DEVELOPMENTAL NEUROPATHOLOGY OF PERINATAL BRAIN DAMAGE AND THE PATHOGENESIS OF EPILEPSY

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PREFACE

As a medical student (Granada University Medical School, Spain), interested in Pediatrics, expended countless hours at the hospital pediatric facilities and got to know many of the children and their medical problems. A particular case, still vivid on my mind, awaken my scientific curiosity. One day, walking and talking with a seven years old child he unexpectedly felt down unconscious with multiple, incontrollable and erratic muscular contractions involving face, body and extremities and salivating. I was overwhelmed thinking it was the child' last hour. At the time, my knowledge of epilepsy was nil. Following the seizures, the child was up, talking and walking with me as if nothing has happened and without any knowledge of the event. What could have caused the brain motor cortex to suddenly discharge that amount of altered activity causing generalized and erratic muscular contractions remains inexplicable.

I migrated to USA, become a Pediatric (Developmental) Pathologist and Director of the Pediatrics Autopsy Service (1962-1999) at the Dartmouth-Hitchcock Medical Center, New Hampshire. I carried out countless postmortem studied of children brains, normal (unaltered) as well as those altered by hemorrhagic, hypoxic-ischemic and/or traumatic damage. With an NIH Fellowship, I spend one year (1967-68) at the Cajal Institute (Madrid, Spain) studying Cajal' old Golgi preparations and learning about the method. Some of my Golgi studies of children' brains have been published: The Human Brain. Prenatal Development and Structure, Springer, Heidelberg, Germany, 2012.

The present monograph explores the developmental neuropathology of selected perinatal cortical injuries through their acute, subacute and chronic stages. Including: a) how an altered neuronal activity evolves in a damaged cortical region; b) how it moves through the cortex (epileptic auras); and c) how it reaches the motor cortex to be discharged as erratic and incontrollable muscular contractions. Understanding these processes should provide insights into the pathogenesis of epilepsy secondary to perinatal brain damage.

Hanover, New Hampshire, USA, 2017.

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1. **INTRODUCTION**

"It is more important to know
the brain that has a disease than
the diseases that affects the brain"

M.M-P

Pediatric epilepsy hallmark is the recurrent, erratic and uncontrollable muscular contractions or seizures. The normal pattern of neuronal activity is also disturbed causing strange sensations or auras. Only epilepsies secondary to perinatal brain damage will be explored herein.

Referring to epilepsy (the Sacred Disease) Hippocrates said: "It is not, in my opinion, any more divine or sacred than any other diseases, but has a natural cause" (1). Twenty-five centuries later, we still want a clear understanding of epilepsy altered neuronal activity, it's moving (auras) through the cortex and its eventual discharge, by the motor cortex, as erratic and incontrollable muscular contractions or seizures. In 1971, Taylor, introduced the term 'cortical dysplasia' to describe the altered cytoarchitecture in the cerebral cortex of 10 epileptic patients and considered the changes the source of refractory epilepsy (2).

The neonatal cerebral cortex is particularly vulnerable to hypoxia, ischemia, circulatory disturbances and trauma. Its vulnerability is caused by the immaturity of its microvascular system (3). Growing and interconnecting capillaries are particularly vulnerable in prematurity, labor difficulties, neonatal asphyxia, infections, respiratory difficulties and trauma (4-8).

The present study explores the developmental neuropathology of different types of cortical injuries (local altered corticogenesis), their impact on regions functionally interconnected with them (distal altered corticogenesis) and the eventual discharge by the motor cortex as seizures. The observations described are from the post-mortem brain studies of children who survived perinatal cortical injuries and later died at different ages (Table 1). The staining procedures used include Golgi, Bodian, neurofilament, myelin and other routine neuropathologic staining.

Any cortical injury results in lesions of variable sizes and locations. These lesions developmental neuropathology is characterized by neuronal, microvascular, axonal and glial alterations, laminar obliterations, reactive gliosis and the presence of large (hypertrophic) neurons and of balloon cells. These cortical alterations are commonly referred as 'acquired cortical dysplasia' (4). Because the damaged cortex is still developing: 'acquired altered corticogenesis' migth be a preferable term (4). Moreover, any cortical injury undergoes developmental transformations that eventually impinge on the brain development of surviving children. Children who survive perinatal cortical injuries often develop neurological sequelae including epilepsy, cerebral palsy, mental retardation, poor school performance and behavioral problems. The relationships between early cortical

TABLE 1. Perinatal Cortical Damage: Clinical & Neuropathological Data

Case	Born	Lifespan	Clinical Data	Stains	Autopsy
I. Acute Stages					
1 9568	21wg	1 hour	Cardiac Electra Malformation	H&E	PVH, PH, WMH & WMD
2 9941	26wg	2 hours	Meconium Aspiration	H&E, Golgi	Focal PVH & WMH
3 9442	22wg	2 hours	RDS	H&E, Golgi	Multifocal PVH
4 9369	24wg	3 hours	RDS	H&E, Bodian	PVH, WMH, PH
5 9460	27wg	4 hours	RDS	H&E, Golgi	PVH, IVH
6 9548	24wg	17 hours	Fetal Transfusion Syndrome	H&E	Atelectasis, PVH
7 7897	30wg	1 day	RDS	H&E, Golgi, Bodian	PVH, IVH, Focal WMD
8 904493	26wg	1 day	RDS	H&E, Golgi	PVH, GMD
9 9953	25wg	3 day	RDS, BPD	H&E, Bodian	PVH, PH, WMD, GMD
II. Subacute Stages					
10 9115	33wg	5 days	RDS, Meconium Aspiration	H&E	Multicystic Enceph., Hydrocephalus, WMD, PVH
11 9345	28wg	5 days	Prenatal WM damage	H&E, Golgi, Myelin, Bodian	Multicystic Enceph., Hydrocephalus
12 9319	26wg	9 days	RDS	H&E, Bodian	PVH, WMD, Brain edema
13 904720	28wg	10 days	Enterocolitis, Peritonitis	H&E, Golgi	PVH
14 9004	38wg	12 days	RDS, Prenatal WM damage	H&E, Golgi	Multicystic Enceph.diopathy, Focal LMH
15 90108	28wg	13 days	BPD	H&E, Golgi	WMD, Brain edema, PH
16 9045	26wg	16 days	RDS, Pneumonia, Hypertermia	H&E, Golgi	WMD, Brain edema, PH
17 9971	29wg	17 days	RDS	H&E, Golgi	Hydrocephalus
18 794190	34wg	18 days	RDS, Microcephaly, Retarded growth	H&E, Golgi	PVH
19 9473	32wg	20 days	Fetal transfusion, Plethoric twin	H&E, Golgi	Multicystic Enceph., LMH, Focal microgyria, WMD
III. Chronic Stages					
20 722143	35wg	26 days	Intestinal atresia, Removed surgically	WMD, Focal GMD	WMD, Focal GMD
21 9273	31wg	26 days	RDS	WMD, Brain edema	WMD, Brain edema
22 9449	30wg	32 days	Prenatal WMD	H&E, Golgi	Multicystic Enceph., WMD
23 9010	39wg	5 weeks	Pneumonia, WMD	H&E	Multicystic Enceph., Hydrocephalus
24 907	24wg	3 months	BPD	H&E, Golgi	WMD, Hydrocephalus
25 913	26wg	8 months	BPD	H&E, Golgi, Bodian	Multicystic encephalopathy, Microgyria, Focal GMD
26 9272	24wg	8 months	BPD	H&E, Golgi, GFAP	Multicystic encephalopathy, Microgyria, WMD, PVL
27 9269	28wg	2.5 years	Epilepsy, Developmental delay	H&E, Golgi, Neurofilament	Pneumocephaly, Ulegyria, Altered corticogenesis
28 8631067	32wg	3 years	Epilepsy	H&E, Golgi, Myelin, GFAP	Hydrocephalus, Frontal lobe biopsy, Altered corticog.
29 88173	38wg	8 years	Drowning, Epilepsy, Mental retardation	H&E, GFAP, Myelin	Pneumocephaly, GM Survival, Altered cortices
30 9317048	Term	10 years-Alive	Epilepsy, Left frontal removal of cyst	H&E, Golgi, Neurofilament	Pneumocephalic Cyst, Frontal lobe
31 9381	35wg	11 years	Epilepsy, Mental, Cerebral Palsy, Pneumonia	H&E, GFAP, Myelin, Neurofilament	Occ. Pneumcephaly, Altered corticogenesis, PVL
32 93343	Term	16 years	Epilepsy, Occipital Lobectomy	H&E, Golgi, Neurofilament	Occipital poirencephaly, Altered corticogenesis
33 93147	3-6/o, Stillton	7 years	S.B., Epilepsy, Mental, Retardation, CP, Blind, No speak	H&E, GFAP, Neuro Element, Myelin	Ext. GM & WM damage, Genes, enceph., Altered Cort.
34 99153	3-mo, Shaken	9 years	S.B., Epilepsy, CP, Blind, No speak, Mental Retardation	H&E, GFAP, Neuro Element, Myelin	Ext. GM & WM damage, Genes. enceph., Altered Cort.

Keys for abbreviations: RDS =Respiratory Distress Syndrome, BPD= Bronchopulmonary Displasia, SIS=Shaken Infant Syndrome, PVH= Periventricular Hemorrhage, PH= Pial Hemorrhage, WMH= White Matter Hemorrhage, IVH= Intraventricular Hemorrhage, GMD= Gray Matter Damage, WMD= White Matter Damage, PVL= Periventricular Leukomalacia.

injuries and any of those neurological sequelae remains essentially unresolved and inadequately investigated (Table 1).

In 1973, Gastaut proposed that epilepsy was "a chronic brain disorders of various etiologies characterized by recurrent seizures due to excessive discharge of cerebral neurons Quoted in Duckett Perinatal Neuropathology (9). I share his opinion but will redefine his term 'discharge' for 'altered discharge' of cerebral neurons. In my opinion, Epilepsy is a developmental disorder resulting from the combination of various interrelated processes. First, any cortical injury (hemorrhagic, hypoxic-ischemic and/or traumatic) results in the destruction of local gray matter (GM) neurons as well as in the survival of others that undergo post-injury morphological as well as functional alterations. Damaged axons regenerate and reestablish new synaptic connections with the surviving neurons altering both their morphology and function. The damaged site microvasculature, radial glia fibers and glial cells will also regenerate. Second, the damaged site altered neuronal activity (local altered corticogenesis) might be transferred through cortical areas morphologically and functionally interconnected with the original injured site, resulting in additional alterations (distant altered corticogenesis). Third, the altered neuronal activity moves (auras) through interconnected cortical regions until it reaches the motor cortex to be discharged as erratic and incontrollable muscular contractions or seizures. The brain motor cortex discharges neuronal activity -normal as well as altered- through controlled or uncontrolled motor activities. Fourth, although the motor cortex discharges the altered neuronal activity, is not affected by it, neither is the source of epilepsy. After the seizures, epilepsy 'per se' disappears until a new altered neuronal activity starts to accumulate at the original damaged site and similar developmental processes are repeated anew. To understand the pathogenesis of epilepsy require neuropathologic studies of the damaged site as well as of regions functionally interconnected with it up to the motor cortex. In my opinion, it is more valuable to understand the brain that has a disease than the diseases that affect the brain. Dead neurons do not cause clinical symptoms but altered (transformed) ones do.

Reasons for our incomplete understanding of the neuropathology of perinatal brain injuries or about their impact on the brain maturation of surviving children are many and complex. Any brain study requires the death of the affected child and its neuropathologic study with appropriate methods, including the Golgi procedure, by a qualified pediatric neuropathologist. The human brain (normal as well as altered) postmortem decomposition is rapid and irreversible and the structure of neurons, fibers, capillaries and neuroglia are quickly disturbed. For Golgi studies, the ideal interval between the child death and the brain postmortem study should range between 2 to 4 hours, rarely achievable for obvious reasons. Moreover, the collaboration of parents, pediatricians, pediatric nurses and a pediatric neuropathologist are necessary for the gathering of fresh postmortem brains. Collecting an adequate number of fresh postmortem children brains (normal as well as altered) requires a life-long commitment and the collaboration of many.

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Any damaged cortical region will influence the post-injury development of areas functionally interconnected with it throughout the cortex. Cortical regions not primarily damaged must be also studied, something seldom done. The cortical location of an abnormal neuronal activity in fMRI and electron-encephalographic studies (e.g. the temporal lobe) could be misleading, because any cortical injury will influence the structural and functional maturations of regions functionally interconnected with it throughout the cortex. The altered neuronal activity travel (auras) through anatomically and functionally interconnected regions up to the motor cortex to be discharged. Epileptic patients should be encouraged to be mindful of these auras because that knowledge could prevent serious accidents, simply by avoiding driving the car.

During my tenure (1962-2000) as Director of the Pediatric Autopsy Services (Dartmouth-Hitchcock Medical Center), I was able of collecting postmortem brains from only 34 children who survive perinatal cortical injuries and died at different ages (Table 1). Their postmortem studies range from hours, days, weeks, months to years after the cortical damage. Therefore, acute, subacute (healing) and chronic (repaired) developmental stages of various types of cortical injuries are included in the study. Some of the chronic cases studied have already developed clinical epilepsy (Table 1). It must be emphasized that the neuropathological observations reported and discussed herein include findings from different cortical injuries, different brains and different children. Despite these objections, the neuropathological data from these 34 cases should provide the bases for additional and much needed studies of the impact of cortical injuries on the maturation of affected children brains. The study represents an attempt for understanding what happen to the child brain following a perinatal cortical injury. The observations provide some insights into the pathogenesis of these children' epilepsy (Table 1).

2. BRAIN INJURIES DEVELOPMENTAL NEUROPATHOLOGY: AN OVERVIEW

It is important to recognize that in addition to the dural (first) and the arachnoids (second) meningeal vascular compartments, the pia anastomotic capillary plexus (PACP) represents a third one and the source of all perforating vessel that enter the cortex during its pre- and post-natal maturations (3, 10). Initially, the arachnoids arteries supplied the pia capillaries, eventually local capillary angiogenesis maintain the PACP expansion that parallels that of the cortex (Figure 1).

It is surprising that pia capillary plexus is not described in classic neuroanatomy and/or embryology' textbooks. The customary removal of the meninges prior to brain examination will carry the PACP with it (10). A microscopic study of the discarded meninges will be necessary to visualize it. The pia capillaries and the cortex external glial limiting membrane (EGLM) are also separated their respective basal laminae (Figure, 3B, C). Sprouting pial capillaries are the only one capable of perforating the cortex EGLM to enter into the nervous tissue (Figure 2). Once they have entered, they are incapable of exiting the nervous tissue (10, 11). Circulatory demands determine which vessels become
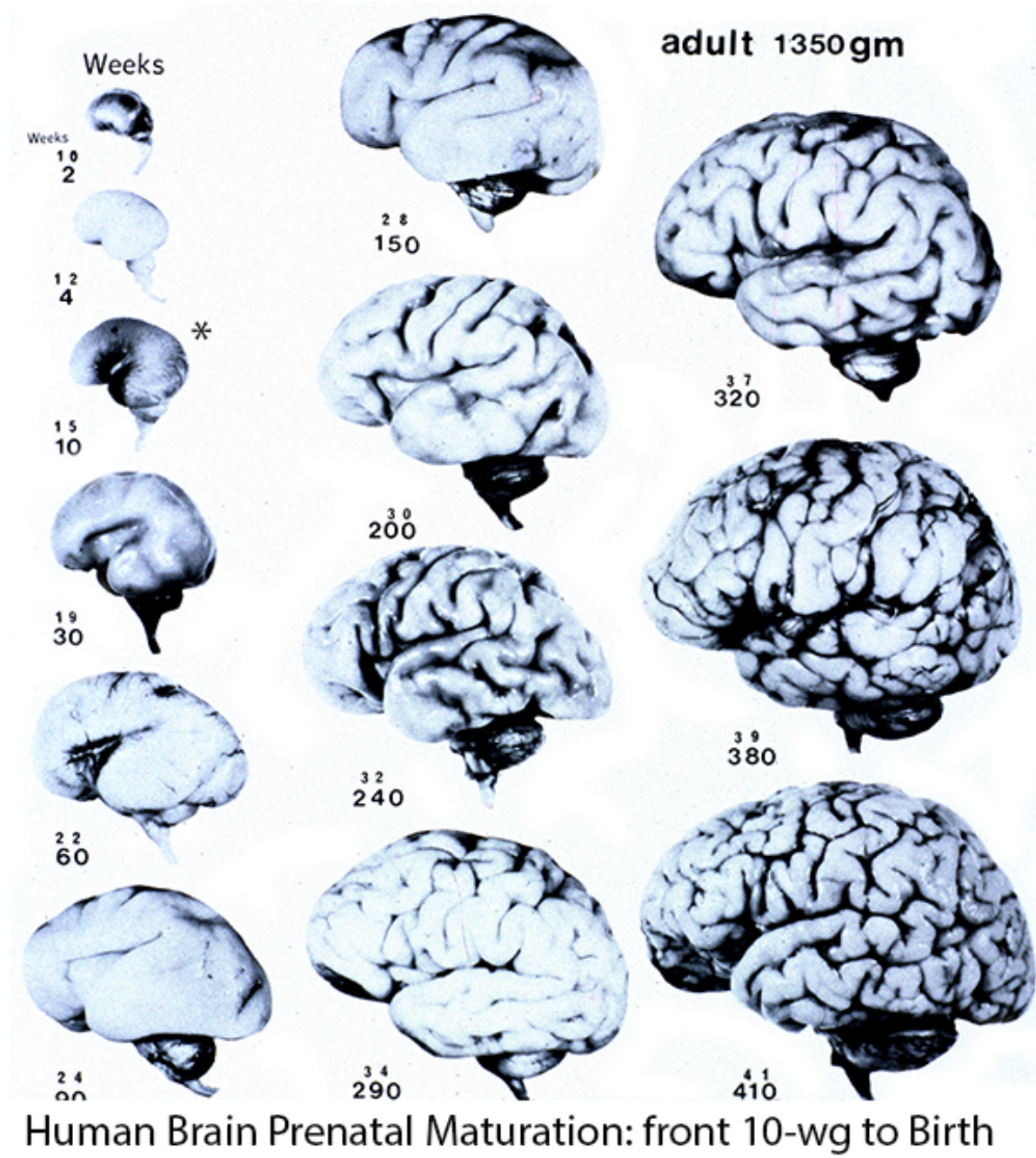
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Figure 1. Photomicrographic montage showing the prenatal development of the human brain from the 10th to the 40th w-g,. The human cerebral cortex starts it developmental maturation around the sixth-seventh week of gestation (not illustrated). Postnatally, the human brain triplicates its size and weight as well as the complexity of its gyral patterns. (Modified, from Larroche, 1977).
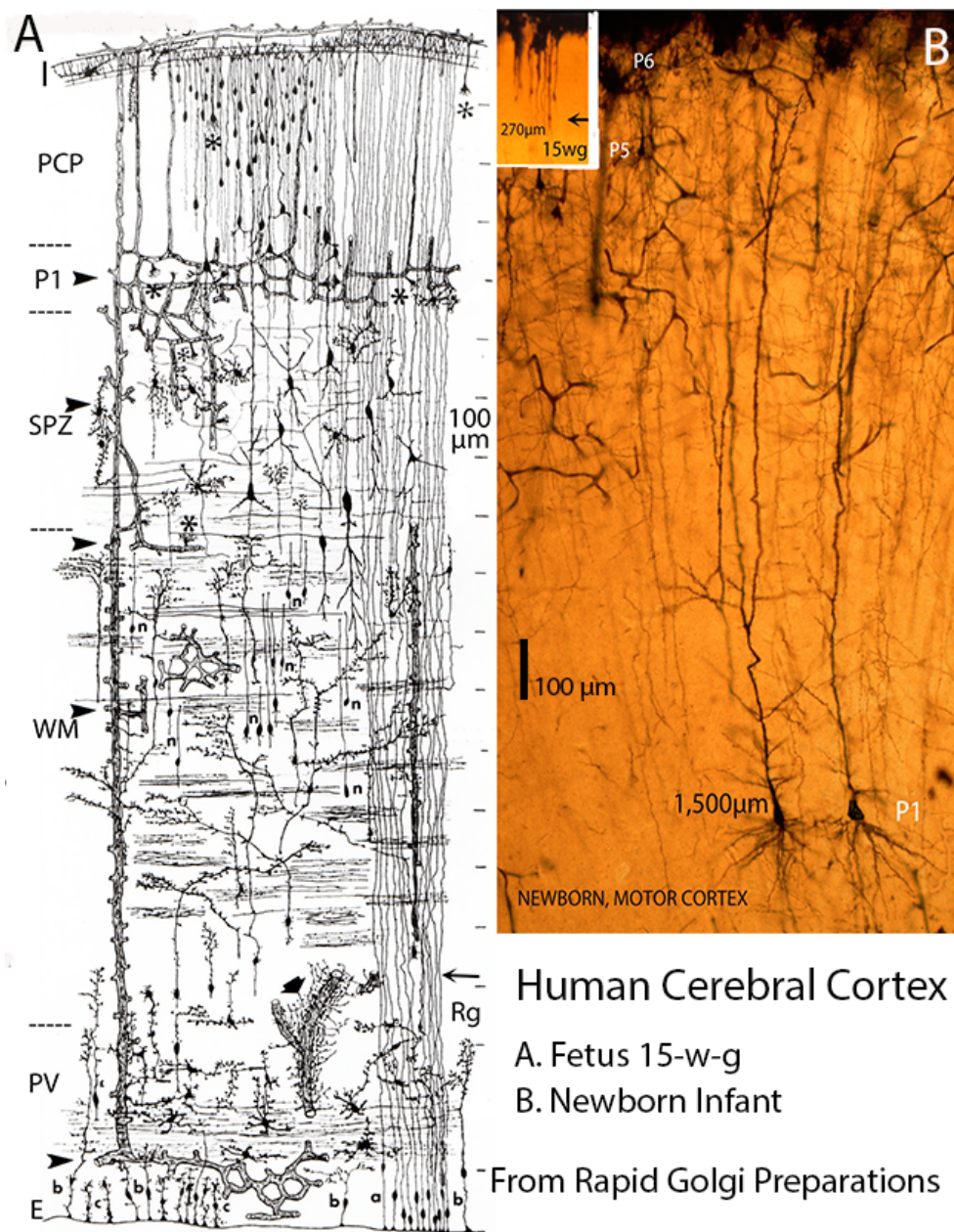
Figure 2. Montage of camera lucida drawings (**A**) from rapid Golgi preparations of the cerebral cortex of 15-w-o fetus of its primary motor region measuring roughly 800 µm in width, 2,000 µm in height and 150 µm in thickness. It illustrates the development and organization of its basic neuronal, microvascular, neuroglia and fibers systems throughout the first lamina (I), the gray matter (GM) pyramidal cell plate (PCP), the subplate region (SP), the white matter (WM), the matrix zone (MZ), the periventricular zone (PVZ) and the ependymal surface (ES). The GM deep pyramidal neurons (P1) have started their ascending functional maturation developing short basal dendrites and a few apical dendritic spines. Concomitantly a new intrinsic capillary plexus (arrowhead) between contiguous perforators is established throughout the P1 region. Radial glial fibers (RDF) extending from ependymal to pial surfaces are also illustrated as well as numerous ascending glial cells precursors throughout the MZ, WM and SP zones. Glial cell precursors advancing processes bend in the direction of corticofugal and/or corticotectal fibers in the WM zone, possibly representing developing fibrous astrocytes and oligodendrocytes. The MZ is characterized by large blood vessels surrounded by bizarre glial processes. A prominent band of horizontal fibers, of unknown origin and function, is recognized throughout the PVZ. **B.** Rapid Golgi preparations of the motor cortex of a newborn infant and of a 15-w-o fetus (inset) illustrating the size, morphology and functional maturation of their deepest and older pyramidal cells. At 15-w-g, the deepest pyramidal neurons (inset) measure about 270 µm, have started their ascending maturation by developing short basal dendrites and a few spines in their apical dendrites. The remaining pyramidal neurons are still immature with smooth somas and apical dendrites anchored to the first lamina. By birth time, the deep pyramidal neurons (P1) measure 1500 to

