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The biophysics of neuronal growth

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Abstract
For a long time, neuroscience has focused on biochemical, molecular biological and electrophysiological aspects of neuronal physiology and pathology. However, there is a growing body of evidence indicating the importance of physical stimuli for neuronal growth and development. In this review we briefly summarize the historical background of neurobiophysics and give an overview over the current understanding of neuronal growth from a physics perspective. We show how biophysics has so far contributed to a better understanding of neuronal growth and discuss current inconsistencies. Finally, we speculate how biophysics may contribute to the successful treatment of lesions to the central nervous system, which have been considered incurable until very recently.

(Some figures in this article are in colour only in the electronic version)

This article was invited by E Frey.

Contents

Glossary

Astrocytes	Most abundant glial cell type in the mammalian CNS, star-shaped.
Axon	The dominating neuronal process, can reach a length of several meters, microtubule plus ends are pointing toward the growth cone.
CNS	Central nervous system, consisting of brain, spinal cord and retina.
Dendrite	Fine, branched neuronal process, receives synaptic input from other neurons, mixed microtubule polarity.
Ectoderm	One of the three embryonic germ layers, gives rise to epidermis (skin) and nervous system.
Filopodium	Actin-rich spike-like protrusion from the lamellipodium, part of the growth cone.
Glial cell	Principal cell type in the nervous system; fulfills a multitude of functions including maintenance of extracellular homeostasis, pH, provision of nutrition for neurons and involvement in neuronal information processing.

Growth cone	Leading tip of growing nerve cell process, highly motile structure densely packed with cytoskeletal elements. Determines growth direction and speed.
Guidance cue	Signal that orients migrating and growing cells; can be gradient of diffusible molecules, molecules bound to cellular or extracellular surfaces, physical cues such as substrate topology, electrical and optical cues, mechanical cues.
Lamellipodium	Flat, fanned-out projection at the end of a neuronal process, part of the growth cone.
Neurite	General term for neuronal process if it is not clear whether axon or dendrite.
Neuron	Principal cell type in the nervous system; excitable cell that processes and transmits information by electrochemical signaling.
Mesoderm	One of the three embryonic germ layers, gives rise to bone, connective tissue, muscle and blood cells.
Molecular clutch	Complex of proteins coupling the actin cytoskeleton to the extracellular matrix, allows force transduction and modulates retrograde flow in growth cones.
Pioneer axons	First axons that seek out correct path to target according to all available guidance cues; following axons use pioneer axons as primary guidance cue.
PNS	Peripheral nervous system, consisting of cranial and spinal nerves, intramural nervous system including receptors and effector organs, such as motor end-plates and ganglia.
Radial glial cell (RGC)	Crucial cell in the developing CNS, involved in neurogenesis and neuronal guidance; has two long radial processes that connect tissue surfaces; radial phenotype is typically transient but may persist in certain CNS regions.
(Dendritic) spine	Small membranous protrusion from a dendrite, typically receives input from a single synapse of an axon.
Synapse	Contact between neurons where a signal is transferred from one neuron to the next.

1. Introduction

1.1. General comments

With the discovery of neurons and their distinct parts over 100 years ago, an important contribution of physical impacts on the morphology, growth and normal functioning of nervous tissue and cells seemed likely. However, due to a lack of appropriate techniques to study physical material properties and the effect of physical stimuli on individual cells or even cell parts, these ideas remained speculation for the longest time. In the interim, science has focused on biochemical aspects of neuronal growth. However, a key component of neuronal growth is motion, and motion cannot be understood without considering physical concepts such as forces. Furthermore, the neuronal environment is highly complex, consisting of tissues of varying stiffness. These differently stiff tissues may constitute obstacles in the way of growing nerve cell processes or even serve as a cue determining orientation and velocity of their growth.

Here we report how physics has so far contributed to the progress in understanding neuronal growth. We briefly introduce the biological background required to understand the very special environmental conditions nerve cells encounter, summarize existing data about physical properties of nervous tissue and cells, and present current knowledge about the impact of physical stimuli on neuronal behavior with an emphasis on mechanical stimuli. Finally, we speculate how biophysics could help improving treatment strategies of nerve lesions in future, which to date is one of the biggest challenges in medicine.

1.2. Biological background

The vertebrate nervous system can be divided topographically into central (CNS) and peripheral nervous system (PNS). According to their anatomical location, brain, spinal cord and retina constitute the CNS, while the PNS comprises the cranial and spinal nerves as well as the intramural nervous system including their receptors and effector organs, such as motor end-plates and ganglia. An important—mechanical—difference between CNS and PNS is their mechanical environment. While the PNS is not particularly protected from mechanical trauma, all parts of the CNS are encased by an 'exoskeleton', i.e. the brain by the skull, the spinal cord by the vertebrae and the retina by the sclera. As a consequence of the stiff envelopes, growth and expansion of the CNS for instance during embryonic development or in case of edema are limited. For example, the time frame of CNS tissue growth is different from that of its exoskeleton, which contributes to the folding of the brain [1]. Further, due to the production of large amounts of liquid in the various cavities (ventricles, central canal and vitreous body in brain, spinal cord and retina, respectively), central nervous tissue is already exposed to a pressure exceeding the barometric pressure [2, 3]. This pressure even increases in the case of edema, which quickly becomes deleterious for the CNS within its exoskeleton [4, 5]. These large-scale mechanical aspects of brain function are relatively well understood and we will focus more on microscopic, cellular aspects.

The basic building blocks of the nervous system are *neurons* and *glial cells*. Neurons are highly specialized, excitable cells that process and transmit information by electrochemical signaling. Typical neurons consist of a cell body, or soma, which contains the nucleus and other organelles, one *axon*, which is the dominating cell process that can reach a length of several meters, *dendrites*, which are fine, branched cell processes that receive synaptic input from other neurons, and presynaptic terminals [6] (figure 1). Axons and dendrites *in vitro* are collectively referred to as processes or neurites. Contacts between neurons are called *synapses*, where a signal is transferred from one neuron to the other one. The spatial distribution of neuronal cell bodies and processes leads to

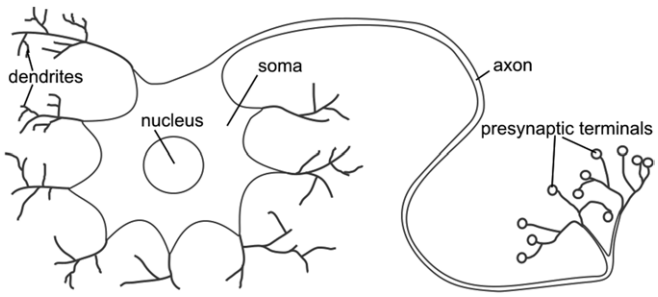

Figure 1. Typical morphology of a neuron.
Typical neurons consist of a cell body, or soma, which contains the nucleus and other organelles, dendrites, which are fine, branched cell processes that receive synaptic input from other neurons, one axon, which is the dominating cell process that can reach a length of several meters, and presynaptic terminals [6]. Axons are often surrounded by a myelin sheath—built by oligodendrocytes and Schwann cells in CNS and PNS, respectively—that insulates them and accelerates action potential propagation.

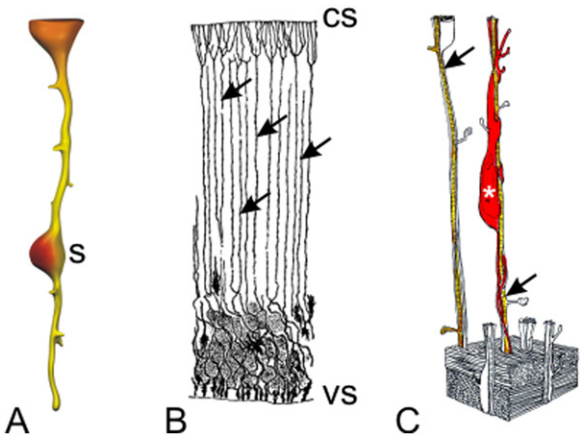

Figure 2. Radial glial cells (RGCs).
(*A*) RGCs are tubular-shaped cells with two long radial processes emanating from the soma (s). The color shows the stiffness distribution along RGCs in the adult retina (from yellow = compliant to red = stiff) [27]: the radial processes are significantly more compliant than any other cellular structure in the tissue. (Courtesy of Dr Jens Grosche.) (*B*) RGCs (arrows) span the entire thickness of central nervous tissue and connect the ventricular (vs) with the cortical surface (cs). (Adapted from Ramón y Cajal (1909) [249].) (*C*) During development, RGCs give rise to neurons and serve as their guides: a neuron (asterisk) is migrating along an RGC (arrows). (Adapted from Rakic (2003) [45] and reprinted with permission from Wiley InterScience.)

regional features in the CNS on both the macroscopic and microscopic length scale. Tissue rich of neuronal somata—the gray matter—appears darker than tissue densely packed with mostly myelinated axons, which is called white matter (the myelin amount determines the whiteness). While in the brain white matter is found in its deep parts, in the spinal cord white matter is confined to the superficial region. Glial cells, which have phylogenetically evolved with the increasing complexity of the nervous system [7], fulfill a multitude of different functions. Classically, they were thought to provide a mechanical scaffold to neurons (the Greek *glia* actually means putty or glue) [6, 8]. Glial cells are responsible for the maintenance of the extracellular homeostasis [9], help to relay light through the retina as optical waveguides [10], and are even involved in neuronal information processing [11], to name but a few functions. Additionally, they contribute to the blood–brain barrier, which is found in most parts of the CNS but not in the PNS.

