

Developmental Neuropathology and Impact of Perinatal Brain Damage. III: Gray Matter Lesions of the Neocortex

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Abstract. The evolving neuropathology of primarily undamaged cortical regions adjacent to the injured site has been studied in 36 infants who survived a variety of perinatally acquired encephalopathies (microgyrias, ulegyrias, multicystic encephalopathies, porencephalies, and hydranencephalies) and later died of unrelated causes. Their survival times range from hours, days, weeks, or months, to several years. Ten of these children developed epilepsy, 2 developed cerebral palsy, and several were neurologically and mentally impaired. In all cases studied, the undamaged cortex adjacent to the injured site survives, retains its intrinsic vasculature, and is capable of continuing differentiation. However, its postinjury development is characterized by progressive alterations compatible with acquired cortical dysplasia that affects the structural and functional differentiation of its neurons, synaptic profiles, fiber distribution, glial elements, and vasculature. The synaptic profiles of many neurons are transformed by an increased number of intrinsic loci that replace extrinsic ones vacated by the destruction of afferent fibers. The intrinsic fibers of layer I and some Cajal-Retzius cells survive even in severe lesions and may be capable of interconnecting cortical regions that have lost other type of connections. Some intrinsic neurons undergo postinjury structural and functional hypertrophy, acquire new morphologic and functional features, and achieve a large size (meganeurons). Probably, these meganeurons acquire their structural and functional hypertrophy by partial endomitotic DNA and/or RNA reduplication (polyploidy). These postinjury alterations are not static but ongoing processes that continue to affect the structural and functional differentiation of the still developing cortex and may eventually influence the neurologic and cognitive maturation of affected children. This study proposes that, in acquired encephalopathies, the progressive postinjury reorganization of the undamaged cortex and its consequences (acquired cortical dysplasia), rather than the original lesion, represent the main underlying mechanism in the pathogenesis of ensuing neurological sequelae, such as, epilepsy, cerebral palsy, dyslexia, cognitive impairment, and/or poor school performance.

Key Words: Acquired; Cortical; Dysplasia; Reorganization; Encephalopathies; Epilepsy.

INTRODUCTION

Neonatal encephalopathies constitute a heterogeneous group of genetic and/or acquired brain disorders and are characterized by variable severity and distribution, multifactorial etiology, unsolved pathogenesis and, often, poor clinical outcome (1-13). The present study explores the evolving neuropathology of selected perinatally acquired lesions affecting (directly or indirectly) the cortical gray matter (GM) and the subsequent (postinjury) impact on the cortical maturation of affected infants. Perinatally-acquired GM lesions are associated with premature birth, respiratory difficulties, circulatory disturbances, neonatal asphyxia, fetal transfusion syndrome of monozygous twin, infections, trauma, and/or labor complications (2-6, 13). They are often caused by hemorrhagic and/or hypoxic-ischemic injuries, or by a combination of both. They can occur prenatally, during birth, and/or postnatally. Infants who survive perinatally acquired GM lesions often develop devastating and costly neurological sequelae, including epilepsy, cerebral palsy, dyslexia, cognitive impairment, poor school performance, and/or minimal brain damage (13-18).

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This work has been supported by a Jacob Javits Neuroscientist Investigator Award, the NINCDS, Grant NS-22897, and the NIH.

While the available information on the clinical, radiological (imaging), and pathological features of acquired GM lesions is adequate, their impact on the still developing infant neocortex and/or their possible role in the pathogenesis of ensuing neurological sequelae have not been adequately investigated. To understand the outcome of any type of neonatal encephalopathy, it is necessary to establish the direct damage on the still developing neocortex, as well as the indirect or postinjury impact on its subsequent structural and functional differentiation. Four working hypotheses will be tested in this study: 1) that any perinatally-acquired GM lesion affect the subsequent differentiation of primarily undamaged cortical regions adjacent to the injured site; 2) that the postinjury differentiation of these regions will be altered; 3) that these local postinjury alterations will have repercussions throughout the developing neocortex; and, 4) that the postinjury altered reorganization of primarily undamaged GM regions, rather than the original lesion itself, play a crucial role in the pathogenesis of ensuing neurological sequelae.

This study explores the evolving neuropathology of selected GM lesions and their impact on the subsequent differentiation (maturation) of cortical regions adjacent to the injured site in surviving infants. This study evaluates some of the repair mechanisms of an injured developing neocortex, which are poorly understood. This study is the third of a 3-part investigation that has explored the neuropathology, impact, and clinical outcome of perinatally

acquired encephalopathies in surviving infants. The neuropathology and impact of hemorrhagic (Part I) and of white matter (Part II) lesions have been reported previously (17-18).

MATERIAL AND METHODS

Thirty-six cases of infants who survived documented perinatally acquired brain lesions and who later died for a variety of unrelated reasons have been selected from the pediatric autopsy records of the Dartmouth-Hitchcock Medical Center. Pertinent clinical and pathological data of these cases are summarized in the Table. The survival time of these infants ranges from hours, days, weeks, or months, to several years. Of the selected cases, 10 infants developed epilepsy, 2 developed cerebral palsy, and several were neurologically and mentally impaired. The interval between cortical damage and the clinical manifestation of a neurological disorder is usually long, ranging from 2 to 3 yr. The study of this material has permitted us to evaluate the direct effect of selected lesions upon the developing neocortex; to evaluate the evolving neuropathologic of each lesion through its acute, subacute (healing), and chronic (repaired) stages; and, to evaluate the repair mechanisms of a still developing neocortex.

The following neurohistologic procedures have been used: 1) routine stains (hematoxylin & eosin [H&E], Nissl, luxol-fast-blue, and Bodian), 2) some immunohistochemical stains, including glial fibrillary acidic protein (GFAP), SMA-32 non-phosphorylated neurofilament protein (NF), and synaptophysin (SY), and, 3) the rapid Golgi (RG) method. The combined use of these special procedures is required to identify some aspects of the postinjury reorganization of primarily undamaged GM regions adjacent to a lesion. Some postinjury alterations are unrecognizable with routine procedures. NF and RG stains have been essential in demonstrating the survival of the intrinsic fibers of layer I, of Cajal-Retzius (C-R) cells, and of some residual white matter (WM) fibers in severe cortical damage in acquired ulegyria, microgyria, multicystic encephalopathy, porencephaly, and hydranencephaly. The RG method has been essential in demonstrating the abnormal morphology of some postinjury-transformed neurons. The various procedures used are described elsewhere (19-22).

RESULTS

Perinatally acquired cortical lesions involving the GM are quite variable in location, severity, and distribution. They may be focal, segmental, diffused, and/or rather extensive. In most cases both the GM and underlying WM are involved (microgyrias, multicystic encephalopathies, ulegyrias, porencephalies, and hydranencephalies); in some cases, layer I and the upper cortical layers are involved (leptomeningeal heterotopias); and, in a few cases, only the GM seems to be primarily damaged (layer III segmental necrosis).

All cases studied are characterized by the survival of primarily undamaged cortical regions adjacent to the original injured site. These regions retain their intrinsic vascularization and remain capable of continuing their

postinjury differentiation. The postinjury differentiation of these regions is invariably altered and their overall structural and functional organization is modified. The postinjury differentiation of the cortical regions further away from the damaged site are less affected than proximal ones. The overall cytoarchitecture, laminations, and the placement and distribution of neurons, fibers, glia cells, and blood vessels appear to be unaffected and normal gyral patterns are often preserved. Relatively normal gyral patterns may even be preserved overlaying extensive cortical lesions (18). The preservation of gyral patterns implies the late (perinatal) occurrence of some encephalopathies. On the other hand, the cytoarchitecture of the undamaged GM contiguous to the injured site is invariably and severely altered. These alterations are the result of postinjury transformations of still developing neurons, fiber systems, synaptic profiles, glia elements, and blood vessels. These alterations are compatible with acquired cortical dysplasia. The elucidation of these alterations and the evaluation of their impact on the subsequent maturation of the still developing neocortex are the main objectives of this study.

Pathologic features that are common to most types of acquired cortical dysplasias, such as reactive gliosis, are described first. Pathologic features typical of some neonatal encephalopathies are described separately, including acquired microgyrias (cases 22, 23, 24, 25, 26, 28, 29), ulegyrias (cases 26, 29, 31, 32, 36), leptomeningeal heterotopias (cases 15, 19, 24, 25, 28, 31, 34), multicystic encephalopathies (cases 22, 23, 26, 27, 28), porencephalies (cases 33, 34, 35, 36), and hydranencephalies (cases 29, 31). Some of the cases studied show more than 1 type of cortical lesion (Table).

Glial Reparation (Gliosis) in GM Lesions

The repair of WM lesions is characterized by the removal of the damaged tissue by macrophages followed by cystic and/or trabeculated cavitation of the injured site with minimal reactive gliosis (18). In contrast, the repair of GM lesions is characterized by the replacement of the damaged tissue by reactive gliosis and the absence of cavitation (Fig. 1A-E). In severe cortical lesions involving the WM and GM, the residual cavitation may extend up to the level of layer I.

In general, the presence of cortical gliosis (glial scarring) implies a previous GM lesion and reflects its location and extent. The frequently used term "nonspecific gliosis" should be abandoned because any reactive gliosis is an indication of a previous GM injury, including minimal damage. GM gliosis (glial scarring) could be minimal, focal, segmental (Fig. 1B), and/or diffused (Fig. 1C). To determine the presence, location, and extent of GM gliosis, the use of GFAP preparations is indispensable. These preparations are needed to establish the type of astrocytes participating in the reparative gliosis as well

as the age of the scar. Minimal reactive gliosis may not be recognized with routine methods.

The reparative gliosis (glial scarring) of acquired GM lesions is essentially composed of reactive fibrous astrocytes, which differentiate locally in response to tissue damage. These reactive astrocytes are characterized by numerous long, unbranched, and smooth radiating filaments (Fig. 1F). These glial filaments are often oriented perpendicular to the pial surface, and some may reach it (Fig. 1F). The glial filaments of these reactive astrocytes lack terminal vascular endfeet. The morphologic features of these reactive fibrous astrocytes are clearly demonstrated in GFAP and RG preparations (Fig. 1D-F). As the glial scar ages, its reactive astrocytes become smaller and less active, their filaments become more tightly packed, and the GFAP stain becomes less intense (Fig. 2C). Old glial scars may also have scattered hypertrophic astrocytes. Throughout the glial scarring, the anastomotic capillary plexus of the GM may be locally altered. The specific protoplasmic astrocytes of the GM do not appear to participate in reparative gliosis. However, old GM lesions may have large protoplasmic astrocytes with numerous vascular endfeet and morphologic features that resemble those of developing embryonic astrocytes (23).

Two special types of reactive gliosis observed in some acquired GM lesions need special mention, namely, layer I (subpial) gliosis and the GM/WM border gliosis. Some GM lesions are characterized by subpial gliosis involving layer I, which is essentially composed of small reactive astrocytes probably derived from the specific astrocytes of layer I and a few scattered fibrous astrocytes. In layer I gliosis, the reactive astrocytes are small with tightly packed glial filaments that often run parallel to the pial surface (Figs. 1D, 2C, D). Occasionally, these reactive astrocytes invade and obliterate the perivascular compartment of local Virchow-Robin spaces and may accompany the perforating vessels into the cortex for a considerable distance. Some of the reactive astrocytes may even reach the vessels of the underlying WM. The presence of astrocytes within the Virchow-Robin space implies local damage and disruption of their glial wall allowing their penetration into the perivascular space. The reactive fibrous astrocytes of layer I gliosis are characterized by long descending glial filaments that invade the upper layers of the cortex (Fig. 1D). Often, these fibrous astrocytes are quite numerous and form a prominent subpial glial wall with long descending filaments, which may reach lengths of 300 to 500 μm (Fig. 1D). In layer I gliosis, the intensity of the GFAP stain also decreases with increasing age of the scar (Fig. 2C).

Reactive gliosis at the GM/WM border is prominent in some GM lesions (Fig. 1E). It is composed of reactive fibrous astrocytes with long (300 to 500 μm) ascending filaments that invade the lower cortical laminations. This

border gliosis is present in cases without obvious underlying GM lesions (24) and also in cases with subcortical WM damage. When both layer I and GM/WM border gliosis coincide in the same region they appear as a mirror images of each other (Fig. 1D, E). The pathogenesis of layer I and/or GM/WM border gliosis is not clear. Both types, especially layer I gliosis, have been described in epilepsy (5, 7, 12). These 2 types of gliosis could represent examples of persistent reparative gliosis that continue to occur in old GM lesions and/or in some minimal GM lesions.

Postinjury Cortical Reorganization in GM Lesions

The most important aspect of perinatally acquired encephalopathies is the survival of primarily undamaged GM regions in and around the original injured site that are capable of continuing their postinjury differentiation. If the infant survives perinatal brain damage, the postinjury structural and functional differentiation of these primarily undamaged GM regions will invariably be altered, resulting in acquired cortical dysplasia. The postinjury alterations of cortical regions closer to the damaged site are more prominent than distant ones, those of smaller areas are more severe than larger ones, and those at border areas between damaged and undamaged regions are always severe. These postinjury alterations are not static, but ongoing processes that will continue to affect the structural and functional maturation of the developing neocortex, possibly for as long as the child is alive. Eventually, these alterations could influence the child's neurological and cognitive development.

