

Neuronal Traction Force: From Growth Cone Motility to Neuronal Development, Migration, and Regeneration

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Abstract

Neurons are mechanically active cells that continuously interact with their extracellular environment during development, migration, axon extension, and regeneration. Among neuronal mechanical variables, traction force provides an informative interfacial readout because it reflects how cytoskeletal dynamics and adhesion are converted into forces exerted on the surrounding environment. This review summarizes the biomechanical basis of neuronal traction force, clarifies its relationship with tension and adhesion, and discusses traction force microscopy as a major method for mapping neuronal force output. We then review direct traction-force evidence related to growth cone advance, molecular-clutch-mediated force transmission, mechanical microenvironment sensing, neuronal migration, and discuss how force-based regulation is related to regeneration-associated growth. Current direct evidence is strongest for growth cones and migrating neurons, whereas mature synaptic remodeling, disease-related contexts, and regeneration models remain supported mainly by indirect mechanobiological evidence or externally applied force paradigms. Future studies combining three-dimensional models, traction readouts, molecular perturbation, and functional imaging will be important for defining how neuronal force generation contributes to nervous system development, adaptation, and repair.

Keywords

neurons; traction force; growth cone; molecular clutch; mechanical microenvironment; neural regeneration

1 Introduction

For decades, neuronal behavior has been interpreted primarily through the lenses of molecular signaling and electrical activity. However, accumulating evidence indicates that neurons function as active mechanical units that continuously sense and respond to mechanical information, rather than as purely "passive" cells devoted only to receiving and transmitting electrical signals. During nervous system development, tissue homeostasis, and disease, tissue stiffness, extracellular matrix composition, geometric constraints, and forces exerted by surrounding cells can all influence neuronal morphology, migration, and functional state (Pillai and Franze, 2024, Motz et al., 2021, Javier-Torrent et al., 2021, Chighizola et al., 2019). Therefore, incorporating mechanical factors into the interpretive framework of neuronal behavior has become an important direction at the interface of neuroscience and biomechanics (Feng et al., 2025, Kang et al., 2025, Yu and Li, 2024, Su et al., 2024, Liu et al., 2024).

Among the many mechanical variables involved, neuronal traction force is particularly important because it captures the mechanical output generated when neurons transmit cytoskeletal forces to the extracellular environment through adhesive contacts. Early studies showed that growth cones can actively pull on their substrate and that externally applied tension can promote neurite elongation, establishing force as an integral component of axonal growth rather than a secondary consequence of morphology change (Bray, 1984, Lamoureux et al., 1989, Heidemann et al., 1990, Zheng et al., 1991).

Mechanistically, neuronal traction force is generated through the coordinated action of actin polymerization, myosin-based contractility, retrograde actin flow, and adhesion-mediated cytoskeletal coupling. Studies of apCAM (Suter and Forscher, 2001), L1-CAM (Nishimura et al., 2003), N-cadherin (Bard et al., 2008), and shootin1 (Shimada et al., 2008, Toriyama et al., 2013) have progressively linked molecular adhesion systems to force transmission and growth cone motility. These findings provide a molecular basis for understanding how chemical guidance, cytoskeletal organization, and traction-force generation are integrated during neuronal growth.

However, research on neuronal traction force remains fragmented, and previous reviews have usually addressed neuronal mechanobiology, growth cone mechanics, molecular clutch models, or neural regeneration as relatively separate topics. In contrast, the present review uses traction force as a unifying interfacial mechanical readout that links molecular force transmission, cytoskeletal dynamics, mechanosensing, neuronal migration, and regeneration-associated growth. This perspective is useful because traction force lies between molecular-

scale coupling events and cell-scale neuronal behaviors: it reports how actin retrograde flow, myosin-dependent contractility, adhesion-clutch engagement, and extracellular mechanical cues are converted into measurable mechanical output. Therefore, rather than organizing the field around individual molecules, assays, or biological processes alone, this review aims to provide a traction-centered framework for understanding how neurons generate, transmit, and respond to force during development, migration, and regeneration. At the same time, we explicitly distinguish conclusions supported by direct traction-force measurements from those based on indirect mechanobiological inference, because the strength of evidence varies substantially across growth cones, neuronal migration, branching, synaptic remodeling, disease-related contexts, and regeneration.

2 Biomechanical Basis of Neuronal Traction Force and Traction Force Microscopy (TFM)

In studies of neuronal mechanobiology, traction stress, traction force, tension, and adhesion are closely related but refer to distinct physical processes. Neuronal traction stress usually denotes the local mechanical output formed at the contact interface when neurons transmit endogenous cytoskeletal forces to the outside world through adhesive sites on the substrate or surrounding environment. When the emphasis is on the distribution per unit area, the term traction stress is commonly used; when the emphasis is on the resultant integrated force, traction force is more appropriate (Athamneh and Suter, 2015, Suter and Miller, 2011, Miller and Suter, 2018). By contrast, tension emphasizes tensile load borne or transmitted along the long axis within neurites or axons, whereas adhesion emphasizes the binding of membrane receptors to the extracellular matrix or to molecules on neighboring cell surfaces, as well as the coupling of this binding to the intracellular cytoskeleton. These concepts are interrelated but not interchangeable. In what follows, "traction stress/traction force" will refer to interfacial output, "tension" to internal axonal load, and "adhesion" to the interfacial condition on which force transmission depends.

Mechanistically, neuronal traction force arises when intracellular cytoskeletal forces are transmitted to the extracellular environment through adhesive contacts. In growth cones, actin polymerization, myosin II-mediated contractility, retrograde F-actin flow, and adhesion-molecule coupling provide the cellular basis for traction generation (Suter and Forscher, 2001, Bridgman et al., 2001, Burnette et al., 2008, Kerstein et al., 2015). This force-transmission

framework explains why traction force is closely related to, but still distinct from, axonal tension and adhesion.

Early micromanipulation studies established that neurite elongation and growth cone advance are closely associated with mechanical tension and local pulling forces, providing the conceptual basis for quantitative measurements of neuronal force output (Bray, 1984, Lamoureux et al., 1989, Heidemann et al., 1990). Among current approaches, traction force microscopy (TFM) is particularly useful for estimating neuron–substrate traction because it maps substrate deformation induced by cells cultured on compliant, bead-embedded substrates. In a typical TFM workflow, fluorescent bead images are acquired in a deformed state with the neuron attached and in a reference state after cell removal. The resulting displacement field is then converted into a traction map through mechanical inversion based on assumptions about substrate elasticity, geometry, and boundary conditions (Athamneh and Suter, 2015, Ghate et al., 2024). This workflow allows neuronal force output to be spatially mapped, quantitatively estimated, and followed over time, making TFM especially suitable for studying growth cone motility, neuronal migration, and local mechanical asymmetry. A schematic overview of the TFM workflow, together with a representative neuronal TFM result from the authors' experiments, is shown in Fig. 1.

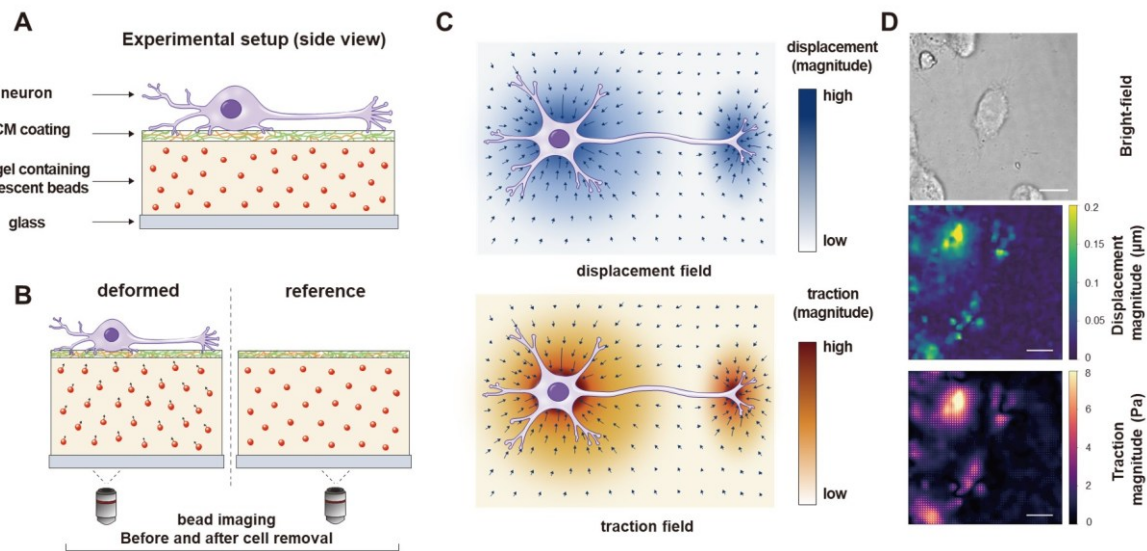


Fig. 1 Schematic workflow and representative output of neuronal traction force microscopy. (A) Schematic side view of a neuron cultured on an extracellular matrix-coated soft elastic gel containing fluorescent beads. Cell-generated traction is transmitted through the ECM coating to deform the bead-embedded substrate. (B) Fluorescent bead images are acquired in the

