# Cerebrospinal Fluid Disorders

## Lifelong Implications

David D. Limbrick Jr.
Jeffrey R. Leonard
*Editors*

Springer

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*Editors*
David D. Limbrick Jr.
Department of Neurological Surgery
Washington University School of Medicine
St. Louis Children's Hospital
St. Louis, MO
USA

Jeffrey R. Leonard
Nationwide Children's Hospital
Department of Neurosurgery
The Ohio State University College
of Medicine
Columbus, OH
USA

ISBN 978-3-319-97927-4 ISBN 978-3-319-97928-1 (eBook)
https://doi.org/10.1007/978-3-319-97928-1

Library of Congress Control Number: 2018959740



This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

# Preface

Disturbances in cerebrospinal fluid (CSF) physiology can occur at any stage across the lifespan, with clinical presentation, natural history, and neurological implications of pathology dependent upon patient age and etiology. While the result is often hydrocephalus, risk factors and biological mechanisms are myriad. In 2018 at least, treatment, however, remains relatively uniform: diversion of cerebrospinal fluid (CSF). While there have been advances in the gold standard treatment for hydrocephalus, the CSF shunt, technical advances in endoscopic third ventriculostomy (ETV) with or without adjuvant choroid plexus cauterization (CPC) have resulted in increased interest in and investigation of these procedures. As neurosurgical methods advance, it is best to view hydrocephalus—regardless of the age of onset—as a lifelong chronic problem. Even with optimal treatment, there remains neurological morbidity and mortality and a significant cost to the evolving healthcare system. Because of this, patients and their families demand rapid improvements in treatment methods for this challenging disease.

This text is designed to provide the current "state of the field" for CSF disorders that occur across the lifespan. The authors of the following chapters are true content experts and provide their accumulated knowledge, experience, and wisdom in addition to a thoughtful view forward. We begin with a discussion of the ventricles themselves, with changes in the ependyma that occur throughout development and as the brain matures and then ages. Pathophysiological changes and their role in hydrocephalus of various etiologies are considered. Alterations in CSF composition, physiology, and flow are also discussed in the context of neurodevelopment and neurodegeneration.

In recent years, we have come to a detailed understanding of the etiologies of hydrocephalus—congenital, acquired, and those observed in the aging brain. This book contains chapters that chronicle the rapidly evolving discoveries that identify the genetic basis of hydrocephalus. Considerable advances have been made in genetic etiologies, and, in many cases, these findings have provided insight into mechanisms underlying the pathogenesis of hydrocephalus. Novel methods in neuroimaging and biochemical biomarkers have also provided novel insights both into the disease itself and into its effects on the nervous system, from infancy through adulthood.

The most common cause of pediatric hydrocephalus in high-income countries is posthemorrhagic hydrocephalus, while globally, in low- and middle-income

countries, postinfectious hydrocephalus predominates. Because of the relevance to public health, both of these disorders are considered in detail in the ensuing chapters. Likewise, full consideration is also given to hydrocephalus-associated spina bifida, which has taken on new significance in the era of fetal myelomeningocele repair and the emerging role of ETV in this setting.

While the causes of hydrocephalus in adults may differ from those that affect children, there is certainly overlap. For example, trauma and brain tumors may cause hydrocephalus in individuals of any age. Other etiologies may converge as well; though often noted in preterm infants (e.g., from hemorrhage in the immature germinal matrix), posthemorrhagic hydrocephalus occurs frequently in adults after subarachnoid hemorrhage or other common forms of intracranial hemorrhage. Perhaps the most vexing form of adult-onset hydrocephalus is idiopathic normal pressure hydrocephalus (iNPH). iNPH deserves special consideration, as its pathophysiology, diagnosis, and treatment remain controversial.

Though simple in principle, the management of hydrocephalus can be exceedingly difficult. Multiloculated hydrocephalus is notoriously challenging, often requiring multiple fenestration procedures and complex shunt configurations. Intracranial hypotension presents a different sort of challenge; the diagnosis and definition of a CSF leak may be more difficult than its treatment.

Over the past decade, there has been increasing emphasis on improving the quality and safety of neurosurgical care for hydrocephalus patients of all ages. Committed experts have built consortia to study hydrocephalus and conduct sophisticated clinical trials to help determine best practices. Others have worked to improve existing shunt designs and catheter components or to develop the next generation of devices to help neurosurgeons treat this chronic disease.

We would like to conclude by thanking each of the contributing authors for their commitment and their efforts in compiling this book. We hope that you find this collection valuable as you build your expertise in this complex disease and its treatment!

Sincerely,

St. Louis, MO, USA — David D. Limbrick Jr.
Columbus, OH, USA — Jeffrey R. Leonard

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# Contributors

**Prince Antwi, BA** Department of Neurosurgery, Yale School of Medicine, New Haven, CT, USA

**Samuel R. Browd, MD, PhD** Seattle Children's Hospital, University of Washington, Department of Neurological Surgery, Seattle, WA, USA

**Shlomi Constantini, MD, MSc** Dana Children's Hospital, Tel-Aviv Medical Center, Pediatric Neurosurgery, Tel Aviv, Israel

**Michael C. Dewan, MD, MSCI** Vanderbilt University Medical Center, Monroe Carell Jr. Children's Hospital at Vanderbilt, Department of Neurological Surgery, Nashville, TN, USA

**William Dobyns, MD** Department of Genetics, University of Washington, Center for Integrative Brain Research, Seattle Children's Research Institute, Seattle, WA, USA

**Dan Doherty, MD, PhD** Department of Pediatrics, University of Washington and Seattle Children's Hospital, Center for Integrative Brain Research, Seattle Children's Research Institute, Seattle, WA, USA

**David L. Dornbos III, MD** Department of Neurological Surgery, The Ohio State University Wexner Medical Center, Columbus, OH, USA

**Per Kristian Eide, MD, PhD** Oslo University Hospital – Rikshospitalet, Department of Neurosurgery, Oslo, Norway

**Johannes Marthinus Nicolaas Enslin, BPhysT, MBChB, MMed, FCNeurosurg** Red Cross Children's Hospital, Department of Neurosurgery, Cape Town, South Africa

**Anthony Graham Fieggen, MBChB, MSc, MD, FCS(SA)** Division of Neurosurgery, University of Cape Town, Department of Surgery, Cape Town, South Africa

**Charuta Gavankar Furey, MD** Department of Neurosurgery, Yale School of Medicine, New Haven, CT, USA

**Thomas Garton, BS** University of Michigan, Department of Neurosurgery, Ann Arbor, MI, USA

**Maria Montserrat Guerra, PhD** Instituto de Anatomía, Histología y Patología, Facultad de Medicina, Universidad Austral de Chile, Valdivia, Chile

**Mark Halverson, MD** Department of Radiology, Nationwide Children's Hospital, Columbus, OH, USA

**Mark G. Hamilton, MDCM, FRCSC, FAANS** Division of Neurosurgery, Department of Clinical Neuroscience, University of Calgary, Calgary, AB, Canada

**Brian W. Hanak, MD** University of Washington, Neurological Surgery, Seattle, WA, USA

**Prashant Hariharan, MS, MEng** Department of Chemical Engineering and Materials Science, Department of Biomedical Engineering, Wayne State University, Detroit, MI, USA

**Carolyn A. Harris, PhD** Department of Chemical Engineering and Materials Science, Department of Biomedical Engineering, Department of Neurosurgery, Wayne State University Medical School, Detroit, MI, USA

**Jason S. Hauptman, MD, PhD** Seattle Children's Hospital, University of Washington, Department of Neurosurgery, Seattle, WA, USA

**Albert M. Isaacs, MD** Division of Biology and Biomedical Sciences, Washington University in St. Louis, St. Louis, MO, USA

Division of Neurosurgery, Department of Clinical Neuroscience, University of Calgary, Calgary, AB, Canada

**Evan J. Joyce, MD, MS** University of Utah, Department of Neurological Surgery, Salt Lake City, UT, USA

**Kristopher Thomas Kahle, MD, PhD** Departments of Neurosurgery, Pediatrics, and Cellular and Molecular Physiology, Yale School of Medicine, New Haven, CT, USA

