The function of mechanical tension in neuronal and network development

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Received 6th January 2010, Accepted 22nd January 2010
First published as an Advance Article on the web 25th February 2010
DOI: 10.1039/b927402b

A bewildering series of dynamic processes participate in the proper development of the complex architecture of the nervous system. Recent years have seen a growing interest in the role of mechanical forces in neural development. This is an exciting field of multidisciplinary study that encompasses biology, physics and engineering and enjoys both conceptual and technical recent advances in all of these areas. Here I present an update of very recent work on several related questions, including the role of neurite and axonal tension in the development of single neuron morphology, the effects of mechanical cues from the substrate, the role of tension in axonal pruning and synaptogenesis, and more. Particular emphasis is placed on the very recent and exciting shift from descriptive mechanics to a possible role for tension forces in neuronal and network function.

Mechanical forces and the development of neuronal morphology

It is by now well established that the growth cones of developing neurites generate tensile forces.[1,2] This is well exemplified in a recent study of *Aplysia* neurons, which utilized the atomic force microscope (AFM) to investigate the properties of growth cones.[3] It was found that a dense packing of actin filaments provides the growth cone with its increased mechanical stiffness that supports and transduces tension. Recent reports confirm previous work and suggest that the tension generated along newly-formed neurites affects all aspects of neuronal morphology (Fig. 1), from the shape of the neuronal somata[4] to the local geometry of the bifurcation of neurites into their sub-branches.[5]

While mechanical forces have a role as regulators of axonal development in early embryonic stages, this regulatory role is also not lost during later stages of morphological plasticity in the nervous system.[6] This phenomenon is of great significance in the rapid expansion of the nervous system during animal growth. It may also facilitate attempts to repair the damaged nervous system.[6] In a recent study, Pfister *et al.*[7,8] tested the ability of integrated axons to endure escalating rates of stretch. They found that axon tracts could be stretch-grown at rates of 8 mm/d, while still maintaining their morphology and density of organelles and cytoskeletal constituents. The importance of the latter is shown, for example, in the recent work of Yang and Saif,[9] who found that the strongly linear, reversible and repeatable response of fibroblasts to stretch is fully accounted for by actin filaments.

Ample detailed recent studies have investigated the interaction between cyto- mechanics and the cytoskeleton in cells in general (*e.g.* ref. 10–12), and specifically in neurons.[13] Beyond the important role of the cytoskeleton in direct tensile force generation, it is also instrumental in mechanotransduction, *i.e.* in channelling the signals associated with mechanical forces in the periphery of the cell to the nucleus and, ultimately, to gene activity. This coupling mechanism was recently reviewed by Wang *et al.*[14] An alternative mechanism of mechanotransduction can be inferred from the work of Franze *et al.*,[15] which suggests a transient calcium influx through stretch-activated ion channels in the membrane of growth cones in response to mechanical stress.

An important but rarely addressed point is that of the ability of neuronal processes to sustain mechanical stress without losing their functional properties. In this respect, Pfister *et al.*[8] reported that stretch-growth did not alter sodium channel activation, inactivation and recovery, or potassium channel activation, with the axons' overall ability to transmit active signals being maintained. The functional aspects of neuromechanics are further elaborated upon below.

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Insight, innovation, integration

The development of the brain is a complex dynamical process. Complete understanding of this process will be achieved only by taking into account the effects of physical–mechanical forces. This is a field of multidisciplinary study that takes advantage of both, conceptual and technical advances in biology, physics and engineering. Recent years have seen significant contributions, reviewed herein, including the role of axonal tension in the development of neuronal morphology, the effects of mechanical cues from the substrate, the role of tension in axonal pruning and synaptogenesis, and more. Coming years will see more emphasis put on the role of mechanical forces in brain and nervous system function. Work in this direction will benefit much from recent advances in material science and nano-technology.

