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Review

Mechanics in the nervous system: From development to disease

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SUMMARY

Physical forces are ubiquitous in biological processes across scales and diverse contexts. This review highlights the significance of mechanical forces in nervous system development, homeostasis, and disease. We provide an overview of mechanical signals present in the nervous system and delve into mechanotransduction mechanisms translating these mechanical cues into biochemical signals. During development, mechanical cues regulate a plethora of processes, including cell proliferation, differentiation, migration, network formation, and cortex folding. Forces then continue exerting their influence on physiological processes, such as neuronal activity, glial cell function, and the interplay between these different cell types. Notably, changes in tissue mechanics manifest in neurodegenerative diseases and brain tumors, potentially offering new diagnostic and therapeutic target opportunities. Understanding the role of cellular forces and tissue mechanics in nervous system physiology and pathology adds a new facet to neurobiology, shedding new light on many processes that remain incompletely understood.

THE FORCE IS STRONG IN CELLS OF THE NERVOUS SYSTEM

Animal tissue cells, including neurons and glia, constantly and actively exert forces on their environment. This is evident when cells navigate through tissue, such as during axon growth or microglial chemotaxis. In order to move, cells exert pushing and pulling forces on their immediate surroundings. For example, during brain development, growth cones at the tip of advancing axons pull themselves and their axons forward,[1,2] and in the absence of dominant growth cones, axons can also push their way through the tissue.[3] The forces exerted by growth cones on their axons also generate tension along the axon,[4] which may regulate axon fasciculation (i.e., bundling),[5] much like a zipper closes when sufficient force is applied. Ultimately, it is mechanical forces that lead to cortical folding in the brain of large mammalian species.[6–8] In essence, forces drive motion at every scale, from the subcellular to the tissue level.

In homeostasis, forces can be perceived by specialized sensory neurons and their associated structures. For example, different types of mechanoreceptors in our skin detect tiny tissue deformations, thus enabling the sense of touch. Similarly, hair cells in the inner ear and the lateral line system of fish transduce pressure (sound) waves into electrical signals, whereas proprioceptors located in the musculoskeletal system sense and respond to various kinematic parameters, fine-tuning body posture and movement. However, apart from these specialized mechanosensors, every other nervous system cell also mechanically interacts with their surrounding tissue. Even in the absence of external forces or discernible motion, stationary tissue cells, such as neural progenitor cells, mature neurons, or quiescent astrocytes, actively probe their mechanical environment.

These cells possess a contractile actomyosin cytoskeleton, which generates and maintains a baseline tension—a force directed in parallel to the cell surface. The actin cytoskeleton is coupled to the external environment via transmembrane proteins such as integrins and cadherins. Through this linkage, forces generated by actomyosin are transmitted to the cell membrane and neighboring cells and/or the extracellular matrix (ECM). Concurrently, neighboring cells exert forces back, and the ECM may resist cellular forces to varying degrees depending on its mechanical properties. Despite cells sensing and responding to such mechanical signals, the complex mechanical interactions between nervous system cells and their environment are often overlooked, in part due to a lack of tools and techniques.

Neuro-mechanobiology has gained significant traction only in recent years, with the merging of the physical with the life sciences and the resulting development of new methods for measuring and manipulating cellular forces and tissue mechanics, coupled with the discovery of crucial mechano-responsive proteins. Examples of such proteins include mechanosensitive ion channels (MSCs) of the Piezo family[9] and the transcriptional regulator yes-associated protein (YAP).[10] In this review, we highlight recent advances in the field, unveiling

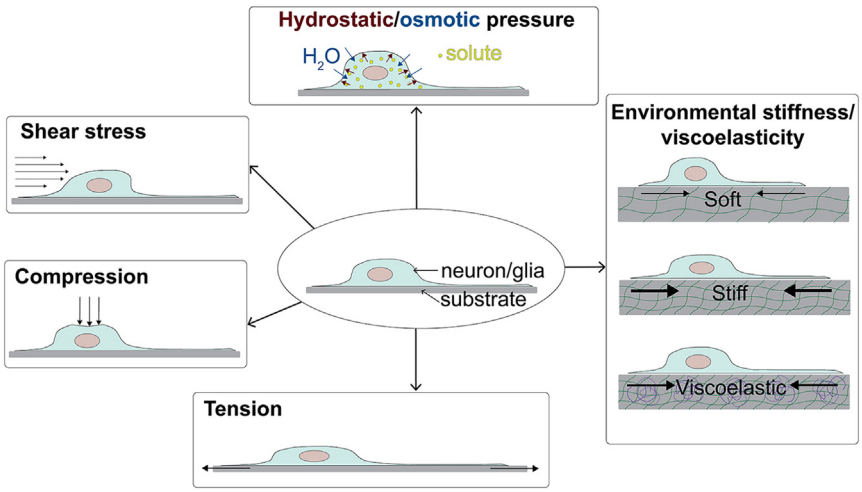

Figure 1. Mechanical signals encountered by nervous system cells

Schematic representation of active external forces (left and middle) and passive mechanical properties of the environment (right) experienced by cells: tension, compression, shear stress, hydrostatic/osmotic pressure, and environmental stiffness/viscoelasticity. Closed arrows indicate directions of forces. Arrow thickness in right panel indicates the magnitude of forces (i.e., on stiffer substrates, cells generate higher forces than on softer substrates).

compelling contributions of mechanical signals to the development, normal function, and pathology of the nervous system. Specifically, our focus lies on cells that are not directly associated with any mechanical senses.

MECHANICAL SIGNALS ENCOUNTERED BY NERVOUS SYSTEM CELLS

Neurons and glial cells constantly encounter diverse mechanical cues (Figure 1). They actively explore the passive mechanical properties of their environment, including tissue stiffness, tissue viscosity, and, in the case of motile cells, the available space. Simultaneously, they passively experience forces generated actively by neighboring cells, fluid flows, or larger-scale tissue movements.

Passive material properties of CNS tissue

What is being measured?

Overall, the brain and spinal cord are among the softest tissues in our body.[11] Central nervous system (CNS) tissue is mechanically heterogeneous, with certain regions being stiffer or softer, or more or less viscous, than others.[12–21] These stiffness heterogeneities result in distinct patterns of mechanical signals, analogous to the chemical signal patterns that shape CNS development and repair.

Brain tissue progressively stiffens during development, throughout maturation, and into adulthood,[12,18,22–26] where brain stiffness plateaus. However, these stiffness changes occur non-monotonically, and in humans and mice, brain softens again from ~40 years[27] and ~10 months of age,[26] respectively. Moreover, mechanical properties of brain tissue also undergo changes in many diseases and after injury (see mechanics in pathological processes section). However, whether these mechanical changes are merely an accompanying feature that can be used for diagnosis or if they are causally linked to the onset and progression of the disease[28] remains to be determined.

Importantly, there is not one single value that characterizes the mechanical properties of CNS tissue. Tissue is viscoelastic, behaving both elastically (i.e., it stores the applied energy and resumes its shape after being deformed) and viscously (i.e., the applied energy is dissipated or lost through viscous flow). Elastic stiffness is usually characterized by an elastic (storage) modulus (Pa), whereas viscosity (Pa·s) is represented by the loss modulus (Pa). Furthermore, water, a major component of tissues, flows through the network of cells and ECM, influenced by the tissue's local porosity, impacting mechanical properties and measurements as well. Finally, motile cells may encounter tissues with varying degrees of connectivity. Loosely connected tissue allows for easier movement through it, whereas densely packed tissue may restrict cell motility, resulting in steric hindrance. We will primarily focus on tissue stiffness as an important mechanical parameter that regulates cell function.

Viscoelastic materials are characterized by time-dependent deformation, i.e., when a constant force is applied, the material's deformation increases with time. Hence, brain tissue feels softer when forces are applied on a slower timescale compared with when forces are applied more rapidly.[28,29] For example, when brain tissue stiffness is assessed by applying a small stress (i.e., force per area) at the seconds timescale (at a frequency of ~1 Hz), such as during atomic force microscopy (AFM) measurements, it has an elastic modulus on the order of ~100 Pa. If the same tissue is measured at a higher frequency, for example, using magnetic resonance elastography (MRE) (see also the subsection on brain tumors) or ultrasound-based shear-wave elasticity imaging, it appears much stiffer, with an elastic modulus on the order of ~10 kPa.[26,27] In short, the rate of force application can affect the perceived stiffness of brain tissue.

A key factor contributing to this difference in behavior is that, on slower timescales, the energy applied through the measuring device can be dissipated by viscous flow—the material has time to relax, and water can flow away from the region where the force is applied. However, at faster timescales, this is not the case, and the material increasingly resists the applied forces.