**John R. W. Kestle, MD** Primary Children's Hospital, University of Utah, Department of Neurosurgery, Salt Lake City, UT, USA

**Abhaya V. Kulkarni, MD, PhD** Division of Neurosurgery, Hospital for Sick Children, University of Toronto, Toronto, ON, Canada

**Annie Laquerriere, MD, PhD** Pathology Laboratory, Rouen University Hospital and Inserm Unit 1245, Team Genetics and Pathophysiology of Neurodevelopmental Disorders, IRIB, Rouen, France

**Jeffrey R. Leonard, MD** Nationwide Children's Hospital, Department of Neurosurgery, The Ohio State University College of Medicine, Columbus, OH, USA

**David D. Limbrick Jr., MD, PhD** Department of Neurological Surgery, Washington University School of Medicine, St. Louis Children's Hospital, St. Louis, MO, USA

**William B. Lo, MBBChir, FRCS(SN), FEBNS** Division of Neurosurgery, Hospital for Sick Children, University of Toronto, Toronto, ON, Canada

**Barry R. Lutz, PhD** Department of Bioengineering, University of Washington, Seattle, WA, USA

**Francesco T. Mangano, DO** Division of Pediatric Neurosurgery, Cincinnati Children's Hospital Medical Center, Department of Neurological Surgery, University of Cincinnati College of Medicine, Cincinnati, OH, USA

**John M. McGregor, MD** Department of Neurological Surgery, The Ohio State University Wexner Medical Center, Columbus, OH, USA

**Jason Milton, DO, PharmD, MBA** Department of Neurosurgery, OhioHealth, Columbus, OH, USA

**Nicholas Musgrave, BS** The Ohio State University Medical Center, Columbus, OH, USA

**Robert P. Naftel, MD** Monroe Carell Jr. Children's Hospital at Vanderbilt, Neurological Surgery, Division of Pediatric Neurosurgery, Nashville, TN, USA

**Shahid M. Nimjee, MD, PhD** Department of Neurological Surgery, The Ohio State University Wexner Medical Center, Columbus, OH, USA

**Eduardo Ortega, MD** Hospital Regional de Valdivia, Unidad de Neurocirugía, Instituto de Neurociencias Clínicas, Medical School, Valdivia, Chile

**Smruti K. Patel, MD** Division of Pediatric Neurosurgery, Cincinnati Children's Hospital Medical Center, Department of Neurological Surgery, University of Cincinnati College of Medicine, Cincinnati, OH, USA

**Jonathan A. Pindrik, MD** Division of Pediatric Neurosurgery, Department of Neurological Surgery, Nationwide Children's Hospital, The Ohio State University College of Medicine, Columbus, OH, USA

**Ciarán J. Powers, MD, PhD** Department of Neurological Surgery, The Ohio State University Wexner Medical Center, Columbus, OH, USA

**Geir Ringstad, MD** Oslo University Hospital – Rikshospitalet, Department of Radiology, Oslo, Norway

**Jay Riva-Cambrin, MD, MSc, FRCS(C)** Alberta Children's Hospital, Department of Clinical Neurosciences, Calgary, AB, Canada

**Esteban M. Rodríguez, MD, PhD** Instituto de Anatomía, Histología y Patología, Facultad de Medicina, Universidad Austral de Chile, Valdivia, Chile

**Jonathan Roth, MD** Dana Children's Hospital, Tel-Aviv Medical Center, Pediatric Neurosurgery, Tel Aviv, Israel

**Varun Shah, BS** The Ohio State University Medical Center, Columbus, OH, USA

**Ammar Shaikhouni, MD, PhD** Department of Neurological Surgery, The Ohio State University Wexner Medical Center, Columbus, OH, USA

**H. Wayne Slone, MD** Department of Radiology, The Ohio State University Wexner Medical Center, Columbus, OH, USA

**Luke G. F. Smith, MD** Department of Neurological Surgery, The Ohio State University Wexner Medical Center, Columbus, OH, USA

**Eric Anthony Sribnick, MD, PhD** Division of Neurosurgery, Nationwide Children's Hospital, Department of Neurosurgery, The Ohio State University College of Medicine, Columbus, OH, USA

**Jennifer M. Strahle, MD** Washington Univeristy in St. Louis, Department of Neurosurgery, St. Louis, MO, USA

**Nathaniel Toop, MD** Department of Neurological Surgery, The Ohio State University Wexner Medical Center, Columbus, OH, USA

**Hannah Tully, MD, MSc** Department of Neurology, University of Washington and Seattle Children's Hospital, Center for Integrative Brain Research, Seattle Children's Research Institute, Seattle, WA, USA

**Shawn M. Vuong, MD** Division of Pediatric Neurosurgery, Cincinnati Children's Hospital Medical Center, Department of Neurological Surgery, University of Cincinnati College of Medicine, Cincinnati, OH, USA

**John C. Wellons III, MD, MSPH** Monroe Carell Jr. Children's Hospital at Vanderbilt, Neurological Surgery, Division of Pediatric Neurosurgery, Nashville, TN, USA

**William E. Whitehead, MD** Texas Children's Hospital, Department of Neurosurgery, Houston, TX, USA

**Michael A. Williams, MD** Departments of Neurology and Neurological Surgery, University of Washington School of Medicine, Seattle, WA, USA

**Patrick P. Youssef, MD** Department of Neurological Surgery, The Ohio State University Wexner Medical Center, Columbus, OH, USA

**Weihong Yuan, PhD** Department of Radiology, University of Cincinnati, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

# Part I

# Normal and Abnormal CSF Physiology

# Physiopathology of Foetal Onset Hydrocephalus

1

Esteban M. Rodríguez, Maria Montserrat Guerra, and Eduardo Ortega

## Abbreviations

| | |
|---|---|
| AQP4 | Aquaporin 4 |
| CSF | Cerebrospinal fluid |
| GW | Gestational week |
| NPC | Neural progenitor cell |
| NSC | Neural stem cells |
| SA | Sylvius aqueduct |
| SVZ | Subventricular zone |
| VZ | Ventricular zone |

## Balanced View of CSF Physiology

### Aiming to a Balanced View of CSF Physiology

Several functions have been ascribed to the cerebrospinal fluid (CSF), including protection to the brain, excretion of metabolites, homeostasis of the brain chemical environment, and as a transport pathway between different brain areas [18, 80, 98, 108]. These various functions, coupled with its rapid turnover, perpetual formation,

E. M. Rodríguez (✉) · M. M. Guerra
Instituto de Anatomía, Histología y Patología, Facultad de Medicina, Universidad Austral de Chile, Valdivia, Chile
e-mail: erodrigu@uach.cl

E. Ortega
Hospital Regional de Valdivia, Unidad de Neurocirugía, Instituto de Neurociencias Clínicas, Medical School, Valdivia, Chile


D. D. Limbrick, J. R. Leonard (eds.), *Cerebrospinal Fluid Disorders*,
https://doi.org/10.1007/978-3-319-97928-1_1

and continuous circulation and absorption have led to consider the CSF as the "third circulation," as first referred to by Cushing [16].

For decades, the CSF was regarded as a water solution of ions and few other components, such as glucose and vitamins. The concept of waste drainage was also associated to the "physiology" of CSF. Furthermore, the functional significance of the complex structure of the ventricular and subarachnoid compartments and the multiple populations of cell types lining discrete areas of the ventricular walls were, and still are, overlooked or neglected.

When the cerebrospinal fluid-contacting neurons were discovered, the concept that the CSF could be a pathway for signal molecules started to develop. This idea was strongly substantiated by the demonstration that the choroid plexus is a true gland that, in addition to transport water and ions, it also has the capacity to transport peptides and proteins from blood to CSF and to synthesize and secrete into the CSF a series of biologically active molecules [14, 78, 98]. Although the series of peptides, proteins, and neurotransmitters detected in the CSF using different methods increased throughout three decades, it was the analysis by mass spectrometry that suddenly revealed the enormous complexity of the molecular composition of the CSF.

