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# The mechanical control of nervous system development

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## Summary

The development of the nervous system has so far, to a large extent, been considered in the context of biochemistry, molecular biology and genetics. However, there is growing evidence that many biological systems also integrate mechanical information when making decisions during differentiation, growth, proliferation, migration and general function. Based on recent findings, I hypothesize that several steps during nervous system development, including neural progenitor cell differentiation, neuronal migration, axon extension and the folding of the brain, rely on or are even driven by mechanical cues and forces.

**Key words: Mechanics, Mechanosensitivity, Mechanotaxis, Mechanotransduction, Stiffness, Force, Tension, Brain folding**

## Introduction

Many processes in development involve growth and motion on different length and time scales. All of these processes are driven by forces; the development of organisms and organ systems would not proceed without mechanics. For example, during neuronal development, neurons migrate and extend immature processes (neurites), which become axons and dendrites. Axons then grow in two different phases, both of which are distinguished by the nature of the forces that drive the growth. In the first phase, growth cones at the tips of axonal processes actively exert forces on their environment (Betz et al., 2011), thus pulling on the processes (Lamoureux et al., 1989). In a second phase, after connecting with their target tissue, axons may be passively pulled by the increasing distance between target and nervous tissue, resulting in considerable growth in length, a process referred to as stretch growth (Weiss, 1941). Once the final connectivity is established, tension may develop along neuronal axons, which may be involved in neuronal network formation and the folding of the brain. Apart from this direct requirement of forces for developmental processes, which has been studied to some degree in the past, the mechanical interaction of cells with their environment may add an additional level of control to several processes in the developing nervous system, including progenitor cell differentiation and cellular guidance.

The idea of an important contribution of mechanics to the development of the nervous system has been around for more than a century. However, recent decades have seen only little progress in this field compared with other (e.g. electrophysiology, molecular biology or genetics-based) areas of neuroscience. Progress often depends on the availability of appropriate methodology. Only recently has the increasing involvement of physical and engineering approaches in interdisciplinary studies of biological systems led to the development of new techniques and conceptual approaches that can be used to quantitatively probe and control relevant mechanical parameters, such as cell and tissue stiffness, cellular forces, and tension. In recent years, such tissue mechanics-based studies have resulted in an increasing awareness of the importance of physical parameters, particularly in developmental biology, where cell systems constantly undergo dramatic rearrangements. These rearrangements naturally rely on forces acting on cells (without which there would be no motion) and the resistance of cells and cell groups to these forces, which depends on their viscoelastic properties and determines, for example, where cells are placed within a tissue. These fundamental and important parameters have so far been ignored to a large extent, but it is clear that a consideration of these parameters could provide a new understanding of developmental processes in general.

Here, I focus on the potential involvement of mechanics in the development of the nervous system.

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## A brief overview of biomechanics and measurements

The mechanical interactions between a cell and its environment depend on the forces acting on and exerted by the cell, the mechanical properties of the cell and its environment, and the coupling between them. Below, I highlight some mechanical features of cells and tissues, and briefly explain how mechanical properties and forces can be measured.

### Cellular forces and tension

Most, if not all, tissue cells, including neurons and glial cells, exert forces on their environment. Current techniques to determine cellular traction forces measure the strain $\gamma$ (i.e. deformation; see Glossary, Box 1) of a substrate of known compliance in order to calculate the stress $\sigma$ (i.e. force $F$ per contact area $A$; see Glossary, Box 1) exerted by cells. These substrates are chosen to be linearly elastic (see Glossary, Box 1), which means that $\gamma \propto \sigma$. By contrast, many biological materials tend to be non-linearly elastic (see Glossary, Box 1). In traction-force microscopy (Munevar et al., 2001; Betz et al., 2011; Koch et al., 2012), a compliant substrate is deformed by cells and deformation fields are tracked using fluorescent nanoparticles embedded within the substrate. In an alternative approach, stiffer elastomeric substrates are structured as arrays of needle-like posts (Tan et al., 2003). Cells are cultured on these substrates and the deflection of the needle-like posts can be measured. On a smaller scale, intracellular forces can be measured using Förster resonance energy transfer (FRET)-based force sensors (Grashoff et al., 2010). Here, a short elastic domain is inserted between two fluorophores that undergo FRET; this tension sensor module is inserted into vinculin, a protein connecting the actin cytoskeleton with cell adhesion molecules (integrins). Because FRET efficiency decreases under tension, piconewton forces across vinculin can be measured.

In contrast to other cell types, neurons extend long processes, which are under mechanical tension (i.e. a pulling force; see Glossary, Box 1) (Bray, 1979; Heidemann and Buxbaum, 1994;

**Box 1. Glossary: terminology of mechanical parameters**

**Elastic modulus.** The ratio of stress σ to strain γ; a constant describing a material's resistance to deformation. Unit: Pa.

**Elasticity.** The property of a material to deform in response to a force and to return to its original state once the force is removed.

**Linear elasticity.** Stress and strain are proportional; the elastic modulus is independent of the strain.

**Non-linear elasticity.** Biological materials usually show non-linear elasticity. Their elastic modulus changes with strain. Cytoskeletal and extracellular matrix networks, for example, stiffen when they are increasingly deformed.

