

Mechanotransduction in Neuronal Cell Development and Functioning

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Abstract

Although many details remain still elusive, it became increasingly evident in recent years that mechanosensing of microenvironmental biophysical cues and subsequent mechanotransduction are strongly involved in the regulation of neuronal cell development and functioning. This review gives an overview about the current understanding of brain and neuronal cell mechanobiology and how it impacts on neurogenesis, neuronal migration, differentiation, and maturation. Therein; we are focussing particularly on the events in the cell/microenvironment interface and the decisive extracellular matrix (ECM) parameters (i.e. rigidity and nanometric spatial organisation of adhesion sites) that modulate integrin adhesion complex-based mechanosensing and mechanotransductive signalling. It will also be outlined how biomaterial approaches mimicking essential ECM features help to understand these processes and how they can be used to control and guide neuronal cell behaviour by providing appropriate biophysical cues. In addition, principal biophysical methods will be highlighted that have been crucial for the study of neuronal mechanobiology.

1. Introduction

For a long time research on neuronal cell development and functioning was principally concentrated on the influence of biochemical factors, but in recent years accumulating evidence made clear that taking a biophysical perspective on these processes is intriguing and promising for various reasons. One outstanding attribute of neuronal cells is the extreme polarisation and compartmentalisation that is taking place during neuronal differentiation and maturation. It requires highly coordinated and dynamic cytoskeletal actions and cell/microenvironment interactions to realise neuronal migration, neurite- and synaptogenesis, as well as neural network formation and plasticity (Flynn, 2013),(Kerstein et al., 2015),(Leterrier et al., 2017),(Park and Goda, 2016),(Lilja and Ivaska, 2018). Developing neurons possess growth cones which are sensory compartments at the tip of neurites, build for the exploration of the microenvironment by their capacity to integrate chemical and mechanical cues (Chan and Odde, 2008),(Lowery and Van Vactor, 2009),(Myers et al., 2011),(Vitriol and Zheng, 2012),(Franze et al., 2013),(Kerstein et al., 2015), and specialised to operate in the soft brain tissue (Chan and Odde, 2008),(Betz et al., 2011),(Kerstein et al., 2015). Also when neurons reached their terminal differentiation and maturation stage with a complex morphology characterised by an axon and several dendrites, equipped with numerous fine structures such as synapses and spines, they maintain a remarkable plasticity to enable the processing of incoming information. This plasticity is highly dependent on integrin-mediated interaction with the microenvironment (Park and Goda, 2016),(Lilja and Ivaska, 2018).

Biophysical aspects, and in particular integrin-mediated mechanotransductive processes, involved in the regulation of neuronal cell development and functioning, will be a focus of this review. Special attention will be drawn to what is happening in the cell/microenvironment interface as these events are, literally and functionally, at the base of the mechanotransductive signalling and its impact on cellular behaviour. Throughout the review various examples of bioengineering approaches are indicated that were useful to gain insight into the influence of mechanotransduction on neuronal differentiation and/or exploit mechanotransductive mechanisms to guide neuronal cell behaviour in a controlled manner. Finally, this review will highlight some methods that are used to study biophysical aspects of (neuronal) cells and their environment. For further reading, the reader will be pointed to reviews that accentuate specific aspects of the different arguments in more detail.

2. Microenvironmental Cues Influencing Neuronal Cell Development

2.1 Biophysical, structural and compositional peculiarities of the extracellular matrix of the central nervous system

The extracellular matrix (ECM) of the central nervous system (CNS) has some distinctive and unique features regarding mechanics, structure and composition that differ substantially from the ECM of other organs and tissues (**Fig. 1A-D**).

Albeit regional mechanical heterogeneity within brain and spinal cord compartments has been reported (Elkin et al., 2007),(Christ et al., 2010),(Koser et al., 2015),(Antonovaite et al., 2018) (**Fig. 1B**), the CNS has as a general softness compared to other tissues (Franze et al., 2013),(Barnes et al., 2017) (**Fig. 1C**). Interesting observations in this regard are the stiffening of the human brain tissue due to ageing (Sack et al., 2011) and changes in mechanical properties in neurodegenerative diseases (e.g., in multiple sclerosis, amyotrophic lateral sclerosis and Alzheimer's disease) and brain cancer (e.g., in glioblastoma) (Tyler, 2012),(Barnes et al., 2017),(Tanner, 2018) (**Fig. 1C**).

The ECM composition of the CNS is furthermore characterised by a high abundance of hyaluronic acid and other glycosaminoglycans, glycoproteins (such as e.g. tenascins, reelin or laminins, the latter particularly in the region

of the blood-brain-barrier) and proteoglycans (such as e.g. lectican family members) (**Fig. 1A**) which are highly intertwined at the nanoscale (**Fig. 1D**). The non-fibrillar type IV collagen is present in the brain ECM but the amount of fibrillary proteins (such as e.g. collagen I) is instead relatively low (Ruoslahti, 1996),(Dityatev et al., 2010b),(Lau et al., 2013).

Apart from the interstitial neural matrix the brain ECM possesses also some special partitions with specific tasks and locations. In the subventricular zone of the adult brain, heparan sulphate proteoglycan and laminin-rich structures called fractones can be found close to the brain blood vessels that serve as neural stem cell niches and neurogenic zones (Kerever et al., 2007),(Mercier, 2016) (**Fig. 1A**). Another particular brain ECM structure is the perineuronal net; a specialised scaffold surrounding the neuronal cell body and proximal processes of many neurons in the CNS, that is essential for the synaptic structure and regulates synaptic plasticity (especially crucial components, such as tenascin-C and -R, brevican and neurocan) (Pizzorusso et al., 2002),(Geissler et al., 2013),(Sorg et al., 2016) (**Fig. 1A,D**).

ECM constituents and in particular integrin/microenvironment interactions are indeed strongly involved in all steps of neuronal development (Long and Huttner, 2019) which will be further outlined throughout the review, in particular in paragraph 3.2. Furthermore, alterations in the brain ECM composition and organisation have been found in neurodegeneration and brain cancer (Bonneh-Barkay and Wiley, 2009),(Lau et al., 2013),(Miyata and Kitagawa, 2017),(Barnes et al., 2017),(Tanner, 2018).

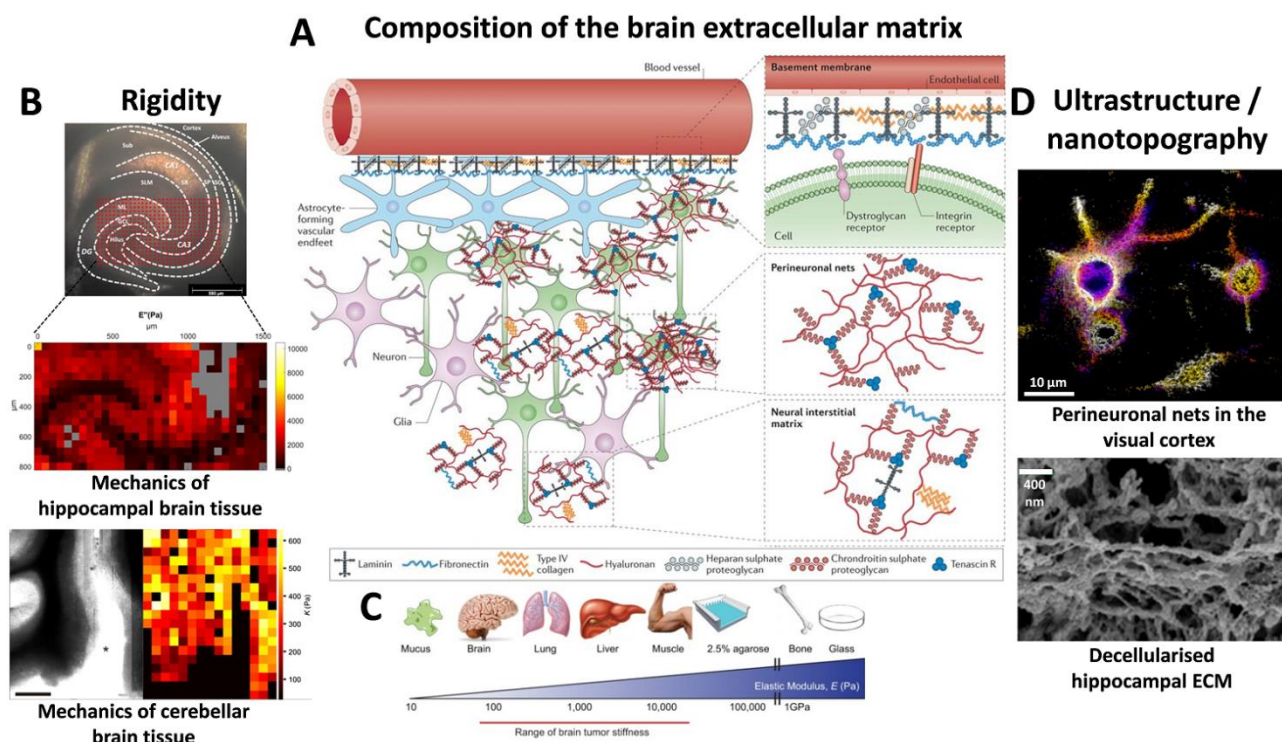


Fig. 1 Compositional, mechanical and structural features of the brain extracellular matrix