Hence, it is crucial to select the appropriate measurement parameters based on the problem being studied. For instance, when investigating how cells sense mechanical properties of tissue ("mechanosensing"), forces should be applied to the tissue at a timescale similar to that at which cells probe their mechanical environment, typically within seconds to minutes. On the other hand, computational studies on how the brain deforms

during the initial impact leading to traumatic brain injuries, where the head (and thus the brain) experiences a sudden, very quick external mechanical impact, should be based on mechanical brain properties assessed at these rapid timescales (~kHz and above).

The local mechanical properties of CNS tissue depend on the mechanical properties of local tissue constituents, including neurons, glial cells, ECM, and blood vessels. Glial cells, for instance, are about twice as soft as their neighboring neurons.[29] Thus, a region with a higher proportion of glia will likely be softer overall. Furthermore, different ECM components have different mechanical properties, which may explain why the neurogenic niche in rodents, which is rich in the ECM-cross-linking enzyme transglutaminase,[30] is stiffer than the remaining brain parenchyma.[19] Alterations in any of these components, both in composition or arrangement, could account for alterations in CNS tissue mechanics during development and disease.[12,18,23–25,31]

Additionally, the density of cells has been shown to correlate with local CNS tissue stiffness; however, this relationship is not straightforward. In embryonic *Xenopus* brains, regions with higher stiffness strongly correlate with high cell densities,[20] and perturbing mitosis leads to a decrease in both cell density and tissue stiffness.[25] However, in developing chick somites, cell densities increase only after the tissue has stiffened,[32] and in the mouse hippocampus, cell densities negatively correlate with tissue stiffness.[33] The discrepancies in the relationship between cell density and local CNS stiffness could be the result of various cell-intrinsic factors, such as cell type and stage, the mechanical properties of individual cells, e.g., their cytoskeletal composition and arrangement, as well as the adhesion strength (i.e., coupling) between the cells. All in all, the study of how tissue microarchitecture affects local tissue mechanical properties is an active field of research, with more questions than answers at present.

How to measure tissue mechanics?

Obtaining measurements of brain tissue mechanics is further complicated by a dearth of available methods. Shear rheometers, commonly available in most engineering and materials science departments, can be used to reliably measure the viscoelastic properties of CNS tissue slices. However, these measurements have limited spatial resolution (~cm) and tell us very little about properties at the cellular or molecular scale. A much higher resolution can be achieved using AFM-based indentation measurements, which have become a gold standard in the field.[34,35] AFM applies forces at time and length scales relevant to cellular mechanosensing and can generate stiffness (and more recently, viscoelasticity) maps of tissues *in vivo* and *ex vivo* at cellular resolution.[15,20,25,36] One important limitation of this method, however, is that measurements are restricted purely to the surface of a material. As such, to probe mechanics deeper within tissues, non-contact methods are required. MRE and Brillouin microscopy are recent techniques that indirectly assess mechanical properties such as shear-wave speeds and longitudinal moduli, respectively, within tissues. Although MRE and Brillouin measurements may correlate with direct mechanical tests (e.g., by AFM),[13,37–39] more work is needed to fully understand how the measured parameters relate to tissue stiffness and viscosity as sensed by cells.

To summarize, the mechanical properties of neural tissue are complex and depend on several measurement parameters, including the frequency (i.e., speed) at which the tissue is probed and the applied strain (i.e., the tissue deformation as a function of how much force is applied).[28] As different methods work at different length and timescales, results obtained from the same tissue may vary significantly, depending on the method and parameters used. However, when the mechanical properties of CNS tissue within the same system are assessed using similar approaches, the results are highly reproducible, demonstrating that cells encounter robust time- and location-specific mechanical signals.

Another factor contributing to the wide variation in CNS mechanics data in the literature is sample preparation. Tissue samples need to be very fresh, with measurements conducted within a few hours post-mortem and maintained in physiological buffer solution at the right temperature, as their mechanical properties may otherwise change quickly.[34] Freezing, drying, or fixing of tissues, even if only partial, will massively alter their mechanical properties and fail to preserve relative local differences in tissue stiffness. However, after excluding these studies, the remaining body of literature is largely consistent.

Active forces experienced by CNS cells

Motile CNS cells, including microglia and neuronal growth cones, exert forces on their environment[1,2,40,41] and pull on adjacent cells.[8,42,43] Furthermore, growth cones pull on their own axons, and axons themselves are contractile.[1,4,5,41,44–50] This means that axons are under tension from early growth and even after connections are established. Additionally, dendrites generate tensile forces, which, for example, pull on the overlying nose skin cells in the developing zebrafish, triggering nostril opening.[51] On a larger scale, it has long been demonstrated that air or fluid flow or pulsating blood vessels generate forces that may be perceived by CNS cells.[52,53] Ultimately, tissue movements, such as those occurring during cortex folding and brain growth, are driven by and generate forces.[6,54] These forces are transmitted to cells and the ECM within the tissue. Overall, these active forces are also sensed by CNS cells and contribute to regulating their migration, morphology, and overall function.

MECHANISMS OF MECHANOTRANSDUCTION

Mechanotransduction is the process by which cells convert mechanical forces into biochemical or electrical signals. Cellular forces are generated largely by actin polymerization and the interaction of actin with myosin motors, which leads to contractility and a smaller degree by microtubule polymerization and dynein-based microtubule sliding (for recent reviews on neuronal forces see Franze[1] and Miller and Suter[41]). These intracellular forces are transmitted through the cell membrane to the ECM and neighboring cells via protein complexes containing cell adhesion molecules (CAMs) such as integrins and cadherins, respectively. Each protein in this linear force chain, from actin to adapter proteins, (e.g., talin and vinculin), to adhesion molecules to ECM, experiences the same force. If this force surpasses intramolecular forces, it may lead to conformational changes in any of these proteins. Because the function of a

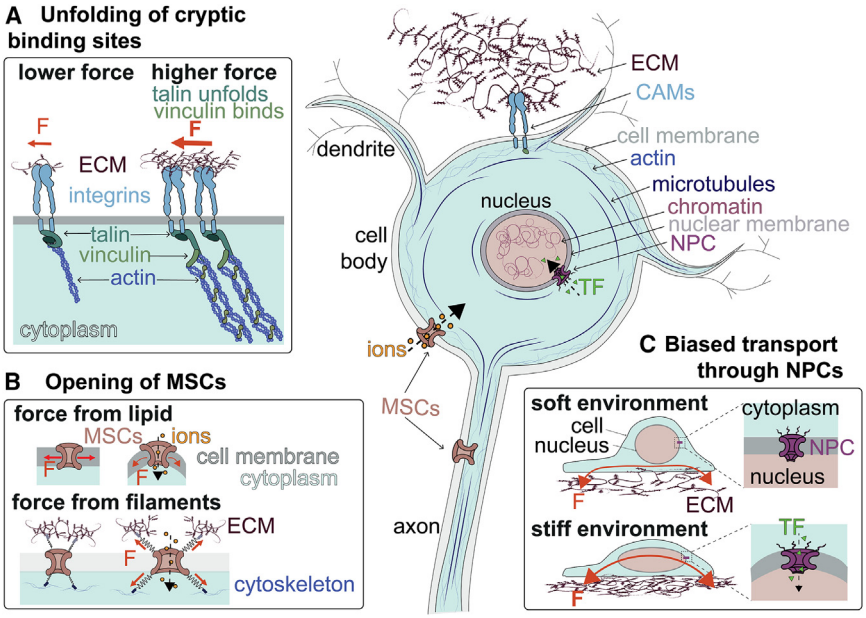

Figure 2. Mechanisms of mechanotransduction

(A) Unfolding of cryptic binding sites. Talin, for example, which connects actin to integrins, has cryptic binding sites for vinculin. At low force regimes, vinculin cannot bind to talin, while at high forces, talin domains unfold and vinculin can bind, linking talin to more actin via vinculin, thus reinforcing traction forces.

(B) Opening of mechanosensitive ion channels (MSCs) allows an influx of ions along their concentration gradients, leading to biochemical alterations within the cell. Forces can be transmitted directly through alterations in the lipid bilayer (force from lipids) or by linkage via auxiliary proteins such as the extracellular matrix (ECM) or cytoskeleton (force from filaments).

