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Neurofluids – deep inspiration, cilia and preloading of the astrocytic network

Hans C Ludwig (orcid.org/0000-0001-8744-9510)[1], Steffi Dreha-Kulaczewski (https://orcid.org/0000-0003-4951-3176)[2] and Hans C Bock (https://orcid.org/0000-0002-6656-6053)[1]

1. Department of Neurosurgery, Division of Pediatric Neurosurgery, University Medical Center Göttingen, Robert-Koch-Str. 40, 37075 Göttingen, Germany
2. Department of Pediatrics and Adolescent Medicine, Division of Pediatric Neurology, University Medical Center Göttingen, Robert-Koch-Str. 40, 37075 Göttingen, Germany

Corresponding author: Hans C. Ludwig (hludwig@med.uni-goettingen.de)

Search strategy and selection criteria

We searched PubMed, Crossref Search and Web of Science for reports published in English from Jan 1, 1995 to December 31, 2020. The search terms “hydrocephalus”, or “hydrocephalic” were combined with many terms for epidemiology, pathophysiology, etiologies, diagnosis, management such as “aquaporin”, “cerebro spinal fluid”, “glymphatic”, “sleep”, “head down tilt”, “nitric oxide”, “amyloid”, “neurovascular coupling”, “autoregulation”. In addition to the search results, we also hand searched the references of relevant articles retrieved by search strategy.

Key points

- In vivo real time flow MR-imaging facilitates the study of human CSF pathways without gating and quenching of slow frequency breathing induced signals.
- Recent findings challenge historical views about CSF circulation and call for a physiologic concept that takes the interplay of macroscopic fluid flow and microscopic drainage pathways into account.
- Novel insights into brain fluids flow at ependymal surfaces, cilia sensing, aquaporin and pericapillary spaces are rising.
- Ependymal cilia sensing uses signal pathways modulated by nitric oxide and amyloid-ß.
- Neurofluid flow and waste clearance into the astrocytic network enabling fluid flow towards pericapillary Virchow-Robin spaces is mediated by aquaporin receptors and tuned by neurovascular coupling.
- Brain disorders arising from disturbed cerebrospinal fluid (CSF) circulation constitute a major proportion of human CNS diseases.

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Abstract

With the advent of real-time MRI, the motion and passage of cerebrospinal fluid can be visualized without gating and exclusion of low-frequency waves. This imaging modality gives insights into low-volume, rapidly oscillating cardiac-driven movement as well as sustained, high-volume, slowly oscillating inspiration-driven movement.

Inspiration means a spontaneous or artificial increase in the intrathoracic dimensions independent of body position. Alterations in thoracic diameter enable the thoracic and spinal epidural venous compartments to be emptied and filled, producing an upward surge of cerebrospinal fluid inside the spine during inspiration; this surge counterbalances the downward pooling of venous blood toward the heart.

Real-time MRI, as a macroscale in vivo observation method, could expand our knowledge of neurofluid dynamics, including how astrocytic fluid preloading is adjusted and how brain buoyancy and turgor are maintained in different postures and zero gravity.

Along with these macroscale findings, new microscale insights into aquaporin-mediated fluid transfer, its sensing by cilia and its tuning by nitric oxide will be reviewed. By incorporating clinical knowledge spanning several disciplines, certain disorders—congenital hydrocephalus with Chiari malformation, idiopathic intracranial hypertension and adult idiopathic hydrocephalus—are interpreted and reviewed according to current concepts, from the basics of the interrelated systems to their pathology.

Key words

Real-time MR imaging, CSF, cilia sensing, aquaporin, nitric oxide, amyloid-ß, glymphatic system, hydrocephalus

Main text

Introduction

We are currently witnessing the revision of refuted theories and the development of new concepts to understand the pathophysiology of hydrocephalus (HC), which was classified over 100 years ago by Dandy (1919) into the categories of malabsorptive and occlusive HC. This classification defines the choroid plexus as the main secretion site for cerebrospinal fluid (CSF) and assumes that resorption can take place anywhere in the basal cisterns, in the pacchionian granulations or along nerve sheaths and lymphatic vessels of the meninges. This classification is still the basis for education and training of medical students and residents within neurology and related disciplines today. It also serves as the rationale for decision making in therapeutic strategies for neuroendoscopic or shunt-related treatment procedures. Although this classification has been questioned by several authors over the last few decades and continues to face challenges today, the Dandy concept is still popular[1].

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The reason for such traditional adherence to established concepts despite new knowledge is the gap between experimental findings or theoretical considerations and its slow implementation into everyday treatment and care.
Recent research arguing against the traditional Dandy concept was presented by the work of the Croatian group of Orescovic, Bulat and Klarica. They demonstrated zero net CSF flow through and out of the cannulated aqueduct in a cat model for over 2 hours[2]. Nevertheless, these results are difficult to translate into daily hydrocephalus care[3].
We ourselves presented further data demonstrating an inspiration-driven upward surge of CSF as an exchange maneuver, counterbalancing the intrathoracic pooling of venous blood during inspiration in a manner that fulfills the requirement of the Monro-Kellie doctrine[4,5]. This equation states that drained venous blood is replaced upstream by the same volume of CSF and vice versa. The exchange volume is quite high, accounting for 20% of the total hemodynamic output of the heart.
We have previously attributed a regulatory capacity to the human aqueduct, facilitating flow through its narrow structure, with the conducted flow varying as the 4th power of the radius according to the Hagen-Poiseuille equation[6,7]. This unique aqueductal architecture might provide the modulatory function of a nozzle, with form following function[6] and vice versa. Even complete closure might occur, induced by a large, cardiac-driven oscillation of the fluid compartment above the level of the narrow aqueduct. A low-pressure level beneath the aqueduct in a standing position results in an apparatus resembling a gravity-assisted valve to fulfill the requirements of differential CSF supply in the supine and upright positions. The results and accepted understandings of experimental approaches to the CSF dynamic system could be transferred into daily HC care by analyzing different mechanisms of fluid trapping in occlusive hydrocephalus[7]. Characteristic MRI findings and intraoperative video recordings could be summarized as an occlusive mechanism where centripetal upward CSF flow passes through the aqueduct from bottom to top without restrictions but becomes trapped inside the aqueduct by internal membranes or stenosis, resulting in a characteristic ventricular enlargement above and a downward bowing of the floor of the third ventricle[7,8].

The main obstacle to gaining direct clinical insight regarding treatment adaptation and optimization from recent research findings[9] is the perception that they often refer to animal models or physical simulations hardly comparable to human physiology.
One crucial reason for the controversy of results from animal models might be based on differences in the specifics of the aqueductal architecture and its function and setup regarding the organization of hydrostatic efforts in relation to gravity. The aqueduct in rats is oriented in a horizontal position combining a valve and guiding system equipped with a central wire, the Reissner fiber, consisting of spondin fibrils that connect the roof of the third ventricle with the sacral tip of the spinal canal[10,11]. Moreover, CSF flow in rats is dependent on CSF movements induced by motile cilia, which create a steady fluid movement above the ventricular ependyma and choroid plexus in correspondence to body movements and passive propagation of CSF[12-14].
It is therefore highly important to reanalyze the main elements essential for CSF flow propagation and the necessary ciliary sensing ability and to derive new insight into related disorders. Certain aspects discussed here are still conceptual, based on real-time flow MRI with derivatives supported by the literature.

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Ciliary function

Different genetically based congenital aberrations of ciliary function are known to cause syndromic HC in rats, mice and humans. In humans, there are several genetic aberrations associated with HC, such as Kartagener syndrome[15] and flagellar disorders[16], but it seems that humans are less dependent than rodents on ciliary motility for the propulsion of CSF. Nevertheless, ciliary pathology might be involved in a failure of flow sensing and signal transduction[12]. Knowledge from animal-based HC studies might therefore be restricted and should be transferred to human physiology with caution.

It appears necessary to resume focus on different human environments and the hydrostatic context as well as on disturbances of the participating systems through a deductive analytical approach.

We have previously described possible causal mechanisms for space flight disease, which represents a subtype of hydrocephalus featuring enlarged ventricles and basal cisternal CSF compartments even several months after landing [17]. Symptomatic space flight disease presents with headache, nausea, vomiting and papilledema, affecting up to 60% of astronauts and cosmonauts sometimes even causing permanent visual disturbances[18]. We interpreted the influence of zero gravity as facilitation of the inspiration-related upward flow of CSF from the spine to the cranium with a simultaneous lack of the hydrostatic counterforce[19].

