utero* revealed abnormal dendritic orientation and patterning *(10)* and in vitro exposure to MAM can disrupt microtubule assembly *(11).* Therefore, altered MAP2 expression levels in dysplastic neurons may be indicative of some type of developmental aberration in the etiology of cortical dysplasia.

Additional cytoskeletal proteins, such as nestin and MAP1, serve as useful markers for cytoskeletal maturity and a number of laboratories used these proteins to examine potential developmental aberrations in CD. It is generally believed that a better understanding of how the malformed brain forms would provide insight into how the malformed brain functions. The normal expression and function of these proteins is as follows: (1) nestin, an intermediate filament protein, is tran-

siently expressed during the neurulation stage *(12,13)* and (2) MAP1 expression is downregulated during cortical development and this protein is thought to modulate microtubule assembly, which in turn can affect dendritic outgrowth and neuronal migration. Increased expression of both nestin and MAP1 in dysplastic tissue from patients with focal CD *(14)* supports the hypothesis that dysplastic cells are immature and suggests that cortical malformations arise from a defect in cell differentiation or commitment. More general cytoskeletal changes were found using single-cell gene analysis in tissue obtained from patients with focal CD. In these studies, Crino and colleagues reported that dysplastic balloon cells exhibit increased expression of intermediate filament genes, e.g., neurofilament (NF) isoforms (NFL, NFM, and NFH), desmin, and peripherin *(9).* NF antibody staining in New Zealand Black and BXSB autoimmune mice, an animal model featuring ectopic neurons in neocortical layer I, also indicates an abnormal distribution of neurofilaments *(14).* Cytoskeletal abnormalities may be be acting in other malformations of cortical development aside from CD. For example, mutations in the *filamin 1* gene have been identified in patients with periventricular heterotopias. Filamin 1 is a phosphoprotein that may link the actin cytoskeleton with membrane receptors and is particularly enriched in the developing brain, where it appears to be necessary for proper cortical migration *(15).*

These are intriguing observations because cytoskeletal components are instrumental in the movement of growth cones during early phases of neurodevelopment. For example, actin filaments are extremely dynamic and orchestrate the navigational path of filopodia and subsequent movement of neurons to their final cortical destinations. Similarly, microtubules provide a "highway" for the cellular motors (dynein and kinesin) that are needed for the physical advancement of growth cones *(16).* A disruption in this machinery could result in abnormal cellular movement and the placement of neurons in incorrect brain regions. Neurons that fail to migrate to their correct cortical location, presumably as a result of a disruption in the normal neurodevelopmental processes, would set up a brain structure with the potential for abnormal synaptic communication and an excitation-inhibition imbalance.

Gene Mutations in the Malformed Brain

With recent advancements in human gene mapping we now know that a variety of mutations result in the development of a cortical malformation. For example, malformations are often encountered in patients with the multisystemic neurocutaneous (and often epileptogenic) syndrome of tuberous sclerosis complex (TSC). Two recently identified gene mutations, TSC1 and TSC2, are associated with TSC. TSC genes encode for the proteins hamartin and tuberin, respectively *(1,2,17).* Tuberin appears to function as a GAP (GTPase activating protein) that inhibits the Ras-related family of small GTP-binding proteins such as Rap1 and Rab5. Tuberin inhibits the G_1/S phase transition in the cell cycle and promotes entry into the G_0 phase. Less is known about hamartin, but it is thought to regulate cell adhesion. Both proteins also act as tumor suppressors *(17,18).* Recent evidence in *Drosophila* has shown that TSC1 and TSC2 form a complex both in vitro and in vivo and play a part in cell growth and proliferation. Cells mutant for TSC1 have increased size and overexpression of TSC genes affects cellular proliferation/size both at the cellular and organ level. Genetic studies further suggest that TSC1 and TSC2 act as negative regulators of the insulin-signaling pathways regulating cellular growth *(19,20).*

Lissencephaly, a malformation-associated epileptic disorder characterized by the absence or decrease of gyri, has been linked to a mutation in the LIS1 gene that encodes PAFAH1B1

(platelet activating factor-acetylhydrolase [PAFAH] brain isoform Ib). PAFAH1B1, a noncatalytic subunit of PAFAH, regulates the level of platelet activating factor in the brain and has several WD40 repeats, suggesting its involvement in multiple protein-protein interactions. PAFAH, for example, forms a complex with the centrosome and can interact with microtubule (MT) binding proteins such as dynein; therefore, it is implicated in microtubule dynamics *(2,21).*

