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Morphomechanics: Transforming Tubes into Organs

Larry A. Taber
Department of Biomedical Engineering, Washington University, St. Louis, MO 63130, USA, tel: (314) 935-8544

Larry A. Taber: lat@wustl.edu

Abstract

After decades focusing on the molecular and genetic aspects of organogenesis, researchers are showing renewed interest in the physical mechanisms that create organs. This review deals with the mechanical processes involved in constructing the heart and brain, concentrating primarily on cardiac looping, shaping of the primitive brain tube, and folding of the cerebral cortex. Recent studies suggest that differential growth drives large-scale shape changes in all three problems, causing the heart and brain tubes to bend and the cerebral cortex to buckle. Relatively local changes in form involve other mechanisms such as differential contraction. Understanding the mechanics of organogenesis is central to determining the link between genetics and the biophysical creation of form and structure.

Introduction

During embryonic development, many organs begin as simple tubes. Some of these organs (e.g., lungs and kidneys) eventually become a network of branched tubes, while others (e.g., heart and brain) develop into complex structures that no longer bear much resemblance to tubes. Although much is now known about the physical mechanisms that drive many of the fundamental processes of morphogenesis [1,2,3], how specific processes are integrated to create specific organs remains poorly understood. The importance of proper organ formation is clear, as without properly functioning organs, the embryo usually does not survive.

This review focuses on mechanical aspects of heart and brain development. Both of these organs are initially simple tubes that bend, twist, and remodel into their mature forms. Branching morphogenesis, which is central to the development of organs such as the lungs and kidneys, is not considered here. After providing a brief background for each problem, we discuss current thinking on each topic, as well as some of the remaining unanswered questions. We emphasize similarities in heart and brain development, as nature may use comparable means to create other organs.

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It is important to note that, during the past few decades, most work has focused on molecular and genetic aspects of development. Hence, the current state of knowledge is several years old for some of the topics discussed herein. One objective of this review is to stimulate new interest in these important and challenging problems of organ morphomechanics.

Cardiac Morphogenesis

The heart has long fascinated developmental biologists. The heart is initially a relatively straight tubular structure comprised of three layers: an inner endothelium (endoderm); a relatively thick middle layer of extracellular matrix (cardiac jelly, CJ); and a two-cell-thick outer layer of myocardium [4]. During the fourth week of development in human or days 2–3 in chick, the heart tube (HT) loops into a curved tube that subsequently divides (septates) into four chambers [5,6]. The heart also undergoes changes in internal structure, including the formation of valves and highly organized myofibrils, allowing it to pump increasing amounts of blood to the rapidly growing embryo [7,8].

Cardiac Looping

Looping of the heart represents the first large-scale morphogenetic event that breaks left-right symmetry in the vertebrate embryo. During this process, the HT first becomes c-shaped (c-looping) and then s-shaped (s-looping) [5].

During c-looping, the HT simultaneously bends ventrally and twists rightward [5] (Fig. 1A). In a remarkable master's thesis written more than 60 years ago, Butler (JK Butler, M.S. Thesis, University of Texas, 1952) showed that bending is caused by forces generated within the HT. He also speculated that torsion depends "on factors associated with the attachment of the heart to the body of the embryo." Recent studies generally support these early observations.

As the HT bends, the original ventral and dorsal sides become the convex outer curvature (OC) and concave inner curvature (IC) of the curved tube, respectively. Investigators have proposed and tested numerous possible mechanisms for the bending component of c-looping [4]. These include buckling of the HT as it outgrows its allotted space [9], regionally constrained longitudinal stretching of the HT caused by CJ swelling [10], differential hyperplastic myocardial growth [11], active cell-shape changes in the myocardium [12,13,14], differential cytoskeletal contraction [15], and forces exerted on the HT by the rupturing dorsal mesocardium [15]. With the possible exception of active cell-shape change, none of these hypotheses have survived experimental scrutiny unscathed [4,13].

Recent studies suggest another possibility. Soufan et al. [16**] have found significant increases in myocardial cell size on the ventral side of the HT during c-looping (Fig. 1B), which would be consistent with bending driven by differential *hypertrophic* growth. This finding was unexpected, because it had been commonly thought that the heart grows primarily by hyperplasia before birth and hypertrophy after birth [17]. Motivated by these new results, Shi et al. [18**] reexamined the differential growth hypothesis using computational modeling and experiments on isolated chick hearts. Their model shows that

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the gradient in cardiomyocyte growth measured by Soufan et al. [16**] is capable of generating the degree of bending, as well as the changes in myocardial stress and strain distributions, observed experimentally (Fig. 1C).