Under normal terrestrial gravity conditions, a similar condition exists only in the supine position and is promoted by deep and enduring inspiration during stages II and III of non-REM (NREM) sleep[20].

The similarities of CSF movements during NREM sleep compared to our studies of healthy young individuals by real-time MRI (RT-MRI) indicate a strongly regulated upward CSF movement restricted to periods of vigilant forced inspiration or during NREM sleep with sustained deep respiration[20].

The persistently altered dimensions of the CSF compartments even months after landing and termination of zero gravity imply a structural reorganization of the ventricular architecture and the periventricular microstructure, which requires involvement of certain sensing elements and specific receptor interplay. This interplay might be challenged by the endurance of space flight microgravity in contrast to the terrestrial environment, in which strong upward CSF movement can be observed only sporadically during single phases of NREM sleep and during phases of spontaneous forced breathing [5 21]. The propulsion of CSF primarily by deep breathing, as opposed to the small and fast oscillations driven by the heartbeat, was a common observation in several MRI experiments, in which physiological cardiac gating by real-time methods enabled the effect of the pulse to be excluded [22, 23, 24]. The review at hand will focus on these elements and provisions and will identify related concepts.

Sleep and CSF

Recently published experiments with healthy sleeping individuals inside an MRI scanner demonstrated upward CSF flow through the IVth ventricle restricted to deep NREM sleep. This upward flow coincided with slow electroencephalogram (EEG) waves and a cortical brain oxygenation level-dependent (BOLD) signal, pointing towards high cortical metabolic O_2 consumption. A completely different signal has been measured and documented during REM sleep, featuring high cortical electric activity and coincidental EEG signals typical of upregulated mental and neuronal processes and even muscular activity such as lid closure or tongue, mimic or limb movements. During these repeated REM periods, which must alternate with NREM phases for

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regular and healthy sleep performance, no sustainable CSF flow and BOLD signals have been registered, even not during upregulated cortical electric activity [20]. Previously and without integrating EEG and BOLD signals into the experimental setup, it has been reported that adrenergic signaling is important for sleep and awake state and amyloid-β (Aß) clearance[25] during sleep.

The question that arises is whether cortical electric activity as a concomitant expression of the elevated BOLD signal is the underlying reason for the steady and deep breathing rhythm or whether NREM-induced, sustainable inspiration breathing patterns provide sufficient CSF flow, sensed and processed into neuronal cortical activity by ependymal cilia. This steady flow detection and sensing is a unique mechanism of different epithelial tissues operating as an interposed signal to register and read out of different membrane coupled receptor bindings and propagation of its effector pathway throughout the epithelial cellular units (Fig. 1). One of the binding sites for growth factors and signals from the extracellular liquid environment are cilia-coupled membrane receptors, which have to integrate signals from extracellular flow excitation into intracellular checkpoints for regular function[26]. These checkpoints are linked to basic pathways of inflammation, redox balance and energy supply by the Toll-like receptor (TLR) family[27], transient receptor potential vanilloid 4 cation channel (TRPV4) [28] and nitric oxide (NO)[29].

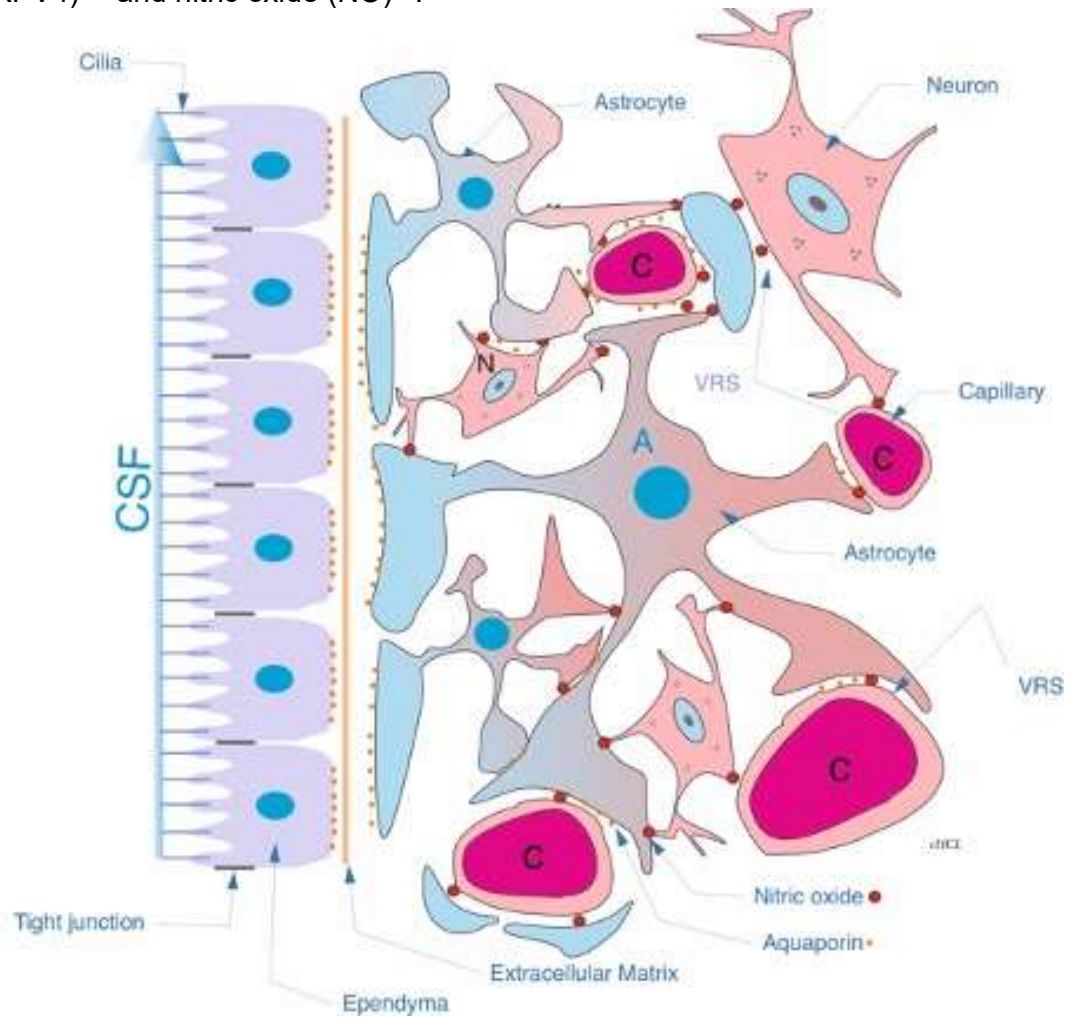

Figure 1: Schematic drawing of the astrocytic network. Cilia deflection, nitric oxide signaling and neurovascular coupling for the enhancement of CSF and interstitial fluid exchange from

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ventricular and subventricular to the Virchow-Robin space (VRS). Subependymal extracellular matrix of the basilar membrane. Ependymal cell layer with tight junctions. Drawn according to Jiménez et al. [122].

Ciliary dysfunction

Primary and motile cilia cover the surface of the ependyma and choroid plexus epithelium. Since evidence exists for the flow-sensing capability of primary cilia, a growing list of disturbances and diseases induced by a dysfunction of this mechanism have been described. Several groups have focused on these organelles, their function and disturbances related to CSF disorders. The research group of Kortcuogly *et al*. has investigated the impact of differently expressed cilia patterns on CSF flow characteristics inside the third ventricle of rats[30]. These cilia are able to promote and conduct the synergistic activity of coupled cell matrices by integrating a circadian genetic mechanism[31]. Cilia even protect the underlying intracellular actin skeleton, which stabilizes cilia and maintains constant tissue shape and architecture[32]. Coupled by Ca^{++} signaling, cilia are able to combine different ependymal cells and synchronize them as a functional unit in the form of pinwheels, which are functional groups of cilia-bearing ependymal cells[33]. The main sensing function of cilia is believed to be the detection and surveillance of fluid flow[34].