(C) Biased transport of transcription factors between the cytoplasm and the nucleus through nuclear pore complexes (NPCs). On stiffer substrates, cells are more spread, deforming the nucleus, leading to a change in nuclear envelope geometry and permeability of the NPC to transcription factors such as YAP. *F*: denotes forces. Closed red arrows indicate direction of force, closed dashed arrows indicate flow direction of ions/transcription factors through a channel/pore. CAMs, cell adhesion molecules; TF, transcription factor.

protein is governed by its shape, such conformational changes can lead to functional changes. These force-induced changes in activity may lead to side effects in proteins that are not primary mechanosensors, such as N-methyl-D-aspartate (NMDA) receptors.[55] However, many proteins in CNS cells are meant to sense and respond directly to mechanical signals. In this section, we briefly highlight the currently best-studied mechanosensitive proteins and mechanotransduction mechanisms that are relevant to neurons and glial cells (Figure 2).

Unfolding of cryptic binding sites

One way in which such conformational changes may impact protein function is through the unfolding of cryptic binding sites (Figure 2A). One of the best-studied examples in mechanobiology is the interaction of the two cytoskeletal proteins vinculin and talin. In its relaxed state, talin, which connects actin filaments to integrins, has several cryptic binding sites for vinculin. Thus, in the absence of forces, vinculin cannot bind to talin. However, forces above a certain threshold led to the unfolding of talin domains and the exposure of these binding sites.[56] When talin is unfolded, vinculin can bind to talin, indirectly linking talin to more actin through vinculin's actin-binding sites. This reinforces the link between actin and talin, enabling the generation and transmission of larger forces.[57] The increased forces generate a positive feedback loop, leading to further unfolding of talins, reinforcing the connection between talin and vinculin, further leading to more binding, etc.

Stiffer substrates provide more traction than softer ones, enabling a faster buildup of forces.[58] Furthermore, integrins form catch bonds with their ECM binding partners,[59] and the lifetimes of these bonds increase with the tensile force applied. Hence, on soft substrates with a stiffness below a cell type-specific threshold value, the lifetime of integrin bonds is shorter than the time needed to build up sufficient force to unfold talin. However, above this threshold, the longer lifetimes of integrin bonds allow for the buildup of sufficiently high forces to unfold talin, which in turn leads to increased vinculin recruitment, actin engagement, reinforcement, and growth of adhesion sites, thus ultimately amplifying the mechanical signal of "stiffness." As a consequence, the (traction) forces exerted by neurons and glial cells increase on stiffer substrates.[60–65] Depletion of vinculin leads to the loss of this mechanoresponse in neurons, rendering them insensitive to substrate stiffness.[64]

Further proteins found at adhesion sites, which are involved in mechanotransduction cascades, include the tyrosine kinases Src kinase and focal adhesion kinase (FAK), potentially linking mechanical signaling to receptor-mediated chemical signaling cascades.[66]

Opening of MSCs

MSCs are pore-forming transmembrane proteins that sense and respond to mechanical stimuli, such as stretch, bending, torsion, pressure, shear, osmotic forces, and touch, by changing the opening probability of their pore (Figure 2B). They are among the most rapid signal transducers known, converting mechanical signals into relevant biochemical signals within tens of milliseconds.[67] The activation of MSCs is thought to be mediated either by forces transmitted directly through the lipid bilayer (membrane-delimited "force from lipid" models)[68] or by auxiliary proteins, such as the ECM and/or the cytoskeleton, which transmit forces to the channel complex ("tethered" models).[69–71] There has been considerable progress in understanding the proposed gating mechanisms and structures of MSCs[72,73]; however, there is still much to learn about the specific mechanical signals that cells respond to *in vivo*.[70,73] Regardless of the activation mechanism, MSCs respond to mechanical forces along the plane of the cell membrane, allowing an influx of, usually, cations e.g., Na^+, K^+, and Ca^{2+}, through the channel along their concentration gradients.

MSCs identified in the nervous system include the epithelial sodium channel (ENaC)/degenerin (DEG) channels, transient receptor potential (TRP) channels, TWIK-related potassium channel (TREK) and TWIK-related arachidonic acid activated potassium channel (TRAAK) which are members of the two-pore domain K^+ (K_2P) ion channels family, and Piezo channels. However, with the exception of Piezo1 and Piezo2, it is not clear whether in vertebrates these channels are primary mechanosensors or rather activated downstream in mechanotransduction cascades. Furthermore, although TRP, K_2P, and ENaC/DEG channels affect nervous system function, these channels not only respond to mechanical cues but also to molecular signals and changes in pH and temperature. Thus, isolating the effects of mechanical signaling from these other modalities is complex. Piezo channels, on the other hand, exclusively respond to mechanical signals, providing an advantage in mechanostransduction studies, as the responses to mechanical cues are not confounded by other signals.

Mechanosensing through Piezo channels plays a diverse and critical role in the nervous system, from early development to adult function, and potentially also in neurological disorders (for a recent review, see Delmas et al.[74]). Piezo is a non-selective cation channel, which is largely thought to be activated by an increase in membrane tension.[75] This notion is based on *in vitro* and *in silico* studies, and the absence of binding sites with the cytoskeleton and the ECM. However, although Piezo1 activity increases with larger actomyosin-based traction forces on stiffer substrates,[76] membrane tension in axons does not.[60] Recently identified interactions of Piezo1 with cadherins[77] may potentially explain this discrepancy, or an increase in membrane tension could be restricted to the immediate vicinity of the force-transmitting adhesion sites. How Piezo channels are activated *in vivo* will need to be addressed in future work.

Experimentally addressing the mechanosensitivity of cells is not straightforward. There are a few drugs available impacting MSC activity. Currently, there are a small number of synthetic agonists—Yoda1, Jedi1, and Jedi2—specific to Piezo1,[78,79] whereas there is no equivalent agonist available for Piezo2. These agonists should only be applied on short timescales, as they allow cations to flow along their concentration gradients, depleting membrane potentials on longer timescales. MSC inhibitors, such as gadolinium ions (Gd^{3+}), ruthenium red, and the spider venom peptide GsMTx4, are typically non-specific. They exert their effects on multiple ion channel classes and mostly act indirectly by altering membrane mechanics[80,81] rather than directly or specifically blocking the channel. Genetic manipulations such as knocking down/out or overexpressing various MSCs have vastly increased our understanding of the role of these channels in diverse biological systems. Nonetheless, the question often remains what the origins and nature of the native forces within a cell are, which activate Piezo and other mechanosensors.

Biased transport of TFs through NPCs

Another important mechanism of mechanotransduction is through mechanically regulated shuttling of transcription factors (TFs) between the cytoplasm and the nucleus[10] (Figure 2C). These TFs are active when inside the nucleus but not when outside of it.

On stiffer substrates, cellular traction forces increase (see above), resulting in more cell spreading and thus greater deformation of the nucleus.[82] This nuclear deformation leads to a change in curvature of the nuclear envelope, thereby altering the geometry and, thus, permeability of nuclear pore complexes (NPCs). Permeability changes of NPCs depend on the cargo's molecular weight and its affinity for nuclear transport receptors. The molecular-weight-dependent increase in NPC permeability is larger for passive diffusion than for facilitated nucleocytoplasmic transport, leading to a force-dependent bias in cargo transport into or out of the nucleus,[83] thus linking tissue stiffness to transcriptional regulation via the regulation of cellular forces.

The first transcriptional co-regulators whose activity was identified to be controlled by the stiffness of the substrate on which cells grew were YAP and transcriptional coactivator with PDZ-binding motif (TAZ).[10] Although YAP was initially recognized as a downstream regulator of organ growth in the Hippo pathway, it was then found to be mechanically activated independently of Hippo signaling.[10] Additionally, although other mechano-responsive TFs have been identified,[83,84] YAP has remained a prominent focus in cellular mechanotransduction studies since its discovery in 2011.

Although this force-dependent regulation of TF activity may be less critical for processes occurring in the axons of neurons—far away from the nucleus—it may constitute an important mechanism regulating the function of CNS cells without distinct axons. We provide several examples in the following subsection.

Further mechanisms of mechanotransduction

In addition to these well-established mechanosensitive structures and mechanotransduction mechanisms, there are many other ways to translate mechanical cues into an intracellular, biochemical response.[85] For instance, primary cilia found in most cell types are potential mechanosensitive structures,[86] and membrane reservoirs containing specific receptors could be pulled open by forces, facilitating targeted chemical signaling cascades if the appropriate signaling molecules are present. Membrane reservoirs could also regulate membrane tension and thus, indirectly, the activity of MSCs. Furthermore, compression and tension of polymers, such as F-actin or microtubules, change their chemical potential.[87] Consequently, post-translational modifications of cytoskeletal proteins may be regulated by and/or regulate mechanical signals. For example, microtubule acetylation has recently been identified as a crucial component in cellular mechanotransduction events.[88–90] As a final example, mechanosensitive G-protein-coupled receptors have been suggested to be involved in detecting shear stress and/or cell swelling/stretch,[91] although the exact mechanotransduction mechanisms are still poorly understood. These examples represent a fraction of the structures involved in cellular responses to mechanical signals, and further investigation will shed light on their intricate interactions.