1600 μm in length and have thousands of apical dendritic spines, some collateral and terminal dendrites whiting the first lamina. (From: Marín-Padilla, 2011).

arterial and which one's venous perforators. In the newborn cortex, each exiting venous perforators is surrounded by 8 to 10 entering arterial ones. The ratio between entering arterial and exiting venous perforators changes and respond to the developing brain functional demands. In non-cortical regions, axons are also capable of entering as well as exiting the nervous tissue (11).

The EGLM perforation by pia capillaries is a complex developmental process (11). At the entrance site, vascular and EGLM basal laminae fused. Pia capillaries endothelial cell filopodia enter the nervous tissue by perforating through the fused basal laminae (Figure 3A-3E). At the entrance site, vascular and EGLM basal laminae fuse forming a funnel that accompanies the vessel into the nervous tissue while remaining open to the meningeal interstitial (Figure 3C). This funnel, the 'Virchow-Robin Compartment (V-RC)' maintains the perforating vessel extrinsic of the nervous tissue (Figure 3D, 3E). V-RCs remain open, for life, to the meningeal interstitium and serve as the cortex GM sole drainage system (12). Lacking a proper lymphatic system, the V-RCs assume the physiological task of removing functional debris from the cortex. Every day, especially during night sleep, macrophages (glial and mesodermal) remove debris and residual neurotransmitters and carry them into local V-RCs and into the meningeal interstitium for final elimination through the arachnoidal vessels perivascular lymphatics. In chronic encephalopathies (Alzheimer and post-traumatic), the amount of cellular debris increases considerable impairing the V-RCs drainage capability (12). The W-RCs are filled to capacity by debris carrying macrophages, barring the entrance of additional ones. Macrophages incapable of entering local V-RCs die in situ liberating their proteolythic cargo. These proteolythic deposits (plaques) cause inflammatory responses and additional neuronal damages. Over time, these unresolved processes sowed the cerebral cortex of necrotic foci (plaques) of death neurons, axonic fibers, macrophages and denatured proteins (amyloid). These residual plaques cause additional damage to neurons resulting in progressive dementia (12).

During the cortex pre- and post-natal maturations, pial capillaries perforate the cortex EGLM at regular intervals, ranging from 400 to 600 micrometers (11). This intervascular distance remains unchanged during the cortex pre- and post-natal maturations (Figure 4A). Emerging capillaries from contiguous perforating vessels puncture through the V-RC outer glial wall, enter into the nervous tissue and establish a short-link anastomotic plexus of intrinsic capillaries between them (Figure 4B). The capillary perforation of V-RCs outer glial wall is a process similar to the original perforation of the EGLM by pial capillaries (11). GM intrinsic capillaries are also incapable of re-entering V-RCs by perforating through their outer glia wall. They are also incapable of exiting the cortex. Functional demands determine their arterial and/or venous nature. Local neurons, axons terminals, synaptic contacts and protoplasmic astrocytes reside within the spaces of the intrinsic capillary plexus.

The GM intrinsic capillaries represent the so-called blood-brain-barrier, are covered by a single basal lamina and respond to the local neuron functional demands. The functional activity of local neurons, mediated through protoplasmic astrocytes, controls the
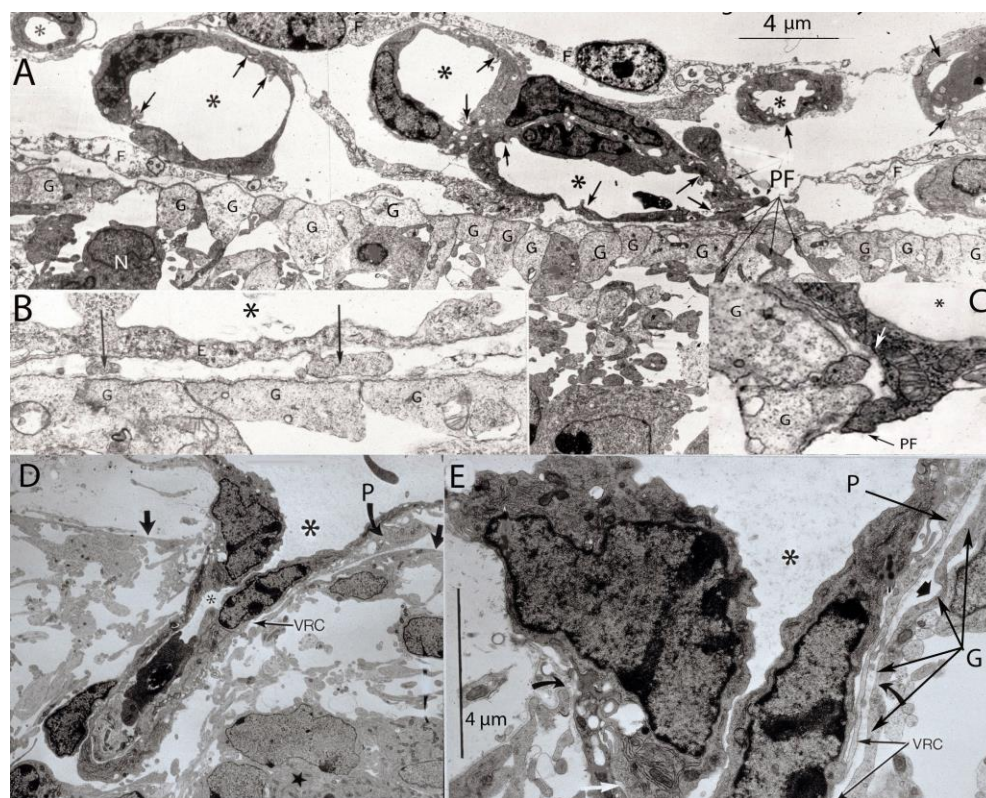

Figure 3. Montage of electron photomicrographs showing the fundamental steps in the perforation and entrance of pial capillaries into the embryonic cerebral cortex. **A.** View of the embryonic cortex covered by the external glial limiting membrane (EGLM) composed of adjoining glial end feet covered by basal lamina manufactured by them. Growing pia capillaries (*) establish a pial anastomotic capillary plexus (PACP) from which all perforating vessels that enter into the cerebral cortex originate. The PACP system represents a third meningeal vascular compartment in addition to the arachnoids vessels (second) and the dural venous sinuses (third). In addition to pia capillaries, macrophages, fibroblasts, collagen fibers and cerebrospinal fluid are also PACP components. **B.** View of the endothelial cells filopodia contacts with the cortex EGLM with fusion of both basal laminae (arrows). **C.** At the capillary entrance site, vascular and EGLM basal laminae fused forming a funnel that accompany the perforating filopodia into the nervous tissue while remaining open to the meningeal interstitial, known as the Virchow-Robing Compartment (V-RC). **D**. Detail of the entrance of a perforating vessel into the nervous tissue. **E**, High power view of the perforating (*) capillary showing the fusion of the cortex and capillary basal laminae and extension of the surface EGLM forming a funnel that accompanies the vessel into the nervous tissue, known as Virchows-Robin Compartment (V-RC). New glial cell endfeet are progressively incorporated into it as the vessel penetrates the nervous tissue. The presence of a meningeal cell, possibly a pericytes (P), within the V-RC perivascular space is also illustrated. (From: Marín-Padilla, 2011, 2012).

blood flow between contiguous perforators (10). Functional magnetic resonance image studies (fMRI) are based on this property of GM intrinsic capillaries. The intrinsic capillaries plexus between contiguous perforating vessels their local neurons, axons terminals and protoplasmic astrocytes represent independent functional centers (10).

The similar distance between perforating vessels through the cortex GM and hence the width of the intrinsic capillary plexus represents a biological constant needed for the local neuron's functional activities. It might also represent the optimal distance for oxygen

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diffusion between perforators as well as the distance inflammatory cells could travel between contiguous perforators. The intrinsic capillary plexus width is comparable to Mountcastle's functional vertical columns, suggesting additional functional roles (13). The number of perforating vessels and that of intrinsic capillaries is considerably greater through the GM than through the WM These vascular differences determine the distinct developmental neuropathology and outcome of GM versus WM perinatal damage (6, 7).
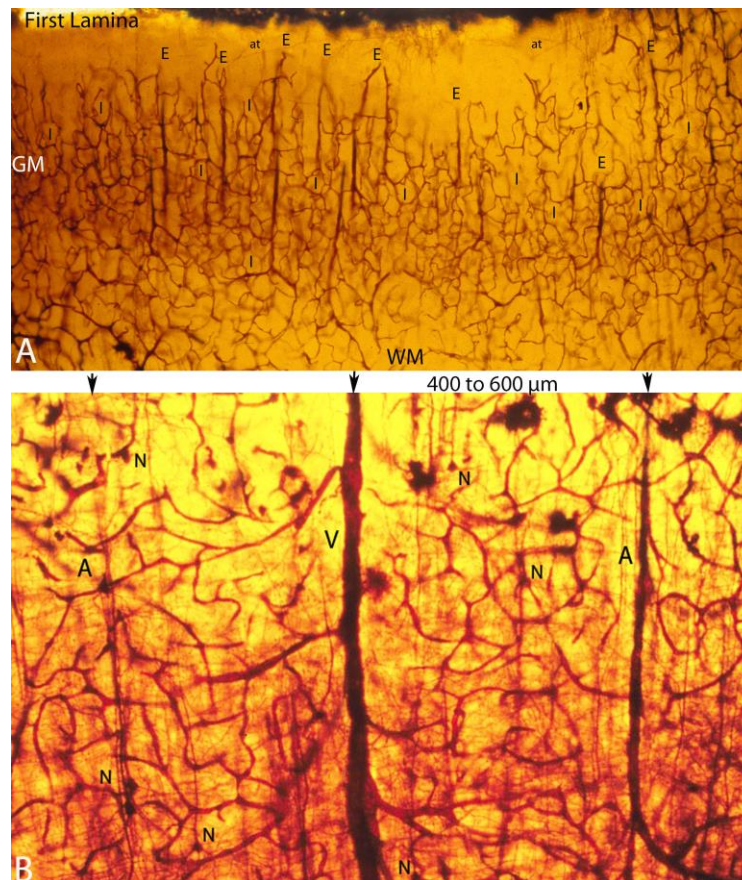
Figure 4. Color photomicrographs of the motor cortex of a 2.5-month old infant, showing its extrinsic (E) and intrinsic (I) microvascular compartments, from Golgi preparations. A. Photomicrograph of the infant motor cortex showing the extrinsic (E) and the intrinsic (I) microvascular compartments composition and interrelationships throughout the gray matter. The equidistant distance (400 to 600 micrometers) between perforating vessels and hence the width of the intrinsic anastomotic capillary plexus, between them, remain essentially unchanged through the cerebral cortex prenatal and postnatal maturations. This distance is considered to represent a biological constant needed for the functional activity of the gray matter (GM) neurons between the perforating vessels. Few of the GM perforators penetrate the white matter (WM) establishing a large anastomotic venues plexus among them. B. Detail, at a higher magnification, of the intrinsic capillary anastomotic plexus established between contiguous arterial (A) and venous (V) perforators and a few scattered neurons (N). The GM intrinsic capillaries that enter the nervous tissue, are incapable of reentering the V-RCs. Circulatory dynamics and functional demands will determine their arterial and/or venous nature. During the brain pre- and post-natal maturations, the ratio of arterial versus venous capillaries between contiguous perforators changes in response to functional demands. The rectangular section of the infant motor cortex illustrated in B, measures roughly 380 x 240 micrometers. (From: Marín-Padilla, 2015)

The cortex microvascular system has dual venous drainages systems: a rapid one for the GM, where most neurons reside, and a slower one for the WM, with fewer neurons (14). In the GM blood circulates rapidly, constantly and independently entering through arterial

perforators and exiting through contiguous venous ones. In the WM blood circulates slowly through an extensive anastomotic venule's plexus between its few arterial perforators. The WM blood drains into the periventricular venous plexus and through the Rosenthal' veins into the brain ventral venous circle. The ampulla of Galen drains the ventral venous circle into the dural venous sinuses.

Extensive hemorrhagic, hypoxic/ischemic and/or traumatic brain injuries are incompatible with life and provide no information concerning their developmental neuropathology or impact on the child maturing brain. On the other hand, children with less extensive cortical injuries might survive for days, weeks, months and even years, and eventually died for unrelated causes (Table 1). Only from these few unfortunate cases, the developmental neuropathology of perinatal cortical injuries could be studied and their impact on brain maturation evaluated.

Unfortunately, to study the neuropathology of any cortical lesion the affected child must die, and postmortem brain studies must be carried out as soon as possible with appropriate staining techniques, including the Golgi procedure. The gathering of an appropriate postmortem material of aged cortical lesions is difficult, time consuming and require the collaboration of parents, pediatricians, nurses, hospital personal and of a qualified pediatric neuropathologist. These prerequisites are rarely attainable and, hence the developmental neuropathology of perinatal cortical injuries has been seldom investigated. The developmental neuropathology of selected perinatal cortical injuries caused by hemorrhagic, hypoxic-ischemic and/or traumatic injuries are explored herein throughout their acute, subacute and chronic stages (Table 1).

3. HEMORRHAGIC INJURIES NEUROPATHOLOGY

A variety of hemorrhagic lesions have been described in the brain of children born prematurely suffering from perinatal hypoxia, asphyxia, labor complications, respiratory difficulties, circulatory disturbances and/or trauma. They include extradural, subdural, subpial, intracortical, periventricular and intraventricular hemorrhages. The neuropathology of these hemorrhagic injuries is well known, however that on surviving children is not. Neither is known their possible role in the pathogenesis of ensuing neurological sequelae, such as epilepsy.

The developmental neuropathology of only two types of hemorrhagic injuries are explored herein: those involving the subpial and the periventricular (germinal matrix) regions (Table 2). These regions are distinguished by the special vulnerability of either their growing (subpial region) and/or regressing (periventricular region) blood vessels. The brains of children who survive these hemorrhagic injuries undergo postinjury developmental transformations and their clinical outcome is often associated with epilepsy (15-24).

In subpial hemorrhages, the cortex external glia limiting membrane (EGLM) is often damaged resulting in leptomeningeal heterotopias. In periventricular hemorrhages the

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radial destruction of radial glia interrupts the ascending migration toward the developing cortex of both neuronal and glial precursors. The dislodge precursor cells evolve into periventricular heterotopias. Both leptomeningeal and periventricular heterotopias have been described in epilepsy.