The postinjury alterations of the surviving GM have been investigated in 4 types of perinatally acquired encephalopathies: 1) microgyrias; 2) ulegyras; 3) extensive GM damage associated with severe WM lesions; and 4) minimal GM damage of regions faraway from the original lesion. Each case studied represents a stage of the ongoing postinjury reorganization of the surviving GM adjacent to a particular cortical lesion, which has occurred days, weeks, months, and/or years earlier (Table). Although pathologically these neonatal encephalopathies differ from each other, the postinjury reorganization of the surviving GM, clinical outcome, and ensuing neurological sequelae are essentially similar in all of them, due, in part, to the utilization of common repair mechanisms and developmental pathways.

Acquired Microgyrias

Acquired microgyrias are often observed in the cortex overlying some porencephalies, multicystic encephalopathies, and hydranencephalies (4, 7, 8, 12-14, 18). The retention of already formed gyral patterns implies a late occurrence for these disorders. Acquired microgyrias imply direct damage to the developing GM above the WM lesion. These acquired cortical lesions are characterized

TABLE
Perinatal Neocortical Damage: Clinical and Pathological Data. (Revised from Table 1 in references 17 and 18)

| Case | Born | Lived | Clinical findings | Stains | Autopsy findings (neocortex) |
|--------------------------------------|-------|---------|--|----------------------------|---|
| I. Acute Stages | | | | | |
| 1 | 21 wg | 1 h | Cardiac Ebstein HE malformation | PVH, PH | Focal WMH & GMH |
| 9368 | P | | | | |
| 2 | 26 wg | 2 h | RDS | HE & Golgi, Bodian | PVH, Focal WMH |
| 8941 | P | | | | |
| 3 | 22 wg | 2 h | Aspiration meconium | HE & Golgi | Early multifocal PVH, PH |
| 8442 | P | | | | |
| 4 | 24 wg | 3 h | RDS | HE, Bodian | Early multifocal PVH, Focal WMH, PH |
| 9379 | P | | | | |
| 5 | 27 wg | 4 h | RDS | HE & Golgi | PVH, IVH |
| 8460 | P | | | | |
| 6 | 24 wg | 17 h | Fetal transfusion syndrome | HE | Anemic twin, Early WMD, Plethoric twin, Severe WMD, PVH & IVH |
| 9548 | P | | | | |
| 7 | 30 wg | 1 day | RDS | HE & Golgi, Bodian | Early PVH, IVH, PH, Focal WMD & GMD |
| 8937 | P | | | | |
| 8 | 26 wg | 1 day | RDS | HE & Golgi | PVH, Focal PVL, GMD |
| 804493 | P | | | | |
| 9 | 25 wg | 3 days | RDS, Early BPD | HE, Bodian | Early PVH, PH, IVH, Focal WMH & GMH |
| 9353 | P | | | | |
| II. Subacute (healing) Stages | | | | | |
| 10 | 32 wg | 4 days | Plethoric twin, Fetal transfusion, Prenatal WM damage | HE, GFAP, Neurofilament | Multicystic encephalopathy, Healed WMD, Surviving GM, Neocortical dysplasia |
| 9478 | P | | | | |
| 11 | 33 wg | 5 days | RDS, Aspiration meconium | HE | Multicystic encephalopathy, PVL, WMD, Mild hydrocephalus |
| 91135 | P | | | | |
| 12 | 28 wg | 5 days | RDS, Prenatal WM damage | HE & Golgi, Myelin, Bodian | LMH, Focal PH, Necrosis WM, Multicystic encephalopathy, Acute hydrocephalus |
| 8845 | P | | | | |
| 13 | 26 wg | 9 days | RDS, BPD | HE, Bodian | PVH, Cerebral edema, WMD, Necrosis WM axons |
| 89129 | P | | | | |
| 14 | 28 wg | 10 days | Enterocolitis, Peritonitis | HE & Golgi | PVH, Early PVL |
| 804720 | P | | | | |
| 15 | 38 wg | 12 days | RDS, BPD, Prenatal WM damage | HE & Golgi | Multicystic encephalopathy, WMD, Early PVL, Focal LMH, Surviving GM. |
| 9054 | P | | | | |
| 16 | 28 wg | 15 days | BPD | HE & Golgi | WMD, Cerebral edema, PH, GMH, IVH |
| 90108 | P | | | | |
| 17 | 26 wg | 16 days | RDS, Pneumonia, Hypothermia | HE & Golgi | WMD, Subpial edema, PH, Early PVL, Hydrocephalus |
| 9045 | P | | | | |
| 18 | 29 wg | 17 days | RDS, Early BPD | HE & Golgi | Recent MH, Early PVL |
| 8971 | P | | | | |
| 19 | 34 wg | 18 days | RDS, Microcephaly, Retarded growth, Prenatal WM damage | HE & Golgi | Multicystic encephalopathy, Extensive LMH, Microgyria |
| 794170 | P | | | | |
| 20 | 35 wg | 26 days | GI atresia surgically treated, BPD | HE & Golgi | Focal WMD & GMD |
| 722143 | P | | | | |
| 21 | 31 wg | 26 days | RDS, BPD | HE, Myelin | Focal WMD and edema, GMD |
| 83273 | P | | | | |

TABLE (Continued)

| Case | Born | Lived | Clinical findings | Stains | Autopsy findings (neocortex) |
|--------------------------------|-------------------------------|-----------------|---|--|---|
| III. Chronic (repaired) Stages | | | | | |
| 22 9449 | 30 wg PCD | 32 days | Prenatal WM damage | HE & Golgi, Neurofilament | Multicystic encephalopathy, Healed WMD, Surviving GM |
| 23 9210 | 39 wg PCD | 5 weeks | Prenatal WM damage, Pneumonia, BPD | HE, GFAP | Focal microgyria, PVL, WMD, Multicystic encephalopathy, WHD, Hydrocephalus ex-vacuo |
| 24 907 | 28 wg PCD | 3 m | Perinatal brain damage, Developmental delay, BPD | HE & Golgi, Myelin, GFAP, Neurofilament | WMD, PVL, Surviving GM, Hydrocephalus ex-vacuo, LMH, Neocortical dysplasia |
| 25 9832 | 27 wg PCD | 4 m | Perinatal brain damage, Severe BPD | HE & Golgi, Neurofilament, GFAP | Multicystic encephalopathy, Extensive WMD, LMH, Focal neocortical dysplasia |
| 26 96137 | 26 wg PCD | 7 m | Perinatal brain damage, Developmental delay, BPD, Seizures | HE & GFAP, Neurofilament | Multicystic encephalopathy, Ulegyria, WMD, GMD, Neocortical dysplasia |
| 27 8615 | 26 wg PCD | 8 m | Perinatal brain damage, Developmental delay, BPD | HE & Golgi, Bodian | Multicystic encephalopathy, Focal microgyria, PVL, Hydrocephalus ex-vacuo, Neocortical dysplasia, HN |
| 28 88272 | 24 wg PCD | 8 m | Perinatal brain damage, Developmental delay, BPD, Seizures | HE & Golgi, GFAP, Myelin, Neurofilament | GMD & WMD, PVL, LMH, Multicystic encephalopathy, Microgyria, Hydrocephalus, Neocortical dysplasia, HN |
| 29 93209 | 28 wg PCD | 2.5 years | Developmental delay, Status epilepticus | HE & Golgi, GFAP, Neurofilament | WMD, Focal ulegyria, Ex-vacuo hydrocephaly, Neocortical dysplasia, HN |
| 30 8511067 | 32 wg PCD | 5 years, Alive | BPD, Cerebral palsy, Epilepsy, Mental retardation | HE & Golgi, Myelin, GFAP | Frontal lobe Bx. (2.5 years), GMD, LMH, Neocortical dysplasia, HN |
| 31 98147 | Term Shaken 11 days PCD | 7 years | Postnatal brain damage, Cerebral palsy, Blindness, Epilepsy, Mental retardation | HE, Myelin, GFAP, Bodian, Synaptophysin, Neurofilament | Fronto-temporal porencephaly, Hydrocephalus ex-vacuo, Ulegyria, PVL, Focal LMH, Neocortical dysplasia, HN |
| 32 88175 | 35 wg PCD | 8 years | Drowning, Prenatal WM damage, Drowning, Mental retardation, Epilepsy | Myelin, HE, Neurofilament, Bodian, HE, GFAP | Parieto-occipital porencephaly, Surviving GM, PVL, Extensive LMH, Focal ulegyria, Neocortical dysplasia, HN |
| 33 9517048 | Term PCD | 10 years, Alive | Postnatal brain damage, Left frontal trauma, Lobectomy, Epilepsy | HE, Golgi, Neurofilament | Left frontal GMD and WMD, Surviving GM, Multicystic encephalopathy, Porencephaly, Neocortical dysplasia, HN |
| 34 9381 | 37 wg | 11 years | Postnatal brain damage, Cerebral palsy, Epilepsy, Mental retardation | HE, GFAP, Myelin, Neurofilament | Parieto-occipital porencephaly, PVL, LMH, Surviving GM, Neocortical dysplasia, HN |
| 35 97173 | Term PCD | 11 years | Peritonitis, Cerebral Palsy, Mental retardation, Epilepsy | HE, GFAP, Neurofilament | Left parieto-temporo-occipital porencephaly, Surviving GM, Atrophy corpus callosum |
| 36 93343 | Term | 16 years, Alive | Postnatal brain damage, Epilepsy, Lobectomy | HE, Golgi, GFAP, Neurofilament | Right occipital porencephaly, Neocortical dysplasia, HN, Focal ulegyria |

Key: PCD = perinatal cortical damage; RDS = respiratory distress syndrome; BPD = broncho-pulmonary dysplasia; HE = hematoxylin & eosin; PVL = periventricular leukomalacia; PVH = periventricular hemorrhage; IVH = intraventricular hemorrhage; PH = pial hemorrhages; WM = white matter; GM = gray matter; WMH = white matter hemorrhage; GMH = gray matter hemorrhage; WMD = white matter damage; GMD = gray matter damage; HN = hypertrophic neurons; LMH = leptomeningeal heterotopia; GFAP = glial fibrillary acidic proteins stain.

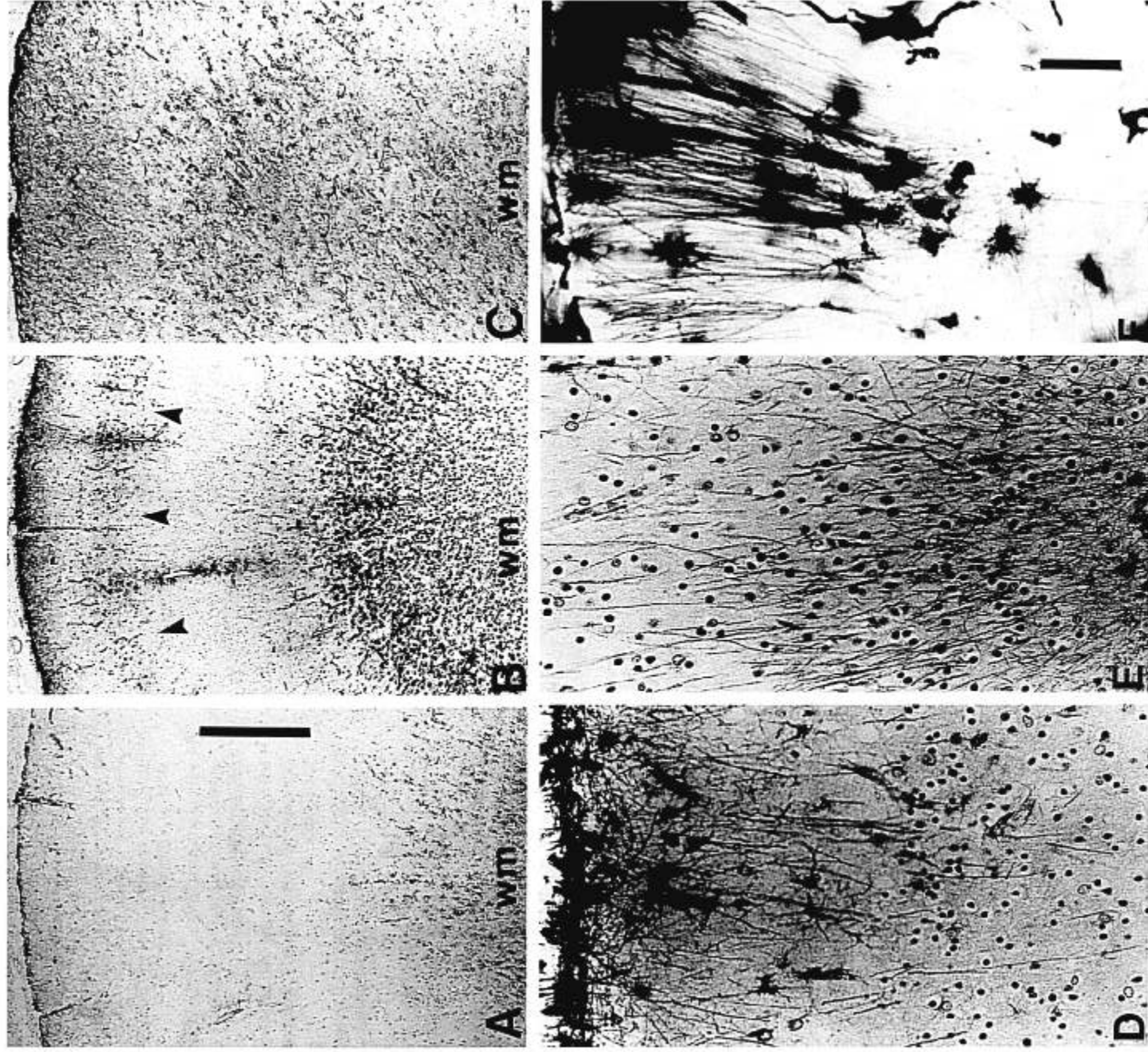


Fig. 1. Composite figure of GFAP and RG preparations illustrating various aspects of the reactive gliosis observed in perinatally acquired cortical lesions involving the gray matter. (A) Degree of glial staining in a normal undamaged cortical region. (B) Degree of reactive gliosis of a partially damaged cortical region showing layer I, GM/WM border, and segmental layer II-III (arrowheads) gliosis. (C) Degree of diffuse gliosis of a severely damaged cortical region. (D) View of layer I gliosis with the long descending glial filaments of reactive fibrous astrocytes. (E) Detail of the long ascending glial filaments of GM/WM border gliosis. (F) Detail of the morphologic features of reactive fibrous astrocytes from RG preparations of cortical gliosis. Figures A-C are from case 24 and D, E from case 28. The pial surface is at the top of all figures and the upper edge of the white matter (WM) is visible in some of them. Bars: A, B, C = 250 μm; D, E, F = 100 μm.