(A) The cartoon illustrates principal components of the brain extracellular matrix (ECM). Image with permission from Lau et al. (Lau et al., 2013), Copyright (2013) Springer Nature. (B) Two examples of the mechanical properties (elastic modulus) in different brain compartments (top: mouse hippocampus, bottom: rat cerebellum, scale bar: 400 μ m) are shown that were obtained by atomic force microscopy-based recordings. Images from Antonovaite et al. (Antonovaite et al., 2018), and with permission from Christ et al. (Christ et al., 2010), Copyright (2010) Elsevier. (C) The graphic highlights the general softness of the brain tissue in comparison to other body tissues. Image with permission from Barnes et al. (Barnes et al., 2017), Copyright (2017) Company of Biologists LTD. (D) The upper image demonstrates a

STORM super-resolution recording of perineuronal nets (chondroitin sulphate proteoglycans stained with *Wisteria floribunda* agglutinin-Dy749P1). Image courtesy of Xiaowei Zhuang (Harvard University, Cambridge, MA, USA). The lower image shows a scanning electron microscopic recording of the configuration of decellularised hippocampal extracellular matrix. Image from Tajerian et al. (Tajerian et al., 2018).

Decellularised brain ECM (an example can be seen in **Fig. 1D**) that are applied as substrates (either as 2D coating or 3D hydrogel) foster neurite outgrowth (Medberry et al., 2013) and functional neural network formation (Lam et al., 2019) better than equivalent decellularised ECM substrates from other tissues. A recent study furthermore shows that decellularised brain ECM increases the neuronal reprogramming efficiency of mouse embryonic fibroblasts into induced neurons, compared to e.g. 2D laminin-coated substrates. The promotive effect was already observable when the brain-derived ECM was also presented as a 2D coating, but strongly pronounced when it was applied as a 3D hydrogel environment (Jin et al., 2018). This emphasised the importance of appropriate microenvironmental biophysical and topographical cues, even in the presence of the same biochemical components.

The ECM building blocks and their interactions/crosslinking define the biophysical configuration of the intricate meshwork and determine its specific mechanical and structural properties, i.e. rigidity and nanotopography (Gasiorowski et al., 2013), (Young et al., 2016) (**Fig. 1A-D**). In the next paragraphs it will be detailed how these two principal biophysical cues deriving from the *in vivo* microenvironment, or from engineered biomaterials that are mimicking these pivotal features, impact on the neuronal cell behaviour and functioning.

2.2 Rigidity

Generally speaking, the interaction of (neural) stem cells (shown for embryonal and induced pluripotent stem cells, as well as foetal and adult neural stem or progenitor cells) with substrates that possess brain-like rigidity (usually ≤ 1 kPa elastic modulus, **Fig. 1C**) favours neuronal viability (Georges et al., 2006) and directs their fate towards neuronal lineage commitment (Saha et al., 2008), (Leipzig and Shoichet, 2009), (Teixeira et al., 2009), (Banerjee et al., 2009), (Seidlits et al., 2010), (Keung et al., 2011), (Keung et al., 2012), (Keung et al., 2013), (Franze et al., 2013), (Mammadov et al., 2013), (Musah et al., 2014), (Sun et al., 2014).

Numerous studies furthermore indicated a promotive effect of softer substrates on neurite outgrowth for a variety of neuronal cell types and different gel materials used as substrates (Balgude et al., 2001), (Willits and Skornia, 2004), (Kostic et al., 2007), (Jiang et al., 2008), (Teixeira et al., 2009), (Sundararaghavan et al., 2009), (Cheng et al., 2011), (Man et al., 2011), (Koch et al., 2012), (Hopkins et al., 2013), (Franze et al., 2013), (Kerstein et al., 2015), (Mosley et al., 2017). However, it should be mentioned that also some conflicting results regarding neuron sensitivity towards substrate rigidity have been reported; in some studies (e.g. with PC12 cells (Leach et al., 2007), cortical neurons (Norman and Aranda-Espinoza, 2010) or hippocampal neurons (Koch et al., 2012)) neurite outgrowth was insensitive to the tested mechanical substrate properties, or in one case for cortical neurons an even stronger outgrowth on stiffer substrates was noted (Stabenfeldt and LaPlaca, 2011). Furthermore, it has been shown that the mechanosensitivity varies between different neuronal cell types (Koch et al., 2012). Certainly the plethora of utilised combinations between substrate materials and ligands/adhesive agents (e.g. (hydro)gels made from agarose, collagen I, polyacrylamide, polydimethylsiloxane, silk fibroin, polyethylene glycol, or methylcellulose, functionalised with often varying concentrations of collagen I, laminin, fibronectin, matrigel, or poly-lysine; taking into account only some of the cited references) complicates a comparison of the results. The contradictory effects could be due to not considered aspects, such as structural differences in porosity, crosslinking and mesh size, which could have led to changes in topographical parameters. The latter affect also strongly neurite outgrowth, as will be highlighted in the next paragraph.

2.3 Topography

A widely demonstrated impact of accordingly designed anisotropic micro- or nanotopographical features on neuronal cell behaviour (shown for various neuronal cell types and (neural) stem cells during neuronal differentiation) is the alignment of cell polarity and neurite/axon outgrowth (Hoffman-Kim et al., 2010),(Kim et al., 2013),(Simitzi et al., 2017), e.g. along ridges (Rajnicek et al., 1997),(Johansson et al., 2006),(Ferrari et al., 2010),(Lee et al., 2010),(Ferrari et al., 2011),(Bédier et al., 2012),(Yang et al., 2013),(Yang et al., 2014),(Baek et al., 2018), electrospun fibres (Xie et al., 2009),(Lim et al., 2010),(Wang et al., 2010),(Gertz et al., 2010),(Smith Callahan et al., 2013), pillar arrays (M. Park et al., 2016) or elliptical cones (Simitzi et al., 2015). This contact guidance was attributed to focal adhesion confinement and alignment (Ferrari et al., 2010),(Ferrari et al., 2011),(Tonazzini et al., 2013),(Yang et al., 2014),(Baek et al., 2018), favouring in this manner the effective neurite outgrowth in a specific direction. This phenomena is of high biomedical interest and already exploited to improve nerve guidance conduits utilised to promote the regeneration of peripheral nerve cells (Hoffman-Kim et al., 2010),(Sarker et al., 2018).

However, the potential impact of appropriate nanotopographical features goes beyond these more geometrically guiding effects; since they can also influence the neuronal program, i.e. gene/protein expression and differentiation as specific instructive cues (Xie et al., 2009),(Lee et al., 2010),(Lim et al., 2010),(Yang et al., 2013),(Smith Callahan et al., 2013),(Yang et al., 2014); similar to the effects described for soft substrates. In fact, even isotropic and disordered nanotopographies (made by quite different methods and materials, such as e.g. silica nanobeads, carbon nanotubes, silicon nanowires, assembled zirconia nanoclusters, nanorough glass surfaces generated by reactive-ion etching or platinum-coated polystyrene nanopattern) have been reported to promote neuritogenesis, neuronal differentiation and neural network maturation (Migliorini et al., 2011),(Kang et al., 2012),(Fabbro et al., 2012),(Bugnicourt et al., 2014),(Schulte et al., 2016c),(Schulte et al., 2016b),(Chen et al., 2018),(Baek et al., 2018),(Schulte et al., 2018). Nanotopographies with a suitable dimensionality have the ability to modulate integrin adhesion complexes (IAC) in a way that impacts on neuronal cell decision making, programming and fate (Yang et al., 2013),(Yang et al., 2014),(Schulte et al., 2016c),(Schulte et al., 2016b),(Maffioli et al., 2017),(Chen et al., 2018),(Baek et al., 2018).