Congenital inherited disturbances of ciliary function, such as Bardet-Biedl syndrome, are related to the clinical disorder of disturbed renal water reabsorption. It could be described as causing severe disturbances of cilia assembly and disassembly and thereby alter Wnt signaling[35,36], a highly important intracellular checkpoint of the hedgehog pathway. Another severe ciliopathy causes polycystic kidney disease (PKD), which leads to profound destruction of the kidney parenchyma by cystic tubular reformation induced by dysfunctional cilia-conducted signal processing[37]. In PKD, intraflagellar transport (IFT) is disturbed by mutated proteins, including cystin (cpk mouse), polycystin 1 and 2 (PC-1 and 2), encoded by Pkd1 and 2 and mutated in autosomal dominant PKD (ADPKD); fibrocystin, mutated in autosomal recessive PKD (ARPKD); and nephrocystin and inversin, mutated in nephronophthisis[38]. In our view on hydrocephalus-related disorders and to regain arguments for interaction between ciliary flow sensing of CSF movements and consequences for concomitant structural organization and control, it is important to mention that disturbed ciliary function and processing is able to induce aberrations of the regular tubular kidney tissue pattern. The underlying evolutionarily derived system of cilia processes adjustments of tissue properties, such as planar cell polarity and left-right and apical-sacral organization of the neural tube, is phylogenetically widely distributed[39].

In this view, it is no surprise that the identical genes pkd1 and pkd2, which encode renal tubular tissue integrity, are involved in the organization of planar cell polarity in neural tube progenitor cells and early brain ventricular organization[38,40]. Endothelial primary sensing cilia are related to atherosclerosis in areas of blood flow disturbances, where they act as biomechanical flow sensors of endothelial function[41]. Inside the CSF compartments cilia-based processing of ependymal polarity, its maintenance and function in normal and disturbed organisms has already been described. In rat dysfunctional cilia, disturbances of the intraflagellar transport (IFT) gene encode alterations of the plexus and ventricular ependyma[42]. Mammalian motile cilia arrange ependymal orientation during development by hydrostatic forces and fluid flow[34]. Structural defects of cilia of the choroid plexus, subfornical area and ependyma induce

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ventriculomegaly[43]. In deionizing brain radiation due to antitumor therapy with concomitant hydrocephalus, it could be demonstrated that the ependymal cells of the ventricles and aqueduct have lost their cilia, and hydrocephalus is a frequent post radiation complication[44]. It could be shown in rat tail suspended animals as a model for zero gravity that disturbances of ependymal cilia were the underlying reason dependent on the rate of repeated simulation of weightlessness[45]. In addition to the described alterations of choroidal cGMP levels[46], several authors could already report structural alterations of choroidal cell polarity, vesicle turnover and cilia expression during the late nineties in space-flight-exposed rats as well as in hindlimb-suspended rats as a corrective modulation of CSF secretion in microgravity[47,48,49]. Davet *et al*. reported in detail on structural ventricular and choroidal readaptation after space flight or hindlimb suspension. The authors identified altered expression of ezrin, a protein related to laminin-aquaporin binding, and observed enhanced expression of microvilli, ciliary protrusion and vesicular density as a hint of elevated turnover of the apical ependymal cell structures[49]. They concluded that even after several days of normal terrestrial gravity, the alterations remained in place and could not revert to baseline. This was demonstrated once more in rats several years later by another group of authors who focused on structural alterations of the apical microvilli as an adaptation process[50].

Cilia and metabolism

To interpret the different observations under reduced gravity, one might focus on the alterations of venous and arterial blood flow and alterations of the CSF in the first place. In several human models of ground-based space flight simulations during SPACECOT studies, the pathophysiology of hemodynamics and ventricular size modulations were studied under hypercapnia as an ideal model for real space flight. Compared to supine baseline positioning, head-down tilt (HDT) in ambient air resulted in a significant increase in lateral ventricular volume ($p=0.03$). Cerebral blood flow, however, decreased under HDT in the presence of either ambient air or 0.5% CO_2 ($p=0.002$ and $p=0.01$, respectively). This effect was partially reversed by acute 3% CO_2 exposure. Following HDT under ambient air exposure to 3% CO_2, increased aqueductal cerebral spinal fluid velocity amplitude ($p=0.01$) and lateral ventricle CSF mean diffusivity ($p=0.001$) occurred[51].

These different results posed the question of whether altered hemodynamics, for example, by modifications of the cerebral redox situation together with structural elements of the ependyma, are able to interplay and serve as the included interrelated pathway[52]. In this pathway, nitric oxide (NO) and cGMP are essential for the regulation of cerebral blood flow (CBF) and its neurovascular coupling[53,54], and both mediators are related to the activity of motile cilia in several human and animal tissues[55].

Neurovascular coupling

Neurovascular coupling is the basic regulator of metabolism based on the energy demands of neuronal function. The coupling requires a concomitant interplay between the metabolic state of a neuron and the serving capillary network section surrounded

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by an astrocytic process and its interrelated metabolic pathways [56,57,58]. The interplay might be part of the resting state network [59].

Neurons close to the lateral but not the medial corpus callosum can be stained with antibodies directed against $NADPH^+$ and neuronal NO synthase (nNOS), mostly in direct contact with ependymal layers and in close vicinity to the CSF space. The authors raised the question of whether the close spatial relationship implements the possibility that these neurons are a major source of NO during neural activity. The somatic, dendritic and axonal processes of many $NADPH^+$/nNOS neurons were closely associated with intracallosal blood vessels. As NO is a potent vasodilator, these findings strongly suggest that NO-positive neurons transduce neuronal signals into vascular responses in selected corpus callosal regions, thus giving rise to hemodynamic changes detectable by neuroimaging[60]. This could imply that enhanced CSF flow detected by sensing cilia serves as an important signal that is transmitted by astrocytic end feet [53] to the vascular network (Fig. 1 and 2). Enhancing neurovascular coupling by sufficient CSF flow could be the underlying pathway further conducting inspiration-induced sustainable CSF movements upwards into a neuronal signal that elevates metabolic activity and an enhanced BOLD signal during regular NREM sleep. Sleep disturbances on the contrary side have been identified to be related to altered NO levels[61]. As a consequence of apnea disorders[62], modified sleep homeostasis is linked to NO mediation[63]. Due to this close relationship between sleep, NO mediation and CSF flow, it is not surprising that disturbed sleep performance can immediately be detected by upregulated tau and amyloid beta (Aβ), even in healthy patients, after a single night of sleep deprivation[64, 65]. Sleep deprivation and circadian disturbances are able to induce Aβ and tau accumulation by impairing glymphatic flow[66]. HDT of –12° under microgravity-simulating conditions was able to significantly alter sleep quality and brain perfusion in a recent experimental study of physiology during space flight[67]. In another recent MRI study focusing on sleep and the dimension of the perivascular Virchow-Robin space (VRS) as parts of the glymphatic system[68], it could be shown in rats that sleep and VRS dimensions were negatively correlated. These results further enhance the link between sleep, interstitial or perivascular fluid flow and waste metabolite clearance[69]. This relation is dependent on the astrocytic aquaporin-4 (AQP4) outfit and in this aspect from several genetic variations[70].

