Cerebral Cortical Dysplasia Associated with Pediatric Epilepsy. Review of Neuropathologic Features and Proposal for a Grading System

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Key Words: Brain development; Cortical dysplasia; NCAMS; Neuronal migration; Pediatric epilepsy; Radial glia; SAMS.

INTRODUCTION

Cortical dysplasia (CD), in the broadest sense, describes a spectrum of pathologic changes reflecting a disturbance in the process of its development. Neocortical development can roughly be considered to be the result of the overlapping processes of proliferation, migration, terminal differentiation and programmed cell death (PCD) of neuronal precursors and neurons, synapse elimination, dendritic arborization and cortical remodelling (1). Abnormalities of these processes result in abnormalities of cortical architecture and (by inference) its electrophysiological properties. CD manifests clinically by producing seizures, developmental delay and focal neurologic deficits. These seizures are often intractable and may be life-threatening (2). Compared to other structural lesions of the CNS that cause epilepsy, CD tends to affect younger patients, has a less favorable prognosis, and shows more frequent extra-temporal lobe involvement (3).

Neocortical malformations have usually been considered to be disorders of neural migration which have variously been classified with regard to morphology or putative etiology. CD has been considered a subtype of this group of disorders. In this paper, we wish to broaden the concept of CD to include the full range of neuronal migration disorders (NMD) and to reclassify it as a spectrum of pathologic changes reflecting disturbances of normal developmental processes. Extremes within this spectrum would include the most subtle and the most severe forms of malformation. We define CD, therefore, as a spectrum of derangements in development of the neocortex associated with a range of morphologic features and with multiple putative etiologic factors, including genetic and environmental influences.

Seventy-seven patients with CD have undergone cortical resections ranging from partial lobectomies to hemispherectomies for the control of intractable seizures at UCLA-CHS. The majority of these patients were part of the Pediatric Epilepsy Surgery Program between 1986 and 1994. Based upon this experience we propose that CD can be characterized with regard to nine specific and easily identifiable microscopic abnormalities: 1) cortical laminar disorganization, 2) single heterotopic white matter neurons, 3) neurons in the cortical molecular layer, 4) persistent remnants of the subpial granular cell layer (SGL), 5) marginal glioneuronal heterotopia, 6) polymicrogyria (PMG), 7) white matter neuronal heterotopia, 8) neuronal cytomegaly with associated cytoskeletal abnormalities, and 9) balloon cell change.

These microscopic features can be used as the basis for a grading system for CD. The microscopic changes reflect disruption of the normal developmental processes and can be used as relative markers for the time of the disruption during intrauterine life. Each of the microscopic features can be hypothesized as having occurred during a specified time window, based on theoretical and empirical evidence, as well as data gathered from experimental animal models. We will demonstrate that grading CD with regard to these features can be used to stratify the dysplasia into "categories" reflecting early, mid and late developmental disruptions, which correlate with the resultant clinical severity of disease. The rationale for this grading system will be discussed, as will the biologic significance of each of the characteristic features.

We will review the normal process of neocortical development and identify the points of susceptibility at which the error or errors (genetic, environmental, or both) resulting in CD can manifest. We will then review the biological significance of each of the associated features, exploring the empirical observations as well as data obtained from experimental models. Finally, we will examine likely etiologies of CD, reviewing the evidence for genetic and environmental causes.

METHODS

We reviewed specimens from 77 patients (38 male, 39 female) who underwent surgical cortical resections for intractable seizures between 1986 and 1994. (Four patients underwent two resections.) Tissue specimens from the resections were immersion-fixed in 10% formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin, Klüver-Barrera, Bielschowsky, Weil and cresyl violet stains, as required. Immunohistochemical studies on some of this material have been reported elsewhere (4-7) and (where relevant) will be described.
The number of sections reviewed in each case ranged from four to 38, based upon the size of the specimen. All cases were reviewed by two neuropathologists (HVV at the time of initial resection, PSM in a retrospective fashion). The specimens were evaluated for the presence of the specified microscopic features as well as any other abnormal findings. The features were noted and the specimens were graded as follows: severe-presence of balloon cells and/or neuronal cytomegaly (± cytosomal abnormalities); moderate—presence of PMG and/or white matter neuronal heterotopia, but not balloon cells or neuronal cytomegaly; mild—no evidence of balloon cells, neuronal cytomegaly, PMG or white matter neuronal heterotopia. Clinical histories were obtained from patient charts by two of the investigators (PSM and LPN). In addition to demographic information, clinical information pertinent to seizure frequency, developmental delay, neurologic deficit and age at surgical presentation was obtained and scored as follows. Seizure frequency was subgrouped by number of seizures per day: 0–5, 6–20, or >20. (This was assessed by using the maximum number of preoperative seizures per day listed in the chart.) Developmental delay was subjectively assessed (using neuropsychological evaluations when possible) and scored on a 1–4 scale with 1 = none, 2 = mild, 3 = moderate and 4 = severe. Neurologic deficit was scored as either present or absent.

RESULTS

The patients ranged in age (at the time of resection) from 3 weeks to 35 years (mean age 7.8 years). All patients underwent resections for attempted surgical control of intractable seizures. The series includes two patients with Sturge-Weber disease, one with clinically manifest tuberous sclerosis (TS) and one with Aicardi syndrome. Twenty-six patients underwent a hemispherectomy (three or more lobes), 19 underwent partial hemispherectomy (two or more lobes) and 32 patients had lobectomy or partial lobectomy. The frontal lobe was resected in 30 cases, temporal lobe in 64, parietal in 35, and occipital in 28.