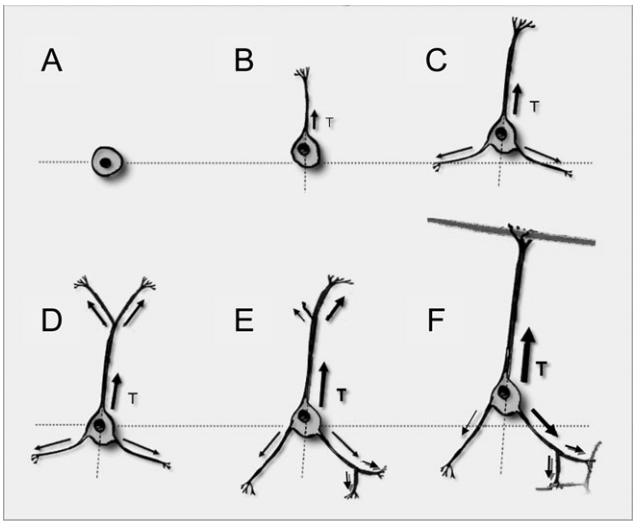

Fig. 1 A toy model (schematic diagram modified from Hanein *et al.*[4]) demonstrating processes in neuronal development, in which mechanical tension plays a role: A–C. Neurite initiation and growth and the shape of the neuronal soma. D–F. Neurite branching (including effects on branching angles), and branch pruning or survival. Neuronal migration in the direction of the major branch is also shown; the different sizes of the arrows reflect the level of induced tension.

Mechanical interactions with the environment

The interactions of cells with their environment, both cellular and non-cellular, have important implications for the development of specific cell morphologies.[16,17] In the case of neurons this may affect the development of normal, as well as pathological, wiring diagrams. In their natural settings neurons grow in a very inhomogeneous mechanical environment. Hence, in addition to the much studied biochemical cues, which are well known to control axonal guidance, neurons are also susceptible to various mechanical stimuli. It has been shown that neurons continuously probe their mechanical environment and have clear preferences for certain features of the substrate, including topographical features. Johansson *et al.*[18] report that neuronal processes preferred to grow on ridge edges and elevations in the patterns rather than in grooves (see also Lee *et al.*[19]). A further example was reported by Georges *et al.*,[20] who link matrix compliance to growth behaviour, adhesion and morphology of primary cortical neurons and astrocytes. Those authors have shown that neurons have a bias for growth on soft materials (whereas astrocytes spread and adhere better to stiff materials). Franze *et al.*[15] report that a growth cone's ability to detectably deform its environment is proportional to the forces exerted by the growth cone. Hence, active mechanosensitive probing of the surrounding may provide positive stimuli for neuronal growth in a soft environment, while more rigid contacts will deliver a negative feedback.

In addition to matrix compliance, neurons have a clear preference for complex three-dimensional structures. In particular, neurons (and several other cell types) appear to adhere to and grow extremely well on surfaces with nanoscale topography.[18,21] The mechanisms involved in the above are not yet fully understood. A step in this direction was attempted by Sorkin *et al.*,[22] who explicitly demonstrated the propensity of neuronal processes to entangle around features of rough surfaces by utilizing a substrate composed of pristine carbon nanotube (CNT) islands (Fig. 2). Neurons were found bound and preferentially anchored to the rough surfaces; moreover, the morphology of the neuronal processes on the small isolated islands of the high density CNT were found to be conspicuously curled and entangled. The reported attachment

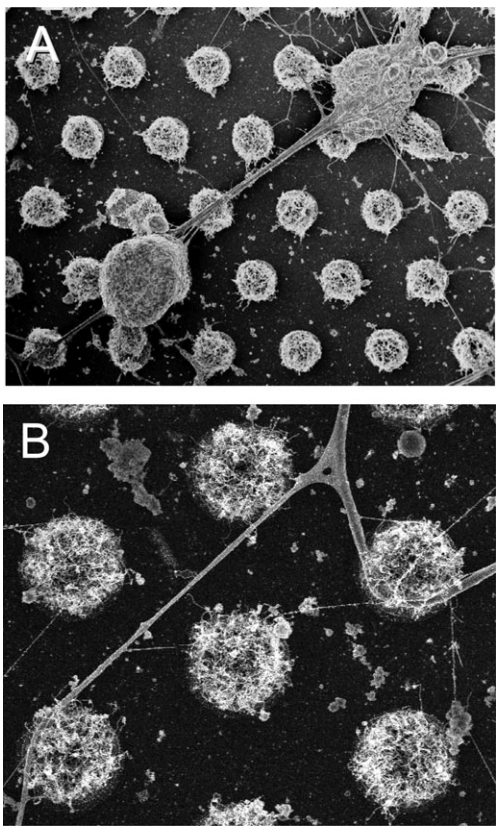

Fig. 2 Locust neurons cultured on substrates composed of pristine carbon nanotube (CNT) islands. The CNT islands are a prefered anchorage site for the neurons (A) and are instrumental in tension generation along the strongly-attached neurites (B). Scale bars: 10 μm and 30 μm in A and B respectively. Images taken from the work of Hanein and Ayali.[4,22,23,30]