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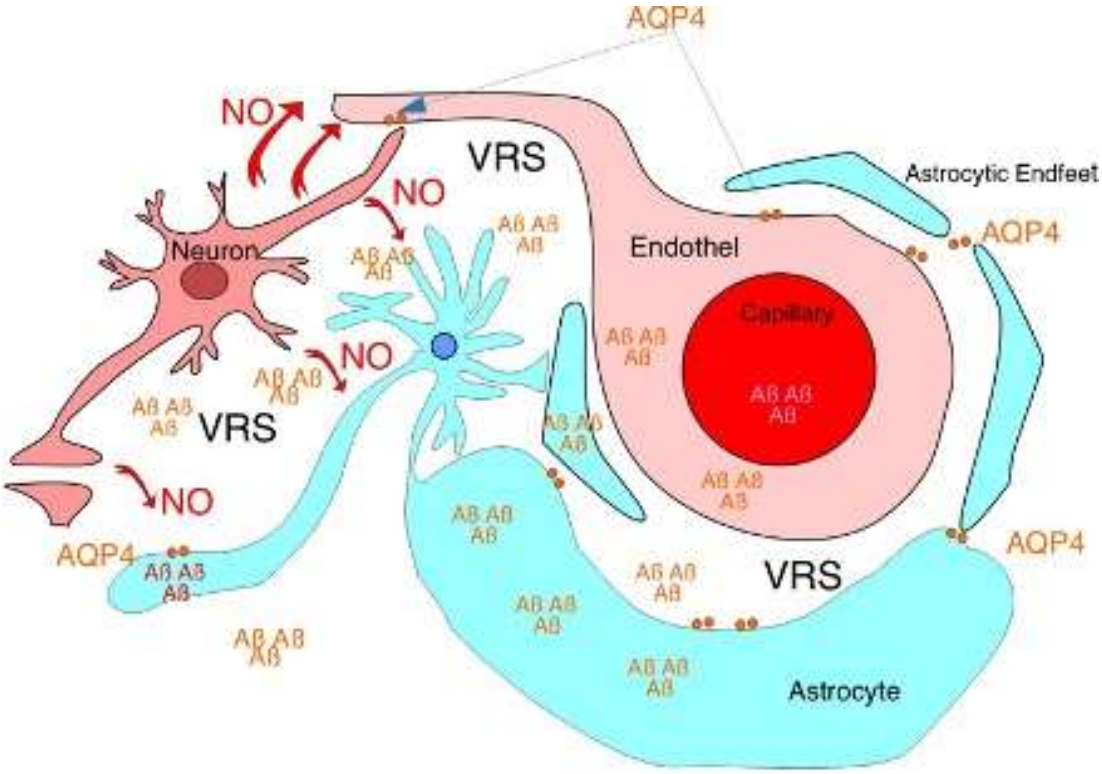

Figure 2: Schematic drawing of the neuronal amyloid beta (Aβ) waste turnover from the neurons to the astrocytic network passing into the Virchow-Robin space (VRS) for clearance into the capillary bed. Enhancement by neuronal nitric oxide (NO) diffuses freely and is transmitted through aquaporin 4 (AQP4) canals.

There is still an ongoing discussion in the literature regarding whether the glymphatic system truly exists and is able to fulfill these differently allocated functions of waste clearance and lymphatic capabilities. Some authors question whether waste clearance is possible by diffusion alone[71]. Others doubt that a necessary long-range network for convective transport through the parenchymal gray matter exists[72]. The advocates of the glymphatic theory argue for the proposed fluid transport in brain parenchyma and a major role for AQP4 in cerebrospinal fluid (CSF)–interstitial fluid exchange under normal physiological conditions[73,74]. Before resuming the glymphatic system[75] as the main CSF handling structure [76], the anatomical and physiological assumptions of the underlying astrocytic network should be recapitulated.

The astrocytic network

Previously, astrocytes were considered “bystanders” with the sole purpose of clearing out neuronal perisynaptic Aβ accumulation. Moreover, astrocytes are able to synthesize Aβ themselves and accumulate the metabolite in their cytoplasmic network, thereby establishing an interconnected network system (Fig. 2). With this function, the astrocytic network is necessary for the protection of neurons for the establishment of the blood-brain barrier as well as the conduction and distribution of waste products

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through AQP4 canals. Perisynaptic deposits of Aβ can severely damage the maintenance of the network, which has the intriguing capacity for permanent buildup, turnover, self-establishment and renewal. This capacity is mainly provided by the waste metabolites themselves, identifying Aβ as the main promotor[77]. Aβ is a common and physiologically occurring antimicrobial substance[78]. The promoting metabolite together with the efforts of neurovascular coupling use AQP4 connectivity as conduits for NO as the main redox metabolite and Aβ as the main waste-clearing signal. This could lead to the mechanism by which astrocytes are induced by their recycled neuronal Aβ to produce their own Aβ as an enhancement and activation signal. Armato *et al*. outlined this unique mechanism, which might result in suicide overproduction of Aβ and the coupled metabolite NO, leading to Aβ overload[77]. The physiological purpose behind this mechanism is seemingly to enhance the establishment and recruitment of larger distributing and clearance network capacities to expand the contact zone with the adjacent pericapillary VRS as a sink for waste products (Fig. 1 and 2). The choroid plexus was once thought to serve as a bystander system in the clearance of Aβ. This system was shown to become corrupted or dysfunctional regarding the clearance ability of Aβ, as could be demonstrated in patients with normal-pressure hydrocephalus (NPH)[79].

The astrocytic network is further enhanced by Ca^{++}-sensing proteins and hypoxia inducible factor (HIF) as pathway mediators for three distinct VEGF ligands[80]. As long as the astrocytic network can be maintained in a regular mechanism without extreme Aβ overload and fueled by enough energy supply without disturbances of the redox state, glymphatic waste clearance might be functionally preserved.

The differences in systemic lymphoid functions dependent on similar but distinct AQP isoform receptors in varying body organs and tissues might not be as high as assumed. In the brain parenchyma, this astrocytic network is connected to the CSF, compartmentalized and organized by extracellular basal membranes and its main equipment component AQP4, oriented and expressed in a specific pattern polarized at the basal and apical astrocytic membrane constituents (Fig. 1). It seems that astrocyte-produced Aβ serves as a mediator of VEGF enhancement for angiogenic purposes[81]. Armato *et al*. (2013) indicated that stimulating neuronal activity in this region increases blood flow through the expanded vascular network and with it blood oxygen-dependent (BOLD) fMRI signaling by NO liberation. However, as the brain converts to Alzheimer's disease (AD), the excessive accumulation of Aβ and the resulting production of large amounts of NO will severely perforate the blood vessels and break through the blood-brain barrier[77]. This pathway connects neuronal activity-elevated function on demand by vascular-neuronal coupling[82] and enhances the waste clearance network in an impressive way. The network might function properly, as long as no disturbances are mainly driven by a failure in AQP4 balance, ATP diminution or Aβ overload by altered sleep and recovery capacity. These disturbances could lead to unphysiological Aβ deposition and network breakdown. In that situation, unphysiological, damaging amounts of NO might be liberated and lead to breakdown of membranes, perforation and damage of the blood vessel tree with disastrous consequences for the brain function inducing dementia. Therapeutics aiming at the pharmacological principles of Aβ accumulation alone, with antibody therapies designed to target Aβ, might not be successful without addressing and including the complete network system. Another restriction is the study of the glymphatic system in vitro, a setting in which the directional recycling path among ependymal CSF-contacting surfaces, the astrocytic AQP4-equipped network and pericapillary spaces cannot be supplied. In this network, neurons and astrocytes play a distinctive role in capillary constricting processes mediated by glial metabotropic glutamate receptor

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signaling and its neuronal counterpart on the other side of the pathway, in which excitatory glutamate binding increases nNOS function with NO liberation. This enhancement increases capillary effector signaling, where NO leads to cGMP elevation, capillary dilation and blood flow elevation[53]. The necessary enzyme outfit is present in normal astrocytes but shows even enhanced expression in human glioma cells. Here, environmental damage and breakdown of the blood-brain barrier enhancing blood and substrate supply as well as edema formation are accompanied by the grade of malignancy. In malignant astrocytoma and cerebral metastasis, the expression of NOS isozymes nNOS, ecNOS and iNOS, ecto-nucleotidase (CD73) for cGMP liberation, VEGF as the most potent angiogenetic factor and the extent of the surrounding brain edema volumes are significantly correlated[83,84]. The same mechanism as in glioma edema has been reported for stroke pathophysiology by immediate disturbances of energy metabolism, NO depletion, K^+ elevation and enlargement of perivascular VRS, causing immediate inflow of CSF and brain edema by swelling of astrocytic end feet (Fig. 3)[85,86].