Doublecortin (DCX) is a gene mutation associated with X-linked lissencephaly and subcortical laminar heterotopia ("double cortex" syndrome). Mutations in the DCX gene in male patients result in a lissencephaly phenotype that is similar to that caused by mutation of the LIS1 gene. However, heterozygous DCX mutations in women show a double cortex phenotype known as subcortical band heterotopia; this probably arises from the mosaic state due to random X-inactivation in females. Doublecortin is a microtubule-associated protein (MAP) and has been shown to bind microtubules and promote their polymerization *(2,22).*

A number of interesting genetic mouse models of cortical disorganization exist and provide further insight into the molecular mechanisms underlying MCD and epileptogenesis *(1,2,19,23,24).* The *reeler* mouse has an autosomal mutation that results in abnormal cortical lamination, e.g., an inverted neocortex with the outer layers assuming a more interior position *(1).* Reelin, the defective protein in the *reeler* mouse, is a large glycoprotein secreted by early-born neurons in the marginal zone of the immature CNS. The Reelin signaling pathway seems to be involved in proper radial organization and layering of postmitotic neurons in the CNS *(22–24). Reelin* mutations have been identified in two families with members who have recessive lissencephaly; these patients also exhibit cerebellar malformations *(25).* The *scrambler* mutant, whose phenotype is similar to that of the *reeler* mouse, has a mutation in Disabled-1, a tyrosine-kinase adapter protein that belongs to the Reelin pathway *(22,24).* A recently developed *p35* knockout mouse exhibits a similar cortical phenotype. The *p35* gene encodes a brain-specific activator of cyclin-dependent kinase 5, a protein necessary for proper neuronal migration *(2,25).* Inactivation of Cdk in mice results in profuse defects in neuronal migration in several parts of the central nervous system (CNS), including the cerebral cortex and the hippocampus. *p35* knockout mice exhibit cortical disorganization, hippocampal granule cell dispersion similar to that observed in epileptic patients *(26,27),* and display a spontaneous seizure phenotype. The available information strongly suggests that proper development of an organized cortical structure requires a complex series of molecular events—disruption of any of these varied steps could result in a malformed brain and a predisposition to the development of epilepsy.

Synaptic Changes in the Malformed Brain

Epileptic activity derives from abnormal electrical discharge in the brain *(28).* In the case of cortical malformations, aberrant electrical activity could result from improper connections among dysplastic neurons (synaptic dysgenesis) or from synaptic changes that occur on the postsynaptic cell itself (receptor alterations). Several studies have reported abnormalities in the expression of postsynaptic neurotransmitter receptor subtypes in human epileptic specimens featuring dysplasia *(29–31).* A specific role for glutamate (the primary excitatory neurotransmitter) in generating hyperexcitability within a malformed brain is suggested by a recent finding that NMDA-type glutamate receptors are differentially expressed. In particular, an NMDA subunit (NR2), is aberrantly expressed in dysplastic cortical regions of patients with focal CD. In

the adult cortex, NR1 NMDA-type subunits normally form a homodimer that produces a relatively small NMDA current. The presence of NR2 subunits (rather than NR1 homodimers) could lead to the formation of an NR1/NR2 heterodimer. This is an important molecular observation with potential functional relevance because NR2 homodimers are nonfunctional, but a NR1/NR2 heterodimer can pass excitatory currents that are up to 60 times greater than that observed with homomeric NR1 channels *(29).* Simply stated, this atypical glutamate channel expression pattern could explain the hyperexcitability associated with dysplastic tissue. Support for such a role in epileptogenesis comes from correlative work showing increased NR1/NR2 co-assembly in resected epileptogenic regions from patients with focal CD *(30).* Again, animal model findings are consistent with the idea that postsynaptic receptor changes occur in dysplastic brain regions. In the neonatal freeze-lesion model of polymicrogyria, changes in pharmacological sensitivity to GABA and NMDA receptor antagonists suggest altered subunit compositions for these receptors *(32).* In the MAM model of nodular heterotopia, NR1 glutamate receptor subunit expression is decreased and GluR2 flip subunit expression is increased *(33).*

Interestingly, Crino et al. *(31),* using single-cell mRNA analysis and micro-dissection of individual neurons in tissue samples obtained from epileptic patients with focal CD, reported that glutamate receptor changes are unique to dysplastic cells in neocortex but cannot be generalized to include heterotopic cells displaced to the subcortical white matter. The distinct gene-expression profiles between these two cell types included differential expression of the glutamate receptor subunits (GluR1, NR_{2B}, and NR_{2C}) as well as $GABA_A$ receptor subunits ($R_{\alpha 1}$, $R_{\alpha 2}$ and $R_{\beta 2}$). Although these studies suggest that heterotopic and dysplastic cells could behave differently (owing to their aberrant expression of receptor proteins regulating excitation-inhibition), more evidence is needed to understand how the overall pattern of gene expression in specific neuronal subpopulations in a disorganized cortex translates into epileptogenesis.