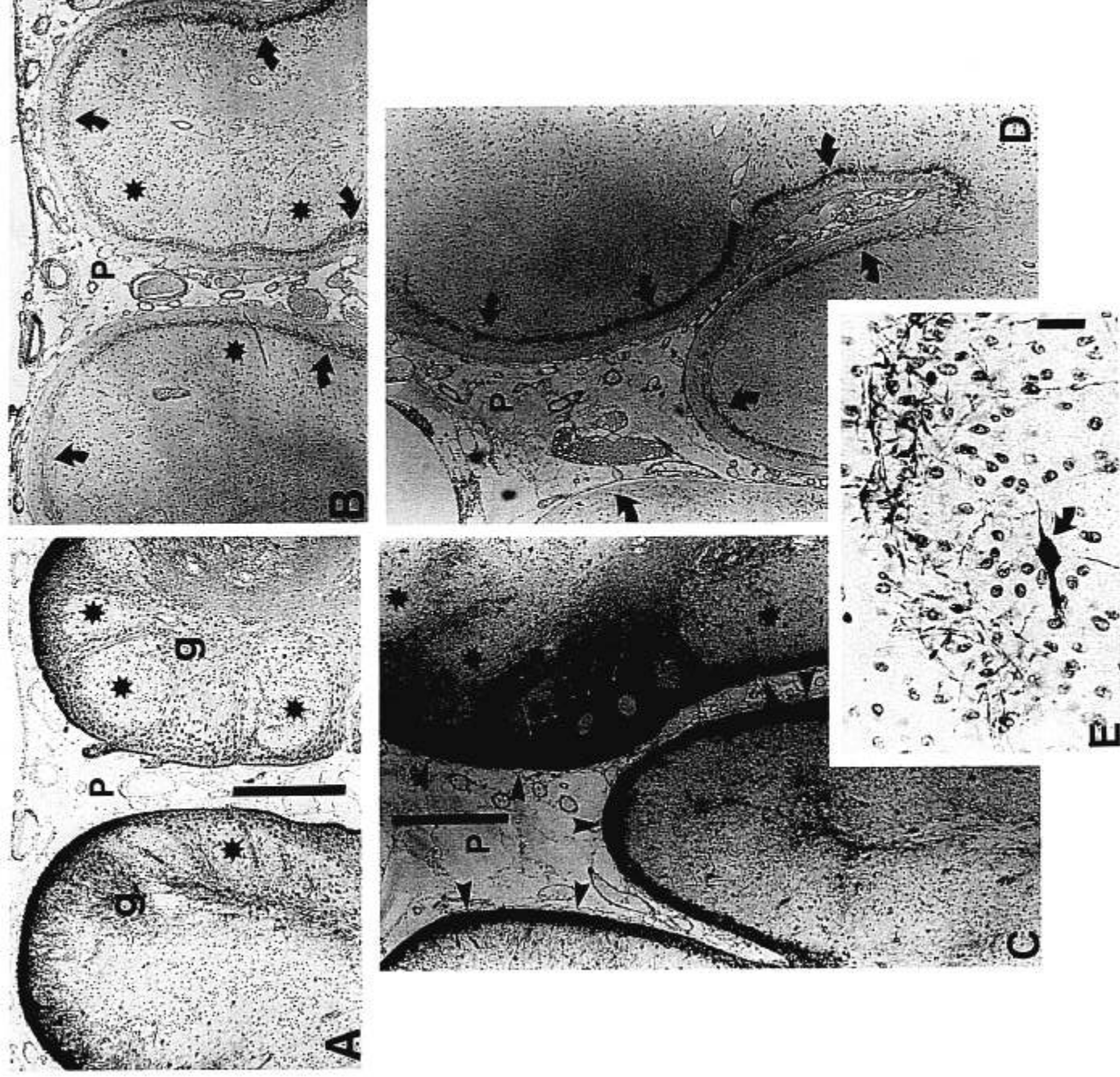


Fig. 2. Views of contiguous affected microgyri stained with GFAP (A, C) and NF (B, D) procedures illustrating simultaneously various pathologic features of perinatally acquired microgyria (case 28). (A, C) Illustrate the survival of islands of gray matter tissue (asterisks) surrounded by reactive gliosis (g) and extensive layer I gliosis (arrowheads) of affected microgyri. (B, D) Illustrate the survival of the intrinsic fibers of layer I interconnecting contiguous affected microgyri (arrows), of a few Cajal-Retzius cells, and of a few islands of gray matter (asterisks) tissue. (E) Detail of a surviving Cajal-Retzius cell (arrow) and of some intrinsic fibers of layer I (NF preparations). Despite the extensive subpial gliosis (C, arrowheads), the C-R cells and layer I (B, D, arrows) are capable of interconnecting contiguous microgyri. The intrinsic fibers of layer I (B, D), the C-R cells (E), and the intrinsic fibers of the surviving gray matter are strongly NF⁺ suggesting an active functional activity. The compactness, small size of the reactive astrocytes, and the lesser intensity of GFAP stain characteristic of old glial scarring (g) can be appreciated in A and C. The meninges and pial surface (P) are indicated. (see also Fig. 4). Bars: A-D = 500 μ m; E = 100 μ m.

by size reduction of the affected gyri, sulci expansion, cytoarchitectural disorganization, reactive gliosis, often layer I gliosis, focal neuronal, fibrillar and microvascular alterations, partial obliteration of layer I (marginal heterotopia), and severe attenuation of the underlying WM (Figs. 2A–D, 3A).

In the microgyric cortex, the number of entering and exiting fibers is significantly reduced due to anterograde and retrograde degeneration of corticopetal and corticofugal fibers destroyed by the underlying WM lesion. The destruction of afferent and efferent fibers leaves the microgyric cortex partially deprived of sensory inputs and unable to reach distant functional targets. Moreover, the degeneration of corticopetal fibers results in the vacancy of many extrinsic synaptic loci, which may be replaced by a compensatory increase in the number of intrinsic ones. This postinjury synaptic reorganization affects the structural and functional differentiation of many neurons within the microgyric cortex and increases its intrinsic neuropil. The destruction of corticofugal fibers transforms projective pyramidal neurons of the microgyric into local-circuit interneurons with an intracortical distribution of their axonic collaterals (18).

Throughout the microgyric cortex, areas of GM of variable size survive practically embedded within the reactive gliosis (Figs. 2A–D, 3A). Small islands of GM retain a few intrinsic neurons and a rich intrinsic neuropil. Despite the extensive GM damage and the marked reactive gliosis, the intrinsic fibers of layer I survive interconnecting contiguous affected microgyri (Fig. 2B, D). Moreover, the presence of surviving C-R cells has been demonstrated with both NF (Fig. 2E) and RG preparations (Fig. 4A). Areas of GM remain functionally interconnected by the intrinsic fibers of layer I and residual WM fibers throughout the microgyric cortex (Fig. 2B, D). Similarly, the apical dendrites of surviving pyramidal cells within the microgyric GM remain functionally interconnected by the long axons of C-R cells (25–27). These functional interconnections contribute to both the survival and the postinjury reorganization of GM throughout the microgyric cortex. The survival of the intrinsic elements of layer I through contiguous affected microgyri is an important observation not previously recognized with significant clinical implications.

NF and RG preparations have confirmed the altered cytoarchitecture of the microgyric cortex. This attenuated cortex is characterized by cellular disorganization (Fig. 3A), complex intrinsic neuropil, and, more importantly, the presence of both atrophic (Figs. 3B, C, 4A), as well as large hypertrophic and strongly NF⁺ neurons (Figs. 3A, D, E, 4A). While atrophic neurons are found at all levels of the microgyric cortex (Fig. 4A), large hypertrophic neurons are only found occasionally (Fig. 3D, E). Atrophic neurons are characterized by their small size, short dendrites with a few spines, and by an axon which

often has ascending collaterals (Figs. 3B, C, 4A). Some atrophic neurons show terminal dendritic changes suggesting progressive centripetal degeneration (Fig. 4A). Other atrophic neurons resemble pyramidal cells with a retracting apical dendrite that seems to have lost contacts with layer I (Fig. 4A). In contrast, hypertrophic neurons are characterized by their large size, long and irregular dendrites covered with spines, and by an axon that branches intracortically (Fig. 3D, E). Hypertrophic neurons may be found at all cortical levels but they are more prominent in layers II and III (Fig. 3A). Both atrophic and hypertrophic neurons are considered to be postinjury transformed cells that have responded to alteration of the intrinsic circuitry of affected microgyri. Through the establishment of reciprocal interconnections, the functional activity of these postinjury transformed neurons could influence the differentiation of proximal, as well as of distant regions of the developing neocortex.

The WM underlying the microgyric cortex is markedly reduced and may be cystic (Fig. 3A). However, in some areas bundles of myelinated and strongly NF⁺ fibers have survived (Fig. 3A). These myelinated fibers, probably composed of afferent and efferent fibers, run within antero-posterior fascicles. These residual WM fibers may be capable of functionally interconnecting the microgyric cortex with other cortical and/or subcortical regions. The survival of these WM fibers could also play a role in the discharge of both normal as well as abnormal (epileptic) impulses from the postinjury transformed microgyric cortex.

Despite partial sensory deprivation and inability to reach some functional targets, the surviving GM within the microgyric cortex undergoes progressive cytoarchitectural reorganization compatible with an acquired cortical dysplasia secondary to perinatal brain damage. This type of acquired cortical dysplasia could cause cortical dysfunction, influence the neurological and cognitive development of affected children, and, eventually, play a role in the pathogenesis of ensuing neurological sequelae.

Acquired Ulegyrias

This unique type of neonatal encephalopathy occurs when the GM through the depth of a sulcus is severely damaged while that of its corresponding gyral crowns is spared (Fig. 5A). Ulegyrias, often observed in multicystic encephalopathies, are invariably associated with the destruction of the underlying WM around the sulcus (Fig. 5A). Probably, the vascular compromise of the sulcus embedded within the WM lesion and the vascular sparing of the gyral crowns supplied directly by leptomeningeal perforating vessels participate in the pathogenesis of this disorder. In the event of a vascular compromise (sulcal edema) caused by the WM injury, the neocortex unique microvascular organization will better protect the GM of