In the last decades, it has been unravelled that mechanotransductive processes are at the basis of these biophysical cue effects on (neuronal) cell development. The next paragraphs will therefore focus on how the cellular mechanotransductive machinery actually senses and interprets microenvironmental biophysical features in **3.1 (Fig. 2A-D)**, highlighting in particular also what is known about the neuronal context in **3.2 (Fig. 3)**.

3. Mechanosensing in the Cell/Microenvironment Interface and Neuronal Mechanotransductive Processes and Signalling

The adhesive structures in the cell/microenvironment interface that enable the cell to perceive the biophysical configuration of its microenvironment and to translate the information into appropriate cellular responses are highly intricate. We will concentrate on the fundamental mechanotransducers of the cells, i.e. integrin adhesion complexes (IAC), although various cell surface receptors are known to contribute to mechanosensing and -transduction (such as GPI-anchored proteins (Kalappurakkal et al., 2019) (e.g. uPAR (Ferraris et al., 2014),(Schulte et al., 2016a)), CD44 (Seidlits et al., 2010),(Kim and Kumar, 2014) syndecans (Bass et al., 2007),(Morgan et al., 2013), or receptor tyrosine

kinases (Yang et al., 2016)), which often however cooperate in some way with integrins. First we will detail processes of mechanosensing in the cell/microenvironment interface in general (**Fig. 2A-C**) and the decisive extracellular matrix parameters that influence them (**Fig. 2D**).

3.1 Mechanosensing and -transduction

The integrin family consists of heterodimeric transmembrane receptors, always build up by one α - (18 types exist) and one β -subunit (8 types) that can assemble in 24 different combinations. They possess large extracellular domains and short cytoplasmic tails (with the exception of $\alpha 6 \beta 4$ integrin that has a longer β -subunit tail). Despite some redundancy, the different integrin heterodimers have distinct binding specificities to ligands (such as e.g. the RGD motif) present in the numerous proteins of the ECM (or in some cases receptors in the membrane of other cells) rendering possible the versatility of integrin signalling and its broad involvement in many cell biological events. However, there are common features of integrin-mediated cell adhesion realising mechanosensing and interpretation of the microenvironment by mechanotransductive processes (Changade and Sheetz, 2017),(Gauthier and Roca-Cusachs, 2018),(Sun et al., 2019),(Kechagia et al., 2019) (**Fig. 2A-D**).

Integrin activation; i.e. the transition from low to high ligand affinity state by changing the integrin conformation from bent and closed to extended and open with separated cytoplasmic tails (Shattil et al., 2010),(Zhu et al., 2013), can either be induced and/or stabilised by integrin ligand binding itself (outside-in signalling), by intracellular events (inside-out signalling, often through signals arriving from G-protein coupled receptors) (Sun et al., 2019),(Kechagia et al., 2019), or by modulation of the membrane tension (Wang and Ha, 2013),(Ferraris et al., 2014),(Paszek et al., 2014),(Schulte et al., 2016a),(Gauthier and Roca-Cusachs, 2018). In any case, the adaptor proteins talin and kindlin are recruited to the cytoplasmic tail of the integrin β -subunit which is essential for integrin activation (Jiang et al., 2003),(Theodosiou et al., 2016). The glycocalyx; a pericellular sugar coat that surrounds the cell membrane and is attached to proteoglycans, glycolipids and -proteins, is another important player in the cell/microenvironment interface that influences integrin properties. Its compression in the vicinity of integrin/substrate binding sites leads to mechanical loading of the integrins through force application towards the cell membrane. The compressed glycocalyx acts furthermore as a steric kinetic trap that impacts on lateral integrin diffusion and promotes integrin clustering (Paszek et al., 2014). These initial processes are independent of actomyosin contraction (Choi et al., 2008),(Wang and Ha, 2013),(Changade et al., 2015) (**Fig. 2A**).

However, the talin rod can bind to filamentous actin (f-actin) which connects the ECM to the actin cytoskeleton in the nascent adhesions (Jiang et al., 2003); engaging in this manner also the molecular clutch by linking the integrins to the retrograde actin flow and its forces generated by actin polymerisation and actomyosin contraction (Chan and Odde, 2008),(Zhang et al., 2008),(Schulte et al., 2016a) (**Fig. 2A**). An interesting historic side note in the context of this review is the fact that the molecular clutch hypothesis was first developed (Mitchison and Kirschner, 1988) and decisively elaborated (Chan and Odde, 2008) studying neuronal growth cones. However, whether this initial structure disassembles immediately or instead is reinforced and matures by recruitment of further proteins and integrin clustering depends on the extent of force loading within the molecular clutch (Wang and Ha, 2013),(Oria et al., 2017) (**Fig. 2A-B**). At sufficient force loading, different stabilising events can take place. Forces in the low pN range are sufficient to maintain integrins in their extended conformation (which can happen very quickly in less than a second) (Strohmeyer et al., 2017),(Li and Springer, 2017)). At forces in the order of tens of pN the ECM/integrin binding can further strengthen by catch bond formation, shown e.g. for $\alpha 1 \beta 5$ and $\alpha V \beta 3$ integrin, increasing thus the lifetime of the bond (Kong et al., 2009),(Chen et al., 2017). Talin can be activated by stretching of the talin rod (starting from forces of

~5 pN to tens of pN) which leads to unfolding of cryptic binding sites for vinculin, first near the membrane and integrins and later closer to the f-actin. Vinculin is recruited to these uncovered sites, leading to its movement towards f-actin at higher force loading (>5-25 pN). During these events vinculin is activated itself and its tail forms a catch bond with f-actin (maximally stable at ~8 pN), stabilising thereby additionally the nascent adhesions (del Rio et al., 2009),(Grashoff et al., 2010),(Ciobanasu et al., 2014),(Yao et al., 2014),(Case et al., 2015),(Elosegui-Artola et al., 2016),(Huang et al., 2017) (**Fig. 2A**). This force-dependent reinforcement and the consequential increase in lifetime allows the recruitment of further essential IAC components (Carisey et al., 2013),(Case et al., 2015). The forming IAC organise into modular nanometric units (with dimensions of ~80-120 nm containing 20-50 integrins (Changede et al., 2015)) with a stratified nanoarchitecture composed of 3 layers, i.e. an integrin signalling layer (containing e.g. paxillin, integrin-linked kinase (ILK), focal adhesion kinase (FAK), p130Cas, and src), a force transduction layer (mainly talin and vinculin) and an actin regulatory layer (e.g., f-actin, α -actinin, zyxin, or VASP) (Case et al., 2015) (**Fig. 2B-C**). In further maturation steps the modules can group into structures with increasing dimensions, i.e. first in focal complexes then focal adhesions, but their dimension and especially composition are quite versatile depending on the cell biological context. The recruitment of many adaptor and signalling proteins; such as e.g. paxillin, ILK, FAK, src, p130cas, PAK, or ERK, transforms the IAC into signalling hubs capable of controlling and influencing cell signalling, decision making and fate in many ways. Integrin downstream signalling controls, e.g., actin cytoskeletal dynamics through modulation of RhoGTPase activity (in particular RhoA, Rac1, and Cdc42) impacting in this way also on the localisation of mechanosensitive transcription factors, such as e.g. YAP/TAZ, or proliferation and differentiation by activation of the ERK/MAPK pathway (Sun et al., 2019),(Kechagia et al., 2019),(Humphries et al., 2019),(Green and Brown, 2019). The remodelling of the cytoskeleton can furthermore lead to alterations in the nuclear architecture by its connection via the LINC (Linker of Nuclear and Cytoskeleton) complex which affects the spatial chromosome organisation and gene expression by mechanoregulatory transcription factors (Uhler and Shivashankar, 2017).