deformed state with the cell attached and in the reference state after cell removal. The displacement of embedded beads between these two states provides the basis for TFM analysis. (C) Representative schematic displacement and traction fields reconstructed from bead displacement. Arrows indicate the direction of substrate displacement or traction, whereas color maps indicate relative displacement or traction magnitude. (D) Representative neuronal TFM results, showing the bright-field image, displacement magnitude map, and traction magnitude map of a cultured neuron. The color bars indicate displacement magnitude in μm and traction magnitude in Pa. Scale bars, 10 μm . The representative neuronal TFM result in Fig. 1D is unpublished data from the authors' laboratory.

At the same time, neuronal TFM should be interpreted as an inference-based rather than a direct force-measurement method. The calculated traction field depends strongly on the inversion algorithm, regularization strategy, substrate parameters, bead-displacement accuracy, and image-registration quality. Fourier-transform traction cytometry provides an efficient approach for reconstructing traction fields on planar, linearly elastic substrates, but it may smooth highly localized force peaks depending on the regularization parameters used. Finite-element-based TFM can better accommodate complex geometries, boundary conditions, or non-uniform material properties, but it requires more detailed modeling choices and is more sensitive to assumptions about substrate mechanics. These issues are especially important in neuronal systems because growth cones, thin axons, and small adhesion sites often generate weak and spatially confined forces. Therefore, local traction peaks in small growth cones may approach the practical detection limit of conventional TFM and can be affected by bead density, optical resolution, displacement noise, and the signal-to-noise ratio of the reference/deformed image pair. Because the effective detection limit of TFM is not a fixed value, but depends on substrate stiffness and thickness, bead density, imaging resolution, displacement-detection accuracy, regularization strength, and the selected traction metric, a universal numerical threshold cannot be defined across studies. This is particularly relevant for neuronal forces in the sub-nN to nN range, where small errors in bead displacement or image registration may substantially influence reconstructed local traction peaks. For this reason, neuronal TFM studies should report not only traction or stress values, but also the experimental and computational parameters that determine measurement sensitivity and signal-to-noise performance.

Another important limitation is dimensionality. Most neuronal TFM studies have been performed on two-dimensional planar substrates, where the reconstructed traction field mainly

reflects basal force transmission at the neuron–substrate interface. However, neurons in vivo interact with soft, viscoelastic, heterogeneous, and three-dimensional environments. In three-dimensional matrices, organoids, tissue slices, or developing brain tissue, force transmission may be distributed through surrounding extracellular matrix fibers, neighboring cells, and out-of-plane components that are not captured by standard 2D TFM. Therefore, conclusions derived from 2D TFM should not be automatically generalized to three-dimensional neuronal environments without additional validation. Emerging 3D force-mapping strategies will be important for testing whether the same traction-generating mechanisms observed in growth cones and migrating neurons on planar substrates operate in more physiological settings.

Because different force-measurement approaches capture different mechanical variables and operate at different force and spatial scales, their results should not be treated as interchangeable. To clarify the methodological scope of this review, commonly used force-measurement approaches in neuronal mechanobiology are compared in Table 1, with emphasis on their main readouts, approximate force scale, spatial information, strengths, and limitations (Athamneh and Suter, 2015; Kilinc et al., 2015; Kerstein et al., 2015; Kumarasinghe et al., 2022).

Table 1. Comparison of force-measurement approaches used in neuronal mechanobiology.

Method	Main readout	Typical force scale / sensitivity	Spatial information	Strengths in neuronal studies	Key limitations
Traction force microscopy (TFM)	Substrate deformation reconstructed into traction stress or traction force maps	Usually reported as pN/ μm^2 or Pa-level traction stress; sensitivity depends on substrate, imaging, and inversion parameters.	Full-field 2D traction maps; emerging 3D TFM can provide volumetric force information	Enables spatial and temporal mapping of growth-cone and migration-associated traction; compatible with live-cell imaging and molecular perturbation	Inference-based rather than direct force measurement; sensitive to noise, image registration, substrate parameters, and regularization; standard 2D TFM does not capture all 3D or out-of-plane forces
Micropillar arrays	Deflection of calibrated elastomeric pillars converted into local forces	Typically nN-scale cellular or subcellular forces, depending on pillar stiffness and geometry	Discrete force vectors at pillar positions	Provides relatively direct force estimates with simple mechanical calibration; useful for local pulling forces	Discrete sampling rather than continuous maps; artificial topography may affect growth-cone behavior and adhesion organization
Force-calibrated microneedles / glass fibers	Deflection of a calibrated flexible probe during pulling or contact	Typically nN-scale forces; range depends on probe stiffness	Local force at the probe–cell contact site	Useful for measuring or applying defined pulling forces to neurites, axons, or growth cones	Local and perturbative; does not provide full-field traction distribution; contact geometry can influence the measured response
Optical tweezers	Force applied to or measured through trapped beads or probes	Typically pN-scale forces	Highly localized force application or measurement	Allows controlled local mechanical perturbation with high force sensitivity; useful for probing membrane, receptor, or bead-coupled interactions	Requires bead/probe attachment; limited to local measurements; optical setup and photodamage considerations may constrain live neuronal experiments

Method	Main readout	Typical force scale / sensitivity	Spatial information	Strengths in neuronal studies	Key limitations
Atomic force microscopy (AFM)	Cantilever deflection during indentation, pulling, or contact	pN–nN range depending on cantilever and mode	Local mechanical measurement at the probe contact region	Useful for measuring neuronal stiffness, membrane/cortical mechanics, and local mechanical response	Primarily measures indentation or contact mechanics rather than endogenous full-field traction; probe contact can perturb soft neuronal structures
Molecular tension probes	Force-dependent fluorescence or molecular extension across selected receptor–ligand linkages	Typically pN-scale molecular forces, depending on probe design	Molecular-scale or adhesion-site-level force readout	Reports receptor-specific force transmission and can connect molecular clutch engagement to mechanical load	Usually reports forces across selected molecular linkages rather than total cell-generated traction; requires specialized probe design and calibration

Note. The force scales listed here are approximate and depend on probe design, substrate stiffness, calibration strategy, imaging conditions, and analysis workflow. These approaches are therefore complementary rather than interchangeable.

In the context of this review, we use “direct traction-force evidence” mainly for studies that reconstruct substrate deformation into traction maps or otherwise quantify cell-generated pulling forces, whereas molecular, cytoskeletal, adhesion, or morphological data without direct force readouts are interpreted as indirect mechanobiological evidence. Representative studies and their quantitative and methodological characteristics are summarized in Table 2.

Table 2. Representative studies of neuronal traction-force readouts, with emphasis on TFM-based approaches.

