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Mechanobiology of Neural Development

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Abstract

As the brain develops, proliferating cells organize into structures, differentiate, migrate, extrude long processes, and connect with other cells. These biological processes produce mechanical forces that further shape cellular dynamics and organ patterning. A major unanswered question in developmental biology is how the mechanical forces produced during development are detected and transduced by cells to impact biochemical and genetic programs of development. This gap in knowledge stems from a lack of understanding of the molecular players of cellular mechanics and an absence of techniques for measuring and manipulating mechanical forces in tissue. In this review article we examine recent advances that are beginning to clear these bottlenecks, and highlight results from new approaches that reveal the role of mechanical forces in neurodevelopmental processes.

Keywords

Neural development; brain morphogenesis; mechanical forces; developmental biology; biomechanics; mechanotransduction

Introduction

A developing embryo grows, folds, and contorts in a series of complex but reproducible events to generate all organs of the human body. Each organ is shaped to the unique architecture necessary to support its function. Failure in this process results in spontaneous abortion or lifelong developmental defects. Thus, understanding organ patterning is critical to preventing and treating developmental disorders. Traditionally the field of developmental

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Conflict of Interest

The authors declare no conflict of interest.

biology has focused on genetics and chemical signaling; however, emerging evidence shows that mechanical cues are as important as genetic and chemical cues.

D'Arcy Thompson proposed in a seminal treatise over a hundred years ago that organismal growth is governed by physical and mechanical principles [1]. Empirical observations in embryology provided evidence that mechanical cues were important for development [2]. However, the daunting technical challenges associated with studying physical forces in living tissue, and the lack of a molecular understanding of how mechanical forces are generated and transduced by cells, prohibited an in depth examination. Furthermore, with the advent of molecular biology the focus in the field of developmental biology through the 20th century shifted to understanding molecular and genetic mechanisms. In the last two decades, the area of developmental mechanics - the study of how forces arise during embryo development and how they shape organismal growth - has gathered momentum.

Vertebrate brain development begins when ectodermal cells are induced into the neural lineage, forming the neural plate, which folds over to generate the neural tube. The tube elongates and bends, then dilates and constricts at specific points. The neural stem/progenitor cells (NSPCs) lining hollow cavities called ventricles proliferate and then differentiate. The differentiated cells migrate, connect with each other, and generate specialized neural signaling centers. The biological processes that give rise to the brain and spinal cord are all dynamic processes that produce mechanical stresses and strains, which in turn affect cell behavior and organ patterning (Fig. 1).

Recent experiments employing novel bioengineering principles and methodologies have demonstrated the importance of mechanical cues in cell fate and differentiation. These findings generated new principles of neural development, and motivated the development of novel approaches for measurement and manipulation of mechanical cues in developing neural tissue. In this review, we examine developmental mechanics of the brain in mammalian and non-mammalian organisms, highlight the latest developments in this area and identify key areas of investigation spawned by these recent findings. We regret not being able to cite several related studies due to space constraints, and refer the interested reader to other review articles wherever possible.

Neural Tube Closure

The first major mechanical event in the development of the nervous system is neural tube closure (NTC). This is mediated by actomyosin force generation at the neural plate's apical surface, which becomes the apical border once the neural tube closes. The neural plate bends at three specific locations called hinge points. The two ends of the folding neural plate then meet, and "zipper" the tube closed (Fig. 2). Failure in this process causes neural tube defects such as anencephaly and spina bifida.

Galea et al. described the complex dynamics of mechanical forces during mammalian NTC [3]. They showed that laser ablation of the closing neural tube at a single point was sufficient to re-open a portion of the neural tube longitudinally. Furthermore, after this re-opening, tissues distant from the neural tube underwent expansions or compressions, suggesting a

mechanical coupling of these tissues with the closing neural tube through the actin cytoskeleton [3].