TABLE 2. Perinatal Cortical Damage Developmental Neuropathology

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 A. Hemorrhagic Injuries

    1. Subpial Hemorrhages with EGLM Damage

        a) Leptomeningeal Heterotopias

    2. Periventricular Hemorrhages with Radial Glial Fibers Damage

        a) Periventricular Heterotopias

B. White Matter Hypoxic-Ischemic Damage with Functional Disconnections of

        a) Overlying Gray Matter

        b) Subcortical Centers, including the Thalamus

C. Traumatic Injuries

        a) Shaken Infant Syndrome

D. Gray Matter Damage

        a) Acquired Altered Corticogenesis

The cortex subpial region includes numerous perforating pial capillaries that enter into the nervous tissue, at regular intervals, by perforating through its EGLM. First lamina growing and interconnecting capillaries consume considerable amount of energy (oxygen) and are particularly vulnerable to its deficiency. The first lamina contains the expanding terminals dendrites of all pyramidal neurons and the axon terminals of Cajal-Retzius cells (C-RCs) that extend throughout the entire cortex establishing functional contacts with the terminal dendrites of all GM pyramidal neurons (25). Also, first lamina has scattered smaller neurons and numerous special astrocytes (comet cells) that contribute endfeet to the cortex expanding EGLM (25).

The first lamina is the first stratum to develop in the mammalian cerebral cortex and its primordial composition resembles the premammalian cortical organization of reptiles (10). In humans, its formation starts around the 6th-7th week of gestation preceding the formation of the cortical (pyramidal) plate formation (10). The pyramidal cells plate form around the 8th week of gestation separating first lamina elements from subplate zone ones. The C-RCs long horizontal axons orchestrate the ascending and inside-outside placement of all pyramidal neurons. Despite different opinions, C-R cells survive and continue to play important functional roles in the developing cortex (26, 27). The GM is formed by the inside-

outside incorporation of pyramidal cells, arriving via radial glial fibers. The cortex inhibitory neurons develop later and have a different extracortical origin (28).

The functional maturation of GM neurons and its microvascular system are ascending and concomitant processes that start around the 16th weight-gestation, proceed up to birth and continue postnatally (28). From the start of cortical development up to mid-gestation, radial glia fibers are the sole endfeet' contributors to the cortex expanding EGLM. During late gestation, radial glial cells fibers start to disappear and are barely recognizable by birth time. The diminishing radial glia fibers are unable of providing the needed glial cells endfeet for the cortex expanding EGLM as well as for the GM growing microvascular system (10). Late in gestation, a new source of glial cells endfeet is needed. In my opinion, the so-called ephemeral subpial granular layer of Ranke (SGR) will be the new source of glial cells for the cortex expanding EGLM and for the GM growing microvascular system (14). Its formation starts around the 16th week of gestation, reaches its greater expansion by the 26th and is barely recognizable by the 35th (29). The extraordinary expansion of the human cortex GM and EGLM, compared with that of other mammals, explain the prominence of its SGR. Undoubtedly, other mammalian species also have SGRs with similar developmental functions but with a less obvious presence.

The SGR is formed by a late ependymal to pia migration of glia cell precursors via radial glial fibers. The late transportation of glial cells precursors may be the radial glial fibers last developmental function (29). The SGR undifferentiated cells become the main source of first lamina special astrocytes that will supply the needed endfeet for the cortex expanding EGLM. First lamina special astrocytes (comet cells) are small cells with several ascending processes that provide endfeet for the expanding EGLM and a few descending ones that reach into the underlying GM (Figure 5A-5E). Also, first lamina special astrocytes become the precursor of the GM protoplasmic astrocytes that supply endfeet for its developing microvasculature (Figures 5, 6). First lamina astrocytes descending processes establish contacts with the GM growing capillaries (Figure 5A, arrows). Losing their EGLM attachment and reabsorbing their trailing processes They are transformed into GM' protoplasmic astrocytes (Figure 6A, B). The GM vascularization is an ascending and stratified process and the distance between first lamina and its growing capillaries remains essentially constant (29).

First lamina astrocytes descend and become protoplasmic astrocytes during the GM ascending vascularization. Later in development and postnatally, both first lamina astrocytes and GM protoplasmic astrocytes regenerate in-situ and contribute endfeet to both the expanding EGLM and its microvascular system. Other different opinions have been expressed concerning the SGR nature and developmental role (29).
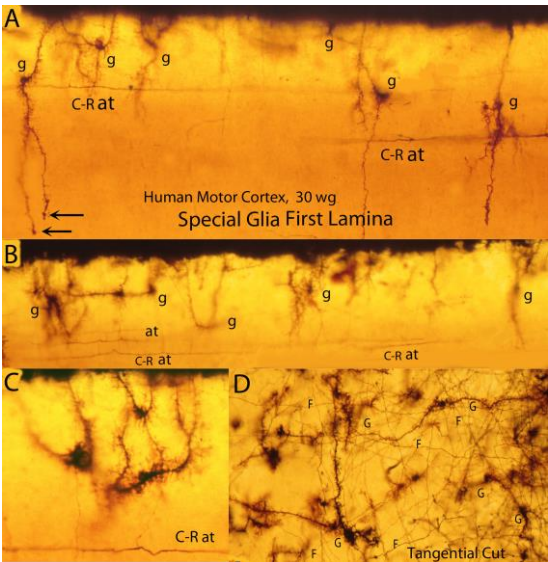
Figure 5. Color photomicrographs from the motor cortex of a 30-w-g infant showing the first lamina special astrocytes (comet cells) variable morphology, from Golgi and GFAP preparations. A. Illustrate first lamina astrocytes with ascending processes that contribute endfeet for the maintenance of cortex EGLM. Some of them also have descending processes that contribute endfeet (arrows) to GM blood vessels. Cajal-Retzius cells horizontal axons (C-R at) are also illustrated. B. Further views of first lamina special astrocytes variable morphology. C. Higher power views of a first lamina special astrocyte with ascending processes contributing endfeet to the cortex EGLM. D. First lamina tangential view showing, from this perspective, the variable morphology of its special astrocytes among Cajal-Retzius crisscrossing axons terminals. Glial fibrillary acidic protein (GFAP) preparation of the cortex first lamina show the large number of special astrocytes and their uninterrupted endfeet' contribution to the EGLM. (From: Marín-Padilla, 1995).
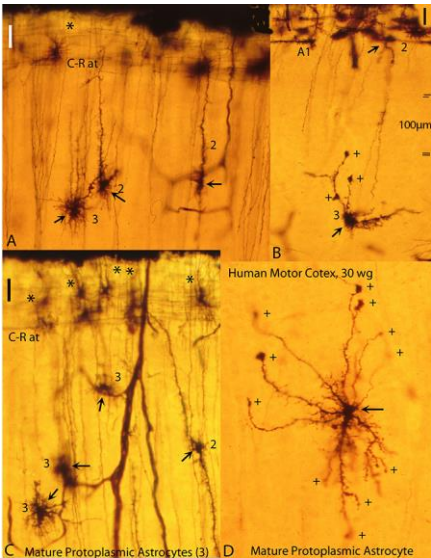

Figure 6. Montage of color photomicrographs showing various developmental stages of GM protoplasmic astrocytes evolving from first lamina special astrocytes, from rapid Golgi preparations of newborn infant's motor cortex. A. Two developing GM protoplasmic with trailing processes still attached to the EGLM (2) and a mature one (3) already disconnected. A few first lamina astrocytes are also illustrated. B. A matured protoplasmic astrocyte (3) with long processes with terminal endfeet (+) contacting local blood vessels. C. Views of immature protoplasmic astrocytes (2) with trailing processes and a mature one (3) among GM vessels. A few first lamina astrocytes (*) are also illustrated. D. Mature GM protoplasmic astrocyte (arrow) with long bifurcating branches that ends in endfeet (+) vascular contacts. The vascular territory of a single GM protoplasmic astrocyte may be quite large. (From: Marín-Padilla, 1995).

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A. Subpial Hemorrhagic Injury with EGLM Damage.

The subpial hemorrhages occur because its sprouting, perforating and interconnecting capillaries are particularly vulnerable to anoxia. The subpial region components, include: a) the meninges pial anastomotic capillary plexus (PACP) from which all perforating vessels that enter the cortex originate; b) the cortex external glial limiting membrane (EGLM) composed of adjoining glial endfeet that separates and protects the nervous tissue from surrounding ones; and, c) the first lamina neuronal, axonic fibers and glial components. Perinatal subpial hemorrhagic injuries range from small (microscopic) to medium to extensive ones and, often, are multiple (Figure 7A). Subpial hemorrhages damage the cortex EGLM and the underlying GM neurons (Figure 7A, 7C, 7E). Large subpial hemorrhages are destructive lesions, often incompatible with life and offer no information concerning their developmental neuropathology. In contrast, smaller bleeds are compatible with life and hence , their neuropathology could be explored. Depending on the children' survival, subpial hemorrhages go through acute, subacute (healing) and chronic (repaired) stages.

Acute Injury. Subpial damage earliest feature is edema of the EGLM glial endfeet with elongation of local perforating vessels (Figure 7B, 7D, arrows). If the edema persists or increases, the rupture of local perforating vessels causes the subpial hemorrhage (Figure, 7C, E, arrows). Phagocytic microglial and macrophages invade the damage site and start removing the necrotic debris. Reactive fibrous astrocytes with long unbranched processes are also recognized through the damage region (Figure 8B). The size of subpial damage depends on that of the hemorrhage and ranges from microscopic to rather extensive ones.

Subacute (healing) Injury. Regenerating new blood vessels re-vascularize the damaged region establishing a new anastomotic microvascular system (Figure 8B). Post-inflammatory new blood vessels may lack the functional properties of the cortex intrinsic capillaries and may respond inadequately to local functional demands (14). The amputated terminals of afferent axons and radial glial fibers regenerate throughout the damage site (Figure, 8A, B). These regenerating fibers are characterized by fine and long searching filopodia and by growth cones (Figure 8B). The regenerating radial glial fibers supply new endfeet for the reconstruction of the damaged EGLM. The EGLM reparation could occur either at the cortex surface and/or within the meningeal space (Figure 8B). First lamina reactive gliosis and glial scars have been described in epilepsy.

The size of EGLM disruptions ranges from microscopic to rather extensive ones (Figure 9A-9F). The EGLM rupture result in the exodus of underlying GM elements (neurons, axonic fibers, radial glial fibers, blood vessels and glia cells) into the subarachnoid space (Figure 9A-9F). Displaced cortical elements within the meningeal space survive, reestablish

new functional interconnections and develop a new interconnecting microvascular system. The resulting lesion is known as local leptomeningeal heterotopias (LMHs). LMHs become permanent features in the brain of affected children and are often associated with epilepsy (Figures 9, 10, 11). Dendrotomized pyramidal neurons survive and are transformed into irregular stellate neurons (Figure 8A, 9B). The regenerating axonic terminals re-establish new post-injury functional connections with the dendrotomized pyramidal neurons and new post-injury functional activities are reestablished throughout the damage region. The number of displaced cortical neurons into the meningeal space depends on the size of the EGLM disruption (Figure 9, 11).
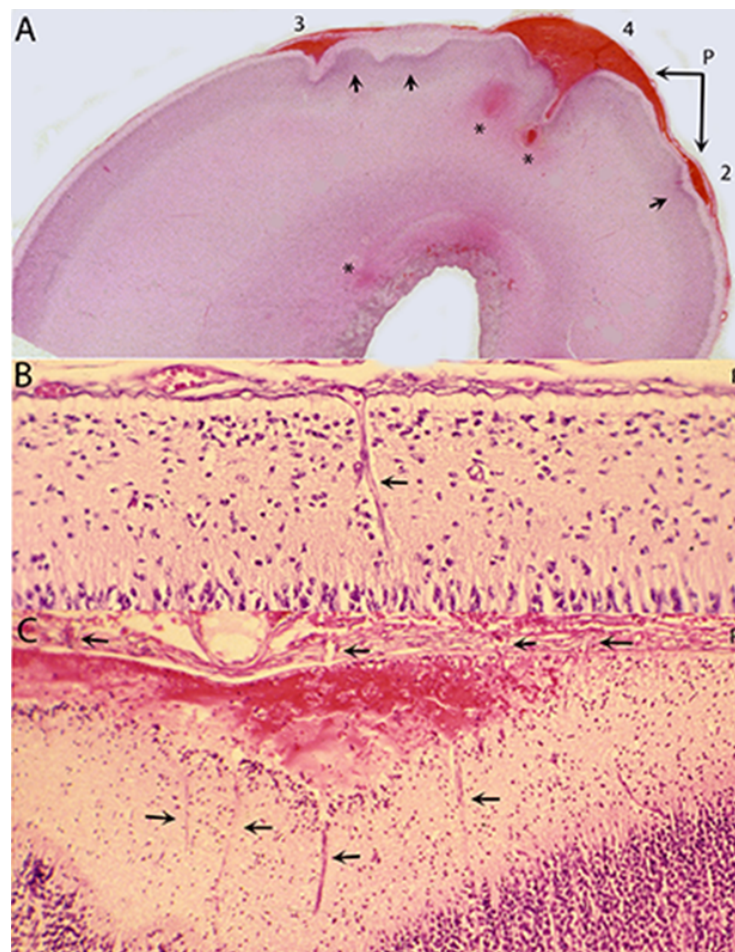
Figure 7. Color photomicrographs from postmortem brain H&E studies of premature infants with respiratory difficulties and subpial hemorrhagic damage. A. General view of the motor cortex with subpial hemorrhages ranging from microscopic to larger one from a 28-w-o premature infant with respiratory difficulties. In most hemorrhage the cortex EGLM is still intact (P) while the underlying gray matter (arrows) is often affected and deformed. B. The earlier sign of subpial damage is edema of the endfeet of radial glial fibers with the elongation (arrow) of local perforating vessels. C. If the edema persists and/or increases, the rupture of local perforating vessels (arrows) causes the subpial hemorrhages. Large subpial hemorrhages will injure the underlying gray matter, dendrotomize local pyramidal neurons terminal dendrites, amputate the local radial glial fibers and axons terminals and could damage the cortex external glial limiting membrane (EGLM) resulting in additional developmental neuropathologies. (From: Marín-Padilla, 1996).
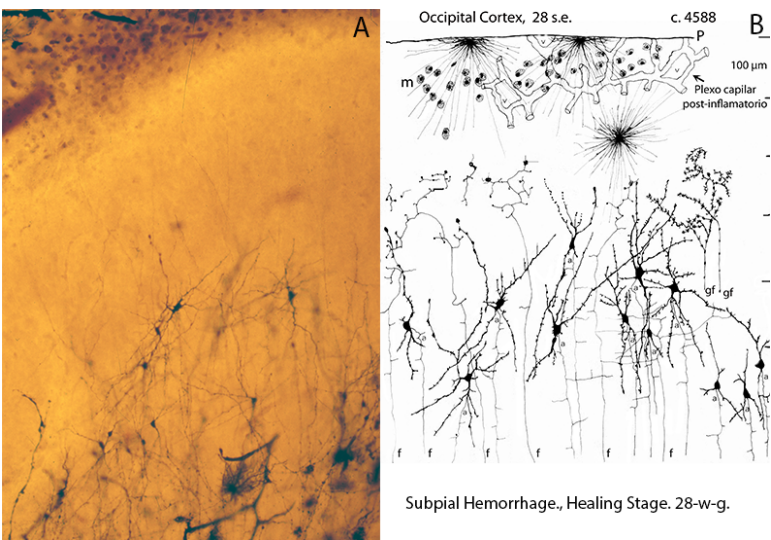
Figure 8. Color photomicrograph of a Golgi preparation (A) and a camera lucida drawing (B) illustrating some aspect of a subacute (healing) subpial hemorrhagic injury developmental neuropathology, from the occipital cortex of a 28-w-g premature infant. A. Hemosiderin carrying macrophages fills the damaged site and several pyramidal neurons with dendrotomized apical dendrites. Dendrotomized pyramidal neurons survive and are transformed into stellate ones. B. Camera lucida drawings of the injured site showing: the stellate morphology of dendrotomized pyramidal neurons, the early regeneration a distribution of amputated axons and radial glial fibers, a new post-injury anastomotic vascular plexus, macrophage carrying cellular debris and numerous reactive fibrous astrocytes with long unbranched filaments explaining the lesion reactive gliosis. (Case 11, Table, 1).
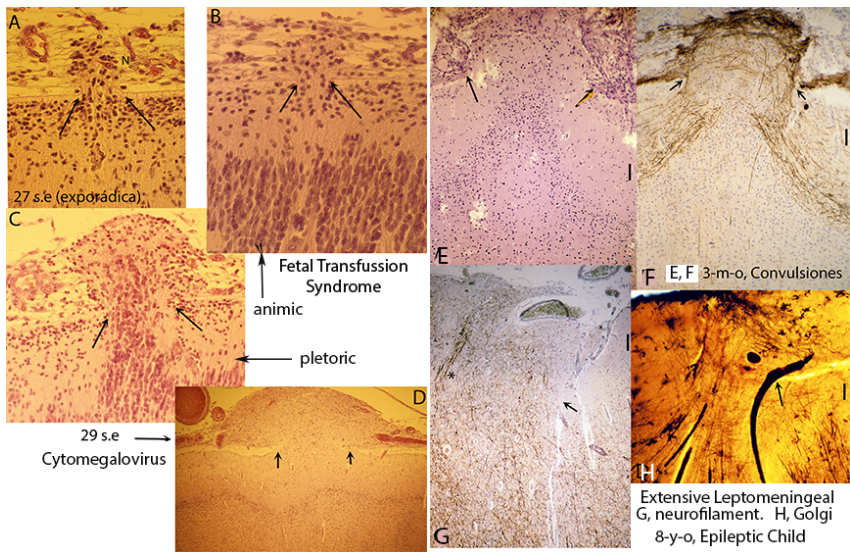

Figure 9. Photomicrographs of various leptomeningeal heterotopias (LMHs) caused by the rupture of the cortex external glial limiting membrane (arrows) with the subsequent exodus of cortical elements into the meningeal space. A. Small incidental LMH with a displaced large cortical neuron into the meningeal space. B. & C. Photomicrographs of the LMHs found in the cortex of monozygotic twins with transfusion syndrome, more extensive and severe in the plethoric one. D. One of several LMHs from the brain of a child with cytomegalovirus infection. E. Hematoxylin and eosin preparations of a LMH from a 3-m-o infant with convulsions who died with severe bronchopulmonary dysplasia. The large displacement of cortical elements into the meninges through the EGLM rupture (arrows) and

