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Review

Why the embryo still matters: CSF and the neuroepithelium as interdependent regulators of embryonic brain growth, morphogenesis and histiogenesis

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ABSTRACT

The key focus of this review is that both the neuroepithelium and embryonic cerebrospinal fluid (CSF) work in an integrated way to promote embryonic brain growth, morphogenesis and histiogenesis. The CSF generates pressure and also contains many biologically powerful trophic factors; both play key roles in early brain development. Accumulation of fluid via an osmotic gradient creates pressure that promotes rapid expansion of the early brain in a developmental regulated way, since the rates of growth differ between the vesicles and for different species. The neuroepithelium and ventricles both contribute to this growth but by different and coordinated mechanisms. The neuroepithelium grows primarily by cell proliferation and at the same time the ventricle expands via hydrostatic pressure generated by active transport of Na^+ and transport or secretion of proteins and proteoglycans that create an osmotic gradient which contribute to the accumulation of fluid inside the sealed brain cavity. Recent evidence shows that the CSF regulates relevant aspects of neuroepithelial behavior such as cell survival, replication and neurogenesis by means of growth factors and morphogens. Here we try to highlight that early brain development requires the coordinated interplay of the CSF contained in the brain cavity with the surrounding neuroepithelium. The information presented is essential in order to understand the earliest phases of brain development and also how neuronal precursor behavior is regulated.

Introduction

Initially, the embryonic brain is a hollow fluid-filled tube. The development of the neural tube involves three distinct phases: formation of the tube (neurulation), polarization of the tube into an anterior expanded brain and posterior spinal cord and histiogenesis of the neuroepithelium throughout. Much attention has been given to the analysis of the mechanisms that form the tube via neurulation as well as the later period of embryonic brain development involving cell differentiation of the neuroepithelium. However, little attention has been given to the phase of early brain development in between these two periods during which time the anterior part of the neural tube, the future brain enlarges many fold. In fact, in human embryos, the brain increases 100,000 fold in volume during this period (Desmond and O'Rahilly, 1981). Not only is the growth immense but it is rapid.

Moreover, most embryological research of the brain and spinal cord comprising the central nervous system (CNS) has focused on the neuroepithelium. This emphasis on the neuroepithelium ignores the existence of the brain ventricles[1] filled with cerebrospinal fluid (CSF) and its role in early brain development. Today several research findings have generated sufficient evidence to support the hypothesis that the CSF is directly involved in early brain development. The main objective of this review is to demonstrate that the bi-dimensional impact of CSF with the neuroepithelium must be taken into account in our global understanding of brain development.

With the aim to expose in an ordered way what is known about the influence of CSF in early brain development, we develop a diagram which illustrates the line of argument in this review. As a general consideration, research, much of which has been developed by the authors and their collaborators, support the idea that CSF contributes to brain development by two general mechanisms:

1. CSF is a main force driving brain growth and morphogenesis during early brain development. Several research findings have shown that the normal growth and morphogenesis of the embryonic brain requires the pressure generated, within a closed ventricular

[1] Although correct embryological phraseology for the embryonic CNS is the neural tube comprised of a cavity or presumptive ventricles, to simplify we use ventricle throughout to describe the cavity for both the embryonic and adult brain. Likewise, we use CSF to describe the fluid for both embryonic and adult brains and use brain to refer to both the neuroepithelium and ventricular space.

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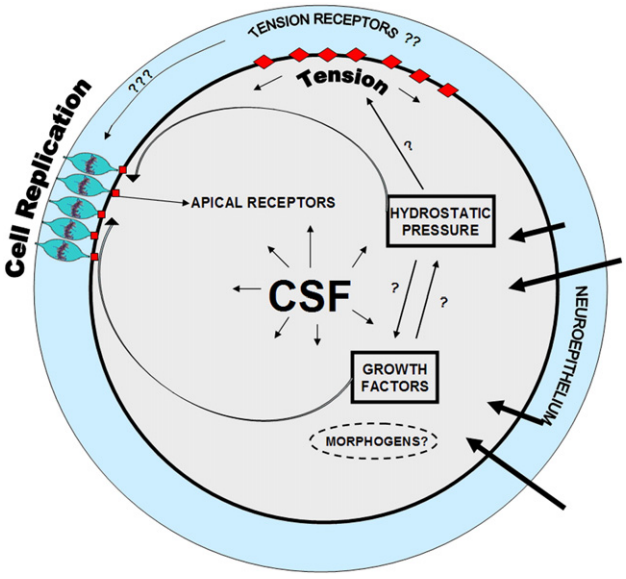

Fig. 1. A schematic diagram based on a transverse section through the midbrain region that explains the interdependence of the interactions of hydrostatic pressure created by the CSF and growth factors within the CSF upon the behavior of the neuroepithelium. Fluid crosses the neuroepithelium via an osmotic gradient (large arrows on right). The CSF generates expansion of the luminal surface indicated by arrows emanating from the word CSF. Growth factors most likely stimulate mitosis of the neuroepithelial cells via apical receptors symbolized by the red boxes on the ventricular surface by the mitotic cells. There may also be a bidirectional influence of growth factors on hydrostatic pressure and vice versa. Hydrostatic pressure may stretch the inner surface of the neuroepithelium and may stimulate mitotic activity via tension receptors such as focal adhesion kinases (FAKs) on the surface or within the neuroepithelial cells.

system, via accumulation of CSF within them, and that this accumulation of CSF within the embryonic brain ventricles occurs via an osmotic gradient. The CSF pressure promotes the expansion of the brain creating a tension state in the neuroepithelium which stimulates cell proliferation and suggests the presence of tension receptors.

2. Recently it has been demonstrated that, at early stages of development, CSF exerts an intense trophic influence on the behavior of neuroepithelial cells, regulating neuroepithelial cell survival, proliferation and differentiation. The interaction of CSF which has a complex composition, including growth factors and morphogens, with the apical surface of neuroepithelial cells has elicited marked influences upon mitosis, apoptosis and differentiation.

We propose as a major thesis of this review that these two components, CSF and neuroepithelium, are totally interdependent working as a functional entity regulating brain growth, morphogenesis and neuroepithelial cellular behavior in early brain development (Fig. 1).

Formation of the CNS

We begin by summarizing briefly the main morphological steps during early formation of the central nervous system (CNS).

There are three embryological tissues, ectoderm, mesoderm and endoderm that form the major tissues and organs of the vertebrate body. Ectoderm, the outer layer of early embryo differentiates into neural ectoderm, neural crest and skin ectoderm.

Once the cells of the neuroectoderm organize into a flat plate along the dorsal surface, they bend into a tube and at the same time become committed primitive neurons. The formation of the tubular CNS from the neural plate (neurulation) has been reviewed extensively (Jacobson, 1981; Jacobson and Gordon, 1976; Smith and