In summary, it appears that differential hypertrophic growth is the primary cause of the bending component of cardiac c-looping, although the other mechanisms (active cell-shape change, dorsal myocardial tension, CJ swelling) may play secondary roles [18**] (Fig. 1C). In fact, looping may involve a combination of several different processes, some of which may be redundant and compensate when others fail [11,19]. Such backup mechanisms, which probably include modified gene expression as well, may explain why congenital defects are more rare than the number of developmental perturbations would suggest [20].

In contrast to bending, which is driven by internally generated forces, the torsional component of c-looping is caused mainly by external loads. In his thesis, Butler suggested that the main twist-causing force is provided by the left omphalomesenteric vein, which grows larger than the right vein and exerts a torque on the heart. Recent research supports this idea, as inducing the right vein to grow larger than the left leads to abnormal leftward looping [21*]. Other results suggest, however, that vein forces provide only a relatively small amount of torsion which determines looping direction, while the splanchnopleure supplies a surface load that pushes the HT into its fully twisted position (Fig. 1D) [22*,23].

This is not the full story, however; multiple redundant mechanisms also may be involved in torsion. For example, some data suggest that asymmetric cell proliferation in the dorsal mesocardium determines looping direction, as cells normally divide faster on the left side of this structure and push the HT rightward [24*]. In addition, Linask and colleagues have shown that a protein called flectin is expressed predominantly on the left side of the HT during rightward looping, while greater expression on the right side leads to abnormal leftward looping [25,26]. More recently, flectin has been identified as a form of myosin II [27], but its function in looping remains unknown. Interestingly, c-looping in the chick does not require contraction, while in zebrafish embryos inhibition of contraction apparently affects looping [28]. Here, it is important to note that significant morphological differences exist between zebrafish and chick (as well as human) hearts [29,30*].

The next phase of looping, termed s-looping, moves the primitive atria from their initial location caudal to the primary HT (future left ventricle) into their ultimate positions anterior to the ventricle [5]. The forces that drive s-looping apparently are exerted by external loads, including those supplied by the brain tube as it bends [31].

Effects of Blood Flow

Some researchers have speculated and continue to speculate that hemodynamic loads affect the early stages of looping [26,32], but most available evidence suggests otherwise. While some studies in zebrafish support a role for blood flow [32], the chick heart undergoes normal c-looping when the heartbeat is blocked [33,34].

On the other hand, studies have shown convincingly that blood flow affects later growth and remodeling of the heart. For example, the embryonic heart has the ability to adapt to changes

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in loading conditions in ways that parallel the mature heart, e.g., pressure overload triggers ventricular hypertrophy [35,36]. Moreover, perturbing pressure and flow leads to abnormalities in patterns of myocardial trabeculation, septation, and valve formation [7,30*, 32,37,38,39,40]. Experiments have linked fluid shear (drag) to the regulation of these processes, possibly through changes in gene expression [41,42,43,44,45]. Relatively little is known about the corresponding morphogenetic mechanisms, but a recent computer model suggests that both fluid pressure and shear stress play major roles in molding the valves [46*].

Brain Morphogenesis

The embryonic brain begins to develop at approximately the same time as the heart.

Shaping of the Primitive Brain Tube

The anterior part of the neural tube expands to create the brain tube (BT), while the posterior portion of the neural tube becomes the spinal cord. Local circumferential constrictions next divide the neuroepithelium of the BT into three primary vesicles called the forebrain, midbrain, and hindbrain. In addition, bilateral evaginations from the forebrain create the optic vesicles, and a series of transient bulges called rhombomeres form along the hindbrain (Fig. 2A).

Recent studies have shown that the mechanism that creates the boundaries between vesicles is species dependent. The primary mechanism in the chick is localized circumferential contraction at the apical (inner) side of the wall, which decreases the BT circumference within the boundary regions (Fig. 2A) [47*]. In zebrafish, on the other hand, radial cellular shortening first generates a local circumferential groove that establishes the midbrain-hindbrain boundary, which is then sharpened by local laminin-dependent basal constriction [48*] (Fig. 2B). The specific biophysical mechanisms that drive these shape changes are not well understood, but actin intensity is highest on the basal side of the boundary region, suggesting that actomyosin contraction generates the constriction.