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the first lamina obliteration are noticeable. F. Neurofilament stained preparation of the LMH (E) showing the massive entrance of axons fibers from the underlying cortex into the meningeal space through the EGLM rupture (arrows). Such a massive deviation of axons fibers into the meningeal space implies functional alterations as well. G & H. Neurofilament (G) and Golgi (H) preparations of extensive LMHs bordering an occipital porencephalic cyst from an 8-y-o epileptic child, whom drawn in the home swimming pool, showing the extensive migration of axons, neurons and blood vessels into the meningeal space. Figures 9 E-H illustrate the developmental neuropathology of LMHs chronic stages. The cortex cytoarchitecture under LMHs is significantly altered with bizarre post-injury neuronal alterations (see also Figures, 10 and 11).
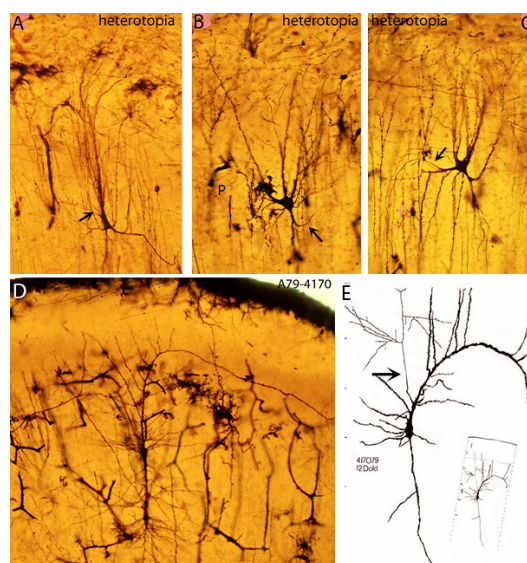

Figure 10. A series of photomicrographs from Golgi preparations showing post-injury altered large hypertrophic GM neurons underlying a LMH from a 34-w-g premature infant (A- E) who lives 18 day and died in respiratory failure. A, B, C. Color photomicrographs of post-injury altered large hypertrophic neurons (second/third cortical strata) with long ascending dendrites that penetrate into the LMH, large somas and axons that originate from one of their dendrites (arrows). A normal (unaffected) pyramidal neuron (P) of the same cortical strata (B) is illustrated for comparison. D. Examples of two (superimposed) post-injury altered hypertrophic neurons from an undamaged cortical region adjacent to the LMH. One of these altered hypertrophic neurons (D) has bizarre ascending dendrites and a long one that bends under the first lamina and extends for a considerable distance. E. Camera lucida drawing of a large post-injury altered hypertrophic neuron, from a region adjacent to the LMH, with a large and curved ascending dendrite with several ascending collaterals, few basal dendrites and a descending one. Its axon (arrow) originates from the main ascending dendrite. These neurons post-injury morphological alterations imply functional alteration as well.
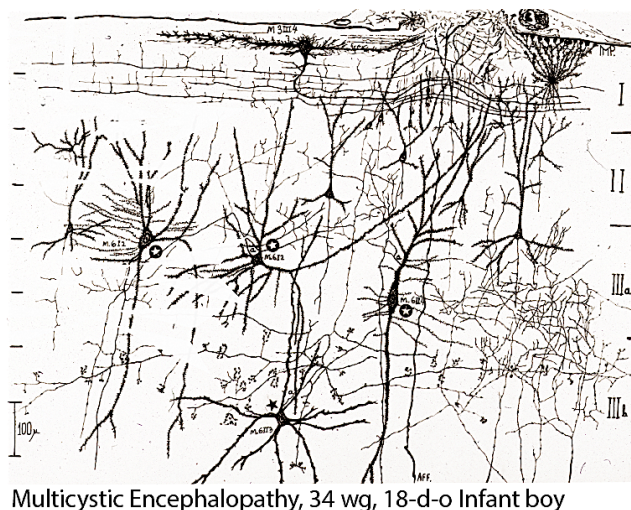

Multicystic Encephalopathy, 34 wg, 18-d-o Infant boy

Figure 11. Composite camera lucida drawing illustrating some aspects of the developmental neuropathology of a small LMH and of the underlying cortical laminae as well as those from adjacent undamaged cortical region, from postmortem Golgi preparations of a 34-w-g, 18-d-o infant with multicystic encephalopathy. Cajal-Retzius cells' long horizontal axons bend under the EGLM rupture without

entering into the LMH. The terminal dendrites of some of post-injury transformed cortical neurons as well as afferent fibers penetrate the LMH. Several large post-injuries altered hypertrophic neurons (white stars) under the LMH as well as from adjacent undamaged cortical regions are illustrated. A post-injury transformed inhibitory basket cells (black arrow) with a large axon spread and numerous perisomatic baskets is also illustrated. The cortex intrinsic and extrinsic neuropil, under the LMH are increased. Unaltered local pyramidal neurons are also drawn for comparison. The cortical neurons post-injury morphological alterations imply functional alterations as well (Case 18, Table 1).

Chronic (repaired) Injury. Below the LMH, the cortex first lamina is obliterated by the displacement of elements from underlying layers and its meningeal demarcation is unrecognizable (Figure 9C, 9E, 9G). The cortex, underlying LMHs, is altered with a variety neuronal, microvascular and axonic alterations and fibrous astrocytes gliosis (Figures 10A-10E and 11). The first and second laminae demarcation also are obliterated (Figure 9G, 9H). EGLM remnants may be recognized within meningeal tissue. GM neurons morphology is altered (Figures 8, 10, 11).

The apical dendrites of transformed pyramidal neurons may be multiple, long and irregular and their original morphology is unrecognizable (Figures 10A-10D, 11). Some neurons assume bizarre, irregular and stellate morphologies (Figure 10A-10C, 11). Some may represent hypertrophic inhibitory basket cells because pericellular nests are found around some neurons (Figure 11). The long terminal dendrites of transformed pyramidal neurons penetrate the leptomeningeal space (Figure 10A-10C). The axon of these transformed hypertrophic neurons often arises from one of the dendrites at a considerable distance from their soma (Figures 10A-10E, 11). The intrinsic neuropil within the LMHs is increased.

EGLM damage has been described in the literature under different terminologies: brain warts, marginal heterotopias, sulci fusion, ulegyria, agyria and pachygyria, to mention a few (Figure, 9). These terminologies are unrelated to the previous EGLM rupture. The term 'leptomeningeal heterotopia' (LMH) secondary to EGLM damage, used herein, may be more appropriated. The LMHs size varies considerably, ranging from microscopic without dysplastic alterations to extensive ones with extensive neuronal alterations including the presence of large hypertrophic neurons. LMHs have been described in epilepsy (4, 28).

A new post-injury microvascular system is re-established throughout the damage region that interconnects first lamina and leptomeningeal elements. Irregular fibrous astrocytes with long unbranched processes contribute to the first lamina reactive gliosis. Pigmented macrophages, fibroblast-like meningeal cells and irregular fibrous astrocytes are also recognized throughout the region. An interesting observation is the bending of C-RCs horizontal axons under the EGLM disruptions by-passing the defect without entering into the meningeal space (Figure 11).

B. Periventricular (Germinal Matrix) Hemorrhagic Injury with Radial Glia Damage

Before describing the developmental neuropathology of periventricular hemorrhages (PVHs), the region neuroanatomy and neurohistology are briefly described. The periventricular matrix zone is composed of innumerable closely packed, undifferentiated and migrating cells and by numerous radial glial fibers. It is vascularized by a few of the GM arterial perforating vessels and by the extensive anastomotic plexus of thin-

walled venules established among them. Early in development, it is crossed by numerous ascending radial glia fibers attached to both ependymal epithelium and the cortex EGLM that serve as guides for the ascending migration of both neuronal and glial precursors (29). The periventricular matrix zone is an ephemeral region that diminish progressively as its cellular precursors migrated into the developing cortex. It disappears, late in development, when all its precursors cells have migrated out of it. The periventricular matrix is the source of both GM neurons and glial precursors for the developing cortex (29).

The matrix zone neurogenesis for GM neurons starts around the 8th w-g and by the 16th w-g, it has essentially terminated. During this time, the developing GM is composed of an increasing number of immature and spineless pyramidal neurons of different sizes anchored to the first lamina by their terminal dendrites and by equidistant perpendicular perforating blood vessels without interconnecting capillaries (Figure 2A). From the 16th to late in gestation, the periventricular matrix zone is a thick, hypercellular and poorly vascularize zone composed of undifferentiated neuronal and glial precursors that ascend into both the WM and GM using radial glial fibers as guides. The zone reaches its greater thickness around the 24th w-g., its thickness diminishes progressively and by the time of birth (40th g-w) is barely recognizable (28). By birth time, a narrow band of undifferentiated cells may still be recognized above the ependymal epithelium. Progressively, the expanding WM occupy the germinal matrix original space.

Also, the periventricular matrix zone is the source of a late ependymal to pia migration of glial cells precursors, via radial glia fibers, that will establish the ephemeral subpial granular layer of Ranke (SGR) throughout the cortex first lamina. Perhaps, this late developmental role explains the radial retention beyond the 17th - 18th w-g, when all GM neurons precursors have already migrated out it.

The WM and the periventricular region are vascularized by a few GM arterial perforators that reach the periventricular region and establish an anastomotic plexus of venules throughout the region (5). As the matrix zone cellular precursors ascend into the developing cortex, its thickness diminishes progressively and consequently many of its venules regress and, eventually, disappear. Regressing matrix venules are particularly vulnerable to perinatal asphyxia. Their rupture is the main source of periventricular hemorrhages (5, 6).

Periventricular hemorrhages (PVHs) are frequent types of bleeds in prematurely born infants and those suffering from perinatal asphyxia. Initially, PVHs are microscopic and multiple with a tendency to coalesce into larger and often devastating ones (Figure, 12D). Their size ranges from microscopic, to small (Figure, 12A, 12D), to rather extensive ones (Figure 12B, 12C, 12D). PVHs could damage the ependymal epithelium resulting in lethal intraventricular hemorrhages (Figure 12B). PVHs etiology and developmental neuropathology have been seldom investigated.

Acute injury. PVHs are destructive lesions and everything involved is rapidly destroyed, including the original injured vessels. By the time most PVHs are studied in postmortem

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brains, it is already too late to demonstrate their original vascular damage. It will be necessary to study the periventricular matrix zone in all children who have died from prematurity or neonatal asphyxia even if the PVH are not yet visible to the naked eye. Neuropathologic studies of the periventricular region of premature infants who have died from asphyxia have demonstrated endothelial cell damage in some matrix venules, possible regressing ones (Figure 12F). The PVH central venule shows focal endothelial cell necrosis with formation of a fibrin thrombus attached to the injured wall (Figure 12F, a, c). Often, the thrombus ejects through the injured vessel wall (Figure 12F, b). The damaged matrix venules are probably regressing at the time. Damage venules are rapidly destroyed by the hemorrhage. PVHs could also damage the ependymal epithelium causing lethal intraventricular hemorrhages (Figure 12B, 12C, 12E).
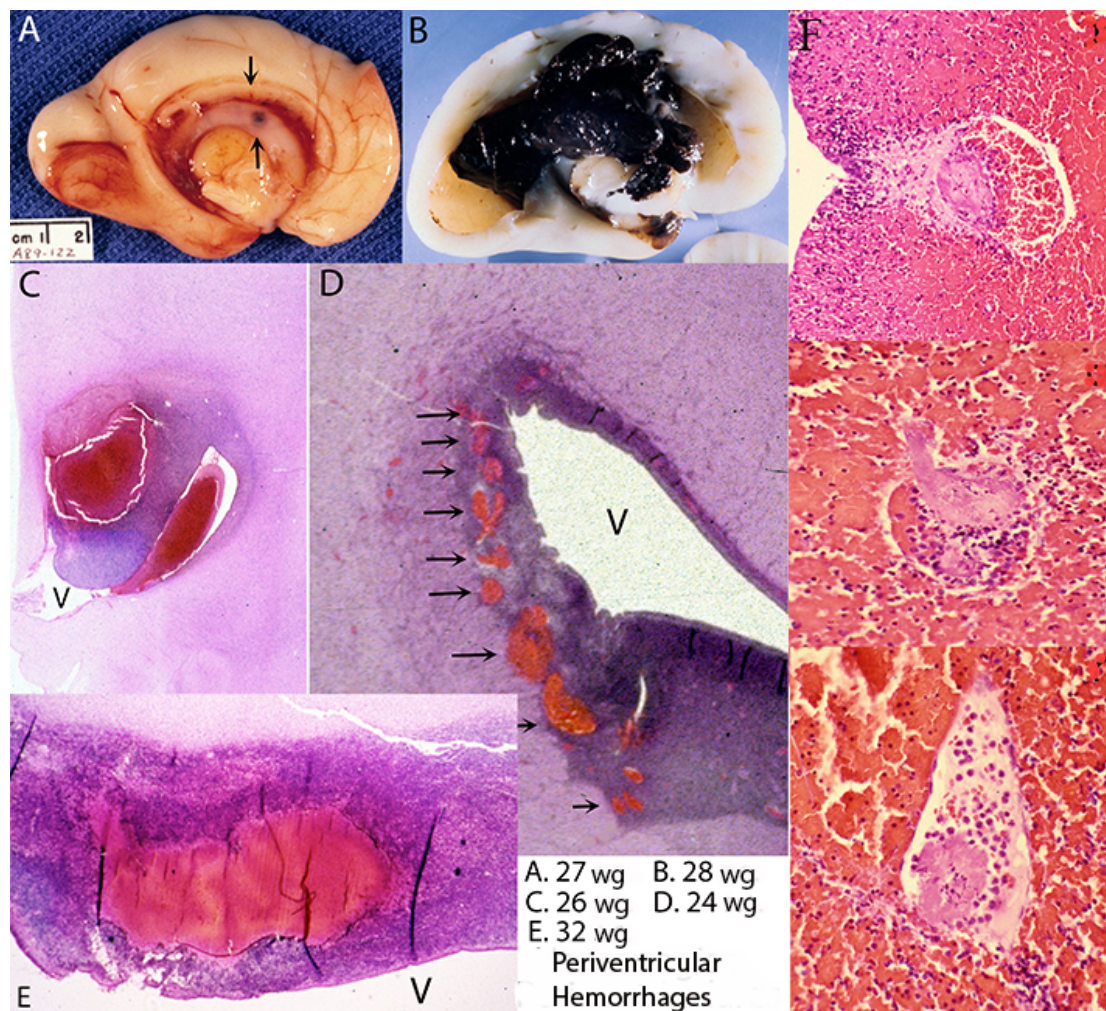

Figure 12. Composite figure illustrating various neuropathological aspects of periventricular hemorrhages (PVHs), including microscopic (**A**) and large (**B**) bleeds as well as lethal intraventricular hemorrhages (**B, C**). **D**. General view of the periventricular matrix zone and ventricle from the post-mortem brain study a 24-w-g premature infant illustrating the multiple origins (arrows) of early PVHs, including their tendency to coalesce into larger ones. Even the smaller PVHs are no useful to investigate their vascular pathogenesis

because the original damaged vessel is already destroyed. **F**. Examples (a, b, c) of the early endothelial cell injury of matrix venules in periventricular hemorrhages not yet visible to the naked eye. The local endothelial cell damage is often accompanied by the formation of a fibrin thrombus attached to the injured vessel wall (a, c). The fibrin thrombus is often ejected (b), with the bleed, through the ruptured vessels wall. (Cases: 4, 5, 8, 11, 21, Table, 1).
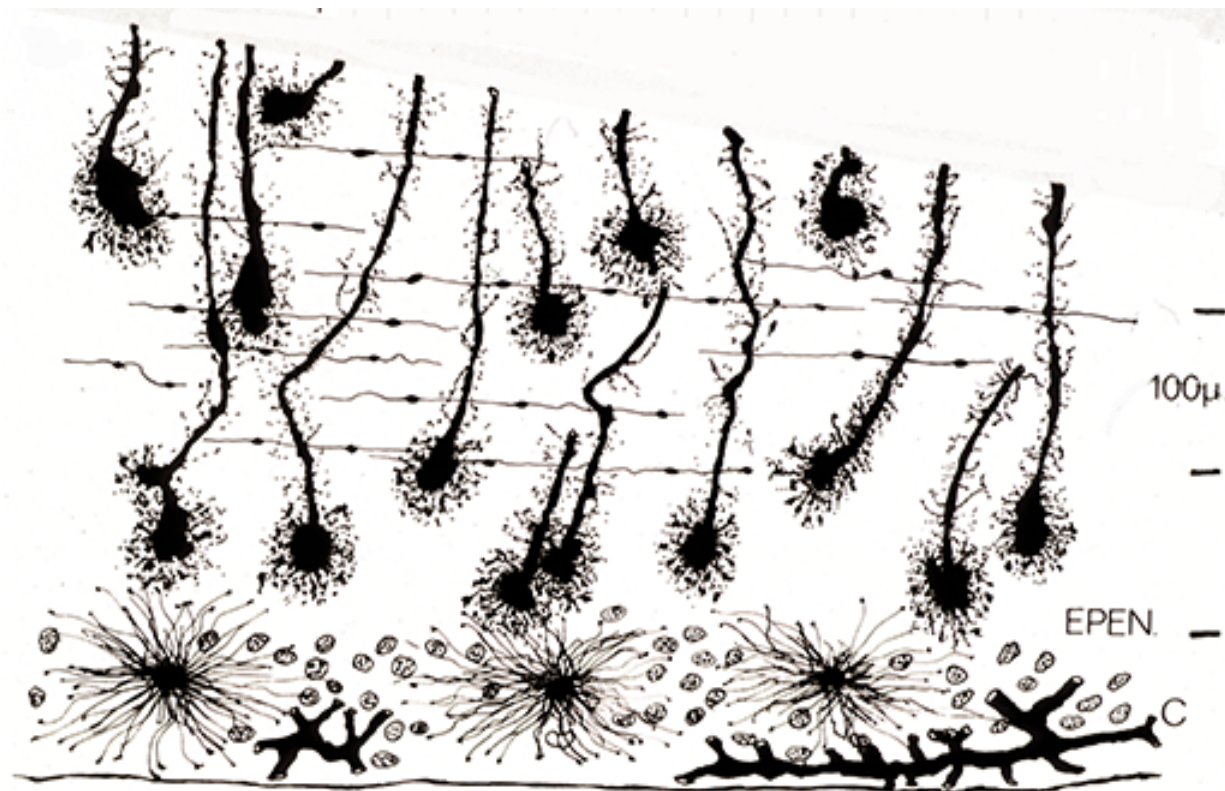

Figure 13. Camera lucida drawings, from an early PVH, showing the retracting stumps (balls) of damaged radial glial fibers, first described by Cajal. The retracting stumps and the fibers of damaged radial glia are covered by fine filopodia, both heavily stained in Golgi preparations. Numerous reactive fibrous astrocyte and macrophages are found throughout the healing ependymal region. A new post-injury anastomotic vascular plexus is also established throughout the healing ependymal region. Scale 100 μm.

Subacute (healing) Injury. The hemorrhagic site is invaded by both microglial and macrophages that rapidly remove the necrotic debris. New blood vessels sprout from undammed perforators and invade the damaged site establishing a post-injury microvascular plexus through the region (Figure 13). Fibrous astrocytes increase throughout the damaged site and contribute to the region reactive gliosis and glia scar (Figure 13). The periventricular fibrous astrocytes often acquire irregular and bizarre morphologies. Rosette-like formations of ependymal cells are also observed through the healing zone.