In recent years, the discovery of aquaporins and other water transporters, all highly selective for water molecules, has again moved the balance to the oversimplified view that CSF physiology refers almost exclusively to water exchange between brain compartments. The glymphatic concept emphasizes the transport of water and waste molecules from the brain parenchyma into subarachnoid space along perivascular pathways of the Virchow Robin spaces, overlooking the fact that this "brain parenchyma" refers to the most superficial region of the brain cortex. It is really disturbing when the physiology of CSF is only associated with the movement of water through the different brain compartment, what leads several authors to talk about CSF secretion when in actuality they are only referring to water transport, completely disregarding the rich heterogeneity of the ventricular walls (circumventricular organs included) and the wealth of signal molecules that use the CSF as a pathway.

## The CSF as a Pathway for a Cross Talk Between Different Periventricular Regions

CSF proteomics is showing a wealth of over 200 proteins [113]. A long series of peptides and neurotransmitters are also present in the CSF. Some of these compounds move by bulk flow from the interstitial fluid of brain parenchyma, many are secreted by neurons, glia, and ependyma into the CSF, others are transported by specific transport systems from blood to ventricular CSF (choroid plexus) while a few of them originate from cells present in the CSF. For many of these compounds, CSF levels bear hardly any relationship to peripheral levels in the blood [98].

The long series of biologically active proteins, peptides, and neurotransmitters present in the CSF reach this fluid through different mechanisms: (1)

Neurotransmitters and their metabolites reach the CSF via the bulk flow of parenchymal fluid. (2) Regulated secretion into the CSF of biologically active compounds by the circumventricular organs (subcommissural organ, pineal gland, choroid plexuses, and median eminence), such as SCO-spondin, basic fibroblast growth factor, melatonin, transthyretin, transthyretin-T4 complex, transthyretin-T3 complex, nerve growth factor (NGF), transforming growth factor-β (TGFβ), vascular endothelial growth factor (VEGF), transferrin and vasopressin [28, 42, 43, 81]. (3) Selective and circadian regulated secretion by CSF-contacting neurons of serotonin and neuropeptides such vasopressin, oxytocin, and somatostatin [80, 99, 100]. (4) Transport of peripheral hormones through the choroid plexus. Most of the transported hormones, such as leptin, prolactin, and thyroxin, have specific targets, mostly the hypothalamus [14, 81]. The concentrations of these neuroactive compounds vary between locations, suggesting they are important for the changes in brain activity that underlie different brain states [98].

Furthermore, a series of findings indicate that cells forming the ventricular walls release into the CSF microvesicles containing signalling and intracellular proteins [13, 22, 32, 55, 96]. Harrington et al. [32] suggested that this bulk flow of nanostructures generates a dispersed signal delivery, of longer duration.

Thus, the early view that the CSF is a medium carrying brain-borne and blood-borne signals to distant targets within the brain [80] has largely been supported by numerous investigations [42, 45, 81, 108]. Worth mentioning here is the much neglected system of CSF-contacting neurons most likely playing receptive functions sensing CSF composition. Most of these neurons are bipolar with the dendritic process reaching the CSF and endowed with a 9 + 0 single cilium [100].

In brief, a good body of evidence is revealing that the dynamic and molecular composition of the CSF and, consequently, the CSF physiology is much more complex and fascinating than the simplistic view held for decades. Signal molecules, either specifically transported from blood to CSF or secreted into the CSF by a series of periventricular structures, use the CSF to reach their targets in the brain. This allows a cross talk between brain regions located beyond the blood-brain-barrier, thus keeping the brain milieu private [29, 98].

## Changing of CSF Composition as It Moves Through the Ventricular System

The ventricular CSF changes its molecular composition as it unidirectionally moves through the various ventricular and subarachnoid compartments (Fig. 1.1a). The choroid plexus of the lateral ventricles, the interstitial fluid of the parenchyma surrounding these ventricles, and axon endings secreting into these cavities are the source of molecules forming this “first” fluid. At the third ventricle, new compounds are added to the CSF by hypothalamic neurons, the pineal gland, and the local choroid plexus [42, 69, 80]. When entering the Sylvius aqueduct (SA), the CSF is enriched by the secretion of the subcommissural organ [101] (Fig. 1.1a). Consequently, the CSF of the fourth ventricle is different as compared to that of the

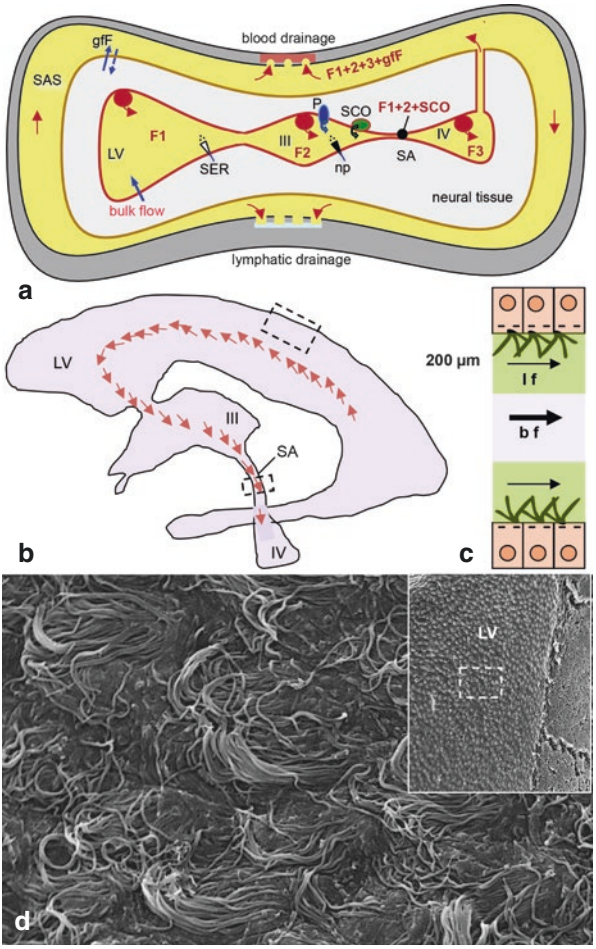



**Fig. 1.1** (**a**) Line drawing depicting the changes in the CSF compositions as it moves throughout the lateral (LV), third (III) and fourth (IV) ventricles. F1 = CSF of the lateral ventricles contains molecules from the bulk flow of the parenchyma and compounds secreted by the choroid plexus and CSF-contacting neurons, such as serotonin (SER). F2 = CSF of the third ventricle contains F1 plus compounds secreted by the choroid plexus and hypothalamic CSF-contacting neurons (np, neuropeptides) and the pineal (P). SA = the Sylvius aqueduct contains F1 + F2 plus the secretion of the subcommissural organ (SCO). F3 = CSF of the fourth ventricle contains F1 + F2 + SA fluid plus compounds secreted by the choroid plexus and CSF-contacting neurons. The CSF of the subarachnoid space contains F1 + F2 + F3 plus molecules from the glymphatic flow (gfF) draining the most superficial region of the brain cortex. (**b**) Line drawing representing the laminar CSF flow (arrows) generated by the cilia beating. (**c**) Scheme representing the laminar (lf) and bulk (bf) of CSF though the Sylvius aqueduct (for orientation see rectangle in **b**). The broken line on top of ependymal cells depicts the negative charges from sialoglycoproteins of the glycocalix. (**d**) Scanning electron microscopy of the lateral wall of a normal rat showing the bundles of cilia of each cells beating in the same direction. *Inset.* Low SEM magnification of a lateral wall of a lateral ventricle (LV). Rectangle frames an area similar to that shown in **d**

lateral ventricles [113]. This partially explains the different protein composition between the CSF collected from the lateral ventricles and that obtained from a subarachnoid compartment [101].