**Poisson's ratio (ν).** Negative ratio of transverse to axial strain. For most materials, $0 \leq \nu \leq 0.5$. Biological materials often have a Poisson's ratio between 0.4 and 0.5; for incompressible materials such as water, ν=0.5.

**Shear modulus (*G*).** Quantifies the elastic resistance of a material to deformation in shear (stress is applied parallel to the surface). Unit: Pa. Can be transformed into Young's modulus using Poisson's ratio: $G=E/(2(1+\nu))$.

**Strain (γ).** Relative deformation of a material under stress. Dimensionless.

**Stress (σ).** The force exerted normalized by the area over which the force is applied. Unit: Pa ($N/m^2=pN/\mu m^2$). Depending on the direction of stress application: compressional, extension or shear stress.

**Tension.** A pulling force (not a stress). Unit: N.

**Viscoelasticity.** Combining viscous and elastic properties. Materials with viscoelastic properties partly recover their initial shape after stress application (elastic contribution) but also continue to flow (or increase strain; viscous contribution) as long as the stress is applied, until they either reach a plateau (viscoelastic solids) or slowly flow (or creep) without limit (viscoelastic liquids or viscoplastic materials). The viscoelastic response of a material to stress depends on the time scale.

**Viscosity (η).** Resistance of a fluid to stress; the ratio of stress σ to strain rate $d\gamma/dt$ (or flow rate). Unit: Pa•s.

**Yield stress ($\sigma_y$).** Maximum stress that can be applied before a sample ruptures.

**Young's modulus (*E*).** Quantifies the elastic resistance of a material to elongation or compression (stress is applied normal to the surface). Unit: Pa. Can be transformed into shear modulus using Poisson's ratio: $E=2G(1+\nu)$.

Pfister et al., 2004; Siechen et al., 2009; Suter and Miller, 2011). This tension may be quantified with calibrated microneedles: forces are applied to neurites, and the change in neurite length and the deflection of the needle (which is proportional to the applied force) are measured (Dennerll et al., 1988). On a larger scale, tissues in developing organisms are also under tension. This cortical tension can be measured using laser ablation, whereby a focused laser beam is used to cut a tissue, and the subsequent relaxation of the tissue is recorded and analyzed (Mayer et al., 2010).

### Measuring cell and tissue mechanics

Biological cells and tissues are generally viscoelastic (see Glossary, Box 1), i.e. they behave partly like a viscous fluid (such as honey) and partly like an elastic (see Glossary, Box 1) solid (such as rubber). As such, their response to an applied force depends on the time scale over which the force is applied: strain will increase with time if a stress is maintained until equilibrium is reached.

Most techniques that measure mechanical cell or tissue properties externally impose stress to the sample and then measure the resultant strain. The ratio of stress and strain (σ/γ) yields an elastic modulus (see Glossary, Box 1), which is a measure of stiffness. If the stress is applied normal (i.e. perpendicular) to the surface (i.e. tensile or compressive forces), the Young's modulus *E* (see Glossary, Box 1) is determined; if the stress is applied in parallel, the shear modulus *G* (see Glossary, Box 1) is measured. In the simplest case, biological samples are assumed to be linear elastic materials, which is often a reasonable assumption for small deformations occurring over short time scales. However, more complex measurements also take different time scales into account and yield frequency-dependent moduli that characterize both elastic and viscous properties (i.e. elasticity and viscosity; see Glossary, Box 1).

There has been a flurry of development of different techniques to allow the measurement of cell mechanics and to allow mechanical manipulation of cells (summarized in Table 1), including atomic force microscopy (AFM), magnetic bead twisting, magnetic tweezers, optical traps, micropipette aspiration, cell poking, and microrheology. Most of these techniques rely on contact and/or are invasive. It should be noted that for non-contact, non-invasive methods (e.g. Brillouin microscopy or magnetic resonance elastography) the spatial resolution is currently not sufficient for studies at a cellular level. Importantly, almost all single cell mechanics methods are difficult, if not impossible, to carry out *in situ*.

Compliant substrates made of various hydrogels or rubbers as well as micropillar arrays have been used to mimic the mechanical properties of tissues in order to study mechanosensitive cell responses *in vitro*. To improve representation of the heterogeneous mechanical properties of biological tissues, stiffness gradients have been introduced to such surfaces using different approaches (Lo et al., 2000; Byfield et al., 2009; Kuo et al., 2012).

Finally, cells need to transmit their forces to their environment in order to move (and to probe the mechanical properties of the environment). This is achieved via adhesion complexes (point contacts in neurons, focal adhesions in glial cells), which couple the force-generating cytoskeleton to the extracellular matrix or other cells. The adhesion strength of individual cells and growth cones can, for example, be quantified using calibrated microneedles (Zheng et al., 1994) and AFM (Krieg et al., 2008; Franze, 2011).

## Mechanical control of early neural development: regulation at the cellular and molecular level

It has long been established that sensory neurons can respond to mechanical stimuli in their environment. Hearing, balance, touch and proprioception are all mechanical senses that are directly mediated by neurons. The majority of cells in the nervous system, however, are usually considered to rely on chemical signals only. Nevertheless, recent *in vitro* studies suggest that many neuronal as well as glial cell types also respond to mechanical cues throughout their development (Fig. 1) (reviewed by Franze and Guck, 2010; Moore and Sheetz, 2011; Franze et al., 2013).

