NEURAL REGENERATION RESEARCH
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Contribution of mechanical forces to structural synaptic plasticity: insights from 3D cellular motility mechanisms

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Cells, tissues, and organs are constantly subjected to the action of mechanical forces from the extracellular environment — and the nervous system is no exception. Cell-intrinsic properties such as membrane lipid composition, abundance of mechanosensors, and cytoskeletal dynamics make cells more or less likely to sense these forces. Intrinsic and extrinsic cues are integrated by cells and this combined information determines the rate and dynamics of membrane protrusion growth or retraction (Yamada and Sixt, 2019). Cell protrusions are extensions of the plasma membrane that play crucial roles in diverse contexts such as cell migration and neuronal synapse formation. In the nervous system, neurons are highly dynamic cells that can change the size and number of their pre- and postsynaptic elements (called synaptic boutons and dendritic spines, respectively), in response to changes in the levels of synaptic activity through a process called plasticity. Synaptic plasticity is a hallmark of the nervous system and is present throughout our lives, being required for functions like memory formation or the learning of new motor skills (Minegishi et al., 2023; Pillai and Franze, 2024).

While extensive research has uncovered key molecular mechanisms and signaling pathways involved in synaptic plasticity, much less is known about the contribution of mechanical forces and physical properties to this process. Neuronal synapses are surrounded by a complex microenvironment composed of other cells and the extracellular matrix (ECM), which likely influences the dynamics of both pre and post synaptic protrusions (Minegishi et al., 2023). To highlight how biomechanics regulate morphological changes occurring during synaptic plasticity, we discuss here the idea that neuronal structural plasticity employs mechanisms akin to those used for cellular motility in other cell types. To move, migrating cells change their shape through diverse mechanisms that depend on the environment, and which can be broadly subdivided into two types: mesenchymal and amoeboid migration.

Mesenchymal migration is characterized by actin-rich protrusions such as lamellipodia and filopodia. These protrusions require adhesion to the substrate and F-actin polymerization at the leading edge to allow forward movement, with concomitant Myosin 2 contractility at the back of the cell to push the cell forward. In contrast to mesenchymal migration, amoeboid migration typically relies on blebs (García-Arcos et al., 2024). Blebs are spherical protrusions of the plasma membrane that arise from places where the plasma membrane detaches from the actin cortex, typically lacking F-actin, and are promoted by conditions of high contractility, high confinement, and low cell adhesion. Unlike other types of cellular protrusions used in cell migration (e.g., lamellipodia), blebs do not rely on actin polymerization to push the plasma membrane forward; instead, they depend on Myosin 2-mediated contractility, which generates hydrostatic pressure and consequently a rapid influx of cytoplasm into these protrusions. In addition, whereas other types of protrusions are associated with high levels of substrate adhesion, blebs form with low adhesion to the substrate. Interestingly, blebs can form even in the absence of external chemical cues (Yamada and Sixt, 2019) highlighting their remarkable mechanical nature. The physical properties of both the actomyosin cytoskeleton and the surrounding 3D environment are key for bleb formation. Contrary to the classical idea that each cell type uses a specific type of migration mode, it is now becoming well-accepted that cells can switch between mesenchymal and amoeboid migration depending on the mechanical characteristics of the surrounding environment (Yamada and Sixt, 2019; Schick and Raz, 2022; García-Arcos et al., 2024). This highlights the role of external cues for cellular behavior.

Similarly to migrating cells, neurons are also exposed to diverse microenvironments. Presynaptic boutons and dendritic spines are embedded in the ECM and in direct contact with other neurons, glia, and/or muscle (Minegishi et al., 2023; Pillai and Franze, 2024). As such, this 3D environment can promote or hinder the growth of pre- and postsynaptic protrusions during plasticity. These changes in neuronal synaptic structure depend on factors such as (1) how tightly packed neurons are, (2) the elasticity or stiffness of the ECM, (3) the degree of adhesion, and (4) how much force the neuron exerts onto its neighbors, to name a few. Most studies have shown that both axonal growth during development and dendritic spine enlargement during plasticity occur by actin-driven growth, initiating from lamellipodia and filopodia-like protrusions (Minegishi et al., 2023). However, the cellular mechanism underlying presynaptic bouton formation in wired neurons or during plasticity has remained unclear until recently.