Whether sufficient force loading within molecular clutch can occur to permit the different steps of reinforcement and IAC maturation depends decisively on critical mechanical and structural microenvironmental parameters; i.e. the substrate rigidity and the spatial organisation of integrin adhesion sites (in particular in terms of ligand spacing, density and distribution, as well as topography) (**Fig. 2D**), respectively their combination (Gauthier and Roca-Cusachs, 2018),(Kechagia et al., 2019). In general, the lower the rigidity the (s)lower is the force loading per integrin. If the rigidity is too low, the integrin/ligand bond lifetime is too short and the initial adhesion is likely to disassemble before the mentioned force thresholds in the molecular clutch can be achieved. Higher rigidities instead enable a stronger force transmission along the ECM/integrin/talin/f-actin axis surpassing the force thresholds and favouring thus IAC reinforcement and maturation processes (Elosegui-Artola et al., 2016) (**Fig. 2A**). Furthermore, it has been observed that IAC maturation is not taking place if the ligand spacing distance exceeds a certain threshold (>60-70 nm) on rigid substrates (Arnold et al., 2004). Until very recently, this was attributed to a potential direct measurement of the ligand spacing by an adaptor protein (with talin as a potential candidate) that works as a molecular ruler, but new data indicate instead that the force loading within the molecular clutch is actually the principal decisive factor. Considering both parameters; i.e. rigidity and spatial organisation and distribution of adhesion sites, in combination there are indeed counter-intuitive effects (Oria et al., 2017). Too large ligand spacing distances on high rigidity substrates can increase the force load per integrin to an extremely high, i.e. eventually too high, level which causes an adhesion collapse (Liu et al., 2014),(Oria et al., 2017); most probably due to limitations in the maximal integrin recruitment impeding sufficient force redistribution. On lower substrate rigidities instead an increase in ligand spacing distance can lead to an augmentation of force load per integrin above the critical force thresholds enabling thus IAC

maturation (Oria et al., 2017). However, a minimal adhesion unit of ~3-6 integrins binding ligands in certain vicinity (i.e. in the high tens of nm) promotes integrin clustering, including also the recruitment of unligated integrins. This accentuates the importance of the distribution of the adhesion sites in terms of order, and explains why introducing disorder in the adhesion site distribution (and local differences in ligand spacing) can change again the whole outcome in regards to whether IAC maturation takes place or not (Jiang et al., 2003), (Huang et al., 2009), (Schvartzman et al., 2011), (Oria et al., 2017). Recently, it has furthermore been shown that nanotopographical features impact on cell migration with involvement of actomyosin and Rho activity (J. Park et al., 2016) (**Fig. 2A-D**).

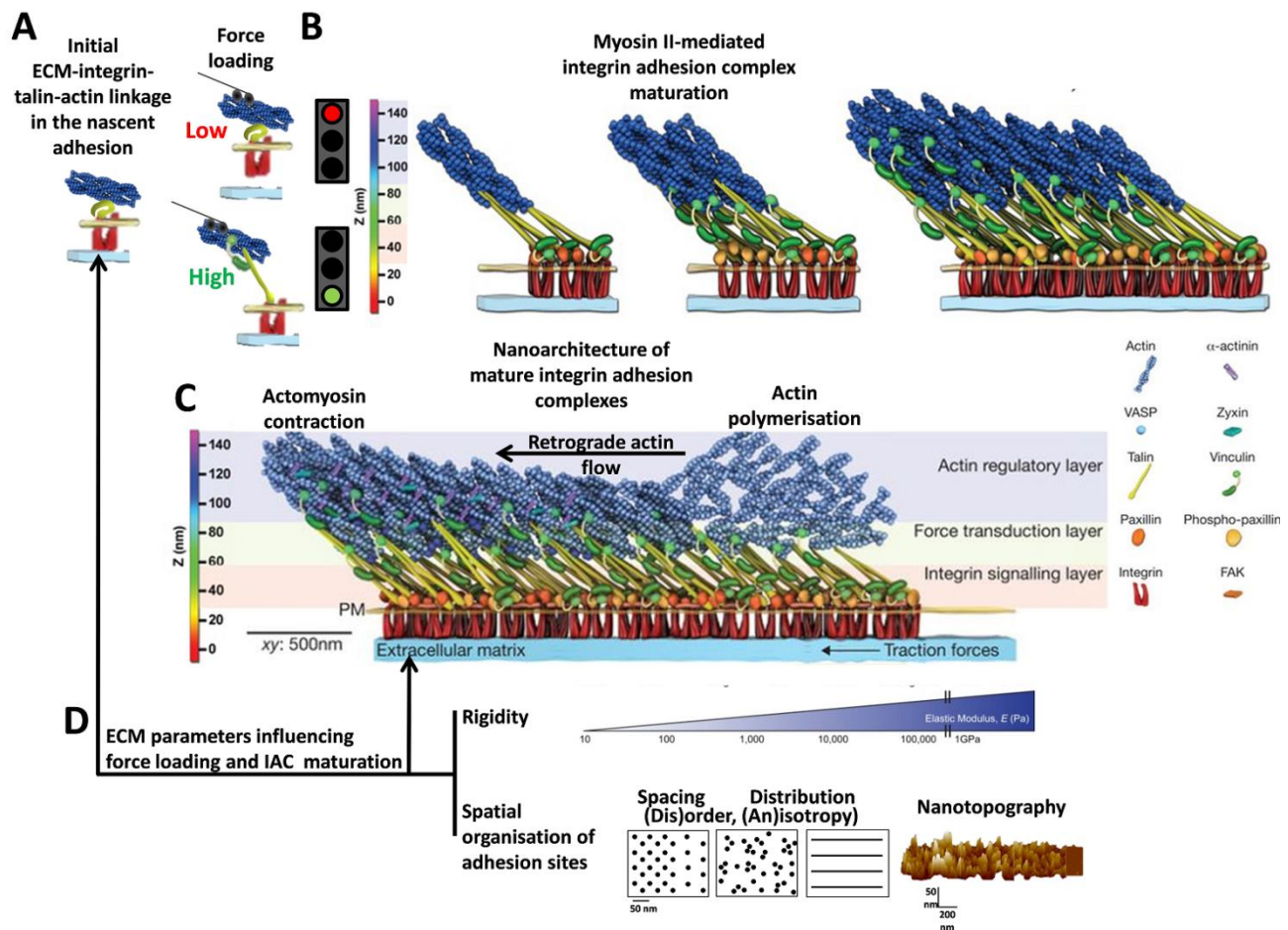


Fig. 2 Integrin-mediated mechanosensing and mechanotransductive sequence with influencing parameters of the extracellular matrix

(A) The cartoon illustrates the initial ECM-integrin-talin-actin linkage in the nascent adhesions and how the force loading within this molecular clutch determines whether this structure disassembles (in case of too low force loading) or reinforces (in case of sufficient force loading) and (B) matures into integrin adhesion complexes (IAC) by recruitment of further proteins. (C) In this graphic the stratified nanoarchitecture of mature IAC with its different layers is shown. (D) The extent of force loading and IAC maturation is determined by biophysical cues of the extracellular matrix, in particular the rigidity and the spatial organisation of the integrin adhesion sites (in terms of spacing, distribution and nanotopography). Further details on IAC maturation are outlined in paragraph 3.1.

The figure contains adapted elements of images with permission from Case et al. (Case et al., 2015) and Case and Waterman (Case and Waterman, 2015), Copyright (2015) Springer Nature; Barnes et al. (Barnes et al., 2017), Copyright (2017) Company of Biologists LTD.; and an adapted element with permission from Borghi et al. (Borghi et al., 2018), Copyright (2018) American Chemical Society.

Actually these processes in the cell/microenvironment interface are even more complex with various further levels of regulation, such as e.g. mechanisms to modulate the cell surface availability of integrins by endocytosis (clathrin-dependent or –independent (CLIC/GEEC) routes), trafficking/recycling, or degradation, as well as talin competitors/inhibitors or talin cleavage. The interested reader can find further details on certain aspects in recent reviews of Gauthier and Roca-Cusachs (Gauthier and Roca-Cusachs, 2018), Green and Brown (Green and Brown, 2019), Humphries et al. (Humphries et al., 2019), Sun et al. (Sun et al., 2019) and Kechagia et al. (Kechagia et al., 2019).

These events in the cell/microenvironment interface contribute essentially to the regulation of many cell biological behaviours. The next paragraph will outline the current understanding about the involvement of the mechanotransductive pathway in neuronal cell behaviour (some examples are highlighted in **Fig. 3**). Considering the aforementioned local heterogeneity and complexity of biophysical cues in the brain ECM (**Fig. 1**), these insights are also highly relevant in regards to the optimisation of biomaterial approaches that are based on exploitation of mechanotransductive mechanisms (**Fig. 4**).