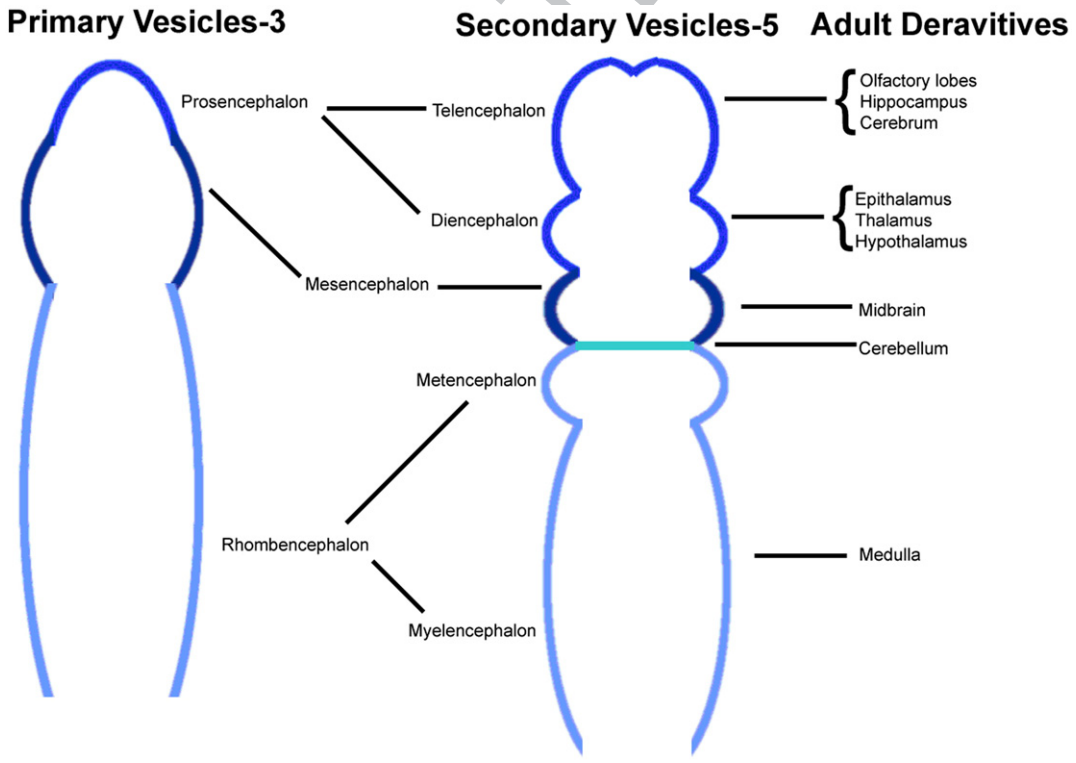

Fig. 2. A schematic diagram of the dorsal view of embryonic brain vesicles. Initially the CNS has three vesicles that then form five vesicles from which all of the adult derivatives develop. Only a few of the adult derivatives are shown in the last column. The three vesicles have been colored different shades of blue to illustrate the specific derivatives of the prosencephalon and rhombencephalon. Note that the cerebellum forms from the posterior part of the mesencephalon and anterior part of the metencephalon. (Modified from Bailly-Cuif and Wassef, 1995).

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Schoenwolf, 1997; Keller, 2002; Ybot-Gonzalez et al., 2007). This tube differentiates into five unique morphological precursors (telencephalon, diencephalon, mesencephalon, telencephalon and myelencephalon) that ultimately form all of the adult brain and spinal cord structures. The five vesicles literally form all of the structures of the adult CNS (Fig. 2).

Much attention continues to be given to gene regulation of both the rostral–caudal and dorsal–ventral gradients in early brain development establishing positional information for neuroepithelial cells (Litingtung and Chiang, 2000; Liu and Joyner, 2001; Robertson et al., 2003; Parada et al., 2005a). However, equally important is the impact of these gradients on the growth and morphology of the brain. Gradients of gene expression may result in changes in cell size, shape and distribution that influence morphology. Patterns of gene expression may also influence neuroepithelial cell sensitivity to environmental cues and spatial information. It is quite clear that what does happen during this period of brain development is that the neural tube rapidly increases in size and changes its morphology. The brain forms many bulges. It bends and rotates as well as becoming greatly enlarged atop the cylindrical narrow spinal cord. This growth and change in brain morphology and histiogenesis are directly related to CSF (Fig. 3).

CSF positive pressure, a key mechanism in brain growth

In this section we first develop some theoretical considerations about how mechanical forces such as hydrostatic pressure and mechanical tension must be considered as relevant driving forces in CNS development. This theory is necessary in order to explain mechanisms that mediate CNS development in the embryonic brain. Hydrostatic pressure produces a physical (volumetric) event within the ventricles. The data will show evidence of a positive pressure inside brain ventricles that serves as a causal connection between hydrostatic pressure and brain growth and morphogenesis. The establishment of a closed fluid compartment within the brain allows fluid accumulation via an osmotic gradient.

Biomechanical considerations relative to embryonic brain growth and morphogenesis

In this age of molecular approaches to biological problems, the value of the mechanical and physical properties of tissues and cells often becomes overlooked. Years ago, D'Arcy Thompson published his classic analysis of how tension and pressure can interact with structural anisotropies and asymmetries to determine the shape of biological structures (Thompson, 1917). He applied his theories to several animal and plant structures, but never to the brain. Eighty years later, Van Essen applied these same principles of growth and form to explain much about the morphogenesis of the CNS (Van Essen, 1997). He emphasizes that "mechanical tension working against internally generated hydrostatic pressure is a major driving force for many aspects of CNS morphogenesis". Most of Van Essen's analysis of ion directed morphogenesis in the CNS is based on the anisotopies in the orientation of the axons, dendrites and glial processes of the neuroepithelium that when under tension impart elasticity to the tissue. He notes what many experimental embryologists have witnessed about the brain in living embryos; namely, that the neuroepithelium springs back to its original position after transient deformation. Moreover, since the neuroepithelial cells are under tension and lack a rigid framework, the only thing that keeps the brain from collapsing into a smaller structure is the hydrostatic pressure created by the CSF. It has been shown that reducing the intra-lumenal pressure reduces the tangential growth of the neuroepithelium (Desmond, 1985; Desmond and Jacobson, 1977).

Van Essen uses physical forces and anisotropies to explain the folding of the cerebral cortex and hypothesizes that morphogenesis can be explained mainly by tension and does not necessarily require elaborate molecular instructions. In our opinion more studies are needed to understand the mechanical properties of the CNS in the living embryo. However, today it is known that many molecular events are involved in regulating morphogenesis during CNS development. Thus, more needs to be understood about the interplay between mechanical forces and molecular regulators of CNS morphogenesis during embryogenesis. It is with this view that we provide the following analysis of early embryonic brain growth and morphogenesis.

Measured brain growth

The growth of the early embryonic brain has been measured and described for only a few species, for example, chick, rat, and human (Desmond and Jacobson, 1977; Desmond and O'Rahilly, 1982; Levitan and Desmond, 2008; Pacheco et al., 1986; De Paz, 1999). These studies demonstrate that initially brain growth is very rapid and that ventricle growth plays a key role (Fig. 4). In 48 h the chick embryo brain increases 30-fold with the ventricles contributing 70% to this dramatic increase (Desmond and Jacobson, 1977; Pacheco et al., 1986). The rates of growth differ between the different vesicles and for different species. In both the rat and human, the forebrain grows the fastest but in chicks the mesencephalon grows fastest (Desmond and O'Rahilly, 1981; De Paz, 1999; Levitan and Desmond, 2008) suggesting a phylogenetic and ontogenetic regulation of this process. Both the neuroepithelium and ventricles contribute to this growth but by different mechanisms. The neuroepithelium grows primarily by cell proliferation and the ventricles expand via hydrostatic pressure created by the fluid within. The data clearly show that the tissue and ventricles work co-operatively in this early period of brain growth and that CSF plays a key role in the coordination of the two mechanisms. Moreover, the ventricular fluid and tissue no doubt regulate brain morphogenesis while the brain is expanding during early development. However, since less is known about the processes involved in shaping the brain during this period, we have chosen to focus on what is known about its growth. Nevertheless, shaping or sculpting of the brain is also an interesting phenomenon and needs extensive research in the future.

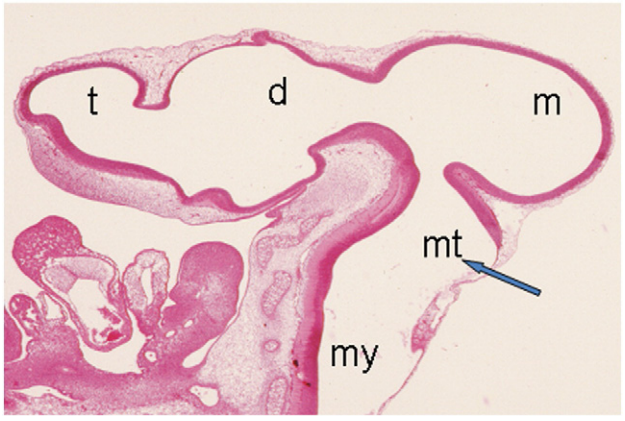

Fig. 3. A sagittal section of a HH stage 23–24 chick embryo showing the huge ventricles within the thin neuroepithelium. At this stage, the neuroepithelium is mainly a one cell thick pseudostratified epithelium. All five vesicles are present (t = telencephalon; d = diencephalon; m = mesencephalon; mt = metencephalon; and my = myelencephalon). Note the small dorsal evagination of the diencephalon which is the pineal and the thin roof of the metencephalon (arrow).