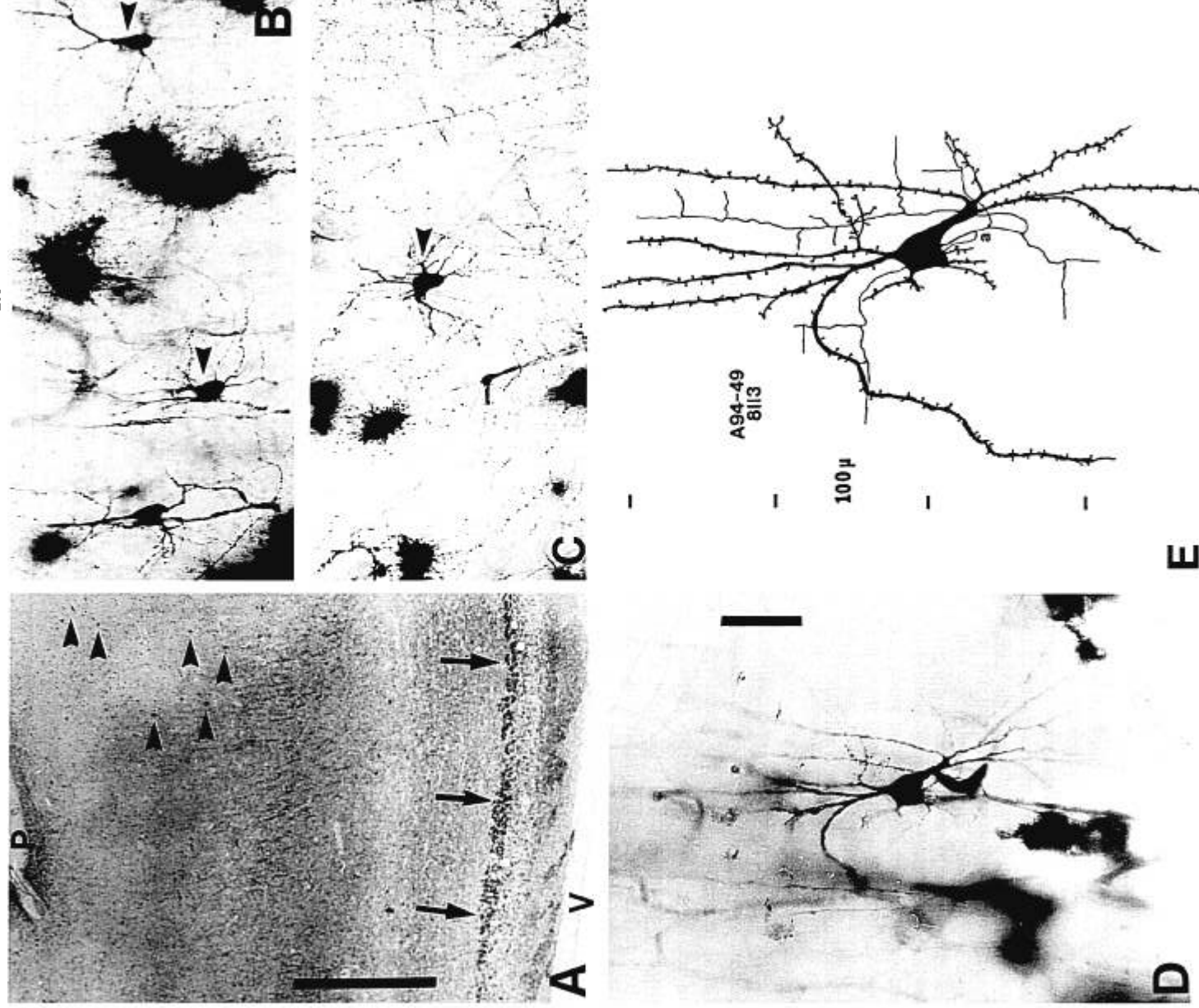


Fig. 3. Composite figure of NF and RG preparations from a perinatally acquired microgyria (case 22). (A) An overview of the microgyric cortex with isolated hypertrophic meganeurons (arrowheads), attenuated white matter, and the survival of an antero-posterior bundle of myelinated and NF⁺ fibers (arrows). (B, C) Views from RG preparations of various atrophic neurons (arrowheads) and some degree of reactive gliosis (dark stained areas). (D) Detail from RG preparations of the abnormal morphology of a hypertrophic meganeuron. (E) Camera lucida drawing of the meganeuron depicted in (D) illustrating its large deformed body, several short and long ascending and descending spiny dendrites, and the axon (a) distributed intracortically. The abnormal morphology of atrophic and hypertrophic neurons is considered to be postinjury acquired. The pial surface (P) is at the top and the ventricular (V) one at the bottom of all figures. Bars: A = 250 μm ; B-D = 100 μm ; E scale = 100 μm .

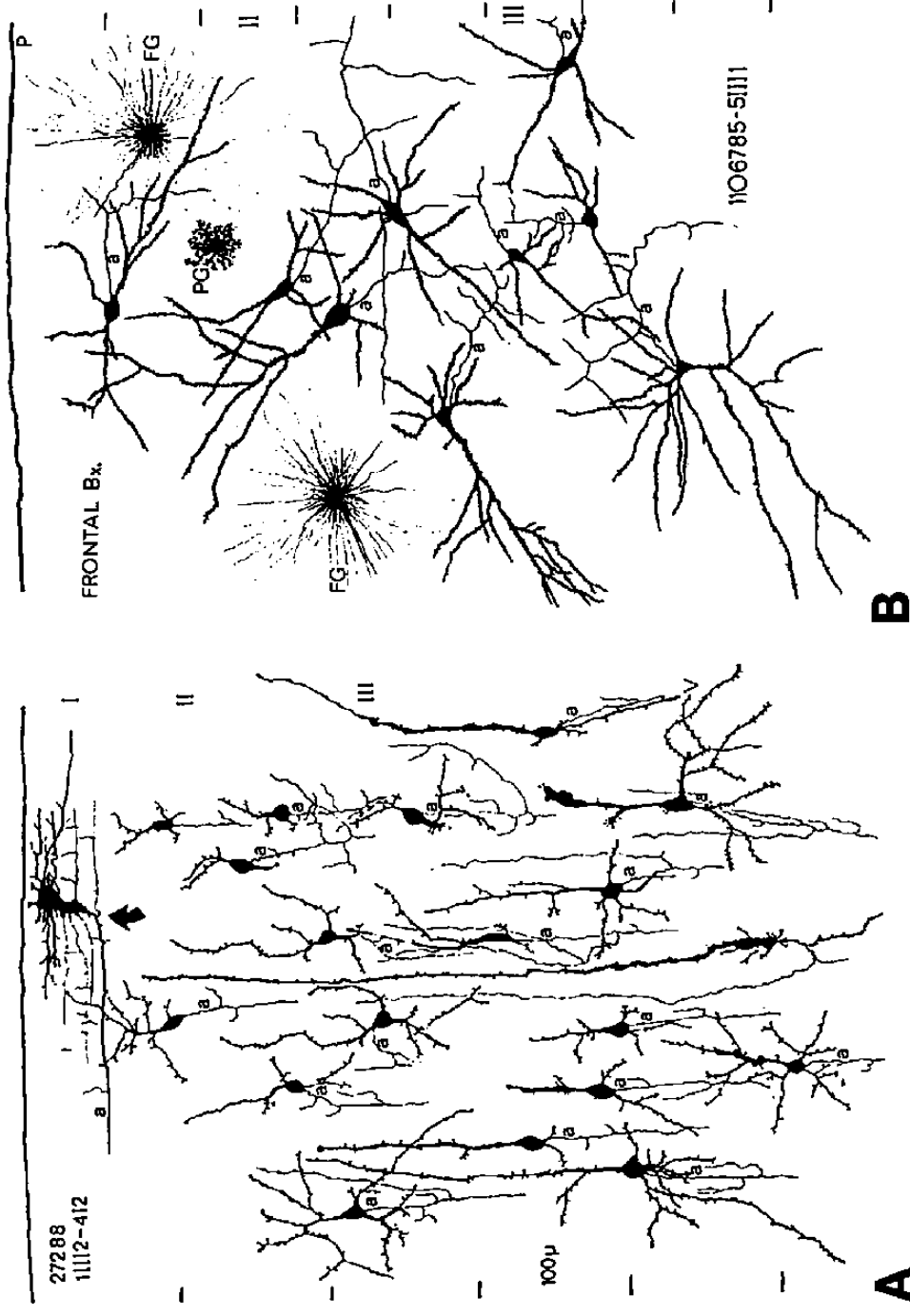


Fig. 4. Composite figure of camera lucida drawings from RG preparations illustrating the neuronal anomalies and organization (A) of a perinatally acquired microgyria (case 28) and of a primarily undamaged cortical region (B) adjacent to a small marginal (layer I) heterotopia (case 30). (A) Detail of the dysplastic cortex of a microgyrus with neuronal disorganization, poor neuronal development (atrophy), and dendritic alterations suggesting degenerative changes. Some neurons are postinjury transformed into small stellate cells, others show alterations suggesting centrifugal dendritic reabsorption, while still others appear to be pyramidal cells which have lost their contact with layer I, perhaps by progressive distal reabsorption. Many of these postinjury transformed neurons have intracortically distributed axons (a) with ascending collaterals. The surviving Cajal-Retzius cells (arrow) have retained their typical morphologic features and long horizontal axons which are recognized throughout the affected microgyric cortex (see also Fig. 2B, D). (B) Detail of a primarily undamaged cortex adjacent to a small marginal (layer I) heterotopia (case 30) showing laminar obliteration, neuronal disorganization, and marked disorientation of dendrites of many neurons which seem to have lost their normal orientation to layer I (see also Fig. 10). This dendritic disorientation is considered to be postinjury acquired to alterations of the region intrinsic circuitry and to the lack of response to layer I developmental cues altered by the marginal heterotopia. This primarily undamaged cortex also shows a mild increase in the number of reactive fibrous astrocytes (FG) as well as nonreactive protoplasmic astrocytes (PG). Scales = 100 μ m.

the gyral crown than that of sulcus deeper region (18, 28–33).

The clinical significance of ulegyrias is the survival of the GM of contiguous gyral crowns, which is practically isolated by the near complete destruction of their corresponding sulci (Fig. 5A). Excepting for the few residual WM fibers running through fiber-carrying residual trabeculae (Fig. 5A, B), the number of afferent and efferent fibers entering and exiting the surviving GM of the gyral crowns is markedly reduced. If the infant survives this

type of lesion, the GM of the gyral crowns (despite structural and functional isolation) survives and undergoes progressive postinjury reorganization compatible with acquired cortical dysplasia secondary to perinatal brain damage. Throughout the depth of affected sulci, the cortex has been reduced to a thin gliovascular membrane with scattered mineralized neurons, lagging macrophages, and extensive reactive gliosis (Fig. 5A–C). This residual cortex is covered by meninges with underlying extensive layer I gliosis and lined by the residual WM

cavity with a few attached trabeculae (Fig. 5A, B). Despite its extensive damage and gliosis, NF and RG preparations have demonstrated the survival of the intrinsic fibers of layer I and of some C-R cells throughout the entire length of the damaged sulcus (Fig. 5A-C). The survival of the intrinsic fibers of layer I throughout the damaged sulcus capable of interconnecting contiguous gyral crown is an important observation not previously recognized. In addition, a few residual WM fibers also survive in the residual cortex of affected sulci. These residual WM fibers seem to be connected with those of fiber-carrying trabeculae. Both the intrinsic fibers of layer I and the residual WM fibers are strongly NF⁺. The survival of layer I and WM fibers capable of interconnecting the isolated GM of contiguous gyral crowns contribute to their survival as well as to their progressive postinjury reorganization.

The surviving GM of the gyral crowns is also characterized by postinjury dysplastic changes, which are more pronounced at its borders with the necrotic sulcus (Fig. 5D-G). Prominent postinjury alterations include cytoarchitectural disorganization, obliteration of laminae, complex intrinsic neuropil, frequent obliteration of layer I by marginal heterotopias, preservation of layer I intrinsic fibers and C-R cells (Fig. 5B, C), marked attenuation of the underlying WM often reduced to a few fiber-carrying trabeculae (Fig. 5A), and the presence of both atrophic and hypertrophic NF⁺ neurons (Fig. 5D, E, G) and scattered dead mineralized neurons (Fig. 5C). Surviving atrophic neurons are characterized by their small size, short spiny dendrites, and intracortically distributed axon. Hypertrophic neurons are characterized by their large size, long, irregular, and spiny dendrites, an axon that often arises from a dendrite and branches intracortically, and by their strong NF⁺ reaction (Fig. 5F, G). Hypertrophic neurons are found either isolated and/or in small groups of 2 to 3 cells (Fig. 5D). Both atrophic and hypertrophic neurons are considered to represent postinjury transformed cells that have responded to alterations of the region intrinsic neuropil.

Therefore, in ulegyria the GM of contiguous gyral crowns are reciprocally interconnected by the intrinsic fibers of layer I, the long axons of C-R cells, and the few residual WM fibers which, undoubtedly, contribute to their survival and progressive postinjury reorganization. Through the establishment of reciprocal interconnections with other cortical regions, the postinjury acquired cortical dysplasia of the gyral crowns could influence the neurological and cognitive development of affected children and, eventually, play a role in the pathogenesis of ensuing neurological sequelae.

Acquired Severe GM Damage

In some perinatally acquired encephalopathies, the GM may be extensively damaged particularly throughout the

center of the lesion (Figs. 6A, 7A-C). This type of severe GM damage, often found in some porencephalies, hydranencephalies, and multicystic encephalopathies, is always associated with extensive WM damage. Invariably, the necrotic process involves the subcortical WM and lower cortical layers. In some cases, the necrotic process advances upward, progressively involving the entire cortex, which is often reduced to a thin gliotic membrane (Figs. 6, 7). If the infant survives this type of severe cortical damage, residual areas of GM and WM tissue survive and undergo progressive postinjury transformations compatible with acquired cortical dysplasia secondary to perinatal brain damage.

The damaged WM is often reduced to a few gliovascular and fiber-carrying trabeculae within a residual cavity lined by a thin periventricular gliotic band (Figs. 6A, 7A-C). This periventricular gliosis is composed of tightly packed reactive astrocytes with glial filaments running parallel to the ventricular wall and of scattered foci of ependymal cells. The ventricular system is invariably expanded by the ex-vacuo hydrocephalus caused by the massive WM damage (Figs. 6, 7).

