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Totally Tubular: The Mystery behind Function and Origin of the Brain Ventricular System

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Summary

A unique feature of the vertebrate brain is the brain ventricular system, a series of connected cavities which are filled with cerebrospinal fluid (CSF) and surrounded by neuroepithelium. While CSF is critical for both adult brain function and embryonic brain development, neither development nor function of the brain ventricular system is fully understood. In this review, we discuss the mystery of why vertebrate brains have ventricles, and whence they originate. The brain ventricular system develops from the lumen of the neural tube, as the neuroepithelium undergoes morphogenesis. The molecular mechanisms underlying this ontogeny are described. We discuss possible functions of both adult and embryonic brain ventricles, as well as major brain defects that are associated with CSF and brain ventricular abnormalities. We conclude that vertebrates have taken advantage of their neural tube to form the essential brain ventricular system.

Keywords

brain ventricle; neural tube; CSF; morphogenesis; neural tube defects

1) What is the brain ventricular system?

The vertebrate brain has a characteristic and complex three-dimensional structure. One highly conserved aspect of brain structure is the brain ventricular system, a series of connected cavities lying deep within the brain, filled with cerebrospinal fluid (CSF) (Fig 1) (1). The ventricles and the CSF they contain, together with the surrounding neuroepithelium and associated secretory structures, form the brain ventricular system. The brain ventricles were first described over two thousand years ago, when it was believed that higher mental functioning resided within them (reviewed in (2)). While this belief was incorrect, the function of the brain ventricles is, indeed, very important and under intense study.

In the adult human brain, there are four connected ventricles: two lateral ventricles within the cerebrum, a third ventricle within the diencephalon, and a fourth ventricle lying between the cerebellum and pons (Fig 1) (3). The lateral ventricles are connected to the third ventricle, which is linked to the fourth ventricle via the cerebral aqueduct. In turn, the fourth ventricle joins to the spinal cord canal and the subarachnoid space that envelops the brain. The adult human brain contains about 140 ml of CSF, of which approximately 20 ml is within the ventricles and the remainder is surrounding the brain (1). Adult CSF is produced mainly by the choroid plexuses, highly vascular structures located within the ventricles (Fig 1) (4), and some CSF may be produced by cells lining the ventricles (5). The choroid

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plexuses produce about 500 ml per day, suggesting that the fluid is exchanged 3–4 times each day (1). CSF flow forms a circulatory system and is believed to flow from the lateral ventricles to the third and fourth (Fig 1), and then out into the subarachnoid space where it is absorbed into the hematopoietic circulatory and lymphatic systems (1). Control of CSF flow is thought to originate in pressure gradients produced by secretion and by beating cilia with uniform orientation located on the ependymal epithelium that lines the ventricles (6).

What does the brain ventricular system do? The notion of a circulatory system deep within the brain suggests functions analogous to the hematopoietic circulatory system, including transport of nutrients and wastes, and these functions have been attributed to the adult CSF. In addition, CSF protects the adult brain from physical trauma (7). As we will discuss later in this review, recent data suggests that in both the embryo and adult brain, CSF may additionally carry signaling molecules that regulate neurogenesis and survival (8,9).

2) Conservation of embryonic and adult brain ventricular systems

The vertebrate embryonic brain originates from a columnar epithelium that comprises the neural plate (10). In humans, the neural plate develops early in the fourth week after fertilization, and later that week, completes neurulation to form the neural tube (11). The fundamental mechanisms of neurulation appear to be largely conserved throughout vertebrates (12). Subsequently, the ends (neuropores) of the tube close, the anterior portion of the tube becomes the brain, and the posterior becomes the spinal cord. In most animals, towards the end of neurulation, the future brain begins to undergo a series of stereotypical constrictions, bends, and expansions, to subdivide into the primary embryonic “brain vesicles”, forming the future forebrain, midbrain, and hindbrain (Fig 2A,B) (10). In teleosts, however, shaping of the brain occurs after neurulation (13), and thus the morphogenetic events of neurulation and brain shaping are separable.

The cavities of the brain vesicles are filled with CSF and form the embryonic forebrain, midbrain, and hindbrain ventricles (Fig 2C). Following early brain ventricle shaping and initial inflation, the ventricles undergo massive expansion, with ventricle volume increasing significantly faster than brain tissue growth (14). In mammals and chick, spinal cord occlusion transiently seals off the brain ventricular space directly preceding this expansion period (15,16), which may then allow intraluminal pressure to promote ventricle enlargement. It is not known whether this process occurs in other vertebrates. In most vertebrates, the embryonic forebrain ventricle splits into the two lateral ventricles and the third ventricle. The midbrain ventricle becomes the narrow cerebral aqueduct which connects the third and fourth ventricles, and the hindbrain ventricle becomes the fourth ventricle (Fig 2C) (10).

While the gross anatomical development of the ventricles is well-documented, the molecular mechanisms underlying this development remain poorly understood. However, several vertebrate systems have been useful in understanding development and function of the brain ventricles, including chicken and rat. Recent work has demonstrated that the zebrafish is a valuable system to study formation of the embryonic brain ventricles, due to the ability to image live embryos at single cell resolution, and through isolation of genetic mutants (13,17–19).