Positive pressure within the embryonic brain ventricle: causal connection with brain growth and morphogenesis

Positive pressure within the embryonic brain ventricles has been measured in the chick embryo (Jelinek and Pexieder, 1968, 1970; Gato

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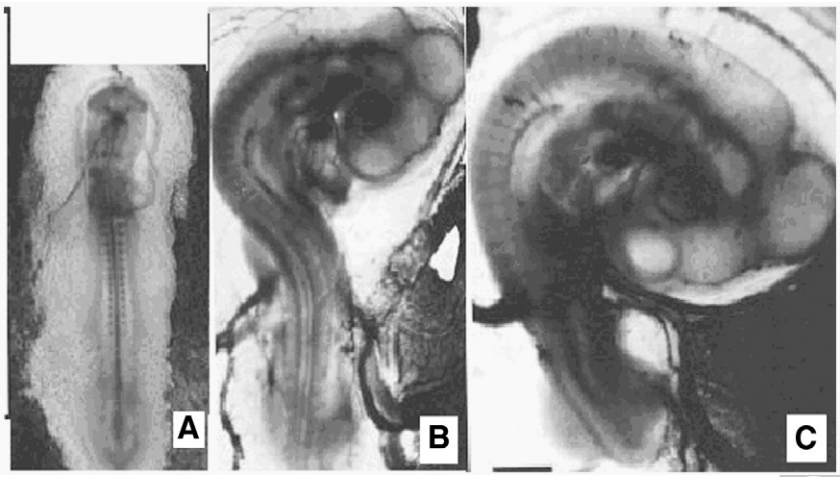

Fig. 4. Photomicrographs of three living chick embryos taken at the same magnification. The embryo on the left (A) is a dorsal view of a HH stage 12 (47 h), the one in the middle (B) a lateral view of a HH stage 18 (67 h) and the one on the right (C) is a lateral view of a HH stage 24 (71 h). In only 24 h the brain has increased 85 fold. Note how the head has bent and rotated 90° to the right. Also, note how the brain vesicles appear expanded like a balloon. (Modified from Fig. 1 in Desmond and Jacobson, 1977.)

et al., 1998; Desmond et al., 2005) and exhibits a 10% increase per embryonic stage during the period of rapid brain enlargement (Desmond et al., 2005). Jelinek and Pexieder (1968, 1970) reported that the brain collapsed upon removing CSF. While their report suggested that intra-luminal pressure might play a role in early brain growth, the causal connection was directly established by Desmond and Jacobson, 1977 and Desmond, 1985. Using chick embryos, they drained the ventricles of CSF for 24 h thus decreasing the intra-luminal pressure. They found that growth was significantly decreased, morphogenesis was disrupted, and that the neuroepithelial tissue and cell number was reduced by 50%. The tissue volume and cell number of sham operated controls (solid rods used instead of hollow tubes) did not differ significantly from the values for non-manipulated controls. This experiment demonstrated that cerebrospinal fluid pressure directs expansion of the ventricles, and strongly suggests that this CSF pressure is directly involved in normal morphogenesis and neuroepithelial cell proliferation. The experiment also shows that accumulation of the cerebrospinal fluid in the ventricles of the neural tube generates pressure because the tube is sealed from the outside. This raises three relevant questions: how is the tube closed? Does creation of a closed system result in an increase in brain expansion? How does the fluid accumulated within this closed ventricle generate the pressure?

Establishment and maintenance of a closed fluid compartment

At the end of neurulation in birds, mice and rats, the neural folds fuse initially at the mesencephalon, then anteriorly in mice and rats and posteriorly in birds in a zipper-like fashion. In both mammals and birds, the anterior neuropore closes before the posterior one resulting in a transitory period in which the brain ventricles expand when the tube is apparently open since the posterior neuropore is still open (Van Straaten et al., 1996; Smith and Schoenwolf, 1997; Copp, 2005). Desmond et al. (1982, 1985, 1984a,b, 1986) have shown that the neural tube in chick embryos is transiently sealed during the period of rapid enlargement by occlusion of the neurocoel in a region that parallels the somites beginning just posterior to the heart. Occlusion is transitory beginning at stage 11, reopening at stage 14+ (Schoenwolf and Desmond, 1986) and occurs coincident with completion of neurulation (Desmond and Field, 1992).

This occlusion creates a closed fluid system cranial to the presumptive spinal cord during a time in development that the posterior neuropore is still open. Not only does the occlusion prevent flow of fluid posteriorly but physiological experiments using dye injection show that the fluid does not cross the neuroepithelium (Desmond and Schoenwolf, 1985) (Fig. 5) Moreover, recent experiments by Desmond and Levitan, 2002, showed that brain expansion is directly dependent upon occlusion. By experimentally occluding the neurocoel of chick embryos prior to when it occurs naturally, they showed that the brains of the experimental embryos grew significantly larger than the brains of non-occluded controls during the first 5 h following the artificial seal. In the next 7 h, the brains of the embryos in which occlusion occurred naturally grew significantly larger that the brains of the embryos with precociously occluded neurocoels.

The dependence of brain expansion upon occlusion has only been demonstrated in the chick embryo. However, occlusion has been described for humans, rats, mice and salamanders (Freeman, 1972; Desmond, 1982; Desmond and Schoenwolf, 1984, 1985; Schoenwolf and Desmond, 1984a,b, 1986; Desmond and Field, 1992). Occlusion requires 2nd messengers such as Ca^{2+}, calmodulin and cAMP (Desmond et al., 1993) but does not involve typical inter-cellular attachments like interdigitations, tight junctions or abundant cell surface materials (Schoenwolf and Desmond, 1984a). However, occlusion does appear to require n-cadherin (LaConti et al., 2004).

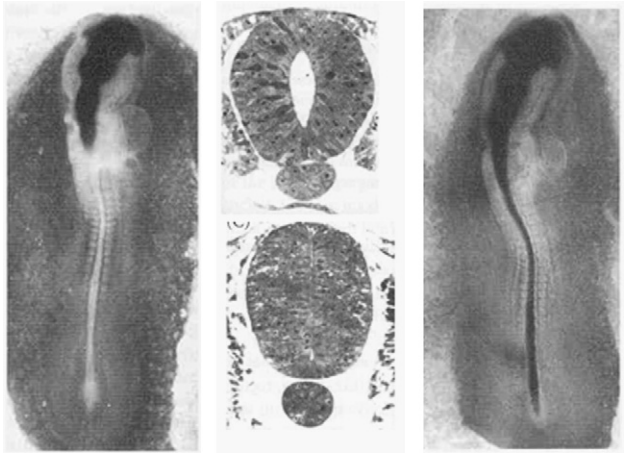

Fig. 5. Occlusion of the spinal neurocoel shown by dye injection into the midbrain of living embryos (HH stage 11, dorsal view) and also in transverse histological sections. The dye fails to flow past the anterior level of the heart when the neurocoel is occluded (left photo) whereas it flows throughout the extent of the spinal cord in non-occluded neurocoels (right photo). In transverse sections, the medial walls of the neuroepithelium are closely apposed with no ventricle apparent in occluded neurocoels (bottom center). (Figure adapted from the Eighth Edition of "Developmental Biology" by Gilbert).

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Accumulation of fluid within the ventricles via an osmotic gradient

Once the embryonic neural tube is closed, cerebrospinal fluid accumulates within its ventricle generating a positive pressure and a key question arises as to what mechanisms are involved in the genesis of cerebrospinal fluid accumulation. At least four possibilities exist based on physiological principles which include: (1) direct passive diffusion of water via hydrostatic pressure created by blood flow; (2) direct passive diffusion of water via water-channels or aquaporins; (3) active transport of Na^+ into the ventricles via Na+–K+ ATPase pumps; and (4) transport or secretion of proteins and proteoglycans into the ventricles. The last two mechanisms can work together in creating osmotic gradients.

Recent genetic screens with zebrafish suggest that brain expansion is dependent to some degree upon hydrostatic pressure. These studies clearly showed that ventricle formation occurs independent of heart circulation but that complete inflation of the ventricles requires a beating heart (Scheir et al., 1996; Lowery and Sive, 2005). However, whether the expansion of the brain ventricles in higher vertebrates, such as birds and mammals requires heart circulation yet remains to be demonstrated.

Direct water transport via aquaporins does not seem to be the mechanism of fluid accumulation during early brain expansion since aquaporins have only been demonstrated in both bird and mammalian embryos during the development of the choroid plexus which occurs at a much later time in development than the initial period of brain expansion (Johansson et al., 2005; Nico et al., 2001).

Experiments support the fact that Na^+ crosses the neuroepithelium via Na+–K+ ATPase pumps. Oubain, treated embryos (blocks the Na+–K+ ATPase pumps) had smaller ventricles than controls (Li and Desmond, 1991). On the other hand, the increase of Na^+ in the ventricles (induced by β-D-xyloside which increases the free chains of chondroitin sulfate and free Na^+) leads to hyper-expanded ventricles (Alonso et al., 1998). More recently, ventricle expansion was shown not to occur in the *snakehead* mutant in zebrafish which is most likely due to impaired ion transport (Lowery and Sive, 2005).