No.	Biological context	Method and mechanical environment	Quantitative force or stiffness information	Perturbation / variable	Main finding and evidence interpretation	Ref.
1	Baseline biomechanics of growth cones; developing neuronal growth cones	Growth-cone rheological/mechanical analysis plus traction-force measurements; 2D substrate-based setting	Elastic modulus: 106 ± 21 Pa; estimated internal stress ~ 30 pN/ μm^2 ; weak traction/stress scale confirmed by traction measurements.	Growth-cone mechanics and retrograde actin flow	Growth cones behave as soft and weak force generators. Evidence level: direct mechanical/traction-related measurement.	(Betz et al., 2011)
2	Substrate rigidity sensing; DRG versus hippocampal neurons	TFM on laminin-coated polyacrylamide substrates; 2D culture; soft/intermediate/rigid substrates	Substrate stiffness: ~ 300 , 1000 , and 5000 Pa. Representative peak stress: DRG, 53.0 ± 3.3 Pa; hippocampal, 15.9 ± 1.5 Pa.	Substrate stiffness, neuronal type, time in culture, myosin II inhibition	Traction forces increase with substrate stiffness and differ by neuronal type; DRG growth cones exert stronger forces than hippocampal growth cones. Evidence level: direct TFM.	(Koch et al., 2012)

No.	Biological context	Method and mechanical environment	Quantitative force or stiffness information	Perturbation / variable	Main finding and evidence interpretation	Ref.
3	Peripheral traction vs. neurite tension; regenerating Aplysia bag cell neurons	TFM/substrate-deformation analysis of Aplysia growth cones; 2D deformable substrate	Mean neurite tension: 3.1 ± 1.6 nN; mean total scalar force: 6.5 ± 3.8 nN.	Growth cone area and time-dependent traction dynamics	Local traction dynamics and global neurite tension are related but not equivalent; much force is locally balanced within the growth cone. Evidence level: direct TFM/force analysis.	(Hyland et al., 2014)
4	Guidance cue to force conversion; cultured rat hippocampal neurons	TFM on compliant bead-embedded substrates; 2D culture; L1-CAM-related adhesion context	Netrin-1 increased growth-cone traction; reported mainly as relative traction enhancement rather than a comparable scalar value.	Netrin-1, Pak1-mediated shootin1 phosphorylation, shootin1 perturbation	Chemical guidance cues can be converted into measurable traction output through the Pak1–shootin1 pathway. Evidence level: direct TFM plus molecular perturbation.	(Toriyama et al., 2013)
5	Molecular clutch in axon outgrowth; cultured hippocampal neurons	TFM on L1-CAM-Fc-coated polyacrylamide gels embedded with fluorescent beads; 2D culture	Netrin-1 enhanced traction; cortactin knockdown or shootin1–cortactin disruption reduced traction. Absolute comparable scalar value not reported.	Netrin-1, cortactin RNAi, shootin1–cortactin interaction, rescue experiments	Shootin1–cortactin interaction strengthens F-actin–adhesion coupling and promotes traction force generation. Evidence level: direct TFM plus molecular perturbation.	(Kubo et al., 2015)
6	Migration force map; migratory cerebellar granule neurons	TFM on deformable substrates; 2D migratory neuron assay	TFM mapped three traction/contraction centers; no single comparable scalar force value was emphasized.	Neuronal migration stage, myosin II activity, leading/trailing process dynamics	TFM identified three contraction centers in distal leading process, proximal leading process, and trailing process. Evidence level: direct TFM.	(Jiang et al., 2015)
7	Mechanical clutch in neuronal migration; migrating olfactory interneurons	TFM in explant-derived migrating neuroblasts; semi-3D or substrate-supported migration context	Shootin1b loss reduced traction stress; reported shootin1 KO value: 4.53 ± 0.66 pN/ μm^2 .	Shootin1b loss, L1-CAM/cortactin coupling, F-actin retrograde flow	Shootin1b couples F-actin retrograde flow to adhesion and promotes leading-process extension and neuronal migration. Evidence level: direct TFM plus molecular perturbation.	(Minegishi et al., 2018)
8	Nuclear translocation during migration; cerebellar granule neurons	TFM during neuronal migration; 2D culture; local traction analysis near the proximal leading process	Local traction patterns and force dipoles were linked to nucleokinesis; no single comparable scalar force value was emphasized.	Physical hindrance of nuclear translocation, local leading-process swelling, myosin II-related force generation	Local traction in the proximal leading process can pull the nucleus during nucleokinesis. Evidence level: direct TFM linked to a specific migration step.	(Umeshima et al., 2019)
9	Growth-cone clutch regulator; chick spinal neurons	TFM combined with growth-cone adhesion and cytoskeletal analyses; 2D culture	Fmn2 depletion reduced growth-cone traction-stress generation; reported mainly as relative reduction.	Fmn2 morpholino/knockdown, adhesion stability, F-actin retrograde flow	Fmn2 stabilizes point contacts and is required for growth-cone traction stress generation. Evidence level: direct TFM plus molecular perturbation.	(Ghate et al., 2020)
10	Mechanosensitive clutch interface; cultured mouse hippocampal neurons	Traction/deformation analysis with L1-laminin clutch assays; 2D substrate setting	Laminin-coated PA gels with different stiffness conditions; growth-cone traction stresses described as $< \sim 80$ pN/ μm^2 .	Substrate stiffness, L1-laminin interaction, clutch engagement	ECM mechanics regulate axon outgrowth through mechanosensitive clutch engagement rather than through bulk stiffness alone. Evidence level: direct force-related readouts plus molecular/adhesion perturbation.	(Abe et al., 2021)

No.	Biological context	Method and mechanical environment	Quantitative force or stiffness information	Perturbation / variable	Main finding and evidence interpretation	Ref.
11	Soft-tissue-like chemotropism; rodent hippocampal neurons on soft hydrogels	TFM on 0.1 kPa hydrogels; 2D soft-substrate assay	Substrate stiffness: 0.1 kPa; growth-cone force generation was small and reduced by drebrin or paxillin perturbation.	BDNF-bound stripes, drebrin phosphorylation, paxillin–drebrin association, myosin II/Src inhibition, siRNA perturbations	BDNF-induced growth-cone turning and force generation require drebrin phosphorylation and paxillin–drebrin association in soft environments. Evidence level: direct TFM plus molecular perturbation.	(Chen et al., 2022)
12	Soft physiological environment and microtubule reinforcement; hiPSC-derived cortical i3Neurons	High-resolution TFM in soft microenvironments; human iPSC-derived neurons	High-resolution TFM showed small and transient traction forces in soft brain-like microenvironments.	DCX loss, microtubule reinforcement, BDNF gradient, soft environment	DCX supports productive growth-cone advance by reinforcing microtubules when adhesion-mediated traction is weak and transient. Evidence level: direct high-resolution TFM plus cytoskeletal perturbation.	(Dema et al., 2024)
13	Substrate deformation as predictor of advance; Aplysia growth cones	AFM with apCAM-coated colloidal cantilever and force-calibrated glass microneedles; local adhesion-mediated growth cone advance	Adhesion-mediated Aplysia growth-cone forces were in the 1–100 nN range.	Probe stiffness, apCAM/adhesion ligand, adhesion-mediated growth cone advance	Productive advance depends on when and where substrate-cytoskeletal coupling is established, not simply on peak force amplitude. Evidence level: direct calibrated local force measurement.	(Athamneh et al., 2015)
14	Netrin-1/DCC clutch during haptotaxis; cultured hippocampal neurons	TFM/substrate bead displacement on polylysine or netrin-1-coated substrates; 2D haptotaxis assay	Netrin-1 substrate: 11.9 ± 1.1 pN/ μm^2 ; polylysine substrate: 6.1 ± 1.5 pN/ μm^2 .	Substrate-bound netrin-1, DCC, shootin1a, grip/slip state of DCC	DCC–shootin1a adhesion clutch mediates netrin-1-dependent traction-force transmission during axonal haptotaxis. Evidence level: direct TFM/substrate-deformation measurement plus molecular perturbation.	(Qiu et al., 2024)

Abbreviations: BDNF, brain-derived neurotrophic factor; DCX, doublecortin; DCC, deleted in colorectal cancer; DRG, dorsal root ganglion; ECM, extracellular matrix; hiPSC, human induced pluripotent stem cell; TFM, traction force microscopy.

3 Traction Force in the Growth Cone: Generation, Distribution, and Function

Having distinguished traction force, tension, and adhesion, the growth cone becomes the central model for observing interfacial mechanical output from neurons. It is both the leading structure that senses external guidance cues and substrate properties and the effector that converts intracellular cytoskeletal dynamics into external mechanical output. The growth cone therefore functions as a true mechano-chemical transducer, converting external cues into changes in adhesion, cytoskeletal organization, and force-generation state that ultimately determine whether the neuron advances, pauses, or turns (Pillai and Franze, 2024, Franze, 2020, Kerstein et al., 2015, McCormick and Gupton, 2020).