The response of nervous tissue cells to mechanical stimuli is particularly interesting with respect to their mechanical environment. Adult nervous tissue is mechanically inhomogeneous (Elkin et al., 2007; Green et al., 2008; Christ et al., 2010); [for recent reviews on brain mechanics, see (Chatelin et al., 2010; Franze and Guck, 2010; Franze et al., 2013)]. Moreover, the stiffness of adult brain tissue changes with age (Sack et al., 2009), suggesting that already during development the mechanical properties of nervous tissue are prone to

**Table 1. Cell mechanics measurement tools**

| Technique | Forces | Main applications | Advantages | Disadvantages |
|---|---|---|---|---|
| Atomic force microscopy (AFM) | Compressive and tensile forces (pN-mN range) | Molecular, cellular and tissue stiffness measurements, protein unfolding, cell adhesion measurements, stress application to biological samples, surface scanning | High spatial and temporal resolution, combination with other techniques, working range over several scales | Restricted to surfaces, not high throughput |
| Cell poking | Compressive forces (nN-μN range) | Cell stiffness measurements | Easy to set up | Restricted to surfaces, limited force and spatial resolution |
| Magnetic bead twisting | Shear forces (pN-nN range) | Cell rheology, stress application to cell surface receptors | High throughput, good force resolution | Restricted to surfaces, binding may cause secondary effects |
| Magnetic tweezers | Tensile forces (pN-nN range) | Cell stiffness measurements | Easy to set up | Requires magnetic beads to be taken up by or bound to cells |
| Micropipette aspiration | Tensile forces (tens of pN–μN range) | Cell stiffness, membrane tension measurements | Easy to set up | Limited spatial and force resolution |
| Microrheology | Passive method (no forces actively applied) | Cell rheology | Easy to set up, high throughput, *in vivo* measurements possible | Position of the particles difficult to control |
| Optical stretcher | Tensile forces (pN range) | Cell deformation assays | High throughput, contact-free | Limited spatial resolution and force, heating of samples |
| Optical tweezers | Tensile, compressive, shear forces (pN range) | Stress application to cells and molecules | High temporal and spatial resolution | Limited force, often μm-sized beads have to be attached to the sample, heating of samples |

alteration, and cells encounter different mechanical cues depending on location and developmental stage.

## Neurogenesis

The first event in the development of the nervous system to which mechanics could significantly contribute is the maturation of neural precursor cells. During cortical development, radial glial cells first give rise to neurons, and at later stages they differentiate into glial cells (Götz and Huttner, 2005). Studies have shown that when mesenchymal stem cells are cultured on deformable substrates, compliant matrices, in contrast to stiffer substrates, promote differentiation into a neuronal phenotype (Engler et al., 2006; Keung et al., 2012) (Fig. 1). Brain tissue is one of the softest tissues in our body, and it stiffens with age. While the mechanical properties of brain tissue have never been measured at different developmental stages, it is conceivable that it starts stiffening already early during development, and at a certain developmental stage cortical tissue stiffness might exceed a critical threshold, thus contributing to the shift from neurogenesis to gliogenesis.

In support of this hypothesis, on compliant substrates neuronal growth is promoted over that of glial cells (Georges et al., 2006). Furthermore, in reeler mice, which lack the extracellular matrix glycoprotein reelin, an increase in the number of glial fibrillary acidic protein (GFAP)-positive astrocytes is accompanied by a decrease in the number of newly generated neurons (Zhao et al., 2007), and enhanced GFAP expression in retinal glial cells leads to their stiffening (Lu et al., 2011). The enhanced GFAP expression