Several investigators have suggested that Na^+ exits the CSF to the outside creating a trans-neuroepithelial electric potential that appears to direct normal morphogenesis (Hotary and Robinson, 1991; Shi and Borgens, 1994; Borgens and Shi, 1995). Other evidence (Sedlacek, 1975; Alonso et al., 1998) shows that a high concentration of Na^+ remains inside the ventricular system and that this high concentration of intra-ventricular Na^+ is probably associated with CSF proteoglycans as has been reported for many biological systems particularly in the extra-cellular matrix (ECM) (Comper and Laurent, 1978).

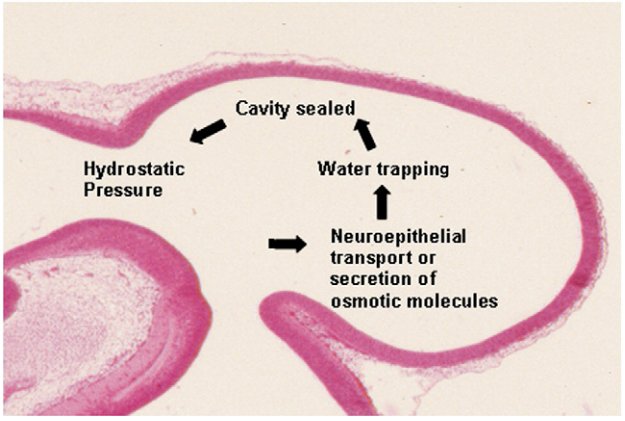

Fig. 6. A composite diagram including a sagittal section of a HH 23–24 chick embryo mesencephlon, inside which is a schematic flow chart summarizing that osmotic active molecules transported across or secreted by the neuroepithelium into the CSF of a closed ventricular system of the embryonic CNS generate hydrostatic pressure within the ventricle. This pressure expands the ventricle outward and thus the neuroepithelium surrounding the ventricle.

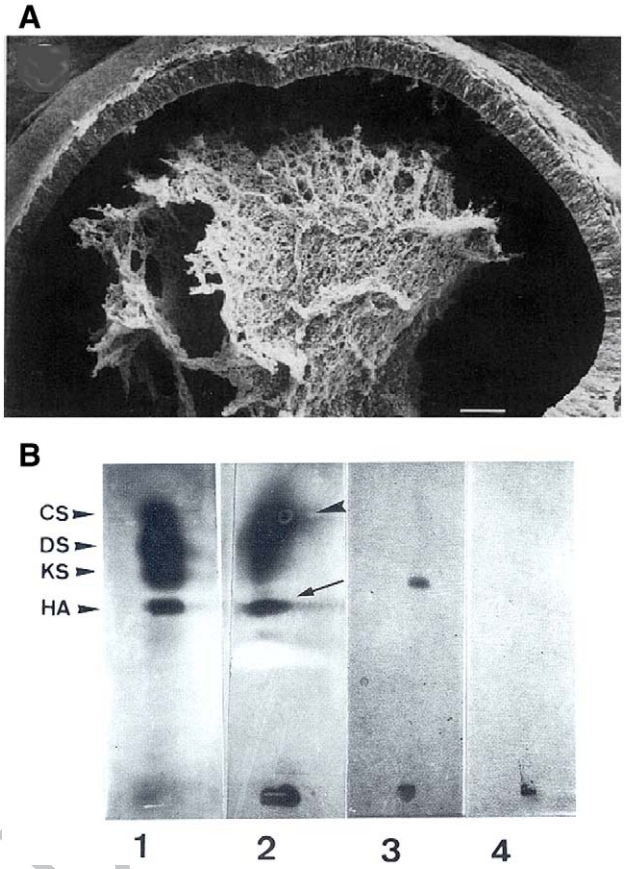

Fig. 7. (A) A SEM of a fractured HH stage 20 chick embryo mesencephalon (lateral view) showing precipitated material within the ventricle after fixation with Carnoys fixative. (Adapted from Fig. 1ff in A. Gato et al., 1993.) (B) An electrophoretic separation and identification of chondroitin sulfate (CS) and hyaluronic acid (HA) as major proteoglycans present within the CSF of a HH stage 23 chick embryo. Lane 1 represents standards, with CS = chondroitin sulfate, DS = dermatan sulfate, KS = keratan sulfate and HA = hyaluronic acid. Lane 2: Electrophoretic separation of the neural tube components and Lanes 3 and 4: shows the sensitivity of these components to chondroitinase AC (just digest chondroitin/dermatan sulphate proteoglycans: lane 3) and chondroitinase ABC (Digest also hyaluronic acid: lane 4) (Adapted from Fig. 5 in M. I. Alonso et al., 1998.)

Gato et al. 1993 demonstrated that osmosis is the mechanism responsible for the accumulation of fluid inside the ventricles and the subsequent genesis of CSF pressure (Fig. 6). They propose that osmotic components enter the ventricles from the outside by crossing the neuroepithelium or that they are directly secreted into the ventricles by the neuroepithelial cells setting up an osmotic gradient between the inside and outside, then water passes along the gradient and accumulates in the sealed ventricle generating hydrostatic pressure. Support for this hypothesis comes from the finding of a precipitable material inside the brain ventricle of chick and rat embryos morphologically compatible with an extracellular matrix and also the presence of morphological features such as secretory vesicles and prominent golgi apparatus in the apical portion of the neuroepithelial cells compatible with the secretory activity (Gato et al., 1993).

In addition, Gato et al. have also shown that in chick and rat embryos, the presence in CSF of powerful osmotic molecules such as proteoglycans in the CSF contributing to the osmotic gradient that work together with Na^+ as essential elements in the genesis and regulation of CSF pressure (Alonso et al., 1998, 1999, 2000; Gato et al., 2004) (Figs. 7A,B). Proteoglycans have a high negative charge created by the large amount of COO^- and SO_3^- radicals that retain high amounts of positive ions (Galligani et al., 1975) The experimental support for

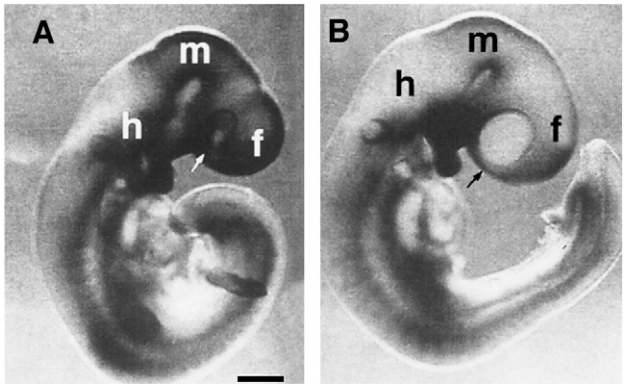

Fig. 8. Lateral macroscopic views of a 11.7 day-old rat embryo control (left) with a β-D-xyloside-treated animal (right). Note the hyper-expanded brain at the fore (f), mid (m) and hindbrain (h) of the treated embryo in contrast to the control. (Adapted from Figs. 3a and b in M. I. Alonso et al., 1999.)

the relationship between proteoglycans — Na^+ osmotic power and the genesis of CSF hydrostatic pressure in the brain ventricle of chick and rat embryos came from Alonso et al. (1998, 1999). They injected β-D-xyloside into the subgerminal layer of the neuroepithelium of chick and rat embryo brains which increased the concentration of both proteoglycans and Na^+ into the CSF and consequently the hydrostatic pressure in brain ventricles. Both the rat and chick embryo brains expanded significantly more than control embryos (Fig. 8).

CSF regulates relevant aspects of neuroepithelial cell behavior

Thus far, the works cited provide evidence that the CSF regulates brain growth and morphogenesis via mechanical mechanisms such as fluid pressure. However, experiments from both Desmond and Gato viewed collectively show that the CSF plays other relevant roles in early brain development. Releasing CSF via intubation resulted in a 50% reduction of cells in the chick neuroepithelium (Desmond and Jacobson, 1977 and Desmond, 1982) and treating neuroepithelial explants *in vitro* with and without CSF, showed that the neuroepithelium needs the presence of CSF to be self-sufficient in cellular survival, replication and differentiation (Gato et al., 2005) An over-arching conclusion from these respective findings is that rather than continue to interpret embryonic brain growth and morphogenesis based on the different properties of CSF, physical and biological, it is time to focus on the inter-dependence of mechanical and biological factors in controlling embryonic brain growth, differentiation and morphogenesis.

In the next part of this review, we first emphasize how the CSF positive pressure is involved in the control of the mitotic behavior of neuroepithelial cells in chick embryos. Then we summarize how CSF influences neuroepithelial cell behavior by means of biologically active components. Then, in order to clarify how CSF exerts this influence we discuss the macromolecular origin and composition of the CSF especially proteins including growth factors and morphogens. And finally we analyze how CSF exerts some of its biological actions upon the replication and differentiation of the cells within the neuroepithelium.