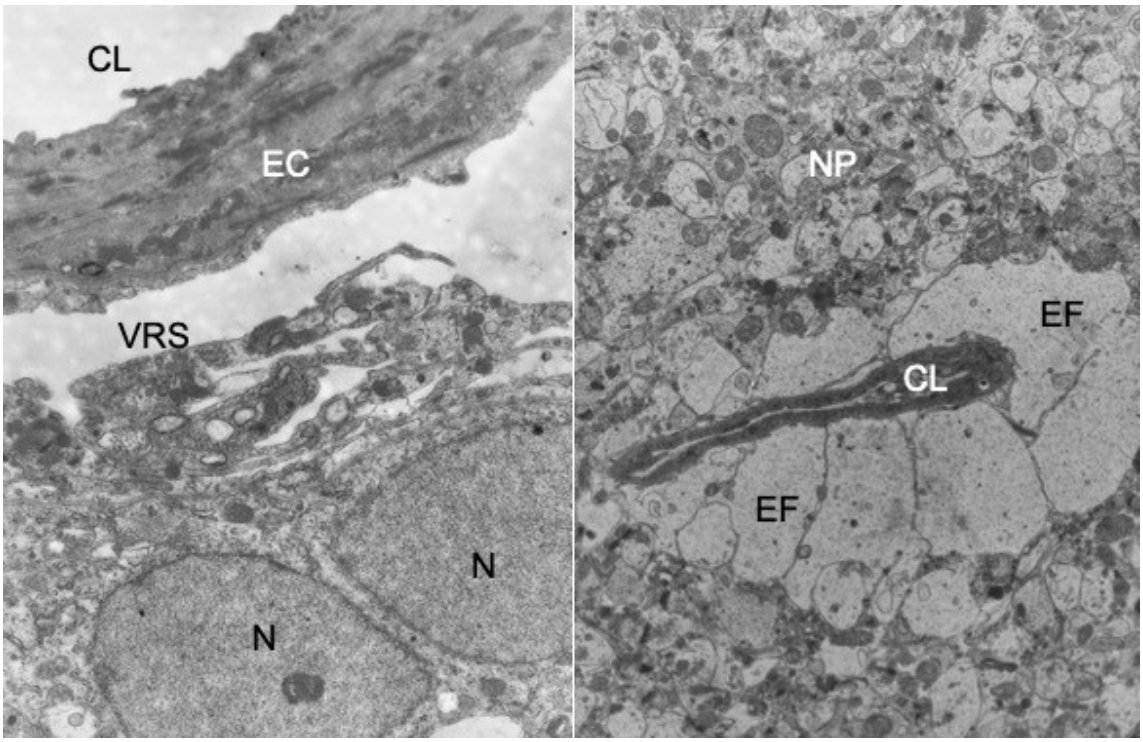

Figure 3: HO:YAG-LASER-(Holmium:Yttrium Aluminum Garnet- LASER, 2.0 microns) induced thermal hypoxic lesion of rat cortex studying LASER-tissue interaction in vivo. Enlargement of the pericapillary Virchow-Robin space (left) and swelling of pericapillary astrocytic end feet (EF) (right) depicting perifocal brain edema induction by electron microscopy, V=3.600 (left) and V=5.900 (right). The perivascular space is extended on the left, the capillary lumen (CL) and VRS are obstructed (right); nucleus (N), endothelial cell (EC)[86].

All these mechanisms in infectious or tumorous disorders or under healthy conditions use the astrocytic network draining into the VRS as a major transmission space for the most important physiological cascades to enable the regulation and fine tuning of blood supply for the most delicate energy metabolism with glucose and oxygen for regular brain function[87]. The glymphatic network serves as a first line for the demands of

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energy metabolism by neurovascular coupling and secondary as a waste clearance system. As a powerful metabotropic partner in this pathway, NO can pass the network freely by diffusing through membranes but could even be conducted and distributed by AQP4 canals. The second metabolite is Aβ as the main substance, which forms and establishes the structural network pattern[77]. The network is dependent on adequate preloading by ventricular CSF sources, obviously exclusively during NREM sleep with a sustained breathing-induced upward surge. This occurs in balance with venous blood flowing downwards into the epidural venous system, which is void of valves and always kept open by steady-state negative transmural pressure[88].

The permanently ongoing assembly of the astrocytic conducting and glymphatic network depends on Aβ uptake from neurons and production by astrocytes themselves, as well as the polarized expression of AQP4 canals localized at the apical surfaces facing the CSF on one side and the VRS membranes on the other side, to respond to and fulfill the requirements of a stable and healthy blood-brain barrier[89]. The fluid flow and exchange in this network is independent from systemic blood pressure, as mentioned by Nakada previously[89], a prerequisite for cerebral autoregulation. Therefore, the network must be fueled and loaded by sufficient fluid flow from the CSF compartments on one side of the network to drain back into the postcapillary system and peridural parasagittal lymphatic structures[90], which has been withdrawn from the precapillary compartments on the other side in a turnover system (Fig. 1).

To establish and maintain this system, AQP channels are structurally connected to the extracellular matrix by several matrix proteins, such as dystroglycans, focal adhesion protein (FAK) and proline rich tyrosine kinase 2 (PYK2)[91], and this outfit is enhanced under malignant glioma conditions to gain an advantage in energy competition by enhancing angiogenesis through the tyrosine kinase pathway[92].

Disturbances of the astrocytic network and NO-mediated fluid flow

Disturbances and disorders of this intriguing astrocytic network could be induced by disturbances and irritations of the structural network itself or by malfunction of the excitatory metabolites and pathways. On one side of this unique imbalance, astrocytic overload with Aβ from perisynaptic compartments leads to damage to astrocytic processes, breakdown of the blood-brain barrier, overloading astrocytic Aβ synthesis and structural tissue breakdown, disturbing the main waste clearance process. It is therefore highly pathognomonic that partial loss of endothelial NO leads to increased cerebrovascular Aβ along with compensatory mechanisms to protect vascular integrity [93]. On the other side of this damage, the energy supply by dysregulation of NO-conducted and mediated capillary tuning does not take place, and NO cannot be propagated any more by the network to distant capillary segments[53]. Both mechanisms involve CSF and interstitial fluid as well as pericapillary fluid volumes, and the mechanism depends on its filling and preload fluid supply. Energy metabolism is dependent on the redox state as the main adjustment mechanism. Moreover, the coupling is dependent on the interplay between the conversion of angiotensin II to angiotensin (1-7) by angiotensin converting enzyme 2 (ACE2) and is transmitted by endothelial NOS expression and NO liberation[94]. We have outlined above that sleeping performance with sufficient periods of NREM phases coincides with sustained, apnea-free breathing and adequate capillary coupling as indicated by a strong BOLD signal[64].

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The redox state could be altered by relevant hemodynamic changes in HDT, apnea periods, hypertensio[95], atherosclerosis and diabetic disorders. Latter conditions may lead to disturbed mitochondrial function recently described in biopsies of NPH patients[96]. The ACE2 system, on the other hand, has been recently identified as a condition initiating and mediating strokes in the cerebral manifestations of COVID-19[97].

Particulate matter and astrocytic network toxicity

Another serious mechanism disturbing the redox state came into focus during the last two decades; this mechanism consists of cerebral enrichment of environmental particulate matter (PM), which has been described by several authors. PM with a size of or below 2.5 microns is a result of traffic pollution in highly industrialized countries and can easily be taken up through the nasal olfactory bulb. Recently, it was reported that PM is strongly enhanced in rat and dog brains from high traffic areas of Los Angeles or Mexico City[98]. Such enrichment of PM in perivascular spaces of the astrocytic network is able to induce severely damaging oxygen products by free radicals and to disturb the fine regulation of the redox state as a checkpoint for several different pathways. In mice from air-polluted metropolitan regions of Los Angeles, severe damage could be documented close to the corpus callosum[99]. Proteomic studies in rats could demonstrate disturbances in astrocytic and mitochondrial homeostasis inducing redox alterations and neurodegeneration[100]. PM entering the brain by the olfactory bulb can destroy the blood-brain barrier by astrocytic damage using the oxygen pathway and may induce AD and dementia[101,102] as well as multiple sclerosis in children[103]. In this context, it is of great importance that epidemiologic studies were able to identify a high risk of dementia disorders in subjects who resided less than 300 m from a high-traffic highway[104] or in geographic areas with high ambient air pollution[105]. The effect on the brain from long-term individual exposure to elevated air pollution is an inflammation-like pathology resembling AD[106]. This pathology can be detected even in younger individuals, including children, who live in cities with heavy PM pollution[107,108,109,110,111]. Astrocytes seemed to be the main target of such disturbances[112] by oxygenic stress induction and ferroptotic mechanisms[113]. Recent findings of PM on the fetal side of the human placenta suggest that PM reaches the fetal brain during its highly vulnerable developmental period of periventricular astrocytic structures. The grade of prematurity and preterm birth was related to the load with PM in the investigated specimen[114]. This seems to be crucial for air pollution by PM but is a steady growing research interest for a similar facultative pathology induced by nanoplastic in fish[115] and mice[116]. Nanoplastics could enter rat placenta and fetal rat brain[117], zebrafish offspring by placental tissue passage and affect the cerebral neural tissue of the F0 and F1 generations by altering the cellular respiratory system and inducing oxygenic mitochondrial dysfunction. To date, neurotoxicity by nanoplastics has been documented for nematodes[118], mussels [119] and marine fish[120], fetal rat but not in humans so far.