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Amir Ayali (born 1963) received his PhD from the Hebrew University of Jerusalem in 1995. He went on to become a postdoctoral fellow at the Section of Neurobiology and Behavior, Cornell University. In 1999 he returned to Israel and started his own group at the Department of Zoology, Tel Aviv University. His interests and research span from behavior and neural mechanisms for behavior to neuronal networks and nervous systems development and plasticity.

mechanism was also found to be relevant to cell–cell interactions.[22] This mechanical anchorage of neuronal processes and the generation of tension along neurites have, in turn, important implications for neuronal and network development (as described below).

Recently, Greenboum *et al.*[23] utilized the above properties of mechanical interactions of neurons with their environment to achieve a unique bio-chip electrode system. The strong mechanical interactions between the neurons and the CNTs are used to position and stabilize the cells and the network and facilitate an optimized interface between the highly-conductive CNTs and the neurons for long-term electrical recordings.

The studies presented above are closely related to new approaches to clinical therapy based on the fabrication of microenvironments for axon guidance and neuronal regeneration. These have been the focus of much recent work.[24,25] In a recent review Norman *et al.*[26] describe biomaterial properties and signalling mechanisms involved in the fabrication of optimal axonal guidance platforms. They emphasize that a thorough understanding of the mechanical properties of the substrate and neuronal mechanotaxis is an essential step toward tissue engineering (see also Yu *et al.*[27]). Hence, the success of these approaches is strongly dependent on our ability to explain how physical forces and mechanical structures contribute to the active material properties of nerve cells and neural tissues, as well as how the impact of these forces affects neuronal information processing and decision-making.[28]

From neuronal and network form to nervous system function

Tension forces affect the function of neurons and the nervous system by way of their contribution to various aspects of neuronal morphology, as described above. However, the mechanical forces have been suggested in recent reports to further influence key processes, instrumental in the development of a functional nervous system. These include affecting the specific functional wiring diagram of neuronal networks, the formation and maintenance of active synapses (synaptogenesis and synaptic plasticity), and more. A related challenge, only very recently tackled, is that of correlating previous and novel *in vitro* results to observations, as well as experimental manipulations that demonstrate a role for mechanical tension in intact animals.

In a recent review Luo and O'Leary[29] present axon pruning, the selective elimination of axon and dendrite branches and synapses, as a key process in neuronal plasticity, both during normal development of the nervous system as well as in response to injury or disease. They review the potential cellular and molecular mechanisms that underlie these phenomena. Anava *et al.*[30] have utilized the recently developed CNT substrate mentioned above in order to examine in detail the development and branching pattern of cultured insect neurons, as well as the geometry of their interactions with the substrate. They identified key early developmental steps preceding and regulating neuronal interconnections. These are based on the mechanical attachment of neuronal branches to their targets and on the resulting induced tension, which serves as a signal for survival of the axonal branch (or for branch pruning, Fig. 1D–F), and also for the subsequent formation of synapses. Franze *et al.*[15] explored more closely the mechanism involved in axonal branch pruning and suggest, as mentioned above, that local mechanical stress (above a distinct threshold) results in a calcium influx through mechanosensitive, stretch-activated, ion channels, with subsequent neurite retraction. Hence the build-up of mechanical tension in developing axons is instrumental in axonal pruning and probably also in synaptogenesis, two fundamental processes in developing functional networks, traditionally predominantly attributed to biochemical and activity-dependent mechanisms.

A further important step towards connecting mechanical forces and nervous system functional aspects was recently demonstrated by Siechen *et al.*,[31] who showed that neuromuscular synapses employ mechanical tension as a signal to modulate vesicle accumulation and synaptic plasticity. Traditionally, neurotransmitter vesicle accumulation at presynaptic terminals has been again attributed primarily, if not exclusively, to biochemical signalling processes originating from the post-synaptic cell. In a set of very elegant *in vivo* experiments on the embryonic *Drosophila* nervous system, Siechen *et al.*[31] showed that vesicle clustering at the neuromuscular presynaptic terminal depends on mechanical tension within the axons. Using micromechanical force sensors, those authors further showed that embryonic axons that have formed neuromuscular junctions maintain a rather constant rest tension. The cells actively resisted applied experimental perturbations of this endogenous mechanical force.