CSF positive pressure influences neuroepithelial cell behavior

A great reduction in neuroepithelial cell density has been correlated with a loss of tension across the neuroepithelium maintained by the hydrostatic pressure. Conversely, when Desmond et al., 2005 increased the intra-luminal pressure for 1 h in chick embryonic brains, the mitotic density of the neuroepithelium was significantly greater compared to controls. This finding is similar to the much earlier finding of Abercrombie, 1970 who showed an increase in mitotic activity of cells under tension (stretch) in cell culture. Physiologists have long recognized the relationship between internal pressure and vessel expansion by stating that the distending tension in the wall of a vessel at any given pressure is directly proportional to its radius and elastic limit (law of LaPlace after Gardner, 1973). Complementary experimental approaches must now be developed to demonstrate how cellular tension is able to modify cellular behavior.

Cellular tension across the neuroepithelium created by the pressure of the CSF may be detected via tension receptors similar to mechanosensors that have been demonstrated in fibroblasts (Wang et al., 2001). These mechanosensors consist of focal adhesion kinases (FAKs) that appear to respond to increasing tension in the substrate by stimulating the assembling and disassembliy of stress fibers via detection by the integrins on the cell membrane. FAK-null fibroblasts are unable to reorganize focal adhesions in response to push and pulling tensions exerted by the substrate whereas WT cells are unable to do so. FAKs have not been detected in the embryonic neuroepithelium as of yet but have been detected in keratocytes of the epidermis (Schober et al., 2007) and cardiac muscle cells (Tosrsoni et al., 2003) to name but a few.

CSF influences neuroepithelial cell behavior by means of biologically active components

In the last few years, Gato et al. have developed a research line based on the hypothesis that CSF is able to induce specific changes in neuroepithelial cell behavior on the basis of its molecular composition. Their research has focused on the protein composition of CSF, the trophic effect of molecules in the CSF upon neuroepithelial growth and differentiation, and mechanisms by which the molecules exert their biological action. They have shown that CSF is directly involved in neuroepithelial cell behavior by using organotypic cultures of mesencephalon tissue from chick embryos in presence or absence of CSF (Gato et al., 1998, 2005) (Fig. 9). They further showed that CSF also is involved in the mesencephalic expression of the Otx2 gene when the chick mesencephalon was cultured with the isthmus (Parada, et al., 2005a). Current work developed in the Gato laboratory show that the trophic influence of CSF upon neuroepithelial precursors are also extensive in mice and rats. These findings support the idea that the cellular behavior of the neuroepithelium is not self-sufficient but relies upon the CSF suggesting to us that the CSF and neuroepithelium are interdependent and work together as a functional unit.

This idea is in agreement with research findings of several different laboratories describing the influence of CSF on the behavior of precursor neurons over their lifetime. Particularly relevant is the research of Miyan et al., who have shown that during fetal stages, CSF is able to support survival and replication in rat cortical precursor cells. These authors show that CSF composition and properties change during fetal stages exhibiting the highest mitogenic activity at 19–20 days of development. Another interesting theory proposed by these authors is that CSF composition changes as CSF moves from the lateral ventricles to the subarachnoid space by the sequential addition of components from the different choroid plexus. In fact, in the subarachnoid space, CSF has been related with cortical stratification via reelin synthesis by Cajal–Retzius cells (Miyan et al., 2006; Salehi and Mashayekhi, 2006). For the adult brain, Sawamato et al., 2006 reports the influence of CSF on the migration of newborn neurons from the subventricular zone to the olfactory bulb. These research findings highlight the crucial role of CSF to brain functionality during life. Moreover they highlight the fact that CSF may have different properties in different brain locations as well as during different periods of the lifetime of the brain, i.e., embryonic, fetal, and adult.

The protein composition of CSF

As we have stated before, proteoglycans and ions are major components of the embryonic CSF. However, the most studied

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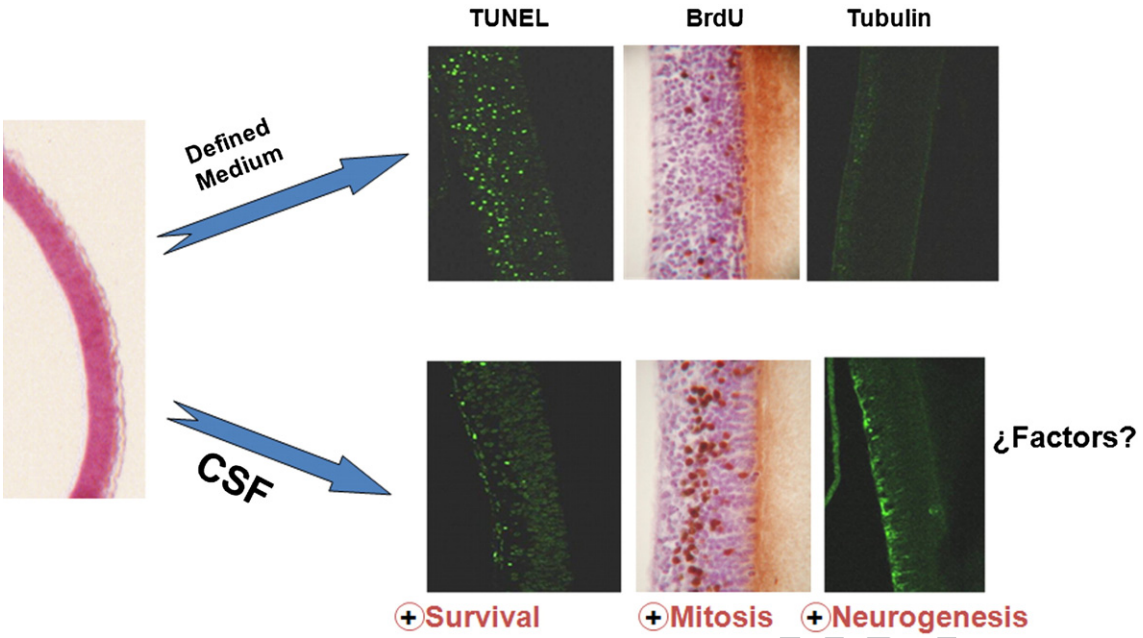

Fig. 9. A series of photomicrographs of a part of the neuroepithelium of the neuroepithelium in the roof of the mesencephalon of a stage 20 chick embryo cultured *in vitro* to compare the effect of CSF treatment with defined medium. Note that in CSF treated explants, there is a decrease in apoptosis (TUNEL) (left set), and an increase in both mitotic (BrdU positive cells) activity (middle set) and neuronal (tubulin positive cells) differentiation (right set). Scale bar in TUNEL and tubulin images: 30 μm, and in BrdU images 50 μm.

components of CSF both at the embryonic and fetal stages are proteins. Birge et al., 1974 and Dziegielewska et al., 1980b demonstrated that CSF in the chick embryo is as high as 30-fold richer in proteins compared to adult CSF. The protein concentration in embryonic CSF has been studied in several species. In chick and sheep it increases progressively during the late embryonic period while diminishing sometimes at the fetal stage (Dziegielewska et al., 1980a; Checiu et al., 1984; Fielitz et al., 1984). In rats, however, this decrease does not occur until after birth (Dziegielewska et al., 1981), suggesting that phylogenetic differences play a role in CSF maturation. The presence of albumin, fetuin alpha-fetoprotein, transferrin, and lipoproteins have been demonstrated during the early fetal stage in sheep CSF (Dziegielewska et al., 1980a,b). The first three represent 70–80% of all CSF proteins, a percentage which diminishes in the late fetal period. The presence of alpha-fetoprotein, albumin, transferrin, IgG, and alpha 1-antitrypsin was also described in rats (Dziegielewska et al., 1981). In rats, the alpha-fetoprotein and albumin account for more than 50% of the total.

Gato et al., 2004 analyzed the entire protein composition of the embryonic chick CSF. They showed a complex protein pattern with several protein fractions with different molecular weights and concentrations. The authors identified 21 different protein fractions showing a stable ontogenic pattern during embryonic and fetal development and most of these proteins were also present in the embryonic serum. The conclusion of the Gato study was that CSF components could have high biological value. More recently, a collaboration between the Bueno lab at the University of Barcelona and the Gato lab have reported an extensive protenomic analysis of chick and rat embryonic CSF with the identification of several proteins including extracellular matrix, enzymes, proteoglycans and apolipoproteins among others many of which could have high biological value (Parada et al., 2005b, 2006). Recently, the protein analysis of CSF during development has been compared with CSF of healthy and adult brains in people with neurodegenerative diseases (Parada et al., 2007).