Additionally, recent work suggests there are variations in tissue stiffness during NTC: the zippering points are softer than the immediately surrounding tissue of the neural tube [4]. This work utilizes a new non-invasive technique called Brillouin microscopy, which provides stiffness measurements in 3D, as opposed to the traditional 2D surface stiffness measurements via Atomic Force Microscopy (AFM). If this observation holds across different species, it would be interesting to examine whether the observed stiffness differences during NTC are an epiphenomenon of the process, or whether they are key for its normal progression.

Neural Crest Cell Migration

The first major migratory event during development of the nervous system involves neural crest (NC) cells. This specialized neural stem cell population migrates away from the neural tube over relatively long distances and generates diverse structures such as cartilage, peripheral nerves, and smooth muscles.

NC migration is guided by chemotaxis [5]. However, substrate mechanical cues also play an important role in cellular migration [6,7]. A recent study by Barriga et al. examined the contribution of tissue stiffness to the migratory patterns of the NC *in vivo* [8 ••]. They showed that the initiation of NC migration is regulated by stiffening of the surrounding tissue. When the stiffness of the tissue was mechanically or pharmacologically perturbed, NC migration did not commence. Moreover, the stiffness cues were largely found to arise from changes in cellular density [8 ••]. Additionally, Scarpa et al. found that when NC migration begins, adhesion forces between cells reduce and traction forces applied to the substrate at the leading edge of the NC increases [9]. The few NC cells that initially migrate (termed “leader cells”) appear transcriptionally different from those that follow [10], suggesting a functional difference between leader and follower cells. It remains to be determined whether leader and follower cells differ in their response to the mechanical environment.

Neural Progenitor Proliferation

Once the neural tube closes, fluid mechanical cues become a powerful determinant of the continual development of the nervous system. In a classic experiment almost half a century ago, Desmond and Jacobson showed that relieving pressure in the brain ventricle of 3-day old chick embryos for a few hours results in a collapse of the entire central nervous system [11]. The intervention also reduced proliferation of the neural stem/progenitor cells (NSPCs). This experiment elegantly demonstrated that ventricular pressure is a defining aspect of early brain development. Although the molecular mechanism associated with this process in embryonic development is unclear, work is beginning to identify the molecular signaling involved. Desmond et al. identified focal adhesion kinase (FAK) as part of the mechanoresponsive signaling pathway that reacts to increased ventricular pressure [12]. They found that artificially increasing neural tube pressure by increasing intraluminal osmolarity caused activated FAK to localize to the apical border [12].

Recent work has now shown shear flow to be another important regulator of NPSC proliferation. The epithelial sodium channel (ENaC) was found to be a direct mechanotransducer of CSF shear flow in adult mouse NSPCs [13]. Through time-lapse imaging, the Gotz group found that ENaC allowed the influx of sodium ions into the cell in response to CSF flow. When this channel was functionally inhibited, NSPC proliferation decreased [13]. These studies set the stage for further investigations to understand the magnitude, temporal dynamics, and origin of the fluid mechanics in neural development.

Neural Progenitor Differentiation

The neural tube is composed of radial glial cells (RGCs), a class of NSPCs. These bipolar cells span the neuroepithelium, extending processes from the apical to the basal surface, with the cell bodies staggered to form a pseudostratified layer. These cells initially divide rapidly to expand the NSPC pool, and then differentiate first into neurons, then into glial cells. Studies examining mechanical regulation of NSPC fate have largely focused on substrate stiffness *in vitro*, in part due to the ease of tuning this mechanical cue using polymer chemistry [14–19].

Seminal work from the Discher group demonstrated that mesenchymal stem cell lineage choice is powerfully affected by substrate stiffness: soft substrates promoted neurogenic differentiation, moderately stiff substrates induced myogenic differentiation, and hard substrates triggered osteogenic differentiation [20]. This mechanosensitive lineage specification was found to require cellular contractility through Myosin II motors, suggesting the model that stem cells use Myosin II-mediated traction forces to probe substrate stiffness [20]. This paradigm-shifting study highlighted the importance of mechanical cues for stem cell fate and launched the new field of stem cell mechanobiology.