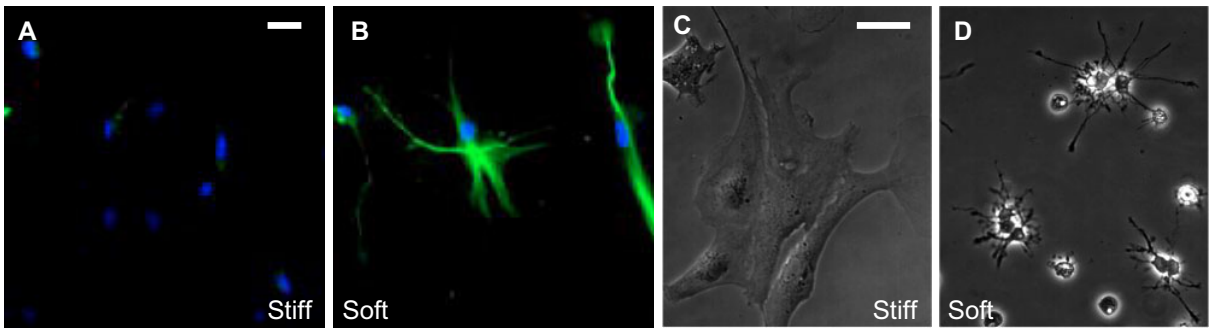


**Fig. 1. Mechanosensitivity of nervous tissue cells.** (**A**,**B**) Only when mesenchymal stem cells are cultured on 'soft' neurogenic substrates with a compliance similar to that of brain tissue do they assume a neuronal phenotype and express the neuronal cytoskeletal marker β3-tubulin (green). Nuclei are shown in blue. Adapted from Engler et al. (Engler et al., 2006) with permission from Elsevier. (**C**) When primary glial cells (astrocytes) are cultured on substrates with a stiffness comparable to that of muscle tissue ('stiff'), they spread and assume a morphology similar to that observed when they are cultured on tissue culture plastics. (**D**) However, when the same cells in the same chemical environment are cultured on softer substrates with a compliance similar to that of brain tissue ('soft'), their cellular morphology changes drastically, and they extend star-like processes and resemble their *in vivo* appearance. Images in C,D courtesy of Pouria Moshayedi. Scale bars: in A, 5 μm for A,B; in C, 10 μm for C,D.

in the mouse mutants could thus lead to an increase in tissue stiffness that causes, or at least contributes to, the observed decrease in neurogenesis.

Apart from passive mechanical tissue properties, active forces (e.g. tension in the tissue) might also influence neuronal development. For example, the folding of the mammalian cortex, which itself is driven by forces (see below), leads to different tissue layer dimensions and mechanical stress distributions within the crowns and fundi of gyri (outward folds) (Bok, 1959; Welker, 1990; Xu et al., 2009). Neuroblasts start to differentiate earlier in gyral crowns than in fundi, they increase in size and shape earlier, and the degree of elaboration of their dendrites is significantly more extensive (Welker, 1990), indicating that mechanical stress might be involved in progenitor cell development. In agreement with this hypothesis, mechanical tension *in vitro* drives neural stem cell differentiation towards mature neuronal cells (Chang et al., 2013).

### Neuron-glia interactions

By contrast, the differentiation of Schwann cells and oligodendrocyte precursor cells, which are glial cells responsible for providing the myelin sheath for neurons, increases with stiffness (Cai et al., 2012; Jagielska et al., 2012). The interaction of neurons and glial cells, for example during myelin sheath or synapse formation, might therefore also be influenced by mechanical signaling: neurons, which usually grow well on soft substrates (Georges et al., 2006), are stiffer than their neighboring glial cells (Lu et al., 2006), which, by contrast, seem to grow preferentially on stiffer substrates (Georges et al., 2006; Moshayedi et al., 2010). Astrocytes, for example, spread more on stiffer substrates, and their F-actin cytoskeleton is more organized compared with compliant surfaces (Georges et al., 2006; Moshayedi et al., 2010) (Fig. 1). The opposing mechanical properties and preferences of neurons and glial cells might attract them towards each other, and they might explain why neurons in mixed cultures often grow on top of glial cells.

### Neuronal migration and axonal growth

In addition to well-established chemical signaling, the speed and direction of neuronal and growth cone migration (see below) also depend on the mechanical interaction between cells and their environment. For example, mechanical tension along neurites has been suggested to contribute to the directionality of migrating neurons (Hanein et al., 2011).

The migration of fibroblasts (and some other non-neural cell types) has been shown to be guided by stiffness gradients in their substrate (‘mechanotaxis’) *in vitro* (Lo et al., 2000). Neurons as well as growth cones during axonal pathfinding are likely to encounter environments with different mechanical properties as they migrate *in situ*. In support of this, stiffness gradients have recently been reported in CNS tissue (Elkin et al., 2007; Franze et al., 2011). Thus, although compelling evidence is still missing, it seems likely that neurons in the developing nervous system might be guided by mechanical signals, in addition to the battery of established chemical cues.

After neurons have arrived at their destination, they send out immature processes. One of these processes turns into an axon, which usually grows over long distances, whereas the others become dendrites. Forces (tension) might not only be involved in the generation of axons (Bray, 1984). Many neuronal cell types adapt their morphology, and particularly the number, length and branching patterns of their neurites, to the stiffness of their substrate *in vitro*, including mammalian dorsal root ganglion cells, spinal cord and hippocampal neurons, but not always cortical neurons (Georges et al., 2006; Jiang et al., 2008; Norman and Aranda-Espinoza, 2010; Koch et al., 2012). Neurite outgrowth is a mechanical process, and as such it might well be influenced by the interaction between neurites and the mechanical environment *in vivo*.

### Growth cone motility

Forces during neuronal growth are generated by growth cones, which are the leading tips of developing axons and dendrites. They are highly motile structures that determine the speed and direction of outgrowth. Growth cones are densely packed with actin filaments, which are polymerized at their leading edge. At the same time, myosin II motors, which are concentrated at the central zone of the growth cone, pull on actin filaments. These myosin-based forces, together with forces arising from actin polymerization, give rise to the well-studied retrograde actin flow observed in neurons (Medeiros et al., 2006). The actin cytoskeleton is also coupled to the substrate via point contacts, which are made up of protein complexes containing integrins, vinculin, talin and many others (Renaudin et al., 1999). These point contacts form molecular ‘clutches’ (Suter and Forscher, 1998), which allow growth cones to transmit forces to their substrate, which may lead to its deformation (Franze et al., 2009; Betz et al., 2011; Koch et al., 2012). Accordingly, inhibition of actin polymerization leads to a reduction in the maximum force and velocity of growth cone protrusion, and a reduction in membrane stiffness results in larger forces and increased velocity (Amin et al., 2012). Thus, forces exerted by neurons can be controlled by controlling actin polymerization and myosin activity. Furthermore, interactions between actin filaments and microtubules, which modify stress distributions in the growth cone, are required for growth cone motility and turning (Geraldo and Gordon-Weeks, 2009). Growth cone traction forces finally oppose the tension that is acting along neurites (Bray, 1979; Dennerll et al., 1988; Heidemann and Buxbaum, 1994; Ayali, 2010; Suter and Miller, 2011) (see below). While the mechanisms of force application are comparatively well understood, how mechanical input is translated into an intracellular, biochemical response (‘mechanotransduction’) is currently ill defined.