Gross Pathologic Findings

The specimens showed a range of abnormalities. Five specimens exhibited hemimegalencephaly (HME) (one with lissencephaly), and one specimen exhibited extensive pachygria (4). Two specimens showed gross PMG, and one exhibited macroscopic neuronal heterotopia in the white matter. Nineteen specimens revealed focal cortical thickening with loss of the normal cortex white matter demarcation. Forty of the specimens showed no macroscopic pathologic alterations. In addition to gross examination, whole mount sections are frequently useful in evaluation of these specimens (Fig. 1).

Microscopic Findings

Cortical laminar disorganization (Fig. 2A, B) was found in all 77 cases. This consisted of an irregular arrangement of the cortical neurons (often focal) with a loss of the normal laminar organization and irregular neuronal clustering. Neurons exhibiting abnormal polarity with misdirected apical dendrites were frequently encountered. Single heterotopic neurons in the white matter (Fig. 3A) were noted in 76 of 77 patients, consisting of clearly identifiable neurons located singly and in small groups. These heterotopic neurons were most prominent in regions of overlying cortical disarray. Increased cellularity of the cortical molecular layer with scattered identifiable neurons therein was noted in 56 patients (Fig. 3B). Small cells arranged in rows in a subpial distribution consistent with remnants of the SGL (Fig. 4A) were noted in 51 specimens, usually in a patchy focal distribution. Marginal glioneuronal heterotopia consisting of ex crescences of neuroglial tissue exuding through the pia into the subarachnoid space (Figs. 4B, 5A, B) were noted in 23 patients. Microscopic neuronal heterotopia consisting of islands of disorganized neurons within the subcortical white matter (Fig. 6) were found in 32 patients. Foci of PMG (usually unlayered) (Fig. 11) were present in 11 patients, including three of the four with HME. None of the patients with foci of PMG showed evidence of destructive lesions.

Neuronal cytomegaly characterized as large, irregularly shaped neurons was noted in resections from 43 patients. These large, irregularly shaped neurons (Figs. 7, 8A, B) were clearly distinct from normal Betz cells. In 40 of the patients, these cytomegaly neurons exhibited cytosomal abnormalities including neurofibrillary tangle-like cytoplasmic inclusions (Fig. 8) (5–7), irregular clumping of Nissl substance, and cytoplasmic vacuolization. Balloon cells with eccentric nuclei and abundant opalescent eosinophilic cytoplasm, similar to some of the cells seen in cortical tubers of TS (Figs. 9A, B, 10A, B) were seen in resections from 17 patients. All of the cases with neocortical balloon cells also exhibited neuronal cytomegaly with cytosomal abnormalities. Chastin’s subpial gliosis was present in 75 of the specimens.

Other pathologic findings included evidence of destructive lesions in 12 cases. These consisted of otherwise typical cystic infarcts and large foci of cystic encephalomalacia sometimes involving white matter, calcification and ferrugination of neurons. None of these were found in association with the 11 specimens containing PMG.

Seventeen resection specimens showed evidence of nonspecific gliosis and mild nonspecific inflammation consisting of rare microglial nodules with minimally increased inflammatory cells and perivascular cuffing. One resection specimen showed changes consistent with Rasmussen’s encephalitis (in addition to the malformative abnormalities).

GRADING OF THE LESIONS

The proposed grading system is based on the hypothesis that the microscopic features reflect events occurring within discrete time windows during brain development.
and can therefore be used to stratify CD into lesions resulting from early, mid and late developmental disruptions (which correlate, respectively, with mild, moderate and severe CD). Balloon cells and neuronal cytomegaly represent early developmental disruptions, while PMG and microscopic neuronal heterotopia represent intermediate events. Marginal glioneuronal heterotopia, persistent SGL, neurons in the cortical molecular layer and

Fig. 1. Representative whole mount sections of a specimen from a child with severe CD and HME. Top panel shows the presence of multiple neuronal heterotopia. Note small islands of neurons in the white matter, predominantly in a periventricular location, i.e. nodular heterotopia (arrowheads). Also note the presence of laminar neuronal heterotopia (arrow) giving the appearance of a “double cortex.” Lower panel shows another region in which there is a “blurring” of the normal cortex–white matter junction (arrow), as well as multiple neuronal heterotopia (arrowheads). (Klüver-Barrera, ×2.6.)

Fig. 2. (A) Cortical laminar disorganization exhibiting loss of the normal pattern of orderly cortical lamination. Note irregular clustering of neurons in layers 2 and 3 of the neocortex. (Klüver-Barrera, ×70.) (B) High magnification section of the deep neocortex, exhibiting irregular clustering of neurons, many of which are enlarged. Note the apical dendrites, many of which show abnormal polarity. Subcortical white matter is at the bottom of the figure. (Klüver-Barrera, ×520.)

Fig. 3. (A) Heterotopic neurons (e.g. as indicated by arrows) scattered in the subcortical white matter. (H and E, ×520.) (B) Section of the cortical molecular layer exhibiting increased cellularity with scattered heterotopic neurons (arrowheads). (Klüver-Barrera, ×200.)