NSPC lineage choice into neurons or glia is also modulated by substrate stiffness [21–23]. Keung et al showed that the Rho GTPases RhoA and Cdc42 were important for NSPC specification, and also identified RhoA as important for neurogenesis in the adult rat brain [24]. Recently, dynamic hydrogels have been used to resolve temporal dynamics of this fate decision [25]. By reversing the stiffness of substrates at different time points, Rammensee et al. identified a time window of 12–36 hours in which NSPCs were receptive to stiffness cues that informed fate decisions. Reversal of substrate stiffness within this time window resulted in the reversal of fate commitment; however, after this time window passed, NSPCs were generally committed to their fate [25].

Our group discovered that Piezo1, a mechanically-activated ion channel, transduces substrate stiffness to direct the mechanosensitive lineage specification of NSPCs [23]. Specifically, cell-generated traction forces elicit Piezo1 Ca^{2+} flickers in spatially localized hotspots, suggesting a model wherein cells generate traction forces to probe substrate stiffness, and Piezo1 tranduces these traction forces into spatially-regulated Ca^{2+} flickers to determine cell fate [26]. When Piezo1 was pharmacologically inhibited or genetically knocked down, human brain-derived fetal cortical NSPCs (hNSPCs) showed a decrease in neurogenesis and an increase in astrogenesis [23]. Interestingly, this result differed from a previous report from adult rat hippocampal neural stem cells, which showed increased

neuron formation on soft substrates versus stiff substrates [21,23], suggesting that the biological origin of the stem cells may influence how they respond to mechanics.

Piezo1 also regulated the nucleo-cytoplasmic localization of the transcriptional co-activator Yap in hNSPCs: Yap was excluded from the nucleus on soft substrates (which elicited reduced Piezo1 activity) and also when Piezo1 was knocked down [23]. This suggests that Piezo1-mediated Yap signaling may be involved in shaping the mechanical response of NSPCs. In rat, adult hippocampal neural stem cells, Rammensee et al showed that Yap was involved in stiffness-mediated neurogenesis, but not through the Yap nucleo-cytoplasmic localization mechanism. Rather, they found that Yap interacts with B-catenin on stiff substrates to negatively regulate neurogenesis [25]. While the two studies demonstrate a role for Yap in neural stem cells, they suggest that the underlying mechanisms may differ with neural stem cells from different brain regions, developmental stages, or species.

These *in vitro* studies conducted over the course of a decade uncovered the importance of mechanics in stem cells of the neural lineage and identified key underlying mechanisms. However, an open question remained as to whether tissue stiffness played a role *in vivo*. While studies have yet to examine the role of stiffness on NSPC differentiation in the embryonic brain, recent work by Segel et al. showed how stiffness affects proliferation and differentiation of adult rat oligodendrocyte progenitor cells (OPCs). They showed that the rat brain stiffens with age, and found that OPCs in stiffer adult brains have significantly lower rates of proliferation and differentiation than OPCs in softer newborn brains, an observation recapitulated *in vitro* with hard and soft substrates [27 ••]. Furthermore, this “ageing” effect was reversible if the stiffer environments were softened pharmacologically *in vivo*. Consistent with our group’s findings, they also observed that matrix stiffness is transduced by Piezo1.

Many *in vitro* studies have used stiffness ranges on the scale of thousands of pascals, whereas the *in vivo* stiffness ranges in adult and embryonic brains are much smaller, on the scale of a few hundred pascals [28–31]. Interestingly, the study by Segel et al. suggests that even small stiffness ranges seem to powerfully affect cell behavior [27 ••]. A study by Kjell et al found that one of the neurogenic niches in the adult mouse brain, the subventricular zone, is 100 Pa stiffer than surrounding tissues [32 •]. To test whether this small difference was sufficient to induce higher rates of neurogenesis, they placed NSPCs onto gels that only differed by 100 Pa in stiffness and found that the stiffer gel resulted in double the rate of neuroblast formation.