Integrating different signals

Ultimately, the various mechanisms discussed synergistically contribute to cellular responses. Contractile forces generated by the actomyosin cytoskeleton are transmitted toward (1) adhesion sites, where they are transmitted to the extracellular

environment; (2) membrane via linking proteins such as ezrin, radixin, and moesin (ERM proteins), potentially influencing membrane tension and MSC activity; and (3) the nucleus via linker of nucleoskeleton and cytoskeleton (LINC) complexes containing nesprin and other linking proteins, potentially impacting TF shuffling. Adhesion proteins, Piezo1, YAP, and others are thus often activated collectively,[89,92–95] and their activity may be interconnected. For example, depleting Piezo1 in cells cultured on glass leads to more nuclear exclusion of YAP.[92] The interactions of different mechanotransduction mechanisms could fine-tune the cellular response to mechanical cues.

In addition to mechanical signals, cells are also exposed to a plethora of chemical cues, such as signaling molecules, and need to integrate these different signals. In the end, mechanical and chemical signals converge on shared intracellular downstream signaling pathways, influencing common gene regulatory networks, and thus potentially affecting similar gene expression patterns and functions of CNS cells.[96–98] For example, the activities of the tyrosine kinases' FAK and Src kinase are regulated both chemically and mechanically,[66] as are intracellular levels of the second messenger Ca^{2+}.

On one hand, mechanical signals can modulate intracellular and extracellular chemical signaling. For instance, inhibiting Piezo1 increases the secretion of pro-inflammatory mediators from microglia.[99] Conversely, activating Piezo1 *in vitro* inhibits the release of cytokines and chemokines, such as interleukin (IL)-1β, IL-6, and tumor necrosis factor alpha (TNF-α), from lipopolysaccharide (LPS)-stimulated astrocytes[100] and microglia.[101] These findings suggest that Piezo1 activity could decrease neuroinflammation—potentially through Ca^{2+} signaling—by inhibiting the nuclear factor (NF)-κB inflammatory signaling pathway.

On the other hand, chemical signals may modulate mechanical signaling. When advancing growth cones of commissural neurons encounter immobilized netrin-1, a well-established chemical axon guidance cue, traction forces increase,[102] likely through p21-activated kinase 1 (Pak1-mediated phosphorylation of the "clutch" protein shootin1, which couples F-actin to the adhesion molecule L1CAM.[103,104] Similarly, microglial traction forces and their migratory bias toward stiffer substrates ("durotaxis") increase after exposure to LPS,[62] a potent immune activator.

Understanding the integration of chemical and mechanical signals in CNS cells will be key to understanding CNS development and disease and should thus be a focus of future research. In the following sections, we will review recent evidence for the role of mechanics in regulating CNS development, homeostasis, and disease.

MECHANICS IN NERVOUS SYSTEM DEVELOPMENT

Given the dynamic interplay between the physical environment, cellular mechanosensing, gene transcription, and cell function (reviewed in Wagh et al.[105]), mechanics unsurprisingly plays an important role in shaping nervous system development. In this section, we will explore how mechanics affects neural stem cell proliferation, differentiation, migration, axon growth and connection, and cortex folding (Figure 3).

Proliferation

The proliferation rate of neural stem cells cultured in 3D substrates inversely scales with the stiffness of the substrate.[106] After 7 days in culture, neural stem cell numbers increased 14-fold in soft substrates (~0.2 kPa), whereas in stiff substrates (~20 kPa), the number of cells merely doubled. Similarly, brain organoids encapsulated in stiffer (~3.6 kPa) gels showed more restricted growth, forming fewer neuroepithelial buds compared with organoids embedded in gels that were 30× softer, and in stiffer environments, cells shifted from a proliferative to a more differentiated state.[107]

Although substrate stiffness likely contributes to this mechano-response, the observed behavior might largely be a consequence of the available space. As cells proliferate, their total volume increases, causing the colony to expand as long as space is available. In a confined 3D environment, proliferating cells will push against the surrounding substrate, thus generating pressure (this pressure may be important in cortex folding, elaborated below). In a softer 3D environment, space can be generated easily by shifting the material boundary (i.e., by deforming the matrix) through the pressure generated by growth, facilitating cell proliferation. In contrast, in a stiffer environment, growth pressure leads to less deformation of the surrounding matrix and hence to less space available for cells to grow into.

Additionally, in the yeast *Saccharomyces cerevisiae*, growth-induced pressure leads to increased osmotic pressure and macromolecular crowding within the cell, potentially hindering protein expression and diminishing cell growth.[108] This mechanism is likely to be evolutionarily conserved and could contribute to reduced neural cell proliferation in stiffer environments.

These phenomena could explain the preference for proliferation in softer 3D *in vitro* environments. Hence, substrate softness may serve as a permissive cue, rather than an instructive one, in cell proliferation. This implies that brain tissue stiffness should not exceed a certain critical value to maintain an environment conducive to cell proliferation. However, it is important to note that a feedback loop may exist, as demonstrated in the developing *Xenopus* brain, where cell proliferation leads to an increase in tissue stiffness.[25]

In addition to environmental stiffness, cells in the developing nervous system are also subjected to fluid flow-based shear forces. An ENaC-dependent mechanism was shown to induce the proliferation of adult neural stem cells in the murine subependymal zone in response to fluid flow.[109]

Differentiation

Following the initial proliferative divisions of neural stem cells, asymmetric divisions occur, generating more stem cells, non-stem cell progenitors, neurons, and eventually glia.[110] Whether progenitor cells differentiate into neurons or glia may be influenced, in part, by the mechanical properties of the environment, at least *in vitro*. For example, induced neural stem cells exhibited a preference for differentiating into neurons on softer gels and glia on stiffer gels.[111,112] As brain tissue stiffness increases throughout development,[12,18,22–25] one could speculate whether this alteration in stiffness affects the shift from neurogenesis to gliogenesis during brain development. Interestingly, when cultured on soft gels, rat adult hippocampal neural stem cells

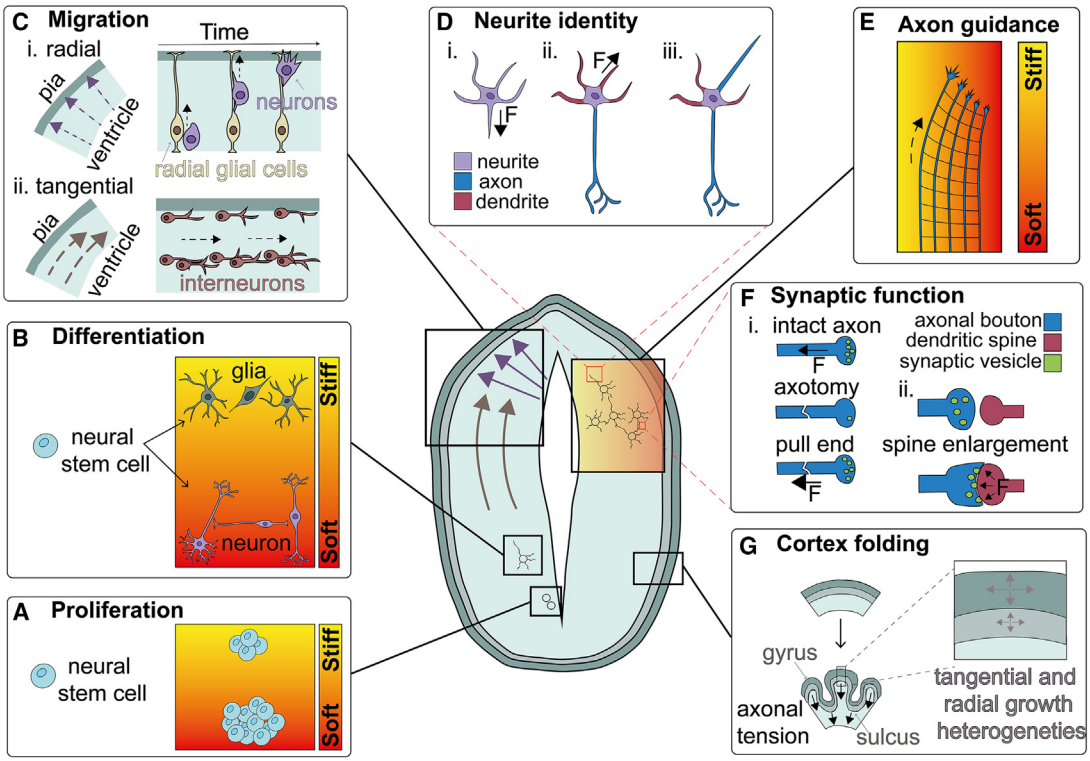

Figure 3. Mechanical regulation of developmental processes in the nervous system

(A) *In vitro*, neural stem cells are more proliferative in softer than in stiffer substrates.