Commonalities in dysfunction of astrocytic network homeostasis

Adaptation of the different experimental and observational results into the clinical setting of CSF-related disorders must integrate the complete structural and functional system of the astrocytic network:

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(1) Adequate filling and preload of the astrocytic network with CSF moving upward from spinal to ventricular compartments in balance with venous blood at the beginning of the inspiration-induced CSF turnover, accompanied by the process described in (2).
(2) Concomitant ciliary sensing of the aqueductal passage of CSF flow and preload status during sustained NREM phases in deep sleep.
(3) Ependymal uptake and conductance by sufficiently interconnected AQP4-bearing astrocytic processes organized and stabilized in concomitant different basal lamina structures.
(4) Nitric oxide-mediated coupling of vascular dilation and Aβ-waste loaded fluid uptake into the VRS for further vascular drainage or recycling purposes.

From the perspective of pediatric neurosurgery and neurology, several disorders are closely linked to some of the available experimental models and to disturbances of any of these different involved parts of the system.

Disorders of CSF and the astrocytic network

The Hyh mouse model

At the beginning of this overview on neurological deteriorations, one of the main facilities for preload sensing should be outlined focusing on a mouse model, in which basic and severe disturbances of cilia function account for severe lifelong hydrocephalic disability. In the model of the genetic hop gait mouse, an inherited failure of ciliary sensing occurs by mutation of the SNAP gene, and the resulting cilia disassembly leads to a complete loss of all cilia bearing ependymal cells with catastrophic consequences for the subventricular zone and the underlying astrocytic network. The disorder has similarities to posthemorrhagic hydrocephalus (PHHC), in which blood intake by germinal matrix bleeding[121,122] induces regional or nearly complete loss of ependyma with its known sequela of hydrocephalus, lifelong shunt dependency, periventricular white matter disease, cognitive dysfunction and disability correlating with the severity of prematurity. In PHHC, ependyma and subventricular zone integrity are dependent on healthy cilia function and fluid sensing worked by the NO–cGMP pathway[123,124]. Blood intake into this system by germinal matrix bleeding induces ciliary dysfunction and breakdown of the epithelial tissue pattern[125].

Modern therapeutic approaches for this disorder might be derived by early lavage of the ventricular system for rescue of the ependymal epithelia and regaining as much ciliary function as possible. This method has recently been introduced by several groups and is currently under investigation by an intercontinental prospective study (TROPHY study under the auspices of the International Federation of Neuroendoscopy-IFNE http://trophy-registry.org)[126]. Another similar underlying mechanism for ependymal loss in autosomal recessive Ccdc 39 gene mutant mice is the altered turnover of L1CAM and loss of dynein and outer parts of the motile cilia,

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which leads to hydrocephalus[127]. Congenital neural tube defects share similar pathosequelae.

Chiari malformation and syringomyelia

Chiari malformation is a disease that can occur in isolation (type I) or concomitantly with spina bifida aperta (type II); in this disease, ciliary dysfunction occurs due to loss of ependyma and CSF obstruction. Most children benefit from neuroendoscopic approaches for reestablishment of CSF dynamics or from decompressive approaches targeting flow obstructions caused by hindbrain herniation (Fig. 4).

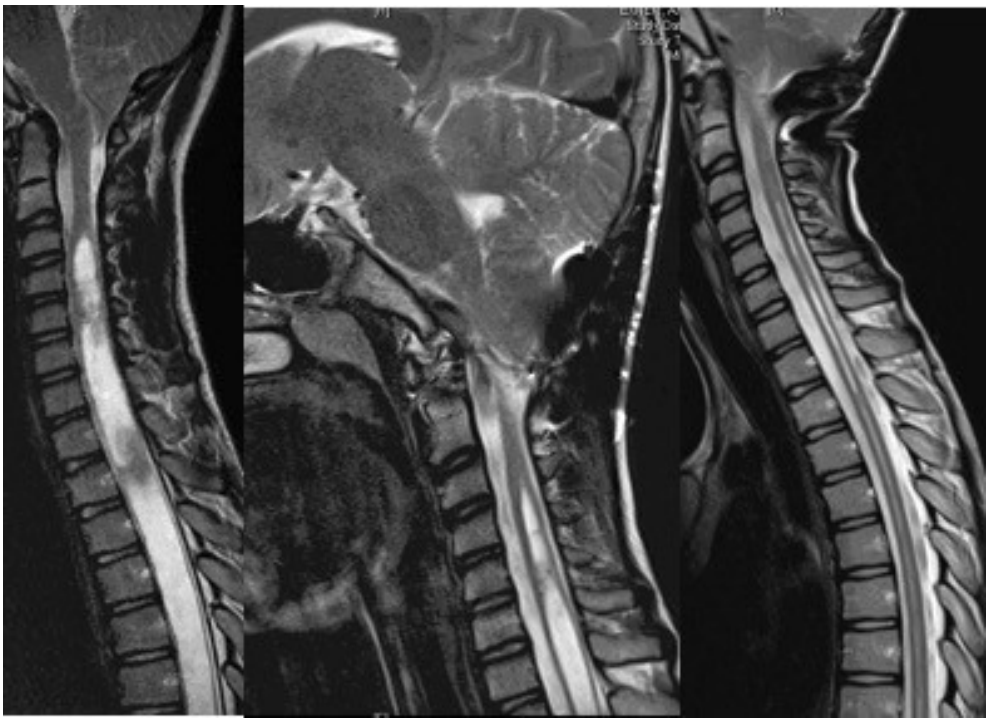

Figure 4: Chiari type I malformation, tonsillar herniation, syringomyelia before (left), 5 months (center) and 15 months after (right) foraminal decompression surgery. Nearly complete reduction of the syrinx volume.

In syringomyelia, a disorder that is often concomitant with spina bifida and meningomyelocele (MMC), centripetal CSF movements and flow are disturbed, and the widening of the medullary central canal is a result of sometimes large cystic holocord enlargement and concomitant myelopathy (Fig. 4). To reflect similarities with the widening of the ureter in the event of ciliary dysfunction caused by planar cell polarity disruption, syringomyelia might also be transmitted by a failure in the sensing capacity of the ependymal surface of the central canal or a missense by the absence of enough fluid flow. In children with tethered cord syndrome, featuring mechanical fixation of the cord by lipoma or scar tissue, a myelopathic lesion occurs, with clinical consequences for gait, back pain, spinal scoliotic development, skin somatosensation or bladder/bowel function. The necessary microsurgical untethering of the cord surface mostly leads to recuperation by reestablishing the physiological breathing- and cardiac-driven cord movements that recruit the CSF flow and ciliary sensing inside the central canal of the spinal cord. This recuperation can be observed in cases with sacral tethered cord (Fig. 5) or hindbrain herniation accompanying cervical syringomyelia, in

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which surgical untethering and decompression of the hindbrain and medullary structures can lead to an impressive recuperation from myelopathy or neurophysiological dysfunctions.