The dominating glial cell type in the PNS is Schwann cells. Glial cells in the CNS can be classified according to their size in macro- and microglia. Macroglial cells, as well as all neurons, arise from the embryonic *ectoderm*. However, microglia, which are responsible for unspecific immunoresponse in the CNS, originate from monocytes, which are white blood cells stemming from the *mesoderm*. CNS macroglia can generally be divided into astrocytes, which in the brain constitute the most abundant glial cell type, oligodendrocytes, which build the myelin sheet enclosing neuronal processes to enable fast signal transmission, and ependymocytes, which line the ventricles and the central canal.

Before the maturation of these macroglia, radial glial cells (RGCs) are dominating the developing nervous tissue. RGCs possess two long radial processes connecting outer and inner surfaces of the tissue. The radial phenotype is typically transient but may persist in certain CNS regions: examples include Bergmann glia in the adult cerebellum and Müller glial cells in the retina. Embryonic RGCs give rise to neurons and later to astrocytes and ependymocytes [12], and during cortical development newborn neurons migrate along those RGCs [13] (figure 2). Furthermore, axons growing along superficial tissue layers may turn at right angle and also follow radial glial fibers [14], which might be significant in the current context as discussed below.

During process formation, neurons send out the leading tips of their processes in a directed fashion. These tips, which are called growth cones (figure 3), are highly motile structures that provide the machinery to move forward and a mechanism to provide traction on their path. They also possess detectors of *guidance cues* that translate environmental cues into directional movement and thus guide the neuronal process in a spatially biased way toward their destination [15] (see section 2). Whether a neuron elongates an axon or dendrites is regulated by extrinsic and intrinsic signals [16]. Generally, outgrowth of dendrites often occurs subsequent to that of axons, and in some cases dendritic differentiation may start only after the axon has established connection with its target [17]. While the axon largely continues growing toward its destination, the dendritic tree grows by the addition and elongation of branches [18]. However, the final branching pattern of neurons is not only established by branch addition and maintenance but also by branch retraction and elimination [19]. Despite certain differences in surface receptors [20] and the composition of their cytoskeleton (mainly microtubules) (cf section 2.1), growth cone-mediated outgrowth appears to be similar in axons and dendrites [18]. First, the growth cone's leading edge protrudes by actin polymerization-driven elongation, after which an increasing number of microtubules invades these protrusions, a process called 'engorgement'. Finally, the proximal part of the growth cone consolidates [21] (for a more detailed explanation of the cytoskeleton see section 2.1). The growth direction is determined through

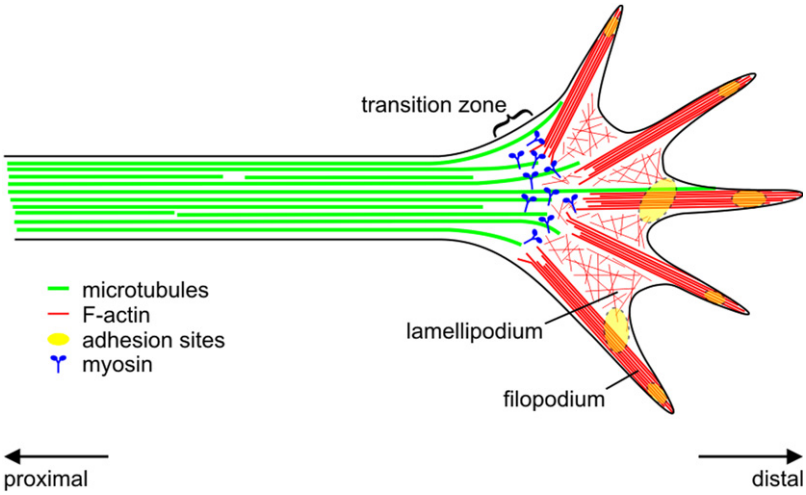

Figure 3. Ultrastructure of a neuronal growth cone.
Growth cones, which are the distal tips of advancing neuronal processes, consist of a flat region called lamellipodium and spike-like protrusions called filopodia. They are actin-filled structures with densely packed actin bundles inside the filopodia. Apart from actin, also microtubules may extend into filopodia. Microtubules extend the full length of a neurite and splay apart in the growth cone. In axons, all microtubules are arranged in parallel with their plus ends pointing distally; in dendrites, microtubule polarities are mixed. The cytoskeleton is coupled to the environment via transmembrane protein complexes ('molecular clutches'), which enable growth cones to exert forces on their substrate. Plus end actin assembly takes part at the growth cone's leading edge, pushing the membrane forward. At the same time, myosin II motors, which are concentrated in the transition zone of the growth cone, permanently pull actin filaments proximally, thus creating a 'retrograde flow'. This retrograde actin flow together with cell adhesion results in the generation of traction forces, which produce tension on the neurite, and which are involved in neuronal guidance.

the detection of guidance cues by appropriate sensors in the growth cone [22]. Subsequent signal transduction pathways link the activation of these sensors to a reorganization of the cytoskeleton, which finally causes the neurons' reorientation [21, 23, 24]. Importantly, growth cones are autonomous and contain all the machinery needed to sense and respond to their specific pathways and targets; they are able to move and extend *in vitro* and *in vivo* even when their connection to the soma is cut off [25, 26].

Guidance cues (also see section 2.3) are classically understood to be either gradients of diffusible molecules [28] or molecules bound to cellular or extracellular surfaces that orient migrating and growing cells. They can either be attractive, repulsive or both [22]. Growth cones are for instance known to turn toward gradients of netrins and ephrins [23, 29–32] and also toward morphogens such as Sonic hedgehog [33]. Prominent examples of negative guidance cues include semaphorins and Slits [34, 35]. However, the neuronal response to these guidance cues is complex and depends on other factors; netrins and ephrins for instance may also repel neurites [36–38]. In addition to these guidance signals other cues including growth factors [39], neurotransmitters [40, 41], glial cells [13, 42–45] and extracellular matrix (ECM) proteins [46, 47] may directly influence neuronal growth and migration. It is likely that these extracellular signals are transduced via the Rho family of GTPases, which include CDC42, Rac and Rho [48], and which dynamically affect both axon and dendrite structure [18].

Generally, a growth cone's response to a certain guidance cue depends on several factors. For instance, the substrate neurons are growing on may decide whether a growth cone is attracted to or repelled by a chemical signal. Laminin, which is an extracellular molecule, turns growth cone attraction by netrin-1 into repulsion [38]. A neuron's age may influence this response as well [49]. In both cases, this change in response is accompanied by a change in intracellular cAMP levels, which appears to be crucial for the switching of the turning direction [36, 39]. Cyclic nucleotide signaling has also been shown to modulate the activity of calcium channels in growth cones [50]. Calcium seems to be a key player in the translation of chemical signals into a directional change of neurite outgrowth. Local changes in the intracellular calcium concentration may change growth cone dynamics and finally cause its turning or collapse [41, 51, 52]. Of note, all of these well-accepted guidance cues are essentially chemical in nature and fairly well understood. The focus of this review is on recent evidence that also physical, and mostly mechanical, cues influence neuronal growth.