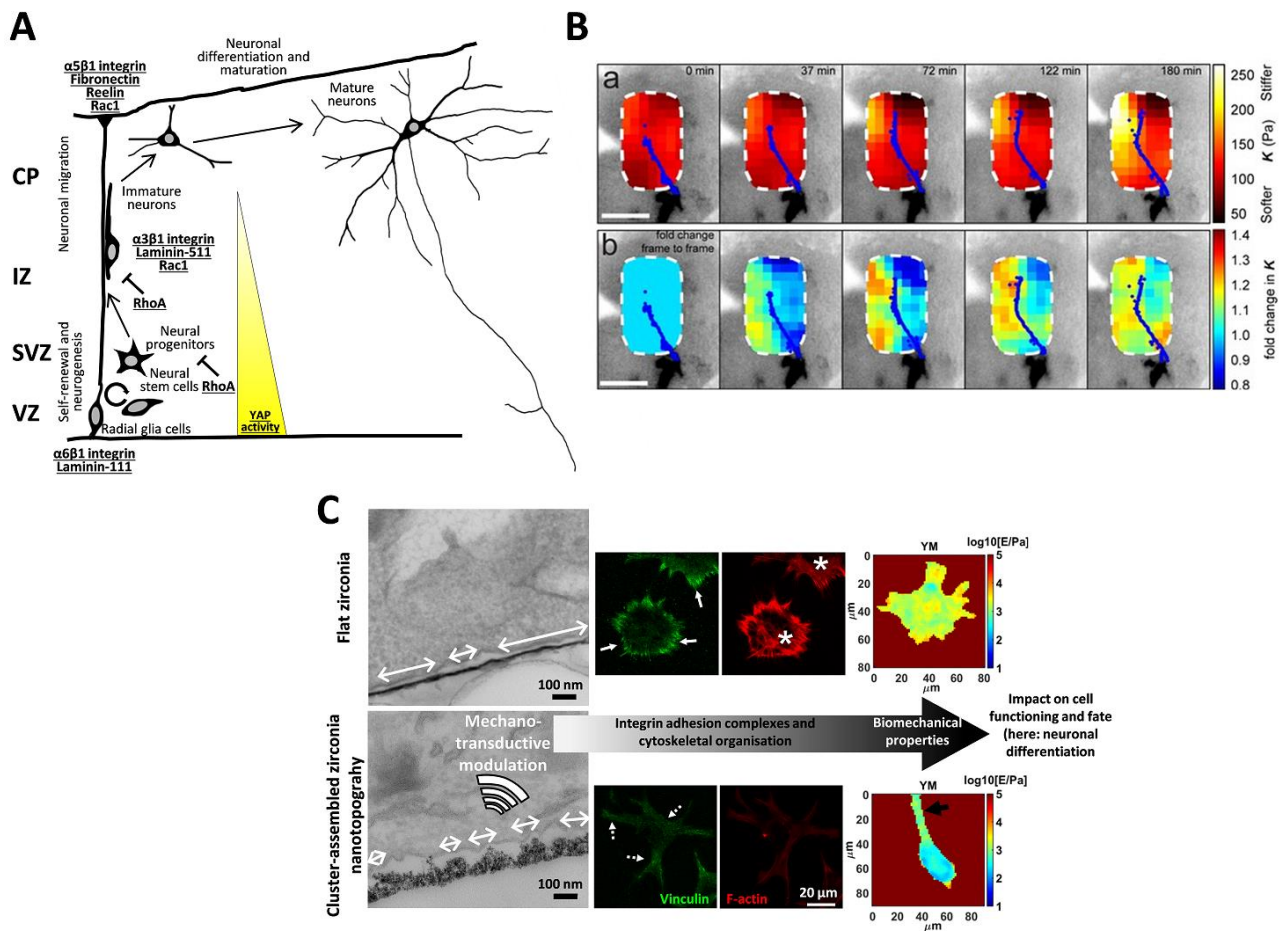


Fig. 3 Mechanotransductive processes in neuronal development and functioning

(A) The graphic illustrates the different phases of neuronal cell development (in this case during cortex formation) starting from self-renewal and neurogenesis, passing to neuronal migration, neuritogenesis, and ending with terminal differentiation and maturation with synaptogenesis and network integration (VZ: Ventricular zone, SVZ: Subventricular zone, IZ: Intermediate zone, CP: Cortical plate). Extracellular matrix and cellular proteins related to mechanotransductive processes that are known to influence different phases of these events are indicated in bold and underlined. Further details can be found in paragraph 3.2. The figure contains elements of an image from Schulte et al. (Schulte et al., 2016b). (B) The panel shows *in vivo* mechanosensitivity of a growing axon in the *Xenopus* brain. The colour code indicates (a) the stiffness (elastic modulus) of the brain tissue measured by atomic force microscopy-based

recordings or (b) the stiffness changes over time in the same region. The fluorescently labelled axon was tracked and outlined in blue and documents the directed axon movement towards the softer region (Scale bars = 100 μm). The image has been reproduced from Thompson et al. (Thompson et al., n.d.). (C) The panel demonstrates the modulations along the mechanotransductive sequence induced by the interaction of the neuron-like PC12 cells with ECM-mimicking nanotopographical zirconia substrates produced by the nanofabrication technique supersonic cluster beam deposition, compared to flat zirconia surfaces. In the transmission electron images it can be seen that the cells interact only with the apical part of the nanotopographical asperities which restricts the dimension of the nanometric adhesion sites (indicated by the white arrows) to smaller sizes with respect to the situation on flat zirconia. Also the integrin adhesion complexes (vinculin staining in green recorded by TIRF microscopy) remain of small dimensions (focal contact/point contact size, see white arrows with dashed lines) whereas mature focal adhesions form only on the flat substrate (see white arrows). Consequently, on the nanostructured zirconia no stress fibre formation (epifluorescence of phalloidin staining in red) can be noted, while there are abundant stress fibres on the flat zirconia (examples marked by white asterisks). The cells on the nanotopographical substrate are softer than on the flat surface (quantified by atomic microscopy-based analysis, the colour code indicates the Young's modulus (YM)) and neurite outgrowth was visible (black arrow). The image was adapted from Schulte et al. (Schulte et al., 2017), Copyright (2017) American Chemical Society.

3.2 Mechanotransductive processes and signalling in neuronal cell development and functioning

Albeit many details remain elusive, it is evident that the ECM configuration and integrin-mediated mechanosensing/-transduction participate in the control of neuronal cell development and functioning at all stages (Franze et al., 2013),(Stukel and Willits, 2015),(Park and Goda, 2016),(Barnes et al., 2017),(Lilja and Ivaska, 2018),(Long and Huttner, 2019),(Xu et al., 2019) (**Fig. 3-4**).

Integrin/microenvironment interactions and RhoGTPase signalling play an important role during neurogenesis and the subsequent neuronal long-range migration when the neuronal progenitors move towards the final destination and terminally differentiate into neurons (Tate et al., 2004),(Fietz et al., 2012),(Long and Huttner, 2019),(Xu et al., 2019). The regulation of neural progenitor proliferation in the stem cell niche during neurogenesis depends on $\alpha\beta 1$ integrin-mediated laminin (predominantly laminin-111) binding which activates MAPK signalling (Campos et al., 2004),(Flanagan et al., 2006),(Haubst et al., 2006),(Lathia et al., 2007),(Ma et al., 2008),(Shen et al., 2008),(Long and Huttner, 2019). Interestingly, it has been shown that the mechanosensitive protein YAP sustains the proliferation of neural progenitors and negatively regulates their neuronal differentiation (shown for the postnatal mouse retina (Zhang et al., 2012) and chicken neural tube (Cao et al., 2008)). Recently, it was furthermore demonstrated that $\alpha\beta 1$ integrin/laminin 511 interaction-dependent YAP activation fosters survival of immature dopaminergic midbrain neurons in their niche (Zhang et al., 2017) (**Fig. 3A**).