A striking feature of growth cone traction force is that it is not uniformly distributed across the entire contact interface, but instead exhibits marked spatial heterogeneity. Classical experiments showed that growing growth cones can actively pull on the substrate (Lamoureux et al., 1989, Heidemann et al., 1990), while higher-resolution studies revealed that this pulling is typically concentrated at the growth cone periphery and lateral edges rather than being evenly spread across the entire ventral surface. Betz and colleagues described growth cones overall as "soft and weak force generators," suggesting that they do not advance by sustained, large-amplitude pulling, but rather by local, compliant mechanical modules that probe the environment and drive forward movement (Betz et al., 2011). Time-series analyses in *Aplysia* and DRG neurons further indicated that, although instantaneous traction maps are complex, one can still extract a relatively stable average contractile pattern that moves with the growth cone (Hyland et al., 2014, Polackwich et al., 2015).

Growth cone traction force is also a dynamically remodeling force field. High-temporal-resolution analyses have shown that growth cone traction force consists of short-lived local peaks together with a smaller number of longer-lasting stress peaks, consistent with a repeated cycle of "grip-release-regrip" in the growth cone (Polackwich et al., 2015). Earlier studies of actin flow found that the rate of growth cone advance is inversely correlated with retrograde F-actin flow: when the growth cone advances along a given interaction axis, retrograde flow along that axis is selectively slowed (Lin and Forscher, 1995). Importantly, direct measurements of substrate deformation in *Aplysia* growth cones further showed that local substrate deformation can predict subsequent advance, reinforcing the view that productive outgrowth depends on effective substrate-cytoskeletal coupling in space and time rather than on force magnitude alone (Athamneh et al., 2015).

These findings suggest that productive growth cone advance depends less on maximal force amplitude than on spatiotemporally coordinated force transmission. Local traction must be generated at appropriate adhesive sites, persist long enough to engage the substrate, and remain coordinated with retrograde F-actin flow and microtubule invasion. Thus, TFM is most informative when traction maps are interpreted together with adhesion dynamics, cytoskeletal organization, and growth cone behavior (Lin and Forscher, 1995, Athamneh et al., 2015, Hyland et al., 2014, Polackwich et al., 2015, Kerstein et al., 2015).

Growth cone turning further illustrates the spatial significance of traction force. Moore and colleagues showed that immobilized netrin-1 is sufficient to reorient axons and is accompanied by local traction generation (Moore et al., 2009). Nichol and colleagues further demonstrated

that guidance can occur through local coupling between retrograde F-actin flow and point-contact adhesions, without requiring a global alteration in actin flow (Nichol et al., 2016). Studies of L1-CAM grip/slip dynamics also showed that asymmetric traction can arise within the growth cone and drive haptotactic turning at a laminin/polylysine boundary (Abe et al., 2018, Abe et al., 2021). Thus, growth cone turning is best understood as a mechanically lateralized process in which local asymmetry of force transmission biases directional movement.

Together, studies of growth cone advance and turning show that traction force is a spatially organized and dynamically regulated component of growth cone motility. This raises the mechanistic question addressed in the next section: how intracellular cytoskeletal forces are captured by adhesion complexes and transmitted to the substrate.

4 How Force is Transmitted to the Substrate: Adhesion Complexes and the Molecular Clutch

The previous section emphasized the spatiotemporal manifestations of growth cone traction force. This section turns to a more mechanistic question: how are the internal forces generated by the cytoskeleton stably transmitted to the external substrate? In the neuronal field, the classical explanation is the molecular clutch model. The key point of this model is not any single protein, but rather that the retrogradely flowing actin network must be mechanically coupled, via specific linkage modules, to membrane receptors and their external ligands before internal cytoskeletal motion can truly be "engaged" and converted into measurable traction force (Kerstein et al., 2015, Suter and Forscher, 2001, Miller and Suter, 2018).

In this sense, adhesion complexes constitute the essential interface required for traction generation, rather than merely serving as passive background conditions. Point contacts in growth cones are viewed as specialized adhesion structures composed of multiple molecules involved in integrin-mediated adhesion and cytoskeletal coupling; these sites serve not only as mechanical linkages but also as nodes for signal integration and local stabilization (Renaudin et al., 1999). FAK is one of the most important hubs in this network, regulating pathfinding and point-contact dynamics downstream of integrin-mediated adhesion, both of which are required for growth cone advance and turning (Robles and Gomez, 2006, Myers and Gomez, 2011). Although these structures are related to focal adhesions in non-neuronal cells, growth-cone adhesions are more commonly described as point-contact adhesions because of their small size, rapid turnover, and close association with filopodia and actin retrograde flow (Renaudin et al., 1999, Robles and Gomez, 2006, Kerstein et al., 2015). To illustrate these adhesion structures

more directly, Fig. 2 includes representative Paxillin-GFP-labeled point-contact adhesion dynamics adapted from a previously published open-access work (Kerstein et al., 2015).

At the molecular level, the apCAM system was foundational. Suter and Forscher clearly proposed in *Aplysia* growth cones that apCAM is not merely a surface adhesion molecule, but also participates in the transmission of traction force, and that its linkage to the cytoskeleton is regulated by Src family kinases (Suter and Forscher, 2001). Subsequently, L1-CAM, ankyrinB, N-cadherin, and shootin1 were incorporated into this pathway. Nishimura and colleagues showed that L1-dependent neuritogenesis requires ankyrinB-mediated coupling of L1-CAM to retrograde actin flow (Nishimura et al., 2003), while Bard and colleagues demonstrated a direct molecular clutch between N-cadherin adhesions and actin flow that drives growth cone migration (Bard et al., 2008). The significance of these studies lies not merely in providing a list of molecules, but in progressively resolving the phenomenon that "neurons pull" into a dissectable and regulatable process of interfacial coupling.

Research on shootin1 further developed this mechanism into a closed loop linking chemical signals to mechanical output. Shimada and colleagues found that shootin1 can interact with both actin retrograde flow and L1-CAM, thereby strengthening the connection between them and promoting axon outgrowth (Shimada et al., 2008). Toriyama and colleagues then showed that netrin-1 enhances growth cone traction force through Pak1-mediated phosphorylation of shootin1 (Toriyama et al., 2013). Fmn2 was subsequently identified as another key clutch regulator; by stabilizing point contacts and strengthening actin-substrate coupling, it promotes the generation of traction force by growth cones (Ghate et al., 2020). Recent work has extended this logic to the netrin receptor DCC: substrate-bound netrin-1 can function through a DCC-shootin1a adhesion-clutch, increase the grip state of DCC on the substrate, and enhance traction-force transmission during axonal haptotaxis (Qiu et al., 2024). These findings make clear that the molecular clutch is not a static connector, but a dynamic interface continuously regulated by upstream signals.

The L1-CAM grip/slip model proposed by Abe and colleagues refined regulation of the molecular clutch at the level of dynamics. L1-CAM movement on the substrate is not fixed, but switches between grip and slip states; when the proportion of time spent in the grip state increases, growth cone traction is enhanced and turning toward that side becomes more likely (Abe et al., 2018). Subsequent work further showed that the L1-laminin clutch interface itself is mechanosensitive (Abe et al., 2021). Thus, traction force should be understood as a highly regulatable force-transmission process with a clear molecular basis, rather than as a purely

phenomenological readout. The major mechanisms by which growth cones generate traction and transmit force through adhesion-based molecular clutch systems are summarized in Fig. 2.