In a recent study, Fernandes and colleagues proposed blebbing as a key mechanism for the formation of new presynaptic boutons in wired motor neurons at the *Drosophila* neuromuscular junction (NMJ) (Fernandes et al., 2023). The authors demonstrated that these boutons emerge rapidly as large round expansions of the neuronal membrane, mostly devoid of F-actin and showing signs of plasma membrane detachment from the actin cortex — all hallmarks of blebs (**Figure 1A**, top). In contrast to what has been shown for neuronal growth cone expansion and dendritic spine growth during embryonic development (Pillai and Franze, 2024), boutons at the NMJ primarily originate from pre-existing boutons in synaptic areas, rather than growing from actin-rich filopodia or lamellipodia that normally do not emanate from synapses (Fernandes et al., 2023). In the context of amoeboid cell migration, bleb formation is preferred under conditions of high and asymmetric Myosin 2 contractility. Consistent with this, the study by Fernandes et al. (2023) showed that Myosin 2 accumulates at the base of emerging synaptic boutons at the early stages of their formation and influences the speed of new bouton formation. These observations further support the hypothesis that presynaptic bouton plasticity can occur through a bleb-like mechanism and challenge the classical view of synapse formation.

Confined environments have been shown to promote bleb formation in migrating cells. For instance, experimentally confining various cell types, from *Dictyostelium* to mammalian cells, leads to an increase in blebbing (Schick and Raz, 2022; García-Arcos et al., 2024). In the context of presynaptic bouton formation, Fernandes et al. hypothesized that muscle contraction could play a mechanical role by providing physical confinement to the motor neurons. Indeed, they showed that inhibiting muscle contraction, either mechanically or chemically, led to a significant reduction in the number of new boutons formed in response to acute stimulation. This effect was observed even in low Myosin 2 activity conditions, suggesting that muscle contraction, and thus physical confinement are pivotal for bouton formation via blebbing in this context (Fernandes et al., 2023). In the brain, despite lacking muscle-induced forces, presynaptic boutons are surrounded by other cells, such as neurons, astrocytes, oligodendrocytes, and microglia, as well as by ECM, which might be able to induce compressive forces and confinement (**Figure 1A**, bottom; Minegishi et al., 2023). A recent study in the mammalian brain revealed that dendritic spines can exert mechanical forces on presynaptic boutons (**Figure 1B**; Ucar et al., 2021). Using elegant mechanical manipulations and optogenetics, these authors showed that stimulated dendritic spines expand and push against their presynaptic partners, enhancing neurotransmitter release. Notably, these expanding dendritic spines exert forces onto boutons comparable to those produced by smooth muscle contraction (Ucar et al., 2021). Cells are also able to modulate the physical properties of their surrounding environment, namely the stiffness and viscoelasticity, by promoting the remodeling of the ECM. While mesenchymal cells and actin-driven protrusion migration are associated with extensive ECM remodeling, amoeboid-bleb migration can occur without major ECM degradation (Yamada and Sixt, 2019). It is still unclear how some cells can migrate through complex matrices without the involvement of proteolytic degradation of the ECM but one hypothesis is that these cells can mechanically push or deform ECM fibrils (García-Arcos et al., 2024). In neurons, ECM components surround dendritic spines and presynaptic boutons, further extending into the synaptic cleft. In the brain, modulation of the ECM has been shown to be critical for synaptic plasticity and maintenance of long-term potentiation. This occurs through activity-dependent secretion of cathepsin B by dendritic spines which leads to the activation of matrix metalloproteinases, resulting in ECM remodeling (**Figure 1C**; Wang et al., 2008; Padamsey et al., 2017). At the Drosophila NMJ, matrix metalloproteinase secretion has also been shown to participate in the orchestration of synapse development (Rushton et al., 2020), but whether the mechanical component of ECM remodeling is important remains unknown.