The surviving cortex above the center of the lesion is often reduced to a thin gliotic membrane with islands of surviving GM tissue (Figs. 6A, 7A-C). This residual gliotic cortex is covered by meninges, vascularized by leptomeningeal vessels, and may have a few attached WM trabeculae (Figs. 6A, 7A). The surviving islands of GM tissue are composed of a few intrinsic neurons embedded within a rich intrinsic neuropil surrounded by reactive gliosis (Fig. 7B-D). Despite the extensive necrosis and gliosis, NF and RG preparations of this residual cortex have demonstrated the survival of both the intrinsic fibers of layer I and of a few WM fibers throughout the entire length of the affected region (Figs. 6A-C, 7A, C). Some C-R cells have also survived in this gliotic cortex (Fig. 6C). The intrinsic fibers of layer I and the few remaining WM fibers are capable of interconnecting the areas of surviving GM tissue through the center of the lesion and also with the better preserved GM at both ends of the gliotic cortex. The preservation of these functional interconnections contributes to the survival of the residual GM tissue and to its subsequent postinjury reorganization. NF and RG preparations of the better preserved GM at the edges of the severely damaged cortex have also shown dysplastic changes that are more pronounced at its border with the gliotic cortex. These postinjury alterations include cellular and fibrillar disorganization (Fig. 6D), laminar obliteration, focal obliteration of layer I (Fig. 6D), complex intrinsic neuropil (Figs. 6D, E; 7D, E), diffused cortical gliosis, layer I gliosis (Fig. 6A-C, 7A, C), and the presence of atrophic and hypertrophic intrinsic neurons (Figs. 6F, 7E, F). Atrophic neurons are

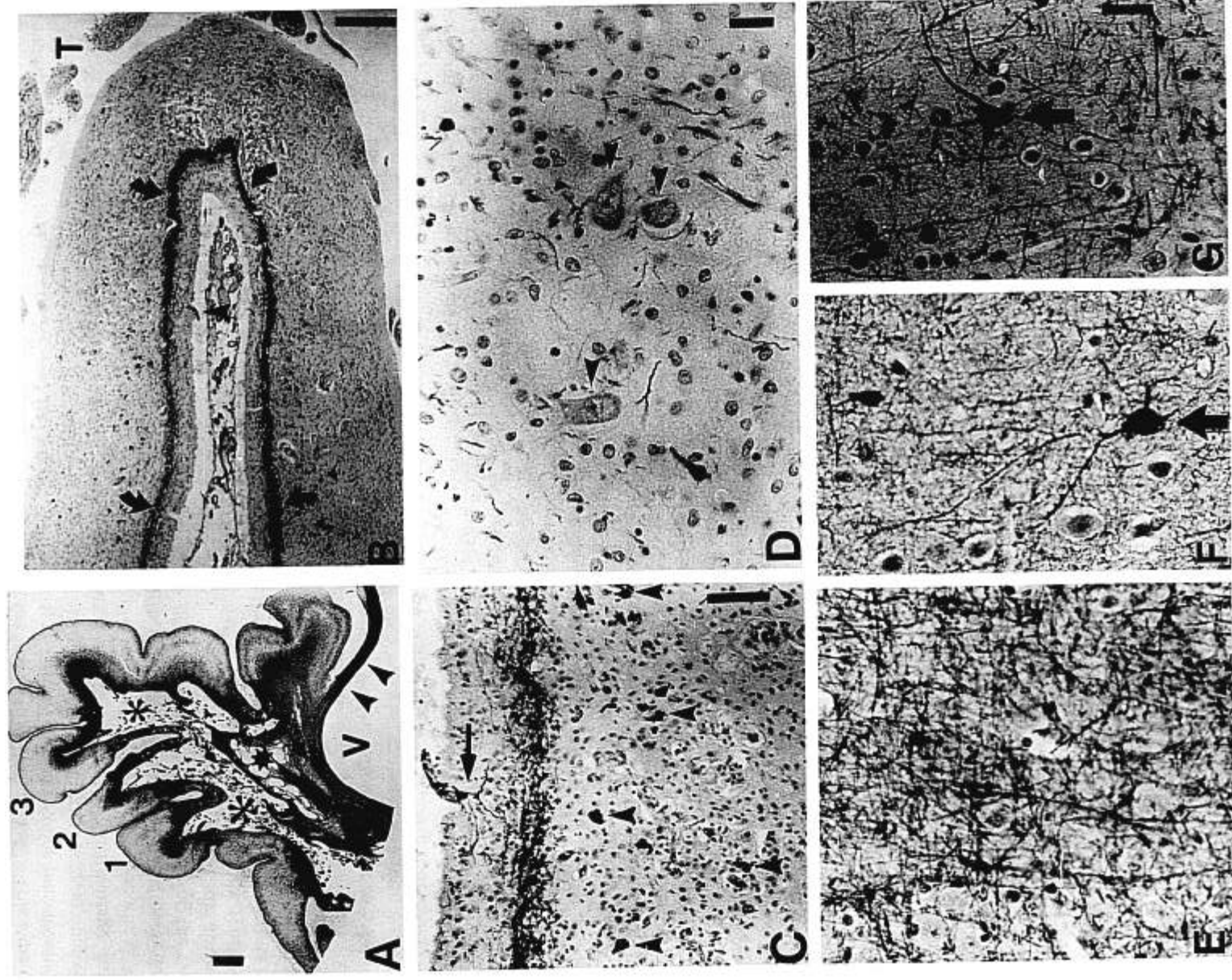


Fig. 5. Composite figure from NF preparations of acquired ulegyria (case 26). (A) Overview of the lesion illustrating the cortical survival through 3 consecutive gyral crowns (1, 2, 3), near complete destruction of the corresponding sulci, remote damage of the underlying white matter involving the sulci, residual white matter trabeculate cavity (asterisks), a more recent white matter damage (star), marked attenuation of the corpus callosum (arrowheads), and ventricular (V) expansion due to a vacuo hydrocephalus. (B, C) Low and high magnifications illustrating the extensive damage of an affected sulcus reduced to a thin membrane with extensive reactive gliosis, scattered dead mineralized neurons (C, arrowheads), survival of the intrinsic fibers of layer I throughout its entire length (C, arrows), survival of some Cajal-Retzius cells (B arrowhead, C arrow), and a few attached fiber-carrying trabeculae (B, T). (D) Detail of the better preserved gray matter of the gyral crowns showing a group of

characterized by a few short dendrites and an axon distributed intracortically. Hypertrophic neurons are characterized by their large size, several long and irregular spiny dendrites, an ascending and/or descending axon which often arises from one of the main dendrites and branches intracortically, and by their strong NF⁺ reaction (Figs. 6F, 7E, F). Isolated hypertrophic neurons are found at all cortical levels but they are more prominent in upper layers where they contrast with the small neuronal size of these regions.

Some of these hypertrophic neurons are parvalbumin positive, which gives support to the idea that they may represent postinjury transformed inhibitory neurons. The stellate morphology and long dendrites of some hypertrophic neurons resemble those of the basket cells. Moreover, NF and RG preparations have demonstrated prominent axosomatic synapses around the body of some pyramidal cells of the better-preserved GM at the lesion edges. Similar types of alterations involving inhibitory basket cells have been recently described (34-37). Hypertrophic cells are considered to be intrinsic (possibly inhibitory) neurons that have responded to postinjury alterations of the intrinsic circuitry of the affected region by structural and functional hypertrophy (20-22, 34). The large size and strong NF⁺ reaction of these hypertrophic neurons give support to this idea. The possibility that a secondary hypertrophy of some basket cells could also be induced by the need to control the excessive (epileptic) firing of projective pyramidal cells should be investigated.

In some cases, NF and RG preparations have demonstrated the presence of abnormal bundles of myelinated and strongly NF⁺ fibers running through the better preserved GM at the lesion edges (Fig. 6D, E). These fiber bundles cross the dysplastic cortex vertically and/or horizontally, often in the proximity of layer I, run within anteroposterior fascicles, and may be composed of both corticopetal and corticofugal fibers (Fig. 6D, E). These fibers may be capable of interconnecting the dysplastic cortex with other cortical and/or subcortical regions and of carrying both normal as well as abnormal (epileptic) impulses from it. In some of these cases (cases 32 and 34) the postinjury acquired cortical dysplasia was already associated with the clinical manifestations of epilepsy (Table).

Acquired Minimal GM Damage

The combined use of various staining procedures (RG, NF, GFAP, PV, and SY) has shown postinjury alterations in primarily undamaged cortical regions far from the injured site, as well as in regions adjacent to small cortical lesions (e.g. marginal heterotopias). Often, these alterations are unrecognizable in routine preparations. These postinjury alterations include partial obliteration of layer I, cytoarchitectural disorganization, partial laminar obliteration, columnar and/or circular arrangement of neurons around cell-free zones, focal reparative gliosis, and the presence of isolated and strongly NF⁺ hypertrophic neurons. The abnormal morphology of hypertrophic neurons can only be recognized using RG preparations (Figs. 3E, 4A, B, 8A-D, 9A, B, 10A, B).

RG preparations of the GM adjacent to, but not in direct contact with, a small marginal heterotopia (case 19) have demonstrated the presence of large hypertrophic neurons (Figs. 8C, D, 9A, B). These neurons are characterized by their large size (meganeurons), long and irregular spiny dendrites, an axon that often arises from one of the dendrites and branches intracortically, and by their strong NF⁺ reaction (Fig. 9A, B). They are often located in the center of cell-free areas within a complex intrinsic neuropil. They are prominent in layer II-III where they contrast with the small neuronal size of these laminae (Figs. 8D, 9A). Some of their long dendrites penetrate and branch within layer I as if they were responding to inputs running subpially (Figs. 8C, D, 9A, B). Their large size, long dendrites, and strong NF⁺ reaction suggest postinjury acquired structural and functional hypertrophy. Similar types of hypertrophic meganeurons have been found immediately below acquired leptomenigeal heterotopias (38).

RG preparations of the undamaged GM adjacent to a small marginal heterotopia (from a brain biopsy, case 30) have shown dendritic disorientation of many neurons which is unrecognizable with routine methods (Figs. 4B, 8A). This frontal lobe brain biopsy was obtained 3 yr after the neonatal brain injury during the surgical implantation of a ventricular catheter for the treatment of hydrocephalus. The child, who is still alive at 6 yr of age, is developmentally and cognitively impaired and epileptic. The dendritic disorientation of these neurons, which were growing at the time of injury, reflect a postinjury developmental response to local postinjury alterations of the

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3 hypertrophic meganeurons (H&E, arrowheads). 3 views (E, F, G) of the better preserve gray matter of the gyral crowns showing complex intrinsic neuropil (E) and the presence of isolated hypertrophic meganeurons (F, G, arrows). The intrinsic fibers of layer I (A-C) interconnect functionally the gray matter of contiguous gyral crowns which have survive despite the extensive necrosis of their corresponding sulci. The hypertrophic meganeurons (F, G), the intrinsic fibers of layer I (B, C), and the intrinsic fibers of the gray matter (E-G) are strongly NF⁺ suggesting active functional activity. Bars: A = 1 mm; B = 250 μ m; D-G = 100 μ m.