3) Molecular and cellular mechanisms of embryonic brain ventricular system development

Crucial to ventricle formation is the neuroepithelium that surrounds the brain ventricles. The embryonic brain ventricular space directly reflects the position and shape of the surrounding

neuroepithelium, and thus, development of the brain ventricles depends upon coordination of several aspects of neuroepithelial development. First, the neuroepithelium is patterned along the anteroposterior and dorsoventral axes, which allows correct positioning of the ventricles and directs downstream morphogenesis of the brain tissue. Second, the neuroepithelium undergoes stereotypical and conserved morphogenesis, regulated cell proliferation, and cell death, in order to shape the brain and the ventricular cavities. Finally, the neuroepithelium secretes the initial embryonic CSF (eCSF) to inflate the ventricles. These processes are described in more detail below.

Positioning the brain ventricles

Initial brain patterning along the anteroposterior and dorsoventral axes occurs before and during neurulation, such that by the neural tube stage, embryonic brain tissue is subdivided into distinct gene expression domains (20). Thus, patterning genes regulate the precise positioning of the brain ventricles, including the characteristic and conserved constrictions and bends within each brain region (Fig 2B). Patterning genes may play a proximal role in neuroepithelial morphogenesis (Fig 3), by directly controlling cytoskeletal machinery. Conversely, patterning genes may play a distal role, required for early tissue specification, which later leads to downstream changes in the neuroepithelium, and, thus, brain ventricle morphology.

Multiple publications document changes in patterning gene expression that result in changes in brain morphology, but it is generally unclear whether any of these genes directly regulate brain morphogenesis. For example, zebrafish *ace* embryos, that are mutant for *fgf8* function, show severely abnormal midbrain morphology, including incorrect shaping of the brain ventricles and absence of the midbrain-hindbrain boundary (21). However, it is uncertain whether Fgf signaling acts at the time of morphogenesis, or whether earlier patterning defines cell types that later undergo morphogenesis. A similar example involves *zic1* and *zic4*, transcription factors which regulate both cell fate specification and proliferation in the zebrafish hindbrain and control hindbrain ventricle formation, but at a time that is not yet known (22). The ventral neural signaling morphogen Sonic Hedgehog (Shh) may have a proximal role in chick brain ventricle expansion (23). Shh is secreted from the notochord, which underlies the midline of the neural tube, and later, from the floorplate. Separating the notochord from the brain, after initial patterning events have occurred, prevents brain ventricle expansion, reduces cell proliferation, and increases cell death (23), suggesting that Shh may play a role in ventricle formation. Shh signaling from the notochord is also required for the regulation of neuroepithelial shape in mice, affecting the location of dorsoventral hinge-points (24). These limited data indicate that a significant future challenge is to connect patterning mechanisms with neuroepithelial morphogenesis.

Morphogenesis of the brain epithelium

In order for brain morphogenesis and associated ventricle formation to occur, an intact, cohesive neuroepithelium must form, with the appropriate junctions. The neuroepithelium must be correctly shaped, requiring a functional cytoskeleton and extracellular matrix. Additionally, regionally-restricted cell proliferation, and perhaps cell death, may further modify the shape of the brain ventricles.

Forming the epithelium—The embryonic vertebrate neuroepithelium comprises a sheet of cells that become connected by apically localized adherens and tight junctions (25–27). These junctions hold the cells together to form a functional unit and also form a barrier between the inside and outside of the neural tube. After the neural tube has formed, the apical surface of the neuroepithelium faces the lumen (the ventricles), and the basal surface is on the outside of the tube (Fig 3A). In most vertebrates, shaping of the brain tube begins

during neurulation, as the lateral hinge-points form (Fig 3B). These hinge-points persist in the later embryonic brain and help shape the ventricles. In the zebrafish neural tube, brain ventricle morphogenesis occurs after neurulation is complete (Fig 3C). Thus, the fish model, unlike other vertebrates, offers the potential to separate processes controlling neurulation from those controlling later brain ventricle morphogenesis.

Information from zebrafish mutants has been highly informative with regard to the role of specific junctional components during brain development. In the N-cadherin mutant, *parachute*, junctions fail to form and the neuroepithelium falls apart, prior to brain ventricle development (Fig 3Di) (28,29). Consistent data has been obtained from chick (30). As N-cadherin is an integral member of neuroepithelial junctions and is expressed throughout brain morphogenesis (31), it may be required at all stages of brain ventricle morphogenesis.

Mutation in the apical adherens junction-associated component, Mpp5, a MAGUK protein (32), leads to a different neuroepithelial phenotype. Zebrafish *nagie oko* mutants, which lack Mpp5 function, form an intact neural tube, but sections through the tube show that apical junctions are disorganized (Fig 3Dii) (13). The neuroepithelial midline does not form or subsequently separate normally, leading to an absence of brain ventricles (Fig 3Dii). Additionally, apically-located hinge-points within the ventricles do not form (13). Further experiments will be able to address whether Mpp5 is required both during neurulation and later during ventricle morphogenesis.

In the zebrafish *heart and soul* mutant, which has a null mutation in *prkci* (corresponding to *protein kinase C iota*) (33), neuroepithelial junctions appear to be normal, but brain ventricle inflation does not occur uniformly throughout the tube (Fig 3Diii) (18). These data suggest that some other aspect of neuroepithelial function is abnormal in *prki* mutants, perhaps cell-cell coordination, junction barrier formation, or cytoskeletal remodeling.