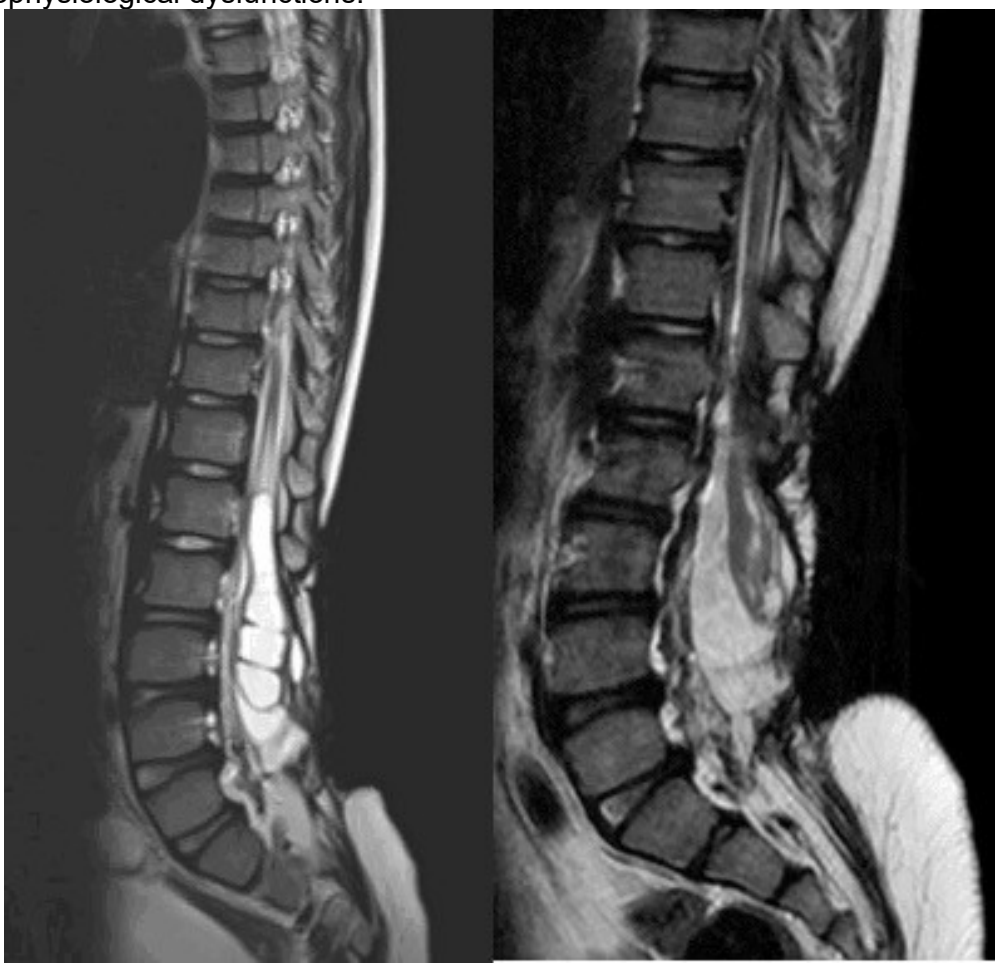

Figure 5: Syringomyelia of the conus medullaris caused by secondary tethered cord syndrome in a 6-year-old boy with open MMC before (left) and 2 years after (right) microsurgical detethering. Reduction of the syrinx volume inside the medullary cone.

Idiopathic intracranial hypertension

Idiopathic intracranial hypertension (IIH) is a disorder typically affecting teenagers and young adults, often females with obesity and high body mass index (BMI). Patients present with headaches, blurred vision and chronic degeneration of the optic nerve by papilledema if no adequate and timely therapy is offered. For diagnosis, typical findings include MRI imaging with pathognomonic small ventricle size together with a high intradural pressure (above 25 cm H_2O) measured by lumbar puncture.

IIH can be related to pathologies of the large intracranial veins of the posterior fossa, for example, by atresia or thrombotic occlusion of the sinus transversus. The main therapeutic issue consists of aiming diminution of the CSF production rate by carbohydrase antagonists and fat burning measures. The acute risk for the optic nerve can be assessed by serial lumbar punctures collecting 20-25 ml CSF each. It has been outlined that the commonly used medications acetazolamide and topiramate are pharmaceuticals that antagonize the AQP1 and AQP4 channels[128,129], the main water-conducting membrane pore of the choroid plexus and ependymal basal membrane. In

our view of the related and underlying pathology, it should be noted that a high BMI together with avoidance of motor activity and sports tends to hamper the necessary upwards centripetal motion of CSF and cause a slight backlog and decreased preload volume of the astrocytic network. Papilledema is then induced by an increasing gradient between intraocular and intracranial pressure, which affects the gradient through the lamina cribrosa. This is the unique anatomical structure that separates the optic nerve fibers on their way from the retina to the chiasma at the posterior pole of the optic globe[130].

The underlying pathophysiology of IIH seems to indicate a deficiency of upward CSF motion and might reflect a deficit of CSF preloading for the astrocytic network that provides brain turgor and buoyancy. This hypothesis is affirmed by the observation that real intracranial measurements of elevated intracranial pressure (ICP) have been reported very sparsely[131], whereas the commonly elevated intradural spinal pressure might reflect a lack of upward CSF movement, with consequent spinal intradural pooling. Implantation of a ventriculoperitoneal shunt for CSF diversion is required to protect the optic nerve and prevent secondary amaurosis. The symptoms of IIH resemble those of pineal cysts, with headaches, blurred vision, sleep disturbances and obstruction of the aqueduct; therefore, patients often show associated slit-like ventricles. Melatonin supplementation sometimes helps; its effect could be interpreted as sleep induction providing sufficient sustained NREM phases with adequate sleep performance. In severe cases, the pineal cyst can be microsurgically fenestrated by a supracerebellar infratentorial approach, restoring sufficient upward CSF movement.

Idiopathic NPH, dementia and AD

Idiopathic NPH (iNPH) affects approximately 2-4% of people over 65 years of age, reaching 6% above 80 years. The patients suffer from gait disturbances, urinary incontinence and dementia according to the triad of Hakim[132]. The disorder has an untreated mortality, which leads to a hazard ratio of 3.8 compared to the healthy group[132]. The common explanation addressing the underlying pathophysiology of iNPH proposes a higher ICP pulsatility related to atherosclerotic alterations and a sort of pulsatile hammer mechanism diminishing the periventricular brain parenchyma and inducing atrophy and degeneration with ventriculomegaly. However, experiments intending to prove the magnitude of this pulsatile effect through the aqueduct have found this effect to be too small for considerable efficacy[133]. Although ICP overnight measurements in iNPH patients have shown a normal amplitude pressure level, a higher pulsatility has been reported underlying the assumed mechanism, contradictory to the above cited observations. [134] Today, diagnostic workup mainly consists of tapping 30-40 ml CSF by lumbar puncture, simulating shunt therapy. In responders, gait usually improves significantly immediately after puncture. Clinically overcome is a triple overnight measurement with ICP recordings and detection of typical Lundberg B-waves escalating to plateau waves of 15 to 20 minutes duration and highly pathologic levels of 30-50 mm Hg until spontaneous relaxation and return to normal values are retained (Fig. 6). The real explanation for that pressure level course as well as the pathophysiology behind iNPH are not well understood. iNPH and AD have remarkably similar symptoms, and dementia is one of the main features of the Hakim triad, suggesting a deeper relationship[135]. Therefore, studies have been performed in which shunted iNPH patients were biopsied, and histopathology showed a high prevalence (19%) for Alzheimer's typical staining pattern with neuritic plaques, amyloid angiopathy and protein tangles. This prevalence had a high prognostic value for the success of shunt diversion therapy. Further observations in a cohort of 1140 healthy Japanese individuals showed that the first feature in still asymptomatic men is ventriculomegaly

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as a precursor sign[136]. Similar investigations using phase contrast MRI have been repeated by other groups[137]. To explain the underlying pathophysiology, CSF flow studies have been undertaken[138]. These studies are based on cardiac-gated MRI phase contrast and could demonstrate that the strength of the flow void signal (Fig. 7) through the aqueduct predicts the success rate by shunt therapy. These authors also explain iNPH as a two-hit phenomenon, in which the first hit consists of a chronic subclinical ventricular enlargement during childhood turning into the development of iNPH later in life[139]. Analyzing these hypotheses in light of the recently and above summarized findings on the astrocytic network and NO-dependent fluid flow enhancement, it can be estimated that in the development of a clinical manifestation of iNPH, a failure in cilia detection and NO liberation occurs with the consequences of architectural reorganization of ventricular and aqueductal width and dimensions. Aqueductal enlargement leads to insufficient tuning and adjustment capabilities for preload and fluid requirements. Ultimately, these observations stand for the equivalence of pathological Lundberg wave measurements, whereas ICP mean values were not elevated[140] but were elevated in single but insufficient stroke volumes, revealing the underlying aqueductal insufficiency (Fig. 7). In the long term, the results include not only ventricular enlargement and widening of the aqueductal diameter with strong void signals (Fig. 7) but also AQP4 expression and polarization become involved and experience significant changes[141]. In iNPH and AD, AQP4 expression is diminished and correlates with elevated CSF-Aβ- levels[142], as long-term alteration indicates a systemic failure of the astrocytic network and its glymphatic waste clearance.