During mammalian cerebellar cortex development, neuronal progenitors leave the (sub)ventricular zone after neurogenesis and undergo a multipolar-bipolar transition before they start to migrate along the radial glia cell (RGC) fibres. The cytoskeletal and morphological changes of this transition are orchestrated by a complex spatiotemporal regulation of RhoGTPase activity (Konno et al., 2005),(Xu et al., 2019). Later on, the brain ECM glycoprotein reelin is decisively involved in the control of neuronal cell adhesiveness. It regulates another shift in neuronal migration mode, by inhibiting the bipolar $\alpha\beta 1$ integrin-dependent migration of the immature neurons along the RGC (Anton et al., 1999),(Hong et al., 2000),(Dulabon et al., 2000),(Schmid et al., 2004) and activating instead the RGC-independent $\alpha\beta 1$ integrin/fibronectin binding-dependent terminal translocation during lamination of the developing neocortex (Sekine et al., 2012). Dynamic and spatiotemporally coordinated RhoGTPase signalling (Xu et al., 2019), actomyosin activity and traction forces (Solecki et al., 2009),(Jiang et al., 2015) are required to enable neuronal migration. Loss of Rac1 in the forebrain leads to apoptosis of neural progenitors and new-born neurons, as well as to defects in migratory competence,

axonal guidance and terminal neuronal differentiation (Chen et al., 2007),(Chen et al., 2009). Lowering RhoA activity instead fosters neuronal lineage commitment (Keung et al., 2011), as well as neurite initiation and outgrowth, in many neuronal cell models (Yamaguchi et al., 2001),(Dergham et al., 2002),(Da Silva et al., 2003),(Fournier et al., 2003),(Schulte et al., 2010),(Gu et al., 2013). In line with this, RhoGTPase signalling has been found to be involved in various biomaterial-induced (either rigidity or topography-based approaches) effects on neuronal differentiation (Georges et al., 2006),(Saha et al., 2008),(Teixeira et al., 2009),(Seidlits et al., 2010),(Keung et al., 2011),(Yang et al., 2014),(Schulte et al., 2016c),(Maffioli et al., 2017),(Chen et al., 2018) (**Fig. 3A**).

Recently, *in vivo* data obtained in the developing *Xenopus* brain demonstrated that retinal ganglion cell axons are sensitive to rapidly changing mechanical properties within the brain tissue that lead to stiffness gradients, causing a directional growth of the axons towards softer areas (Koser et al., 2016),(Thompson et al., n.d.) (**Fig. 3B**). The directional collective migration of *Xenopus* neural crest cells can instead be induced by stiffening of the underlying head mesoderm via integrin-dependent mechanosensing (Barriga et al., 2018).

Interesting insights on the processes in the neuron/microenvironment interface and mechanotransductive signalling were obtained from studies that use biomaterials with topographical surfaces (examples in **Fig. 4C**). Nanotopographical features that restrict the maturation of IAC to dimensions beneath focal adhesion size and consequentially decrease also stress fibre formation (Bugnicourt et al., 2014),(Schulte et al., 2016c),(Baek et al., 2018),(Baek et al., 2019) and cell rigidity (Schulte et al., 2016c) foster neuronal differentiation (**Fig. 3C**). These types of neuron/nanotopography interactions modulated the expression and phosphorylation levels of proteins that are known to be important components of the IAC and mechanotransductive machinery/signalling sequence (e.g., FAK phosphorylation and ILK signalling) (Schulte et al., 2016c),(Schulte et al., 2016b),(Maffioli et al., 2017),(Baek et al., 2018). A study performed with a microtopographical substrates indicated that the ubiquitin E3a ligase; a protein which targets several integrin signalling/mechanotransduction-related proteins (such as, e.g., src family members) for degradation and whose deficiency leads to the neurodevelopmental disorder Angelman syndrome, is involved neurite contact guidance and neuronal topography sensing (Tonazzini et al., 2016). Furthermore, it has been reported that nanotopography- and soft substrate-promoted neuronal differentiation was accompanied by an increase in Ser127 phosphorylation of YAP and its cytoplasmic retention in a actin cytoskeleton-dependent manner (Musah et al., 2014),(Sun et al., 2014),(Baek et al., 2018).

Consistent with their function as explorative compartments of developing neurons, neurite growth cones are particularly influenced and controlled by mechanotransductive processes. The extent of molecular clutch engagement to the retrograde actin flow-generated forces regulates neurite/axon guidance and pathfinding. Within growth cones dynamic and small (focal complex size) integrin-mediated interaction sites are formed with microenvironmental cues, called point contacts. Their spatiotemporal dynamics are tightly governed by a balanced signalling interplay involving diverse IAC signalling-related components, such as RhoGTPases, src, PAK and FAK (Robles et al., 2005),(Woo and Gomez, 2006),(Medeiros et al., 2006),(Myers et al., 2011),(Vitriol and Zheng, 2012),(Santiago-Medina et al., 2013),(Kerstein et al., 2015),(Nichol et al., 2016) (**Fig. 4B**, on the right). Growth cones are soft structures (with low elastic modulus around hundreds of Pa and a tension in the range of hundreds of pN (Betz et al., 2011)) and produce relatively weak forces (compared to other cell types, such as e.g. fibroblasts or epithelial cells). The traction forces are in the range of tens of pN per μm^2 , executed in particular in the peripheral regions growth cone (such as filopodia and lamellipodial edges) (Hällström et al., 2010),(Betz et al., 2011), (Koch et al., 2012),(Franze et al., 2013),(O'Toole et al., 2015), and largely myosin II-dependent (Bridgman et al., 2001). Peripheral neurons seem to generate stronger traction

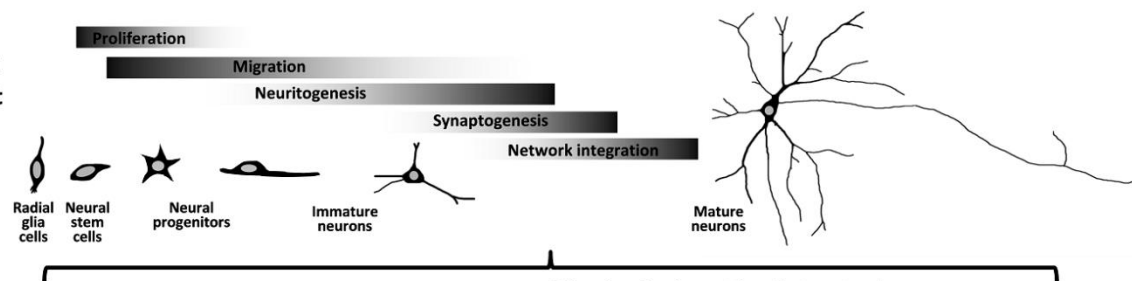
forces than CNS neurons, though (Koch et al., 2012). The protrusive forces, measured for retinal ganglion cell growth cones, are in the order of 100 pN (Fuhs et al., 2013).

Also later on during neuronal maturation, mechanoregulatory processes are potentially highly relevant in controlling the spatiotemporal dynamics of synaptogenesis and activity-dependent synapse plasticity. Postsynaptic terminals of synapses are rich in mechanotransductively active components, such as cell adhesion molecules and regulators of actin dynamics and organisation (e.g., cofilin, drebrin α -actinin and cortactin) (McGeachie et al., 2011), (Sheng and Kim, 2011), (Kilinc, 2018). Cofilin, e.g., is strongly recruited to dendritic spines during long-term potentiation and involved in the remodelling of the synapse structure (Bosch et al., 2014). The configuration and composition of the ECM and dynamic integrin/ECM (dis)engagement modulates the activity-dependent functional plasticity of synaptic connectivity and neural circuitry (Chavis and Westbrook, 2001), (Dityatev et al., 2010a), (McGeachie et al., 2011), (Orlando et al., 2012), (Kerrisk et al., 2013), (Bikbaev et al., 2015), (Park and Goda, 2016), (Kilinc, 2018).

More details about brain ECM or RhoGTPase signalling during neuronal development are outlined in reviews by Long and Huttner (Long and Huttner, 2019), respectively Xu et al. (Xu et al., 2019). Brain tissue mechanics are covered in detail by Franze et al. (Franze et al., 2013) and Barnes et al. (Barnes et al., 2017). The involvement of integrins in synapse formation and plasticity is highlighted in reviews from Park and Goda (Park and Goda, 2016) and Lilja and Ivaska (Lilja and Ivaska, 2018).