Shaping the neuroepithelium—The neuroepithelium undergoes stereotypical constrictions and bends that shape the brain ventricles. Mechanisms required for shaping the neuroepithelium include midline separation, cytoskeletal shape changes, and extracellular matrix function.

Midline separation: In zebrafish, after neurulation, the neural tube is closed, without a luminal space or morphologically visible midline (29) (Fig 3Cii). Soon after, a midline separating the left and right sides of the tube appears, with apposition of apical surfaces on either side (Fig 3Ciii). The absence of a visible luminal space upon neural tube closure is not unique to zebrafish, as transient occlusion occurs in other model systems as well. For example in *Xenopus*, after neural tube closure, the lumen disappears, to reappear as cells are rearranged (34), while in the chick spinal cord, the lumen is occluded concomitant with ventricular expansion (35). Subsequently, the tissue at the midline separates to form the ventricular spaces (Fig 3Civ). Part of this opening is due to secretion of eCSF, however, analysis of zebrafish mutants suggests that initial ventricle inflation requires some additional process that results in separation of the left and right sides of the tube (18). We have called this latter process “midline separation” (18). Several brain mutants that correspond to apicobasal polarity/junction proteins (*prkci/heart and soul*, *crb2/oko meduzy*, *epb41l5/ mosaic eyes*) show a morphologically distinct midline, based on actin staining, but the ventricles only inflate partially, and in places, appear to remain shut (18). These data indicate that correct apicobasal polarity, junctions, and a functional epithelium are required for midline separation. One possible reason for this defect is that the apical surfaces of the mutant neuroepithelia are unusually adhesive, while another may be that mutant neuroepithelial cells are less able than wild-type cells to move or change shape.

Cytoskeleton: Shaping the neuroepithelium and brain ventricles requires constrictions and bends in specific locations (Fig 3Aii,iii), often involving individual cell shape changes and cytoskeletal rearrangements (36, 37). For example, formation of basally constricted cells and subsequent neuroepithelial bending occurs at the midbrain-hindbrain boundary constriction (Fig 3Aii) (19). Several studies have looked at the cytoskeleton during neurulation and hinge-point formation in frog and chick. The hinge-points that form during neurulation in these animals, persist later to shape the brain ventricles. The actin binding protein, Shroom, which influences both actin polymerization and microtubule behavior, is required for apical constriction at hinge-points in *Xenopus* and mice (38, 39). Members of the Ena/VASP family coordinate cytoskeletal dynamics during *Xenopus* neurulation, including apical constriction within the plate, cell elongation, and cell-cell adhesion (40).

Extracellular matrix: Another component of the neuroepithelium that is required for brain shaping is the extracellular matrix (ECM), located at both apical and basal sides of the neuroepithelium. The ECM may play a mechanical role by providing structural support, allowing a changing epithelium to bend and hold its shape, as a unit. The ECM may also play a crucial signaling role, interacting with apical and basolateral junctions and the cytoskeletal machinery to change the shape of cells (41,42). A recent study from our laboratory has shown that formation of the zebrafish midbrain-hindbrain boundary constriction is caused by basal constriction of neuroepithelial cells and is dependent on laminin in the basement membrane (19). Fibronectin is also required for zebrafish brain ventricle expansion, perhaps by stabilizing neuroepithelial structure (18). The roles that laminin and fibronectin play during zebrafish brain ventricle morphogenesis are consistent with the requirement for ECM in epithelial morphogenesis during rat neurulation (43), chick otic placode invagination (44), and chick lens vesicle formation (45).

ECM components at the apical surface of the neuroepithelium may also be crucial. Chick and rat brain ventricles contain an apical ECM rich in chondroitin sulfate, hyaluronic acid, and other proteoglycans, and these may play a role in brain ventricle formation by promoting neuroepithelial integrity and cell shape changes as well as regulating the eCSF osmolality and intraluminal pressure during brain ventricle inflation (43,46–50).

Regional cell proliferation and cell death—Another neuroepithelial process that may regulate brain morphogenesis and ventricle development is cell proliferation, and it has been suggested that brain ventricle shaping depends upon localized cell proliferation throughout the neural tube (10,14,51). Consistent with this idea, regions of constriction between the quail forebrain and midbrain, as well as between the telencephalic ventricles, have significantly higher numbers of post-mitotic cells than the surrounding tissue (52). Additionally, the midbrain-hindbrain boundary region (MHB) in the zebrafish shows about two-fold less proliferation than surrounding tissues (13). The MHB does not open to form a ventricular space (Fig 3A), but it is not known whether the lower rate of cell proliferation regulates ventricle opening in this region. Addition of a DNA synthesis inhibitor to zebrafish, just before the ventricles open, results in smaller but normally shaped brain ventricles (13), indicating a requirement for cell proliferation in ventricle development, but not necessarily in neuroepithelial shaping. This result is consistent with previous studies in *Xenopus* (53).

Multiple genes are known to regulate region-specific proliferation in the brain. For example, the transcription factor Bf-1 is required for proliferation of telencephalic cells and is essential for normal morphogenesis of the telencephalon in the rat (54). The zebrafish *zic2a* and *zic5* transcription factors, required for cell proliferation in the midbrain, and *zic1* and *zic4*, required for cell proliferation in the hindbrain, have been implicated in formation of

normal midbrain and hindbrain ventricles, respectively (22,55). Despite these data, the mechanism by which proliferation regulates ventricle formation is not clear.