1.3. Historical perspective

For centuries, it has been speculated that forces are crucial for the morphology and functioning of some tissues such as muscles. Correlations between mechanical phenomena and biological forms are, for instance, extensively illustrated in D'Arcy Thompson's classic 'On growth and form' written in 1917 [53]. In contrast, the importance of mechanics for the nervous system has only been appreciated relatively recently. First reports suggesting a contribution of tension to PNS morphogenesis appeared in the second third of the 20th century [54, 55]. However, for the next couple of decades, descriptions of mechanical aspects in nervous tissue physiology and pathology remained sparse. The tension in spinal cord and hindbrain exposed to mechanical stress

caused by changes in position was the first clinically relevant mechanical issue to attract attention [56, 57]. At the end of the 1960s, first measurements of mechanical properties of brain tissue were published [58–60]. Finally, the idea of tension being involved in nervous system morphogenesis was revived in the late 1970s, when pioneering work revealed that neuronal processes *in vitro* are under tension [61]. Another study showed that even mechanical tension alone is sufficient to promote axonal outgrowth [62], confirming previous observations [54, 55]. Subsequently, this 'towed growth' has stimulated much future work by different groups, which will be introduced in section 2.2, and it experiences a current revival.

1.4. Outline

In this review, we will first introduce biophysical aspects of the neuronal structures directly involved in neuronal outgrowth—neurites and their growth cones. We will consider their ultrastructure, thereby identifying possible candidates directing growth, discuss growth cone advance and summarize current knowledge about the mechanical properties of growth cones and neurites. Finally, we will review how physical cues may be involved in neuronal outgrowth and guidance and discuss how these cues may be exploited to support regeneration of injured nervous tissue.

2. Biophysics in neuronal growth

While somata of mature neurons are rather stationary, their dendrites and axons can be highly motile. At the beginning of neuronal development, neuronal processes sprout from the cell body and grow over relatively short distances ($\sim\mu$m) to form synapses with their targets. This first growth phase, which is similar in dendrites and axons, is facilitated by the processes' leading tips, the growth cones. In order to advance, growth cones need to maintain their structure, adhere to their substrate and actively generate forces. Once the network is connected, the whole tissue expands due to the increasing number and size of cells, very likely resulting in further tension acting on the neurites [1, 55, 63]. These tensile forces are the basis for the second phase of (probably mainly) axonal growth—passive stretching by mechanical tension [63]—and are important for the morphology of the adult CNS, contributing for instance to the cortical folding and to the compactness of neural circuitry [1]. Tension eventually decreases in adulthood, resulting in relaxed, undulated axons *in situ* [64, 65]. In the following section, internal structures involved in both phases of neuronal growth are introduced in some detail.

2.1. Neuronal ultrastructure

The inherent mechanical properties of a cell influence its shape and response to mechanical forces. These properties are largely governed by the cytoskeleton [66–70]. It provides structure to the cell, is involved in many active processes, such as cell growth and migration, and is assumed to be the main contributor to a cell's active and passive mechanical properties. The cytoskeleton is a dynamic structural and functional framework that is made up of a complex entangled, transiently cross-linked polymer network. This network is composed of three major types of biopolymers as well as of associated proteins such as motor proteins, cross-linkers and other polymer-associated proteins [71]. The three types of cytoskeletal polymers found in eukaryotic cells are microtubules, actin and intermediate filaments.

The most abundant types of intermediate filaments in the nervous system include neurofilaments, which are found in neurons, and glial fibrillary acidic protein (GFAP) and vimentin, which are both found in glial cells. Neurofilaments, which are flexible, apolar polymers with a persistence length of $\sim$1 μm [72], are the most abundant components of axons. They are very stable and almost completely polymerized within a cell [6], which distinguishes them from actin and microtubules. These other two cytoskeletal components generally feed from and contribute to a large monomer pool in constant polymerization and depolymerization. Like most intermediate filaments, neurofilaments provide mechanical resistance against large deformations [73].

Microtubules are hollow cylindrical structures composed of tubulin dimers, whose polar structure conveys a directionality to the polymer; microtubules grow by the addition of tubulin dimers to their plus end. With a length-dependent persistence length of hundreds of micrometers to some millimeters [74] they can be considered rigid rods inside cells. Microtubules usually exhibit a dynamic instability, i.e. they alternate between slow growth at their plus ends and rapid plus end disassembly ('catastrophe'), which may be followed by the recovery of plus end assembly ('rescue') [75]. This dynamic instability enables microtubules to efficiently probe the intracellular space. In mature axons and dendrites, however, they are organized into a paraxial bundle and are relatively stable. This stability is attributable to microtubule-associated proteins (MAPs) such as MAP1, MAP2 and Tau, which suppress the microtubules' intrinsic dynamic instability. Microtubules extend the full length of a neuron, form a dense parallel array in neurites and generally splay apart when they enter the growth cone. In axons, all microtubules are arranged in parallel with their plus ends pointing distally, thus allowing the orderly transport of cell organelles. In dendrites, however, microtubule polarities are mixed [6, 76].

The actin network, which is crucial for neuronal motility [77], is composed of semiflexible polymers with a persistence length of $\sim$9 μm [78]. They are also polar polymers ('F-actin') made of actin monomers ('G-actin'), wound into a double-stranded helix. Actin constantly polymerizes from the (+) or 'barbed' and depolymerizes from the (−) or pointed ends in a process called treadmilling. In nerve cell processes, most of the F-actin array is organized into a cortical mesh, while there are also some actin filaments arranged paraxially within the axon. Growth cones consist of a flat region called lamellipodium, densely packed with F-actin, and spike-like protrusions, full of actin bundles, called filopodia [48, 79–81] (figure 3). Apart from actin, also microtubules may extend into filopodia [82]. In addition to these three main components of the cytoskeleton, motor proteins and a multitude of other regulatory proteins

associated with the cytoskeleton are important for normal cytoskeletal functioning [83]. Motor proteins found in neurites include the microtubule-associated dyneins and kinesins as well as actin-associated myosins. Myosin II for instance is concentrated in a growth cone's transition zone and—together with actin-network treadmilling—drives the so-called retrograde actin flow [84, 85]. Finally, the cytoskeleton is coupled to a neuron's environment via transmembrane proteins such as integrins and cadherins [81, 86], which provides anchorage and enables growth cones to exert forces on their substrate (cf section 2.2). Complexes of proteins coupling the actin cytoskeleton to the ECM are called *molecular clutches*. The highly complex interaction between actin, microtubules and their associated proteins is crucial for growth cone motility, neuronal process outgrowth and guidance [21, 87–90].

2.2. *Forces in neurite extension*

Different models exist explaining how axons and dendrites elongate. On the one hand, it is a long-standing debate where mass addition occurs during axonal extension [91]. Growth is thought to either happen at the tip of the advancing growth cone ('tip growth') or along the length of the elongating axon ('stretch growth'). Most tip growth models assume mass addition through microtubule polymerization or the addition of new microtubules at the growth cone [21, 92]. When the actin cytoskeleton is disturbed using small amounts of cytochalasin B, neurite extension continues but results in misdirected outgrowth [93, 94], suggesting that microtubules alone can push growth cones forward. These models predict an increase in axonal elongation rate when microtubules are prevented from depolymerization. However, a stabilization of microtubules with taxol slows down elongation [95, 96], thus contradicting tip growth models. Furthermore, mass addition in the tip does not seem to be the driving force behind axonal lengthening [97], because rapidly advancing growth cones are small while pausing growth cones enlarge [98]. Alternatively, stretch growth models assume that mechanical tension along extending neurites [61, 99] leads to their elongation [100]. Lamoureux and colleagues showed that growth cones *in vitro* indeed generate tension by pulling [100]. By attaching neuronal somata to calibrated glass needles and lifting the somata, so that only the growth cone remained attached to the substrate, needle deflections clearly showed an increase in neurite tension with growth cone advance, indicating growth cone pulling to be the reason for neurite elongation. Possible origins of this tension considered in the literature include contractile filopodia [101] (cf section 2.3), cytoplasmic filling of the growth cone from the rear (called cytoplasmic pushing) [102, 103] and cytoskeletal pushing [104].

The tension along neurite branches in *in vitro* networks is maintained through the attachment of neurons to their substrate (cf section 2.1). Here, tension controls the diameter of the neurites and the junction geometry [61, 105]. The tension along PC12 neurites *in vitro* has been determined to be on the order of 300–400 pN [106]. The build-up of mechanical tension furthermore promotes the stabilization of some neurite branches, while it causes retraction or elimination of neurite collaterals, suggesting tension to be a crucial, early step that regulates neuronal interconnections [107].