The reasons for these differences between zebrafish and chicken are unclear, but Filas et al. [49] speculate that interspecies differences in early BT morphology demand different mechanisms. For example, the lumen is initially closed in the zebrafish brain but open in chick and human. Localized contraction is required to open the zebrafish BT [50], which later relaxes so the BT can expand [51].

As in the early heart tube, fluid pressure inside the BT apparently plays a crucial role in growth but not morphogenesis. After the primary vesicles form, the ends of the BT seal and the brain expands rapidly as cerebrospinal fluid (CSF) accumulates in the lumen [52]. Experiments suggest that mitotic rates increase in response to wall stresses generated by rising CSF pressure [53,54]; growth is reduced considerably when the pressure is relieved [55]. However, although differential growth can deepen and sharpen the vesicle boundaries [48*], growth apparently plays a relatively minor role in primary vesicle formation [56].

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Like the heart, the BT bends and (in some species) twists [57,58]. As in the heart, data suggest that differential growth drives bending [59,60,61], whereas forces imposed by extraembryonic membranes cause torsion [57,62]. Differential cell proliferation within the BT [63] and changes in somite shape [64,65] also may play a role in torsion. Interestingly, some investigators have speculated that the direction of cardiac looping determines the direction of brain torsion in the chick [66], as both structures almost always twist in the same direction [67]. However, the specific physical mechanisms of both bending and torsion remain poorly understood for the brain.

Later the forebrain undergoes further subdivision. A circumferential groove divides it into the telencephalon and diencephalon, followed by a longitudinal boundary that divides the anteriorlocated telencephalon into left and right sides that eventually become the cerebral hemispheres [68]. The mechanisms that create these boundaries are unknown but may involve both regional contraction and differential growth driven by the rising CSF pressure.

Cortical Folding

In most large mammals, the cerebral cortex (a thin outer layer of gray matter) develops a convoluted shape consisting of gyri (outward folds) and sulci (inward folds). This process, which occurs during the third trimester in humans, greatly increases the surface area of the cortex and is important for normal brain function [69,70]. While researchers have speculated about the mechanics of cortical folding for decades, interest in this problem has intensified during the last 15 years.

Two main theories have dominated thinking on this topic. According to the *axon tension hypothesis*, axons connecting related regions of the cortex generate tension that pulls these regions together, causing the surface to buckle outward [71**] (Fig. 3A). While this hypothesis has garnered considerable support [72,73], recent measurements of fiber architecture and tissue stress in ferret brains seem to contradict such a mechanism [74*].

In contrast, recent studies have provided compelling evidence supporting the *differential growth hypothesis*, whereby folding is driven by different growth rates between various regions and layers of the brain. According to this mechanism, tangential expansion of the cortex is restricted by slower growing subcortical layers, putting the cortex into a state of compression and causing it to buckle [74*,75]. Computer modeling has shown that this mechanism produces stress distributions that are consistent with experimental results [74*].

Currently available data suggest the following sequence of events. First, neuronal progenitors multiply within the ventricular zone of the brain at genetically determined rates that are higher under future gyri than sulci [76,77**]. Genetic regulation of proliferation ensures a consistent global folding pattern, which is highly conserved within a given species [69,76]. These progenitor cells migrate to the cortex along radially oriented glial fibers [78], which fan out toward the surface as new glia form between old fibers [77**] (Fig. 3B). These cells expand the cortex and generate relatively shallow surface bumps that are precursors to the primary gyri. These bumps grow larger during neuronal differentiation as cell bodies and dendrites grow and further expand the cortex [79**]. Finally, secondary

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folds develop with a more variable pattern influenced by local variations in geometry and mechanical properties [76].

This scheme is consistent with the following findings: (1) cortical folding occurs after neuronal proliferation and migration to the cortex is complete [79**,80,81]; (2) smooth (lissencephalic) brains lack fanning of glial fibers [77**]; (3) gyri undergo more rapid tangential growth than sulci [76,82]; and (4) experimentally accelerated cortical growth can cause normally lissencephalic brains to fold [83*,84,85]. Nevertheless, the differential growth hypothesis may not be consistent with all available data, and the mechanism of cortical folding continues to be debated [69,81].

Conclusions

Some common themes emerge from studies of early heart and brain morphogenesis. For both organs, differential growth and external loads play central roles in large-scale tissue shaping. Differential growth apparently causes most of the bending in both tubular structures, while external loads drive torsion. Differential growth also is prominent in generating local shape changes that occur during later development, such as myocardial trabeculation and cortical folding. Interestingly, the mechanisms that create these tubes in the first place, e.g., active contraction and cell intercalation [2], generally play more minor roles, with one exception being boundary formation between the primary brain vesicles.