## Mechanics during neural circuit formation: tension in neuronal networks

From the initiation of neurite growth, to the establishment of synaptic connections with a target cell, to the formation of stable neuronal networks, neuronal processes are constantly under tension *in vitro* (Bray, 1979; Heidemann and Buxbaum, 1994) and *in vivo* (Gilmour et al., 2004; Siechen et al., 2009; Xu et al., 2010). Tension above or below a certain threshold stimulates neurite extension or retraction, respectively (Fig. 2) (Dennerll et al., 1989). For excellent recent reviews about neuronal tension see Ayali (Ayali, 2010) and Suter and Miller (Suter and Miller, 2011). Such tensile forces are generated and maintained by the growth cone (Lamoureux et al., 1989; Lamoureux et al., 2010), by the interaction of actin and myosin along the neurite (Dennerll et al., 1988), and by target cells pulling on the neurite (Weiss, 1941), and they are potentially involved in many different aspects of the development of the nervous system.

### Towed growth and guidance of axons

As mentioned above, the towing of axons results in tension, which is very likely crucially involved in the second phase of axonal

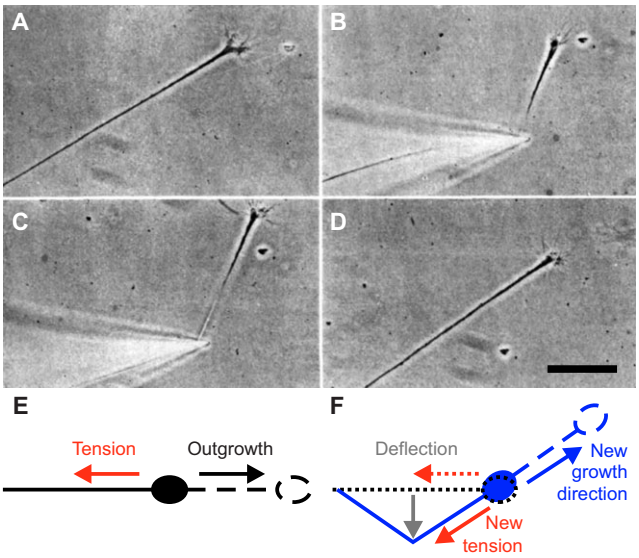


**Fig. 2. Tension along neuronal processes.** (**A**) Neurites are under tension. (**B**,**C**) When they are pulled to one side with a microneedle (B), forces redistribute and, as a consequence, neurites change their direction of growth (C). (**D**) After removing the microneedle, initially relaxed neurites build up tension again, straighten, and resume growth away from the rest of the cell, changing direction again. Scale bar: 50 µm. Adapted with permission (Bray, 1979). (**E**,**F**) Schematic of force distributions in neurite extension. (E) Initially, the growth cone moves (black arrow) in a direction opposite to the tension acting along the neurite (red arrow). (F) When the neurite is deflected (gray arrow), the force redistributes and the neurite changes its outgrowth direction to again oppose the tension.

growth (Weiss, 1941; Bray, 1984; Loverde et al., 2011). Pfister et al. showed that mechanical tension induces extreme 'stretch growth' of integrated axon tracts at remarkable rates and extents (8 mm/day) (Pfister et al., 2004), indicating that axonal lengthening is mainly limited by tension, or rather its relative absence.

Accordingly, when neurons are cultured on a flexible substrate, neurite extension significantly increases with increasing substrate stretching, and neurites preferentially align along the stretch direction (Chang et al., 2013). Similarly, muscle contractions in zebrafish generate mechanical forces that are required for proper pathfinding of sensory axons growing between the muscle and the skin of the fish (Paulus et al., 2009). Another example of the involvement of tension in axonal growth and guidance *in vivo* is exhibited by migrating primordium cells in the developing zebrafish, which not only tow axons of sensory neurons but also guide their pathfinding in this way (Gilmour et al., 2004).