Furthermore, at the interphase brain cortex/subarachnoid space there is a bidirectional flow of CSF and interstitial fluid along the large paravascular spaces that surround the penetrating arteries and the draining veins (Fig. 1.1a). Since water movement along this pathway is mediated by astroglial aquaporin-4 (AQP4) water channels, this paravascular pathway has been termed "glymphatic system" [35, 36]. This pathway facilitates efficient clearance of interstitial solutes, and its failure may lead to neurodegeneration [37].

## Multiciliated Ependyma and CSF Flow

The mechanisms responsible for the CSF circulation are not fully understood. The following factors do play a role: (1) the hydrostatic difference between the production and drainage sites; (2) the pulsations of the cerebral arterial tree; (3) the directional beating of ependymal cilia [18, 106, 107]. The relative contribution of each of these forces is still controversial.

The flow of the CSF throughout the ventricular system involves two different mechanisms, the bulk flow and the laminar flow. Bulk flow is driven by arteriovenous pressure gradients and arterial pulsations. The laminar flow occurs in a thin layer along the walls in a variety of directions [98]. It has been shown that cilia beating is responsible for the laminar flow of CSF, whereas its role in the bulk of CSF taking place in the core of the ventricular cavities is probably insignificant [15, 59, 66, 106]. The cilia beating of the ependyma of the lateral ventricles generate currents as far as 200 μm away from the surface [66] (Fig. 1.1b–d). In the frog brain, about 75% of the CSF within the ventricles is mixed as a result of ciliary activity [66]. Ciliary currents adjacent to the ependyma have been observed in rats, dogs, and humans [109].

The ciliary beating is supported by a sialic acid-induced hydration mantle on the ependymal surface [98]. The frequency of cilia beating is stimulated by the activation of serotonin receptors [68]. Serotonin is released by axon terminals of the suprapendymal serotonergic plexus originated in raphe nuclei [12, 68, 102].

Using computational fluid dynamics, the relative impact of macroscale (choroid plexus pulsation and ventricular wall motion) and microscale (beating of cilia) effects on near-wall CSF dynamics has been investigated [95]. This study revealed a marked effect of the cilia on the near-wall dynamics and directionality but not on the bulk flow. Conversely, the bulk flow alone does not produce any notable directionality of the flow near or on the surface of the lateral ventricles. The authors concluded that in the lateral ventricles, near-wall CSF dynamics is dominated by ependymal cilia action [95]. This confirms early observations that the ciliary movement plays a key role in the maintenance of an adequate CSF flow [31, 109, 111]. The role of the multiciliated ependyma in CSF dynamics is strongly supported by the demonstration that primary cilia dyskinesia, a syndrome that impairs ciliary

activity, leads to the development of hydrocephalus [2, 17, 27, 34, 50, 51, 97]. Shimizu and Koto [93] have suggested that immotility of cilia is of particular importance in narrow canals, such as the Sylvius aqueduct, for the development of hydrocephalus.

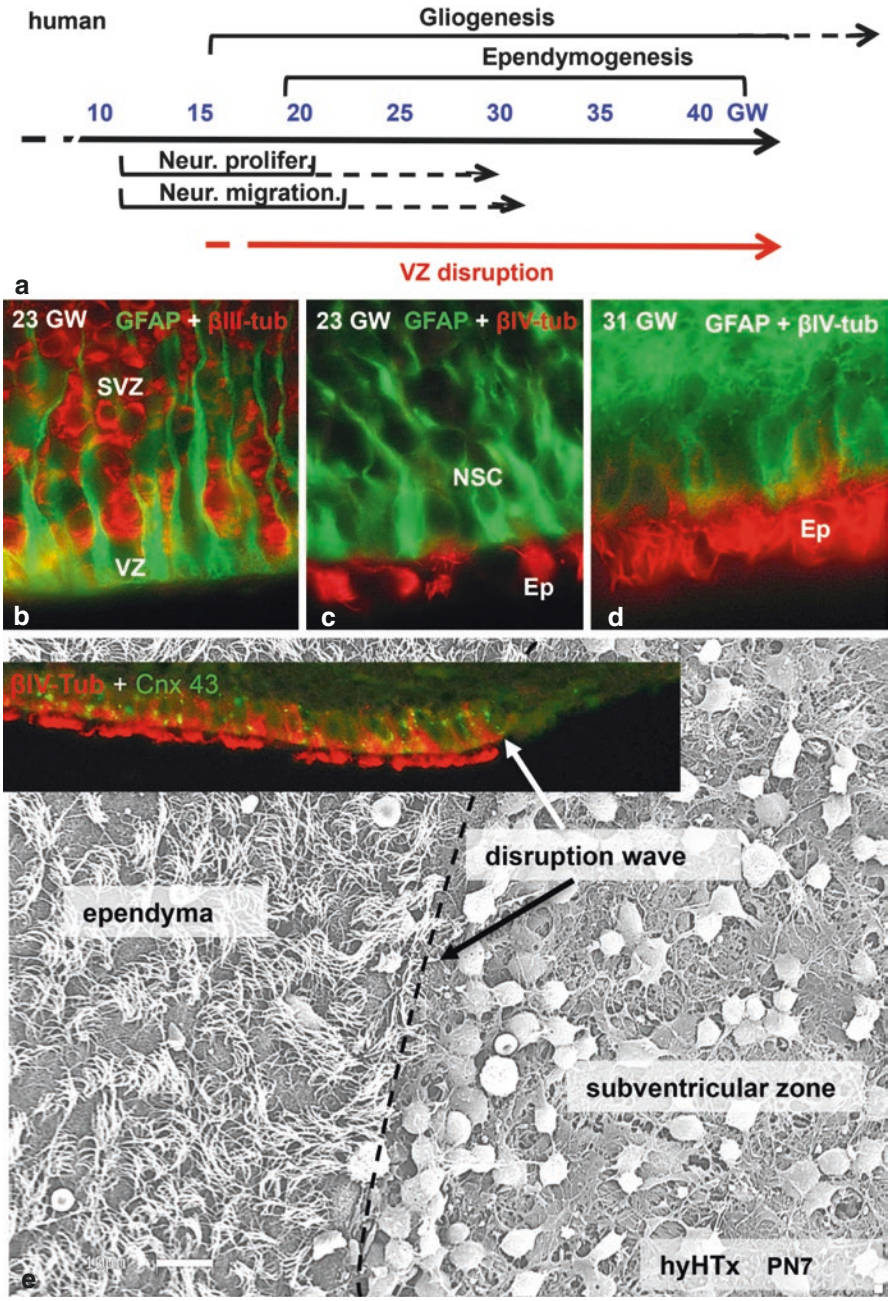
## Human Ependymogenesis

### The Wall of the Lateral Ventricles of the Human Developing Brain Is a Complex and Dynamic Mosaic

Very little, if any, attention has been paid to the complexity of the cell organization of different domains of the ventricular walls. To make it even more complex, such a mosaic undergoes changes during foetal development. This dynamic complexity of the ventricular walls partially resembles that of the ventricular walls of the rodent developing brain. During the last few day of foetal life, the medial wall of the lateral ventricles of the rat is already lined by multiciliated ependyma while the lateral and dorsal walls continue formed by neural stem cells (NSC) [29]. In human embryos, there is also an early division of labour between the medial and the latero-dorsal walls of the lateral ventricles [57]. Such a partition of labour seems an efficient design; while the latter is permanently involved in neurogenesis, the medial wall is progressively engaged in the flow of CSF. Indeed, coupled multiciliated ependymal cells generate the laminar flow of CSF [59, 66] that is essential for the CSF flow along the ventricular system (see above).

In the human developing telencephalon, ependymogenesis starts about the 18th gestational week (GW) (Fig. 1.2a) in the medial wall of the lateral ventricles and progressively continues through the lateral and then to the dorsal walls [57, 83]. Thus, in young foetuses, the lateral wall of the ventricle is fully involved in neurogenesis while the medial wall starts to change its role, from neurogenesis to ependymogenesis. In pre-term foetuses, while the medial wall is fully lined by multiciliated mature ependyma, the lateral wall has mixed populations of cells suggestive of neurogenesis and ependymogenesis.