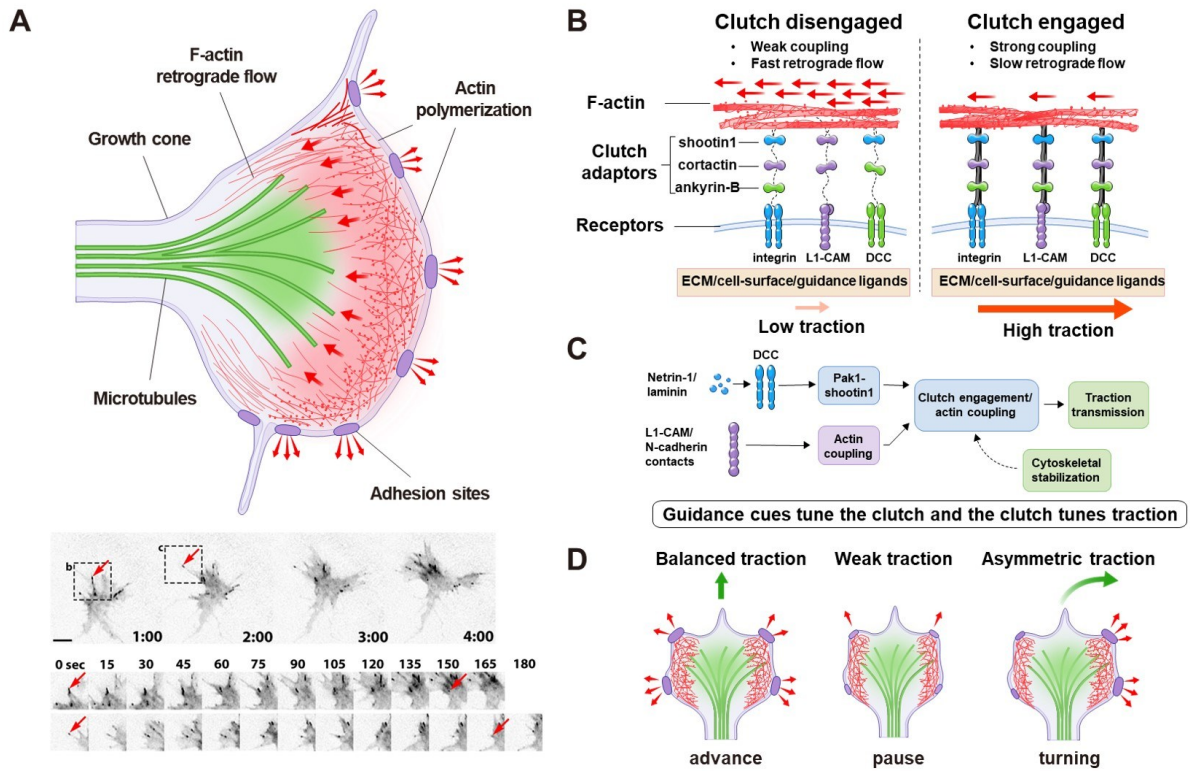


Fig. 2 Growth cone traction generation, point-contact adhesion dynamics, and molecular clutch mechanisms. (A) Schematic growth cone architecture showing F-actin retrograde flow, actin polymerization, microtubule organization, adhesion sites, and spatially localized traction hotspots. The lower inset shows Paxillin-GFP-labeled point-contact adhesion dynamics in a *Xenopus* growth cone, illustrating the transient assembly and disassembly of growth-cone adhesion structures. This inset was adapted from Kerstein, Nichol and Gomez (2015) under the Creative Commons Attribution License (CC BY). (B) In the molecular clutch model, weak cytoskeleton–substrate coupling permits rapid F-actin retrograde flow and low traction transmission, whereas stronger clutch engagement slows retrograde flow and increases traction transmission through adhesion receptors and clutch adaptors. (C) Guidance and adhesion pathways, including netrin-1/DCC, Pak1–shootin1, L1-CAM, N-cadherin, and actin-coupling modules, regulate clutch engagement, cytoskeletal stabilization, and traction output. (D) Different modes of force transmission contribute to growth cone behavior: balanced traction supports advance, insufficient clutch engagement favors pausing, and asymmetric traction biases turning.

5 How the Mechanical Microenvironment Shapes Neuronal Traction Force

Neuronal traction force is not determined solely by the intracellular actomyosin system. Whether the growth cone can convert internal dynamics into effective traction on the outside world also depends on whether the external environment permits such force to be received, transmitted, and fed back. Substrate stiffness, ECM composition, mode of ligand presentation, surface geometry, and spatial confinement can all alter growth-cone adhesion, coupling to retrograde flow, and microtubule advance. Where traction has been directly measured, these environmental variables can influence traction output; in other contexts, their effects on traction remain mechanobiological inferences based on adhesion, cytoskeletal, or morphological changes (Pillai and Franze, 2024, Javier-Torrent et al., 2021, Chighizola et al., 2019). Traction is therefore not an isolated property of the neuron, but an interfacial variable jointly produced by the neuron and its microenvironment.

Among environmental factors, substrate stiffness is one of the variables most directly linked to neuronal traction. Koch and colleagues found, by comparing DRG neurons with hippocampal neurons, that the two respond differently to substrate stiffness: DRG growth cones generate larger traction forces on stiffer substrates, whereas hippocampal neurons show a weaker stiffness response, suggesting intrinsic differences among neuronal types in mechanosensitivity and in the range of their mechanical output (Koch et al., 2012). At the same time, more recent motor-clutch modeling constrained by experimental data suggests that effective substrate coupling can be achieved more rapidly on relatively soft substrates, indicating that the relationship between stiffness and traction is not purely monotonic and may depend on cell type, clutch reinforcement, and the mechanical regime being tested (Pulido Cifuentes et al., 2024). Further *in vivo* studies have also shown that tissue stiffness and mechanosensitive pathways participate in axon growth during development, indicating that stiffness is not merely a materials-science concept *in vitro*, but a genuine instructive variable in nervous system development (Javier-Torrent et al., 2021, Koser et al., 2016).

ECM composition more directly determines what the growth cone can grip and how it grips it. Laminin is one of the most classical ECM ligands in neuronal research. Rivas and colleagues long ago demonstrated that acute addition of laminin can markedly alter growth cone morphology and accelerate growth cone advance within minutes (Rivas et al., 1992). More specifically, studies of the L1-laminin clutch have shown that laminin acts as a mechanical interface that directly changes grip/slip state and the efficiency of traction transmission, beyond its role as a growth-promoting substrate (Abe et al., 2021). In addition, laminin presentation

density itself can alter branching and local adhesive organization, suggesting that ECM density is a mechanical variable that neurons can "read" (Liu et al., 2013).

Geometric structure and spatial confinement likewise reshape neurite growth directionality and potential force-transmission patterns. Micropatterns and topography do not simply constrain which path neurites can follow; rather, at the scale of the growth cone they alter contact area, edge curvature, the distribution of adhesion sites, and the manner in which symmetry is broken (Clark et al., 1993, Fozdar et al., 2010). Smirnov and colleagues further showed that, in narrow channels, overall growth cone advance velocity may not decrease dramatically, but growth cone morphology and its response to changing constraints are clearly altered, suggesting that spatial confinement may shape growth behavior by redistributing adhesion sites, cytoskeletal organization, and potential force-transmission regions (Smirnov et al., 2014).

Most current studies of neuronal traction are still based on two-dimensional culture systems, whereas neurons in vivo reside in environments that are three-dimensional, soft, viscoelastic, and highly heterogeneous. The difference between two-dimensional and three-dimensional settings involves far more than the addition of one extra dimension, because it entails wholesale changes in ligand presentation, force-dissipation pathways, and constraints on cell morphology. This distinction is not merely theoretical: recent work showed that CNS axons in three-dimensional matrices can adopt amoeboid and adhesion-independent growth modes, implying that the force-transmission logic inferred from two-dimensional adhesive substrates cannot always be transferred directly to 3D settings without revision (Santos et al., 2020). Therefore, extending the study of neuronal traction force into three-dimensional systems, organoids, and even in vivo contexts will be an essential next step for understanding this mechanical phenotype (Pillai and Franze, 2024, Motz et al., 2021, Javier-Torrent et al., 2021). The principal ways in which the mechanical microenvironment regulates neuronal traction and growth behavior are summarized in Fig. 3.