Fig. 4. (A) Residual SGL consisting of small round cells arranged in a row beneath the pial surface (arrows). (H and E, ×200.) (B) Marginal glioneuronal heterotopia. A fragment of neuroglial tissue protrudes through the pial surface into the subarachnoid space. Note presence of the residual SGL beneath the pial surface (arrowheads). (H and E, ×200.)

Fig. 5. Marginal glioneuronal heterotopia protruding into the subarachnoid space (A) (H and E, ×80.) Higher magnification view (B) demonstrates the presence of neurons within this disorganized neuroglial tissue. (H and E, ×520.)

single heterotopic white matter neurons reflect late gestational (or early postnatal) events. Cortical laminar disorganization, the defining feature of CD, suggests a failure of the preceding developmental process and is therefore a late event (Fig. 12).

Twenty-one patients were graded as having mild cortical dysplasia, 13 patients were graded as being moderate, and 43 were deemed severe. The severe cases included all five patients with HME, four patients with destructive lesions, the patient with TS, Aicardi syndrome, and one of the patients with Sturge-Weber syndrome. The patients graded as having moderate CD included five patients with destructive lesions and one of the patients with Sturge-Weber syndrome. The mild CD patients included three with destructive lesions.

The pathologic grade of these resection specimens was then compared to the clinical data obtained for age of surgical presentation, seizure frequency, developmental delay and neurologic deficit. Using an analysis for categorical association between ordinal variables (9), a statistically significant positive correlation is obtained between pathologic grade and seizure frequency (p = 0.008, gamma statistic value = 0.381) (Fig. 13). Similarly, a statistically significant negative correlation is obtained between pathologic grade and age at resection (p = 0.003, gamma statistic value = -0.438) (Fig. 14). A weak positive trend between pathologic grade and developmental delay was noted, although it was not statistically significant. No clear association between pathologic grade and neurologic deficit was established.

The statistically significant correlations between pathologic grade and seizure frequency, as well as age, are highly suggestive but need to be interpreted with caution. Subjective parameters such as seizure frequency and developmental delay are difficult to standardize and are fraught with interpretational differences. However, in the absence of more objective parameters, these are currently the standard clinical measures used. It will be necessary to analyze clinicopathologic data in a rigorous prospective fashion.

OVERVIEW OF CORTICAL DEVELOPMENT

At approximately 4 weeks of gestational age, the neural tube forms with a simple pseudostratified neuroepithelium, which then proliferates and continues to surround the developing ventricular system, eventually becoming the ventricular zone. Neuroblast migration then begins and by approximately 6 weeks, the ventricular zone, intermediate zone and marginal zone of the cortical mantle can be appreciated as distinct (1).

The ventricular zone can be described as a mosaic of precursors which will give rise to neurons, astrocytes and oligodendroglia, as demonstrated using retroviral markers (10). Rakic (11) originally suggested the concept of a protocortex in which an architectonic map or plan of the future cortex is laid out in this zone even at such an early developmental stage. The protomaps of the neocortex become translated into architectonic areas as neuronal precursors migrate along radial fibers into the cortex to form ontogenetic columns. Extrinsic factors also influence formation of the cortex within the constraints of this protomap (12).

Formation of the neocortex occurs in five stages (13). The initial cortical plate is first noted at 7–10 weeks of gestation. A primary condensation of this cortical plate occurs as it compacta. At 11–13 weeks, a bilaminar appearance is noted at the cortical plate, followed at 13–15 weeks by a secondary condensation. Condensation into a six-layered cortex is noted at approximately 16 weeks. Neuroblasts continue to migrate through the intermediate zone (future white matter) to the cortex, a process that can continue up to a few months after birth (13). Neurons of the deepest cortical layers are the first to radially migrate, while the neuroblasts destined for the more superficial cortical layers migrate past them (14).

Since neuroblasts are formed in the region of the ven-

Fig. 6. Multiple nodular neuronal heterotopia present in the white matter. Note the presence of small islands of disorganized neurons in the white matter (arrows), predominantly in a periventricular location—ventricular surface is present at top of the micrograph. (Kluver-Barrera, ×45.)

Fig. 7. Neuronal cylomegaly. Single large neuron with irregular clumping of the Nissl substance and neurofilibrillary tangle-like cytoplasmic inclusion (arrow). Note adjacent balloon cells (arrowheads). (H and E, ×910.)

Fig. 8. Low magnification view of the deep cortex with abundant neurons exhibiting neurofilibrillary tangle-like cytoplasmic inclusions (A) (Bielschowsky, ×200.) Higher magnification view (B) shows these cytoplasmic neurofilamentous “tangles” in a number of enlarged neurons: (Bielschowsky, ×560.)

Fig. 9. Balloon cell change. Section of corticectomy from a child with severe CD shows balloon cells at the cortex—white matter junction (A) (H and E, ×200); (B) (H and E, ×520.)