Spatially-regulated cell death (apoptosis) may also contribute to brain shaping (56,57). Blocking programmed cell death in mice, by loss of caspase function, causes an overgrowth of brain tissue and obstructed brain ventricles (58). Conversely, mouse mutants which show more cell death than wild type also show reductions in the amount of brain tissue and over-expansion of the ventricles (59). However, localized cell death is not apparent during initial shaping of the zebrafish brain ventricles (13), suggesting that apoptosis may not play a role at these stages.

Inflation of the brain ventricles

A key process in brain ventricle formation is secretion of embryonic cerebrospinal fluid (eCSF) into the ventricular lumen (Fig 4). In zebrafish, formation of eCSF requires the Na^+K^+ATPase ion pump (13). Zebrafish embryos lacking Na^+K^+ATPase activity fail to inflate the brain ventricles (13), and this pump likely forms an osmotic gradient required for fluid movement into the ventricle lumen (60,61). Studies in chick and rat embryos show that proteoglycans in the eCSF, secreted by the neuroepithelium, also regulate fluid movement into, and size of, the brain ventricles (Fig 4) (46,48,49).

Classic studies in chick embryos have suggested that intraluminal pressure resulting from the accumulation of eCSF is necessary for normal brain development, and consistently, intubation of the chick embryonic hindbrain ventricle results in a collapse of the ventricles (62–64). While the choroid plexus plays a major role in CSF production in the adult, its role during embryonic brain ventricle development is less clear. However, when the brain ventricles initially fill with fluid and later expand, the choroid plexus has not yet formed. In humans, brain ventricles inflate several weeks prior to choroid plexus formation (14). In zebrafish, the ventricles begin inflating at 19 hours post fertilization (hpf) and choroid plexus is not formed until approximately 48 hpf (65). Thus, there must be some other source of eCSF besides the choroid plexus during embryonic CSF production, and the neuroepithelial tissue surrounding the ventricles may play a large role.

4) Brain ventricle function

In this section, we review the functions of CSF in the adult and, particularly, in the embryo (Fig 4,5A).

Adult functions

The functions of CSF in the adult include protection, nutrient transport, and waste removal. These functions were first attributed over a hundred years ago, and there is undoubtedly more to learn about the mechanisms underlying each of them, as well as their importance for adult brain function. Several unstudied aspects of the ventricles and CSF remain. For example, a circumventricular system of neurons sends dendrites and axons into the ventricular space (66). The function of these neurons is unknown, but it seems reasonable to suggest they may sense factors in the CSF or secrete neurotransmitters into the CSF. More recently, it has been suggested that the brain ventricles play a role in controlling homeostatic, hormonal, and signaling mechanisms involved in brain function (8,9). Significant evidence indicates that growth factors and other signals circulate within the CSF and have an effect on brain function (4,67). Thus, there is strong evidence that gonadotropin-releasing hormone, released into CSF, directly affects sexual behavior in sheep (68). Recent work has shown that cilia-mediated CSF flow in the lateral ventricles directs migration of developing neurons in the adult rat brain (69), indicating that fluid flow

may be a crucial function of the brain ventricles. Overall, these data indicate that the CSF within adult brain ventricles plays complex roles in brain function.

Embryonic functions

Requirement for eCSF in neuroepithelial survival and proliferation—A role for the eCSF has been considered only recently, but significant data indicate that this fluid regulates neuronal proliferation and determination. In humans, for several weeks after neural tube closure, the embryonic brain primarily comprises proliferating pluripotent neuroepithelial cells, considered to be the first neural stem cells (14). Neuroepithelial proliferation occurs almost exclusively at the ventricular surface (70), and contact with eCSF and the factors it contains may be a prerequisite for production of early, pluripotent neuroepithelial cells (14) (Fig 4,5A). Only a few neuronal progenitor populations undergo mitosis distal to the ventricles (for example (71)), and these cells are fate-restricted and generated late in development. Moreover, there is a striking correlation between brain ventricle size and amount of neuronal cell proliferation within the corresponding periventricular region (14) – thus, the bigger the ventricle, the greater the amount of subsequent cell proliferation. Consistent with these observations, drainage of eCSF leads to reduced cell proliferation and increased apoptosis in the developing chick brain (72), indicating that eCSF is necessary for normal neuronal development.

Role of fluid pressure on brain development—One mechanism by which eCSF may regulate brain development is through creating pressure within the brain ventricles (Fig 4) (73). Desmond and colleagues observed a 50-fold increase in intraluminal pressure during chick brain ventricle expansion and showed that artificially increasing pressure via saline injection increases neuroepithelial mitoses (73). Although the mechanism by which fluid pressure affects brain development is not understood, these data are consistent with the development of other organ systems. For example, in the zebrafish heart, blood flow modifies the morphology of the atrial and ventricular lumens (74) and stimulates valve morphogenesis (75), and in tissue culture, cell stretching increases cell proliferation (76).

Factors contained in eCSF—In addition to fluid pressure, significant data indicate that factors within the eCSF are pivotal in brain ventricle development and function. Embryonic CSF has a complex protein composition that differs substantially from adult CSF. While adult CSF has only trace amounts of protein, with detectable levels usually indicating infection, damage, or other pathology (1), eCSF is protein-rich, with a changing composition during development and between ventricles (77–79). Proteomic analyses of human, rat, mouse, and chick eCSF have identified approximately 200 different proteins, including signaling and growth factors, extracellular matrix proteins, transport and carrier proteins, and enzymes and proteases (77–79).