Another mechanical cue, substrate stretch, has also been found to play a role in NSPC lineage choice. NSPCs on substrates that were stretched preferentially increased oligodendrocyte specification [33]. Further studies will be required to evaluate the interplay of different mechanical cues in modulating NSPC differentiation.

Neuronal Migration

Newly-formed neurons must position themselves appropriately in the developing brain. They typically do so through two modalities of migration. The first modality, radial migration, which is the major form of neuronal migration in the cerebrum, occurs when

neurons migrate perpendicular to the ventricular surface along the projections of RGCs to their appropriate layer. A subset of neurons, many of which are inhibitory interneurons from the ganglionic eminences, display another form of migration, tangential migration, whereby the cells move parallel to the ventricular surface (Fig. 2).

During migration, traction forces are generated by the cell to allow translocation [34]. Using traction force microscopy, Jiang et al. demonstrated that migrating neurons *in vitro* exhibit three centers of traction force generation, one in the trailing process, and two in the leading process - one near the growth cone and the other near the soma [35]. Neurons must translocate their soma during migration, which was thought to involve both “pulling forces” at the leading process, and “pushing forces” from the trailing process of the neuron. However, Jiang et al. only found pulling forces, and no evidence of pushing forces in migrating neurons [35]. It will be interesting to examine to what extent *in vivo* mechanisms reflect the *in vitro* observations.

Neuronal Wiring

Once neurons migrate to their final destination, axons extend and connect to their target. This process, termed axon guidance or pathfinding, is tightly regulated since precise targeting of axonal processes is critical to the generation of normal neural circuits. While work initially focused on the biochemical aspects of this process, studies have demonstrated mechanical cues to also be vital *in vitro [36]*. However, until recently not much was known regarding the extent to which these findings are relevant *in vivo*.

Koser et al. showed the presence of differential stiffnesses in the developing frog brain. Their work suggests that stiff tissues dictate a straight axonal trajectory, stiffness gradients guide axon turning toward softer tissues, and softer tissues inform axons to slow down and splay out [37]. They also showed that Piezo1 transduces these matrix stiffness cues to inform the axonal pathfinding. Either disruption of normal stiffnesses in the brain or inhibition of Piezo1 resulted in abnormal axonal outgrowth [37]. A follow-up study by the same group showed that cell density is responsible for producing differences in tissue stiffness [38 ••].

A new mechanism of wiring termed “retrograde axon growth” was recently proposed, wherein the synaptic end of the axon is fixed in place and the cell body migrates away to its destination [39]. Breau et al. show in zebrafish olfactory placode, that cells are compressed by neighboring cells, forcing the neuronal soma away from the axon tip and initiating the growth of the axon in a retrograde manner [39]. This is a fascinating new mechanism that requires the coordinated mechanical action of neighboring cells. It will be important to explore whether this is conserved in other animal model systems.

Gyrification

An aspect of neural development unique to a subset of mammals is cortical folding, thought to occur to increase the total surface area of the cerebral cortex and has been appreciated as a highly mechanical process [40]. While many studies have looked at gyrification using animal models such as ferrets and nonhuman primates and computational modeling, new studies have experimentally modeled this process *in vitro* to examine the mechanics of the process in greater detail.

Karzbrun et al, using human brain organoids, highlighted the importance of cytoskeletal forces in forming gyri, the “ridges” of the cerebral cortex [41 ••]. Upon pharmacologically inhibiting force-generating myosin motors, they found a reduction in the curvature of the brain organoid folds, suggesting these cell-generated forces maintain the appropriate shape of gyri [41 ••]. Additionally, AFM-measurements of gyri in human organotypic cultures show that the nascent gyri are stiffer, while the sulci, the furrows of the cerebral cortex, are softer by a few hundred pascals [31]. More studies will be required to determine whether this effect is an epiphenomenon of the process, or helps guide the process forward.