Bray showed that the external application of mechanical tension alone is sufficient to initiate axonal outgrowth [62] (cf section 1.3). Tension applied to neurites and exceeding a threshold causes their initiation and/or elongation; forces below this threshold viscoelastically deform the neurites without growth. In CNS neurons, this force threshold is significantly lower [108–110] than in PNS neurons [111, 112]. The elongation rate of towed neurites from both CNS and PNS nerve cells increases proportionally to the magnitude of tension; an increase in tension of 10 nN results in an increase in the elongation rate of about 1–1.5 μm h^{-1} [108, 109, 112, 113]. Processes of hippocampal neurons resulting from towed growth assume axon-like characteristics, suggesting tension to be a key player in axonal specification [114]. Mechanical tension can even induce extreme 'stretch growth' of integrated axon tracts, a mechanism with potential for future medical applications [63, 115, 116] (see section 4). Here, elongation occurs by viscoelastic stretching and, in a loosely coupled step, by mass addition along the axon [117]. On the other hand, the application of low tensions may lead to neurite retraction, which takes place *in vitro* [61, 99, 106, 109, 111, 118] and *in vivo* [19, 119, 120].

Axonal elongation in response to force application has been modeled by different groups. Dennerll described the viscoelasticity of the axon with a Burgers element, which is composed of a spring, a Voigt element, and a dashpot in series [111]. This model accounted for the experimentally observed three steps in the axonal response to applied mechanical tension: initial elastic stretch, a subsequent period of delayed stretching and finally elongation at a constant rate. According to the O'Toole model, which is an extension of the Dennerll model to the entire axon, each segment of the axon behaves as a Burgers element [97]. Here, the axon is treated as a viscoelastic fluid, and forces exerted at the growth cone are dissipated by adhesions along the axon. This model excellently fits experimental data in which the velocity profile of docked mitochondria in stretched neurites was linear in unattached regions and strongly nonlinear in attached neurite regions: the tension-driven anterograde movement of the cytoskeleton within neurites diminished with distance from the growth cone [97, 121] (figure 4).

The model predicts that the elongation rate of an axon attached to the substrate is proportional to $F_0/(G\eta)^{1/2}$ [97], where F_0 is the constant tension, G, the growth dashpot parameter characterizing an axon's response to distally applied forces, is the product of the axon's axial viscosity $g = (3.6 \pm 2.4) \times 10^6$ Pa s and the cross-sectional area of the axon, A. The constant of friction, η which quantifies the interaction between axon and substrate, is determined by the strength and number of adhesions (g and η characterize the resistance to flow and thus have dimensions of viscosity). Thus, an increase in viscosity due to microtubule polymerization leads to a decrease in elongation velocity, explaining the decreased elongation rate of neurites treated with taxol [95, 96] mentioned earlier. Consequently, tip growth might simply be a special case of axonal stretching where

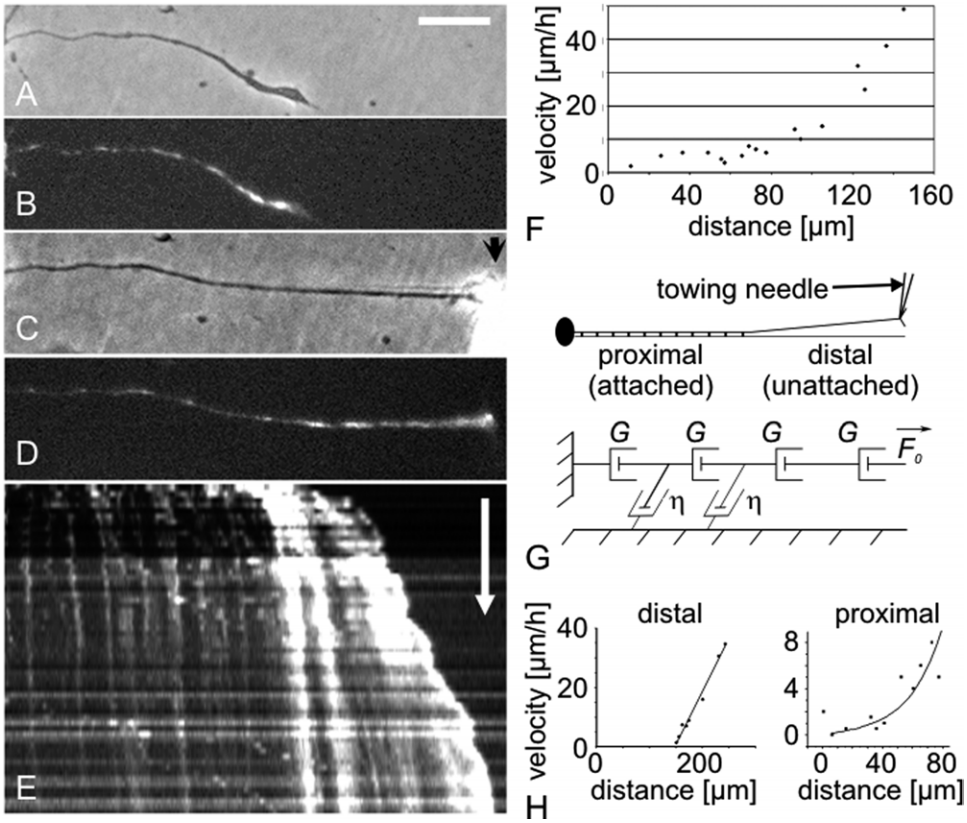

Figure 4. O'Toole model describing axonal elongation in response to constant tension.
(*A*) Neurite before force application, fluorescently labeled mitochondria are visible in (*B*). (*C*), (*D*) After force application with a calibrated glass needle attached to the growth cone (arrow in (*C*)), the neurite elongates. (*E*) The kymograph shows the mitochondrial distribution during a tow; arrow = 30 min. Scale bar: 20 μm. (*F*) During such an experiment, the velocity of mitochondria increases nonlinearly with increasing distance from the soma. (*G*), (*H*) This behavior can be modeled by treating the axon as a viscoelastic fluid, where forces exerted at the growth cone are dissipated by adhesions along the axon. (Adapted from O'Toole *et al* (2008) [97] and reprinted with permission from Elsevier.)

stretching is restricted to the growth cone because of strong axonal adhesions, high viscosity or large thickness of the axon or low force generation in the growth cone [97]. Hence, tension may—similar to a second messenger—act as a regulator of different phases in axonal development: axonal initiation, growth cone-mediated elongation, branching, growth after the growth cone reaches its target and axonal retraction [107, 122]. Neuromuscular synapses employ mechanical tension even as a signal to modulate the clustering of neurotransmitter vesicles and synaptic plasticity [123].

The neurons' cytoskeleton is important for the maintenance of the tension; while actin with its associated proteins, particularly myosin, supports neurite tension, microtubules and its associated proteins oppose it [106, 111]. Neurite retraction may result from myosin-mediated forces on the F-actin array, while these forces are counterbalanced by dynein-mediated forces acting between the F-actin and microtubule arrays [89]. Again, calcium plays a key role in regulating neurite elongation by regulating the balance of such cytoskeletal dynamics [120, 124–126].

In conclusion, growth cones pull on their neurites, thus creating mechanical tension; the extent of neurite lengthening depends on this tension, on the neurite's viscoelastic properties and on its environment. However, even if filopodia have been reported to be the neuronal structures exerting the largest traction forces [127], it has been shown that filopodial contraction—as originally assumed—cannot account for growth cone advancement [102, 103]. We will explain growth cone motility and the physics involved in it in more detail in the following section.

2.3. Growth cone motility

Growth cones are highly motile structures. Actin is continuously polymerizing at their leading edge, and at the same time it is retracted by a myosin-driven centripetal retrograde F-actin flow [84, 85, 128–130] (cf section 2.1). The actin polymerization is controlled by a stochastic bistable process [131]. As a consequence, the growth cone's leading edge switches randomly between extension and retraction [132]; filopodial and lamellipodial motion can essentially be understood as a random process in which errors are corrected by efficient feedback loops [133]. During neuronal development, stochasticity controls numerous intra- and extracellular processes such as biochemical reactions in small subcellular structures and the pattern of weak external signals [131, 134]. Accordingly, the growth direction of a neurite is determined by the tendency to grow toward some particular location (deterministic component driven by gradients) and the noise in the growth angle, i.e. a random deviation from