**Fig. 1.2** (**a**) Panel summarizing the timetable of key events in the developing human brain. The bulk of neural proliferation and neural migration occurs between 12 and 22 GW; then both processes decrease progressively. The process of ependymogenesis starts at about 18th GW and is completed after birth. Gliogenesis starts at about the 15th GW and continues for several months after birth. In the hydrocephalic, human brain disruption of the ventricular zone starts at about 17 GW (*red arrow*). (**b**, **c**) Photomicrographs of a hydrocephalic foetus (23 GW) illustrating the VZ formed by two types of cells: radial GFAP-positive cells (NSC) and radial GFAP/βIV-tubulin positive cells. The subventricular zone (SVZ) contains βIII-tubulin+ progenitor cells. (**d**) The VZ of a 31 GW hydrocephalic foetus appears formed by multiciliated βIV-tubulin+ ependyma (Ep). (**e**) Hydrocephalic HTx rat, PN7. Scanning electron microscopy of the dorsal wall of a lateral ventricle showing the disruption wave, leaving the subventricular zone nude. *Inset.* Similar region with double immunofluorescence for βIV-tubulin (multiciliated ependyma) and connexin 43 (gap junctions). (Source: **a**–**d** from [83])


human
Gliogenesis
Ependymogenesis
10
15
20
25
30
35
40 GW
Neur. prolifer.
Neur. migration.
VZ disruption
a
23 GW GFAP + βIII-tub
SVZ
VZ
b
23 GW GFAP + βIV-tub
NSC
Ep
c
31 GW GFAP + βIV-tub
Ep
d
βIV-Tub + Cnx 43
disruption wave
ependyma
subventricular zone
hyHTx PN7
e

## Cell Types of the VZ. Evidence for an Ependymogenesis Program

According to Rakic [76], the human telencephalic proliferative zone contains considerably complex progenitor cell groups that change during the course of development. In young embryos (5–6 GW) the VZ contains a mixed population of cells; most of them express neural stem markers only (GFAP, GLAST) while others, in addition, also express neuronal markers (βIII-tubulin, MAP-2) indicating their multipotential capacity [114]. Later in development (10–22 GW), vimentin +, GFAP+ cells displaying a long basal process persisted. Proliferation of GFAP+ cells of the ventricular zone (VZ) occurs until the 23rd GW, coinciding with the formation of the ependyma [114]. According to Gould et al. [26], in early pregnancy, the VZ is formed by GFAP+ radial glia/neural stem cells, whereas in late pregnancy, the VZ is formed by GFAP+ ependymal cells with a short basal process,

By use of several markers, Sarnat [85, 86, 88] has followed in human foetuses the temporal and spatial differentiation of cells lining the ventricular walls. In these studies, Sarnat has regarded as ependyma all cells lining the foetal ventricular system. Using this criterion, he concluded that the expression of GFAP and vimentin in foetal ependymal cells follows a regional and temporal distribution [85, 86], with the ependyma of the roof and floor plates being the first to differentiate. During several gestational weeks, GFAP is co-expressed with vimentin in most foetal ependymal cells. At birth, only scattered ependymal cells of the lateral ventricles still express GFAP, and it disappears entirely within the first few weeks of postnatal life [88].

The true process of ependymogenesis in the human remains largely unknown due, to a great extent, to limitations to obtain samples of the ventricular walls from systematically selected regions and selected gestational ages, and to process these samples for different methods. A recent investigation has provided some new evidence on ependymogenesis [57]. Based on the immunoreactivity to GFAP, AQP4, βIV-tubulin, and βIII-tubulin, their morphology (basal process, one or multiple cilia) and their spatial and temporal distribution, we have distinguished seven cell types in the VZ of the lateral ventricles of human foetuses [57, 83]. Type 1 cells with a long radial process, expressing AQP4 in the plasma membrane domain and GFAP throughout the cytoplasm, displaying a single cilium, is the main cell type in the VZ of young foetuses and most likely correspond to NSC (Fig. 1.2b). Type 2 cells are identical to type 1 cells but also express βIV-tubulin, a well-known marker of multiciliated ependyma, suggesting they correspond to NSC that have started to differentiate into ependymal cells (Fig. 1.2c). Cells type 3 through 6 would reflect progressive stages of ependymal differentiation ending in the differentiated multiciliated, βIV-tubulin+, ependyma (type 7) present during the last trimester of foetal life and throughout adulthood (Fig. 1.2d).

## Hydrocephalus

### A Concept

Foetal-onset hydrocephalus is a heterogeneous disease. Genetic and environmental factors, such as vitamin B or folic acid deficiency [40], viral infection of ependyma [44], and prematurity-related germinal matrix and intraventricular haemorrhage [7], contribute to its occurrence.

Numerous investigations in humans and mutant animals have substantiated the view that hydrocephalus is not only a disorder of CSF dynamics but also a brain disorder, and that derivative surgery does not resolve most aspects of the disease [46]. Actually, 80–90% of the neurological impairment of neonates with foetal onset hydrocephalus is not reversed by derivative surgery. *How can we explain the inborn and, so far, irreparable neurological impairment of children born with hydrocephalus?* In 2001, Miyan and his co-workers asked a key question [60]: "Humanity lost: the cost of cortical maldevelopment in hydrocephalus. Is there light ahead?" Although this appealing question has not been responded, there is some light in the horizon. A strong body of evidence indicates that the common past of hydrocephalus and brain maldevelopment starts early in the embryonic life with the disruption of the ventricular (VZ) and subventricular (SVZ) zone. However, the nature, mechanisms, and extent of the brain impairment linked to hydrocephalus are far from been fully unfolded. Certainly, a better treatment of hydrocephalus and the associated neurological impairment will come from a better understanding of the biological basis of the brain abnormalities in hydrocephalus [19, 105]. This view may represent one of the "lost highways" in hydrocephalus research, as described by Jones and Klinge [46].

To have clarity of the timetable of neurogenesis and ependymogenesis in normal rodents and humans seems essential for a better understanding of the early events occurring in foetal onset hydrocephalus.

### Prenatal Neurogenesis. Timetable of Neural Proliferation and Migration, Gliogenesis, and Ependymogenesis

Virtually, all cells of the developing mammalian brain are produced in two germinal zones that form the ventricular walls, the VZ and the SVZ [6, 8, 25, 58, 39, 53]. The VZ is a pseudostratified neuroepithelium that contains multipotent radial glia/stem cells, hereafter called neural stem cells (NSC). NSC line the ventricular lumen and through a long basal process reach the pial surface. A landmark of NSC is their primary cilia that project to the ventricle and are bathed by the foetal CSF [47, 64]. During a fixed period of brain development, NSC divide asymmetrically, with one daughter cell remaining as a NSC and the other becoming a neural progenitor cell (NPC). NPC proliferate and cluster underneath the VZ, forming the so-called

SVZ. NPC differentiate into neuroblasts that start migration using the basal process of NSC as scaffold. In the human, the bulk of neural proliferation and neuroblast migration occurs at a rather short period, between GW 12 and 18 (Fig. 1.2a).

Gliogenesis starts at about the 15th GW and continues for several months after birth. Ependymal cell differentiation starts at about the 18th GW and is completed after birth (Fig. 1.2a) [83, 85, 86].

Over the years, based on our own and other investigators' evidence, we have progressively come to the view that a *disruption of the VZ and SVZ*, in most cases due to genetic defects, triggers onset of congenital hydrocephalus *and* abnormal neurogenesis (Fig. 1.2a, c). We will discuss this evidence below.

### Brain Damage Versus Brain Defects

A distinction must be made between (1) brain *maldevelopment* due to a primary pathology of the VZ that precedes or accompanies onset of hydrocephalus and (2) brain *damage* caused by hydrocephalus. The former occurs during development, and consequently neonates *are born* with a neurological deficit. Brain *damage* is mainly a postnatal acquired defect, essentially caused by ventricular hypertension and abnormal CSF flow and composition.