Consistent with a role for factors in eCSF in promoting neuroepithelial growth, isolated chick and rat embryonic brain cells are not able to replicate or undergo neurogenesis in defined medium, but addition of eCSF to the cultures promotes cell survival, proliferation, and neurogenesis (80,81). Immunodepleting the chick ventricles of Fgf2, a component of eCSF which contributes to regulating neurogenesis, reduces cell proliferation by 50% (82–84). Recent neuroepithelial explant studies indicate roles for eCSF low-density lipoprotein and retinoic acid in promoting neurogenesis in chick, and this is supported by knockout studies in mice (85,86). Parada and colleagues have shown that explants of chick midbrain tissue cultured with basal medium do not express the midbrain markers *otx2* and *fgf8*. However, when cultured with eCSF-supplemented medium, the tissues maintain normal gene expression patterns (87), suggesting that eCSF may regulate this early aspect of brain development. Finally, rat neural cells behave differently in culture depending on the

embryonic age of eCSF applied to the cells (81). Prenatal E21 eCSF can support proliferation of cortical cells from E19, but not E17, embryos (81), suggesting that components within the eCSF regulate neurogenesis in a developmental stage-dependent manner.

Together, these studies indicate that eCSF plays a crucial role in normal development, and understanding its function is likely critical for the success of neural stem cell technology (77,88).

5) Brain defects connected to ventricle abnormalities

Abnormalities in brain ventricle structure and CSF regulation are associated with several common birth defects and often have severe consequences for brain function. These defects highlight the importance of the tubular nervous system, and most appear to relate to an absence of CSF, too much CSF, or CSF of incorrect composition. These abnormalities include cranial neural tube closure defects, hydrocephalus, and neurodevelopmental mental health disorders (Fig 5B). Several of these disorders can also present for the first time in the adult and may be due to long-term accumulation of mild early defects or a sudden late causal event.

Anencephaly

Neural tube defects that result in failure of the tube to close have devastating consequences for brain development and mental function. When the anterior neuropore fails to close, that is, the tube remains open at the front of the brain, a defect called anencephaly results (Fig 5B). Anencephaly leads to a failure of forebrain development, and human fetuses with this condition are either stillborn or die within 24 hours after birth. In this condition, the brain ventricular system is open, eCSF can escape, and the brain is exposed to the outside environment. The outcome of this disorder indicates the importance of the normal brain ventricular environment for brain development. However, it is not known whether the surrounding foreign fluid actively destroys the forebrain (89), or whether the forebrain fails to develop due to absence of factors normally found in the eCSF that are required for neuronal specification and survival.

Schizencephaly

In schizencephaly, a slit or cleft within the brain tissue allows CSF to leave the brain ventricular system (90) (Fig 5B). The cleft can be bilateral (on both sides of the brain) or unilateral, and the walls of the cleft can be apposed (closed lip) or separated (open lip). Schizencephaly results in numerous neurological problems, including seizures, developmental delays, difficulties with language and motor skills, and death. Severity of the problems depends on the size of the defect. For example, open lip bilateral schizencephaly is more severe than the closed lip unilateral form. It is thought that schizencephaly arises during early brain development, and some data suggest that the malformation is due to impaired neuronal migration (90), but the pathology and etiology of schizencephaly are unclear. Although CSF escapes abnormally from the forebrain in both schizencephaly and anencephaly, schizencephaly is by far the less severe of these disorders, suggesting that it arises after the telencephalon is established, and/or that the loss of CSF is not as great as in anencephaly.

Hydrocephalus

Hydrocephalus is characterized by an excess of CSF and over-dilation of the brain ventricles (Fig 5B). It is one of the most common birth defects, occurring in up to 1/500 births (congenital hydrocephalus) (91–93), but it can also arise in children and adults (acquired

hydrocephalus). Congenital hydrocephalus often results in severe disruption of brain development, including decreased neurogenesis (93–95), whereas acquired hydrocephalus damages brain tissue that is already formed. One form of acquired hydrocephalus, prevalent in geriatric patients, is “normal pressure hydrocephalus”. This late onset form leads to progressive mental impairment and gait disturbances, but the underlying cause of the disorder is often unknown and may not be the same as the embryonic form.

It has been suggested that hydrocephalus may result from too much CSF production, too little CSF absorption, impaired CSF flow, and/or abnormal brain shaping leading to blockages of narrow canals that connect the ventricles, especially the cerebral aqueduct (93,95,96). All of these cases would lead to excessive CSF within the brain ventricles and increased intracranial pressure. These abnormal events may be due to environmental insults, such as brain injury, or to genetic defects (believed to account for at least half of all cases (93)). Multiple genes/loci have been implicated in mammalian models of hydrocephalus, many of which correspond to proteins involved in neural development (for example, *otx2*, *rfx4*, *alpha-SNAP*) (93). However, only one gene, the neural cell adhesion molecule *L1CAM*, has been clearly linked to human hydrocephalus, but the mechanism by which *L1CAM* mutation leads to hydrocephalus is unknown. Intriguingly, recent studies have shown that mutations in genes required for development of cilia (that line the brain ventricles) can cause hydrocephalus in both mouse (97) and zebrafish (98,99). This is likely because loss of cilia-induced fluid flow leads to fluid accumulation within the ventricles.