The PVHs most important neuropathological feature is the destruction of local radial glia fibers interrupting the ascending migration of both neuronal and glial precursors to the developing cortex. The dislodged precursors cells form small cellular nodules scattered

throughout the periventricular and WM lower regions. Eventually, these nodules of undifferentiated neuronal and glial precursors become the source of the periventricular glial-neuronal heterotopias, which often described in clinical epilepsy. Large PVHs could also damage the overlying white matter lower regions resulting in additional post-injury neuropathology. The regeneration of the ependymal epithelium reestablishes the region anatomical integrity. Pigmented macrophages, reactive astrocytes with long processes aligned parallel to the ependymal surface and micro-calcifications are also found through the healing zone (Figure 13). An important neuropathological observation in Golgi preparations is the presence, throughout the damaged region, of numerous retracting stumps (balls) of degenerating radial glial fibers (Figure 13). A terminal club-like excrescences with radiating filopodia, first described by Cajal, characterize regression of the radial glia fibers.

Chronic (Repaired) Injury. The repair of small PVHs is a rapid process without significant glial scarring or clinical repercussions. However, the repair of larger PVHs invariably results in permanent neuropathological lesions through the region that would eventually impinge on the maturation of affected children brain. A permanent periventricular scar is formed through the damaged region composed of reactive fibrous astrocytes with long unbranched processes, a new post-inflammatory microvascular system, neuronal and glial cells nodules and rosettes-like nodules of residual ependymal cells (Figure 13). These complex periventricular lesions have been described in the literature with the unfortunate term of 'periventricular leukomalacia' are often associated with epilepsy (30). PVHs most relevant neuropathological feature is the presence of periventricular glio-neuronal heterotopias (Figure 14A, 14B).

Periventricular heterotopias are cellular nodules composed of excitatory and inhibitory neurons, intrinsic and extrinsic axonic fibers and an independent microvascular system (Figure, 14A, B, C). Their proximity to the WM permits the mutual interchange of axonic fibers guarantying their independent functional activity (Figure 14B). Functionally active PVHs may become permanent lesions in the brain of children who survived the injury as well as the source of the altered neuronal activity of epilepsy.

4. PERINATAL WHITE MATTER HYPOXIC-ISCHEMIC INJURIES

The cortex white matter (WM) is composed of different types of fibers (corticipetal, corticofugal, interhemispheric and cortico-cortical) that interconnect various brain regions, a few scattered interstitial neurons and both fibrous astrocytes and oligodendrocytes. Early in development, it is crossed by radial glial fibers that run from ependymal to pial surfaces and serve as guides for ascending neuronal and glial precursors (6). Its interstitial neurons may be remnants of early relay stations between lower brain centers and the cortex.

The WM and tperiventricular matrix zone are vascularized by a few GM arterial perforating vessels that penetrate vertically, reach the periventricular zone and establish an anastomotic plexus throughout the region. Vessels emerging from these few arterial perforators establish an extensive anastomotic plexus of thin-walled venules. In the WM, blood circulates independently but slowly between the few arterial perforators and the extensive anastomotic venules plexus among them. The system drains into the periventricular venous plexus and through the Rosenthal veins into the brain ventral venous circle and through the ampulla of Galen into the dural venous sinus (14).
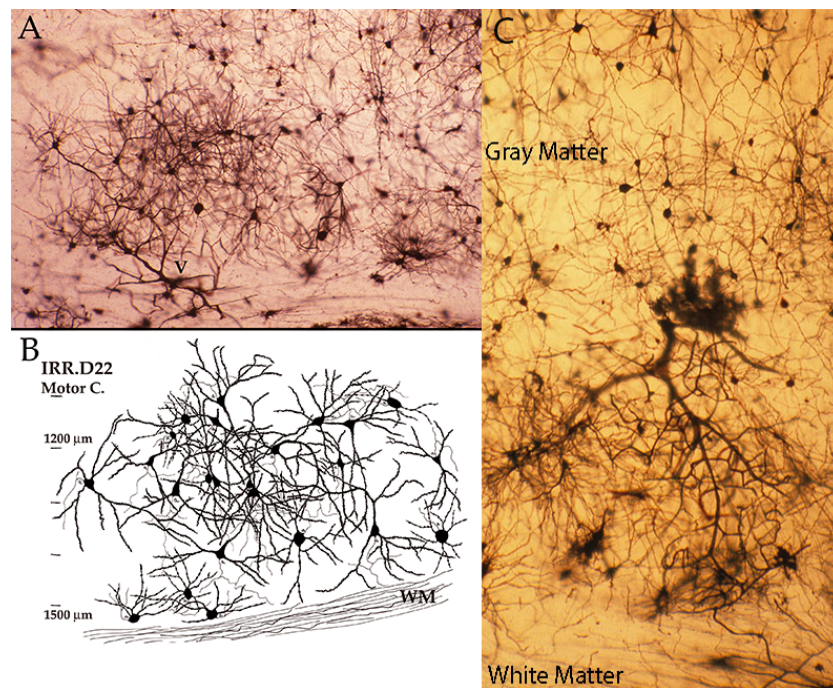

Figure 14. Golgi preparations (**A, C**) and camera lucida drawing (**B**) of experimentally induced periventricular heterotopias in pups of irradiated pregnant rats, showing their post-injury altered neuronal, fibrillar and microvascular composition as well as their close association to the white matter. Periventricular heterotopias secondary to PVHs are cellular nodules composed of both excitatory and inhibitory neurons with both intrinsic interconnections as well as extrinsic ones from the adjacent white matter. Periventricular heterotopias develop their own microvascular system and function as independent structures. **A & B.** Golgi preparation and a camera lucida drawing of irradiation induced nodular periventricular heterotopia showing altered excitatory (larger) and inhibitory (smaller) neurons with bending dendrites within the nodule and its proximity to the white matter permitting the interchange of fibers and functional information. **C.** Golgi preparation of an irradiation induced periventricular heterotopia depicting its independent microvascular system from irradiated rats' pups. (From: Marín-Padilla et al. 2003).

In newborn infants, most WM fibers are still unmyelinated and have collaterals with growth cones moving toward and away from the GM. Its lighter coloration does not become apparent until its fibers are myelinated. Myelinization starts at the end of prenatal life and is completed postnatally. The Cajal-Retzius cells long horizontal axons are among the earliest cortical elements to myelinate. WM fibers start crossing between the cerebral hemispheres around the 12th week of gestation and by the 18th a distinct corpus callosum is recognized.

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The WM, with fewer arterial perforators and an extensive anastomotic venous plexus, is particularly vulnerable in perinatal hypoxic-ischemic injury and the destruction of its fibers is both rapid and extensive (Figures 15, 16, 17). Microglial and macrophages invade the damage region to remove the necrotic debris resulting in small empty spaces that eventually expand into larger cavities (Figure 16, 17C).
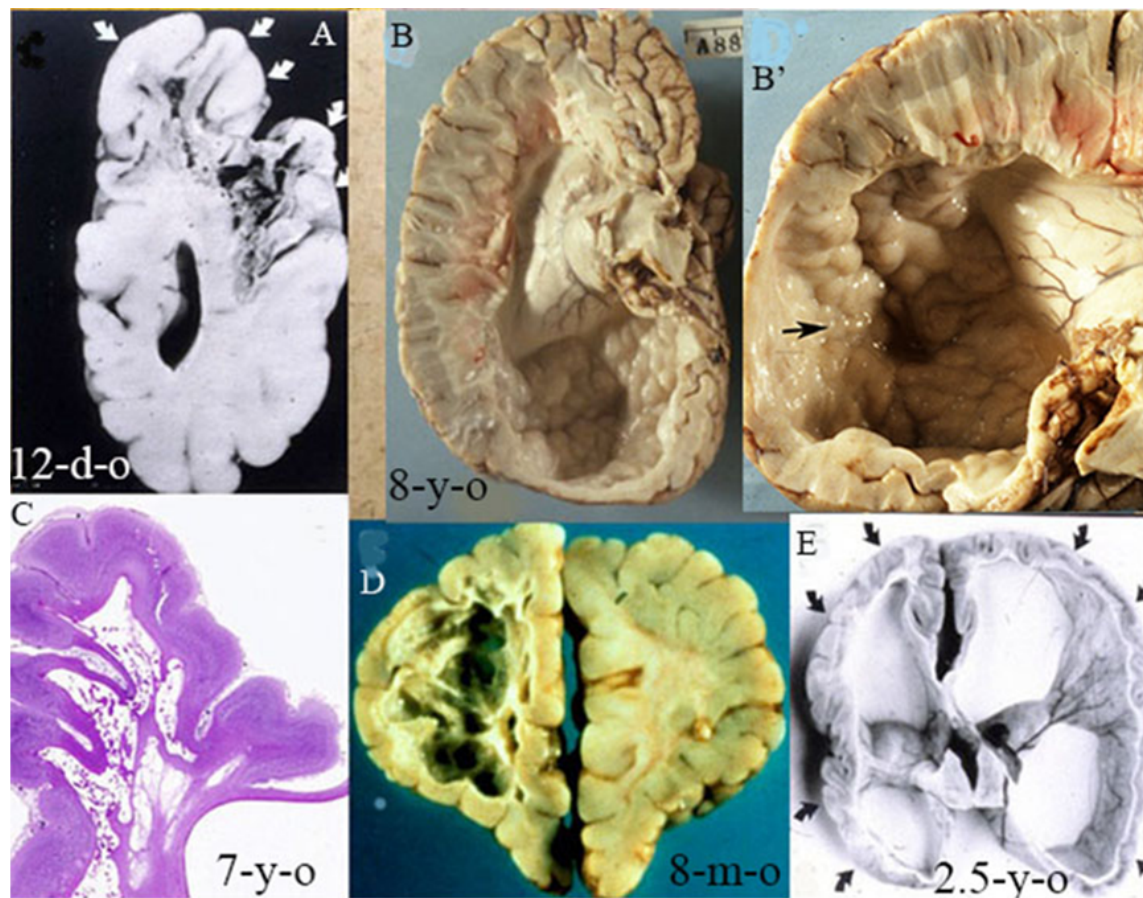
A: Recent WMI; B: Porencephaly; B': Inside view; C: Ulegyria; D: Old WMI;
E; Encephaloclastic Hydranencephaly; B, C, E; Epilepsy; D: Convulsions

Figure. 15. Anatomical color photographs of children' brains with extensive white matter (WM) hypoxic-ischemic infarcts with the survival of the overlying and functionally disconnected GM (arrows) in all of them. **A**. Recent WM infarct with early cavitations and hemorrhages with survival of the overlying disconnected GM from an infant who survival the damage for 12 days. **B**. Extensive unilateral occipital porencephaly with the survival of the disconnected GM, from an 8-y-o epileptic child. **B'**. Inside view of the porencephalic cavity showing isolated rounds structures representing GM sulcus essentially deprived of WM tissue and smaller nodules (arrow) representing periventricular heterotopias. **C.** Damaged brain from a 7-y-o epileptic child with ulegyria, extensive WM damage, glio-vascular trabeculi, significant reduction of the corpus callosum and the survival of the overlying and disconnected GM. **D.** Remote left frontal lobe multicystic encephalopathy with glio-vascular trabeculi with the survival of the overlying GM, from a 8-m-o child with history of convulsions. The unaffected right frontal lobe (illustrated) will have post-injury neuropathological alterations as well, involving its neuronal, fibrillar and microvascular systems. **E.** Bilateral encephaloclastic hydranencephaly with overall survival of the overlying and disconnected GM, from a 2.5-y-o child with convulsions.
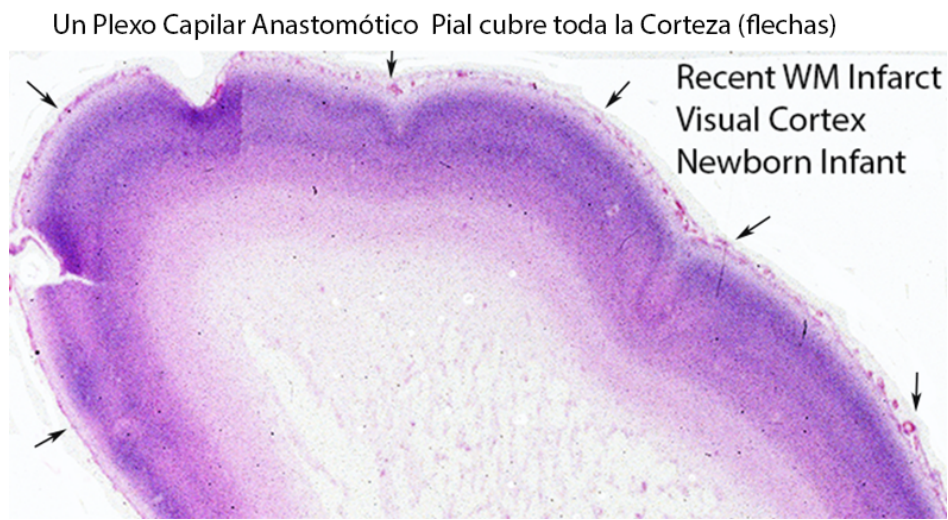
Figure 16. Anatomical color photograph of a recent WM hypoxic-ischemic infarct showing early cavitation and the survival of the overlying and disconnected GM. The GM is still covered by the pial anastomotic capillary plexus (the meninges were not removed) the source of all its perforating vessels. The overlying and disconnected GM has survived the damage. From: a 38-w-g, 12-d-o, infant who died with respiratory distress syndrome. (Case 14, Table. 1).
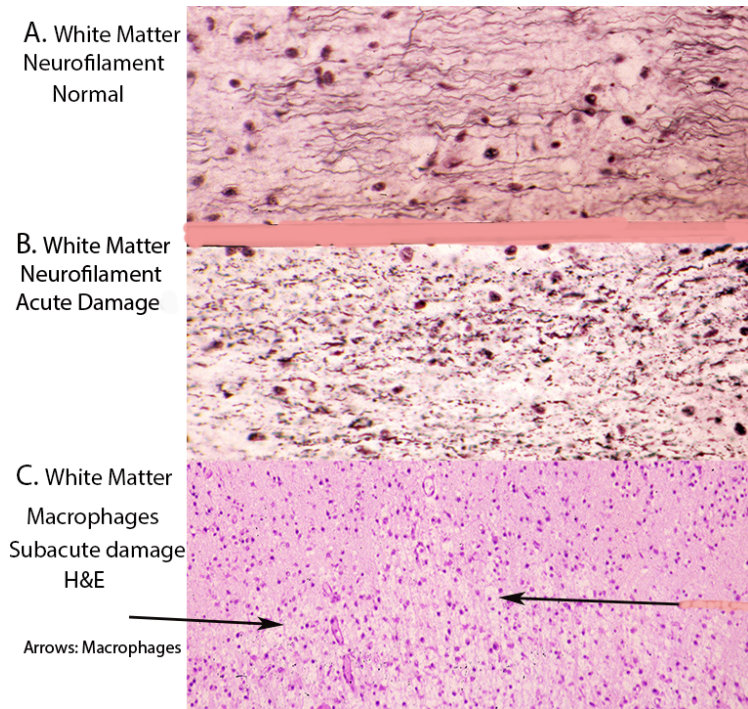

Figure 17. Photomicrographs from neurofilament (**A, B**) and H&E (**C**) preparations of recent WM infarcts illustrating the damage fibers early neuropathological alterations. **A**. Neurofilament preparation of unaffected WM axons showing their fine and wavy appearance as well as some oligodendrocytes. **B**. Detail of the early axon fibers fragmentation from a recent WM infarct. **C.** Hematoxylin and eosin preparations from an acute WM infarct showing several macrophages (arrows) phagocytizing damaged axons fibers. Damaged WM axons are invisible with this stain.

Only WM infarcts with the survival of the overlying and disconnected GM are analyzed herein (Figures 15, 16). Different terminologies have been used to describe these WM infarcts, including: multicyclic encephalopathy, ulegyria, encephaloclastic porencephaly, ex-vacuo hydranencephaly and encephaloclastic hydrocephaly (Figure 15A-15E). These terminologies are meaningless since all of them represent different degrees of WM infarcts with cavitation and the survival of the overlying and functionally disconnected GM (Figure 15). In WM infarcts, the partially disconnected thalamus and hippocampus also survive.

Several questions will be address in this study: a) Do functionally disconnected GM neurons, incapable of receiving information, survive WM infarcts?; b) what happened to the GM disconnected neurons in infants who survive WM infarcts?; c) are the disconnected WM neurons capable of re-establishing intrinsic interconnections among themselves?; d) would these intrinsic interconnections alter their morphology and function?; e) would these transformations of GM neurons impinge on the brain maturation of surviving children?; and, f) would the GM neurons transformations play a role in the pathogenesis of ensuing epilepsy?

I. *WM infarct Developmental Neuropathology*

The infarcted WM tissue developmental neuropathology include, a) the massive destruction of fibers, interstitial neurons and glial cells throughout the damaged region: b) the removal of debris by microglial and macrophages with subsequent cavitation; and c) the damaged WM fibers undergo a series of rapidly succeeding neuropathologic events including edema, necrosis, fragmentation, macrophage reabsorption and cavitation (Figures 15-18).

A softer and poorly outlined area with mild color changes may be the first indication of a WM infarct. As the damage progresses, the infarcted area' borders become well define and focal hemorrhages and small empty spaces start to appear throughout the damaged zone (Figure, 16). The damaged tissue appears as amorphous proteinaceous debris with empty spaces with both intrinsic and extrinsic phagocytic cells (Figures 16, 17C). The early spaces are irregular, without distinct borders and may be filled with fluid and inflammatory cells (Figure 16). As the damage progresses, the original spaces are transformed into cavities lined with minimal reactive gliosis and filled with fluid and inflammatory cells (Figure 15). Gliovascular trabeculae are formed between adjacent cavities (Figure 15D). The WM fiber destruction and cavitation result in a variety of neuropathological entities, such as: multicystic encephalopathy (Figure 15D), porencephaly (Figure 15B, 15B'), ulegyria (Figure 15C), and encephaloclastic hydranencephaly (Figure 15E). The periventricular region may also be infarcted, with or without cavitation, resulting in the so-called periventricular leukomalacia (see above).
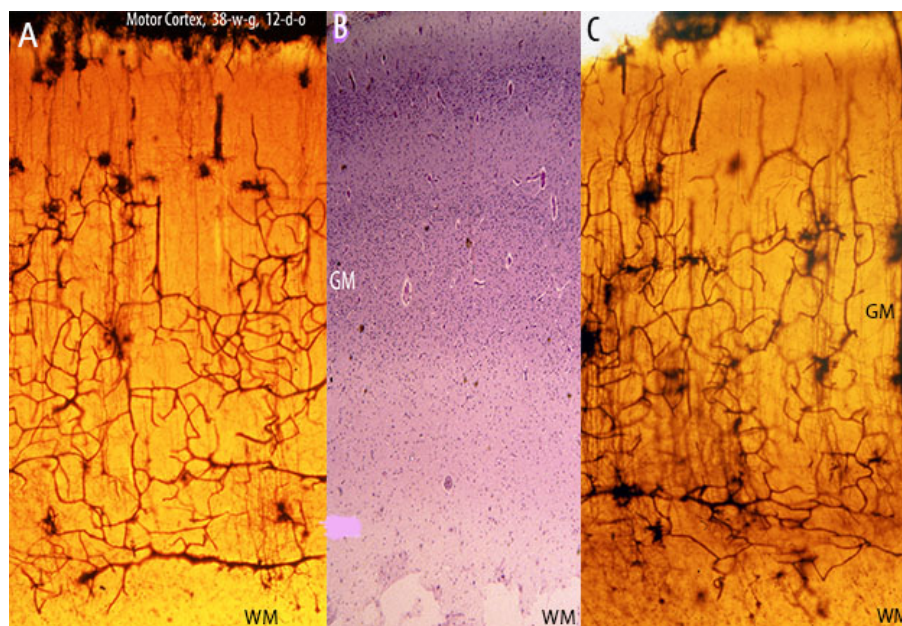
Figure 18. Montage of color photomicrographs from Golgi (**A, C**) and H&E (**B**) preparations showing the survival of the functionally disconnected motor cortex overlying a recent WM infarct. **A, C**. Golgi preparations showing the intact microvascular system of the disconnected GM and the presence of a new post-injury anastomotic vascular plexus bordering the WM infarct that interconnect all arterial and venous perforators, thus, allowing the circulation of blood guarantying both its survival and the post-injury altered functional maturation of its neurons. **B**. Hematoxylin and eosin preparation showing the overlying disconnected GM survival and early cavitations throughout the infarcted WM. Both the cortex overall neuronal cytoarchitecture and laminations are still unaffected throughout the disconnected GM. (Case 14, Table 1).