Another interesting approach to CSF protein composition during development comes from Vio et al., 2000 who has shown that the subcommisural organ (SCO), an ependymal derived gland in the roof of the third ventricle, synthesizes and secretes glycoproteins to the CSF via the apical surface. The precipitate formed comprises Reissner's fiber. These authors have also shown that the SCO is able to secrete other kinds of proteins to the CSF which remain soluble and which could have biological significance such as transthyretin (Montecinos et al., 2005).

These data viewed collectively raise the possibility that during development, the CSF proteins could have three different origins: 1) Transport across the neuroepithelium from an outside source, most likely the serum (Martin et al., 2006); 2) Ubiquitous synthesis and apical secretion from neuroepithelial cells (Gato et al., 1993); and 3) Synthesis and apical secretion from a specific cellular population such as in the SCO or other circumventricular organs.

We do not discuss the development of the choroid plexus and its role as the blood brain barrier (BBB) despite our appreciating that it is indeed an important topic. We have chosen not to include it in this review because we are discussing a period of embryonic brain development prior to when the choroid plexus is most likely functional, i.e, in the fetal stages of mammals and analogous stages in birds. However a relevant question raised is the regulation of CSF composition, taken in account the evidence that many components of embryonic CSF seems to come from outside crossing the neuroepithelium. Martin et al. (2006) demonstrated the specific transport of FGF2 across the chick brain neuroepithelium. Recently Parvas et al. (2008) demonstrated that the neuroepithelial transport of proteins in chick embryos is regulated by specific transcellular routes suggesting that a functional blood-CSF barrier is present in the neuroepithelium before the choroid plexus develops to regulate the composition and properties of CSF during earliest stages of development. A related finding as to how the neuroepithelium might control CSF composition is that membranous exosome-like particles have been demonstrated inside the embryonic brain ventricle (Bachy et al., 2008; Marzesco et al., 2005) suggesting a intensive physiological interchange between CSF and neuroepithelial cells that could be involved in regulation of morphogen and growth factor transduction.

How CSF exerts its biological action

The most striking behavior of the neuroepithelial cells in the embryonic brain during the period of rapid brain growth is their high

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mitotic activity (Desmond, 1982; Desmond et al., 2005) which is one of the characteristics of neuroepithelial cells shown to be controlled by CSF (Gato et al., 2005).

Recently, Martin et al., 2006 have focused on the influence of growth factors in the CSF on neuroepithelial behavior. They demonstrated that FGF2 is present in the CSF of chick embryos and that the immuno-deprivation of the FGF2 activity in the CSF results in a significant decrease in DNA synthetic activity reflecting a marked decrease in cell replication. This study also showed by *in situ* hybridization and PCR that FGF2 mRNA was minimally expressed in the neuroepithelium of chick embryos. However, FGF2 was in the embryonic serum and crossed the neuroepitheliun from the blood to the CSF suggesting that in chick embryos, the FGF2 in the CSF originates in non-neural tissues. In mammalian embryos, the brain neuropeithelium is able to synthesize FGF2 (Raballo et al., 2000) and work is in progress in the Gato laboratory to clarify a possible phylogenetic difference in the origin of CSF growth factors. Mitotic activity of the neuroepithelium as influenced by components of the CSF should be identical in all three brain vesicles. However, local differences exhibited within the neuroepithelium of different vesicles can be explained by differential expression of apical receptors for growth factors (Ozawa et al., 1996; Wilke et al., 1997; Walshe and Mason, 2000).

Another interesting factor relating to CSF is its ability to induce neural differentiation in neuroepithelial precursor cells (Gato et al., 2005). It has been shown that CSF contains retinol and retinol binding protein in chick embryos (Parada et al., 2008) and work is in progress by Gato et al. to clarify how both of these molecules in CSF may be involved in the control of neurogenesis.

Concluding remarks

Finally, we establish the main conclusions with respect to the role of CSF in brain development, and we propose some future lines of research in relation with CSF and embryonic brain development. These suggestions are not meant to be all inclusive but rather points to stimulate further thoughts about such work.

We propose that the embryonic brain at its earliest stages of development has two major components, CSF and NEUROEPITHELIUM, and that they both are totally interdependent working as a functional entity regulating early brain growth, morphogenesis and neuroepithelial cellular behavior. This concept of inter-dependence and co-operativity needs to be appreciated in future studies.

The data presented in this review demonstrate that CSF is involved in two relevant aspects of early brain development: brain growth and morphogenesis and control of neuroepithelial cell behavior.

Brain growth requires the co-ordinated and simultaneous expansion of ventricles and neuroepithelium growth (Fig. 1). The expansion of ventricles is driven by internal hydrostatic pressure generated by an osmotic mechanism controlled by the transport or secretion activity of the neuroepithelial cells. At the same time the neuroepithelium is growing by cell replication. Here we demonstrate that cell replication is regulated by CSF by means of both pressure and biological mechanisms. A most interesting and current question is how these regulating mechanisms inter-relate. Do they function in parallel or in some type of a regulatory cascade to co-operatively stimulate the integrated growth of the ventricle and tissue? CSF control of neuroepithelial cell behavior includes not only cell replication but also cell survival and neuronal differentiation. However, how CSF impacts these parameters remains unknown.

Many important basic biological questions remain unanswered with respect to CSF and early brain development. Some of these questions include: how does intra-luminal pressure regulate cell proliferation in the neuroepithelium? Does it do so by stretching the neuroepithelium which stimulates tension receptors on the apical surface of the cells? (Fig. 1) Are there tension receptors within the neuroepithelium at these early stages of brain expansion similar to focal adhesion kinases (FAKS) located in fibroblasts and known to respond to tension (Schober et al., 2007). Since we have shown independently that cell proliferation is regulated by both pressure and growth factors, an interesting question pertains to how these regulating mechanisms are inter-related? Another relevant but unanswered question is how is the CSF able to induce neurogenesis in neuroepithelial cells. Most likely, such regulation is probably due to the cooperative work of several different factors.

This review is most timely because it coalesces the classic concept about the mechanical role of CSF in embryonic brain development with new views about the influence of CSF upon the behavior of neuroepithelial cells. In considering data supporting both mechanical and biochemical influences upon neuroepithelial behavior and brain growth, we are convinced that the CSF and neuroepithelium are inextricably intertwined as a functional entity. This review is also appropriate because it highlights the role of CSF in early stages of embryonic brain development with the roles attributed to CSF upon fetal and adult brains, allowing us to make evident the real influence of CSF in brain biology along its entire lifetime. Much more research must be done in the near future with respect to CSF during development particularly taking into account that CSF plays a key role in control of neuroepithelial cell behavior. These cells are neural precursor cells, inducing self renewal and neuronal differentiation. Precise knowledge about how these processes are regulated during embryonic brain ontogeny could be the hidden key necessary to activate useful neuroregeneration of precursor neuronal cells within adult brain tissue.

In our opinion, four different areas of embryonic brain research must be addressed so as to focus attention on the inter-dependence of CSF and the neuroepithelium. First, the regulatory mechanisms involved in CSF composition and the genesis of positive pressure as well as their relation to brain morphogenesis needs to be clarified. More precisely, the temporal sequence of pressure and induction of neuroblast proliferation by trophic factors within the CSF needs to be sorted out.

Second, exploration of whether there are mechanosenors like FAKs in the membrane of embryonic neuroepithelial cells that could provide the interface between external pressure and internal micro-assembly of machinery to enable stretching of the cells.

Third, further identification of the biological signals contained in the CSF and their trophic effect on the control of the precursor cell populations within the neuroepithelium needs to be explored.

Fourth, based on the hypothesis that there are stem cell niches in the adult brain (Ehninger and Kempermann, 2007) the value of CSF for the activation of neuronal cell niches within the developing brain needs to be examined. The development of neuroregenerative strategies for adult brain stem cells requires in depth study as to how nueral precursor populations expand and differentiate into neurons. This in depth analysis can come form experiments with the chick and mammalian embryo brain. Such evaluation may well result in useful neuroregenerative strategies.