Another unanswered question is how mechanosensation is achieved in the presynaptic compartment, but a possible mechanism might involve mechanosensitive ion channels, like transient receptor potential and Piezo channels (Pillai and Franze, 2024). These highly conserved channels are activated by cell membrane tension and mechanical deformation, triggering a myriad of signaling pathways, including actomyosin dynamics. In neurons, stretch-activated ion channels are required for axon growth during development (Pillai and Franze, 2024), however, whether they are involved in bouton formation and synaptic plasticity is still unclear. Interestingly, in *Dictyostelium* migrating cells, Piezo ion channels become activated upon mechanical compression and confinement. They are required for bleb formation by promoting calcium influx, potentially enhancing actomyosin contractility and/or cytoplasmic fluidity (Aoki et al., 2021; García-Arcos et al., 2024). Other players involved in mechanosensation and membrane deformation are BAR domain proteins, known to sense and induce membrane curvature in various cell types (Minegishi et al., 2023). A recent study combining experimental approaches with theoretical modeling (Lavi et al., 2019) has proposed that migrating zebrafish germ cells regulate their blebbing activity via BAR protein-induced membrane tension. Several BAR proteins are expressed in adult neurons in the brain and accumulate in the early dendritic protrusions from which dendritic spines grow (Minegishi et al., 2023). Importantly, the loss of these proteins in mice leads to a reduction in the number of

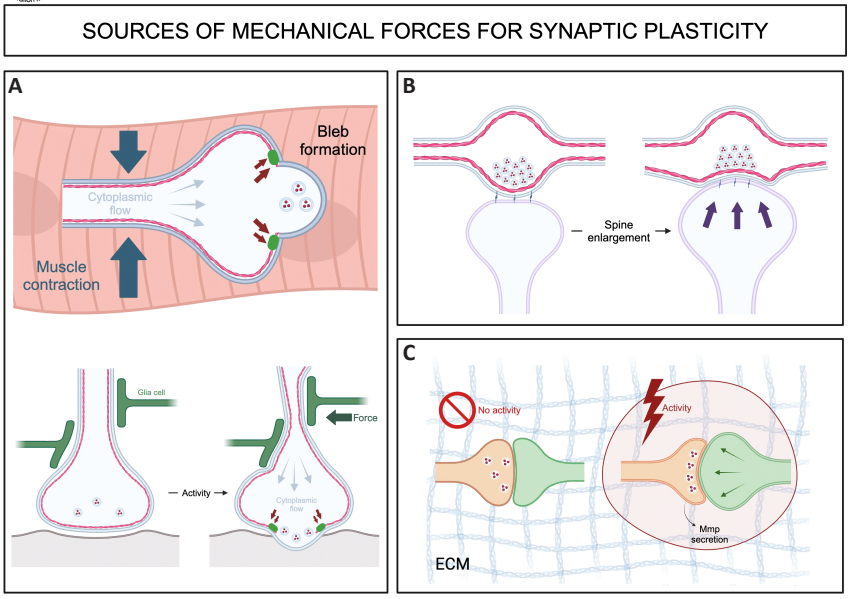

Figure 1 | **Schematics representing possible sources of mechanical forces implicated in structural synaptic plasticity.**
(A) Representation of a new synaptic bouton forming by blebbing coupled with muscle contraction at the *Drosophila* NMJ (as reported by Fernandes et al., 2023). Increased activity induces a local weakening of the actin cortex, priming locations where motor neurons can add new boutons by membrane blebbing. During this process, Fernandes et al. hypothesized that muscle contraction acts through the compression of motor neurons to increase their confinement and cortical tension, facilitating bouton formation (top). Below, an example of how the same principle of mechanical compression facilitating plasticity could, in principle, be used by glia at the NMJ, but also in the brain (bottom image). (B) Schematic of a dendritic spine growing and mechanically compressing the presynaptic bouton, which leads to a transient increase in neurotransmitter release (as shown by Ucar et al. 2021). (C) Schematics of how synaptic activity leads to Mmp secretion and ECM remodeling. This remodeling has been shown to be required for the maintenance of structural changes after long-term potentiation (based on data from Wang et al., 2008 and Padamsey et al., 2017). Created with BioRender.com. ECM: Extracellular matrix; Mmp: matrix metalloproteinase; NMJ: neuromuscular junction.

dendritic spines and to synaptic plasticity-related phenotypes, such as impaired spatial learning and memory. Additionally, mutations in humans are associated with several brain diseases (Minegishi et al., 2023; Pillai and Franze, 2024). Future studies should thus investigate whether mechanosensitive proteins and ion channels play a role in presynaptic bouton formation and plasticity through blebbing mechanisms.