In the upcoming future, we must determine which consequences must be drawn for the underlying mechanisms behind shunt therapy or neuroendoscopic third ventriculostomy in the treatment of iNPH.

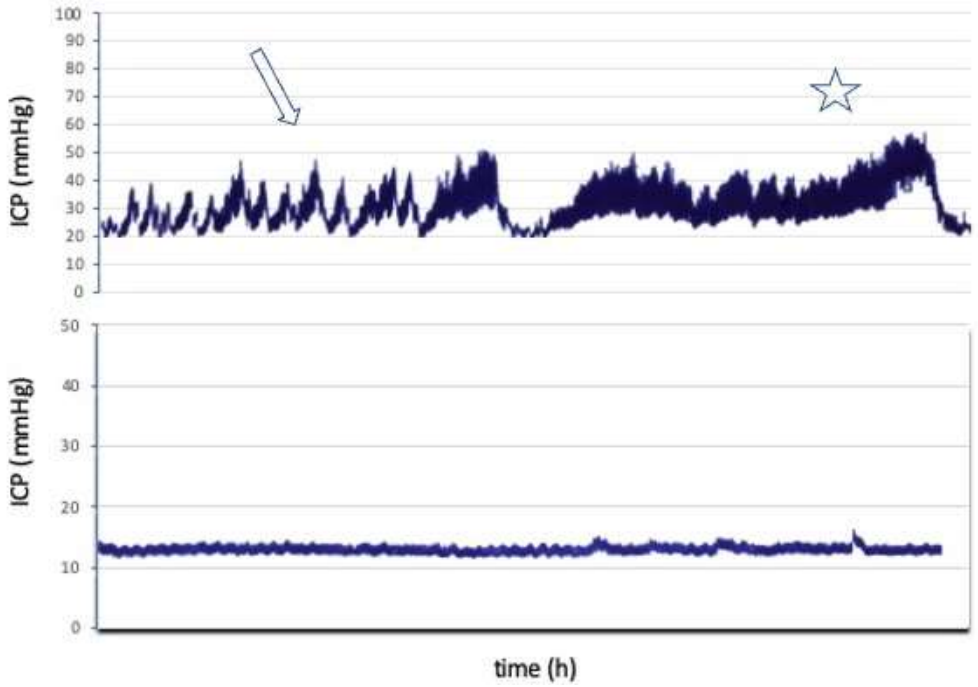

Figure 6: ICP monitoring during sleep in a patient with NPH over 6 hours. Typical B-waves (arrow) and plateaus (asterisk) with ICP elevations above 50 mm Hg, spontaneous

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recuperation before (top) and after insertion of a VP-Shunt (bottom) delineating the normalization of the highly pathological measurements. The waveforms depict disturbed neurovascular coupling.

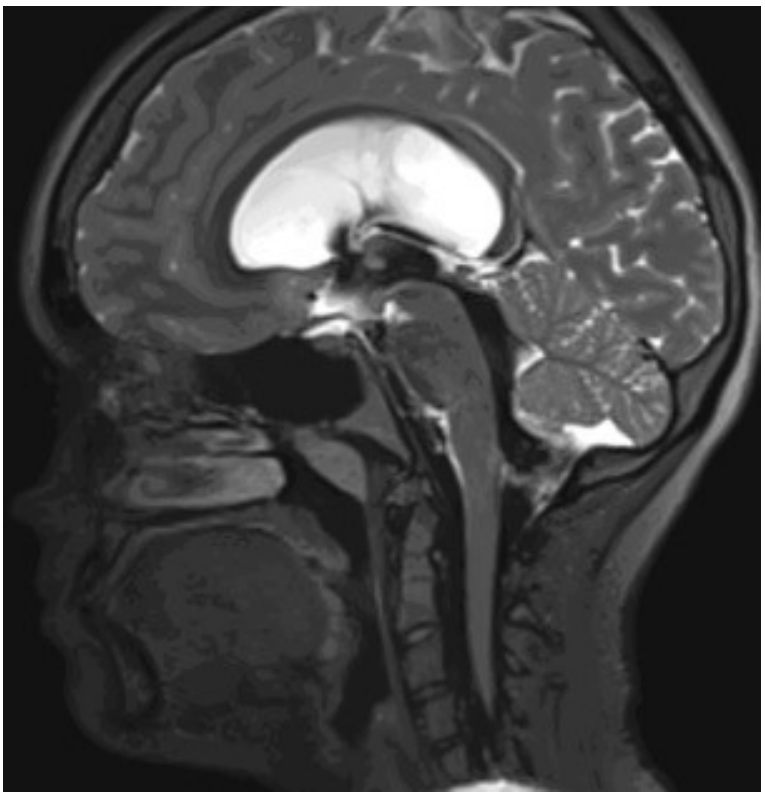

Figure 7: Oscillatory but nonsustainable movements of CSF expressed by strong flow void signaling inside the 4th and 3rd ventricles and aqueduct in a 15-year-old girl suffering from NPH

High-altitude disease

Analyzing the pathologic disturbance of sleep patterns and the redox state behind headaches, brain and pulmonary edema, which can affect mountaineers above 3000m, provides a further elucidating condition under different environmental situations. In this situation or disorder, such as in space flight disease, induced alterations of the CSF preload for proper function of the astrocytic network and its clearance ability occurs. The main common observation leading to headaches and brain edema at high altitude is a specific breathing pattern with periodic breathing intermitted by apnea periods. This is caused by the exclusive inspiration induction by blood CO_2 levels and periodic breathing-induced diminution of CO_2, which is terminated by its elevation again. This can be interpreted as a sleep performance disorder similar to Cheyne Stokes breathing. Therapeutic measurement in high-altitude disease is the carbonic anhydrase antagonist acetazolamide, which modifies the redox state by altering the binding capacity of hemoglobin via pH changes, tuning the system to adapt to high altitude. Importantly, in addition to carbonic anhydrase, AQP4 is an alternative target for acetazolamide. Crystallographic experiments have revealed that blocking AQP4 by this pharmaceutical principle occurs at the

extracellular pore entrance of the AQP4 molecule[129]. This colocalizes in the brain at the perivascular side of astrocytic end feet[143].

Conclusion

In this review, we have discussed the different aspects that act as a prerequisite for a universal brain fluid exchange system beginning with inspiration-induced upward surge of spinal CSF into the ventricles. This CSF dynamic provides enough fluid supply passing the aqueduct in the centripetal flow direction restricted to NREM sleep phases II and III in supine position. The fluid movement upwards is sensed by cilia function and adjusted by aqueductal fine tuning, which allows complete closure during the standing position and a nearly open state during sleep. The CSF supply enables preload entrance into the astrocytic network, which is connected by differential expression of AQP4 canals at the basal and apical membrane protrusions organized and stabilized by extracellular matrix. The interplay of cilia-sensed fluid flow and network-conducted NO liberation enables neuron-vascular coupling on demand, producing the strong BOLD signal that has been measured during NREM sleep by MRI. This interplay could provide a strong fluid exchange and clearance capability dependent on sufficient ventricular CSF supply on one side, the intermediate astrocytic network in between and the pericapillary VRS for uptake of waste and fluid on the other side. This system can be disturbed at certain points, giving rise to several disorders of the CSF exchange system: Chiari malformation, syringomyelia, IIH, iNPH, space flight and high-altitude disease. With this acquired knowledge, derived from our own real time MRI studies, these disorders are now much easier to attribute, which is a requirement for a new vision on hydrocephalus and its adapted therapy.

Author Contributions: HCL and HCB conceived the idea for this review article, drafted the manuscript, and designed and reviewed the entire concept. SDK reviewed the details of the underlying real-time MRI data. HCL, SDK and HCB reviewed the manuscript.

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