Future Directions

With the recent work described above, mechanical forces are now appreciated as crucial for neural development. Yet, the biomechanics of several aspects of brain development are still unclear and ripe for investigation. These include neural induction, neural tube morphogenesis, interkinetic nuclear migration of RGCs [42 •], glial cell migration, and synapse formation and pruning.

Matrix stiffness has emerged as a major regulatory process, likely because its tractability allowed extensive experimentation. Emerging techniques have recently been introduced to probe tissue stiffness *in vivo* in 3D, including the use of lipid droplets [43], and various optical techniques such as Brillouin Microscopy, Optical Coherence Tomography and Magnetic Resonance Elastometry [44]. These non-invasive approaches will provide a more accurate picture of the processes and take stiffness studies to the next level.

Many studies in the past decade have examined mechanical cues in the context of single cells, but it is now crucial to understand these forces in tissues. Furthermore, while much of the work has focused on how tissue mechanics influence developmental processes, it is also important to understand how these forces are generated in the first place and how different forces might influence each other. In some cases cells actively generate force as seen through contractility of the actomyosin cytoskeleton [41 ••]. In other cases, the forces arise as a byproduct of developmental morphogenesis, as seen in the case of cellular density [8,38]. Understanding passive or active forces generated and the interactions between them will be key to provide a comprehensive understanding of development.

Studying mechanical dynamics of neural development *in vivo* is still a challenging feat, especially in mammalian systems. Emerging brain organoid technologies can help bridge the gap. As a system that can recapitulate 3D developmental dynamics *in vitro*, it allows for mechanical measurements and manipulations. Thus, brain organoids can be used to test hypotheses that current technologies render untestable *in vivo* or that are simply not observed in model organisms. Brain organoids have limitations, including the lack of a vascular system, variability of outcomes, and differences in mechanics and metabolism compared to *in vivo* systems; however, several of these will likely be resolved as the technology improves. Questions that might be impossible to answer in organoids or *in vivo* could be modeled computationally, which can suggest future experiments to test new ideas on how mechanical forces shape neural development. Because development cannot be fully understood through the lens of any single discipline, work that integrates diverse fields and experimental systems will be paramount to further our understanding of these processes.

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Abbreviations:

AFM	atomic force microscopy
NC	neural crest
NTC	neural tube closure
NSPC	neural stem/progenitor cell
RGC	radial glial cell
CSF	cerebrospinal fluid