Presynaptic changes have also been reported for dysplastic brain regions. GAP-43, a growth-associated protein, is a phosphoprotein enriched in growth cones and presynaptic terminals *(34).* In the normal brain, GAP-43 levels decline significantly when the growth cone has matured and synaptogenesis is complete. However, in samples from epileptic patients with cortical dysplasia, there is enhanced mRNA expression of this presynaptic protein specifically in large dysplastic neurons. The functional consequence of altered GAP-43 expression could be that dysplastic neurons fail to make appropriate synaptic connections. Although an interesting observation, it is worth noting that initial studies did not report a coincident change in GAP-43 protein levels. Nevertheless, this alteration in GAP-43 expression could be a marker for synaptic remodeling occurring in connections made by dysplastic neurons but further evidence is needed to support this hypothesis.

Conclusion

Molecular analysis of dysplastic neurons in the malformed human brain has revealed several genes that are clearly critical to neurodevelopment and synaptic function (Fig. 1). Animal models, in a limited fashion, have confirmed and extended many of these observations. Further studies, combining cutting-edge molecular and electrophysiological approaches, are needed to explore the precise mechanisms through which these genetic abnormalities lead to a disorganized brain structure with epileptogenic propensities. Additionally, clinical and laboratory studies must continue to identify the potentially large number of genetic defects that may be associated with a dysplastic brain.