Fig. 10. (A) Balloon cells (arrow), which are identical to the cells seen within the tubers of TS, have eccentric nuclei and abundant opaque eosinophilic cytoplasm. (H and E, ×860.) (B) Binucleate balloon cells (arrow) are occasionally seen. (H and E, ×910.)
tricle, yet come to rest in the cortex, migrational mechanisms must be specified. The complex three-dimensional array of the cortex is achieved via a combination of radial and tangential migration. Radial migration refers to the process by which neuroblasts from the ventricular and subventricular zones migrate along radial glia to reach the cortex. Radial glia possess cell bodies in the ventricular or subventricular zones and end-feet on the pial surface (11). This phase of radial extension coincides with an amitotic phase in the life cycle of glia. After neuroblast migration is completed, they return to the mitotic cycle and develop into astrocytes (15, 16). During the migrational process, these radial glia appear to function as a permissive scaffold (17).

The second major mode of migration, complementary to radial migration, is tangential to the pial surface. A subset of neurons migrates orthogonally after migrating radially to form complex three-dimensional neural structures. This mode of migration is considered to be neurophilic, occurring along neuronal processes, rather than gliophilic (18). It occurs widely throughout the development of the CNS (19) and has even been observed to occur in living cortical slices using confocal microscopy (20).

Gray et al (19) used a retroviral labeling technique to assess clonality and migration patterns of neuroblasts. They noted that while the majority of neuroblasts exhibit radial migration and a subset exhibits tangential migration, the position of a small subset of neuroblasts does not conform to this pattern. Walsh and Cepko (21), using similar techniques, showed a predominance of radial migration with some tangential migration and clearly confirmed the presence of neuroblasts exhibiting a non-radial, non-tangential mode of migration. They suggested a number of possible explanations including an early mixing of precursors in the ventricular zone, small separations being amplified by curved radial fibers, and the possibility of neuroblasts "jumping" from one radial fiber to another.

Terminal differentiation of a neuroblast appears to be a multistep process. Rakic (11) suggested that neurons are committed to a specific phenotype, while they still reside within the ventricular zone, around the time of the last mitotic division. This was suggested empirically by the example of the Reeler mouse in which there is a reversal of normal cortical layering. Yet the neuronal phenotype is as expected for the age of the neuroblast, suggesting that a neuroblast is committed to fulfilling a phenotype regardless of its position (22).

McConnell and Kaznowski (23) used heterochronic transplants within embryonic ferrets to study neuronal specification within the ventricular zone. They showed
that laminar determination of the neocortex is a dynamic process involving early programming of phenotypic specification and that there is a window of time in the cell cycle (late S-phase to mitosis) in which neuroblasts are open to environmental cues and can be induced to alter their programmed phenotype, laminar position, and probable subcortical connections. The role of synaptic input in shaping neuronal terminal differentiation has been emphasized by O’Leary (12), who argues that despite the effects of specification exerted in the ventricular zone, functional connections play a large role in area-specific properties of the cortex.

Cell surface properties and extracellular matrix molecules play a crucial role in influencing migration and terminal differentiation of neuroblasts. Edelman and Crossin (24) have shown that neural adhesion and migration are governed by a series of morphoregulatory molecules, cell adhesion molecules (CAMS) and substrate adhesion molecules (SAMS). CAMS are transmembrane molecules bearing extracellular domains which are homologous to domains of the immunoglobulin superfamily (24) and are engaged in homotypic binding. The binding functions interact with the cytoskeleton since they are transmembrane molecules. These molecules are involved in cell adhesion, axon binding, growth cone interactions and other migrational mechanisms. CAMS, SAMS, and extracellular matrix molecules are involved in the regulation of cell shape, motion and process extension (25). These molecules are expressed in a spatio-temporal fashion and are under the control of homeobox-containing genes that are known to govern place-dependent morphology (26, 27).

The temporal levels of expression of the molecules are characteristic of a given anatomic area but are dynamically regulated and subject to local influences. The activ-
ASSOCIATION BETWEEN PATHOLOGIC GRADE AND SEIZURE FREQUENCY

Fig. 13. Association between pathologic grade and seizure frequency. This figure demonstrates the ordinal association—the association of categorical data which are not linearly related—between pathologic grade and seizure frequency. Seizure frequency is stratified into three categories: 0–5/day, 6–20/day, and >20/day, as demonstrated on the X axis. The percentage of each pathologic grade within these categories is shown on the Y axis. (gamma = 0.381, p = 0.008.)

ities of these molecules are further modulated by neural activity itself (28). These morphoregulatory molecules can mediate neuron–neuron interactions (NCAMS), neuron–glia interactions (Ng-CAMS), or neuron–extracellular matrix interactions (17, 29, 30).

Recognizing that the distribution of CAMS in a spatiotemporal arrangement correlates with the fact that cells destined for the same cortical layer are generated at the same time, some investigators have proposed that the end of neuroblast migration may involve recognition of cell surface cues on neurons within the intended lamina, such as the homotypic binding seen with NCAMS (17, 31). Extracellular matrix molecules also appear to be important in cell motion, attraction, repulsion and growth cone migration, while the role of soluble trophic factors in this process has also been noted (32).

PCD is an essential mechanism of normal neural development. In normal development, there is a 25–50% overproduction of neuroblasts, some of which will undergo a physiological PCD (33), as will some glia. PCD appears to be under tight genetic control in the Caenorhabditis (C). elegans model (34). It is an active process which can be blocked by inhibitors of protein synthesis and RNA transcription (35). Ced3 and Ced4 are genes which appear to be implicated in activating PCD, while Ced9, whose human homolog is bcl-2, functions to suppress PCD (34). In addition to strict genetic control, subcortical connections may function in preventing this process. Failure of PCD may lead to mechanical barriers to migration, as well as abnormal numbers of neurons.