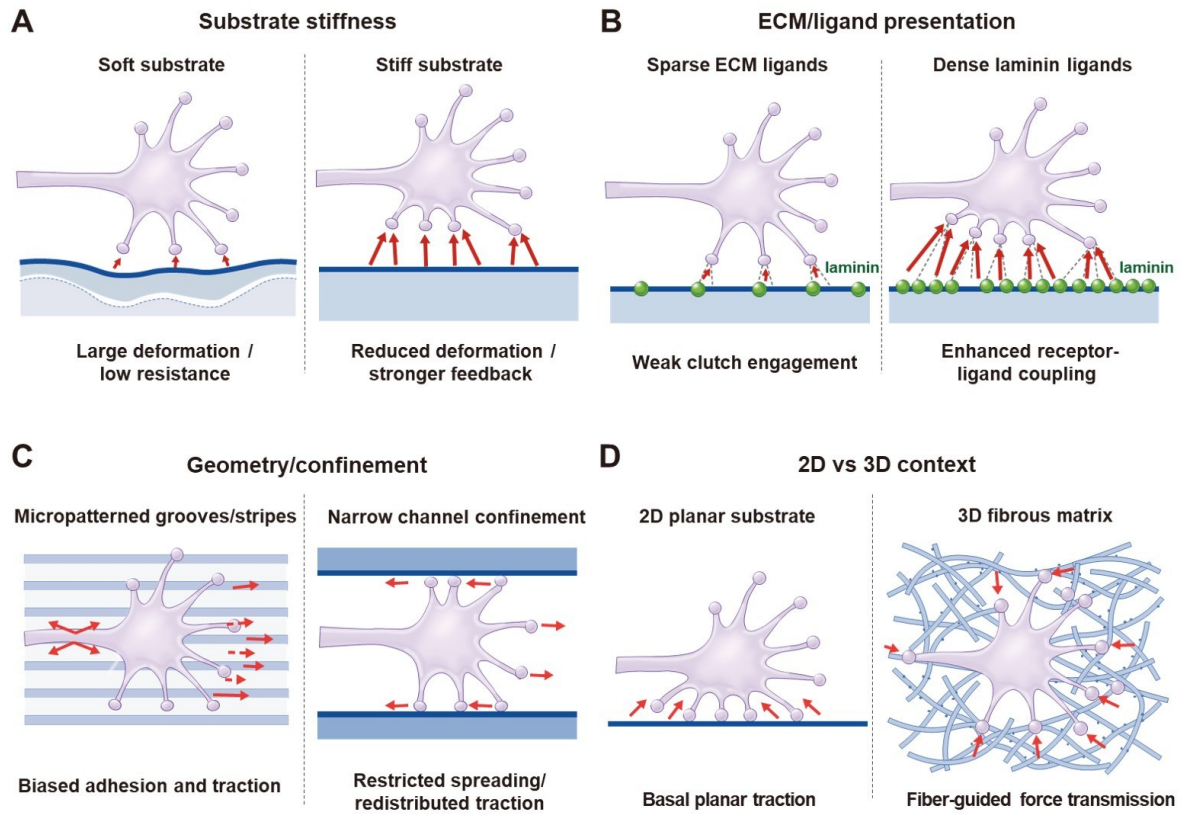


Fig. 3 Mechanical microenvironmental regulation of neuronal traction and growth behavior. (A) Substrate stiffness changes the mechanical resistance against which growth cones transmit force. Soft substrates undergo larger deformation and provide lower resistance, whereas stiff substrates show reduced deformation but stronger mechanical feedback, thereby altering the efficiency of traction transmission. (B) ECM and ligand presentation regulate receptor–ligand coupling and clutch engagement. Sparse ECM ligands weaken clutch engagement and reduce effective force transmission, whereas dense laminin ligands enhance receptor–ligand coupling and promote adhesion-dependent traction transmission. (C) Geometric cues and confinement reshape where adhesions form and where traction is transmitted. Micropatterned grooves or stripes bias adhesion and traction along defined directions, whereas narrow channel confinement restricts growth cone spreading and may redistribute the sites of adhesion and force transmission along the channel boundary. (D) Dimensionality changes the physical route of force transmission. On two-dimensional planar substrates, traction is mainly transmitted through basal adhesive contacts, whereas in three-dimensional fibrous matrices, force transmission is guided by surrounding ECM fibers and may involve different force-dissipation pathways.

6 From Axon Extension to Neuronal Migration and Morphogenesis

During brain development, neurons actively interact not only with the extracellular matrix, but also with radial glia, neighboring cells, and the surrounding tissue architecture, and these interactions contribute to neuronal repositioning, laminar organization, and subsequent morphological development (Pillai and Franze, 2024, Javier-Torrent et al., 2021, Minegishi and Inagaki, 2020). However, compared with the growth cone literature, the strength of evidence here is not uniform. Direct traction-force evidence is relatively strong for neuronal migration, whereas branching and dendritic morphogenesis are still supported mainly by cytoskeletal, adhesion, and morphological data rather than by direct full-field traction measurements.

With regard to neuronal migration, direct and indirect force-related evidence together support the view that the tip of the leading process can function as a growth-cone-like mechanical structure rather than merely a directional leading edge. He and colleagues showed early on that migrating neurons do not move by passive sliding of the soma, but by active pulling at the tip of the leading process; this process depends on myosin II and is accompanied by forward F-actin flow along the leading process (He et al., 2010). Minegishi and colleagues further demonstrated that, in migrating interneurons, shootin1b accumulates in the growth-cone-like structure of the leading process and acts as a molecular clutch through interactions with cortactin and L1-CAM, coupling F-actin retrograde flow to the adhesive substrate and thereby promoting extension of the leading process and subsequent somal translocation (Minegishi et al., 2018). More recently, Nakajima and colleagues identified a bona fide growth-cone-like structure at the tip of migrating neurons in the injured brain and showed that this structure probes extracellular inhibitory cues and helps drive migratory behavior, further strengthening the conceptual link between axonal growth cones and migratory leading processes. Although this study did not directly map traction forces, it strengthens the anatomical and functional analogy between axonal growth cones and migratory leading processes (Nakajima et al., 2024).

Compared with axon elongation, one distinctive feature of neuronal migration is that it must move not only the leading edge but also the relatively bulky soma and nucleus. Thus, traction during migration is not concentrated solely at the extreme front. Using TFM in cultured granule cells, Jiang and colleagues found three distinguishable contraction centers in migrating neurons, located in the distal leading process, proximal leading process, and trailing process, respectively, all of which exhibit high myosin II activity (Jiang et al., 2015). Umeshima and colleagues further showed that local traction force generated in the proximal leading process

can directly trigger nuclear translocation (Umeshima et al., 2019). These findings represent some of the strongest evidence in this area, because they not only propose a mechanical model but also directly link local traction events to specific migratory steps such as somal advance and nuclear movement.

From migration to branching and morphogenesis, local mechanical redistribution remains a plausible and important concept, but this area is currently supported mainly by indirect evidence rather than by a mature chain of quantitative traction measurements. Recent reviews of branching broadly agree that branch formation depends on local actin remodeling, adhesion adjustment, microtubule invasion, and membrane expansion. These processes are mechanically relevant, but they do not by themselves constitute direct evidence of traction-force redistribution (Gallo, 2011, Menon and Gupton, 2018, Pacheco and Gallo, 2016). In axon collateral branch formation, actin patches, filopodia, and microtubule invasion together constitute the cellular sequence of branching, while drebrin coordinates the actin and microtubule systems to promote collateral branch initiation. These studies suggest that local mechanical redistribution may participate in branch initiation and stabilization. However, direct full-field traction measurements demonstrating when, where, and how traction forces are redistributed during branching remain largely unavailable.

Direct traction-force evidence in dendritic morphogenesis is currently lacking or remains extremely limited. The existing literature supports a relatively cautious conclusion: dendrite formation depends on local actin/microtubule coordination, cell adhesion, and branch stabilization, processes that may involve local mechanical organization but have not yet been directly linked to quantified traction-force patterns (Ouzounidis et al., 2023, Seong et al., 2015). Thus, from axon extension to neuronal migration and onward to branching and morphogenesis, a broader mechanobiological continuum can be proposed. Yet the density of evidence is not equivalent across all points on this continuum. A more defensible formulation is that neuronal morphogenesis may depend in part on local cytoskeletal–adhesion remodeling and mechanical redistribution across subcellular regions, but whether this involves measurable redistribution of traction output remains to be directly tested.