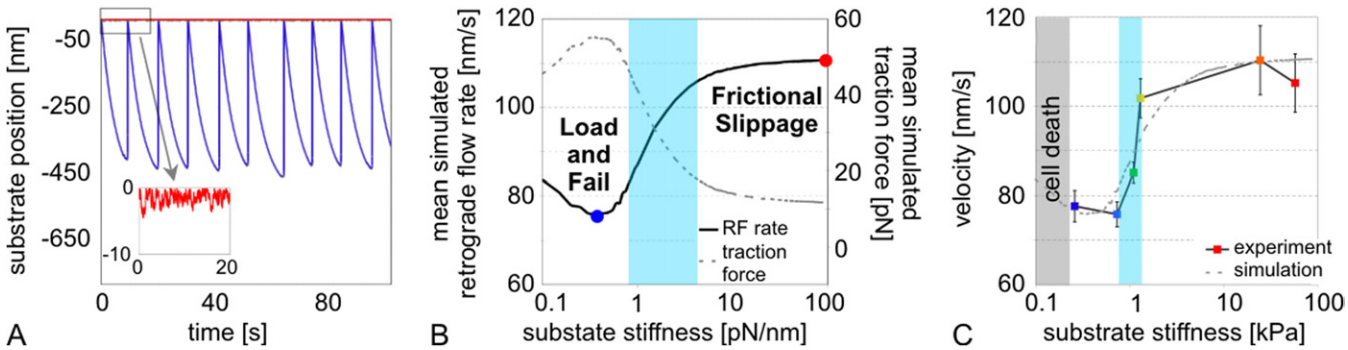

Figure 5. Substrate stiffness-dependent model for motor-clutch motility. (Reprinted from Chan and Odde (2008) [127] with permission from AAAS.) (*A*) For growth cones plated on compliant substrates, the model predicts an oscillatory change in substrate positions due to a 'load-and-fail' dynamics of the motor-clutch (blue line). On stiff substrates, 'frictional slippage' dominates the clutch dynamics (red line). (*B*) According to the model, the 'frictional slippage' regime on stiff substrates is characterized by fast retrograde flow (RF) and low traction forces, while in the 'load-and-fail' regime on soft substrates retrograde flow is slower and traction forces are larger. (*C*) These predictions are well-matched by experiments measuring the retrograde F-actin flow over a range of substrate stiffnesses. An abrupt switch in F-actin dynamics from 'frictional slippage' to 'load-and-fail' dynamics occurs on substrates with an elastic modulus of around $E = 1$ kPa.

the current growth direction (noise component) [135]. It is interesting to note that, despite a noisy environment, growth cones are able to detect chemical signal gradients as small as a single molecule difference across their structure [136]. Moreover, these weak signals are very likely to undergo rapid and large fluctuations. It has been suggested that neurons may exploit 'stochastic filtering' [137] to detect signals below the noise level [131].

Independent of the stochastic processes involved in determining growth direction, there are several models that explain filopodial and growth cone protrusion. Ratchet models [138], such as the 'elastic' Brownian ratchet [139], describe how chemical bond energy is transduced into directed motion without operating in a mechanochemical cycle and directly depending upon nucleotide hydrolysis [138]: the thermal motions of polymerizing actin filaments can generate protrusive forces by rectifying Brownian motion [139]. Actin filaments are considered spring-like wires, which are constantly bending because of thermal energy. When bent away from the cell surface, a G-actin subunit can 'squeeze' in, lengthening the filaments; when straightening against the surface, the restoring force of the filament delivers a propulsive force, thus pushing the filopodial and lamellipodial edge forward [83]. In accordance, switching of 'on/off' states in actin polymerization has been identified as the main determinant of lamellipodial advancement [132]. Each actin filament works as an elastic Brownian ratchet, generating a force of several piconewtons [138–140]. Growth cone lamellipodia have been described to be able to displace beads from an optical trap with a maximum trapping force of 20 pN, while filopodia could not remove beads from traps with a trapping force of 3 pN [133].

In order to advance the growth cone, the motion generated in the actin cytoskeleton needs to be physically linked to the extracellular environment. This is done through a complex assembly of proteins, so-called molecular clutches [79, 141, 142]. This mechanical coupling is thought to create an interface between growth cone and substrate that transmits traction forces to the environment and slows down the retrograde F-actin flow (cf section 2.1), thus allowing actin polymerization to advance the leading edge [127, 142]. In embryonic chick forebrain neurons, a switch in F-actin dynamics from a 'frictional slippage' regime on stiff substrates with fast retrograde flow and low traction forces to an oscillatory 'load-and-fail' dynamics on soft substrates, with slower retrograde flow and higher traction forces, occurs on substrates with an elastic modulus of around 1 kPa [127] (figure 5). The traction force exerted by growth cones is sufficient to deform substrates with a compliance commensurate with their own [118, 143]. Specific interactions between growth cones and surface molecules, such as netrin-1, may modulate the forces exerted by the growth cones [144]. The adhesion force of a neuronal growth cone is comparatively high: the forces required to detach neurons from differently treated plastic surfaces were 3–15 times greater than the typical resting neurite tension, the forces exerted by advancing growth cones or the retraction force; the stress required for growth cone detachment was on the order of 100–200 pN μm^{-2} [145].

Traction forces generated in neuronal growth cones, arising from myosin-driven retrograde F-actin flow in concert with cell adhesion, are also involved in axon guidance [85]. The different guidance mechanisms can act via various pathways, but they probably all converge in local intracellular gradients of second messengers such as cyclic nucleotides [39, 50] and calcium [51, 52, 146, 147], which ultimately result in local changes of the growth cone's actin and microtubule cytoskeleton [21, 82, 87, 148–150]. In particular microtubules seem to be crucial for the determination of growth direction, since the inhibition of microtubule dynamics prevents growth cone turning in response to guidance cues, while their localized stabilization induces turning [151]. Any of the pathways mentioned above can potentially be targeted to control growth and guidance of axons.

3. Neuronal susceptibility to physical cues

During neuronal growth, growth cones navigate through a highly complex environment. They have to integrate a multitude of signals in order to reach their target. As

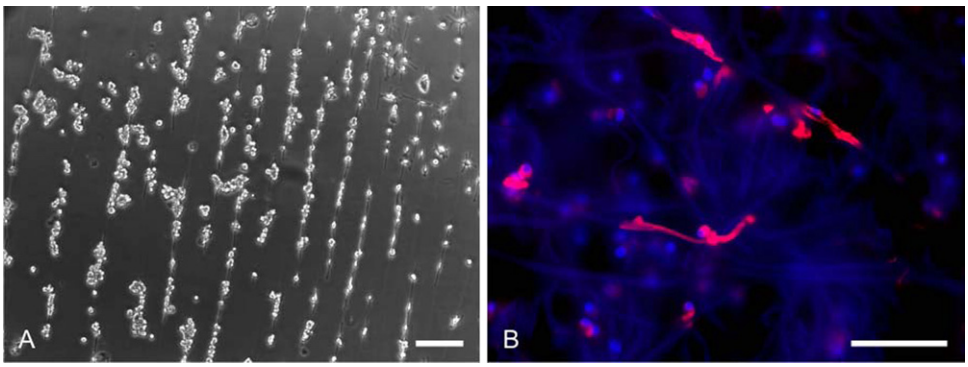

Figure 6. Sterical guidance of astrocytes.
Topological cues, such as creases in the 100 nm range (*A*) or fibers (*B*), are often enough to cause directed outgrowth of cells of the nervous system. (*A*) Astrocytes cultured on polyacrylamide gels with a creased surface. (*B*) Astrocytes grown within a gelatin fiber network. Note how the cells align along the structures. Red: GFAP, blue round structures are cell nuclei, and blue longitudinal structures are gelatin fibers. Scale bars: 60 μm. Images courtesy of Pouria Moshayedi.

introduced in section 1.2, much is known about biochemical guidance cues, which can be in solution or bound to a surface [22, 24, 29, 51]. However, growth cones do not exclusively respond to biochemical cues. Guidance of *pioneer axons* occurs through a variety of mechanisms, including chemotaxis, haptotaxis, contact inhibition and mechanical guidance [152]. For instance, pioneer axons of retinal ganglion cells may retract after both biochemical and mechanical stimulation [153]. In the following section, we will talk about different physical stimuli that may influence neuronal growth and potentially serve neurons as guidance cues. We will focus here on mechanical cues to summarize the rapidly growing body of evidence for neuronal susceptibility to mechanical stimuli.

3.1. Guidance by topological, electrical and optical cues

The topology of the growth substrate belongs to the best-studied physical guidance cues. Contact guidance has been suggested as a crucial mechanism involved in neuronal migration in the developing cortex already in the late 1970s [154]. Physical constraints observed in 3D cultures are similarly encountered *in situ* in the extracellular environment. These sterical constraints can be used to guide neurites [155, 156]. Neurons, and also glial cells, in 2D preferentially extend cell processes along mechanical discontinuities such as grooves or scratches on a substrate [6, 157] (figure 6). Furthermore, aligned but not randomly oriented polymer fiber-based constructs present sub-micrometer scale topographical cues that facilitate the regeneration of peripheral nerves [158]. Thus, topographical cues can influence endogenous nerve growth mechanisms in the absence of exogenous growth promoting signaling molecules or proteins. It was also suggested that the surface energy distribution, through cell–substrate interactions, may trigger neuritogenesis [159].