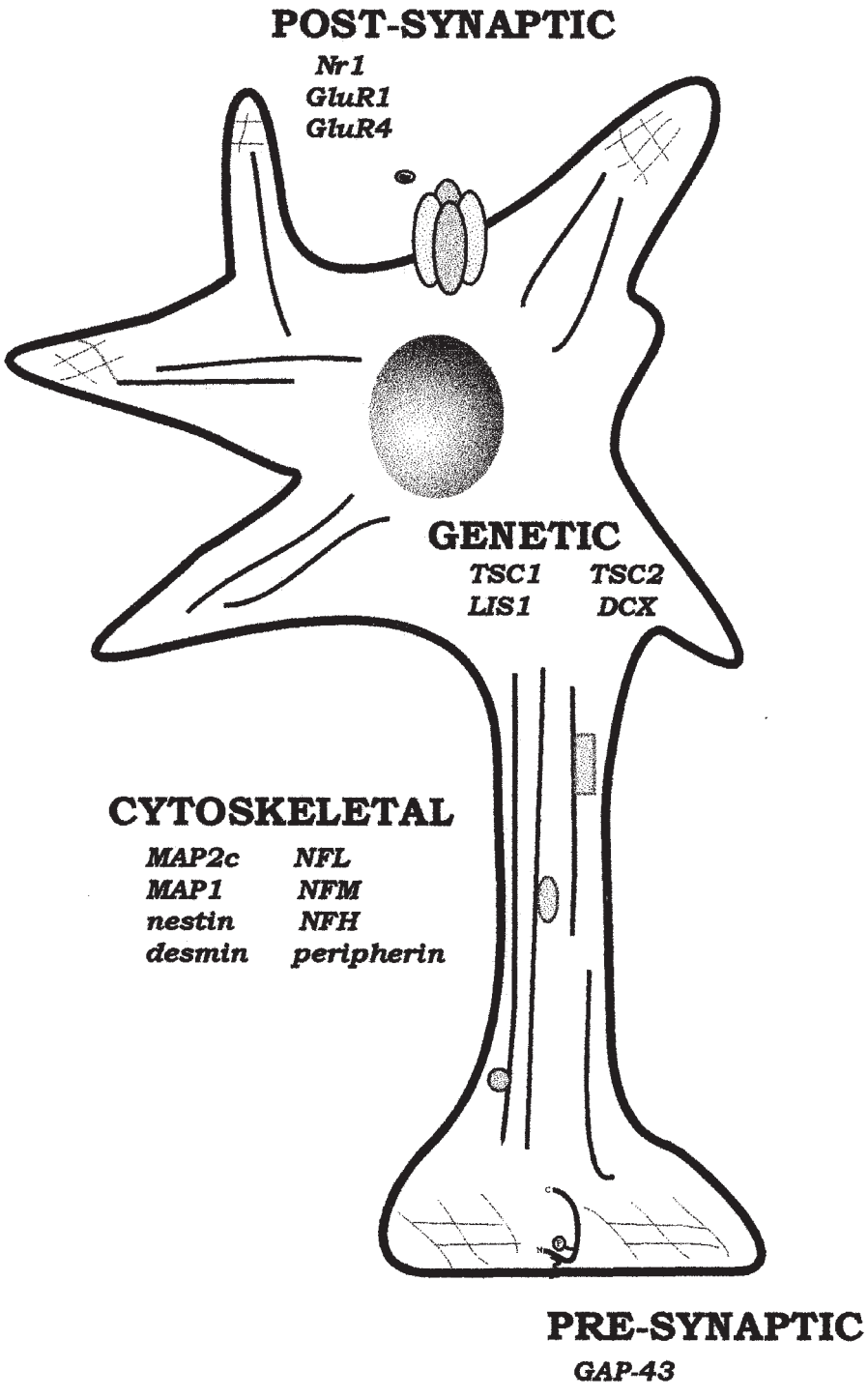

Fig. 1. Summary of the molecular changes that can occur in a dysplastic neuron. Schematic of a dysplastic neuron with a list of the postsynaptic, presynaptic, cytoskeletal, and genetic molecular findings reported, thus far, in dysplastic neurons.

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References

1. Chevassus-au-Louis, N., Baraban, S. C., Gracias, G.-L., and Ben-Ari, Y. (1999) Cortical malformations and epilepsy: new insights from animal models. *Epilepsia* **40,** 811–821.
2. Schwartzkroin, P. A. and Walsh, C. (2000) Cortical malformations and epilepsy. *Mental Retardation Dev. Disabilities* **6,** 268–280.
3. Palmini, A. (2000) Disorders of cortical development. *Curr. Opin. Neurol.* **13,** 183–92.
4. Kuzniecky, R. I. and Barkovich, A. J. (2001) Malformations of cortical development and epilepsy. *Brain Dev.* **23,** 2–11.
5. Palmini, A., Gambardella, A., Andermann, F., Dubeau, F., da Costa, J. C., Olivier, A., et al. (1995) Intrinsic epileptogenicity of human dysplastic cortex as suggested by corticography and surgical results. *Ann. Neurol.* **37,** 476–87.
6. Ohtsuka, Y., Yoshinaga, H., and Kobayashi, K. (2000) Refractory childhood epilepsy and factors related to refractoriness. *Epilepsia* **41,** 14–7.
7. Yamanouchi, H., Zhang, W., Jay, V., and Becker, L. E. (1996) Enhanced expression of microtubule-associated protein 2 in large neurons of cortical dysplasia. *Ann. Neurol.* **39,** 57–61.
8. Duong, T., De Rosa, M. J., Poukens, V., Vinters, H. V., and Fisher, R. S. (1994) Neuronal cytoskeletal abnormalities in human cerebral cortical dysplasia. *Acta Neuropathol* **87,** 493–503.
9. Taylor, J. P., Sater, R., French, J., Baltuch, G., and Crino, P. B. (2001) Transcription of intermediate filament genes is enhanced in focal cortical dysplasia. *Acta Neuropathol.* (Berl) **102,** 141–148.
10. Baraban, S. C., Wenzel, H. J., Hochman, D. W., and Schwartzkroin, P. A. (2000) Characterization of heterotopic cell clusters in the hippocampus of rats exposed to methylazoxymethanol in utero. *Epilepsy Res.* **39,** 87–102.
11. Hoffman, J. R., Boyne, L. J., Levitt, P., and Fischer, I. (1996) Short exposure to methylazoxymethanol causes a long-term inhibition of axonal outgrowth from cultured embryonic rat hippocampal neurons. *J. Neurosci Res.* **46,** 349–359.
12. Crino, P. B., Trojanowski, J. Q., and Eberwine, J. (1997) Internexin, MAP1B, and nestin in cortical dysplasia as markers of developmental maturity. *Acta Neuropathol.* (Berl) **93,** 619–27.
13. Duggal, N., Iskander, S., and Hammond, R. R. (2001) Nestin expression in cortical dysplasia. *J. Neurosurg.* **95,** 459–465.
14. Sherman, G. F., Stone, J. S., Press, D. M., Rosen, G. D., and Galaburda, A. M. (1990) Abnormal architecture and connections disclosed by neurofilament staining in the cerebral cortex of autoimmune mice. *Brain Res* **529,** 202–207.
15. Fox, J. W., Lamperti, E. D., Eksioglu, Y. Z., Hong, S. E., Feng, Y., Graham, D. A., et al. (1998) Mutations in filamin 1 prevent migration of cerebral cortical neurons in human periventricular heterotopia. *Neuron* **21,** 1315–1325.
16. Goldstein, L. S. and Yang, Z. (2000) Microtubule-based transport systems in neurons: the roles of kinesins and dyneins. *Annu. Rev. Neurosci.* **23,** 39–71.
17. Vinters, H. V., Park, S. H., Johnson, M. W., Mischel, P. S., Catania, M., and Kerfoot, C. (1999) Cortical dysplasia, genetic abnormalities and neurocutaneous syndromes. *Dev. Neurosci.* **21,** 248–59.
18. Mizuguchi, M. and Takashima, S. (2001) Neuropathology of tuberous sclerosis. *Brain Dev.* **23,** 508–15.
19. Gao, X. and Pan, D. (2001) TSC1 and TSC2 tumor suppressors antagonize insulin signaling in cell growth. *Genes Dev.* **15,** 1383–92.
20. Potter, C. J., Huang, H., and Xu, T. (2001) Drosophila Tsc1 functions with Tsc2 to antagonize insulin signaling in regulating cell growth, cell proliferation, and organ size. *Cell* **105,** 357–68.
21. Cardoso, C., Leventer, R. J., Dowling, J. J., Ward, H. L., Chung, J., Petras, K. S., et al. (2002) Clinical and molecular basis of classical lissencephaly: mutations in the LIS1 gene (PAFAH1B1). *Hum. Mutat.* **19,** 4–15.
22. Feng, Y. and Walsh, C. A. (2001) Protein-protein interactions, cytoskeletal regulation and neuronal migration. *Nat. Rev. Neurosci.* **2,** 408–416.
23. Allen, K. M. and Walsh, C. A. (1999) Genes that regulate neuronal migration in the cerebral cortex. *Epilepsy Res.* **36,** 143–154.