Brain damage may be associated to regional ischemia, disruption of white matter pathways, and alteration of microenvironment of neural cells [19, 20]. Derivative surgery, the almost exclusive treatment of hydrocephalus today, is aimed to prevent or diminish brain *damage*. It is clear that hydrocephalic patients improve clinically after surgery due to correction of intracranial pressure, improvement in white matter blood flow [19], and probably to resumption of the clearance role of CSF. However, *derivative surgery does not reverse the inborn brain defects*. This has led a study group on hydrocephalus to conclude that "Fifty years after the introduction of shunts for the treatment of hydrocephalus, we must acknowledge that the shunt is not a cure for hydrocephalus" [5].

## Ventricular Zone Disruption

### A Concept and Definitions

For clarity purposes, we shall define the terms used in the present chapter to refer to the ventricular zone. At stages of development when the VZ is mostly formed by neural stem cells (NSC), the acronym VZ will be used. When the VZ is mostly or exclusively formed by multiciliated ependymal cells, the term "ependyma" will be used. The terms "denudation," "disruption," or "loss" will be alternatively used to refer to the disassembling, disorganization, or loss of the VZ cells [81]. A solid body of evidence indicates that radial glial cells are neural stem cells. Throughout the present text, we shall use the term neural stem cells, and its acronym NSC, to refer to the cells forming the embryonic ventricular zone, characterized by a long basal

process, a single 9 + 0 cilium projecting to the ventricle and by expressing certain markers such a nestin [57].

In mutant animals, the disruption of the VZ follows a program that has temporal and spatial patterns, progressing as a "tsunami" wave running from caudal to rostral regions of the developing ventricular system, leaving behind a severe damage (Fig. 1.2e) [29, 41, 74, 81, 103]. A similar process of VZ disruption occurs in human hydrocephalic foetuses [21, 29, 82, 83, 94]. Since the VZ disruption is a continuous process, starting during the embryonic life and continuing during the first postnatal week, the pathology first affects NSC, then the NSC differentiating into ependymal cells and finally the differentiated multiciliated ependyma. These three cell types have distinct phenotypes and certainly play quite different roles. What do they have in common so that the denudation wave will hit them all? Junction proteins appear to be the key to understanding this devastating phenomenon [29].

## A Stormy Intracellular Traffic of Junction Proteins in NSC and Ependymal Cells Leads to Ventricular Zone Disruption

What is the molecular mechanism underlying the VZ disruption occurring in human hydrocephalic foetuses, the HTx rat and in various mutant mice developing hydrocephalus? Overall, a series of findings indicates that disruption of VZ arises from a final common pathway involving alterations of vesicle trafficking, abnormal cell junctions, and loss of VZ integrity [23, 38, 48, 52, 77]. The abnormal localization of N-cadherin and connexin 43 in NSC and ependymal cells and the formation of subependymal rosettes suggest that VZ disruption results from a defect in cell polarity and in cell–cell adhesion of VZ cells. The accumulation of N-cadherin and connexin 43 in the soon-to-detach VZ cells and their virtual absence from the plasma membrane indicate that they are synthesized by the disrupting cells but are not properly transported to the plasma membrane (Fig. 1.3a–d) [29]. The mechanism actually involved in this abnormal expression and translocation of N-cadherin is unknown. The specific disruption of N-cadherin-based junctions is enough to induce ependymal disruption. Indeed, antibodies against chicken N-cadherin injected into the CSF of chick embryos disrupt the VZ, lead to denudation of the SVZ and formation of periventricular rosettes [24]. Similarly, the use of N-cadherin antibodies or synthetic peptides harbouring a cadherin-recognition sequence triggers the detachment of ependymal cells from explants of the dorsal wall of the bovine Sylvius aqueduct [71]. The abnormal localization of connexin 43 in the NSC and ependymal cells could be associated to the faulty localization of N-cadherin. Indeed, it has been reported that gap junction proteins are delivered to the plasma membrane at adherent junction sites [90].

In mutant mice, several gene mutations leading to abnormal trafficking of junction proteins and resulting in VZ disruption have been reported [11, 38, 41, 48, 52, 54, 91]. The nature of the genetic defect in hydrocephalic patients [21, 72, 94] is unknown. It may be postulated that they all carry a defect at one or another point of the pathways assembling adherent and gap junctions.

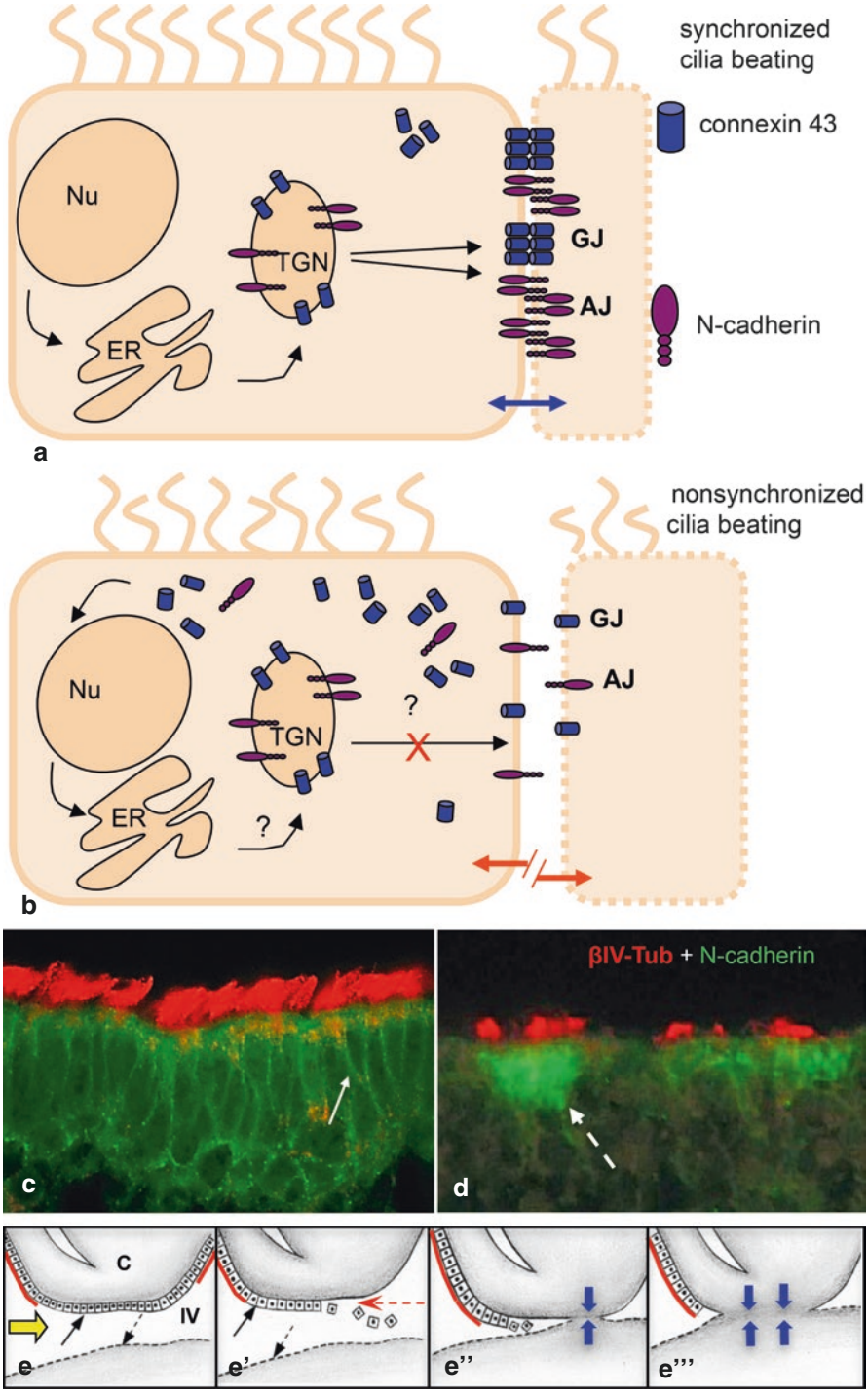

synchronized
cilia beating
connexin 43
Nu
TGN
ER
GJ
AJ
N-cadherin
a
nonsynchronized
cilia beating
Nu
TGN
ER
?
?
GJ
AJ
b
βIV-Tub + N-cadherin
c
d
c
IV
e
e'
e''
e'''