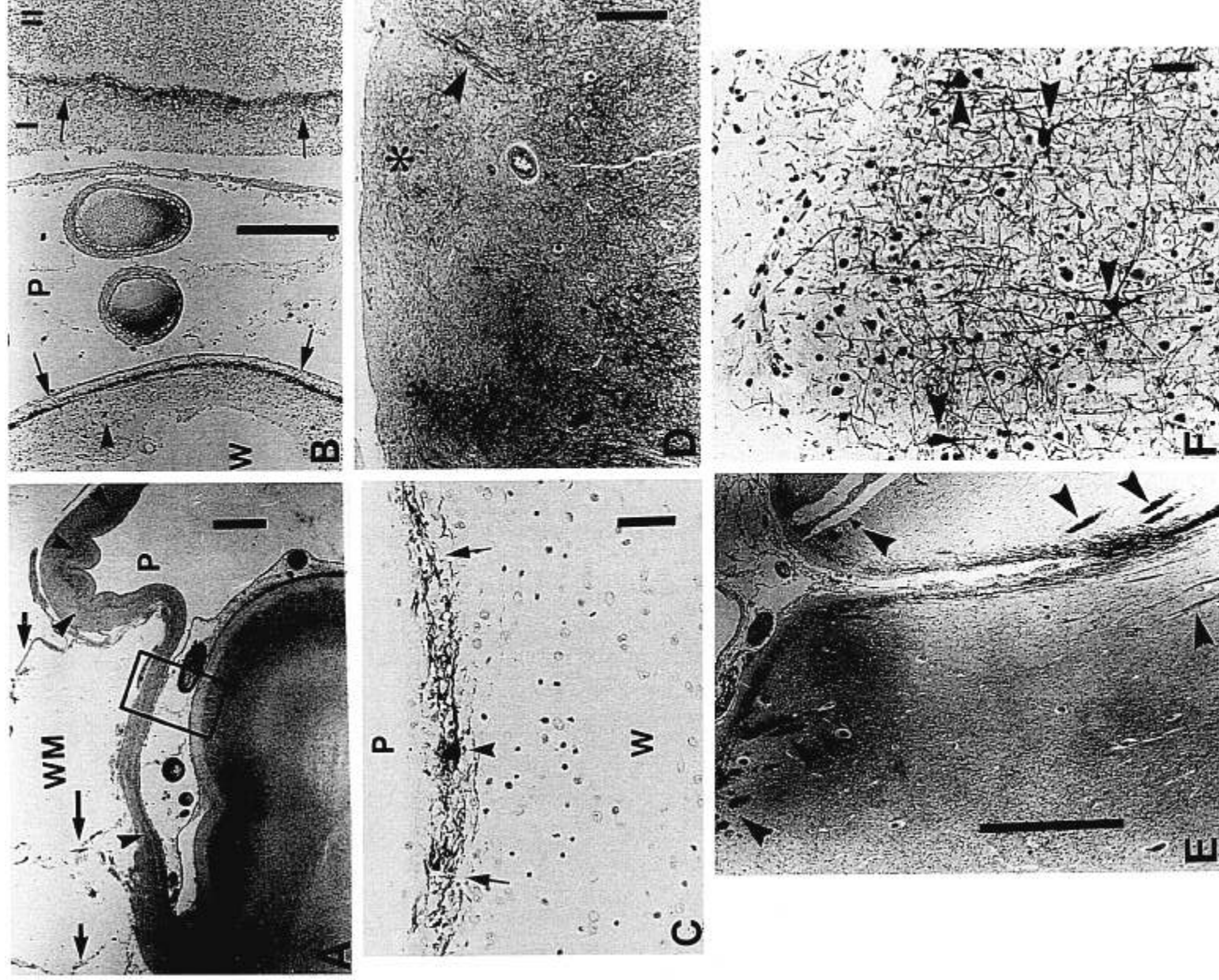


Fig. 6. Composite figure of NF preparations of the residual cortex above a porencephalic cyst (A–C, case 10) and of the better preserved cortex at the lesion edge (D–F, case 32). (A) The extensive cortical damaged through the lesion center (arrowheads) involving practically an entire gyrus and the better preserved cortex of an adjacent gyrus at the lesion edge separated by the meninges (P) of the sulcus. Also illustrated are the residual white matter cavity with fiber-carrying trabeculae (A, arrowheads, B, left arrows) and the contiguous undamaged cortex (B, right arrows) separated by both the damaged cortex (A, arrowheads, B, left arrows) and the meninges (P) of the sulcus. (C) High magnification of the damaged cortex through the lesion center showing the survival of the intrinsic fibers of layer I (arrows) and of some Cajal-Retzius cells (arrowhead). (D) Detail of the better preserved cortex at the lesion edge (case 32) showing layer I

region intrinsic circuitry, possibly caused by the marginal heterotopia. These neurons seem to have lost basic layer I developmental clues (26, 27). Perhaps this child's hydrocephalus implies a more extensive underlying WM injury, which may also have contributed to these dendritic anomalies.

RG preparations of another region of the same brain biopsy have demonstrated the presence of scattered postinjury transformed hypertrophic neurons. These neurons are characterized by their large size (meganeurons), abnormal dendritic morphology, long and irregular spiny dendrites, and an axon that often arises from one of the dendrites and branches intracortically (Figs. 8B, 10A-D). Some of these hypertrophic meganeurons are characterized by very long and irregular spiny dendrites with an unusual distribution, irregular bends, and bizarre terminal dendritic tufts (Figs. 8B, 10B, C). Others are characterized by the unilateral distribution of secondary dendrites, as if they were only responding to impulses arriving from one side of the cell (Figs. 8B, 10A, D). The essential apical dendritic morphology of some of these meganeurons suggests that some of them may represent postinjury transformed pyramidal cells (Fig. 10A, D). The abnormal dendritic morphology of these neurons, which were still differentiating at the time of injury, reflects their response to alterations of the region intrinsic neuropil. These meganeurons are considered to be postinjury transformed neurons that have acquired both structural and functional (strong NF⁺ reaction) hypertrophy. The discharge of these hypertrophic meganeurons could adversely influence the functional activity of adjacent and distant cortical regions, affect the neurological and cognitive development of affected children, and, eventually, play a role in the pathogenesis of ensuing neurological sequelae.

DISCUSSION

The prenatal development of the neocortex is a complex evolving process during which, (under strict developmental constraints [e.g. layer I control of neuronal migration, placement, and morphology (27)] and at specific times) neurons, fibers, glial cells, and blood vessels originate, migrate, differentiate, and establish reciprocal and modifiable structural and functional interrelationships.

This ongoing process continues after birth, probably throughout the life of the individual. The result of this developmental process is the progressive ascending structural and functional organization of a stratified tissue composed of billions of neurons with trillions of modifiable synaptic contacts, supported by innumerable and renewable glial elements, and nourished by a specific and renewable microvasculature (29). This developmental process can, at any time, be interrupted by either genetic and/or acquired damage, and, if the infant survives, the subsequent (postinjury) differentiation of the affected cortex will invariably be altered, resulting in a variety of congenital and/or acquired cortical dysplasias. Genetically induced cortical dysplasias (e.g. anomalies of neuronal migration) have received considerable attention and many neonatal encephalopathies are considered to have a genetic etiology (7, 13, 14, 34-37). On the other hand, perinatally acquired cortical dysplasias, which also cause devastating neurological and cognitive disorders, have received less attention (38). Little is known about the impact of perinatally acquired cortical damage on the subsequent differentiation of the still developing infant neocortex. Genetic and acquired cortical dysplasias should be distinguished from each other, although they may share some pathologic features. These common pathological and clinical features probably reflect similar developmental pathways utilized during the postinjury reorganization of their corresponding affected cortices.

A neuropathologic study of the primarily undamaged, but still developing neocortex of children who survived various types of perinatally acquired encephalopathies (e.g. microgyrias, ulegyras, leptomeningeal heterotopias, multicystic encephalopathies, porencephalies, and hydranencephalies) has been presented. The present study has shown that, invariably, primarily undamaged cortical regions adjacent to the injured site survive, retain their intrinsic vasculature, and undergo progressive postinjury transformations (acquired cortical dysplasia) which affect the structural and functional organization of developing neurons, fibers, glial elements, and microvasculature. Eventually, these progressive postinjury transformations could influence the neurological and cognitive maturation

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obliteration by a marginal heterotopia (asterisks), complex intrinsic neuropil, and the survival of abnormal bundles of myelinated and NF⁺ fibers (arrowhead). (E) Detail of the better preserve cortex at the lesion edge (case 32) showing laminar obliteration, complex intrinsic neuropil, neuronal disorganization, and the presence of abnormal bundles of myelinated and strongly NF⁺ fibers (arrowheads). (F) View of isolated hypertrophic and strongly NF⁺ meganeurons (arrowheads) surrounded by complex intrinsic neuropil. The intrinsic fibers of layer I, Cajal-Retzius cells, intrinsic gray matter fibers, hypertrophic meganeurons, and myelinated fibers are all strongly NF⁺ suggesting active functional activity. The postinjury survival of these myelinated and NF⁺ fibers (8-yr in case 32) could play a significant role in functionally interconnecting this cortex with other cortical and/or subcortical regions and of carrying normal as well as abnormal (epileptic) impulses from it. The pial surface is either at the top of each figure or indicated (P) and the white matter residual cavity (WM) is also indicated. Bars: A = 1 mm; B, E = 500 μm; C, D, F = 100 μm.

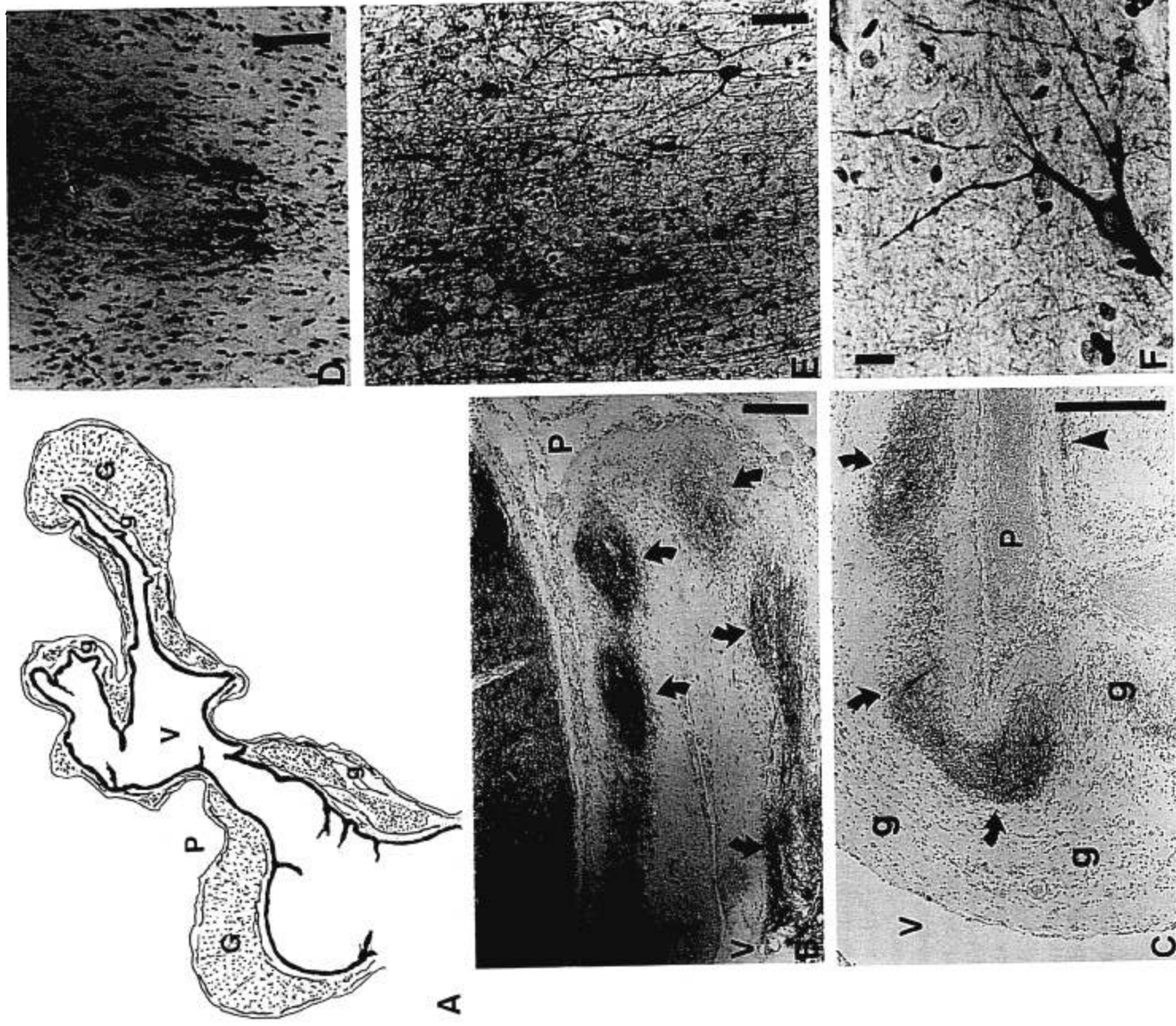


Fig. 7. Composite figure of NF preparations of a frontal porencephalic cyst removed surgically for the treatment of intractable epilepsy (case 33). (A) Schematic drawing of part of the removed cyst showing islands of surviving gray matter tissue (G), marked gliosis (g), collapsed residual white matter cavity (V) with fiber-carrying trabeculae (T), and the pial (P) surface. (B) view of the surgically removed specimen showing a portion of the better preserved cortex adjacent to a severely damaged cortex reduced to a thin membrane with areas of surviving gray matter tissue (arrows) and diffusely gliosis (g), separated from each other by the meninges of an intervening sulcus. (C) high power view of the damaged residual cortex showing small islands of surviving cortex (arrows), intrinsic neuropil with NF⁺ fibers, surviving intrinsic neurons, diffusely gliosis (g), a few surviving intrinsic fibers of layer I, a few residual white matter fibers (arrowheads), the residual white matter cavity (V), and the pial (P) surface. (D) detail of a small island of surviving gray matter tissue, surrounded by diffusely reactive gliosis, composed of intrinsic NF⁺ fibers and a few abnormal intrinsic neurons. (E) detail of the better preserved cortex at the lesion edge showing strong NF⁺ intrinsic fibers and isolated hypertrophic meganeurons. (F) detail of an abnormal strongly NF⁺ hypertrophic meganeuron from the better-preserved cortex. Bars: B, C = 250 μ m; D-F = 100 μ m.

of affected children and play a crucial role in the pathogenesis of ensuing neurological and cognitive disorders, such as epilepsy, cerebral palsy, dyslexia, cognitive impairment, and poor school performance (12-16). Of the cases studied, 10 children have developed epilepsy (cases 26 and 29-36), 2 developed cerebral palsy (cases 31 and 34), while others were neurologically and mentally impaired (Table).