Uncited references

Gilbert, 2006
Shin et al., 2006
Trokovic et al., 2005

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References

Abercrombie, M., 1970. Contact inhibition in tissue culture. In Vitro 6, 128–142.
Alonso, M.I., Gato, A., Moro, J.A., Barbosa, E., 1998. Disruption of proteoglycans in neural tube fluid by beta-D-xyloside alters brain enlargement in chick embryos. Anat. Rec. 252, 499–508.
Alonso, M.I., Gato, A., Moro, J.A., Martin, P., Barbosa, E., 1999. Involvement of sulfated proteoglycans in embryonic brain expansion at earliest stages of development in rat embryos. Cells Tissues Organs 165, 1–9.
Alonso, M.I., Moro, J.A., Martin, P., Barbosa, E., Gato, A., 2000. Enzymatic digestion of neural tube fluid proteoglycans leads to brain growth disruption. Eur. J. Anat. 4 (3), 161–167.
Bachy, I., Kozyraki, R., Wassef, M., 2008. The particles of the embryonic cerebrospinal fluid: how could they influence brain development? Brain Res. Bull. 75, 289–294.
Birge, W.J., Rose, A.D., Haywood, J.R., Doolin, P.F., 1974. Development of the blood-cerebrospinal fluid barrier to proteins and differentiation of cerebrospinal fluid in the chick embryo. Dev. Biol. 41, 245–254.
Borgens, B., Shi, R., 1995. Uncoupling histiogenesis from morphogenesis in the vertebrate embryo by collapse of the transneural tube potential. Dev. Dyn. 203, 456–467.
Checiu, I., Prelipceanu, O., Popescu, O., 1984. The role of cerebrospinal fluid during embryonic development. A biochemical study. Morphol. Embryol. (Bucur) 30, 243–250.
Comper, W.D., Laurent, T.C., 1978. Physiological functions of connective tissue polysaccharides. Physiol. Rev. 58, 255–315.
Copp, A.J., 2005. Neurulation in the cranial region — normal and abnormal. J. Anat. 207, 623–635.
De Paz, F. 1999. Morphometric study of the brain expansion in birds and mammals. Dortoral thesis presented to the Faculty of Medicine, University of Vallalodid, Vallalodid, Spain, 223 pp.
Desmond, M.E., 1982. Description of the occlusion of the lumen of the spinal cord in early human embryos. Anat. Rec. 204, 89–93.
Desmond, M.E., 1985. Reduced number of brain cells in so-called neural overgrowth. Anat. Rec. 212, 195–198.
Desmond, M.E., Field, M.C., 1992. Evaluation of neural groove closure and subsequent initiation of spinal cord occlusion in the chick embryo. J. Comp. Neurol. 319, 246–260.
Desmond, M.E., Jacobson, A.G., 1977. Embryonic brain enlargement requires cerebrospinal fluid pressure. Dev. Biol. 57, 188–198.
Desmond, M.E., Levitan, M.L., 2002. Brain expansion in the chick embryo initiated by experimentally produced occlusion of the spinal neurocoel. Anat. Rec. 268, 147–159.
Desmond, M.E., O'Rahilly, R., 1981. The growth of the human brain during the embryonic period proper. Anat. Embryol. 162, 137–151.
Desmond, M.E., Schoenwolf, A.G., 1985. Timing and positioning of occlusion of the spinal neurocoel in the chick embryo. J. Comp. Neurol. 235, 479–487.
Desmond, M.E., Schoenwolf, A.G., 1986. Evaluation of the roles of intrinsic and extrinsic factors in occlusion of the spinal neurocoel during rapid brain enlargement in the chick embryo. J. Embryol. Exp. Morphol. 97, 26–46.
Desmond, M.E., Duzy, M.J., Federeci, B.D., 1993. Second messenger regulation of occlusion of the spinal neurocoel in the chick embryo. Dev. Dyn. 197, 291–306.
Desmond, M.E., Levitan, M.L., Haas, A.R., 2005. Internal luminal pressure during early chick embryonic brain growth: descriptive and empirical observations. Anat. Rec. 285A, 737–747.
Dziegielewska, K.M., Evans, C.A.N., Fossan, G., Lorscheider, F.L., Malinowska, D.H., Mollgard, K., Reynolds, M.L., Saunders, N.R., Wilkinson, S., 1980a. Proteins in cerebrospinal fluid and plasma of fetal sheep during development. J. Physiol. 300, 441–455.
Dziegielewska, K.M., Evans, C.A.N., Fossan, G., Malinowska, D.H., Mollgard, K., Reynolds, M.L., Saunders, N.R., 1980b. Blood-cerebrospinal fluid transfer of plasma proteins during fetal development in the sheep. J. Physiol. 300, 457–465.
Dziegielewska, K.M., Evans, C.A.N., Lai, P.C.W., Lorscheider, F.L., Malinowska, D.H., Mollgard, K., Saunders, N.R., 1981. Proteins in cerebrospinal fluid and plasma of fetal rats during development. Dev. Biol. 83, 193–200.
Ehninger, D., Kempermann, G., 2007. Neurogenesis in the adult hippocampus. Cell Tissue Res., doi:10.1007/s00441-007-0478-3
Fielitz, W., Esteves, A., Moro, R., 1984. Protein composition of cerebrospinal fluid in the developing chick embryo. Brain Res. 315, 111–115.
Freeman, B.G., 1972. Surface modifications of neural epithelial cells during formation of the neural tube in the rat embryo. J. Embryol. Exp. Morphol. 28, 437–448.
Galligani, L., Hopwood, N., Schwartz, B., Dorfman, A., 1975. Stimulation of synthesis of free chondroitin sulfate chains by β-D-xyloside in cultured cels. J. Biol. Chem. 250, 5400–5406.
Gardner, W.J., 1973. Hydrodynamic mechanisms. The Dysraphic States: Syringomyelia to Anacephaly. Excerpta Medica, Amsterdam, pp. 15–21.
Gato, A., Moro, J.A., Alonso, M.I., Pastor, J.F., Represa, J.J., Barbosa, E., 1993. Chondroitin sulfate proteoglycan and embryonic brain enlargement in the chick. Anat. Embryol. 188, 101–106.
Gato, A., Alonso, M.I., Moro, J.A., Martin, P., Barbosa, E., 1998. Presence of FGF-2 in chick embryo neural tube fluid. Eur. J. Anat. 2, 185–186.
Gato, A., Martin, P., Alonso, M.I., Martin, C., Pulgar, M.A., Moro, J.A., 2004. Analysis of cerebro-spinal fluid protein composition in early developmental stages in chick embryos. J. Exp. Zool. 301A, 280–298.
Gato, A., Moro, J.A., Alonso, M.I., Bueno, D., De La Mano, A., Martin, C., 2005. Embryonic cerebrospinal fluid regulates neuroepithelial survival, proliferation, and neurogenesis in chick embryos. Anat. Rec. 284A, 471–484.
Gilbert, S.F., 2006. Developmental Biology, Eight Edition. Sinauer Associates, Inc., Sunderland, MA, p. 383.
Hotary, K.B., Robinson, K.R., 1991. The neural tube of the *Xenopus* embryo maintains a potential difference across itself. Dev. Brain Res. 59, 65–73.
Jacobson, A.G., 1981. Morphogenesis of the neural plate and tube. In: Connelly, T.G., et al. (Ed.), Morphogenesis and Pattern Formation. Raven Press, New York, pp. 233–263.
Jacobson, A.G., Gordon, R., 1976. Changes in the shape of the developing vertebrate nervous system analyzed experimentally, mathematically and by computer simulation. J. Exp. Zool. 197, 191–246.
Jelinek, R., Pexieder, T., 1968. The pressure of encephalic fluid in chick embryos between the 2nd and 6th day of incubation. Physiol. Bohemoslov. 17, 297–305.
Jelinek, R., Pexieder, T., 1970. Pressure of the CSF and the morphogenesis of the CNS. Folia Morphol. 18, 102–110.
Johansson, P.A., Dziegielewska, K.M., Elk, C.J., Habgood, M.D., Molgard, K., Potter, A., Schuliga, M., Saunders, N.R., 2005. Aquaporin-1 in the choroid plexuses of developing mammalian brain. Cell Tissue Res. 322, 353–364.
Keller, R., 2002. Review: shaping the vertebrate body plan by polarized embryonic cell movements. Science 298 (5600), 1950–1954.
LaConti, J.J., Jablonski, M.G., Desmond, M.E., 2004. N-cadherin maintains occlusion of the chick neural tube. FASEB Abstr. No. LB 10.
Levitan, M. L., Desmond, M. E. 2008. The expansion of the human brain during embryonic rapid brain growth: area analysis and basic modeling. Accepted with revisions.
Li, X.Y., Desmond, M.E., 1991. Modulation of Na+/K+-ATPase pumps in the heart of the chick embryo influences brain expansion. Soc. Neurosci. Abstr. 17, 21.16.
Litingtung, Y., Chiang, C., 2000. Control of shh activity and signalling in the neural tube. Dev. Dyn. 219, 143–154.
Liu, A., Joyner, A.L., 2001. Early anterior/posterior patterning of the midbrain and cerebellum. Annu. Rev. Neurosci. 24, 869–896.
Lowery, L.A., Sive, H., 2005. Initial formation of zebrafish brain ventricles occurs independently of circulation and requires the *nagie oko* and *snakehead/atp1a1a.1* gene products. Development 132, 2057–2067.
Martin, C., Bueno, D., Alonso, M.I., Moro, J.A., Callejo, S., Parada, C., Martin, P., Carnicero, E., Gato, A., 2006. FGF2 plays a key role in embryonic cerebrospinal fluid trophies properties over chick embryo neuroepithelial stem cells. Dev. Biol. 297, 402–416.
Marzesco, A.-M., Janich, P., Wilsch-Brauninger, M., Dubreuil, V., Langenfeld, K., Corbeil, D., Huttner, W.B., 2005. Release of extracellular membrane particles carrying the stem cell marker prominin-1 (CD133) from neural progenitors and other epithelial cells. J. Cell Sci. 118, 2849–2858.
Miyan, J.A., Zendah, M., Mashayekhi, F., Owen-Lynch, P.J., 2006. Cerebrospinal fluid supports viablility and proliferation of cortical cells *in vitro*, mirroring *in vivo* development. Cerebrospinal Fluid Res. 3, 2.
Montecinos, H.A., Richter, H., Caprile, T., Rodriguez, E.M., 2005. Synthesis of transthyretin by the ependymal cells of the subcommisural organ. Cell Tissue Res. 320, 487–499.
Nico, B., Frigeri, A., Nicchia, G.P., Quondamatteo, F., Herken, R., Errede, M., Ribatti, D., Svelto, M., Roncali, L., 2001. Role of aquaporin-4-water channel in the development and integrity of the blood-brain barrier. J. Cell. Sci. 114 (7), 1297–1307.
Ozawa, K., Uruno, T., Miyakawa, K., Seo, M., Imamura, T., 1996. Expression of the fibroblast growth factor family and their receptor family genes during mouse brain development. Mol. Brain Res. 41, 279–288.
Pacheco, M.A., Marks, R.W., Schoenwolf, G.C., Desmond, M.E., 1986. Quantification of the initial phases of rapid brain enlargement in the chick embryo. Am. J. Anat. 175, 403–411.
Parada, C., Martin, C., Alonso, M.I., Moro, J.A., Bueno, D., Gato, A., 2005a. Embryonic cerebrospinal fluid collaborates with the isthmic organizer to regulate mesencephalic gene expression. J. Neurosci. Res. 82, 333–345.
Parada, C., Gato, A., Bueno, D., 2005b. Mammalian embryonic cerebrospinal fluid proteome, has greater Apolipoprotein and enzyme pattern complexity than the avian proteome. J. Proteome Res. 4, 2420–2428.
Parada, C., Gato, A., Aparicio, M., Bueno, D., 2006. Proteome analysis of chick embryonic cerebrospinal fluid. Proteomics 6, 312–320.
Parada, C., Parvas, M., Bueno, D., 2007. Cerebrospinal fluid proteonomes: from neural development to neurodegenerative diseases. Curr. Proteonomics 4, 89–106.
Parvas, M., Rius, M., Bueno, D., 2008. Most of the abundant proteín fractions of embryonic cerebrospinal fluid are produced out of the brain anlagen. Open Proteomisc. J. 1, 1–4.
Raballo, R., Rhee, J., Lyn-Cook, R., Leckman, J.F., Schwartz, M.L., Vaccarino, F.M., 2000. Basic growth factor (Fgf2) is necessary for cell proliferation and neurogenesis in the developing cerebral cortex. J. Neurosci. 20 (13), 5012–5023.