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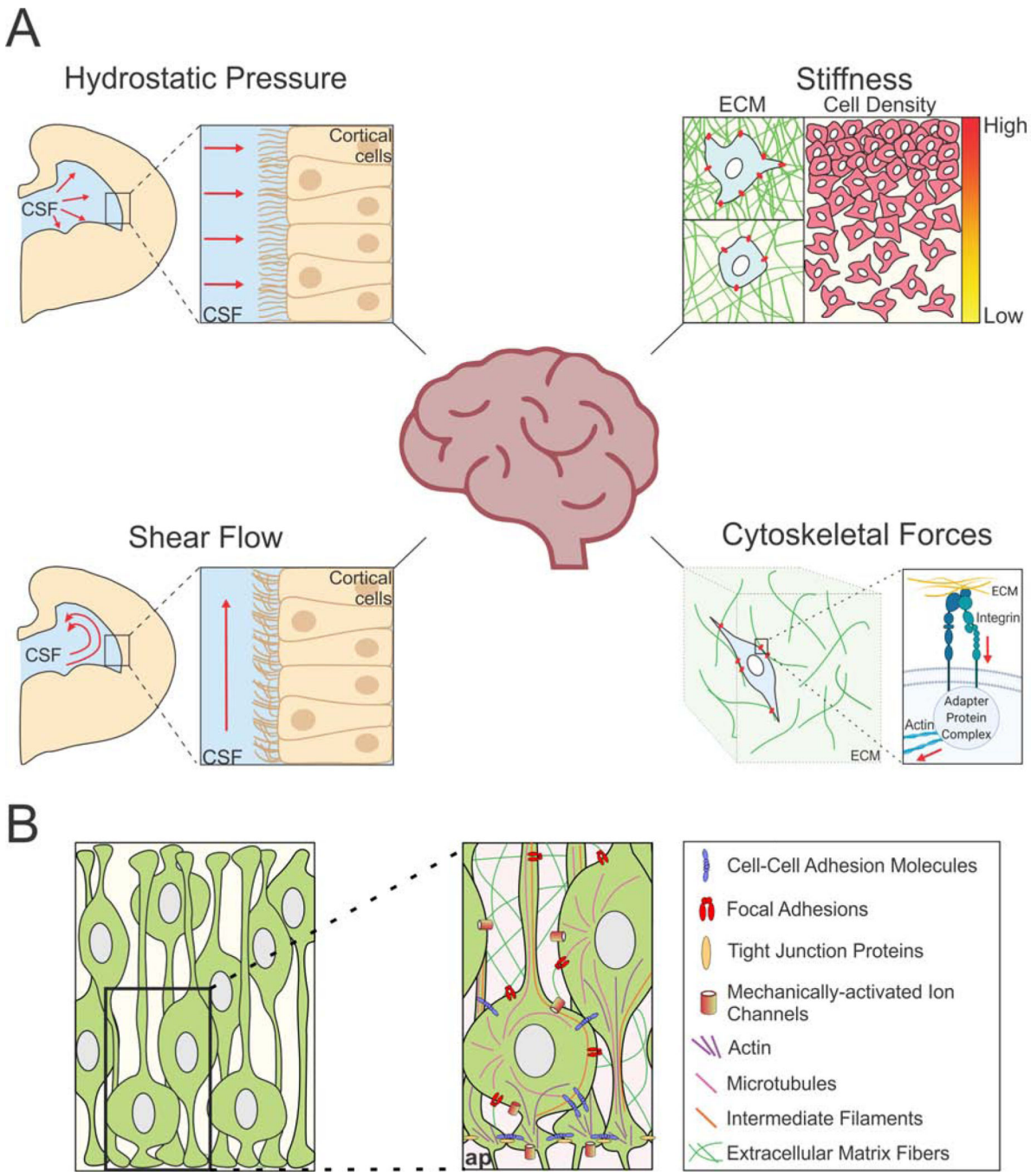

Figure 1. Mechanotransduction in the developing brain.

(A) The developing brain experiences a variety of mechanical cues. Left panels show a schematic of a coronal cross section of half of the developing brain, and the fluid-based forces, hydrostatic pressure **(upper left)** and shear flow **(lower left)** impinging against cells that line the ventricles. Tissue stiffness **(upper right)** is modulated by extracellular matrix components or by cellular density. The actomyosin cytoskeleton **(lower right)** connects to the extracellular matrix through focal adhesions and is integral to cellular mechanotransduction during development. CSF = cerebrospinal fluid, ECM = extracellular

matrix. **(B)** The molecules and cellular structures involved in the mechanotransduction in the developing neuroepithelium. ap = apical border.