Axons injury is recognized in Golgi, Bodian and Neurofilament preparations but not with H&E stain (Figure 17). Damaged WM fibers are broken-down into small and heavily stained fragments (Figure 17A, B). Local microglial, macrophages and some fibrous astrocytes phagocytized the necrotic debris. GFAP stains suggest that astrocytes may be capable of phagocytizing the axonic fragments (Figure 17C). As phagocytic cells abandon the damaged site small empty spaces with tendency to coalesce into larger ones are recognized through the damaged WM (Figure 16). The fibers destroyed in WM infarcts is incommensurable. In WM infarcts nothing remains but empty cavities. The implications that such a massive destruction of WM fibers might have upon the disconnected GM neurons have not been adequately investigated.

II. *Developmental Neuropathology of the GM overlying WM Infarcts*

It is important to emphasize that the disconnected GM neurons overlying WM infarcts survive because they retain their microvascular system essentially intact (Figure, 18A, 18B, 18C). Moreover, a venous anastomotic plexus is established bordering the WM infarct that interconnected the disconnected GM arterial and venous perforators (Figure 18A, 18C). This anastomotic venous plexus allows the circulation of blood throughout the disconnected GM by entering through arterial perforator and exiting through contiguous venous ones (Figure

18). This anastomotic venous plexus guaranties not only the circulation of blood throughout the disconnected GM but also its neurons subsequent structural and functional maturations (Figures 18-21). These ongoing developmental processes could impinge on the maturation of affected children' brains and play a role in the pathogenesis of ensuing epilepsy.

The disconnected GM neurons, overlying WM infarcts, survive because they establish intrinsic functional interconnections among themselves. The synaptic vacancies created by the destruction of WM fibers are reconnected by intrinsic fibers. The neuronal alterations observed throughout the disconnected GM are incommensurable, difficult to describe and/or to classify. To some degree, most of the disconnected GM neurons are altered. Both their dendritic and axonic arborizations are altered and many of them become quite large and hypertrophic. Moreover, the disconnected GM neurons alterations are not static but ongoing processes that continue to evolve and to change throughout the affected children' life. The neuronal transformations also change throughout each lesion acute, subacute (healing) and/or chronic (repaired) stages (Table 1).

Acute Injury. The extend of infarcted WM determines that of the disconnected overlying GM. WM infarcts could affect from small cortical regions to an entire cortical lobe (Figures 15, 16). During WM infarcts acute stages, no obvious neuropathological alterations are yet detectable among the disconnected GM neurons (Figure 19A, 19B). Their overall neuronal morphology, distribution and organization of both excitatory and inhibitory neurons are still unchanged (Figures 18, 19). The axons of all GM projective neurons have been amputated by the WM infarct (Figures 20, 21).

Subacute (healing) Injury. Golgi and neurofilament preparations are essential to demonstrate the post-injury alterations of both neurons and intrinsic fibers throughout the disconnected GM. A generalized reduction of afferent axonic fibers is apparent throughout the GM and the corpus callosum' thickness is significantly reduced (Figure 15C, 15E). Degenerating axons are found through the disconnected GM, representing the anterograde degeneration of corticipetal fibers destroyed by the WM infarct. The consequence of WM fibers destruction is that the overlying GM neurons become functionally disconnected from extrinsic inputs.

An earlier neuropathological observation is an increase of intrinsic fibers, among the disconnected GM neurons, already deprived extrinsic ones (Figure 20). The intrinsic neuropil is composed of recurrent axonic collaterals from axotomized pyramidal neurons and the axon terminal axons of local pyramidal neurons, inhibitory basket, double-tufted and chandeliers cells and other neurons (Figure 20, 21A, 21B). Most disconnected GM neurons become functionally interconnected and undergo structural and functional transformations (Figures 20-25).

The axons of deep axotomized pyramidal neurons undergo both anterograde and retrograde degenerations. Their anterograde degeneration is lost through the underlain infarcted WM and their retrograde degeneration stops at the site of emerging collaterals (Figures 20, 21). The axotomized pyramidal neurons axonic collaterals ascend into the GM and become incorporated into its intrinsic neuropil. Cajal first described the recurrent axonic

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collaterals of both axotomized cortical pyramidal (Figure 21B) and cerebellar Purkinje cells in experimental WM lesions in cat brains. Other collateral from axotomized pyramidal neurons growth horizontally, for a long distance, bordering the WM infarct and might be capable re-establishing distant functional interconnections (Figures 20, 21).
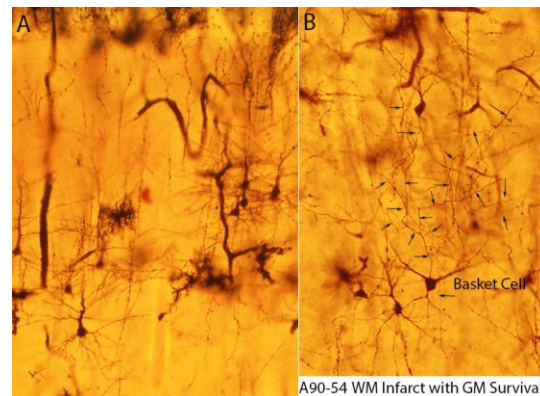

Figure 19. Color photomicrographs from the disconnected motor cortex overlying a recent WM infarct (Figure, 18) illustrating their still unaffected neuronal and microvascular systems **A.** Views of GM disconnected pyramidal neurons, protoplasmic astrocytes, blood capillaries and first lamina especial astrocytes still unaffected. **B.** View of a still undamaged inhibitory basket cell showing the extensive distribution of its ascending axon and collaterals distribution covering the entire depicted region (arrows) as well as some pyramidal neurons and a double-tufted one. Inhibitory basket cells are flat neurons distributed in their entirety (dendrites and axon) within a plane perpendicular the long axis of the gyrus. (Case 14, Table 1).
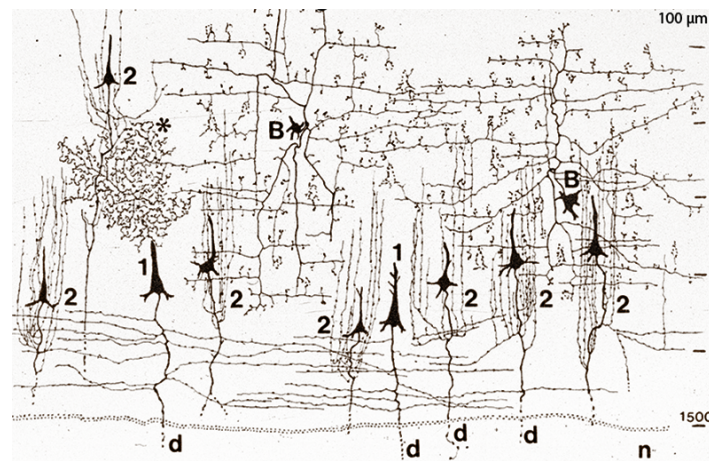

Figure 20. Camera lucida drawings, from Golgi preparations of the cortex of a 38-w-g premature infant who lived 12 days after an extensive WM infarct, showing surviving axotomized pyramidal neurons (1, 2), the extensive distribution of inhibitory basket cells (B) axon terminals and a significant increase (*) of the region intrinsic neuropil. The amputated axons of pyramidal neurons undergo retrograde degeneration that stops at the emergence of collaterals. The axotomized pyramidal neurons' collaterals assume two different post-injury comportments. Some collateral (1) grow horizontally bordering the WM infarct for considerable distances and might be capable of establishing distant post-injury functional interconnections. Other axon collaterals (2) ascend into the GM and become incorporated into its intrinsic neuropil. They will increase the intrinsic functional interconnection among surviving GM neurons. Cajal first described the recurrent axons collaterals of both axotomized pyramidal and Purkinje neurons in experimental WM lesions in the cortex and the cerebellum (Cajal, 1968). However, the disconnected GM most relevant post-injury neuropathological feature is the significant increase of its intrinsic neuropil suggesting an upsurge of functional interconnections among its neurons already deprived of extrinsic inputs. (Case 14, Table 1).
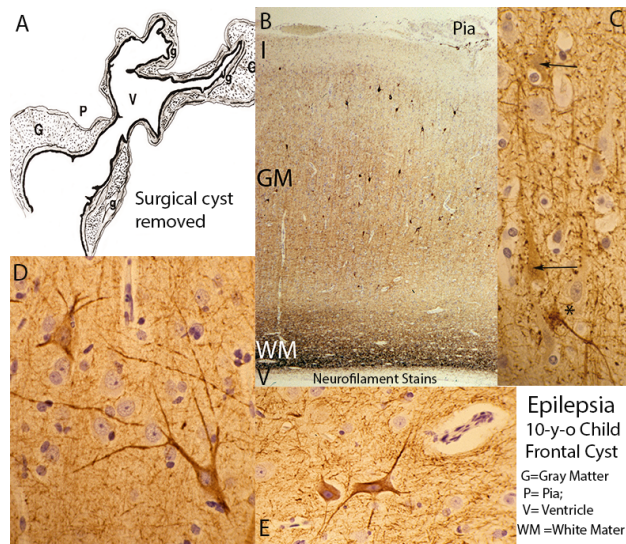
Figure 21. Montage of photomicrographs from a porencephalic cyst of the frontal lobe (removed surgically) of a 10-y-o epileptic child, illustrating post-injury developmental aspects throughout the functionally disconnected and dysplastic GM bordering the cyst. **A.** Camera lucida drawing of the surgically removed cyst showing surviving remnants (g) of disconnected GM tissue a thin band of residual WM tissue with neurofilament positive axons fibers. **B.** Low power view of dysplastic surviving GM tissue with at least 18 post-injury hypertrophic and neurofilament positive neurons through it and a thin band of residual WM. **C, D, E**. Examples of post-injury altered and neurofilament positive hypertrophic neurons from surviving GM foci. Some of the hypertrophic neurons could represent inhibitory basket cells because of numerous neurofilament positive axo-somatic contacts (*) on local pyramidal neurons that are neurofilament negative. (Case 30, Table 1).
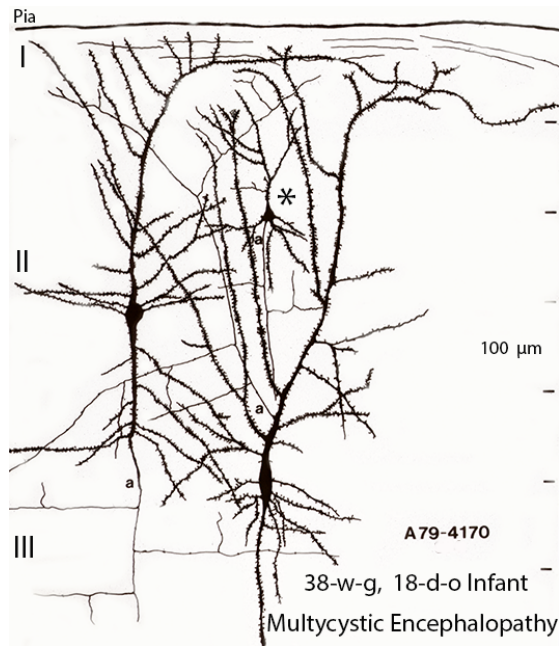

Figure 22. Camera lucida drawings of two post-injury altered large hypertrophic neurons from the disconnected GM overlying a multicystic encephalopathy of a 34-w-g infant who died at 19 days of age with respiratory distress syndrome. Both neurons, located at second and upper third laminae, show bizarre dendritic alterations with long irregular ascending and descending dendrites and axons that originate in one of the dendrites at considerable distance from neuron' soma. The right-side neuron' axon originates at the end of a very long descending basal dendrite. One possible explanation for these bizarre post-injury dendritic morphologies may that they are responding to ongoing contacts with the intrinsic neuropil already deprived of extrinsic inputs. (Case 18, Table, 1).

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Axotomized pyramidal neurons develop new post-injury synaptic profiles (Figures 20, 23, 25). Some od them become quite large (hypertrophic) with long irregular dendrites with extensive axon intrinsic distribution (Figures 22d, 23, 25B). Some of these hypertrophic neurons may be inhibitory basket cells (Figure 25B). Other neurons become smaller (atrophic) with poor dendritic and axonic arborizations. In time, all neurons of the disconnected GM will be transformed structurally and functionally.
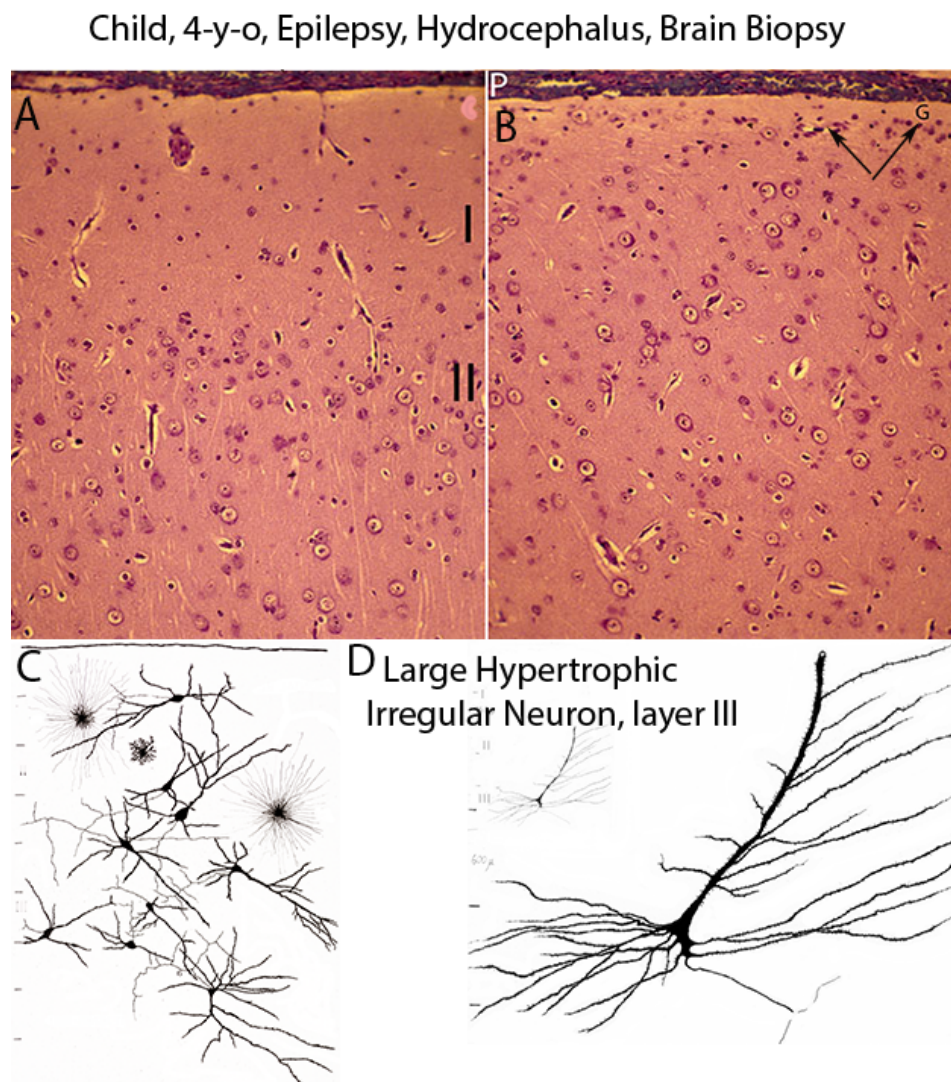

Figure 23. Composite figure with H&E preparations (**A, B**) and camera lucida drawings (**C, D**), from Golgi preparations of a frontal lobe biopsy of a 5-y-o epileptic child with encephaloclastic hydrocephalus. **A.** Section of the normal cortex adjacent to the altered one (for comparison) showing the unaltered cytoarchitecture of the first and second cortical layers. **B.** Section of the altered cortex showing: first lamina obliteration by the displacement of neurons from underlying laminae, the large size of most neurons, the lateral deviation of their apical dendrites and subpial reactive gliosis (arrows) of fibrous astrocytes. **C.** Camera lucida drawing showing the disorientation and dendritic deviations of the displaced neurons and reactive fibrous astrocytes throughout the upper cortical strata. **D.** An example of a post-injury altered hypertrophic neuron (lower layer II) with numerous abnormal basal and apical dendritic collaterals that appear to response preferentially to right side incoming fibers. The dendrites recovered with dendritic spines corroborating their functional activity. The axon of this altered neuron originates from a basal dendrite. (Case 28, Table 1).

The altered dendritic morphology of some of the disconnected GM neurons is quite bizarre (Figure 23). The length, morphology, distribution and number of dendrites of some neurons are significantly altered (Figures 10, 11, 22, 23, 25B). Often, their axon originates from one of the dendrites at a considerable distance from their bodies (Figure 23). These neuronal alterations represent ongoing transformations that continue to evolve throughout the affected children' life. Similar types of hypertrophic and morphologically altered neurons are seen in the GM underlying LMHs caused by subpial hemorrhages (Figures, 10, 11). In both cases, these neurons hypertrophy are, in my opinion, caused by their increase intrinsic interconnectivity.