(B) Neural stem cell fate is also affected by substrate stiffness *in vitro*.

(C) Migration occurs radially from the ventricle toward the pial surface and tangentially along the brain surface, forming the appropriate cortical lamination. *In vitro*, cell migration is regulated by substrate stiffness.

(D) The application of tension to a neurite is sufficient to make it an axon *in vitro* (Di and Dii), and in the presence of an existing axon, an additional axon can be generated by external tension (Dii and Diii).

(E) Axon growth velocity is higher on stiffer substrates. As fasciculated axons are coupled to each other, the faster growing axons on the stiffer side are pulled toward the slower growing axons on the softer side, resulting in turning of an axon bundle exposed to a stiffness gradient *in vitro* and *in vivo*.

(F) Mechanical tension along an axon regulates vesicle clustering at the presynaptic terminal (Fi). Severing the axon decreases tension along the axon, resulting in a loss of vesicle clustering at the neuromuscular junction. Pulling on the severed end is sufficient to restore clustering *in vivo*. Similarly, mechanical pushing of the axonal bouton by dendritic spine enlargement results in an increase of vesicular release from the presynaptic bouton (Fii).

(G) Several mechanical forces contribute to the folding of the brain cortex, including differential expansion of specific brain regions due to heterogeneities in cell proliferation and migration, and tension along axons within the tissue. *F*: denotes forces. Closed filled arrows indicate direction of force, dashed arrows indicate direction of migration.

also preferentially differentiated into neurons on softer substrates and into glia on stiffer ones. In contrast, human neural stem/progenitor cells displayed the opposite behavior,[92] highlighting species or cell-type-specific differences either in mechanotransduction and/or in the readout of conserved mechanotransduction mechanisms.

Various mechanotransduction mechanisms have been identified to impact the fate decisions of neural precursor cells *in vitro*. For example, Piezo1 activity in human neural stem/progenitor cells was lower on softer substrates than on stiffer ones,[92] and perturbations of Piezo1 activity resulted in a decrease in neurogenesis and an increase in astrogenesis.[92] The effect of Piezo1 activity on neural stem cell fate may be mediated by cholesterol biosynthesis.[113]

Moreover, in neural precursor cells cultured on stiff substrates, YAP translocates into the nucleus, whereas on soft substrates, it remains cytoplasmic, and YAP activity has been linked to neuronal differentiation.[92,114,115] Similarly, the TF Olig1, which is involved in oligodendrocyte formation and maturation, is translocated to the nucleus on stiff but not on soft substrates.[84]

The effect of environmental stiffness on differentiation extends beyond the developing nervous system as adult neural stem cells also respond to substrate mechanics.[19,116,117] Neurogenic niches in adult mouse brains are about twice as stiff as non-neurogenic niches.[19] When neural stem cells from these niches were cultured on substrates with stiffness levels corresponding to those of the non-neurogenic and neurogenic niches, higher neuroblast formation was found on the stiffer gel, further supporting the role of mechanics in neural differentiation.[19] Piezo1 is involved in adult neurogenesis and cognitive function; Piezo1-deficient mice have impaired hippocampal volume, learning, and memory functions.[117]

Migration

After neurogenesis, neurons migrate from their proliferative and neurogenic niches throughout the CNS to form the appropriate

lamination and connectivity within the brain.[118–120] This process involves migration along various substrates such as the surrounding ECM, across neighboring neurons, over other axons, and along radial glia cells. These substrates have their intrinsic mechanical properties that likely contribute to directing neuronal migration.

For example, Cajal-Retzius cells, which are some of the first neurons to emerge, develop in various proliferative niches in the brain before migrating to the cortical surface. The marginal zones of the developing mouse cortex, along which these cells migrate, are characterized by stiffness heterogeneities.[121] *In vitro*, Cajal-Retzius cells migrated across longer distances on stiffer substrates. However, Cajal-Retzius cells from different proliferative niches migrated at different rates and exerted different forces on the substrate, even when the stiffness of the substrate was the same. This suggests that cell-intrinsic mechanical properties may also influence migratory patterns, in addition to the mechanical properties of the environment.

Identity and growth

After migrating neurons arrive at their final location, they form connections and networks that are essential for brain development and function. Neurons polarize by developing several projections, including a single axon that transmits information and, typically, several highly branched dendrites that receive information. In this subsection, we explore the role of mechanics in neurite identity (axon vs. dendrite) and growth, whereas the final subsection addresses morphology and connections.

Seminal work from the 1980s demonstrated that the external application of mechanical tension to the neurites of chick sensory ganglion neurons is sufficient to induce axon formation.[122] Further studies in hippocampal neurons revealed that applying tension to other neurites, even in the presence of an existing axon, resulted in the formation of additional axons.[123] However, it remains to be shown whether neurite tension generated by advancing growth cones[1,40] is sufficient to initiate axon formation.

Once the axon is specified, it needs to elongate until it reaches its target, where it stops growing and forms synapses. Comprehensive reviews on the forces involved in axon growth can be found here.[1,40,41] In essence, axon elongation can occur through the following two distinct processes: tip growth, where the growth cone at the tip of an axon exerts forces on its surroundings and pulls the axon forward, and towed growth, whereby the connected axon shaft stretches as the organism grows.

As the growth cone inches forward, its directionality is influenced by a complex interplay of chemical and mechanical cues. Chemo-attractive or -repulsive signals cause asymmetric remodeling of the cytoskeleton and thus traction forces exerted by the growth cone on substrates, thereby changing the direction of force application and hence axon growth. In addition, mechanical cues, such as tissue stiffness, also affect axon turning: when encountering stiffness gradients, axons of *Xenopus* retinal ganglion cells turn toward softer tissues *in vitro* as well as *in vivo*.[20,25] This turning may be a direct consequence of larger neuronal forces on stiffer substrates,[63] leading to torque in axon bundles growing on stiffness gradients.[124] However, the MSC Piezo1 is also involved in regulating axon growth in this system.[20]

MSCs are also involved in stopping axon growth and potentially in axon branching or pruning. Local mechanical stimulation of growth cones may lead to a calcium influx through MSCs, leading to neurite retraction.[125] Different parts of a neuron show different calcium responses, with axons showing higher susceptibility to mechanical stimulation and sustained responses, whereas dendrites exhibit transient responses.[126] Along these lines, EnaC/DEG channels are important for dendritic arbor morphogenesis,[127] and TRP channels may inhibit axon growth,[128] affect axon turning,[129] or promote neurite elongation.[130]

A beautiful *in vivo* example of towed axon growth has recently been identified in the developing zebrafish olfactory placode. After axon tip growth is terminated and synapses have formed, the cell body moves away from the axon terminal, leading to the extension of the axon. The forces driving this mode of axon extension are not cell intrinsic but rather originate from neighboring cells, which push the soma of the neuron away.[42]

Similarly, growing dendrites in the nematode *C. elegans* sense mechanical forces through MSCs of the DEG/ENaC family.[127] Ion currents triggered by the activation of these MSCs are required for proper adhesion and thus force transmission of the dendrites. Inhibiting ENaC/DEG channels resulted in decreased dendritic growth and branching *in vivo*, whereas overexpression of Piezo in these DEG/ENaC mutant worms rescued dendritic growth.[127]

The tension required for axon and dendrite elongation can be maintained *in vitro* and *in vivo* after growth is completed. Reduced axonal tension results in neurite retraction,[4,41] emphasizing the significance of tension in maintaining neurite structure. In the following subsection, we will illuminate the critical role of axon tension in the establishment and maintenance of neural networks.

Neural networks

Mechanical forces are important for diverse processes leading to the formation of neural networks. For example, as briefly mentioned in the introduction, axon zippering and unzippering in growing axon bundles and existing neural networks arise from the competition of axon-axon adhesion and mechanical tension along the axons.[5] Hence, axonal tension is a critical parameter controlling the fasciculation of axon bundles.