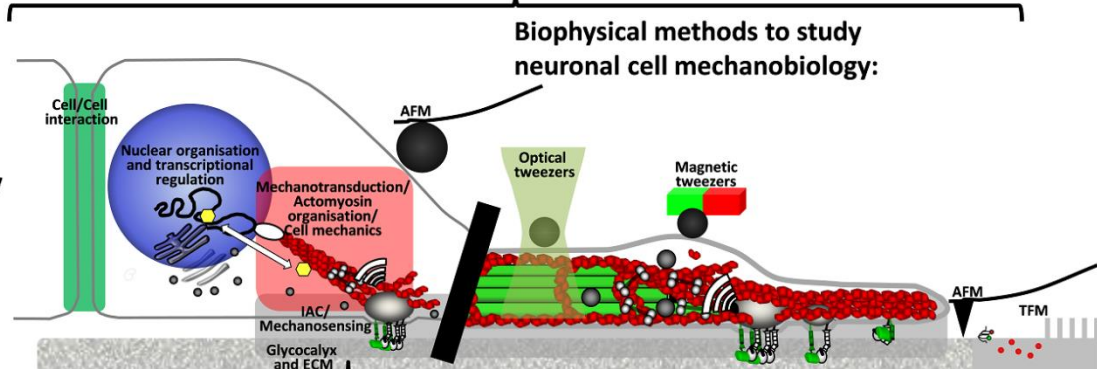
A

Stages of neuronal cell development



B

Neuronal cell mechanobiology



C

Bioengineering approaches to mimic ECM features

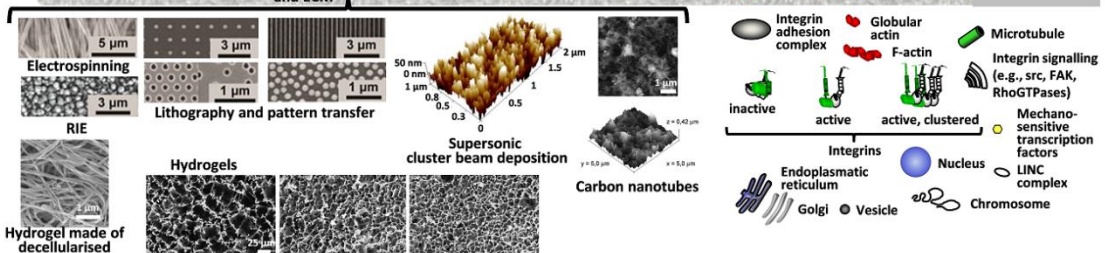


Fig. 4 Biomaterial and biophysical approaches to study and/or control mechanotransductive processes that regulate neuronal cell development and functioning

(A) The scheme illustrates important stages during neuronal cell development. (B) The graphic outlines schematically principal (extra)cellular structures and processes of interest within the integrin adhesion complex-mediated mechanotransductive sequence in neuronal cells (on the right the axon/neurite growth cone is highlighted) and some biophysical methods (AFM: Atomic Force Microscopy, TFM: Traction Force Microscopy) that are used to study them (see also paragraph 4). (C) Examples of different bioengineering approaches are listed that are used for the production of biomaterials which mimic biophysical extracellular matrix (ECM) features, such as hydrogels derived from decellularised brain ECM (Jin et al., 2018), hydrogels made of polymers (in this case polyacrylamide gels with different stiffness ranges, Young's modulus: left: <14.5 kPa, middle: 14.5-29 kPa, right: >29 kPa) (Hadden et al., 2017), electrospinning, lithographic methods and pattern transfer, as well as reactive-ion etching (RIE) (Chen et al., 2014), carbon nanotubes (Cellot et al., 2011) and assembly of zirconia nanocluster by supersonic cluster beam deposition (Schulte et al., 2017). Various approaches are referenced throughout the review in which these types of substrates are applied and exploited to study and guide neuronal cell mechanotransduction and development. (A-C) Together these mechanobiological approaches can contribute to a better understanding of how mechanotransductive processes impact on neuronal cell development and functioning.

The figure contains adapted elements of images with permission from Jin et al. (Jin et al., 2018), Copyright (2018) Springer Nature; Hadden et al. (Hadden et al., 2017), Copyright (2017) National Academy of Sciences; Chen et al. (Chen et al., 2014), Copyright (2014) Elsevier; as well as elements from Cellot et al. (Cellot et al., 2011), Schulte et al. (Schulte et al., 2016c), (Schulte et al., 2016b), and Maffioli et al. (Maffioli et al., 2017).

4. Biophysical Methods to Study Neuronal Mechanobiology

The aim of research in cellular mechanobiology is to understand how biophysical properties of the cells and the surrounding microenvironment are intertwined and influence each other mutually to regulate cell morphology and fate (Fig. 4). In light of this premise, it is fundamental to obtain a precise quantification of the structural and mechanical properties of the microenvironment, cells or tissues. The exploration of mechanotransductive processes relies furthermore on the ability to apply accurately controlled physical stimuli to living cells and to measure the forces of their mechanobiological actions (Iskratsch et al., 2014), in particular also for the understanding of neuronal cell behaviour (Athamneh and Suter, 2015).

The study of biophysical aspects of cells and their microenvironment and/or the nature of their interaction requires specific instrumentation (Fig. 4B). This paragraph will give a short overview about some principal experimental techniques that often were essential to gain the insights into mechanotransductive processes outlined throughout this review.

Optical/Magnetic Tweezers

Optical and magnetic tweezers were amongst the first tools that enabled cell biologists to quantitatively measure the weak forces produced by cells, or to apply corresponding forces to the cells (Iskratsch et al., 2014).

The optical tweezers technique consists in using a suitably functionalised μm -sized dielectric bead as a probe, and exploiting the restoring force generated by the interaction of a laser with the dielectric sphere to control the position of the bead (Fig. 4B). Measuring the probe displacement in experimental conditions provides a measure of the applied force. The bead diameter can range from few hundreds of nm to tens of μm depending on the contact region and the applied pressure required in the experiments. The limitations in bead dimensions are due to the fact that the trapping force decreases with the bead diameter. The tweezers can be calibrated accurately to know how much force is required to remove the trapped bead from its focal centre. The optical traps can generate forces ranging from tens to hundreds of

pN and the effective force constant of the tweezer is typically in the range of pN/m, providing extreme sensitivity to pN forces (Moffitt et al., 2008),(Neuman and Nagy, 2008),(Capitanio and Pavone, 2013),(Siedlik et al., 2016).

The magnetic tweezers approach relies on the same principle of action as the optical tweezer, but in this case magnets are used to position or apply forces on and para-ferromagnetic beads. The advantage of the magnetic control is that many beads can be affected simultaneously (Neuman and Nagy, 2008),(Siedlik et al., 2016),(De Vlaminck and Dekker, 2012),(Le et al., 2016), and it is also possible to induce twisting to the beads, allowing to test different degrees of freedom of the system under investigation (Wang et al., 1993),(Strick et al., 2000). The drawback is that the spatial variation of the field results in a non-uniform force applied to the beads; careful design of the magnetic tweezer apparatus permits nowadays for nearly constant gradient field over more than hundreds of microns (Neuman and Nagy, 2008),(Siedlik et al., 2016),(De Vlaminck and Dekker, 2012),(Le et al., 2016).

An early use of magnetic tweezers in cell biological research led to one of the seminal works for the mechanobiology field by Wang et al. in 1993 (Wang et al., 1993), showing the mechanosensitivity of integrins and mechanotransduction through the actin cytoskeleton. In 1995 Dai and Sheetz (Dai and Sheetz, 1995) characterised the mechanical properties of the neuronal growth cone by means of optical tweezers, demonstrating the role of the actin cytoskeleton in affecting the elastic properties of the membrane. Similar experiments were performed later on to determine the forces exerted by growing filopodia and lamellipodia (Cojoc et al., 2007) or axons (Moore et al., 2009),(Kilinc et al., 2014) during neuronal differentiation, or to compare forces generated by the growth cones of neurons from the central nervous system (hippocampal neurons) and the peripheral nervous system (dorsal root ganglia) (Amin et al., 2013).

Moving the stage in xy-direction or the focus of the laser spot in z-direction in a finely tuned and precisely controlled manner enables also the application of static or oscillatory forces (in the range of a few pN), in order to study e.g. the rheological behaviour of cells. With this method, it was recently demonstrated that mechanical stimuli with pN forces activate calcium channels (Falleroni et al., 2018) and that viscoelastic properties of soma and neurite differ and that in neurites these properties change in dependency of the substrate rigidity (Grevesse et al., 2015).

Additional details on optical and/or magnetic tweezer-based approaches, focussing on applications relevant for biophysics and mechanotransduction, can be found in Capitanio and Pavone (Capitanio and Pavone, 2013), Siedlik et al. (Siedlik et al., 2016) and Le et al. (Le et al., 2016).

Atomic Force Microscopy

AFM belongs to the branch of scanning probe microscopy (SPM). In recent years it has gained increasing importance in the mechanobiology field as an instrument with versatile application modes that allow high resolution morphological imaging (i.e., surface topography) and characterisation of mechanical properties (e.g., stiffness or viscoelasticity) of biological samples, also simultaneously (Haase and Pelling, 2015),(Gavara, 2017),(Alcaraz et al., 2018),(Krieg et al., 2019).