### Network formation

Tension along neurites also influences the shape of neuronal somata (Hanein et al., 2011) and the geometry of neurite branches *in vitro* (Bray, 1979; Shefi et al., 2004) and *in vivo* (Condron and Zinn, 1997). Differential tension along individual branches at a given junction particularly determines the angle between the branches and the branches' diameter. In a similar manner, tension may also influence the final morphology of neuronal networks. Once a neurite is connected to its target, tension promotes its stabilization; at the same time, it causes retraction or elimination of collateral neurites (Anava et al., 2009). Thus, tension might serve as a signal for axonal and dendritic survival, and reduced tension might, therefore, contribute to branch pruning (Franze et al., 2009). Accordingly, the orientation of apical dendrites of pyramidal neurons in the cortex and the degree of their dendritic and axonal arborization depends on their location relative to the curvature of the tissue (Welker, 1990), and thus probably on local tension (Xu et al., 2010). Once the neuronal network is connected, the buildup of mechanical tension will lead to a shortening of the involved neuronal processes, thus contributing to the compactness of neural circuitry (Van Essen, 1997). Finally, mechanical forces may also contribute to the secondary modification of CNS tissue morphology. For example, cellular forces are likely to be involved in the formation of the foveal pit in retinae of some primates, birds, reptiles and fish (Springer and Hendrickson, 2004), and in the folding of the brain (see below).

### Synapse formation and functioning

Tension has also been suggested to contribute to synapse formation (Ayali, 2010). Recent evidence from *in vivo* experiments indicates that tension along axons can be actively regulated by neurons, and it is even involved in synapse functioning. Tension in *Drosophila* axons, for example, contributes to the clustering of neurotransmitter vesicles at presynaptic terminals at the neuromuscular junction (Siechen et al., 2009), and it modulates local and global vesicle dynamics (Ahmed et al., 2012). Hence, mechanical tension in and along neuronal axons might contribute not only to neuronal network formation but ultimately also to regulation of neuronal function.

## Mechanical control at the macroscopic level: brain folding

The folding of the gyrencephalic mammalian cortex is the ultimate mechanical event in CNS development. Cortical folding abnormalities are found in several CNS disorders, such as Williams syndrome, autism and schizophrenia (Van Essen et al., 2006; Nordahl et al., 2007; White and Hilgetag, 2011), indicating the importance of proper force distributions during CNS development.

The degree of cortical folding increases with brain size; the brains of larger animals are usually more convoluted. It is known that deeper cortical layers are thicker in convex gyri than in concave sulci (depressions in the surface of the brain) (Bok, 1959) (Fig. 3). Thus, to conserve the volume of the respective layers of gyri and sulci, neurons in different layers maintain their sizes and relative arrangements and instead adapt their shape, whereas glial cells and blood vessels maintain size and shape and change their relative arrangements. Although it is commonly accepted that intrinsic mechanical forces drive cortical folding, the origin of these forces is still disputed.

Numerous active and passive forces act in and on the cortex over different length and time scales. Sulci and fissures form only after all cortical neurons have been generated and after neuronal migration has been completed (Goldman-Rakic and Rakic, 1984), excluding these events as possible sources of the driving forces. Blood vessels, which run along sulci, can be excluded as well, as their alignment with the sulci occurs secondarily to cortical folding (Welker, 1990). Furthermore, cerebrospinal fluid shows no pressure differential between different brain regions (Welker, 1990), making its involvement unlikely. The skull is also not likely to impose mechanical constraints that are important for gyrification: its ossification starts only after the brain has stopped growing (Welker, 1990), and when different parts of the brain are removed during development, the remaining brain does not expand into the unoccupied regions of the cavity, but its fissuration is unaltered

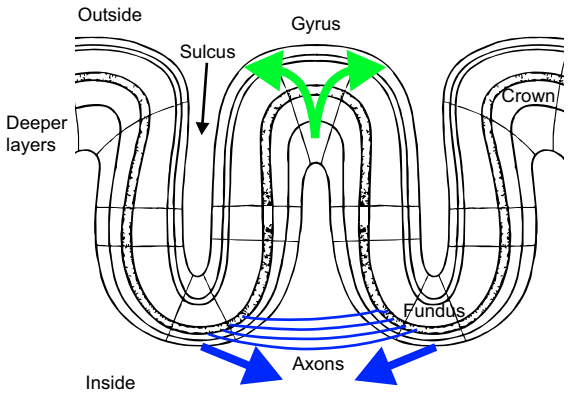


**Fig. 3. Forces in brain folding.** During development of the brain, mechanical forces lead to the folding of the cortex. These forces are currently hypothesized to be either due to differential expansion (green arrows) of certain regions/cell types, or to tension (blue arrows) along neuronal axons (blue). As a consequence of the folding, the deeper cortical layers are thicker in gyri than in sulci. The volume of the cortex is maintained in the respective layers of gyri and sulci (indicated by lines crossing the layers). Image adapted with permission (Bok, 1959).

(Barron, 1950). While these insights disproved some of the older theories about cortical folding, currently there are still two major hypotheses to explain cortical folding (Fig. 3), as discussed below.

### Differential expansion hypothesis

One hypothesis – the differential expansion hypothesis – assumes a central role for compressive forces arising from growth processes during cortical development. In this hypothesis, the tangential expansion of cortical regions, which is driven by the local augmented proliferation of cells and changes in cell sizes and shapes, is assumed to be the driving force for cerebral convolutional development (Mares and Lodin, 1970; Caviness, 1975; Smart and McSherry, 1986; Ronan et al., 2013). Thus, forces driving brain folding are predominantly intracortical. In support of this hypothesis, the experimental reduction of proliferation in the outer subventricular zone leads to a reduction in cortical folding (Reillo et al., 2011). The application of finite element models confirmed that differential cortical growth together with remodeling of the subplate might explain cortical folding and the stress patterns found in brain tissue (Xu et al., 2010). Furthermore, it was shown that removal of the cerebral cortex affects the folding pattern of the remaining brain (Welker, 1990). In such early cortical ablations, gyri and sulci reorient towards the defect. By contrast, the aspiration of basal ganglia and the transection of all thalamocortical connections do not change folding (Welker, 1990).