**Fig. 1.3** Proposed mechanisms underlying ependymal denudation and abnormal CSF flow in the Sylvius aqueduct of spina bifida aperta (SBA) patients. The ependyma of the Sylvius aqueduct (SA) of control foetuses display a normal expression and a normal transport to the plasma membrane of the junction proteins N-cadherin and connexin 43 (**a**). This results in normal gap (GJ) and adherent (AJ) junctions. In the SA of SBA patients, N-cadherin and connexin 43 are expressed but their transport to the plasma membrane is impaired. N-cadherin and connexin 43 are abnormally accumulated in the cytoplasm, whereas functional adherent and gap junctions fail (**b**). All together, this may induce: (i) ependymal denudation, aqueduct stenosis, and CSF obstruction; (ii) nonsynchronized cilia beating, abnormal CSF flow, and may finally contribute to (iii) hydrocephalus. ER, rough endoplasmic reticulum; Nu, cell nucleus; TGN, trans-Golgi network. (**c**, **d**) Pallium of a human hydrocephalic foetus showing zones lined by normal (**c**) or abnormal (**d**) ependyma. In areas of intact ependyma, N-cadherin is localized at the plasma membrane (**c**, full arrow). Close to the disruption front, ependymal cells displayed abnormal expression of N-cadherin (**d**, broken arrows). (**e**–**e**′′′). In *hyh* mice, disruption of the VZ lining the ventral wall of the aqueduct occurs during early foetal life (**e**, broken line). Disruption of the dorsal wall of aqueduct occurs shortly after birth (**e**′, red arrow). Then the ventral and dorsal denuded walls fuse, leading to aqueduct obliteration (**e**′′, **e**′′′, arrows) and hydrocephalus. (Source: **a**, **b** from [94]; **c**, **d** from [29]; **e**–**e**′′ modified after [103])

Nongenetic mechanisms leading to VZ disruption have to be considered also [92, 112]. In fact, lysophosphatidic acid, a blood-borne factor found in intraventricular haemorrhages, binds to receptors expressed by the VZ cells resulting in abnormal N-cadherin trafficking, VZ disruption, and hydrocephalus [112]. The vascular endothelial growth factor is elevated in the CSF of patients with hydrocephalus, and when administered into the CSF of normal rats, it causes alterations of adherent junctions, ependyma disruption, and hydrocephalus [92]. Thus, the possibility that signals from the hydrocephalic CSF may contribute, or even trigger VZ disruption, has to be kept in mind. Furthermore, it should be kept in mind that foetal CSF is the internal milieu of NSC [42]. Interestingly, the CSF of hydrocephalic HTx rats has an abnormal protein composition that contribute to the abnormal neurogenesis occurring in this mutant [56, 61, 62, 101].

## Temporal and Spatial Programs of VZ Disruption

The process of VZ disruption has temporal and spatial patterns. The temporal program implies that disruption starts when the VZ is formed by NSC and finishes when the VZ is formed by multiciliated ependyma. In the mean time, a progressive transition from NSC to multiciliated ependyma occurs. The spatial program discloses that disruption begins in caudal regions of the ventricular system and progresses rostrally to reach the lateral ventricles [41, 74, 103]. Each of the two programs has its own outcomes.

In the temporal program, the early VZ disruption implies the loss of NSC and abnormal neurogenesis, while the late VZ disruption results in the loss of multiciliated ependyma and alterations in the laminar flow of CSF and hydrocephalus [29, 94]. In the *hyh* mutant mouse, the program is turned on at E12 and turned off by the end of the second postnatal week [41, 74, 103]. In the HTx mutant rat, disruption in the telencephalon starts at E19 and finishes at the first postnatal week [29].

In hydrocephalic foetuses, disruption of the VZ in the telencephalon has been shown as early as 16 GW [21, 29].

In the spatial program, the disruption of the VZ of the SA implies aqueduct stenosis/obliteration, alteration of the laminar, and bulk flow of CSF and hydrocephalus. At variance, the disruption of the VZ of the telencephalon leads to abnormal neurogenesis [29, 83].

With the years and based on solid evidence, we have progressively come to the conclusion that *foetal onset hydrocephalus and abnormal neurogenesis are two inseparable phenomena, because they are linked at the etiological level.*

In the pathophysiologic programs of VZ disruption, the loss of NSC and ependyma occurs in *specific regions* of the SA and ventricular walls, and also at *specific stages* of brain development. This explains why only certain brain structures have an abnormal development, which in turn results in a specific neurological impairment.

## Pathophysiology of Foetal Onset Hydrocephalus

### The Complex Cell Organization of the Walls of the Sylvius Aqueduct

The walls of the Sylvius aqueduct of wild-type *hyh* mice are formed by several populations of ependymal cells [103]. Interestingly, in mutant hydrocephalic *hyh* mice, some of these ependymal populations undergo proliferation, others are resistant to denudation whereas others denude [4, 74, 103]. In full-term human foetuses, the dorsal, lateral, and ventral walls of the SA three populations of ependymal cells have been described [94]. The functional significance of three ependymal populations is unclear. However, in spina bifida aperta foetuses, there seems to be an association between ependymal lineages of SA and the observed SA pathology. The ependymal cells lining the ventral wall display a normal subcellular distribution of N-cadherin and connexin 43; these cells do not detach. At variance, the ependymal cells of the lateral SA walls display an abnormal intracellular location of junction proteins and are likely to undergo denudation. The formation of large rosettes is mostly associated to this ependyma [94].

### Ventricular Zone Disruption in the Sylvius Aqueduct, Aqueduct Stenosis/Obliteration, and Noncommunicating Hydrocephalus

In the *hyh* mouse, a programmed disruption of the VZ of the ventral wall of the SA starts early in foetal life (E12.5) and *precedes* the onset of a moderate communicating hydrocephalus. The loss of the ependyma of the dorsal wall of the SA occurring shortly after birth leads to fusion of the denuded ventral and dorsal walls of SA, resulting in aqueduct obliteration and severe hydrocephalus (Fig. 1.3e–e‴) [41, 74, 103]. The phenomenon of VZ denudation associated with the onset of hydrocephalus has also been found in other mutant mice [38, 48, 52, 65, 77].

In human hydrocephalic foetuses, ependymal denudation of SA precedes and probably triggers the onset of hydrocephalus [21, 72, 94]. It can be postulated, on solid grounds, that a primary alteration of the VZ of the aqueduct due to various genetic defects triggers the onset of congenital hydrocephalus.

## Ventricular Zone Disruption in the Sylvius Aqueduct, Loss of Multiciliated Ependyma and Communicating Hydrocephalus

In full-term human foetuses and in the perinatal period of mice the SA is mostly lined by multiciliated ependymal cells [94, 103]. The disruption occurring in this period in hydrocephalic humans and mutant mice implies the loss of multiciliated ependyma. Prior to denudation, the abnormal ependymal cells display abnormalities in the amount and subcellular distribution of N-cadherin and connexin 43 (Fig. 1.3) [94]. Since connexin 43 and N-cadherin co-assemble during their traffic to the plasma membrane [104], the abnormal formation of adherent junctions would also result in abnormal gap junctions. Thus, defects of adherent junctions between ependymal cells in hydrocephalic foetuses could alter gap junction-dependent ependymal physiology prior to, or in the absence of, ependymal disruption. An alteration of the CSF laminar flow through the SA of human hydrocephalic foetuses could be envisaged, even if denudation is confined to small areas of the aqueduct wall and hydrocephalus courses with a patent aqueduct (Fig. 1.3a, b). This could be part of the mechanism resulting in a communicating hydrocephalus.

## At Late Gestational Stages, the Disruption in the Ventricular Zone of the Telencephalon Leads to the Loss of Multiciliated Cells and Likely Alterations in the Laminar CSF Flow

The disruption wave starting in the fourth ventricle, after a few days, reaches the telencephalon; then it continues along the walls of the lateral ventricles following a fixed route but avoiding certain discrete regions that are disruption resistant. This phenomenon occurs in certain mutant animals [41, 103] and part or most of it also occurs in human hydrocephalic foetuses [21, 29] and in premature hydrocephalic foetuses with intraventricular haemorrhage [57].