Previously, it was assumed that the increased intracranial pressure that results from hydrocephalus was the main damaging force to the brain. However, in cases of early onset hydrocephalus, where shunts are used to relieve pressure prior to tissue damage, brain development is still abnormal (94,100). Consistently, direct evidence indicates that abnormal factors within eCSF may be responsible for pathology in certain cases of hydrocephalus. While normal eCSF promotes cell proliferation, eCSF obtained from the enlarged ventricles of the hydrocephalic rat model inhibits cell proliferation in culture (101). Furthermore, abnormalities in eCSF protein content, including reduced proteoglycans, are detectable prior to any morphological brain defects in the rat hydrocephalic model (95). Finally, while cortical periventricular cells in the hydrocephalic rat brain do not divide, they proliferate normally once they are removed from the in vivo environment and cultured in vitro with wild-type eCSF (101). These results all suggest that abnormal regulation of eCSF factors, rather than increased fluid pressure, may be an underlying cause of hydrocephalus-related brain damage.

Neurodevelopmental disorders with altered brain ventricle structure

In addition to neural tube defects and hydrocephalus, a wide range of neurodevelopmental disorders have been correlated with more subtle abnormalities in brain ventricle size and shape (Fig 5B). These include schizophrenia, autism, idiopathic and syndromal mental retardation, fragile X syndrome, Down’s syndrome, attention-deficit-hyperactivity disorder, and other learning disorders (102–111). Even mild ventricle enlargements are associated with developmental abnormalities, including motor and language delays, in the first two years of life (112–117). In addition, ventricular enlargement is one of the earliest and most consistently reported structural brain abnormalities found in schizophrenia (103,110,118–122). Polymorphisms of the *med12 (mediator of RNA polymerase II transcription, subunit 12 homolog)* gene in humans are associated with an increased risk for schizophrenia (123). Interestingly, zebrafish mutants which lack *med12* function show early brain ventricle structure defects, as well as loss of specific neuronal classes (18).

It is not obvious how brain ventricle abnormalities and these mental health disorders are correlated. Does loss of neural tissue lead to ventricle enlargement and altered structure, or

is ventricle enlargement a proximal cause? In several disorders, brain ventricle abnormalities arise during early stages of development (102,120), suggesting that further study of the mechanisms involved in brain ventricle formation may shed light on brain ventricle abnormalities and how to prevent them.

6) Conclusion

Origin of the brain ventricles

The conservation of the vertebrate neural tube raises the question of its origin. In the mid-1800s, it was recognized that tunicate larvae (urochordates) form a tubular nervous system, similar to the vertebrates (discussed in (124)). Subsequent analyses of morphology and gene expression patterns suggest homology between the neural tube in non-vertebrate chordates and the vertebrates (125,126). In larvae of the urochordate, Ciona, a neural tube forms with a very tiny lumen, which later expands anteriorly to form the prosencephalic ventricle (124,127). As in vertebrates, the lumen of the Ciona neural tube is lined with ciliated cells, perhaps indicating a shared function with the vertebrate ventricular system (128). In the cephalochordate, Amphioxus, the brain is also tubular and forms a fluid-filled, anterior cerebral vesicle (126,129), that may be homologous with the vertebrate brain ventricles. Although the function of the urochordate and cephalochordate tubes have been little considered, it is reasonable to suggest that vertebrate ventricular function may have originated in ancestral lineages.

Importance of the vertebrate brain ventricular system, and what's next?

Neither development nor function of the vertebrate brain ventricular system is fully understood in any animal system, and a long list of unanswered questions remains. Underlying these is the assumption that the ventricular system carries out a common set of functions in all vertebrates. Part of this assumption stems from the conserved tubular nervous system, the similarity in embryonic brain morphology across vertebrate groups, and the homologous set of genes known to be required for development and function of the brain in model vertebrates. One significant future challenge is to understand the molecular connection between brain patterning and brain morphogenesis, including ventricle shaping. The precise role of epithelial junctional complexes and the ECM during brain morphogenesis and ventricle formation remain unclear. The connection between cell proliferation and brain morphogenesis is also not understood. The extent to which eCSF governs neuroepithelial fate remains an area of key interest. What is the role of eCSF flow and pressure? What are the roles of the many factors in the eCSF? Does the eCSF primarily govern cell division/proliferation in the brain, or is its primary role to direct formation of specific neuronal or glial subtypes? What is the molecular basis for anencephaly, schizencephaly, and hydrocephalus? Is the connection between ventricular abnormalities and mental health disorders causal? In sum, over the next few years, collecting these data will help piece together the set of mechanisms by which the vertebrate brain ventricular system forms and describe the functional significance of tubular nervous system.