However, disruptions in neuronal proliferation do not always lead to loss of cortical convolutions; failure of cell division often results in smaller brains with preserved sulci and gyri (although on a smaller scale) (Neal et al., 2007). Furthermore, whereas lesions of the frontal lobe result in bilateral changes in sulcal patterns (Goldman and Galkin, 1978), lesions of the occipital lobe produce mainly asymmetric changes (Goldman-Rakic and Rakic, 1984). These differences are difficult to explain solely in terms of local cellular proliferation in the cortex. An important difference between these regions is the prominent callosal connections in the frontal lobe, which are absent in the primary visual areas. It is conceivable that forces could be transferred along these axons (between frontal lobes), suggesting that tension might be required for brain folding. An involvement of long-tract connectivity (intrahemispheric and callosal fibers innervating the cortical plate) in fissure formation would also explain how the disruption of a small part of the cortex results in widespread changes in fissuration of the entire cerebral surface of both hemispheres (Goldman-Rakic and Rakic, 1984).

### Tension hypothesis

It has also been hypothesized that tension along axons in the white matter explains how the cortex folds (Van Essen, 1997). Strong cortico-cortical and weak cortico-subcortical connections were suggested to result in outward folding, whereas the opposite scenario results in inward folding. Or, in other words, more densely interconnected cortical areas tend to buckle together, thus forming a gyrus. An extension of this hypothesis has been suggested recently, taking into account species-specific differences in gray matter connectivity through the white matter (Herculano-Houzel et al., 2010). According to this model, cortical folding is not driven by the gray matter but by tension in the white matter. This idea was supported by morphological data showing the structure and connections of the prefrontal cortices (Hilgetag and Barbas, 2005).

However, recent microdissection assays revealed that, although axons in the developing brain are indeed under significant tension, the patterns of tissue stress are not consistent with the tension-based hypothesis. Tension exists along axons aligned radially inside the developing gyri and circumferentially in subcortical white matter tracts, but tension is not directed across the developing gyri (Xu et al., 2010). The observed relaxation after cutting was suggested to be attributable to enhanced growth in the gray matter compared with white matter (Xu et al., 2009).

In summary, there is currently no theory that can explain all experimental findings and observations relating to cortical folding. Most current approaches favor either the differential expansion or the tension hypothesis, but direct proof for either theory is still lacking. However, these two hypotheses are not mutually exclusive; both mechanisms are likely to contribute together to shaping the brain. Growth in one area of the gray matter could, for example, not only generate compression locally, but also tension on axons of neurons located within this area, which then transmit these forces and pull on a distant part of gray matter. Future experiments will reveal where, when and how local compression and tension along axons provide the forces that drive cortical folding.

## Mechanosensitivity and mechanotransduction

While it is evident that forces and the mechanical properties of neuronal cells and their environment play a key role in the development of the nervous system, it remains unclear how these properties and forces are sensed and transduced by cells to give rise to the appropriate output.

The molecular basis of cell mechanosensitivity, in particular, is still poorly understood. Principally, every cellular element that is involved in transmitting forces to the environment is also exposed to those same forces. These forces across specific proteins can now be measured within cells with piconewton (pN) sensitivity (Grashoff et al., 2010). Such forces will result in strain of the proteins (and membranes), which, if large enough, could cause conformational changes and be the first step in the mechanotransduction cascade. Possible candidates currently discussed as strain sensors include stretch-activated ion channels, caveolae, cryptic binding or phosphorylation sites, cell adhesion sites [including cell adhesion molecules (CAMs) such as integrins and cadherins, proteins linking CAMs to the cytoskeleton such as

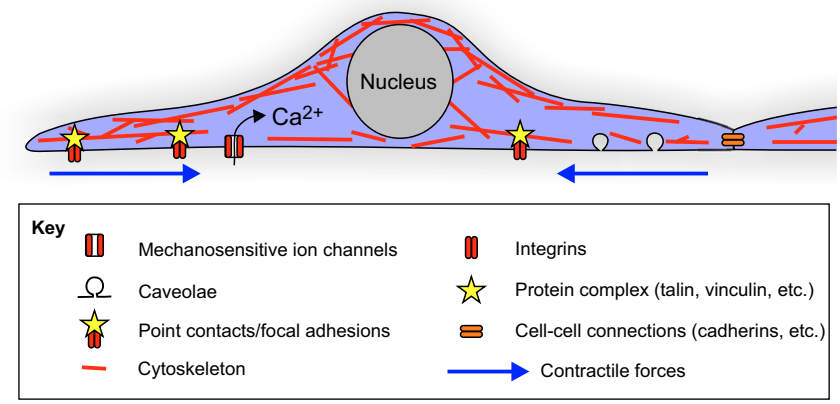


**Fig. 4. Cellular mechanosensitivity.** Every cellular element that is involved in transmitting forces is exposed to the same forces, resulting in strain that could be detected and might serve as the first step in mechanotransduction. Possible strain sensors include stretch-activated ion channels, caveolae, cryptic binding or phosphorylation sites, cell adhesion/connection sites, the cytoskeleton and the nucleus itself. Further possible key players in mechanotransduction include direct physical effects, motor-clutch systems, tension-dependent exo- and endocytosis, or the (slower) activation of transcription factors.