Despite severe cortical damage, extensive destruction of the underlying white matter, partial sensory deprivation, and inability to reach functional targets, these primarily undamaged cortical regions continue to develop, but their postinjury differentiation will be invariably altered. In general, undamaged cortical regions adjacent to an injured site are characterized by cytoarchitectural disorganization, partial obliteration of laminations, gray and white matter attenuation, and some degree of reactive gliosis (glial scarring), including layer I and GM/WM borders. Within this undamaged cortex, some neurons (with appropriate synaptic receptors) respond to local alterations of the intrinsic circuitry with structural and functional hypertrophy, others with atrophy, others develop abnormal dendritic orientation, others fail to respond, while still others die and if not readily removed by macrophages, undergo in-situ mineralization. The intrinsic neuropil of the undamaged cortex is also altered and is characterized by increased intrinsic fibers, which compensate for the destruction of extrinsic ones by the lesion. The synaptic profile of many neurons is also altered and characterized by increased intrinsic synaptic loci that replace extrinsic ones vacated by the destruction of afferent fibers. In some cases, abnormal bundles of myelinated and neurofilament positive fibers have also survived. These abnormal fibers may be capable of interconnecting the undamaged cortex with other cortical and subcortical regions and of carrying normal as well as abnormal (epileptic) impulses.

These postinjury alterations vary considerably from region to region. In general, they are more prominent in regions closer to the injured site than in distant ones, and, also, in smaller surviving cortical areas than in larger ones. Minimal postinjury alterations, often unidentifiable with routine method, are found in cortical regions far from the injured site and/or adjacent to small cortical lesions. Most of these distant alterations represent secondary transformations induced by those of regions proximal to the injured site. During cortical development, these types of secondary alterations will continue to occur as consequences of previous ones. In some cases, the overall structural and functional organization of the entire cerebral cortex may be progressively affected. The identification of many of these postinjury alterations requires, in addition to routine stains, the combined use of various immunohistochemical procedures and, particularly, the

use of the rapid Golgi method for the visualization of the abnormal morphology of postinjury transformed neurons.

The survival of the intrinsic elements of layer I and the presence of large hypertrophic meganeurons, which have been found in most of the cases studied, are significant observations. The intrinsic fibers of layer I and of some Cajal-Retzius cells have survived even through regions where the cortex has been reduced to a gliotic thin membrane (e.g. ulegyras, porencephalies, and hydranencephalies). The survival of these elements may be due, in part, to their subpial location and, hence, accessibility to the pial vascular plexus. These superficial elements are separated from the pial vascular plexus only by the external glial limiting membrane. The intrinsic fibers of layer I extend under the pia surface throughout the entire cortex lacking descending connecting fibers with underlying cortical layers. Therefore, the destruction of lower cortical layers and the underlying white matter will not necessarily affect the intrinsic fibers of layer I. These superficial fibers are capable of interconnecting isolated cortical areas which have lost other types of functional connections destroyed by the original injury. Most of the intrinsic fibers of layer I represent the long axons of Cajal-Retzius cells. These axonic fibers radiate in all directions interconnecting pyramidal cells through adjacent and distant gyri (25-27). The extraordinary length of these axonic fibers allow them to bypass extensive damaged cortical lesions if their original Cajal-Retzius cell have survived. Early in development, layer I also receives nonspecific thalamic fibers which run under the pial surface for considerable distances (27, 39). These afferent fibers ascend through the entire thickness of the neocortex without giving off collaterals and branch within layer I (39). These fibers are relatively few in number due to their developmental dilution and, hence, they are widely separated from each other. Because of these features, the afferent fibers to layer I may or may not be destroyed by an underlying cortical lesion. If they survive, they will also be represented among the intrinsic fibers of layer I. The survival of layer I fibers and that of a few residual WM fibers (from fiber-carrying trabeculae) contribute to the survival of cortical regions isolated by the injury and to their subsequent postinjury reorganization. The survival of these fibers may functionally interconnect the dysplastic cortex with other cortical and subcortical regions.

Another important observation made in most of the cases studied is the presence of isolated hypertrophic meganeurons in postinjury transformed dysplastic cortex. The presence of large neurons has been described in previous studies of cortical dysplasias associated with epilepsy (35-38, 40-45). Meganeurons are characterized by a large often deformed body, abnormal dendritic profile, long and irregular dendrites, numerous synaptic sites (dendritic spines), and an axon that often arises from one of the dendrites and branches intracortically. They are

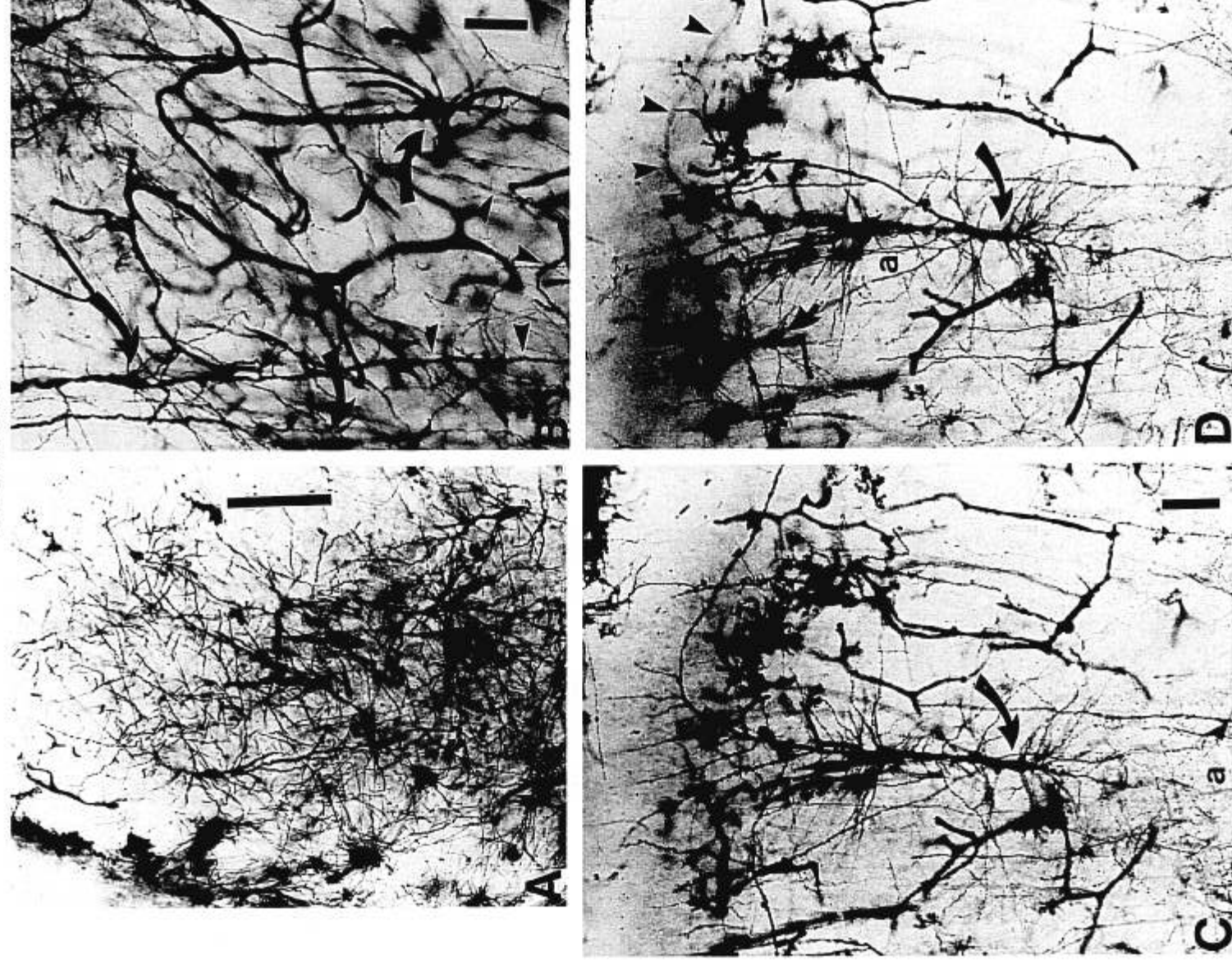


Fig. 8. Composite figure of RG preparations from a primarily undamaged cortical region adjacent to a small acquired marginal (layer I) heterotopia (A, B, case 30) and from the undamaged cortex adjacent to a leptomenigeal heterotopia (C, D, case 19). (A) view of the undamaged cortex below a small marginal heterotopia showing focal cellular, fibrillar, vascular disorganization and the marked disorientation of the dendrites of many of neurons which seem to have lost their basic orientation to layer I. Camera lucida drawings from this cortex illustrating the dendritic disorientation of many neurons is reproduced in Figure 4 (B). (B) view of the undamaged cortex below a small marginal heterotopia showing 3 contiguous hypertrophic meganeurons (arrows) with abnormal body, long and irregular dendrites with anomalous bends and an intracortically distributed axon. Camera lucida drawings of these 3 meganeurons illustrating their location, large size, abnormal dendritic morphology, and relationships to the marginal heterotopia are reproduced in Fig. 10B-D). (C, D) detail of 2 postinjury transformed meganeurons (arrows) from the

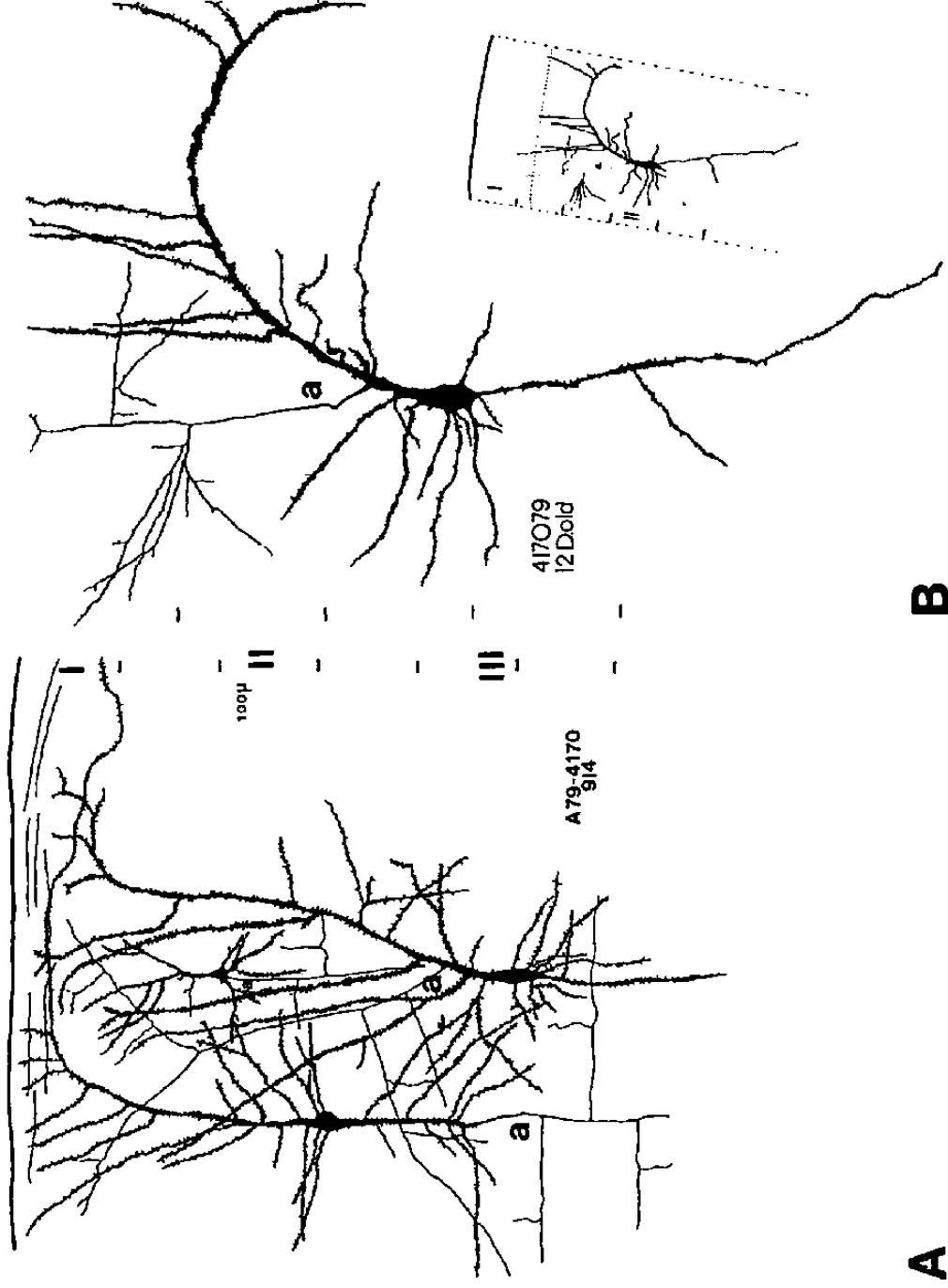
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Fig. 9. Composite figure of camera lucida drawings from RG preparations of the primarily undamaged cortex adjacent to an acquired leptomeningeal heterotopia (case 19). (A) abnormal morphology of 2 hypertrophic meganeurons of layers II and III with abnormal dendritic arborizations characterized by long, irregular and spiny apical dendrites with many ascending collaterals, some reaching and branching in layer I and several basal dendrites with long descending branches with collaterals. Their intracortically distributed axons (a) arise far from the soma. In one neuron the axon arises from a basal dendrite and from the main ascending dendrite in the other neuron. For comparative purposes, the normal size and dendritic morphology of a layer II pyramidal cell is also depicted (see also Fig. 8C, D). (B) view of the abnormal morphology of a hypertrophic meganeuron of layer II characterized by a main ascending dendrite that curves within layer II and sends several collaterals into layer I, a long descending dendrite with fewer collaterals, several basal dendrites, and an ascending axon (a) that arises from the ascending dendrite and sends collateral into layers II and I. The abnormal morphology of these meganeurons is considered to be postinjury acquired. Scales = 100 μ m.

also characterized by a large nucleus and strong neurofilament positive reaction, which suggest an active functional activity. Some meganeurons are parvalbumin positive suggesting an inhibitory function (20–22, 34). Their frequent stellate morphology resembles that of intrinsic basket cells (34). Moreover, complex axosomatic synapses characteristic of basket cells have been found around the body of some pyramidal cells of the transformed

undamaged cortex adjacent to a leptomeningeal heterotopia showing their abnormal morphologic features, large body, long irregular spiny dendrites, some dendrites reach and branch within layer I (arrowheads), and their intracortically distributed axon which often arises from one of the main dendrites. For comparison, notice the normal size and dendritic distribution of a contiguous layer II pyramidal cells (short arrow). Camera lucida drawings of these 2 meganeurons illustrating their abnormal morphology are reproduce in Figure 9 (A). Bars: A–D = 100 μ m.