Neuronal growth can also be guided using electromagnetic fields; in small dc fields, growth cones turn toward the cathode [160, 161]. The turning responses of growth cones induced by gradients of biochemical guidance cues can be significantly altered by applying brief periods of electrical stimulation [162]. Transcutaneous electrical stimulation after crush lesion of the sciatic nerve on the other hand may lead to a delayed regeneration [163]. Finally, extending neurites can be guided by the application of weak optical forces, generated by a focused infrared laser beam placed adjacent to the leading edge of the growth cone [164–166].

The underlying mechanisms of all these physical guidance cues remain unclear. It seems likely that all of them eventually result in influencing the growth cones' cytoskeleton, which determines the direction of growth [21, 87, 148–150] (cf sections 2.2 and 2.3).

3.2. Neuronal mechanosensitivity

In vitro studies of neurons, neuronal networks and nervous tissue slices have mostly been carried out in 2D on either glass or plastic surfaces. However, these surfaces are orders of magnitude stiffer than the normal neuronal environment. Recent studies indicate that neurons and glial cells are actually susceptible to mechanical stimuli from their surroundings so that special consideration has to be given to the mechanical makeup of the environment—a factor that can determine neurite growth.

Culturing primary cortical or spinal cord neurons from the CNS or PC12 cell lines on deformable substrates of different compliance often resulted in changes of their growth characteristics and morphology if compared with growth on tissue culture plastics or glass (short summary provided in table 1). Depending on the culture system and data analysis used, the absolute number of neurons was independent of substrate stiffness [167, 168] or increased with increasing substrate stiffness [169, 170], while the number of neurite branches and/or neurites decreased [171–175], showed a biphasic trend [170] or increased with increasing substrate stiffness [168, 169]. The average neurite length seems not to be influenced by the mechanical properties of the cell culture substrate [167, 168, 170]. More specifically, in a different study the average axon length increased with increasing substrate compliance while the dendrite length was not influenced [169]. This difference in the response of axons and dendrites to mechanical cues in their environment is likely attributable to the differences in their microtubule cytoskeleton. Furthermore, neurons apply greater traction

Table 1. Summary of studies investigating neuronal growth on substrates of different compliance. ↑ increase, ↓ decrease, ↔ no change, ↓↑ decrease followed by increase.

Paper	Cell system	Gel used	Stiffness range (G')	Observations with increasing stiffness
[172] Flanagan *et al* (2002)	Mixed spinal cord cultures	PAA	50–550 Pa	No glia (died), neuronal branches ↓
[173] Gunn *et al* (2005)	PC12 cell line	PEG	E from 20 kPa to 400 kPa	Number of cells with neurites increases with coating concentration and decreases with gel stiffness
[167] Georges *et al* (2006)	Mixed cortical cultures	PAA/fibrin	0.2–9 kPa/ 250–2150 Pa	Astrocyte spreading ↑ (small and round on soft gels), neuronal phenotype unchanged, neurite length ↔, F-actin organization in astrocytes ↑, F-actin structures extending off the length of the neurite ↓, astrocyte area ↑, area independent on coating density (0.05–0.2 mg ml^{-1}), number of astrocytes ↑ in pure and mixed culture, number of neurons ↔ in mixed culture; in co-culture 80% of cells neurons on soft, 45% on stiff PAA, 25% on stiff fibrin, rest astrocytes
[168] Leach *et al* (2007)	PC12 cell line	PAA	7 Pa–18.9 kPa	Number of cells expressing neurites, number of neurites per cell, total neurite length per cell, and branches per mm neurite equal on 190 Pa, 2 kPa, 19 kPa, only lower at 7 Pa.
[170] Jiang *et al* (2007)	Mixed and pure neuronal and astrocyte spinal cord cultures	PAA	0.3–230 kPa	*Mixed cultures*: no oligodendrocytes, neuronal phenotype unchanged, different astrocyte phenotypes, number of neurons ↔, number of vimentin + astrocytes ↑, number of GFAP + astrocytes ↑↓, neurite number ↓↑, neurite length ↔ *Pure neuronal cultures (6–230 kPa)*: number of neurons ↑, neurite number ↓↑, neurite length ↔ *Pure astrocyte cultures*: number of vimentin + astrocytes ↑, number of GFAP + astrocytes ↑↔; Better neuronal survival after glutamate intoxication of mixed cultures on stiffer gels
[169] Jiang *et al* (2008)	Mixed spinal cord cultures (60% are either neurons or GFAP + astrocytes)	DNA cross-linked PAA	6.6–29.8 kPa	Different astrocyte phenotypes, number of neurons ↑, number of astrocytes ↔, dendrite number ↑, dendrite length ↔, axonal length ↓, FAK expression in neuronal cell body ↓

forces and exhibit a slower retrograde actin flow on softer substrates [127] (cf section 2.2). It needs to be noted that the culture systems used in these studies differ sometimes significantly in key parameters such as substrate stiffness and coating, thus making a direct comparison difficult.

When CNS neurons were co-cultured with glial cells, it was mainly the glia cells that responded to the change in substrate stiffness by an increase in area and overall complexity [157, 167, 169, 170]. The neuronal morphology was rather unchanged. However, in these co-cultures, neurons were mostly growing on top of glial cells, which in the CNS are the most compliant cells [27]. Importantly, substrates with stiffness comparable to that of brain tissue select neuronal over glial growth [167] (figure 7).

Thus, the morphological response of neurons to mechanical substrate properties is rather complex. Furthermore, so far only few neuronal cell types have been studied, and it is likely that other neurons respond distinctly to mechanical cues. Generally, neurons seem to preferentially grow on soft substrates with a shear modulus of a few hundred pascals, which is commensurate to CNS tissue stiffness, while glial cells seem to prefer stiffer substrates with a shear modulus of a few kilopascals [157, 167, 176, 177]. This could be the basis for a mechanical structuring of the CNS.

Neural cells also respond to actively applied mechanical stimuli. Cultured neurons and neuron-like cells respond to globally applied shear strains or stresses by changing their mechanical properties, their cytoskeletal composition and their survival rate [178–180]. Concomitantly, the free intracellular calcium concentration rises with increasing strain [181]. When a growth cone is exposed to a mechanical stress that exceeds a certain threshold, it collapses and its neurite retracts in a calcium-dependent manner [118]. Neurites are then either completely withdrawn or grow out again in a different direction, suggesting an involvement of mechanical stimuli in neuronal branch pruning and/or pathfinding. Particularly

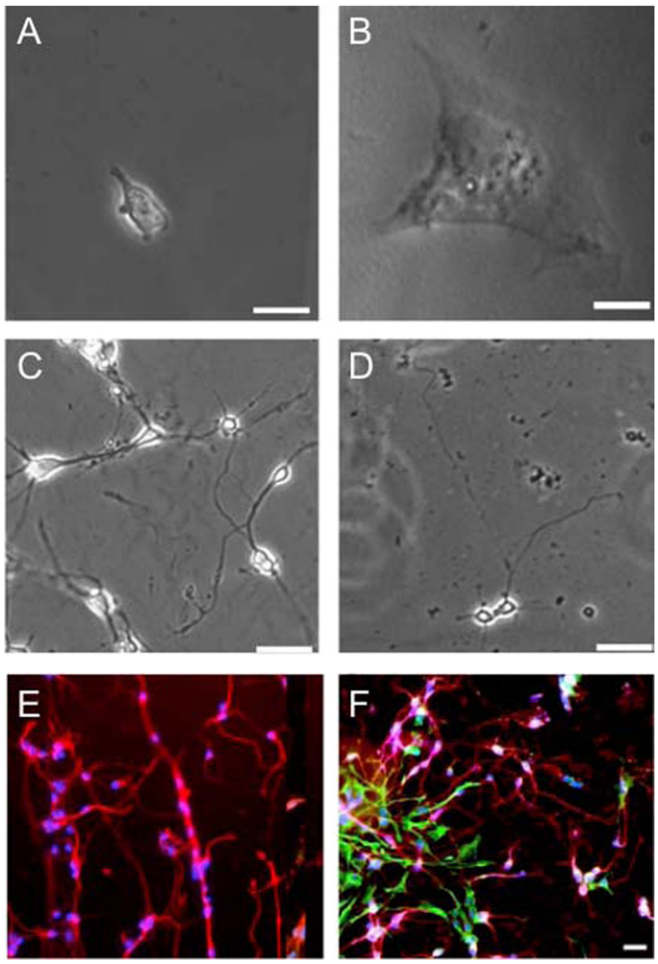

Figure 7. Mixed CNS cultures on substrates of varying stiffness. While astrocytes are smaller and less spread on compliant gels (*A*) as compared with stiff ones (*B*), the neuronal morphology barely changes ((*C*) compliant; (*D*) stiff). (*E*), (*F*) In mixed cultures, astrocytes (green) preferentially grow on stiff substrates. Again, the neuronal morphology (red) is similar on both substrates. It needs to be mentioned that on stiff substrates neurons preferentially grow on top of (compliant) astrocytes. Scale bars: 50 μm (*A*), (*B*), 25 μm (*C*)–(*F*). (Figure adapted from Georges *et al* (2006) [167] reprinted with permission from Elsevier, copyright 2006.) (Colour online.)