These similarities extend to other organs that originate from epithelial tubes. For example, constraints imposed by the mesentery on the growing gut tube causes the gut tube to loop as it grows [86], while differential growth causes internal buckling that generates villi [87**].

Multiple backup mechanisms and complex 3-D changes in shape make studying the physical mechanisms of organogenesis an extremely challenging endeavor. Complete understanding of these problems will require the development of new molecular and genetic tools for targeting specific processes, as well as new image analysis techniques to measure morphogenetic changes in tissue strain and cell shapes in 4-D. Future work also is needed to investigate the role of mechanical feedback and the interactions between mechanics, gene expression, and morphogenesis. Despite the long history, studies of the mechanisms of organogenesis are in some ways just beginning.

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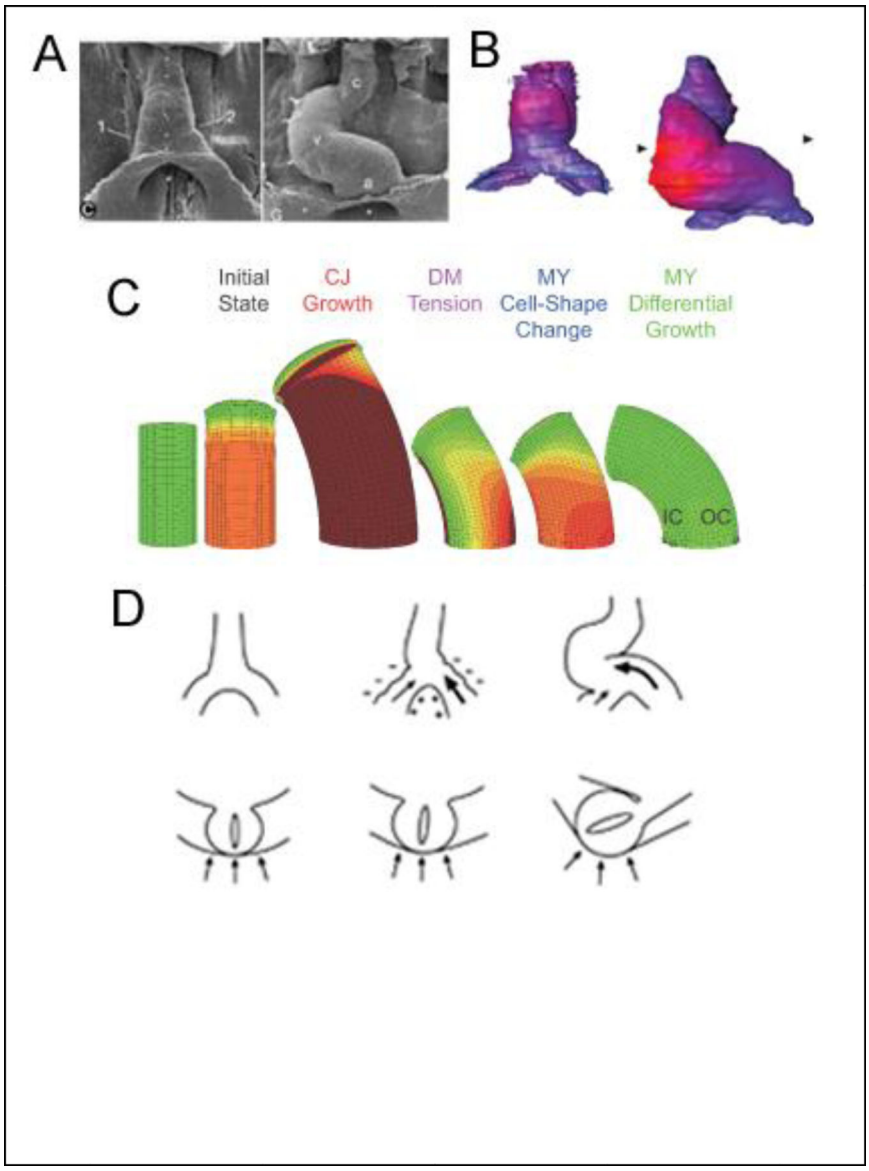