Ciliary beating of ependymal cells is responsible, at least in part, for the laminar flow of CSF occurring on the ventricular surface (see above). Long ago, Worthington and Cathcart [109] concluded that in humans, small areas of ependymal injury and ciliary destruction may affect CSF flow far beyond the region of local damage. During the third trimester of gestation, VZ disruption occurring in hydrocephalic foetuses and in cases with posthaemorrhagic hydrocephalus leaves large areas of the ventricular walls denuded [21, 29, 57]. It seems likely that these local disturbances may impair laminar CSF flow and contribute to the development of hydrocephalus.

## Abnormal Neurogenesis Linked to Foetal Onset Hydrocephalus

### Disruption of the Ventricular Zone of the Telencephalon Is Associated to Abnormal Neurogenesis

In human hydrocephalic foetuses [21, 29], premature infants with posthaemorrhagic hydrocephalus [57], the HTx rat [29] and the *hyh* mouse [23], the VZ disruption results in two neuropathological events: formation of periventricular heterotopias and translocation of NSC/NPC to the CSF (Fig. 1.4a–d).

At regions of disruption where NSC have been lost, the neuroblasts generated in the SVZ no longer have the structural scaffold to migrate and consequently accumulate in periventricular areas forming periventricular heterotopias. In human hydrocephalic foetuses, periventricular heterotopias have been found in young (21 GW) and full-term (40 GW) foetuses, indicating that they were formed early in development and had remained in situ until the end of foetal life and, probably, after birth (Fig. 1.4a, b). Interestingly, a 2-month-old child with a disrupted VZ carried periventricular heterotopias [23]. Humans with disruption in the VZ of the telencephalon carry periventricular heterotopias primarily composed of later-born neurons [23]. Periventricular heterotopias behave as epileptogenic foci [30]. This may explain why 6–30% of hydrocephalic children, including the present case, develop epilepsy that is not solved by CSF drainage surgery [72, 89].

### The Cerebrospinal Fluid Is the Main Fate of the Disrupting NSC/NPC

In hydrocephalic human foetuses [21, 29] and premature infants with posthaemorrhagic hydrocephalus [49], NSC/NPC reach the ventricle at sites of VZ disruption and can be collected from the CSF. Furthermore, cells collected from CSF of two SBA foetuses develop into neurospheres [83].

hydrocephalic foetus, 40 GW, with a large denuded area covered by a layer of glial fibrillary acidic protein (GFAP) positive astrocytes. Periventricular heterotopias (PH) are associated with disruption of the VZ. (**c**) In the HTx rat, disruption of the VZ results in shedding of proliferative neural progenitor cells into the CSF, as shown by injection of BrdU in living animals and tracking the BrdU+ cells in tissue sections (**c**, arrow) and CSF cell pellets (**d**). βIII-tubulin+ or nestin+ cells are present in the cell pellet (**d**). (**e**, **f**) Under proper culture conditions, cells grow forming neurospheres displaying a similar junction pathology than hydrocephalic living animals. In neurospheres from non-affected HTx rat, N-cadherin is located at the plasma membrane (**e**); in neurospheres from hydrocephalic CSF N-cadherin accumulates in the cytoplasm (**f**). (**g**) Line drawing depicting the pathology of ventricular zone (VZ). Whereas disruption in the aqueduct of Sylvius leads to hydrocephalus, VZ disruption in the telencephalon results in abnormal neurogenesis. A cell junction pathology appears to be a final common pathway of multiple genetic and environmental factors that finally result in the disruption of the VZ. (Source: **a**–**g** from [29])

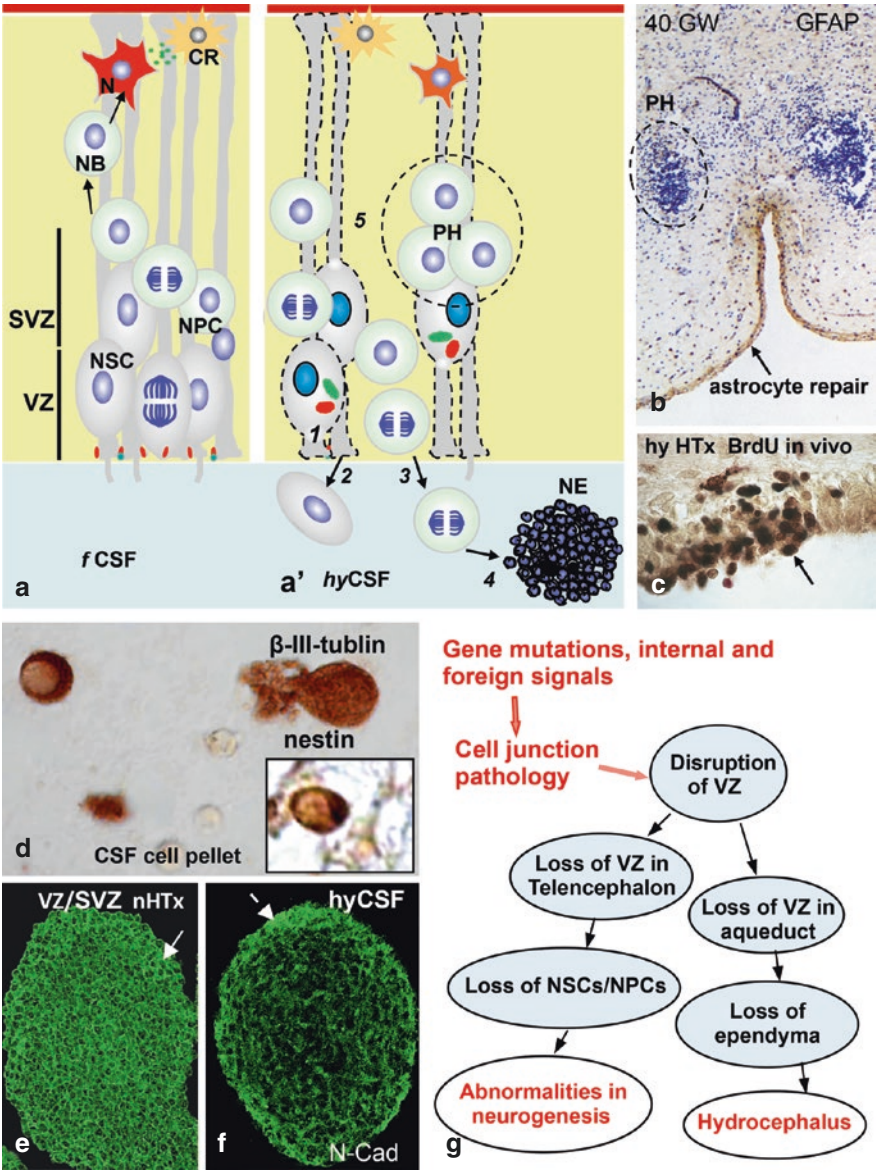



**Fig. 1.4** Neuropathological events associated to the disruption of the ventricular zone in the telencephalon**.** (**a**, **a′**) Line drawings depicting the pathology. (**a**) Neural stem cells (NSC) in the ventricular zone (VZ) proliferate to raise proliferative neural progenitor cells (NPC), which migrate as neuroblasts (NB) along radial processes of NSC and differentiate into neurons (N). NSC are joined by adherent and gap junctions. CR, Cajal-Retzius cell; fCSF, foetal cerebrospinal fluid; SVZ, subventricular zone. (**a′**) Disruption of the VZ results in displacement of NSC [1] and NPC [2] into the CSF [3]. These cells can be collected from the CSF of hydrocephalic rats and cultured. They develop abnormal neurospheres [4]. The absence of the scaffold provided by NSC results in arrested neuroblasts that form periventricular heterotopias (PH) [5]. (**b**) Telencephalon of a human