axons seem to be susceptible to mechanical injury, with rapid stretching being one of the most serious reasons for cell damage. This susceptibility, which is characterized by damage of the axonal cytoskeleton, consequential impairment of axoplasmic transport, axonal swelling and calcium entry into the damaged axons, is attributed to both their highly organized structure and their mechanical properties [182]. Consequences of similar mechanical stimuli for glial cells in culture include astrocyte hypertrophy and an overexpression of GFAP and chondroitin sulfate proteoglycans [183], which is similarly observed in gliotic reactions. RGCs in retinal whole-mounts respond to actively applied tension in the tissue by an increase in intracellular calcium concentration. This calcium rise triggers molecular responses of these glial cells such as the upregulation of transcription factors and basic fibroblast growth factor, which could prevent neuronal damage [184].

To study neuronal growth in 3D environments, mostly compliant materials have been used, such as collagen, fibrin or agarose gels. However, the influence of the hydrogels' mechanical properties on the growth characteristics of neurons has rarely been considered. Currently, only data on mechanics-dependent neurite extension of dorsal root ganglion cells (neurons of the PNS) are available. The rate of neurite extension was inversely correlated with the mechanical stiffness of agarose gels [185], while in collagen gels neurite extension rate peaked at intermediate stiffness [186]. The ability of neurites to cross 3D mechanical barriers formed by the elasticity mismatch of layered agarose gels was strongly decreased [187]. Interestingly, even neural stem cell fate can be directed using mechanical cues [175, 188–193].

A disadvantage of using biopolymers as growth substrate for cells is their biological activity. Cells possess receptors that can specifically bind to biopolymers such as collagen, which in turn may obscure any response due to the mechanical cue. Furthermore, in all gels used in these studies, mechanical parameters are tightly coupled to meshsize. As the gel concentration is increased, the average pore radius decreases exponentially [194], leading to a structural barrier for neurites. This structural barrier may influence neuronal outgrowth much more than the bulk mechanical properties of the gels [186, 195], which in these studies varied only little [185].

Despite the available evidence for neuronal mechanosensitivity, its origin remains poorly understood. A prerequisite for the perception of mechanical material properties is the application of forces on those materials. As discussed in sections 2.2 and 2.3, *in vitro*, there are two different types of forces permanently exerted by neurons: tension along the neurite [61, 99, 109, 118] and contractile forces exerted by the growth cone [118, 127, 143]. However, it remains unclear how the deformation of a neuron's environment caused by these forces is translated into a cellular response.

This response could either be unspecific, driven by simple energetic constraints [196] or caused by 'frictional slippage' [127, 197]. Alternatively, the presence of mechanosensitive ion channels, which increase their opening probability with mechanical tension [198], in the growth cone membrane has been suggested to be involved in neuronal mechanosensitivity [118, 199–203]. An influx of calcium, which has a multifunctional role in directional sensing, cytoskeleton redistribution, traction force generation and relocation of focal adhesions [204, 205], directly or indirectly triggered by the opening of stretch-activated ion channels, appears to be crucially involved in at least some of the responses of mechanosensitive neurons to mechanical stimuli [118, 199, 206, 207]. However, the nature of these putative mechanosensory ion channels is currently still enigmatic. Even if different channel families are discussed, the activation principle should be very similar. Recent experiments indicate that these channels likely belong either to the transient receptor potential (TRP) channel family, to degenerin/epithelial Na^+ channels (DEG/ENaC), and/or to two-pore-domain K^+ (K_{2P}) channels [208–210]. Further work is required to unequivocally demonstrate an involvement of one or more of these channels or any of the other mechanisms in neuronal mechanosensitivity.

Whatever the mechanism, the response of growth cones to mechanical cues in their environment suggests an involvement

of mechanics in neuronal pathfinding. Different types of tissue cells, such as fibroblasts, have been shown to preferentially grow toward stiffer regions on substrates incorporating gradients of mechanical compliance [211, 212]. In contrast, neurons *in vitro* prefer to grow on compliant substrates or are repelled by stiff ones [118, 167, 172]. *In vivo*, neurons grow and migrate along glial cells [13, 14], which in the CNS are significantly more compliant than their environment [27] (cf section 3.3), suggesting an involvement of mechanics in axonal branch pruning and neuronal guidance. Accordingly, it was shown that neurons grown in 3D collagen gels with gradients of mechanical properties send the majority of neurites into the direction of lower stiffness [213]. However, as discussed above, this could be a consequence of the gel's meshsize or its biological activity rather than its mechanical properties. Studies with substrates excluding sterical and specific binding effects will have to shed light on the dependence of directionality of neurite outgrowth on mechanical substrate properties in the future.

3.3. Mechanical properties of nervous tissue and cells

The mechanosensitivity of neurons suggests the mechanical properties of their environment *in situ* to be important. A detailed, quantitative knowledge of these mechanical properties of the neuronal environment is thus desirable to better model, mimic and understand neuronal mechanosensitivity—knowledge that is currently only incompletely available.

Nervous tissue and its constituents (neurons, glial cells and the ECM) are generally nonlinear, viscoelastic materials [27, 176, 214, 215]. Their response to mechanical stress or strain depends on the timescale on which stress or strain are applied. Depending on the mode of testing and on experimental parameters such as age or species, sample preparation, location, *post-mortem* time interval prior to testing, strain or stress magnitudes or loading rates, the shear modulus of nervous tissue is on the order of a few hundred pascals to few kilopascals [27, 167, 216–219]. Thus, nervous tissue is significantly more compliant than other tissues in our body (apart from fat and bone marrow) [220]. Consequently, the comparably stiff dura mater, which envelops brain and spinal cord, may significantly influence measurements if not removed [221]. Furthermore, nervous tissue is strongly heterogeneous and anisotropic, and its mechanical properties differ considerably in different regions [176, 219, 222]. The extent of regional anisotropy correlates with the degree of alignment in the local neuroarchitecture [223]. Adult brain *in vivo* becomes more compliant with time and female brains are stiffer than male ones, so that women are more than a decade 'younger' than men with respect to brain mechanics [224]. Additionally, the mature brain appears to be more compliant than the immature [216, 225]; however, contradictory data show that with increasing age nervous tissue becomes stiffer [226] and more resistant to tensile stress [227].

The mechanical characteristics of biological tissue depend on the mechanical properties of its constituents, which in itself can be quite complex [1] and are summarized in the following. Growing neurites may show a transition from a viscoelastic elongation to active contraction [228]. Neuronal dendritic spines, which are a key component of brain circuitry, exhibit a wide range of rigidities, correlated with morphological characteristics, axonal association and glutamatergic stimulation [229]. Spring constants of PC12 cell neurites have been measured to be about 240 mN m^{-1} [106], neurites of dorsal root ganglion cells are apparently stiffer [111] and forebrain neurons show only a weak elastic behavior [108]. Generally, it seems that processes of CNS neurons show a significantly less elastic behavior than those of PNS neurons [108, 109, 111, 112, 180].