Fig 1. Cardiac looping

(A) Scanning electron micrographs of embryonic chick heart at beginning (left) and end (right) of c-looping (ventral view, stages 10 and 12 of Hamburger and Hamilton [88]). Small dots along the ventral midline of the heart tube move to the outer curvature, illustrating that c-looping consists of ventral bending and rightward torsion. (c = conotruncus; v = ventricle; a = primitive atrium) From [5]. (B) Maps of myocardial cell size at similar stages of development as in (A) (blue = small cells; red = large cells). Dorsal-ventral (inner to outer curvature) gradient in cell size is consistent with a differential growth mechanism for cardiac bending. From [16**]. (C) Computational models for the bending component of c-looping. From the initial state including cardiac jelly (CJ) swelling, the models simulate the following mechanisms: dorsally constrained expansion of the heart tube as CJ continues to grow and swell, dorsal forces exerted on the heart by tension in the rupturing dorsal mesocardium (DM), active cell-shape changes in the myocardium (MY), and differential

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myocardial growth. Only differential growth yields a bending magnitude consistent with experiments. (IC = inner curvature; OC = outer curvature) From [18**]. (D) Schematic of mechanism for torsional component of c-looping (top row = ventral view; bottom row = cross-sectional view). Left: before looping; center: relatively large force exerted by left omphalomesenteric vein (bold arrow) pushes heart tube slightly rightward; right: compression exerted by splanchnopleure (arrows in cross section) enhances rotation of heart tube. From [22*].

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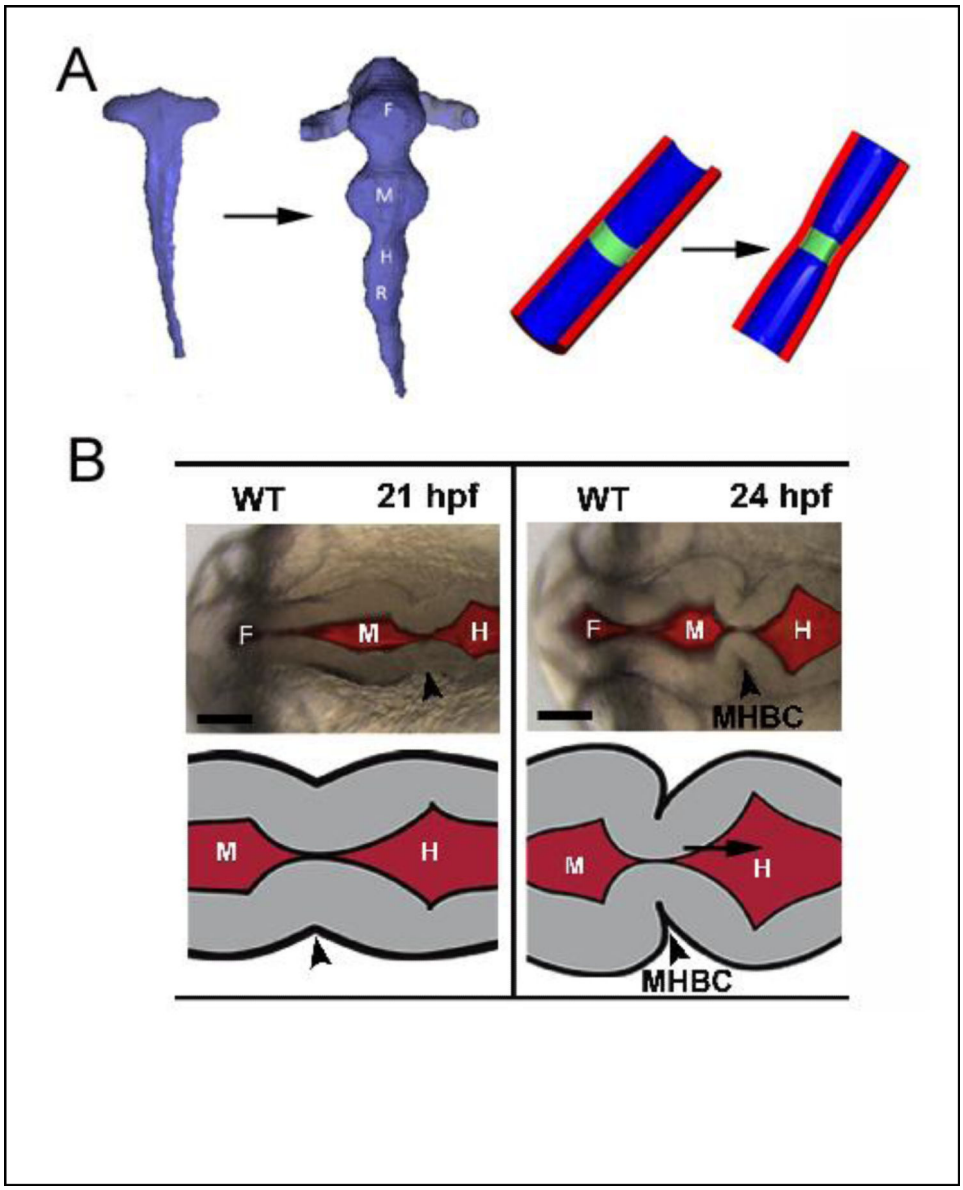