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Robertson, E.J., Norris, D.P., Brennan, J., Bikoff, E.K., 2003. Control of early anterior–posterior patterning in the mouse embryo by TGF-β signalling. Philos. Trans. R. Soc. Lond., B 358, 1351–1358.

Salehi, Z., Mashayekhi, F., 2006. The role of cerebrospinal fluid on neural cell survival in the developing chick cerebral cortex: an *in vivo* study. Eur. J. Neurol. 13, 760–764.

Sawamoto, K., Wicterle, H., Gonzalez-Perez, O., Cholfin, J.A., Yamada, M., Spassky, N., Murcia, N.S., Garcia-Verdugo, J.M., Marin, O., Rubenstein, J.L.R., Tessier-Lavigne, M., Okano, H., Alvarez-Buylia, A.A., 2006. New neurons follow the flow of cerebrospinal fluid in the adult brain. Science 311, 629–632.

Scheir, A.F., Neuhauss, S.C., Harvey, M., Malicki, J., Solnica-Krezel, L., Stainier, D.Y., Zwartkruis, F., Abdelilah, S., Stemple, D.L., Rangini, Z., et al., 1996. Mutations affecting the development of the embryonic zebrafish brain. Development 123, 165–178.

Schober, M., Raghavan, S., Nikolova, M., Polak, L., Pasolli, H.A., Beggs, H.E., Reichardt, L.F., Fuchs, E., 2007. Focal adhesion kinase modulates tension signaling to control actin and focal adhesion dynamics. J. Cell Biol. 178 (6), 667–680.

Schoenwolf, G.C., Desmond, M.E., 1984a. Descriptive studies of occlusion and reopening of the lumen of the spinal cord of the early chick embryo. Anat. Rec. 209, 251–263.

Schoenwolf, G.C., Desmond, M.E., 1984b. Neural tube occlusion precedes rapid brain enlargement. J. Exp. Zool. 230, 405–407.

Schoenwolf, G.C., Desmond, M.E., 1986. Timing and positioning of reopening of the occluded spinal neurocoel in the chick embryo. J. Comp. Neurol. 246, 459–466.

Sedlacek, J., 1975. Some basic chemical compnents of the cerebrospinal fluid in developing chick embryo. Physiol. Bohemoslov. 24 (4), 305–313.

Shi, R., Borgens, B., 1994. Embryonic neuroepithelial sodium transport, the resulting physiological potential, and cranial development. Dev. Biol. 165, 105–116.

Shin, I., Kim, H.J., Lee, J.E., Myung, C.G., 2006. Aquaporin7 expression during perimatal development of mouse brain. Neurosci. Lett. 409, 106–111.

Smith, J.L., Schoenwolf, G.C., 1997. Neurulation: coming to closure. Trends Neurosci. 11, 510–517.

Tosrsoni, A.S., Constancic, S.S., Nadruz Jr., W., Hanks, S.K., Franchini, K.G., 2003. Focal adhesion kinase is activated and mediates the early hypertrophic response to stretch in cardiac myocytes. Circ. Res. 93, 140–147.

Trokovic, R., Jukkola, T., Saarimaki, J., Peltopuro, P., Thorsten, N., Vogt-Weisenhorn, D.M., Trokovic, N., Wurst, W., Partanen, J., 2005. Fgfr1-dependent boundary cells between developing mid- and hindbrain. Dev. Biol. 278, 428–439.

Van Essen, D.C., 1997. A tension-based theory of morphogenesis and compact wiring in the central nervous system. Nature 385, 313–318.

Van Straaten, H.W.M., Janssen, H.C.J.P., Peeters, M.C.E., Copp, A.J., Hekking, J.W.M., 1996. Neural tube closure in the chick embryo is multiphasic. Dev. Dyn. 207, 309–318.

Vio, K., Rodriguez, S., Yulis, C.R., Oliver, C., Rodriguez, E.M., 2000. The subcommissural organ of the rat secretes Reissner's fiber glycoproteins and CSF-soluble proteins reaching the internal ad external CSF compartments. Cerebrospinal Fluid Res. 5, 3 online.

Walshe, J., Mason, I., 2000. Expression of FGFR1, FGFR2, and FGFR3 during early neural development in the chick embryo. Mech. Dev. 90, 103–110.

Wilke, T.A., Gubbels, S., Schwartz, J., Richman, J.M., 1997. Expression of fibroblast growth factor receptors (FGFR1, FGFR2, FGFR3) in the developing head and face. Dev. Dyn. 210, 41–52.

Ybot-Gonzalez, P., Savery, D., Gerrelli, D., Signore, M., Mitchell, C.E., Faux, C.H., Nicholas, D., Greene, E., Copp, A.J., 2007. Convergent extension, planar-cell-polarity signalling and initiation of mouse neural tube closure. Development 134, 789–799.

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