The horizontal axonic collateral of some axotomized pyramidal could reach undamaged cortical regions and reestablish post-injury new functional interconnections. Some might even reach the opposite cerebral hemisphere through the corpus callosum and re-establish distant post-injury functional interconnections. These long functional interconnections could also result in distant neuropathological alterations. The long functional interconnections between the disconnected GM, overlying WM infarcts, and other cortical and subcortical regions remain essentially uninvestigated.
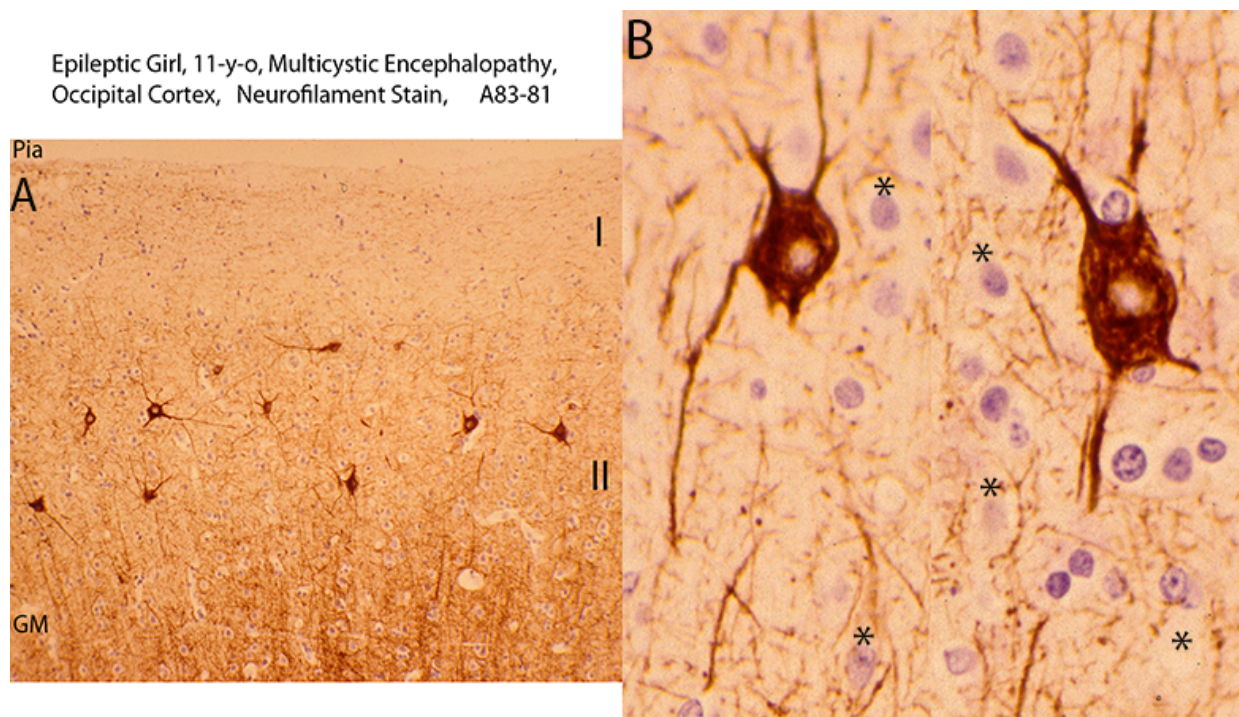

Figure 24. Color photomicrographs (A & B) of neurofilament preparations from the occipital cortex of an 11-y-o girl with multicystic encephalopathy illustrating its post-injury neuronal alterations. **A.** Low power view of the altered occipital cortex showing its first and second laminae with at least 10 large hypertrophic and neurofilament positive altered neurons throughout the second lamina. There is also an increase of neurofilament positive intrinsic fibers throughout the second lamina already deprived of extrinsic ones. **B.** High power microscopic view of the third cortical lamina showing two large hypertrophic neurofilament positive and post-injury altered neurons. Some of these hypertrophic neurons may be inhibitory basket cells suggested by the large number of neurofilament positive axo-somatic terminal around pyramidal neurons (*) that are neurofilament negative. (Case 31, Table 1).
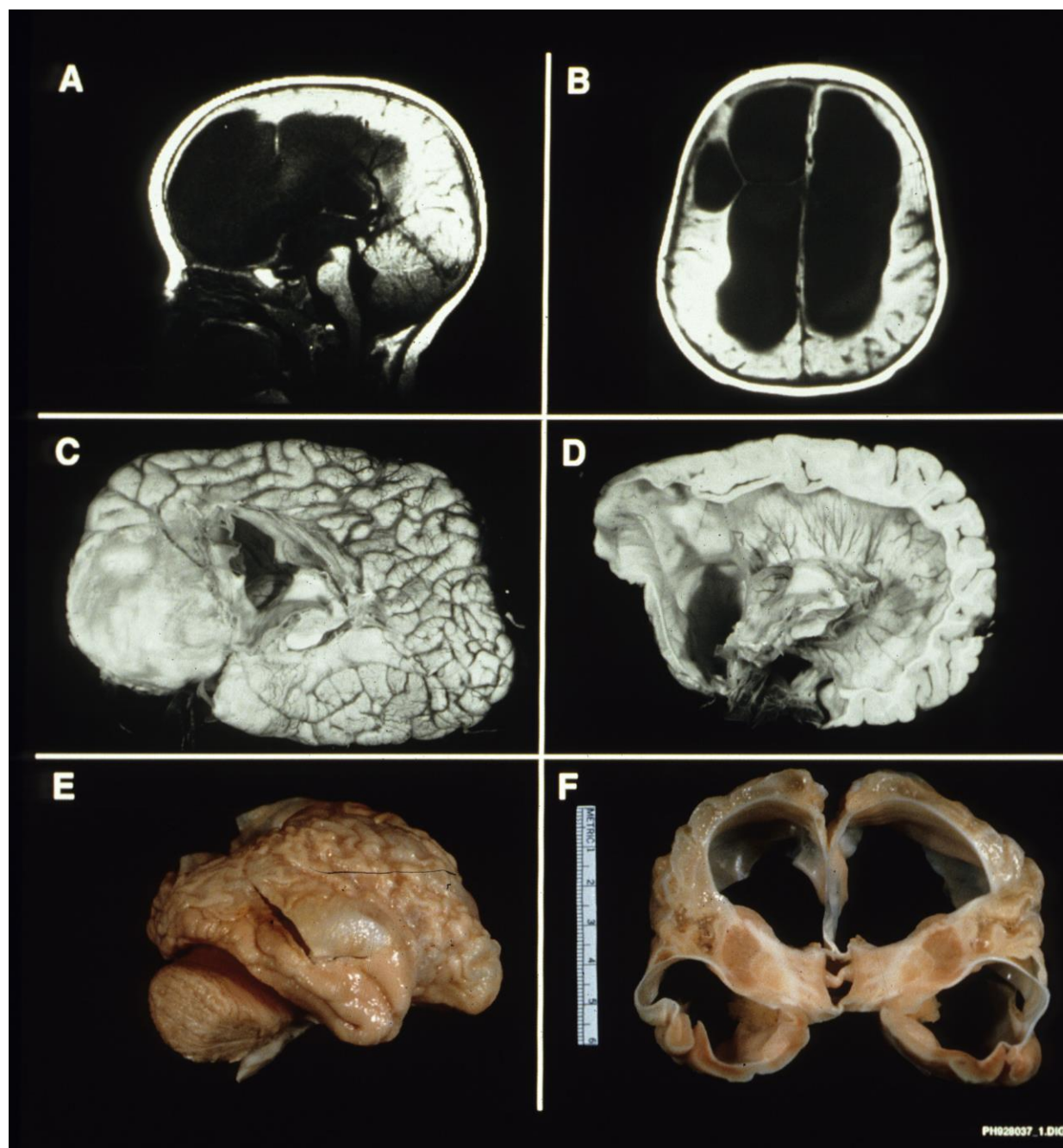
Figure 25. Photographs from MRI (**A, B**) and postmortem (**C-D**) studies of the first Shaken Infant' brain (Case 33, Table 1) showing massive and extensive GM and WM damages. The cortex frontal and temporal lobes are the most severely damage, the parietal lobes are less affected and the occipital lobes (including the primary visual cortex) and cerebellum are probably unaffected by the shaking. These differenced are the result of the roughness of their respective cranial structures and of the cerebellar tentorium flexibility. **E, F.** Postmortem color photographs of the shaken brain (**E**) and of mid-section (**F**) from second SIS infant (Case 34, Table, 1) showing the severity of the cortical lesions and the massive hydrocephalus caused by the extensive damage of GM and WM. Despite the extensive brain damage foci of disconnected GM survive throughout the cortex that will undergo post-injury neuronal (hypertrophy) and fibrillar (increase intrinsic fiber interconnection) alterations.

Some of the basal dendrites of axotomized pyramidal are destroyed by the WM infarct. Axotomized and dendrotomized pyramidal neurons are progressively transformed into short-circuit neurons that continue to undergo cytoarchitectural as well as functional post-injury transformations (Figures 20, 22, 23, 25). Some these transformed GM neurons are heavily stained in neurofilament preparations, perhaps, suggesting functional hypertrophy.

The disconnected GM glial composition is also altered. There is a significant increase in the number of fibrous astrocytes. Fibrous astrocytes are essential WM components, not commonly present in the unaltered GM. The presence of reactive fibrous astrocytes throughout the GM is a common finding in many types of cortical injuries (Figures 8B, 13). The number of first lamina special astrocytes (comet cells) is also increased. They are needed for repairing EGLM ruptures caused the destruction of radial glia fibers by the WM infarct. Focal first lamina reactive gliosis of fibrous astrocytes is also a prominent feature. Protoplasmic astrocytes are essentially unaffected and continue to supply endfeet to the disconnected GM intrinsic microvascular system.

Chronic (Repaired) Injury. The disconnected GM overall cytoarchitectural organization is universally altered and dysplastic and the neuronal alterations are difficult to evaluate. Those illustrated herein represent only those recognized in the few preparations of the disconnected GM studied (Figures 22-25). To recognize the disconnected GM neuronal alterations, the use of Golgi procedure is mandatory. With most routine staining procedures, the altered dendritic and axonic morphology of disconnected GM neurons are invisible. Moreover, these alterations are not static but ongoing GM neurons transformations. A possible explanation for their bizarre dendritic and axonic morphologies is that they represent developmental processes caused by the increasing intrinsic interconnectivity among them.

The first lamina is often obliterated by an increasing number of displaced neurons from lower cortical laminae and its demarcation is often unrecognizable (Figure 24B). A distinctive feature of the disconnected GM overlying WM infarcts is presence of large dysplastic and hypertrophic neurons (Figures 22-25). They include axotomized pyramidal inhibitory basket cells (Figures 22, 23, 25). Neurofilament stains have demonstrated the presence of large inhibitory basket cells as well as the presence of numerous axo-somatic synaptic contacts (baskets) around the body of pyramidal neurons (Figure 25*). Small nodules of undifferentiated neurons and glial cells are often recognized at the GM/WM junction. These nodules could become the precursor of the glio-neuronal heterotopias often described in epilepsy.

A frontal lobe surgical biopsy, from a 5-years old epileptic child with hydrocephalus (possibly encephaloclastic), shows a first lamina obliteration by the displacement of underlying neurons as well as neuronal disorientation (Figure 24A, 24B, arrows). The neurons disorientation reflects their response to the altered local intrinsic neuropil (Figure 24C). Some of the displaced neurons are also hypertrophic (Figure 24B). The normal cytoarchitecture of an adjacent undamaged cortical region is depicted for comparison

(Figure 24A). Golgi preparations of the biopsy show the altered post-injury dendritic morphology of some of these large (hypertrophic) neurons (Figure 24D).

Another finding on the disconnected GM overlying WM infarcts is the columnar aggregation of neurons throughout the upper lamina. Routine neuropathological stains fail to stain the components between the neuronal columns. Quite a different picture emerges using Golgi and/or neurofilament preparations. The interneuronal spaces are composed of numerous interlacing intrinsic axon fibers, dendrites and blood capillaries, all of which are invisible with routine staining procedures. It is possible that the increase intrinsic neuropil might trigger the columnal arrangement. The increase intrinsic neuropil is a neuropathological feature of the disconnected GM, already deprived of extrinsic fibers.

Most large neurons observed in Golgi preparations are also heavily stained in neurofilament preparations suggesting both structural as well as functional hypertrophy (Figures 22, 25). Scattered foci of reactive gliosis of fibrous astrocytes with long unbranched processes are found throughout the disconnected GM including the first lamina (Figure 24B). Figure 25A give a general idea of the large number of transformed GM neurons overlying a WM infarct. The section represents a small area of disconnected GM, removed surgically from an 11-y-o epileptic girl) overlying an occipital cystic encephalopathy. The cortical area (roughly 500 μm² by 6-μm thick) have at least ten large and neurofilament positive neurons (Figure 25A).

It should also be pointed out that the neuronal alterations illustrated and discussed herein represent only those observed in a relatively few (40-50) microscopic sections from large disconnected GM regions overlying WM infarcts. In addition, the post-injury cytoarchitectural and possible functional neuronal alterations described herein are no static but ongoing processes that continue to change during the affected children' life.

In most WM hypoxic-ischemic injuries (hydrocephalus ex-vacuo, porencephalies, multicystic encephalopathies and encephaloclastic hydranencephaly) a narrow band of WM survives under the disconnected GM. This residual WM is composed by a few surviving WM fibers and by the long horizontal axonic collaterals of axotomized pyramidal neurons. This residual WM may be capable of re-establishing local as well as distant interconnections between damaged and undamaged cortical regions and might represent the anatomical substratum needed for the transferring the altered neuronal activity throughout the cortex up to its motor region for its eventual discharge as erratic muscular contractions or seizures. The alterations described herein represent but a small fraction of many possible ones that continue to evolve and to change during the maturation of affected children' brains. These ongoing structural and functional transformations of the disconnected GM neurons could play a significant role in the pathogenesis of ensuing epilepsy and other neurological sequelae following WM infarcts. Cases 28 to 34 have developed clinical epilepsy (Table 1).

5. PERINATAL BRAIN TRAUMATIC INJURIES: SHAKEN INFANT SYSDROME

Undoubtedly, the more severe and extensive traumatic injuries involving the developing infant brain are those associated with the so-called Shaken Infant Syndrome or SIS (31). The developmental neuropathology of the brain injuries of two children who survive SIS, develop epilepsy and later died is explored bellow. If the shaken infant survives (and they often do), he or she will be physically and mentally impaired, unable to speak, often blind and many will require custodian care for life. SIS children develop cerebral palsy, profound motor and cognitive impairments and epilepsy. SIS is considered a criminal act and perpetrators are prosecuted and incarcerated.

I have had the opportunity (with colleagues) of studying the post-injury developmental neuropathology of the damaged brains of two SIS children (Figure 25). The first case (Table 1, case 33) was a 7-years old child found dead in bed in respiratory failure and presumed epileptic seizures. At 11-d-o, the child was admitted to the hospital lethargic with rupture frenulum, tense fontanels, bilateral retinal hemorrhages, eye deviations, facial bruising, healing burn marks in the chest and bloody cerebro-spinal fluid. It was established that the child has been abused and shaken violently by the foster father (later charged and incarcerated). The child developed cerebral palsy, mental retardation, diabetes insipidus and epilepsy. The child was also blind and unable to speak. At six-month of age a ventriculo-peritoneal shunt was placed due to enlarging head circumference. Radiographic studies (CTM and MRIs) documented the extensive cortical injuries and the ensuing post-injury encephaloclastic encephalopathies, later confirmed by post-mortem studies (Figures 25A-25D).

The second child (Table 1, case 34) was the victim of violent shaking at 3-month of age by the mother's boyfriend (later prosecuted and incarcerated). At the hospital admission, the child was semi-comatose with bilateral eyes hemorrhages and unresponsive. Radiological studies demonstrate extensive GM and WM injuries and encephaloclastic hydrocephalus. The child remains essentially unresponsive with cerebral palsy, mental retardation and epilepsy. He was unable to speak and considered to be blind. The child died, at 9 year of age, with bronchopneumonia (Figures 25E, 25F).

SIS' brain injuries are a combination of severe hemorrhagic, hypoxic-ischemic and axonic (shredding) damage involving both the cortex gray (GM) and white (WM) matters. Most traumatic injuries are essentially confined to the cerebral cortex GM and WM matters. Subcortical regions are lees affected but will be secondarily altered by the sensory deprivation resulting from the cortical injuries. The thalamus status marmoratus and the hippocampal sclerosis represent secondary neuropathological alterations resulting from the cortical injuries.

Violent shaken motions of a softer brain again the rigid cranial cavity is the main cause of SIS extensive cortical injuries. Consequently, the more severe injuries are those involving the cortex frontal and temporal lobes. In these regions, the shaken brain is poorly protected again the cranial structures roughness (Figures 25, 26). The parietal lobes are better protected by the smoother surfaces of the parietal bones. The occipital lobes,

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including the primary visual cortex, are the less damaged cortical regions (Figures 25A-25D). They are protected by the occipital bone smoother inner surface and by the cerebellar tentorium. The tentorium also protects the cerebellum that is rarely damaged. But it may be secondarily affected by the sensory deprivation resulting from the damaged motor cortex.
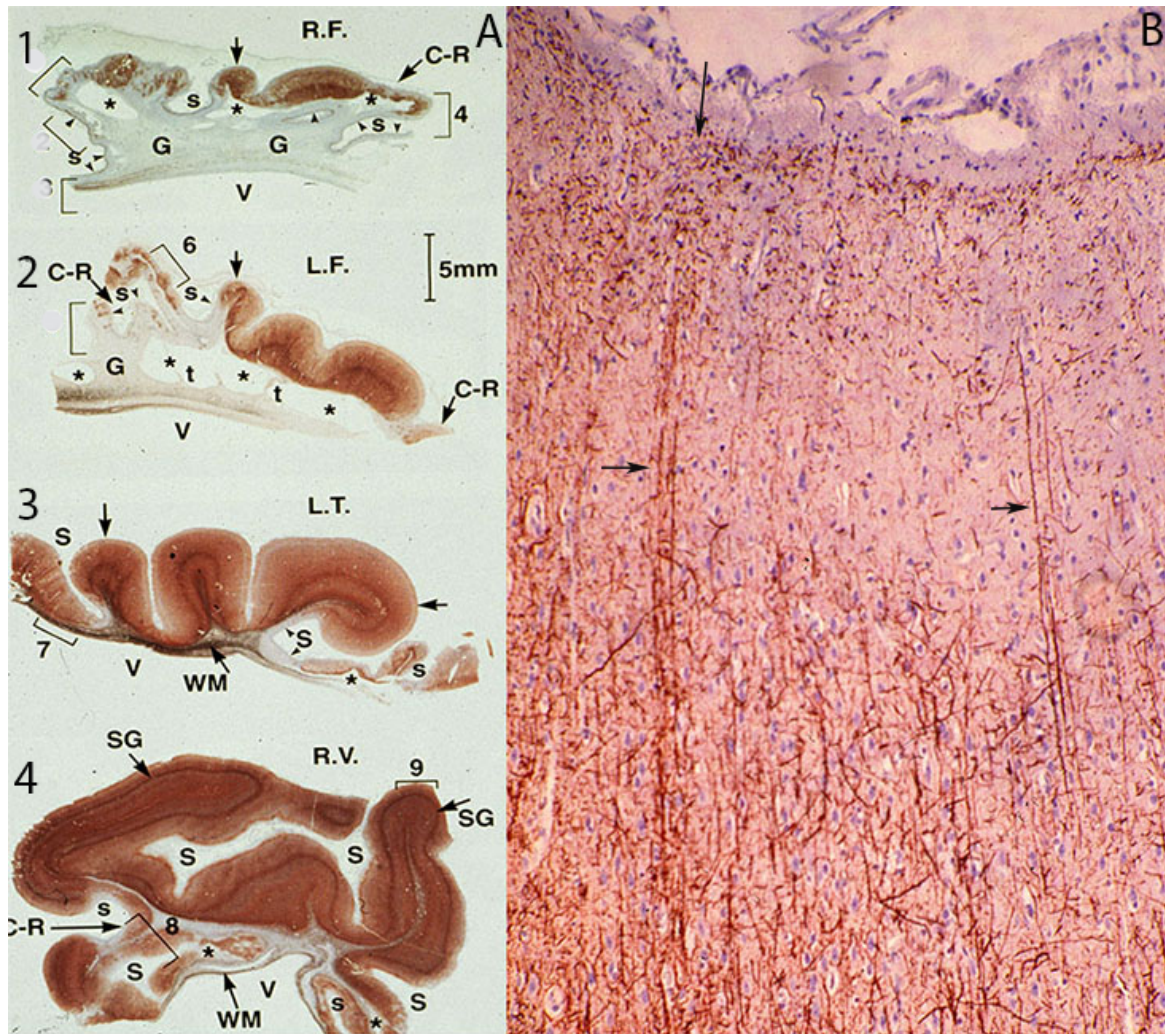