Fig 2. Formation of primary vesicles in brain tube

(A) Chick embryo. Reconstructed brain lumen at stages 10 and 12 (left) and schematic of boundary formation (right). Boundaries between vesicles are created by circumferential actomyosin contraction (green region) at apical side of wall. From [47*]. (B) Zebrafish embryo. Boundary between midbrain and hindbrain (arrowhead) forms in two steps: radial shortening (left) and basal constriction (right) of neuroepithelial cells. (F= forebrain; M = midbrain; H = hindbrain; MHBC = midbrain-hindbrain boundary constriction) From [48*].

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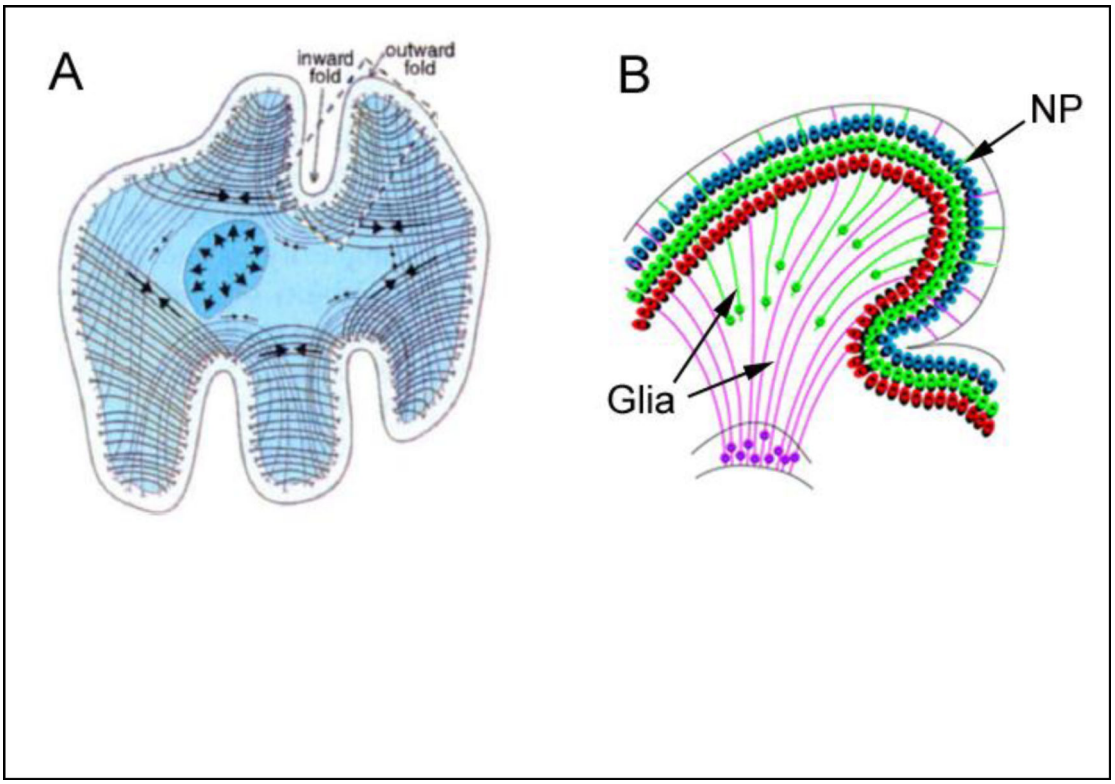

Fig 3. Hypotheses for folding of cerebral cortex

(A) Axon tension. Tension generated by axons (arrows on curved lines) draws interconnected regions together, creating gyri (outward folds). From [71**]. (B) Differential growth. Neural progenitors (NP) migrate and spread out long fan-like glial fibers as they enter the cortex, expanding the surface area and creating a gyrus. From [77**].