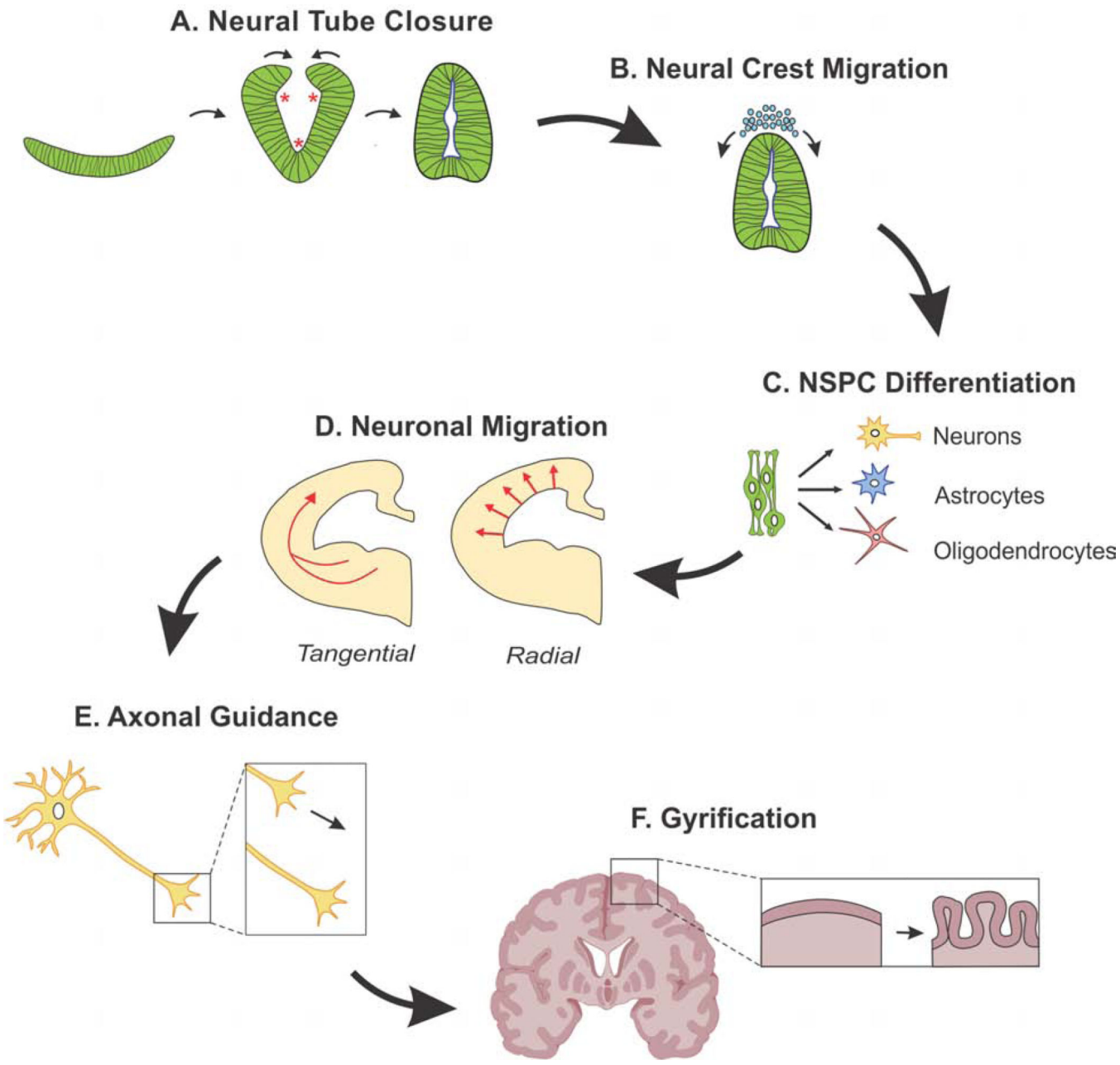

Figure 2. Timeline of mechanical events during neural development

(A) The neural plate bends at three points, called hinge points (asterisks), and closes to form the neural tube.**(B)** Neural crest cells migrate away from the closed neural tube to form a variety of structures. **(C)** Neural stem/progenitor cells (NSPCs) differentiate into neurons, astrocytes, and oligodendrocytes. **(D)** Newly-formed neurons migrate radially or tangentially to their final destination. **(E)** Axons extend from the newborn neurons to form connections throughout the developing brain. **(F)** In some mammals including humans, the cortex folds to increase cortical surface area.