Another aspect of mechanical regulation of presynaptic bouton formation that deserves further investigation is cell adhesion. Cell–cell and cell–substrate adhesion molecules, such as cadherins and integrins respectively, are essential players in mechanotransduction, transmitting intracellular forces generated by the cytoskeleton to neighboring cells and the surrounding environment. Cell–substrate integrin-mediated adhesion is an important regulator of cell protrusion formation. In migrating cells, whereas filopodia and lamellipodia depend on high adhesion to the substrate ECM via focal adhesions, blebs typically form under low adhesion conditions (Schick and Raz, 2022). In fact, inhibiting focal adhesion assembly increases bleb formation in several types of migrating cells. However, the mechanisms are still unclear. One hypothesis is that focal adhesions and the cell cortex compete to recruit the actomyosin cytoskeleton (García-Arcos et al., 2024). Although neurons do not form typical focal adhesions, they can sense and respond to environmental mechanical cues and substrate stiffness via integrins and other typical focal adhesion proteins like Vinculin (Pillai and Franze, 2024). However, how tissue stiffness regulates synaptic plasticity is still largely understudied. It would be worth investigating whether the regulation of cell–ECM complexes plays a role in bleb formation during presynaptic bouton formation. Cell–cell adhesion can also affect bleb formation. In cell migration, it has been shown that cadherin-mediated adhesion restricts bleb formation. For instance, in zebrafish germ cells, E-cadherin confines blebs to the cell leading edge, reinforcing the front-rear axis of these migrating cells (Schick and Raz, 2022). It is thus tempting to speculate that in presynaptic bouton formation, the regulation of the level of adhesion to other neurons and/or glial cells might also play a role in modulating the number and localization of newly forming boutons. It has been shown that N-cadherin and other cell–cell adhesion molecules can regulate dendritic spine formation but their roles in presynaptic plasticity remain unclear (Minegishi et al., 2023).

In summary, blebbing emerges as a novel mechanism for presynaptic bouton formation, akin to the bleb protrusions essential for amoeboid cell migration — a process that is highly reliant on mechanical forces and the 3D external environment. Notably, the conditions that favor bleb formation during cell migration — high confinement, low adhesion, and high contractility — mirror the environment at synaptic terminals. The study by Fernandes et al. (2023) challenges the traditional perspective of bouton formation driven by actin-based protrusions, urging the exploration of blebbing and mechanics in synaptic plasticity in other models. This extends beyond the peripheral nervous system to the brain, where neurons can experience compressive forces from neighboring neurons and glial cells. The parallels between cell migration and synaptic plasticity mechanisms suggest that the knowledge regarding the mechanisms of blebbing in cell migration may offer a framework for better understanding bouton formation. The role of mechanical forces and the 3D extracellular microenvironment in neuronal plasticity also raises the question of whether neurons can alter their remodeling strategy in accordance with the local environment at any given time. This has implications for the prediction and testing of synapse formation in controlled 3D environments, which can contribute to the design of strategies to promote synapse formation in the diseased brain, namely in neurodegenerative disorders where there is a notable loss of synapses.

We would like to thank Anna Hobbiss (Champalimaud Research, Champalimaud Center for the Unknown, Lisbon, Portugal) for critical reading and proofreading of this manuscript.

We would like to acknowledge the fact that many studies that contributed to the conception of this perspective were not cited here due to constraints on the number of references. Review articles were favored but they reflect many research articles critical to the field.

This work was supported by PTDC-01778/2022-NeuroDev3D, iNOVA4Health (UIDB/04462/ 2020 and UIDP/04462/2020), and LS4FUTURE (LA/P/0087/2020).

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Date of submission: May 1, 2024
Date of decision: June 7, 2024
Date of acceptance: June 24, 2024
Date of web publication: July 29, 2024

https://doi.org/10.4103/NRR.NRR-D-24-00498
How to cite this article: *Teodoro RO, Ramos MR, Carvalho L (2025) Contribution of mechanical forces to structural synaptic plasticity: insights from 3D cellular motility mechanisms. Neural Regen Res 20(7):1995-1996.*

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C-Editors: Zhao M, Liu WJ, Qiu Y; T-Editor: Jia Y