Furthermore, in rat hippocampal neurons, membrane tension propagates along axons but not dendrites. High tension at the growth cone promotes rapid axon growth while suppressing branching; conversely, low tension at the growth cone front (e.g., through a physical obstacle) enhances branching and the growth of collateral structures.[131] Thus, the propagation of membrane tension plays a crucial role in controlling the geometry of neurons.

Dendrite branching is also mechanically regulated and increases with substrate stiffness.[132] The resulting branching patterns can be modified through perturbations of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and NMDA receptors or by treating the neurons with astrocyte-conditioned

media, confirming that neurons integrate mechanical and chemical signals.

Once connections are made and neural networks are established, synapse shape and function depend not only on electrical and chemical signals but also on mechanical factors. Mechanical aspects of synaptic structures (e.g., cytoskeletal dynamics, CAMs, and other mechanoregulatory molecules) that potentially affect connectivity and plasticity are reviewed in Kilinc,[133] with a focus on dendritic spine dynamics in Minegishi et al.[134] while the role of forces in synapse function is discussed below.

Cortex folding

At the tissue level, the folding of the cerebral cortex of large mammalian species is a mechanical process.[7] Work on the role of mechanics in cortex folding commenced almost half a century ago.[135] Although the origin of the forces required to fold the brain is still not fully understood, the widespread hypothesis that the cortex folds because the skull imposes a mechanical constraint can be ruled out.[136,137]

Under pulling ("tensile") forces, tissues will stretch, and because they are viscoelastic, sustained tension will lead to permanent growth and relaxation of the mechanical stress. In contrast, under pushing ("compressive") forces, tissues will be compressed. Sustained compression may lead to tissue shrinking, or if the compressive forces are large enough, tissue may buckle (i.e., suddenly bend) to assume an energetically more favorable state.[138]

The prevailing view in the field is that cortex folding results mainly from a "buckling instability," i.e., from a sudden change in shape (i.e., bending) under load. Buckling may occur when the cortex expands at a higher rate than the underlying white matter.[135,138–141] Cell proliferation, astrogenesis, neuronal migration, and likely dendrite formation, which are all crucial events in cortex folding,[118,135,142–148] contribute to the tangential expansion of the cortex. This expansion leads to the generation of compressive forces. Beyond a critical point, a further increase in tissue compression leads to buckling, which is why the cortex folds.[135]

Additional contributions to cortex folding are made by the ECM.[149] As mentioned above, the ECM is an important contributor to local tissue stiffness. For folding to occur, the cortex has to be stiffer than the underlying white matter,[150] potentially explaining why perturbations to the ECM may alter brain folding patterns[149]—as these alterations affect the stiffness ratio of gray and white matter.

Finally, axon tension was also suggested to contribute to cortex folding. Although axon tension is not involved in pulling the base of developing gyri together,[49] as initially hypothesized,[151] it exists in subcortical white matter tracts,[49] where it could enable the propagation of forces across large distances. Such a mechanism could explain why a lesion of a frontal lobe results in bilateral changes in sulcal patterns.[152] The frontal lobes are tightly connected. Thus, lesions in one lobe could cause a relaxation of the connecting axons, leading to a force redistribution in the contralateral lobe and hence altered folding patterns.

For a more in-depth discussion of the mechanisms and forces at play during cortex folding, please see the following excellent reviews: Llinares-Benadero and Borrell,[6] Garcia et al.,[138] and Del-Valle-Anton and Borrell.[153] In summary, many players across length scales—starting from gene expression patterns,[154] local progenitor cell proliferation, neuronal migration, and ECM composition—regulate local mechanical tissue properties and tissue-scale forces in cortex folding. Reciprocally, forces and mechanical tissue properties regulate gene expression patterns, cell proliferation, neuronal migration, and ECM composition.[1,138] How exactly this intricate interplay of molecular and cellular processes as well as cellular and tissue-level forces lead to the folding of the mammalian cerebral cortex will need to be studied in future.

MECHANICS IN HOMEOSTASIS

Once the neuronal network is established, synapses are formed and astrocytes are distributed throughout the CNS, mechanics is still an important regulator of many cell functions. We here provide a few prominent examples.

Neuronal activity

Neuronal activity is regulated by mechanical signals at many levels. For example, in *Drosophila* photoreceptors, the absorption of light leads to conformational changes in the photosensitive molecule rhodopsin, which activates the enzyme phospholipase C, whose activity leads to changes in the physical properties of the cell membrane. Consequently, the microvilli in the photoreceptor cells—and hence the whole ommatidia (compound eye units)—contract in response to light, and the resulting forces lead to the opening of "light-sensitive" ion channels, which actually appear to be mechanosensitive.[155] This exemplifies how mechanical forces can serve as "second messengers" in an otherwise metabotropic signaling pathway. Consistent with this, changes in the mechanical properties of the photoreceptors' cell membranes lead to changes in the speed and sensitivity of phototransduction in *Drosophila*.[156]

Similarly, bright flashes of light induce a rapid shortening of the outer segments of vertebrate photoreceptor cells.[157] In *Xenopus* rod inner segments, the expression of mechanosensitive channels such as transient receptor potential canonical 1 (TRPC1) and Piezo1 is crucial for proper photoreceptor function.[157] These data suggest that mechanosensitivity is an essential component of vertebrate vision as well.

As mentioned above, the activity of NMDA receptors, a neuronal glutamate receptor, may be modulated by mechanical forces.[55] At the level of the neuromuscular junction in *Drosophila*, where information is sent from the nervous system to muscles, synaptic vesicle clustering requires tension (i.e., actively generated forces) along the axon *in vivo*.[50] The activity-dependent structural plasticity of dendritic spines also requires mechanical forces generated by the actomyosin cytoskeleton, which must be properly linked to adhesion sites in the spine to push against the membrane for spine enlargement.[158] Additionally, recent work has shown that applying mechanical forces on the presynaptic terminal increases neurotransmitter release.[159] Extrinsic forces generated by transiently pushing presynaptic boutons in rat brain slices with a glass pipette resulted in an increase of glutamate release and soluble N-ethylmaleimide-sensitive factor activating protein receptor (SNARE) complex assembly. Moreover, inducing postsynaptic spine enlargement through

glutamate uncaging resulted in enhanced SNARE complex assembly and increased glutamate release specifically when the spine was pushed against the presynaptic bouton (Figure 3F).[159]

At the network level, synapse densities and neuronal activity seem to be regulated by substrate stiffness, even if the softest substrates used were two orders of magnitude stiffer than brain tissue.[160] Once neuronal networks are established on similarly stiff substrates, the presynaptic activity, synaptic glutamate concentration, calcium influx, NMDA receptor activity, and postsynaptic activity were all enhanced on even stiffer substrates.[161] Collectively, these findings indicate that mechanical cues actively influence neuronal activity across many levels, thereby shaping function, plasticity, memory, and learning in organisms.

Mechanical glial cell functions in homeostasis

During eye movements, the vertebrate retina is exposed to large forces. Müller cells, the principal glial cells of the vertebrate retina, span the entire thickness of the tissue, connecting all retinal neurons. In addition to numerous metabolic, electrochemical, and optical roles,[162,163] Müller cells also have important mechanical functions. These cells are contractile *in vitro* and *in vivo*[164,165] and actively generate mechanical tension in the healthy retina, promoting tissue cohesion and integrity.[165] Furthermore, Müller cells are also sensitive to tissue-level changes in tension.[166] Mechanical stretch, such as that which might occur during rapid eye movements or trauma (in particular in glaucoma patients[167]), elicits calcium transients in Müller cells, likely mediated by MSCs such as transient receptor potential vanilloid 4 (TRPV4) and TRPC1.[168] These calcium transients lead to early stress responses,[166] enabling the cells to react to the mechanical signal.

Also, microglia, astrocytes, and oligodendrocytes possess MSCs, including Piezo1. Although little is known about the mechanical signals that activate these MSCs in glial cells in healthy conditions, the activation of Piezo1 in astrocytes has been shown to lead to the calcium-mediated release of ATP, thus affecting adult neurogenesis, hippocampal structure, long-term potentiation, learning, and memory *in vivo*.[117] Many mechanosensitive glial cell functions are related to pathological processes though, as described below.

Mechanical interaction between neurons and glial cells: Myelination

Axons may be wrapped in a myelin sheath, which improves the conduction of action potentials, optimizing information transmission along axons. The myelination process involves oligodendrocytes in the CNS and Schwann cells in the PNS extending thin processes, which wrap around axons. Although forces must be involved in the extension of these protrusions and the wrapping process, we still know relatively little about how myelin sheaths form.