Supplemental to PCD, there is a conspicuous elimination of synapses which occurs during development and
is essential to remodelling of the cortex (36). This may be accomplished via different mechanisms; competition for trophic substances has been suggested as one. Synaptic elimination is highly intertwined with the remodelling of cortical connections and is a highly dynamic process (36), demonstrated both in vivo and in vitro (37). Interventions which block spontaneous bioelectrical activity have been shown to result in a decrease in synaptic elimination while increased spontaneous bioelectric activity augments the process (37). This implies a fine-tuning of cortical circuits during remodelling.

Other less well-understood mechanisms play a role in development. These include 1) the subplate neurons, 2) the SGL, and 3) the development of the molecular layer and the glia limitans. Subplate neurons are a subset of the earliest neurons formed, being formed prior to the generation of the neurons of cortical layer six (38, 39). These may serve as temporary targets for ingrowing axon systems and appear to be required for the growth of thalamic axons into the cortical plate (40, 41). Subplate neurons may serve as pioneering neurons of early descending axons and may play a role in enabling cortical plate neurons to find and invade their subcortical targets (40). Finally, subplate neurons participate in the axonal segregation and modelling of the cortex, as illustrated in the occipital cortex (41).

The SGL is first seen in the basal allocortex at 12–13 weeks of gestational age. It migrates from the basal ventricular zone radially to the overlying subpial area, then tangentially over the surface of the neocortex. It disappears between 17–24 weeks, although it may persist focally during the first year of postnatal life (42). The fate of these cells is still under debate. The role of this layer is unclear, although it may serve a barrier function with
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respect to migration (33). The glia limitans is formed jointly by the end-feet of the radial glia abutting the pial surface and the basal lamina of the pia. It too functions as a barrier to migration (33). Choi and Manthias (44) determined that establishment of the glia limitans and the development of the molecular layer are crucial to development of the underlying cortical plate.

Sarnat (33) has pointed out that macroscopic development of the cortex is related to the underlying microscopic processes. The development of convolutions allows for markedly increased surface area enabling the cortex to continue its laminar organization while remaining small enough to fit inside the skull. Richman et al (45) emphasized the function of physical stress generated by imbalance between the space occupied by the superficial cortical layers and the deep cortical layers in formation of the cortical convolutions.

In highlighting certain aspects of neocortical development, we have emphasized the points of susceptibility at which the derangement, or derangements, that result in CD may act. Abnormalities of proliferation, migration, terminal differentiation, PCD and cortical and synaptic remodelling can all produce cytoarchitectural and resultant electrophysiological abnormalities.

DISCUSSION

Classification of Cortical Dysplasia

Malformative lesions of the neocortex have traditionally been classified with regard to morphology or etiology. However, some have argued for a "time-based" classification system, "time" referring to the timing of a putative intrauterine insult. Rakic (46) argued that some malformations of the cortex could be classified, according to his radial unit hypothesis, as being malformations which occur during the first 6 weeks of development and thus have decreased numbers of radial units; for example, lissencephaly and pachygyria. Malformations occurring after 6 weeks could be considered as abnormalities with a normal number of radial units but with an abnormal number of neurons within the ontogenetic columns or with abnormalities of their migration. Sarnat also classifies neuronal migration disorders with respect to the timing of the injury (33). He proposes that there are early migrational defects, occurring usually within the first 8–20 weeks in utero and resulting largely from genetic abnormalities. Migrational defects occurring after 20 weeks of gestation and during postnatal life are classified as late migrational defects, which he proposes are usually acquired.

Morphology-based systems of classification of cortical malformations have, however, been dominant. Friede (47) classified the cortical malformations as: a) agria/lissencephaly/pachygyria, b) heterotopia (laminar and nodular), c) single heterotopic white matter neurons, d) agria-PMG, e) nodular CD, f) verrucous dysplasia, g) glioneu-
in any of these processes will result in abnormal cortical architecture. By itself, it is considered to be a defect occurring late in the process of development.

**Single Heterotopic White Matter Neurons:** Although many neurons still reside in the intermediate zone/white matter in the last trimester of pregnancy and even into postnatal life (13), the phenomenon is accentuated in CD (Fig. 3A). It was present in the vast majority of our patients with CD, and its prevalence has also been noted by Rakic (18) and Wolf et al (50). Meeneke (51) demonstrated, using morphometric techniques, that there is an increase in the number of neurons in the white matter of the inferior frontal gyrus in patients with epilepsy vs controls. It has been suggested that injury to the radial fibers leads to a "stranding" of the migrating neuroblasts within the white matter. Sarnat (33) suggests that if this occurs as an effect on single radial glia, it manifests as single heterotopic white matter neurons, while if a large group of radial glia are affected (as in a vascular injury), then it manifests as a neuronal heterotopia (small islands of disorganized neurons in the white matter). In a cat model using a combination of radioactive thymidine labeling with immunostaining for MAP2 and neuropeptides, Chun and Schatz (38) demonstrated that the interstitial neurons of the white matter are the oldest neurons of the cortex/white matter, and most if not all are derived from subplate neurons. They further noted that there is a decrease in the number of these neurons during development and progressive maturation, which they hypothesize occurs as a result of cell death. Thus, single heterotopic white matter neurons may represent a failure of PCD, as opposed to a failure of normal neuroblast migration.