7 Regeneration, Stretch Growth, and Externally Applied Mechanical Force

Although exogenously applied tension is not identical to endogenous traction force, both lines of work converge on the broader principle that neuronal growth is mechanically regulatable. Regeneration-related studies have further expanded the broader force-based framework of

neuronal growth, although most of this evidence concerns externally applied tension rather than endogenous traction force. Classic tension experiments together with recent reviews indicate that mechanical force can serve not only as a concomitant factor in developmental growth, but also as an exogenous regulatory variable that promotes axon extension and regeneration-associated growth under controlled experimental conditions (Franze, 2020, Bradke, 2022, De Vincentiis et al., 2020). From a historical perspective, this line of thinking is not abrupt. As early as the classic work of Bray, Dennerll, Zheng, and others, externally applied tension had already been shown to induce neurite growth, regulate axonal elongation, and influence neurite initiation (Bray, 1984, Zheng et al., 1991, Dennerll et al., 1989). Lamoureux and colleagues later showed that mechanical tension can even specify axonal fate in a particular process of hippocampal neurons (Lamoureux et al., 2002). The relationships between endogenous traction, externally applied tension, and regeneration-related growth are summarized in Fig. 4.

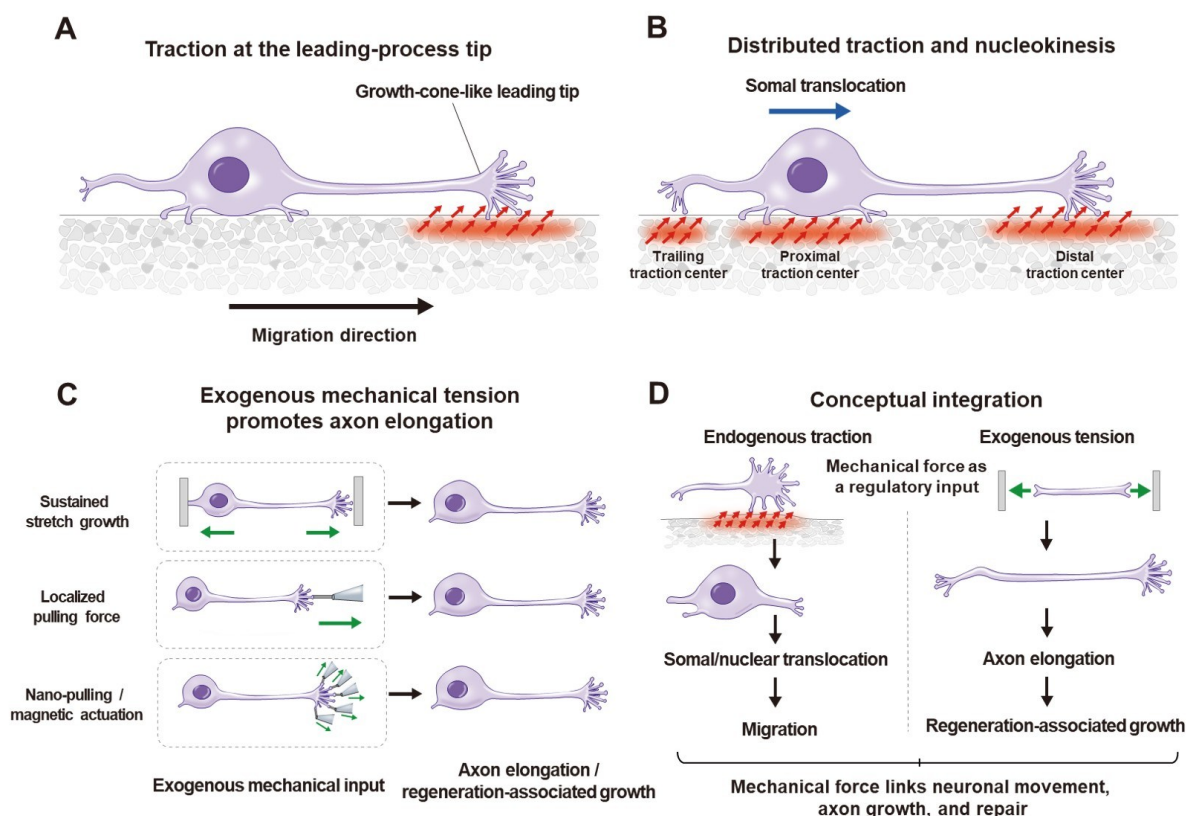


Fig. 4 Mechanical regulation of neuronal migration, axon elongation, and regeneration-associated growth. (A) The leading-process tip of migrating neurons can behave as a growth-cone-like traction-generating structure, producing distal traction that supports directional migration. (B) During neuronal migration, traction is distributed across distal, proximal, and

trailing regions. These spatially organized traction centers contribute to somal translocation and nucleokinesis. (C) Exogenous mechanical inputs, including sustained stretch growth, localized pulling force, and nano-pulling or magnetic actuation, can promote axon elongation under controlled conditions. (D) Conceptual integration of endogenous traction during neuronal migration and exogenous tension during axon elongation or regeneration-associated growth. Together, these examples illustrate how distinct modes of neuronal mechanical regulation link migration-associated force generation to force-regulated axon elongation and repair-related growth.

These studies shifted the interpretation of axon growth from a purely biochemical assembly process toward a model in which mechanical tension contributes actively to elongation and material addition along the axon. Later work pushed this logic further to the level of axon assembly. Reviews have pointed out that increases in axonal length do not simply reflect addition of membrane and cytoskeletal material at the distal tip, but involve tension transmission and material assembly along the entire axonal axis (Heidemann and Bray, 2015, Lamoureux et al., 2010). Corresponding theoretical models likewise emphasize that axon elongation is not a simple geometric stretching process, but a dynamic equilibrium jointly determined by force, viscosity, protein supply, and adhesion (O'Toole et al., 2008).

The concept of stretch growth pushed this idea to a more extreme and clearly defined level. Smith and colleagues first proposed that sustained growth of integrated central nervous system axons can be induced by continuous mechanical tension (Smith et al., 2001). Pfister and colleagues then demonstrated that integrated axon tracts can be stretch-grown in vitro to the centimeter scale, while retaining active electrical signal conduction after stretching (Pfister et al., 2004). Loverde and Pfister further summarized axon stretch growth as an engineerable mechanotransduction platform (Loverde et al., 2011). These results show that sustained tension does not necessarily damage axonal structure; within an appropriate range, it can instead induce genuine growth accompanied by material assembly.

More recent work on nano-pulling has advanced the concept that "externally applied mechanical force promotes axon growth" toward finer, more remote, and lower-amplitude forms of manipulation. A review by De Vincentiis and colleagues pointed out that extremely small external forces can significantly modulate axon growth (De Vincentiis et al., 2020). Subsequent studies have attempted to apply sustained, directionally controllable small forces

using magnetic nanoparticles or magnetically driven platforms, showing that such mechanical stimulation can promote axon elongation, cytoskeletal remodeling, and related transport processes (Falconieri et al., 2023). Thus, the growth-promoting effects of mechanical force are unlikely to reflect only passive elongation by external pulling; instead, they may involve coordinated regulation of the cytoskeleton, axonal transport, and local synthesis.

However, this line of research also requires a clear conceptual boundary. The fact that controlled, low-amplitude, sustained pulling can promote axon extension does not mean that greater tension is always better in clinical repair. A more balanced view is that moderate, controllable mechanical bias may be beneficial, whereas focal or excessive tension could compromise blood supply, aggravate scarring, and impair regeneration. Translational efforts will therefore need to define safe ranges of force amplitude, duration, application site, and tissue tolerance (Bradke, 2022, De Vincentiis et al., 2020, Katiyar et al., 2020).

8 Discussion

Overall, neuronal traction force can be viewed as an important intermediate mechanical variable linking cytoskeletal dynamics, adhesion regulation, and neuronal behavior, rather than as an accessory physical phenomenon (Pillai and Franze, 2024, Motz et al., 2021, Franze, 2020). Its value lies in providing a bridge between molecular mechanisms and cell-scale behavior: it helps explain not only whether neurites extend, but also how neurons convert intracellular actomyosin activity into measurable actions on the external environment. Importantly, the field is better viewed as stratified rather than uniform, with some biological contexts already supported by direct mechanical evidence and others still at the level of plausible mechanobiological extension.