Recent studies revealed that oligodendrocyte precursor and Schwann cell function, as well as myelination, are indeed dependent on substrate stiffness[169–171] and regulated by Piezo1[93,172] and YAP.[173,174] Notably, the aging-related stiffening of the brain increasingly activates Piezo1 in oligodendrocyte precursor cells and thus eventually prevents remyelination in the aged brain.[24]

It was suggested that the internodal lengths of myelin segments may be determined by forces arising from towed growth of axons (see subsection identity and growth),[175] potentially explaining why larger species have longer internodal lengths. However, whether myelinating glial cells are stretched directly during growth or through their tight connections to the axons and/or the ECM remains to be determined. In any case, stretching of oligodendrocyte precursor cells induces nuclear YAP accumulation, which affects oligodendrocyte maturation and morphology *in vivo*.[174] Once myelination is complete, mechanosensitive TRAAK channels are localized exclusively to nodes of Ranvier, the action potential propagating elements of myelinated nerve fibers. The exact function of these channels is still unclear though and warrants further exploration.[176]

MECHANICS IN PATHOLOGICAL PROCESSES

The mechanical properties of neural tissue change during various diseases and following injury. These changes may be an epiphenomenon of molecular and cellular changes occurring during diseases. They may, however, also be key players in the pathogenesis of CNS disorders, contributing to the onset and/or progression of disease. Importantly, not only neurons but also glial cells, which play a pivotal role in many CNS disorders,[177–179] are mechanosensitive. Glia respond to the mechanical properties of their environment and may be (immune)activated when their mechanical environment changes[62,98,180–182] (see subsection integrating different signals). In this section, we review recent advances in our understanding of how mechanical signals might be involved in CNS diseases.

Neurodegenerative diseases

In many neurodegenerative diseases, including Alzheimer's disease (AD),[183,184] Parkinson's disease,[185,186] and multiple sclerosis (MS),[187,188] brain tissue becomes softer *in vivo* (Figures 4A and 4B). This tissue softening correlates with histopathological changes in the tissue, including a decrease in cell numbers, loss of connectivity, and inflammation.[184,192,193]

Many neurodegenerative diseases are characterized by the presence of intracellular and/or extracellular amyloid fibrils (i.e., protein aggregates with a cross-β structure), such as amyloid beta (Aβ) peptide in AD and α-synuclein in Parkinson's disease. Amyloid fibrils are among the stiffest protein fibers known.[194] If they assemble into larger scale structures, such as amyloid plaques in AD, they might constitute stiff objects within an otherwise soft tissue. These structures may be big enough to be mechanically probed by cells (or AFM cantilevers[195]). However, it has yet to be determined whether cells primarily respond to overall tissue softening or localized stiffness of the amyloid plaques.

Either way, mechanosensing by glial cells seems to be an important factor in neurodegenerative diseases. For example, Piezo1 expression is upregulated in AD models either in astrocytes[196] or microglia.[195] Microglia cluster around Aβ plaques in a Piezo1-dependent fashion[195] and could be attracted to the plaques by an attractive gradient in tissue stiffness.[62] Piezo1 activity increases Aβ clearance in microglial cells in both human and mouse models of AD,[195,197] suggesting that this MSC might be critically involved in disease progression and could be an interesting target in future treatment strategies. Additionally, the nuclear translocation of YAP

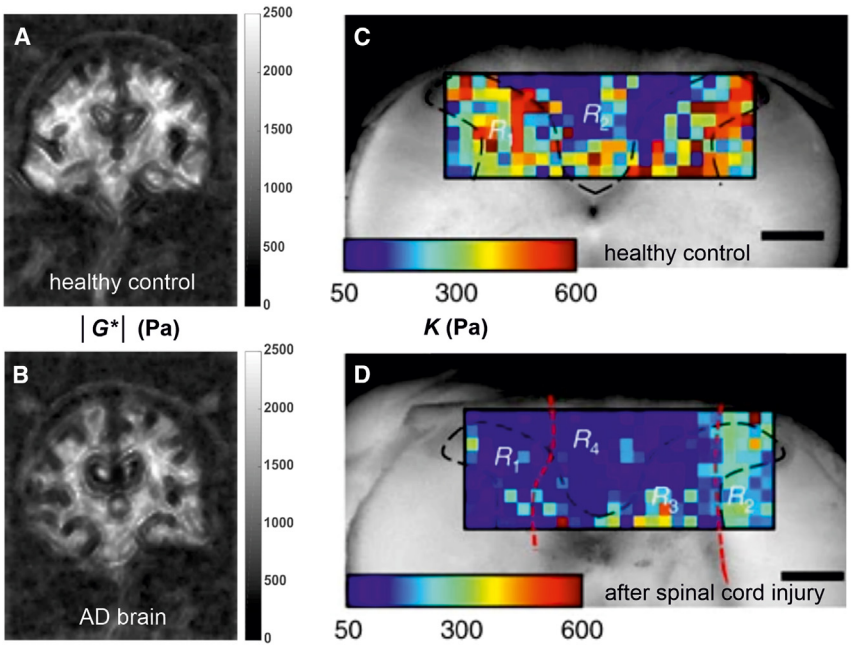

Figure 4. Pathological changes in CNS tissue mechanics

(A and B) Complex shear moduli of human brains measured by magnetic resonance elastography (MRE). Brain tissue of patients with Alzheimer's disease (AD) (B) is softer than that of age-matched healthy controls (A). Adapted from Gerischer et al.,[189] NeuroImage: Clinical, 18, Gerischer et al.,[189] with permission from Elsevier. Considering hippocampal stiffness in in addition to magnetic resonance imaging-based hippocampal volume and mean diffusivity significantly improved diagnostic sensitivity.[189]

(C and D) Elastic moduli of rat spinal cord tissue measured by atomic force microscopy (AFM). In adult rats, where neurons do not normally regenerate, spinal cord tissue significantly softens after injury (D) if compared with healthy tissue (C). Scale bars, 500 μm. Adapted from Moeendarbary et al.[36] and reprinted with permission from *Nature Communications*. In contrast, peripheral nerve tissue in rats and spinal cord tissue in zebrafish, both systems in which neurons do regenerate, stiffen after injury.[190,191]

was recently suggested to be involved in the mechanosensing of microglia in the context of AD.[198]

In contrast to AD, Piezo1 expression is downregulated in the brains of patients with MS.[199] In MS, the demyelination of axons leads to the softening of the tissue,[200] which lacks amyloid fibrils, indicating that the tissue is also soft at the micro-scale and that thus microscale tissue stiffness might regulate Piezo1 expression patterns. In agreement, Piezo1 is upregulated on stiffer substrates *in vitro*.[201] Piezo1 activity leads to a decrease in the proliferation and migration of oligodendrocytes.[199] It furthermore decreases the myelination of axon tracts, and its inactivation was suggested to alleviate demyelination.[172]

In addition, neuronal homeostasis may be mechanically regulated by Aβ. A recent study suggested that Aβ oligomers lead to an increase in membrane tension, thus indirectly activating NMDA and AMPA receptors, leading to excitotoxicity[202] and contributing to AD progression. Hence, AD may also progress through the perturbation of mechanical lipid membrane properties that are mediated by Aβ and sensed by NMDA and AMPA receptors.

In summary, in AD, where Piezo1 activity may be beneficial, stiff plaques may lead to the activation of the MSC. In contrast, in MS, Piezo1 activity seems to be detrimental, and here, stiff plaques potentially required for activating mechanosensing are absent. Based on only these two exemplary neurodegenerative diseases, it is tempting to speculate whether pharmacologically interfering with brain stiffness might help in slowing down disease progression. Further research is required to unravel the complex interplay between mechanics, pathology, and potential therapeutic interventions.

Axon regeneration

Neural tissue stiffness also dramatically changes with traumatic injuries. Similar to other organ systems in our body, neural tissue is replaced by scar-like tissue after significant injury. Typically, collagen deposition leads to a stiffening of the fibrotic scar compared with healthy tissue. In the mammalian peripheral nervous system, where damaged neurons can regenerate, a similar scarring mechanism occurs. Here, collagen expression is significantly increased after crush and transection injuries *in vivo*, and hence, nerve tissue stiffness is increased relative to the stiffness of the healthy nerve.[190] Similarly, spinal cord tissue in zebrafish, where neurons can also fully regenerate, stiffens after injury.[191] The mechanical signature of scar tissue in the mammalian CNS, however, is far more intriguing. In the adult mammalian CNS, where neurons usually fail to regenerate, brain and spinal cord tissue soften after injury[36] (Figures 4C and 4D), although tissue stiffness may revert to healthy values and beyond at chronic time points.[203] These data imply a positive correlation between tissue stiffness and the regenerative capacity of injured neurons

However, it has been shown that, in *Drosophila*, Piezo1 activity, which is likely increased in stiffer tissues,[60,76] actually inhibits axon regeneration through the activation of nitric oxide synthase.[204] Downstream effectors include the cyclic guanosine monophosphate-dependent protein kinase G (PKG) as well as the ataxia-telangiectasia-mutated-and-Rad3-related kinase and checkpoint kinase 1 (Atr-Chek1) pathway.[204,205] Why axons regenerate better in stiffer tissues, whereas Piezo1 activation inhibits their regeneration, remains to be illuminated in future work.