**Neurons in the Molecular Layer:** Meeneke (52) utilized a morphometric analysis to demonstrate a statistically significant increase in the number of neurons within the molecular layer of the cortex in epileptic patients vs controls (Fig. 3B). He considered these as evidence of a slight maldevelopment of the cortex. One possible source for these neurons is a migration from the SGL, which is thought to occur between gestational weeks 17 and 24. Cajal-Retzius neurons are thought to disappear from this region during development. It is still unclear whether these persistent cells represent a failure of migration, an overshoot of migration, or a failure of PCD.

**Persistent SGL:** The persistence of the SGL has been seen in association with many cortical malformations (42). Although it may persist normally in the frontal sulci up to 2–4 months postnatally, its presence is considered a sensitive marker for cortical malformations. Since the inovation of the SGL and the migration of neurons parallels further differentiation of the cortex, it is a sensitive marker for disturbed regional maturation. It is strongly associated with marginal glioneuronal heterotopia and with a hypercellularity of the molecular layer (Fig. 4A, B).

**Marginal Glioneuronal Heterotopia:** Brun (42) defines four basic types of glioneuronal heterotopia: hypoplastic, polyplioid, free nests and brush type. (Although these could technically be considered to be ectopia rather than heterotopia, we will retain the term marginal glioneuronal heterotopia in order to remain consistent with existing nomenclature.) Only the first two have both glia and neurons, and they are the only types associated with CD. They are often found in association with persistent SGL (Fig. 4B), and they tend to occur in the same brain region as the other malformations. Choi and Matthias (44) emphasized the importance of development of the glia laminae and maturation of the molecular layer in development of the underlying cortical plate, implying that failure of this process results in marginal glioneuronal heterotopia and abnormal underlying cortical development. Sarnat (33) suggests that lesions at the pial surface result in injury to the glia laminae and lead to an overmigration of neuroblasts. Radial glia extend through defects in the glia laminae and neuroblasts migrate along these, resulting in marginal glioneuronal heterotopia.

**White Matter Neuronal Heterotopia:** These disorganized masses of neurons in the white matter usually occur in a periventricular position with a nodular morphology (Fig. 6), although rare instances of laminar subcortical bands of heterotopic gray matter have been known to produce the appearance of a "double cortex" (Fig. 1) (47, 50). It has been suggested that these are associated with injury to a group of radial glia leading to a failure of a group of neuroblasts to migrate (33). Alternatively, Rorke (1) suggests that a defect in genes controlling neuronal interactions, neuroblast proliferation and PCD may be causal (see below). Neuronal heterotopia appear to develop at 10–17 weeks of gestational life and have been observed in the children of pregnant women exposed to radiation in this time frame during the bombing of Hiroshima (46). They have also been produced experimentally in embryonic rats by exposure to ionizing radiation (48, 50).

**PMG: PMG consists of small meandering gyri often with bridging of the sulci by fusion of the molecular layers (Fig. 11). It consists of two histological types. The four-layered variant is most frequently considered to result from a destructive lesion which occurs at approximately 20–24 weeks gestation. An unlayered form is thought to result from an insult earlier in development (approximately 13–16 weeks) (48). Whether PMG represents a destructive lesion with secondary malformation or a primary malformative lesion continues to be debated. Empirically, PMG has been noted in association with destructive lesions (53–56). Dvorak et al (57, 58) created an experimental model of PMG in the developing rat with a contact freezing mechanism. They illustrated the mi-
neurosphere to form a focal zone of four-layered microgyric cortex.

Primary malformative etiologies for PMG have also been noted. Rakic (46), in reference to the protomap and radial unit hypothesis, equated the decreased thickness of the cortex with a decreased number of neurons within each ontogenetic column, despite the normal number of proliferative units. Rorke (1) suggests an association of PMG with abnormal migration, terminal differentiation and PCD. In our 77 patients, none of the 12 whose respective specimens exhibited PMG were noted to have associated destructive lesions.

Neuronal Cytomegaly and Cytoskeletal Abnormalities: Enlarged irregular neurons were described in association with seizure-producing focal cortical malformations by Taylor et al (59). These neurons bear an increased complex dendritic arborization as well as an abundance of perisomatic synapses and a paucity of axosomal synapses (4, 5, 60). Increased neuronal size has been associated with an increased DNA and RNA content, increased nuclear volume and increased nucleolar volume. Bignami et al (61) noted a 30% increased DNA content and the threefold increase in nuclear volume. Manz et al (62) noted a 16% increase in DNA, a 40% increase in RNA and a fourfold increase in nuclear volume compared to controls. Both groups concluded that these findings were consistent with neuronal heteropoidy.

Vinters et al (7) noted the presence of agruphylic neurofilbrillary-like tangles and cytoplasmic vacuoles within many of the cytomegaly neurons (Fig. 7), as well as paracrystalline intracytoplasmic structures visible on ultrastructural examination (4). Duong et al (5) demonstrated that these neurofibrillary-like tangles or cytoplasmic neurofilamentous inclusions are, like the neurofibrillary tangles seen in Alzheimer disease (AD), strongly immunoreactive with antibodies to high and medium molecular weight neurofilaments, ubiquitin, and tau. However, they differ from the neurofibrillary tangles of AD in that they are not immunopositive for paired helical filaments (PHF), nor do they show PHF by ultrastructural examination. Both phosphorylated and non-phosphorylated neurofilaments are found within the cell bodies of the cytomegaly neurons, suggesting abnormal phosphorylation of cytoskeletal proteins (5).