The strongest and most coherent evidence chain currently exists in the context of the growth cone. Conceptually, traction force, tension, and adhesion correspond to interfacial output, internal load, and the conditions for force transmission, respectively, and these terms should not be conflated. Mechanistically, actin polymerization, myosin II-mediated contraction, retrograde flow, point-contact dynamics, and clutch molecules together generate a force-transmission system that converts intracellular cytoskeletal dynamics into substrate-directed mechanical output (Franze, 2020, Kerstein et al., 2015, Suter and Forscher, 2001, Nishimura et al., 2003, Bard et al., 2008, Shimada et al., 2008, Toriyama et al., 2013, Ghate et al., 2020, Miller and Suter, 2018). Recent work further strengthens this framework by showing that local substrate deformation can predict subsequent growth cone advance and that additional

guidance-receptor systems can also mediate directional traction-force transmission, thereby extending the clutch framework beyond the classical L1-CAM and N-cadherin paradigms (Athamneh et al., 2015, Qiu et al., 2024).

At the functional level, growth cone advance and neuronal migration remain the best-established application domains of neuronal traction force research. Existing studies consistently show that growth cone traction is spatially heterogeneous, temporally dynamic, and closely associated with advance, pausing, and turning (Franze, 2020, Polackwich et al., 2015, Abe et al., 2018, Abe et al., 2021). Likewise, migrating neurons display traction-generating regions along the leading and proximal processes, and recent work in the injured brain has further reinforced the idea that migratory leading processes contain bona fide growth-cone-like structures that probe extracellular cues and contribute actively to migration (Jiang et al., 2015, Umeshima et al., 2019, Minegishi et al., 2018, Nakajima et al., 2024). By contrast, branching, dendritic morphogenesis, dendritic spine remodeling, and disease-related mechanical alterations should be discussed more cautiously. For branching and mature synaptic remodeling, direct traction-force measurements remain scarce or absent; for neurological disease, direct evidence demonstrating altered neuronal traction force is currently lacking.

Dendritic spine remodeling and disease-related mechanopathology are conceptually relevant to neuronal mechanobiology, but they should not yet be considered established branches of neuronal traction-force research. Existing studies suggest that adhesion-dependent actin coupling, including shootin1a-mediated linkage to N-cadherin and L1-CAM, can contribute to activity-dependent spine enlargement and stabilization (Kastian et al., 2021, Chazeau et al., 2015, Minegishi et al., 2023). However, direct full-field traction measurements comparable to those in growth cones or migrating neurons remain scarce in mature synaptic contexts. Similarly, although abnormal mechanotransduction has been implicated in neurological disorders, direct evidence demonstrating disease-state-dependent alterations in neuronal traction force is currently lacking (Li et al., 2024, Barrantes, 2024). These topics are therefore best framed as future directions rather than as established branches of neuronal traction-force research.

The role of the mechanical microenvironment also deserves a more nuanced interpretation than a simple “stiffer means stronger” model. Substrate stiffness, ECM composition, ligand density, surface geometry, spatial confinement, and dimensionality can reshape neuronal adhesion, cytoskeletal coupling, and, where directly measured, traction output by altering the

efficiency and topology of force transmission (Koch et al., 2012, Koser et al., 2016, Abe et al., 2021, Rivas et al., 1992, Liu et al., 2013, Clark et al., 1993, Fozdar et al., 2010, Smirnov et al., 2014). Moreover, recent modeling suggests that growth cones may couple more efficiently to relatively soft substrates under some conditions (Pulido Cifuentes et al., 2024), while experiments in 3D matrices indicate that CNS axons can adopt amoeboid and adhesion-independent growth modes (Santos et al., 2020). Together, these findings argue that neuronal traction is an interfacial phenotype jointly determined by the neuron, the ligand system, and the mechanical regime in which force is generated and dissipated.

Regeneration-related studies broaden the relevance of neuronal force biology, although most direct evidence in this area concerns externally applied tension or pulling rather than endogenous traction-force mapping. Classic tension experiments, stretch-growth paradigms, and more recent low-force nano-pulling approaches collectively show that mechanical force can function not only as an accompanying variable in developmental growth, but also as an exogenous regulator that promotes axon extension and regeneration-associated growth under controlled experimental conditions (Franze, 2020, Bradke, 2022, De Vincentiis et al., 2020, Lamoureux et al., 2002, Lamoureux et al., 2010, Smith et al., 2001, Pfister et al., 2004, Loverde et al., 2011, Falconieri et al., 2023). These observations support a view of axon growth as a distributed biomechanical process involving tension, adhesion, cytoskeletal assembly, transport, and local synthesis, rather than as an event controlled solely at the distal tip. For this reason, neuronal traction force research has genuine conceptual relevance for neural repair, but this relevance remains partly indirect until endogenous traction-force changes are measured in regeneration models (Bradke, 2022, De Vincentiis et al., 2020, Katiyar et al., 2020).

Several limitations remain prominent. First, the literature is unevenly distributed. The most direct traction-force evidence is concentrated in growth cones and neuronal migration, whereas axon extension and regeneration are supported by a mixture of traction measurements, externally applied tension experiments, and indirect force-related evidence. Evidence in mature neural networks and synaptic homeostasis remains scarce, and direct evidence demonstrating disease-state-dependent alterations in neuronal traction force is currently lacking. Second, most current measurements are still obtained in two-dimensional reductionist systems and therefore do not fully capture the soft, three-dimensional, viscoelastic, and heterogeneous environments in which neurons operate in vivo (Pillai and Franze, 2024, Motz et al., 2021, Santos et al., 2020). Third, substantial differences in cell source, substrate formulation, ECM coating, stiffness range,

and analytical workflow reduce comparability across studies. Finally, although current work can localize where force is generated and suggest how it is transmitted, much less is known about how traction-like mechanical states couple to electrical activity, synaptic transmission, and network function.

For neuronal TFM specifically, several technical issues should be addressed in future studies. First, standardized reporting of substrate stiffness, thickness, bead density, ECM coating, displacement-detection method, inversion algorithm, regularization strategy, and traction metrics would greatly improve comparability across studies. Second, higher-resolution and lower-noise imaging and analysis pipelines are needed to resolve weak and spatially confined forces in small growth cones, thin axons, filopodia-like protrusions, and small adhesion sites. Third, future work should more explicitly compare 2D and 3D force-mapping strategies, because traction reconstructed from basal deformation on planar substrates may not reflect force transmission through fibrous matrices, organoids, tissue slices, or in vivo-like environments. Finally, neuronal TFM should be integrated with calcium imaging, membrane-potential imaging, electrophysiology, molecular perturbation, and quantitative live-cell imaging so that traction maps can be linked not only to morphology and migration, but also to neuronal excitability, synaptic activity, and network-level function.

Accordingly, several broader biological directions appear especially important. First, the field should move beyond two-dimensional single-cell preparations toward three-dimensional matrices, organoids, tissue slices, and in vivo-like systems, not only to improve physiological relevance but also to test whether the same force-transmission principles still hold under these conditions (Pillai and Franze, 2024, Motz et al., 2021, Javier-Torrent et al., 2021, Santos et al., 2020, Nakajima et al., 2024). Second, the scope of the field should expand from developmental growth cones and migrating neurons toward mature neurons, synaptic structural plasticity, and network-level behavior, while maintaining a clear distinction between well-established evidence and emerging hypotheses (Kastian et al., 2021, Chazeau et al., 2015, Minegishi et al., 2023). Third, disease-oriented work should proceed both proactively and cautiously, with particular emphasis on identifying measurable mechanical intermediate phenotypes, including but not limited to traction-force readouts, between cytoskeletal imbalance, synaptic pathology, and abnormal mechanotransduction (Li et al., 2024, Barrantes, 2024).

Overall, research on neuronal traction force has evolved from early observations that neurites can pull into a developing interdisciplinary framework connecting cell mechanics,

developmental neurobiology, and neural repair. The most solid evidence already supports one central idea: neurons are not merely electrical signaling units, but mechanically active cells that continuously exchange forces with their environment. Studying traction force therefore helps explain how neurons extend, navigate, and migrate, while also providing a physically grounded language for discussing regeneration and selected aspects of structural plasticity. With more realistic models and richer multimodal readouts, neuronal traction force research is well positioned to mature from a specialized biomechanical topic into a broader framework for understanding nervous system development, adaptation, and repair.

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COMPLIANCE WITH ETHICS GUIDELINES

Conflict of Interest

Peiyan Duan, Shengyuan Ma, Qian Suo, Lefan Lv, Huijing Jin, Linyong Zhu, Assaf Zemel, Guang-Zhong Yang, and Yuan Feng declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

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