Engineering approaches to promote neuronal regeneration include biomimetic scaffolds for temporarily replacing damaged spinal cord tissue[206,207] and implants like cuffs to facilitate regeneration of peripheral nerves.[208] The aim of such approaches is to promote neuronal regeneration while inhibiting excessive inflammatory reactions. Although both aims can be achieved, at least to some degree, by slow sustained release of different drugs, decreasing the stiffness of the whole implant or its surface has been shown to work similarly well.[98,208] Materials with a stiffness above that of the host tissue leads to the nuclear translocation of YAP in surrounding cells, culminating in

inflammation and scar formation.[208] Future approaches will likely combine appropriate mechanical and chemical signals to facilitate neuronal regeneration in the damaged adult mammalian nervous system.[207]

Brain tumors

Tumors generate compressive forces during growth and expansion, whereas migrating tumor cells exert forces on their environment during metastasis and invasion. Hence, mechanics is likely a significant component of brain tumor biology. In the CNS, the most common and aggressive type of primary tumor is the glioblastoma multiforme (GBM), which has a very poor prognosis for survival. In humans, GBM is generally softer than healthy brain tissue, as measured by MRE *in vivo*, and they are mechanically highly heterogeneous.[209,210] Briefly, MRE assesses tissue stiffness by applying external mechanical vibrations and using magnetic resonance imaging (MRI) to monitor the resulting wave propagation. As waves propagate at different rates in materials of different stiffness (in soft tissues, waves travel more slowly, whereas in stiff tissues, waves travel faster), monitoring wave propagation enables non-invasive tissue stiffness mapping in clinical and pre-clinical settings.

Other types of brain cancer are mechanically similar to GBM.[211] In *Drosophila*, however, glioma tissue is stiffer than healthy brain.[201] Papers reporting GBM in mammals to be stiffer than healthy brain tissue used either frozen, fixed, or dried brain tissue for measurements, calling to question the meaningfulness of the measurements. However, under compression human glioma tissue stiffens more than healthy brain tissue,[212] suggesting that under sufficient tumor growth pressure, brain tumors might indeed be stiffer than healthy brain.

This altered mechanical environment in a tumor may significantly affect tumor cell function. For example, in 2D cultures, stiffer environments enhance the proliferation and tumorigenesis in patient-derived GBM cells through the activation of Wnt/β-catenin signaling[213] and/or epidermal growth factor/ protein kinase B (EGFR/Akt) signaling,[214] as well as GBM aggressiveness.[215,216] Furthermore, GBM cell motility is enhanced on stiffer 2D substrates.[217] In 3D cultures, however, GBM cell proliferation and motility are decreased in stiffer substrates,[218] likely due to steric hindrance (i.e., less available space, due to the smaller mesh size and lower deformability of stiffer substrates). Increased proliferation and motility of GBM cells in 2D can also be triggered by hyaluronic acid via phosphoinositide 3-kinases (PI3K) activation, even on soft substrates,[219] suggesting that the mechanical and chemical signals of the tumor microenvironment can equally regulate the behavior of cancer cells.

Glioma cells themselves are also characterized by heterogeneous mechanical properties. Recurrent GBMs are characterized by high tension and high levels of glycoproteins, which increase the bulkiness of the glycocalyx. This bulky glycocalyx potentiates integrin-based mechanotransduction and tissue tension, thus promoting a stem-cell-like phenotype in GBM cells.[220] Potentially more invasive GBM cells, sampled from the tumor periphery, are characterized by lower adhesive forces and increased motility, compared with GBM cells sampled from the bulk of the same tumor.[221] The most invasive glioma cells are stiffer and generate larger traction forces than less invasive cells, in a microtubule, myosin II, and formin-dependent manner.[222] Although the depletion of myosin IIA impairs tumor invasion, it also increases tumor proliferation in a substrate mechanics-dependent manner.[223]

An exciting recent study revealed that in mice, medulloblastomas are characterized by a gradient in tissue stiffness around blood capillaries.[224] This stiffness gradient is sensed by the MSC Piezo2 in projections of quiescent and slow-cycling $Sox2^+$-expressing tumor cells. The activation of Piezo2 leads to enhanced traction forces and growth of the projections toward the capillaries, which they eventually ensheath. This way, the tumor cells strengthen the blood-tumor barrier, limiting the access of most therapeutic agents. Consequently, Piezo2 knockout compromises the blood-tumor barrier, reduces the quiescence of $Sox2^+$ tumor cells, and enhances the response of the medulloblastoma to chemotherapy,[224] indicating that mechanical signals are an important target for future therapeutic interventions.

In most gliomas, however, Piezo1 (but not Piezo2) is overexpressed, and similar to many other types of solid tumors across different organ systems, Piezo1 abundance inversely correlates with patient survival.[201] Furthermore, Piezo1 is predominantly localized at adhesion sites of GBM stem cell processes. It is upregulated on stiffer substrates, and in *Drosophila*, enhanced Piezo levels correlate with increased brain tissue stiffness, implying a positive feedback loop.[201] Once a tumor has formed, Piezo1 is required for glioma maintenance, growth, and progression. *In vitro*, Piezo1 knockdown abolishes the stiffness-dependent tumor cell proliferation described above. *In vivo*, Piezo1 knockdown inhibits the growth of GBM and significantly prolongs the survival of mice with brain tumors,[201] potentially by disrupting the vicious circle between mechanically induced cell proliferation and increasing tissue stiffness.

OUTLOOK

Every tissue in our body is built from its own set of specific constituents, giving rise to the tissue's distinct mechanical properties. As the local composition and properties of the constituents change during development, aging, and disease, the tissue's local mechanical properties change as well. Thus, throughout the body, tissue mechanics serve as signals that exist without requiring additional energy expenditure—e.g., no extra ATP is needed to generate the signal. Consequently, it is unsurprising that neurons and glial cells evolved mechanisms to detect and respond to mechanical signals.

In this review, we have examined recent advances in our understanding of how mechanical signals regulate nervous system development and homeostasis, and how the same signals may be involved in different pathological processes. Although we primarily focused on tissue stiffness and cellular forces, it is important to acknowledge the presence of other mechanical signals, such as shear forces exerted by fluid flow, steric hindrance due to the crowded environment in tissues, and tissue fluidity (leading, for example, to rearrangements of cells in response to forces).

In contrast to receptor-mediated chemical signaling, mechanical signals, such as tissue stiffness, are not specific. As stiffer substrates provide more traction to cells, cellular forces increase

in stiffer tissues.[62,63] This increase in force directly impacts cell motility. Substrate stiffness thus directly regulates parameters such as cell migration velocity and persistence, and motile CNS cells can even be guided by stiffness gradients.[20,25,62,224] However, due to the lack of specificity, tissue stiffness alone is unlikely to instruct cells on where and when to stop migrating, and on which cells to connect to or interact with. Here, chemical signals are essential. Nevertheless, tissue mechanics may modulate chemical signaling (and vice versa), enhancing the signal-to-noise ratio and robustness of cellular responses to the available signals.

Although significant progress has been made in understanding mechanosensitive proteins and the underlying mechanotransduction cascades, we are still just scratching the surface, and many open questions remain. Future work will show how mechanical and chemical (and potentially electrical[225,226]) signals are integrated by cells to jointly regulate cell function. Knowledge about this integration will be critical for our understanding of developmental processes in the nervous system and will also enable us to gain insights into aging and various disorders.

In certain CNS diseases, mechanical changes of tissues may be disease-specific (“pathognomonic”)[227] and could thus potentially be used in the clinic to support diagnosis.[189] At the same time, incorporating mechanical signals in our understanding of the pathogenesis of CNS disorders will yield deeper insights and potentially lead to the development of new therapeutic interventions targeting, for example, mechanosensing proteins or their downstream effectors, thus improving treatment options.

Overall, mechanical forces are pivotal in numerous biological processes; the study of nervous system mechanobiology, and its integration with more established neurosciences disciplines, will significantly advance our understanding of nervous system function and disease.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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