The probes are attached to an elastic lever (cantilever) that flexes under the interaction forces between the probe and the surface of the sample. The corresponding cantilever deflections are measured by a quadrant photodiode that detects the displacement of a laser reflecting from the exterior part of the cantilever. The probe shape and dimension can be modified according to the needs of the measurement and the information to be obtained. Pyramidal or narrow conical tips are usually exploited for high resolution imaging application (Krieg et al., 2019). More spherical (Indrieri et al., 2011),(Puricelli et al., 2015), or even cylindrical (Rico et al., 2007), probes instead are more suitable for mechanical characterisations of cells, extracellular matrices or tissues due to the fine control of the probe/sample

contact geometry. This system can be run in different modes depending on which structural and/or mechanical parameters of the measured samples are to be characterised (Haase and Pelling, 2015),(Gavara, 2017),(Alcaraz et al., 2018),(Krieg et al., 2019) (**Fig. 4B**).

During the scan, by keeping the cantilever deflection constant through a feedback circuit, the surface morphology of the sample can be reconstructed. This standard imaging application is e.g. widely used to image the nanotopography of cells (also neuronal cells and their compartments, such as growth cones (Parpura et al., 1993),(Grzywa et al., 2006),(Xiong et al., 2009)), natural extracellular matrices (Abrams et al., 2000),(Last et al., 2010),(Gasiorowski et al., 2013), or nanostructured biomaterials (Cellot et al., 2011),(Schulte et al., 2016c),(Schulte et al., 2016b)). It is also possible to apply forces by pushing the probe into the sample. Measuring the flexure of the cantilever through the extent of laser dislocation, the upward force acting on the tip can be calculated and mechanical properties of the sample can be determined. Varying the cantilever geometry allows covering more than six orders of magnitude in force sensitivity (from tens of pN to tens of μ N). The nanomechanical analysis of soft biological samples (such as e.g. cells and many tissues or extracellular matrices) with AFM is still to-date not straightforward. Attention must be drawn to the choice of the right experimental conditions for the application of contact mechanics models (such as Hertz (Hertz, 1882) or Sneddon (Sneddon, 1965)) and also in the data analyses (Puricelli et al., 2015),(Schillers et al., 2017). In the neuroscience field, by means of these types of AFM-based characterisations it was possible to determine the mechanical properties (Young's modulus) of substrates that are able to promote neuronal differentiation processes (Saha et al., 2008),(Leipzig and Shoichet, 2009),(Teixeira et al., 2009),(Banerjee et al., 2009),(Seidlits et al., 2010),(Keung et al., 2012),(Keung et al., 2013),(Musah et al., 2014),(Sun et al., 2014), as well as the mechanics of brain tissue or spinal cord (Elkin et al., 2007),(Christ et al., 2010),(Koser et al., 2016),(Koser et al., 2015),(Antonovaite et al., 2018) (**Fig. 1B** and **Fig. 3B**) and different types of neuronal cells (Lu et al., 2006),(Grzywa et al., 2006),(Spedden et al., 2012),(Schulte et al., 2016c), including changes due to mechanotransductive processes (Koser et al., 2016),(Schulte et al., 2016c),(Thompson et al., n.d.) (as detailed also in precedent paragraphs) (**Fig. 3B,C**).

Instead of applying forces to the cell with the AFM probe to deform the cell in order to analyse the cellular mechanical properties, the AFM can also be used to measure cellular adhesion forces (e.g., integrin/ligand binding (Strohmeyer et al., 2017)) by the force spectroscopy technique. There are two principal approaches to apply this technique. In one case (single-cell force spectroscopy), a cell is attached to a tipless cantilever (using basically the cell as probe) and brought smoothly into contact with a substrate of interest (Taubenberger et al., 2007),(Helenius et al., 2008) or even another cell (Puech et al., 2006). In a second approach, an AFM probe is functionalised in a suitable manner (e.g., with proteins of the ECM) and brought gently into contact with the cell membrane. In both cases after a sufficient time of contact to enable the desired interaction, the probe is then retracted in order to break the generated bonds. From the obtained force versus distance curves; typically exhibiting a complex pattern of sudden jumps and plateaux, it is possible to measure the number of ruptured bonds and the distribution of their strength. Several configurations have been adopted to study cell adhesion and its mechanisms and biomolecular determinants. Depending on the probe and type of functionalisation even adhesion forces at the single molecule level can be measured (single molecule force spectroscopy) (Müller et al., 2009),(Dufrêne et al., 2011).

Further information on AFM methodologies and applications in mechanobiology research is available in more specific reviews from Haase and Pelling (Haase and Pelling, 2015), Gavara (Gavara, 2017), Alcaraz et al. (Alcaraz et al., 2018) or Krieg et al. (Krieg et al., 2019).

Traction Force Microscopy

The first cellular reaction to biophysical stimuli in the microenvironment takes place in the interface between cell membrane (and its embedded receptors) and the ECM.

Traction force microscopy (TFM) aims at measuring the traction forces exerted by the cell towards the substrate it interacts with. This can be achieved by different approaches, either fluorescent microspheres embedded into deformable hydrogel substrates or pillar arrays are used (**Fig. 4B**). When the cells apply forces on these substrates as a consequence the beads or the pillars are displaced. Tracking the displacement microscopically and knowing the stiffness of the hydrogel, respectively pillars, permits an estimate of the applied traction forces and to reconstruct a traction force field. The spatial resolution of TFM is limited by the optical set up and by the relative density of the beads, respectively pillars. Several models have been proposed to accurately convert the strain field into a stress field (Schwarz and Soiné, 2015). In the neuronal context TFM has been used, e.g., to investigate the forces involved in the growth cones of neurons (Chan and Odde, 2008), (Betz et al., 2011) or during neuronal migration (Jiang et al., 2015). A combined TFM/AFM approach was recently applied to study the correlation between cellular rigidity, viscoelasticity, and the contractile prestress, highlighting the role of the actomyosin machinery (Schierbaum et al., 2019).

Another tool to measure the mechanical forces exerted by the cell are molecular tension probes. The functioning of these probes is based on an extendable linker (which can be build up by adequate polymers, or biomacromolecules (oligonucleotide or protein)) flanked by a spectroscopic ruler, consisting of a fluorophore and a quencher (**Fig. 4B**). If a sufficient force is applied to the probe, bonds within the linker region sequentially break and the linker extends leading to a displacement and separation of the fluorophore and quencher. The extent of linker extension is usually measured by fluorescence resonance energy transfer. Knowing the bond strengths inside the linker region, the applied force can be quantified (Jurchenko and Salaita, 2015), (Liu et al., 2017). These tension probes can be utilised as immobilised sensors on a substrate (Liu et al., 2014) or also intracellularly if they are integrated into proteins, an example is the vinculin tension sensor (Grashoff et al., 2010), (Jurchenko and Salaita, 2015).

5. Conclusion and Outlook

In this review we attempted to outline the crucial involvement of mechanobiological aspects in physiological brain and neuronal cell development and functioning. In recent years there are furthermore increasing indications that aberrations in the brain ECM organisation and mechanotransductive processes of neuronal cells strongly contribute to various neuronal pathologies, such as neurodegenerative diseases (e.g., in multiple sclerosis, amyotrophic lateral sclerosis and Alzheimer's disease) and neurodevelopmental disorders (such as, e.g., autism spectrum disorders and schizophrenia) (Tyler, 2012), (Lau et al., 2013), (Franze et al., 2013), (Park and Goda, 2016) (Lilja and Ivaska, 2018), (Barnes et al., 2017), or primary brain cancers (e.g., in glioblastoma) (Barnes et al., 2017), (Tanner, 2018). The research in this field is often still in its infancy, gaining a deeper insight into neuronal cell mechanotransduction by means of biophysical approaches is therefore essential (**Fig. 4**) and will help to understand better how abnormal mechanotransductive processes contribute to the aetiology of brain disorders (Tyler, 2012), (Franze et al., 2013), (Barnes et al., 2017), (Lilja and Ivaska, 2018), (Tanner, 2018). The biomedical significance of research in this direction is underlined, e.g., by a very recent study which suggests a glycocalyx/IAC mechanosignalling feedback loop regulated by tension in glioblastoma multiforme that might be causal for the high recurrence of this tumour after current chemotherapy treatments (Barnes et al., 2018).

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Conflict of Interest

The authors declare to have no conflict of interest.

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