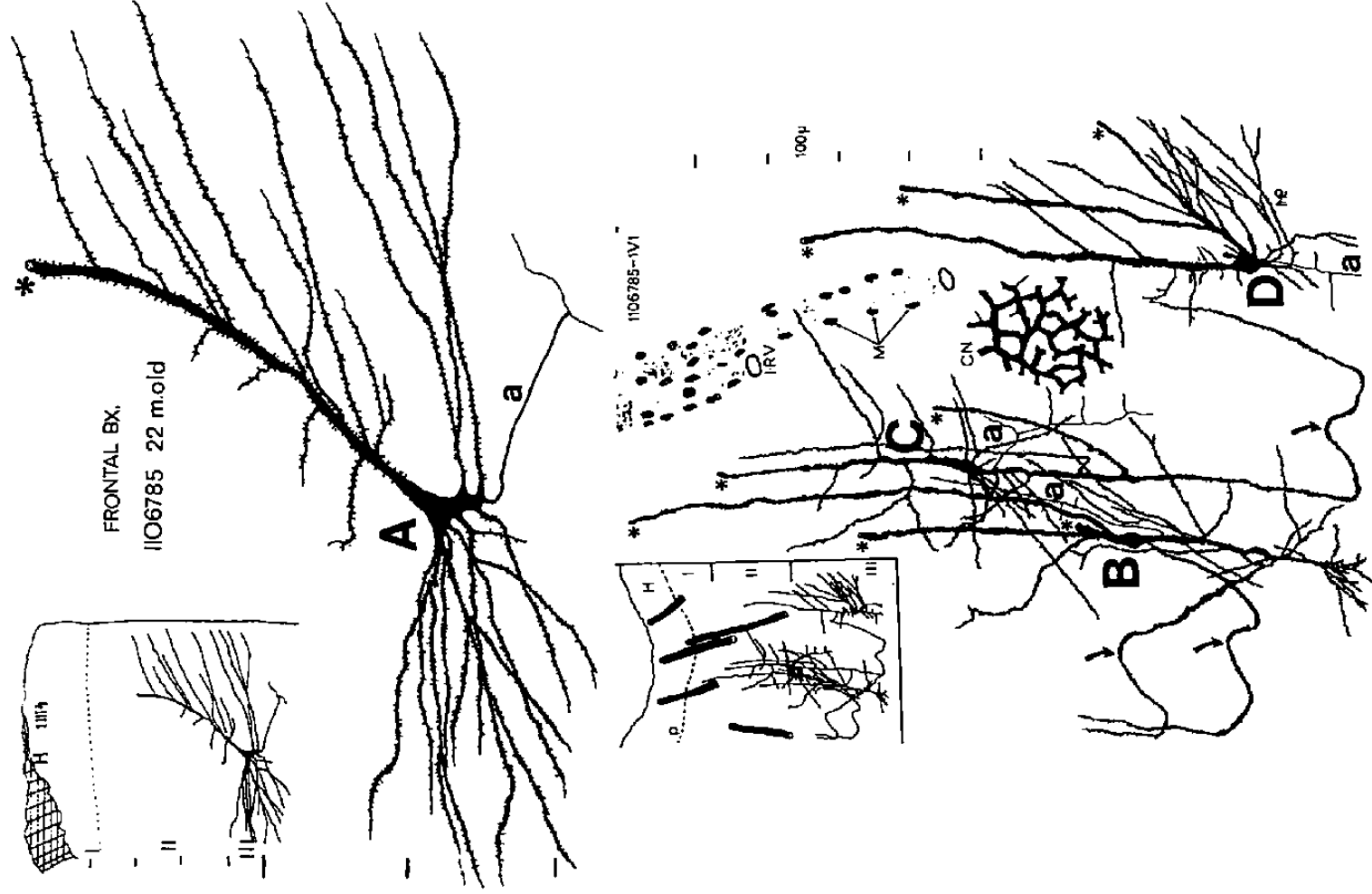


Fig. 10. Composite figure of camera lucida drawings of 4 (A, B, C, D) hypertrophic meganeurons illustrating their postinjury acquired morphology abnormal size and morphology, from RG preparations of a brain biopsy (case 30) adjacent to a small marginal (layer I) heterotopia. The intracortical location (layer II and III), abnormal dendritic morphology, and relationships to the marginal heterotopia (H) of these 3 meganeuron are illustrated in the insets. Also illustrated for comparative purposes are residual postinflammatory vessels (I-R V) surrounded by macrophages (M) and a view of the cortex intrinsic vascular plexus (CN). These meganeurons dendritic arbors are quite irregular with rather long dendrites, their axon (a) branches intracortically and often arises from one dendrite, and their soma is large and often deformed (A). The abnormal dendritic morphology of some of these meganeurons (A, D) seems to respond predominantly to inputs arriving from one side of the cell with most collaterals

dysplastic cortex in this and previous studies (34). Meganeurons are considered to be postinjury transformed intrinsic (possibly inhibitory) neurons, which have responded to increasing functional demands caused by alterations of the region's intrinsic circuitry by both structural and functional hypertrophy.

Based on the large-size of the nucleus of some of these meganeurons, the possibility that they may be polyploidy has been previously suggested (34, 38, 46-48). Developmental disruptions of the nuclear DNA and/or RNA (polyploidy) have been suspected in large neurons found in dysplastic cortices (34, 38, 46-48). In contrast, the present study proposes that meganeurons can also be the result of acquired polyploidy induced by increasing functional demands in some pathologic conditions such as acquired cortical dysplasias secondary to perinatal brain damage. The morphology of CNS neurons is progressively acquired by the interactions of the cell genetic program and environmental clues (51). Therefore, the abnormal morphology of developing neurons can be the result of both genetic and acquired disorders. Abnormalities of the intrinsic circuitry (axonal sprouting) and induced neuronal alterations have been proposed as underlying mechanisms in the pathogenesis of epilepsy (49, 50, 52, 53).

Furthermore, the possibility that some CNS neurons (as cardiac muscle cells) may be capable of responding to increasing functional demands with endomitotic partial DNA and/or RNA reduplication rather than by mitotic division (regeneration), is intriguing. Neurons, as cardiac muscle cells, are endowed for life with the maintenance of large and complex functional cytoplasm capable of undergoing considerable expansion by functional (physiologic and/or pathologic) demands (see addendum). Because these cells are unable to undergo mitotic division (regeneration), they might have retained the capacity to achieve functional hypertrophy and to enlarge their cytoplasm by endomitotic partial reduplication of DNA and/or RNA (polyploidy). The large size, long dendrites, numerous synaptic loci, and huge nucleus of some of these postinjury transformed meganeurons give support to this type of functional hypertrophy. In addition, the possibility that CNS neurons may be capable of partial DNA and/or RNA reduplication (polyploidy) may shed new light on the recently proposed idea concerning neuronal regeneration (by mitotic division) in the adult brain of birds (54) and more recently of mammals—including humans (55). The presence of new nuclear DNA, on

which this proposed regeneration (mitotic division) of adult CNS neurons is based, could have another and different interpretation. Specifically, the presence of new nuclear DNA in some adult CNS neurons may actually reflect acquired polyploidy induced by increasing functional demands from specific physiologic (seasonal birth songs) and/or pathologic (cortical dysplasias) situations. The possibility that some CNS neurons compelled to respond to increasing physiologic and/or pathologic functional demands may be capable of achieving functional hypertrophy by partial reduplication of DNA and/or RNA (polyploidy) should be further investigated.

Finally, it should be emphasized that most of these postinjury alterations are not static but ongoing processes that may continue to modify the structural and functional maturation of the developing neocortex of affected children, probably for as long as they are alive. These ongoing postinjury transformations could eventually modify the differentiation of the entire cerebral cortex and influence the neurological and cognitive maturation of affected children. This study proposes that in perinatally acquired encephalopathies, the postinjury reorganization of the surviving cortex adjacent to the damaged site and its repercussions throughout the developing cortex, rather than the original lesion and/or its glial scarring, are the main underlying mechanism in the pathogenesis of ensuing neurological sequelae. Indirectly, this study also supports the idea that in genetically induced encephalopathies, the inevitable postinjury reorganization of the affected cortex, rather than the genetic injury itself, may also be the main underlying mechanism in the pathogenesis of the ensuing neurological sequelae that occur in genetic disorders.

Conclusions

From this and previous studies (17, 18) concerning the impact of perinatally acquired encephalopathies on the subsequent structural and functional differentiation of the infant developing neocortex, the following conclusions are drawn:

1. The primarily undamaged cortex adjacent to any perinatally acquired lesion survives, retains its intrinsic vasculature, and is able to continue developing. However, its postinjury structural and functional differentiation is invariably and progressively altered.
2. The gray matter overlying white matter lesions, despite partial sensory deprivation and inability to

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expanding in that direction and very few in the opposite one. Other meganeurons (B, C) have very long and irregular spiny dendrites with anomalous bends (arrows) and terminal tufts. While most of these meganeurons are solitary, small groups of 2 or 3 neurons (B, C, D) are frequently found (see also Fig. 5D). The end of the visible dendrite observed within the RG preparation of each neuron is marked by an asterisk. (see also Fig. 8B). Scales = 100 μ m.

reach functional targets, survives, retains its intrinsic vascularization, and undergoes progressive postinjury reorganization. The postinjury reorganization is characterized by neuronal transformations including atrophy, hypertrophy, synaptic profile changes, and reactive gliosis.

3. Some axotomized projective neurons overlying white matter lesions, despite the loss of their functional targets, survive, develop new intracortical axonic profiles, and become progressively transformed into local-circuit interneurons.
4. Some dendrotomized pyramidal cells underlying subpial hemorrhages survive, develop new synaptic profiles, and become progressively transformed into stellate neurons.
5. Some intrinsic neurons of the gray matter adjacent to an injured site undergo postinjury structural and functional hypertrophy characterized by new morphologic features, dendritic and axonic expansion, strongly positive neurofilament reaction, and, possibly, acquired nuclear polyploidy.
6. Both the intrinsic fibers of layer I and some Cajal-Retzius cells survive, even in severe gray matter lesions, and can interconnect functionally adjoining and distant cortical regions that have lost other types of connections.
7. Surviving myelinated and neurofilament positive fibers in primarily undamaged cortical regions may be capable of interconnecting the dysplastic cortex with other cortical and subcortical regions and of carrying normal as well as abnormal (epileptic) impulses from it.
8. These postinjury neuronal, synaptic, and fibrillar alterations are not static but ongoing processes that will continue, perhaps throughout the life of the individual, to affect the structural and functional development of the developing neocortex and influence the neurological and cognitive maturation of affected children.
9. It is proposed that postinjury alterations (neuronal, synaptic, fibrillar), rather than the original lesion and/or its subsequent glial scarring constitute the fundamental underlying mechanism in the pathogenesis of the neurological sequelae (e.g. epilepsy, cerebral palsy, dyslexia, minimal brain damage, cognitive impairment, and poor school performance) that occur following perinatal brain damage.
10. To identify some of these postinjury (neuronal, synaptic, fibrillar) alterations, the combined use of special neurohistological staining procedures (various immunohistochemical stains and, particularly, the rapid Golgi method) is required.

Addendum

After the submission of this work, some papers dealing with neuronal hypertrophy came to my attention. In postmenopausal women, some neurons of the hypothalamus

infundibular (arcuate) nucleus undergo significant hypertrophy and increase the gene expression of neurokinin-B and substance P (Rance, NE and Young, WS. Hypertrophy and increased gene expression of neurons containing neurokinin-B and substance P messenger ribonucleic acids in the hypothalamus of postmenopausal women. *Endocrinology* 1991;128:2239-2247). Similar types of neuronal hypertrophy and increased gene expression also occur in the arcuate nucleus in ovariectomized rats, which can be prevented with the administration of estrogen (Rance, NE and Bruce, TR. Neurokinin-B gene expression is increased in the arcuate nucleus of ovariectomized rats. *Neuroendocrinology* 1994;60:337-345).

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