Figure 26. Montage of photomicrographs from neurofilament stained preparations illustrating the different degree of cortical damaged depending on location. **A**. Views of the severely damaged cortex of the right (**1**) and left (**2**) frontal lobes showing foci of surviving disconnected GM, a few WM residual cavities (*) separated by glio-vascular trabeculi (t), the extensive reactive gliosis (G), the depth of atrophic sulci (S) and a band of residual WM tissue with neurofilament positive fibers, capable of re-establishing long distance functional interconnections. Despite the massive cortical destruction, Cajal-Retzius (C-R) cells were recognized in both cerebral lobes. **3**. General view of the less affected temporal lobe showing the preservation of larger areas of functionally disconnected GM (arrows) above a narrow band of residual WM with neurofilament positive axons overlying the large hydrocephalic ventricle (V) and the depth of some sulci. **4**. View of the primarily unaffected primary visual cortex, showing: the Stria of Gennary (SG), deep sulci (S), the presence of Cajal-Retzius neurons (C-R) and a narrow band of WM tissue with neurofilament positive axons fibers overlying the dilated ventricle (V). **B**. Color photomicrograph of a neurofilament stain preparation of the disconnected GM of the temporal lobe showing vertical bundles of aberrant neurofilament positive fibers (arrows) that reach the first lamina and a noticeable increase in the number of intrinsic fibers throughout the region already deprived of extrinsic ones. Similar upsurge in the number of intrinsic fibers was found throughout many other disconnected GM regions. From: the first child' shaken brain (Case 33, Table 1).
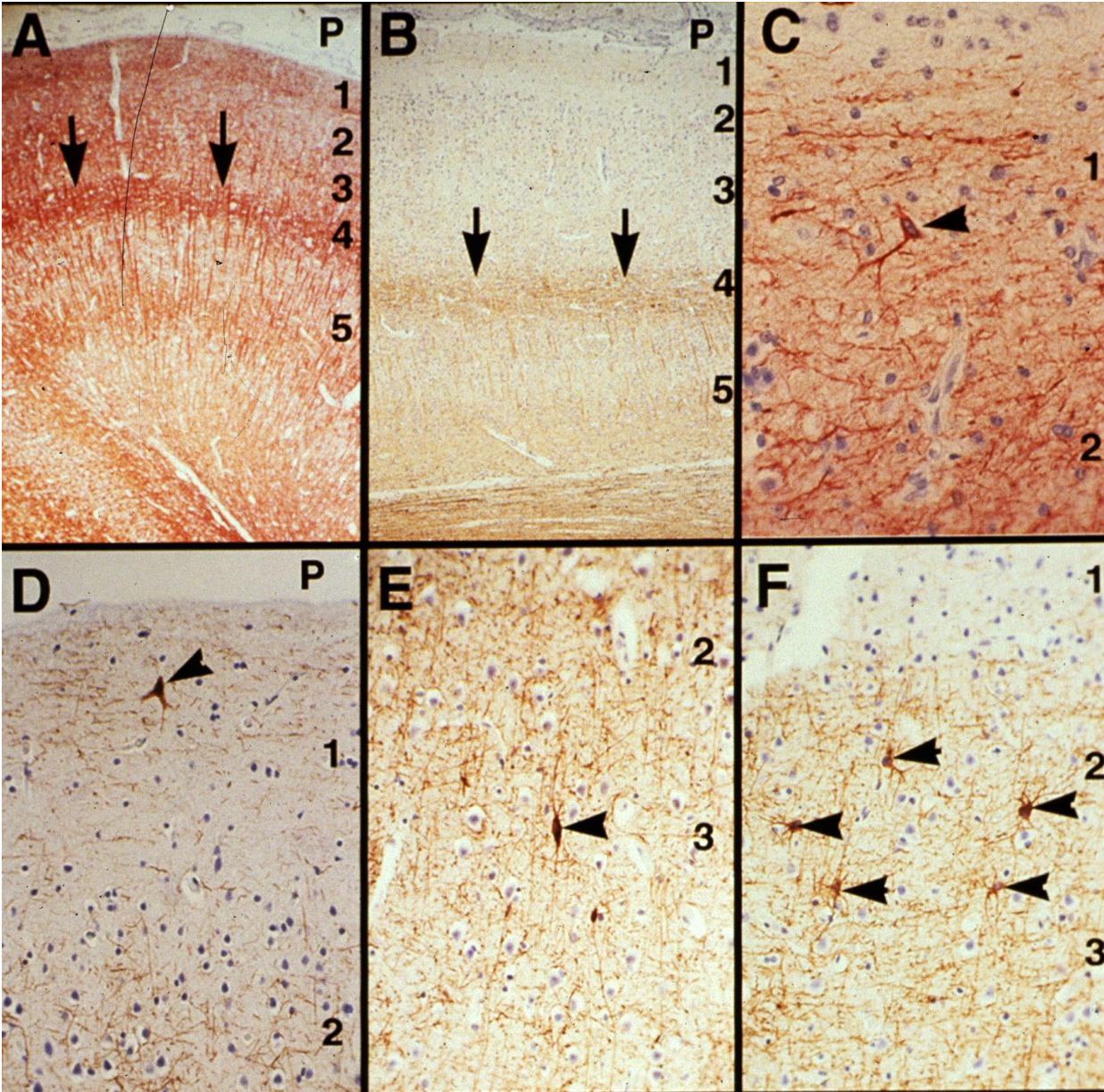
Figure 27. Color photomicrographs (A-F) of neurofilament stained preparations from both SIS children' shaken brains showing some neuropathological aspects of their residual GM. **A** and **B**. Photomicrographs of the primary visual cortex from the second (A) and first (B) SIS children showing the preservation of the Stria of Gennary in their primary visual cortex with neurofilament positive fibers, despite their clinical blindness. **C** and **D**. First lamina neurofilament preparations from the second (**C**) and the first (**D**) SIS brains showing Cajal-Retzius Cells (arrows) and an increase in the number of neurofilament positive intrinsic fibers. **E** and **F**. Neurofilament stain preparations from the second and third cortical laminae showing large hypertrophic neurons with altered morphologies, from the second SIS child. Approximate magnifications: A and B 40x, C - F 250x. (Cases 33 and 34, Table 1).

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The shaken motions cause extensive WM hypoxic-ischemic injuries (infarcts), extensive axonic shredding and ruptures in both hemispheres. The WM tissue is significantly reduced through both hemispheres and the corpus callosum thickness is significantly reduced (Figure 25). The damaged WM tissue involve both hemispheres and is rapidly destroyed and reabsorbed by glial and macrophage phagocytosis (hydrocephalus ex-vacuo) (Figures 25). A narrow band of WM survives under the residual GM in many areas throughout the cortex (Figure 26). The survival of GM neurons throughout the damaged brain have significant clinical implications and could play a role the pathogenesis of epilepsy in SIS cases (31).

The post-mortem neuropathologic studies were carried out in both SIS children formalin-fixed brains. Early CT scans and MRIs studies verified that the original brain lesions include hemorrhagic (blood vessels ruptures), hypoxic-ischemic (reduced blood circulation) damage involving the both GM and WM maters, later confirmed in post-mortem studies (Figures 25-27). The methods used include: H&E, Nissl, luxol-fast-blue, Bielshowsky, Neurofilament protein (DAKO, clone 2F11) and glial fibrillary acidic protein (GFAP).

I. *SIS Developmental Neuropathology.* Post-mortem studies of both SIS brains demonstrated the ongoing developmental neuropathology of the original lesions (Figures 25). The developmental neuropathology of any brain injury continues to evolve and to change for as long of the affected child survives (Figures 25-27). Those described herein represent those found in these children' brains, 7 and 9 years after the original injuries, respectively.

The SIS post-injury neuropathological alterations vary from extensive too mild to minimal depending on their brain location (Figures 25, 26A). In both children, the WM was more severely damaged that the GM. As expected, frontal and temporal lobes are the most severely damaged, parietal lobes are mildly damaged and the occipital lobes, including the primary visual cortex, are the less affected. The cerebelli was also minimally affected (Figure 25). These differences are explained by the roughness of cranial structures and by the protection of the cerebellar tentorium (Figures 25, 26A). The amount of surviving GM in parietal and occipital lobes is greater than in the frontal or temporal lobes (Figure 26A). In most damaged cortical regions, a thin band of WM tissue survives under the residual GM. This surviving WM tissue band is associated with reactive gliosis of fibrous astrocytes and scattered micro calcifications (Figure 27H). The developmental neuropathologies of the brain subcortical regions will not be studied, at this time.

Throughout the cortex, foci of disconnected GM tissue have survived years after the original injury. They survive because they have retained a reduced microvascular system and its connections to the meninges pial capillary plexus (see chapters 2 and 3). The residual and functionally disconnected GM survives because they establish intrinsic functional interconnections among themselves. The surviving GM neurons structural and functional is altered by increasing intrinsic interconnections (Figures 26A, 26B, 27C, 27E, 27F). The

residual GM is dysplastic, lacking distinct laminations and the morphology of most of its neurons is altered. Large neurofilament positive hypertrophic neurons and bundles of aberrant axons fibers are recognized through the residual GM (Figures 26B, 27C-27F).

In the occipital cortex, the GM overall cytoarchitectural organization is better preserve, its laminations are recognized and its intrinsic neuropil, deprived of extrinsic inputs, is increased. The stria of Gennary is recognized in both SIS brains' primary visual cortex (Figures 27A, 27B, arrows). Its presence is somewhat perplexing since both children were considered to be blind. However, the primary visual cortex, functionally disconnected by the WM damage, may be incapable of transmitting visual information to other cortical regions. The SIS original retinal hemorrhages could have also destroyed parts of the retinas and cause the blindness. The preservation of the primary visual cortex in SIS needs to be further investigated.

Both the neuronal and fiber organization of surviving GM are altered and dysplastic in both SIS brains. The surviving GM neurons increasing intrinsic interconnectivity alter both their morphology as well as their function. The increase intrinsic neuropil throughout the disconnected GM is perhaps its most relevant feature. Using neurofilament preparations, the disconnected GM neurons overall morphology and distribution are altered and without distinct demarcations or laminations. First lamina is obliterated by the displacement of underlying neurons (Figures 26A, 26B).

How the altered functional activity of surviving GM neurons move through the residual cortex toward the motor region to be discharged as epileptic seizures remains essentially unexplored and unknown. Two neuropathological observations on the SIS' residual cortex may provide some answers. The first one is the survival of a thin band of residual WM underlying the surviving GM areas throughout the residual cortex (Figure 26A). This WM is composed of both surviving undamaged WM fibers and the axonic collaterals of axotomized GM projective neurons. Eventually; the residual WM long fibers could interconnect the GM foci throughout the cortex. The residual WM fibers and possibly their functional interconnections have survived for 7 and 9 years respectively, the original SIS brains damaged. Their long survival implies that they have remained functionally competent in interconnecting the surviving GM foci throughout the cortex.. These WM fibers long survival could represent the needed anatomical substratum for the transferring of the altered neuronal activity throughout the SIS' brains. We propose that the functional interplay among the surviving GM neurons throughout the cortex, supported by the long WM fibers interconnections, could play a significant role in the pathogenesis of SIS' children epilepsy.

An important neuropathological observation is the long survival of Cajal-Retzius cells (C-RCs) in the residual cortices as well as their long horizontal axons (Figures 26C-26R, 27C, 27D). C–RCs and their neurofilament positive horizontal axons have been recognized in the residual GM of the frontal, temporal, parietal, and visual regions in both SIS brains (Figures 26, 27). The C-RCs long horizontal axon fibers could also establish functional interconnections among the surviving GM foci throughout the cortex (Figure 26A). Their

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long survival (7 and 9 years) in these SIS' children brains implies that they have remained functionally competent and that they are co-participant in the structural and functional neuronal reorganizations (rewiring) of among the surviving GM foci throughout the cortex. Their long survival could also represent the needed anatomical substratum for the transferring of altered neuronal activity throughout the residual cortex up to its motor cortex for discharged (disposal) as erratic muscular activity. We proposed that this mechanism could play a role on the pathogenesis of SIS children epilepsy.

II. *Clinical Impact of SIS Post-injury Developmental Neuropathology.* The most commonly described injuries in shaken brains including: epidural, subdural (basal, para-medial and lateral), cerebral, intraventricular, cervical spinal cord and ocular hemorrhages, cortical hypoxic/ischemic damage and axonal injury, as well as brain and spinal cord contusion and massive cerebral edema. Variations of all of these shaking injuries were observed in our cases.

From a clinical perspective, SIS brains most relevant neuropathological observation may not necessarily be the extensive lesions, but the survival of foci of GM neurons functionally interconnected throughout the residual cortex. The surviving GM ranges from its nearly complete destruction, to scattered foci of dysplastic tissue to the well preserve areas in the occipital lobes including the primary visual cortex. The SIS residual GM neurons may be interconnected by the surviving WM fibers and by the C-RCs long horizontal axons. Their altered neuronal activity may be transferred throughout the residual cortex, reach its motor region and discharge (disposal) as erratic and incontrollable motor activities or seizures.

6. PERINATAL CORTICAL GRAY MATTER INJURIES

The gray matter (GM) neurons functional activity determines the brain overall activity. Most functional activities of the mammalian brain depend on the functional activities of GM neurons. Consequently, the protection of GM neurons is of a paramount biological importance. The cortex GM, where most neurons reside, is protected by a unique microvascular system (14). Most GM neurons alterations described through the second, third and fourth chapters are secondary in nature. On the other hand, primary damage to GM neurons is the role in genetic abnormalities, not studied herein. The mammalian neocortex unique microvascular system has undoubtedly evolved to protect the GM neurons in both normal (unaltered) as well as in altered brain conditions.

The mammalian cortex GM microvascular system is among the riches of the human body. It is composed of equidistant arterial and venous perforating vessels, separated by a distance that ranges between 400 and 600 micrometers, with an intrinsic capillary plexus between them where neurons, axon terminals and glial cell live. The intervascular separation among perforating vessels is maintained throughout the cortex pre- and post-natal maturations (14, 28). During the cortex development maturation the number of additional

perforating vessels parallels its expansion. Each perforating vessels is accompanied by a perivascular Virchow-Robin compartment (W-RC) formed by the fusion, at the vessels entrance, of both vascular and EGLM basal laminae (28). The V-RCs keep the vessels extrinsic to the nervous tissue while remaining open to the meningeal interstitium. Functional demands will determine their arterial and/or venous nature. During cortical development, the ratio between arterial versus venous perforators will change continually in response to functional demands. The V-RCs represent the cerebral cortex sole drainage system (12).

In any type of perinatal cortical damage some neurons GM died while other survive and undergo post-injury structural as well as functional transformation. Their post-injury transformations (alterations) are not only found throughout the injured site but also through cortical regions functionally interconnect with the original damaged site (Chapters 2, 3, 4). The post-injury developmental transformations of GM neurons are not static but ongoing processes that continue to modify both their structure and function for as long as the affected child remains alive and play a significant role in the pathogenesis of ensuing epilepsy and other neurological sequelae.

7. CONCLUSIONS

The development of the cerebral cortex is a complex process during which and under strict developmental and timing constraints neurons, dendrites, axons, blood vessels and glial cells originate, migrate and establish progressive and modifiable structural and functional interrelationships. This controlled process may be interrupted by either acquired and/or genetic insults that will affect the cortex post-injury structural and functional maturations. To understand the developmental neuropathology of these perinatal cortical injuries, it is necessary to study them through their acute, subacute and chronic stages. The difficulty will be in collecting an adequate postmortem brain material from children who survive perinatal cortical injuries and later died at different ages. Collecting this type of postmortem brain material will require a life-long commitment, a qualified Pediatric Neuropathologist as well as the collaborations of many.

The present study was carried out with the proviso that it will necessarily include observations from different cortical lesions, different locations and different aged children. Despite these objections, this study represents an attempt to ascertain what happen to the child' developing brain after a cortical injury that could eventually result in clinical epilepsy. In other words: what is the pathogenesis of epilepsy secondary to perinatal cortical damage. Collecting the necessary postmortem brain material for this type of study will require a life-long commitment, a Pediatric Neuropathologist and the collaboration of many, not easily attainable. Perhaps these difficulties explain why the pathogenesis of epilepsy secondary to perinatal brain damage has been seldom investigated.

This kind of study will require: a) the developmental neuropathology of the original cortical injury (local altered corticogenesis); b) that of cortical regions functionally interconnected with the injured site (distal altered corticogenesis); c) the transferring (auras) of the altered neuronal activity through interconnected cortical regions; and, d) the discharge of the altered neuronal activity by the motor cortex through erratic and

incontrollable muscular contraction or seizures. Once the altered neuronal activity is discharged by the motor cortex, epilepsy 'per se' disappears. New altered neuronal activities stars to build-up at the original injured site and the entire process is repeated once more. Although, epilepsy is not medically curable it can be controlled with medications with excellent results.

The observations described represent an attempt of interconnecting the developmental neuropathology of various types of perinatal cortical injuries with the eventual occurrence of clinical epilepsy. Highlighting that the neuronal activity -normal as well as altered- of any cortical region will be transferred through functionally interconnected cortical regions up the primary motor cortex to be discharged either by normal and/or altered motor activities.

The Golgi color illustrations that accompany this study are exceptional and unique. They illustrate the altered morphology of neurons, dendrites, axons, blood vessels and neuroglia in cortical regions that have survived various types of perinatal injuries. In 1873, Camilo Golgi introduced his method, not as such but as a reaction (*la reazione nera*) and told us that its secret lies in '*probando e reprobando*' (trying and trying against). It is a capricious reaction that lacks a specific methodology. It consists on the fixation of tissue blocks in an osmic acid solution followed by their impregnation in a silver nitrate one. The fixation and impregnation times fluctuate and must be predetermine for each type of tissue and for any age to be studied. Fixed and stained tissue blocks are cut manually with a razor blade and/or with an appropriate instrument. The 150 to 200 micrometers thick sections are placed in glad slides and cover with a drop of Dammar resin. After drying they are ready for studying. The Golgi reaction requires a long-life commitment, the acceptance of failures and patience, the virtue of the less capable, according to Cajal. The Golgi color illustrations speak for themselves and should give support to all the necessary efforts needed to study the damaged brain of children.

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