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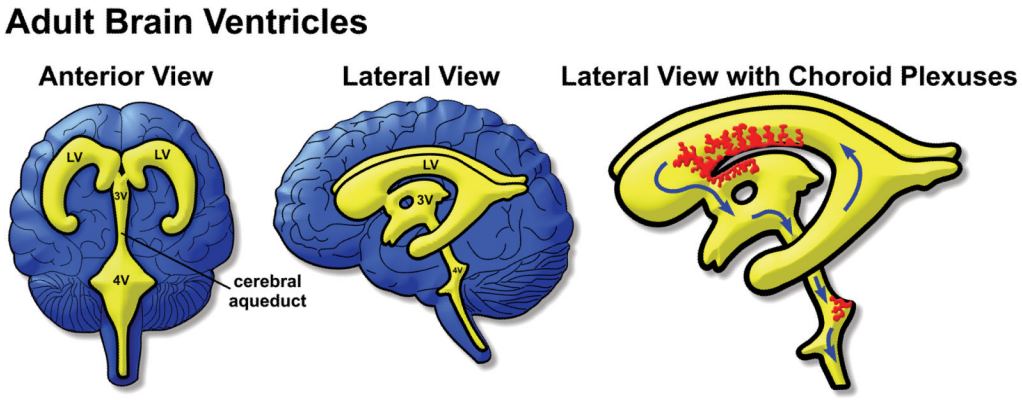

Figure 1. The adult brain ventricular system

Cartoon representation of adult human brain ventricles. Blue represents brain tissue and yellow shows brain ventricles. Choroid plexuses are in red, blue arrows designate direction of CSF flow.

LV: lateral ventricle, 3V: third ventricle, 4V: fourth ventricle.

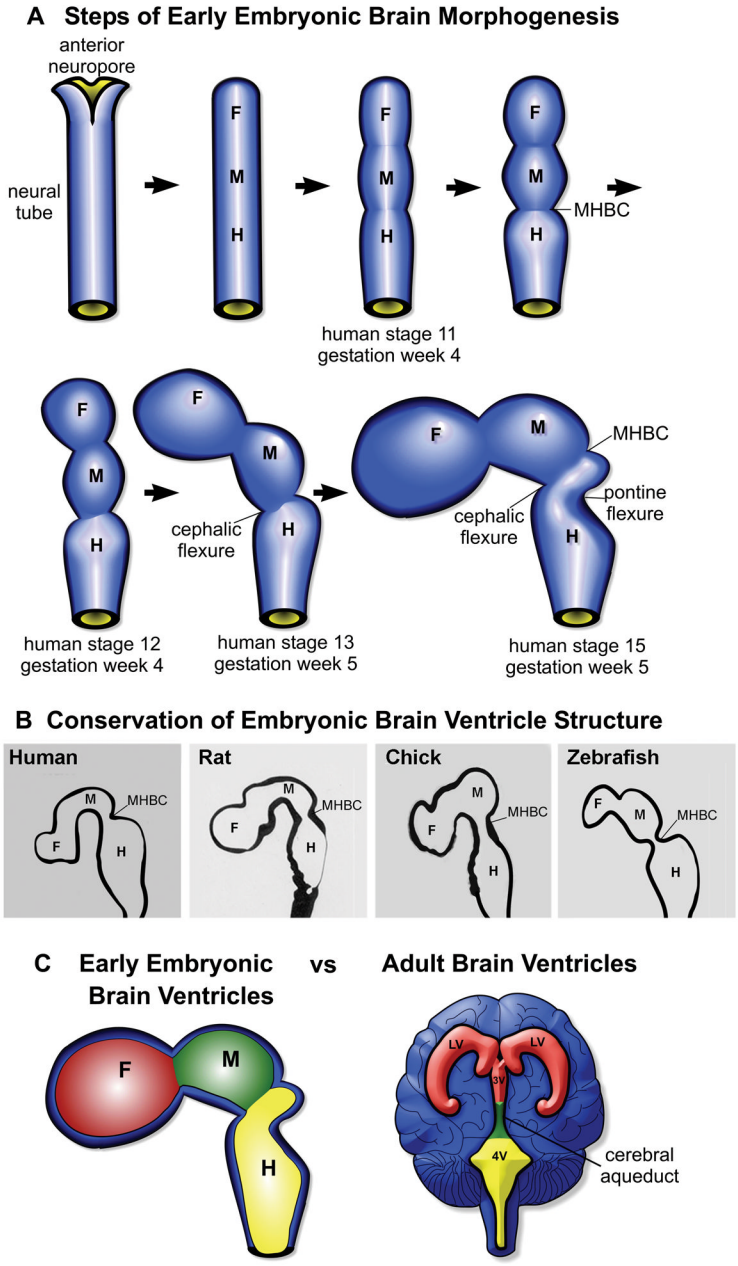

Figure 2. The embryonic brain ventricular system

A. Schematic of vertebrate embryonic brain development. Shown are lateral views of the neural tube as it undergoes early embryonic brain morphogenesis to form the primary brain vesicles.

B. Conservation of embryonic brain ventricle structure. Tracings of embryonic brain ventricles at similar corresponding stages in development, all lateral views. Human embryo brain ventricles, stage 17 (approximately 43 days post fertilization), traced from the Carnegie Embryological Collection. Rat embryo brain ventricles, stage E14 (14 days post fertilization), traced from (130); Chick embryo brain ventricles, stage 16 (approximately 2.5 days post fertilization), traced from (131); Zebrafish embryo brain ventricles, 24 hours post fertilization.

C. Comparison of early embryonic and adult brain ventricles. Colors correspond to the same ventricle regions in the embryo and adult.
Not to scale. F: forebrain (telencephalon plus diencephalon), M: midbrain (mesencephalon), H: hindbrain (rhombencephalon), MHBC: midbrain hindbrain boundary constriction, LV: lateral ventricle, 3V: third ventricle, 4V: fourth ventricle.
In part A, F M H refer to brain vesicles. In parts B and C, F M H refer to ventricles.