24. Lambert de Rouvroit, C. and Goffinet, A. M. (2001) Neuronal migration. *Mech. Dev.* **105,** 47–56.
25. Hong, S. E., Shugart, Y. Y., Huang, D. T., Shahwan, S. A., Grant, P. E., Hourihane, J. O., et al. (2000) Autosomal recessive lissencephaly with cerebellar hypoplasia is associated with human RELN mutations. *Nat. Genet.* **26,** 93–96.
26. Wenzel, H. J., Robbins, C. A., Tsai, L. H., and Schwartzkroin, P. A. (2001) Abnormal morphological and functional organization of the hippocampus in a p35 mutant model of cortical dysplasia associated with spontaneous seizures. *J. Neurosci.* **21,** 983–998.
27. Houser, C. R. (1990) Granule cell dispersion in the dentate gyrus of humans with temporal lobe epilepsy. *Brain Res.* **535,** 195–204.
28. Harrison, T. R. and Fauci, A. S. (1998) *Harrison's principles of internal medicine,* 14th Ed. McGraw-Hill Health Professions Division, New York.
29. Ying, Z., Babb, T. L., Mikuni, N., Najm, I., Drazba, J., and Bingaman, W. (1999) Selective coexpression of NMDAR2A/B and NMDAR1 subunit proteins in dysplastic neurons of human epileptic cortex. *Exp. Neurol.* **159,** 409–418.
30. Najm, I. M., Ying, Z., Babb, T., Mohamed, A., Hadam, J., LaPresto, E., et al. (2000) Epileptogenicity correlated with increased N-methyl-D-aspartate receptor subunit NR2A/B in human focal cortical dysplasia. *Epilepsia* **41,** 971–976.
31. Crino, P. B., Duhaime, A. C., Baltuch, G., and White, R. (2001) Differential expression of glutamate and GABA-A receptor subunit mRNA in cortical dysplasia. *Neurology* **56,** 906–913.
32. Hablitz, J. J. and DeFazio, R. A. (2000) Altered receptor subunit expression in rat neocortical malformations. *Epilepsia* **41,** S82–S85.
33. Rafiki, A., Chevassus-au-Louis, N., Ben-Ari, Y., Khrestchatisky, M., and Represa, A. (1998) Glutamate receptors in dysplasic cortex: an in situ hybridization and immunohistochemistry study in rats with prenatal treatment with methylazoxymethanol. *Brain Res.* **782,** 142–152.
34. Yamanouchi, H., Mizuguchi, M., Oka, A., Takashima, S., Becker, L. E., Eguchi, M., and Nakazato, Y. (2000) Enhanced GAP-43 gene expression in cortical dysplasia. *Neuroreport* **11,** 1815–1819.