vinculin and talin, signaling proteins such as focal adhesion kinase (FAK), and adaptor proteins such as p130Cas], the cytoskeleton and the nucleus itself (Fig. 4). Further possible key players in mechanotransduction include direct physical effects, motor-clutch systems, tension-dependent exo- and endocytosis, and/or the activation of transcription factors. For recent reviews about mechanotransduction in developmental systems see Wozniak and Chen (Wozniak and Chen, 2009) and Zhang and Labouesse (Zhang and Labouesse, 2012).

Mechanotransduction in neurons seems to involve a motor-clutch system (Chan and Odde, 2008), which couples the actin cytoskeleton to the substrate. Talin and vinculin, which link actin filaments to integrins and which are involved in mechanotransduction (Margadant et al., 2011), are likely to be part of such clutches. During axon outgrowth, FAK is mechanically activated, which reinforces interactions between growth cones and the guidance cue netrin 1 (Moore et al., 2012). Netrin 1, in turn, positively regulates traction forces via Pak1-mediated shootin1 phosphorylation, thus promoting actin-substrate coupling, force generation and axon outgrowth (Toriyama et al., 2013). Finally, calcium influx through mechanosensitive ion channels, which also may affect talin, is involved in the neuronal response to mechanical stimuli (Franze et al., 2009; Kerstein et al., 2013).

It is likely that, similar to chemical signaling pathways, more than one mechanism is involved in cellular mechanotransduction. Furthermore, individual mechanical and chemical cues might activate similar or the same downstream signaling pathways and thus interact with each other. For example, when confronted with the chemical attractant netrin 1, advancing neuronal growth cones increased traction forces by an order of magnitude, resulting in redirection of the axon (Moore et al., 2009). Unraveling the molecular events that enable neurons and glial cells to detect and respond to mechanical stimuli will be key to understanding the contribution of mechanical cues to the development of the nervous system.

Mechanics might even be directly involved in signal transduction. Phototransduction in microvillar photoreceptor cells of *Drosophila*, for example, is mediated by a G protein-activated phospholipase C, which hydrolyses the membrane lipid phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)$P_2$ or $PIP_2$] (Hardie and Raghu, 2001). $PIP_2$ hydrolysis has recently been shown to alter the physical properties of the microvillar membrane, most likely by increasing membrane tension and reducing crowding, which results in a contraction of the microvilli (Hardie and Franze, 2012). The light-sensitive transient receptor potential (TRP) channels seem to respond to the mechanical forces generated by $PIP_2$ hydrolysis rather than to chemical messengers, suggesting that mechanics is used as second messenger in metabotropic signal transduction (Hardie and Franze, 2012). TRP channels are also found in many other neuronal and glial cell types, and it is intriguing to speculate whether a similar, mechanical mechanism is involved in TRP channel activation in other parts of the nervous system. Such a mechanism could have tremendous impact on different aspects of the development of the nervous and other organ systems.

## Conclusions

Many events during the development of the nervous system seem to be controlled by mechanics. Forces acting over different length and time scales drive motion and shape changes, and the cellular susceptibility to mechanical stimuli may be exploited as an additional level of control of developmental processes and as a fundamental way of dealing with a changing environment. Understanding mechanics, which is very likely to be intimately linked to biochemistry, will thus be required to gain a more complete picture of development.

In recent years, technological progress has enabled the analysis and measurements of nervous tissue mechanics with ever increasing resolution, as well as providing first insights into neuronal and glial cell mechanosensitivity and mechanotransduction pathways. However, we are only beginning to understand when, where and how mechanical processes take place in the nervous system *in vivo*. Recently developed mechanics techniques need to be combined with cutting edge biological tools to investigate the interplay of mechanics and biochemistry and to illuminate mechanotransduction in more detail. We also need new techniques that take *in vitro* studies a step further. For example, the mechanical 3D environment that cells encounter *in vivo* is currently difficult to reproduce in cell culture systems. Cell cultures with locally and reversibly ‘tuneable’ mechanical properties would be a great asset for the study of cellular mechanosensitivity. Ultimately, mechanics measurements will have to be performed *in vivo*, which poses a big challenge.

Furthermore, we also need to go back and re-visit fundamental questions in nervous system development using newly developed techniques. For example, most textbooks still ascribe a mechanical function to glial cells: to provide structural support to neurons. However, using AFM, glial cells were shown to be twice as compliant as their neighboring neurons (Lu et al., 2006), which should significantly limit the structural support they can offer. An

involvement of mechanical signaling in neuronal development, migration and/or guidance, in signal transduction cascades, and/or in neuronal network formation would revolutionize our understanding of the development of the nervous system.

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