The biological significance of these changes remains uncertain. However, since neuronal phenotype is largely specified around the time of the final mitotic division within the ventricular or subventricular zones (23), this suggests that the derangement responsible for neuronal cytomegaly occurs during a specified time window early in the developmental process. Since cytoskeletal changes occur over time, the significance of these remains less clear. They may be a secondary response to the process of CD, an abnormal effect of neuronal growth factors or growth factor receptors (1). However, neuronal cytomegaly is present in all of the severe CD cases and appears to be the primary phenotypic derangement, while cytoskeletal abnormalities (whose temporal development is more difficult to assess) are more frequently associated in these cytomegaly neurons.

**Ballooning Neuron Change:** Ballooning neurons have nuclei and balanced opalescent eosinophilic cytoplasm (Fig. 10A). They often show binucleation or dysmorphic nuclei (Fig. 10B), are noted to cluster at the cortex/white matter junction (63) (Fig. 9A, B), and ultrastructurally are packed with filaments ranging in size from 400-600 nm with a thickness of 30 nm, interspersed with non-membrane-bound electron-dense helical structures (6). They are noted to have increased AgNORs (silver impregnated nucleolar organizer regions) (64), but not more than reactive astrocytes associated with cellular proliferative activity (65), and a virtual absence of PCNA (proliferating cell nuclear antigen) expression (64). Farrell et al (65) interpreted this finding as a suggestion of heteropority, similar to that seen in the cytomegaly neurons.

The resemblance of these ballooning neurons to cells found within the cortical tubers of TS has suggested the possibility that cases of cortical dysplasia bearing balloon cell change may represent a forme fruste of TS (4, 6, 66, 67). TS is an autosomal dominant syndrome with variable penetrance and a high incidence of sporadic mutations (68). Linkage analysis has implicated possible disease-determining loci at 9q, 11q, 12q, and 16p (69-72). Vinters et al (7) demonstrated dual staining of many cells (including some balloon cells) for both neuronal and glial markers (synaptophysin and GFAP, respectively). This suggests a failure of the cells to commit to a specific phenotype, or "de-differentiation," implying an abnormality occurring in the first trimester of intra-uterine life.

Ronnett et al (73) created a primary cell culture from brain tissue of a patient with HME. It is unclear whether this specimen contained balloon cells. (Ballooned cells were noted within 2 of 5 specimens from patients with HME in our series.) In culture they demonstrated cells with an undifferentiated morphology, but with normal karyotypes and doubling times. They were able to induce neuronal differentiation using isobutyryl-3-methyl xanthine, nerve growth factor, and dibutylcyclic adenosine 3'5'-monophosphate. These cells were thus able to be immunostained for cortical neurotransmitters somatostatin, gamma-amino-butric acid, glutamate, cholecystokinin-8, and vasoactive intestinal polypeptide, while failing to immunolabel for antibodies to adrenergic neurotransmitters. They concluded that the cultured cells represented a group of cortical cells capable of induction of differentiation along neuronal lines, but with some evidence of pluripotentiality. The intriguing possibility remains that at least some of these pluripotent cells were the balloon cells often associated with HME and CD.
Rationale for Proposed Grading System

As we have emphasized, CD represents a spectrum of pathologic change resulting from derangements of the normal developmental process that results in the formation of the cerebral neocortex. Since the time of the developmental derangement is critical in determining the clinical severity of CD, a system which can stratify these changes into early, intermediate and late lesions is desirable. Further, since each of the characteristic pathologic features represents a derangement occurring within a presumed time window, we employ these as a basis for stratification into mild, moderate and severe CD (corresponding to early, intermediate and late lesions). Cortical laminar disorganization is clearly the final step at the end process of development, so this finding in isolation suggests a late lesion. White matter neurons may continue to migrate postnatally, so that their presence also suggests a late aberration. The SGL does not generally disappear until 24 weeks in utero, implying a late time window for persistent remnants. Marginal glioneuronal heterotopia and neurons in the molecular layer require an intact gli limitans and possibly an intact SGL, and so are taken to represent late lesions.

Neuronal heterotopia presuppose an insult occurring during the period of active neuroblast migration. This, in addition to the previously mentioned empiric and experimental evidence, suggests an abnormality occurring at 10–17 weeks of gestation. It has been suggested that four-layered PMG is caused between 20–24 weeks, while unlayered PMG is caused between 13–16 weeks (48). Neuronal cytomegaly implies an abnormality of phenotypic specification, which represents an earlier lesion. Balloon cell change also suggests an early abnormality occurring within pluripotent "stem" cells. On the basis of these features, the lesions can be stratified as mild, moderate or severe, with reference to their time window of occurrence (Fig. 12). Macroscopically, the changes also suggest stratification into discrete time windows, with the gross aberrations resembling similar phases during development.