Primary neurons of hippocampus and retina, while comparatively compliant with a Young's modulus of $E \sim 1$ kPa, have been shown to be about twice as stiff as their neighboring glial cells ($E \sim 400$ Pa) [27], suggesting that glial cells cannot offer any mechanical support to neurons. This is in contradiction to current textbook knowledge, which implies that one of the main functions of glial cells is to provide mechanical support ('support pillars') for neurons. Instead, they might act as compliant, elastic shock absorbing materials surrounding neurons to protect them in the case of mechanical trauma [27]. Nevertheless, in the spinal cord the glial matrix appears to provide significant mechanical support to neurons, suggesting that myelin, ECM and cellular coupling of axons via the surrounding network of glial cells dictates the tensile properties of this tissue [230]. Interestingly, the mechanical properties of RGCs vary in different regions. Despite both of their stem processes being densely packed with cytoskeletal elements [231, 232], they are significantly softer than any other cellular structure in their environment, possessing a shear modulus of around 200 Pa [27] (figure 8). Given the preference of neurons for compliant substrates, the high compliance of RGCs, along which neurons migrate and grow during development [13, 14], might be understood as formerly unknown guidance cue.

4. Outlook: biophysics in neuronal regeneration

PNS and CNS are very different concerning their capability to heal after an injury. In the PNS, proliferating Schwann cells and white blood cells create an environment that supports regeneration of injured neurons, whose ends proximal to the lesion may eventually regrow and restore nerve function. For small gaps, severed nerves can be surgically sutured end-to-end to improve recovery. For larger gaps between the nerve stumps, devices are used to bridge the gap to guide the outgrowing nerve fibers and to prevent the formation of nerve tumors. Devices can be nerve autografts, nerve conduits and tissue-engineered constructs. They are designed in a way to provide an optimal environment to the regenerating neurons; artificial devices ideally incorporate beneficial sterical (e.g. aligned fibers), biological (e.g. Schwann cells), chemical (e.g. growth factors, chemical guidance cues), electrical (e.g. electrodes for stimulation) and mechanical (e.g. materials in preferred stiffness range) cues [233–238]. Here, biophysics can contribute to improving the efficiency of these devices by understanding and exploiting the neuronal susceptibility to physical stimuli, e.g. sterical, electrical and mechanical cues.

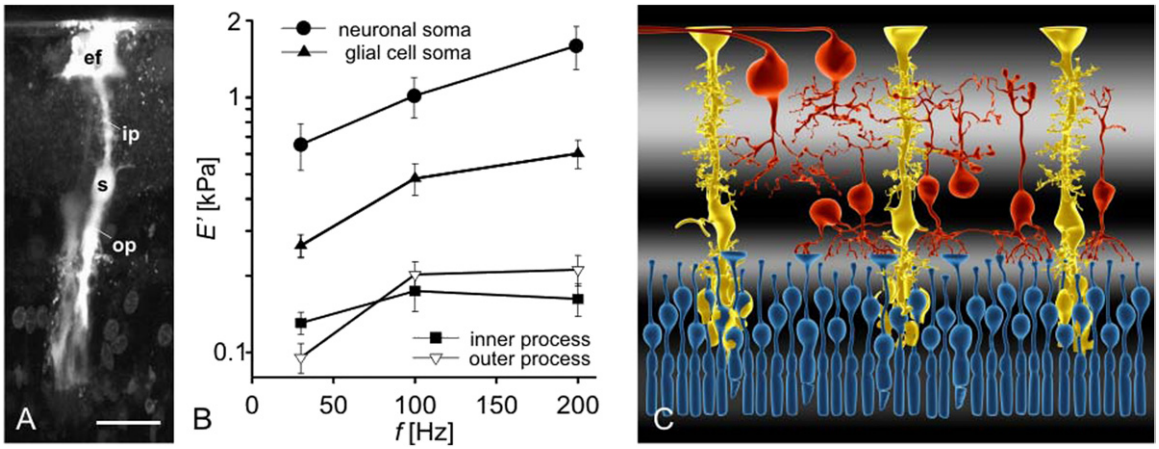

Figure 8. Stiffness distribution in the retina.
(*A*) Radial glial (Müller) cells in the retina consist of an outer (op) and inner (ip) process emanating from the soma (s), the latter terminating in an endfoot (ef). Scale bar: 20 μm. (*B*) Elastic storage modulus of Müller cell parts measured with frequency-dependent scanning force microscopy. Despite being densely packed with cytoskeletal filaments, both Müller cell processes are twice as compliant as the Müller cell soma, and four times as compliant as the neuronal soma. (*C*) Interestingly, in the retina all synapses and most dendrites (red) are restricted to those layers where the glial cells are most compliant. This retinal morphology, together with the neuronal preference for compliant substrates, suggests an involvement of mechanical cues in neuronal pathfinding. (Adapted from Lu *et al* (2006) [27], Copyright (2006) National Academy of Sciences, USA.) (Colour online.)

Regeneration and repair in the mammalian CNS, however, is very limited—even with new treatments under development. When CNS tissue is damaged a glial scar forms primarily by reactive astrocytes, which increase their expression of intermediate filaments and ECM in the vicinity of the disrupted blood–brain barrier [239]. This glial scar is known to inhibit regrowth of axons [240, 241]. Accordingly, scar-modulating treatments have become a leading therapeutic goal in the field of spinal cord injury [242]. While research so far has exclusively focused on the inhibitory effect of molecules present at the site [243], the aspect that the scar tissue also forms a physical barrier to axon regrowth [244] has not been explored.

First, the accumulation of ECM in glial scar tissue [239] probably leads to a decrease in available space, thus building a structural barrier ('steric hindrance') for neurites [186, 195]. Second, with the formation of the glial scar a mechanical barrier could arise, which is impenetrable for neuronal cell processes [187], thus preventing regeneration. And thirdly, it seems sensible that neurons might avoid glial scars because they prefer compliant structures and avoid stiff environments as discussed in this review (cf section 3.2). While data on the mechanical properties of glial scars are currently unavailable, in analogy to fibrotic scars it can be reasonably assumed that they are significantly stiffer than their environment [245]. Whether the increased stiffness of glial scar tissue is due to an increased stiffness of reactive glial cells and/or due to the increased amount of ECM in the scar environment needs to be investigated.

If axons are able to process mechanical guidance cues then it should also be possible to direct and enhance axonal regrowth using appropriate mechanical stimulation. Accordingly, soft hydrogels introduced into lesions show great potential for the treatment of CNS injuries in part because of their mechanical compliance, which supports the growth of neurons without activating glial cell proliferation (cf section 3.2). They may integrate into the parenchyma without major inflammatory responses and induce directed axonal regeneration across the artificial scaffold [237, 246].

In this context, it is intriguing to note that the treatment of glial scars with enzymes such as chondroitinase, which dissolves parts of the ECM, improves functional recovery after spinal cord injuries [247]. If the scars get softer then at least some of the functional recovery might be due to reduced mechanical inhibition of axonal growth.

Overcoming repellent biochemical signaling from the glial scar will always be a major challenge in the treatment of CNS injuries. However, exclusively focusing on biochemical aspects of neuronal regeneration did so far not result in overwhelming therapeutical success. Collecting data about physical properties of glial scars and the structures within them might shed light on formerly unknown problems discussed above. Future therapeutical approaches supporting current therapies, which consider physical problems in CNS nerve regeneration, could then for example aim at softening the glial scar, at a disaggregation of the surrounding ECM, the implementation of integrated axon tracts that were previously 'extreme stretch grown' [63, 115, 116] (cf section 2.2), and the use of properly designed artificial scaffolds, which are compliant and which may contain physical guidance cues in addition to biochemical ones.

5. Summary and conclusions

The idea of an important contribution of physical cues to the morphology, physiology and pathology of the nervous system currently experiences a renaissance. Evidence accumulates that cellular tension is a crucial factor in neuronal development [1, 62, 122]. Mechanical interactions seem not only responsible for axonal growth, the folding of the cortex

and compact wiring but also for the genesis of nervous tissue structure, e.g. the layered structure of the retina [27, 248]. Individual cells—neurons and glial cells alike—feel and respond to the mechanics of their environment [157, 167, 169, 170, 172]. However, the origin of this mechanosensitivity is only beginning to be unraveled. Future work in the field will reveal how neural cells perceive mechanical stimuli, how this information is processed and how exactly cells respond to their complex mechanical environment. Furthermore, most studies investigating physical aspects of neuronal growth are currently carried out *in vitro*. *In vivo* studies need to show whether cellular mechanosensitivity is indeed involved in neuronal guidance, foreign body reactions and the missing regeneration of neurons after injury to the mammalian CNS.

Cells are living in a physical world and obey physical laws. Only the combination of biological, chemical and physical aspects of cellular functioning will allow a comprehensive understanding of the nervous system as an entity. This knowledge will ultimately lead to new strategies for the treatment of CNS injuries and other neural pathologies, which remain one of the biggest challenges in medicine.

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