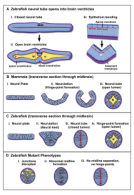

Figure 3. Neuroepithelial morphogenesis during brain ventricle development

A. Schematic showing zebrafish neuroepithelium as it opens into the brain ventricles. After neurulation, the zebrafish neuroepithelium is a closed neural tube (i) connected by apical actin junctions and surrounded by a basement membrane (i,iii). As the brain ventricles open, the neuroepithelium bends in locations of apical constriction (white asterisks) and basal constriction at MHB (ii,iii).

B. Schematic depicting stages of neurulation in mammals, beginning with the columnar epithelium of the neural plate (i). Neurulation and hinge-point formation occur concurrently (ii), resulting in an open neural tube with hinge-points already formed. The lumen remains open and expands after neurulation is complete (iii).

C. Schematic depicting stages of neurulation in zebrafish, beginning with the columnar neural plate (i). Neurulation progresses through a “neural keel” stage (ii) and ends with a closed neural tube (iii). Subsequently, the neural tube opens and forms hinge-points to shape the ventricles (iv).

D. Cartoons of transverse sections of the midbrain ventricle depicting several phenotypes observed when neuroepithelium morphogenesis does not occur normally in zebrafish. When junctions are completely disrupted (i), neurulation does not proceed and ventricle formation is impossible. When the midline does not form correctly (ii), the midline cannot separate to form the ventricles. Some mutants show normal midline formation, but still do not separate at the midline and form hinge-points (iii). F: forebrain ventricle, M: midbrain ventricle, H: hindbrain ventricle, MHB: midbrain-hindbrain boundary.

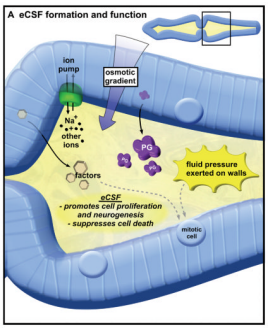

Figure 4. eCSF formation and function during brain ventricle inflation

Cartoon depicting eCSF secretion and function. Inset: dorsal view of embryonic brain, after initial lumen inflation, with enlarged area (hindbrain) boxed. Ion pumps and proteolgycan secretion are thought to form an osmotic gradient regulating fluid flow. Signaling and growth factors are also secreted. Both fluid pressure and growth factors stimulate cell proliferation and gene expression within the surrounding neuroepithelium. Not drawn to scale. PG: proteoglycans. Circular cells at ventricular surface are mitotic cells.

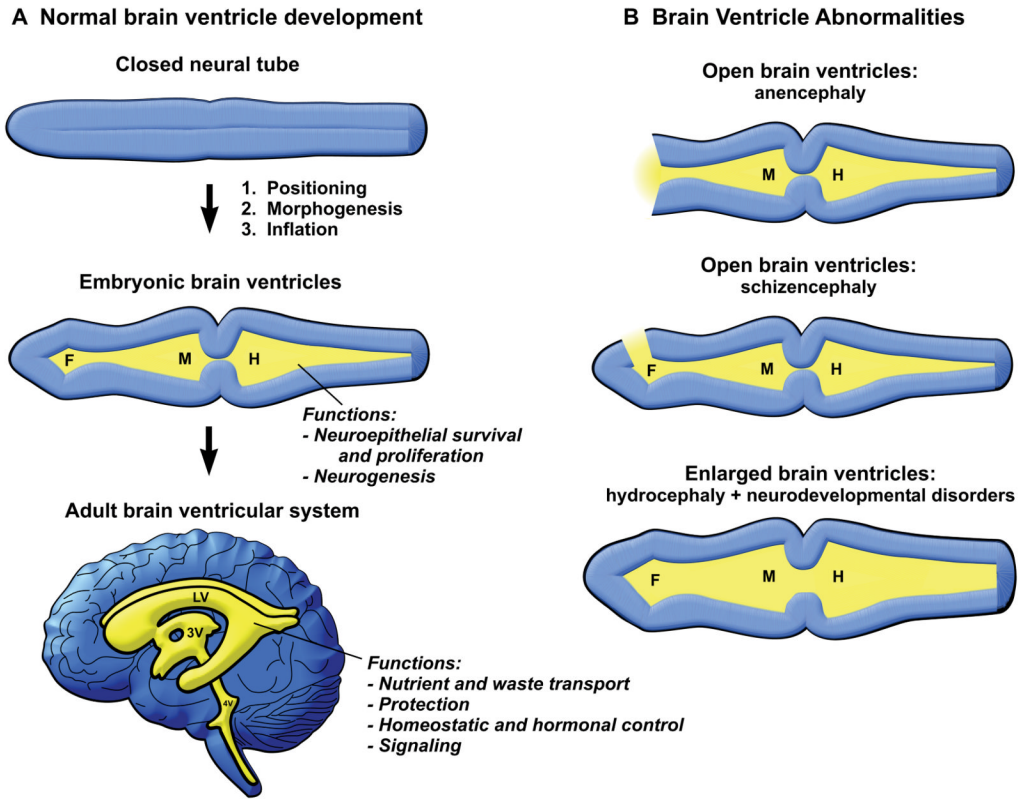

Figure 5. Summary of mechanisms that regulate brain ventricle development

Summary of developmental mechanisms underlying brain ventricle formation, function of the brain ventricular system, and associated abnormalities. See text for details.