Etiologies

Some neocortical malformations appear to have a clearcut genetic basis, although there is an association of others with destructive lesions. In our series, 12 patients with CD also showed evidence of destructive lesions. Sarnat (33) asserts that early migrational defects (<20 weeks) are largely genetic while late migration defects are largely acquired destructive lesions. Jellinger (74), in an autopsy series of patients with infantile spasms, noted that 10% of the patients showed evidence of both a malformative and destructive lesion. He also emphasizes a relationship between the onset of the derangement and its effect on normal development with resultant clinical manifestations. This again highlights the primacy of the time course of the derangement relative to its etiology.

Genetic etiologies for cortical malformations have been proposed on both theoretical and empirical grounds. Rorke (1) proposes that cortical malformations result from a complex disarray of functional interactions of specific genes, trophic factors, adhesion molecules and other surface receptors. She suggests that component features of this group of malformations imply multiple genetic disturbances involving neuron production, PCD, cell specification, neuronal-glial interactions and growth factors and hormones. Much of this hypothetical framework is based upon experimental work in Drosophila (75). An experimental model of a genetic basis for cortical malformations has been demonstrated by Schmahl et al (76).

Mouse small eye syndrome involves a point mutation on chromosome 2 of the mouse resulting in loss of a functional gene product. This locus is identical to a paired box gene (Pax-6) which contains a paired homeobox-like domain. It is involved in developmental control of the CNS and is highly conserved. The gene product is normally expressed in the eyes, forebrain, neural tube, and olfactory epithelium during development. Homozygous mutants show an accumulation of neuroblasts in the intermediate zone, nodular heterotopia, marginal glioneuronal heterotopia and cortical laminar disarray, as well as anophthalmia and absence of nasal cavities. This pattern of malformation corresponds to the developmental and topographic expression of the gene product (76).

In addition to experimental animal models, many empiric observations suggest genetic etiologies for cortical malformations. Patients with Miller-Dieker syndrome, associated with type I lissencephaly, exhibit microdeletions of the 17p13.3 region of chromosome 17 in 90% of cases (77). The deduced amino acid sequence of this region shows significant homology to the beta subunits of heterotrimeric G proteins involved in signal transduction (a mechanism crucial in cerebral development). The association of lissencephaly type II with Walker-Warburg syndrome (48) and with Fukuyama muscular dystrophy with CD (78) is of particular interest since both show an autosomal recessive pattern of inheritance. CD is also associated with neurocutaneous syndromes including TS and Sturge-Weber. As indicated above, the neuropathologic change in severe CD with balloon cells is identical to that seen in cortical tubers of TS. Other descriptions of familial examples of CD have been reported (79).

The finding of destructive lesions in association with CD suggests the possibility of a causal relationship. The association between PMG and destructive lesions of the cortical mantle has been discussed above. Other features of CD can also be produced in animal models by environmental insults. Radiation and toxins have both been associated with the production of white matter heterotopia and marginal glioneuronal heterotopia (48). Of great-
er importance than the nature of the injury is the time in development at which it occurs (80).

Conclusion

CD represents a spectrum of neuropathologic change reflecting a derangement of the normal process of neocortical development. It can be characterized by nine microscopic features: cortical laminar disorganization, single heterotopic white matter neurons, neurons in the molecular layer, persistent SGL, marginal glioneuronal heterotopia, neuronal heterotopia in the white matter, PMG, neuronal cytomegaly with cytakoskeletal abnormalities and balloon cell change. These features reflect events occurring within discrete time windows in development and can therefore be utilized to propose a stratification of the lesions into those occurring with early, intermediate and late gestational events, which in turn correlate with the clinical severity of the epilepsy syndrome. We present 77 patients who underwent cortical resections for intractable seizures and demonstrate how this grading system can be used to characterize the lesions as mild, moderate and severe CD. Further, we show a statistically significant positive correlation between CD grade and seizure frequency (p = 0.008) and a statistically significant negative correlation between grade and age at surgical presentation (p = 0.003).

In a review of the processes of neocortical development, the points of susceptibility involved in the genesis of CD are outlined. Theoretical, empirical and experimental evidence pertinent to each of the pathologic features is reviewed, providing a rationale for this grading system. In summary, all of the malformations of the neocortex show a consistent spectrum of microscopic features representing abnormalities in development. We therefore consider them to represent the response of a developing nervous system with a limited repertoire of responses to alterations which may result from a number of causes.

ACKNOWLEDGMENTS

We thank Alex Brooks, Yan Cheng, and Diana Lenard Secor for technical support, and Carol Appleton for photographic assistance. We also thank Drs. Michael DeRosa, Michael Farrell and Harvey Samat for helpful discussions. We also wish to acknowledge Drs. Donald Guthrie, Gwen Gordon and Fan Zhang for their assistance with statistical analysis.

REFERENCES

27. Jones FS, Prediger EA, Bittner DA, DeRobertis EM, Edelman GM. Cell adhesion molecules as targets for box genes: N-CAM promoter activity is modulated by co-transfection with Hox 2.5 and 2.4. Proc Natl Acad Sci USA 1992;89:2089–90

75. Jan YN, Jan LY. Genes required for specifying cell fates in Dro...
sophila embryonic sensory nervous system. Trends Neurosci 1990; 13:403-8
76. Schnall W, Knoedler S, Faivre J, Davidson D. Defects of neuronal migration and the pathogenesis of cortical malformations are associated with small eye (Sey) in the mouse, a point mutation at the Pax-6 locus. Acta Neuropathol 1993; 86:126-35