Paulus *et al.*[32] have recently investigated whether mechanical forces contribute to axon guidance *in vivo*. They studied the role of mechanical stress generated by muscle contractions in the guidance of zebrafish peripheral Rohon-Beard (RB) sensory axons (Fig. 3). RB peripheral axons extend between the muscle and skin. Different RB axon defects were found in several mutants that affect muscle contraction through different molecular pathways. The severity of these defects appeared to correlate with the extent of muscle contraction loss, and alternative methods of limiting muscle movements caused similar defects. The authors suggest that the mechanical forces generated by muscle contractions are necessary for proper sensory axon pathfinding *in vivo*. The results also provide evidence for the role of mechanical tension in regulating cross-connections between branches of the same neuron (rarely seen *in vivo*).

Moving to a higher neuronal organization level, a prominent feature of most nervous systems is the arrangement of nuclei: neuronal "pools" or dense clusters of functionally-related neurons (good examples of which are the nuclei of the hindbrain: the inferior olivary (ION) and facial motor (fMN) nuclei[33]). To date, the mechanisms underlying neuronal clustering remain uncharacterized. Recent findings by Hanein *et al.* (under review) suggest that the physical forces underlying the clustering phenomenon are very similar to those generating the very general phenomenon of neuronal migration, primarily tension forces along neurites (Fig. 1). Neuronal migration is an intricate process involving a wide range of cellular mechanisms, some of which are still largely unknown. Time-lapse investigation and quantitative vectorial analysis of the forces applied to the neuron by its pulling neurites have revealed that

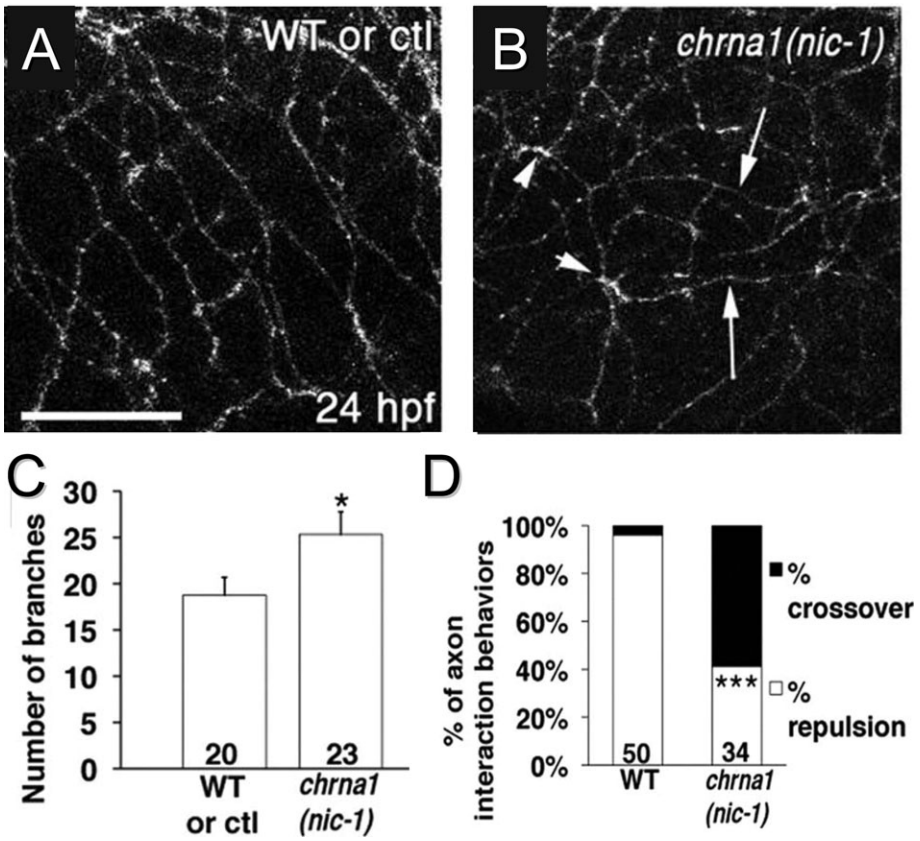

Fig. 3 Blocking muscle contractions in zebrafish embryos causes peripheral RB axon guidance defects (adapted with permission from Paulus *et al.*[32]). A. Lateral view of embryo showing labeled RB neurons. The peripheral RB axons in WT or control treated embryos generally grow ventrally and avoid each other. B, *chrna1* (*nic-1*) embryos (with blocked muscle contraction) show defects in the peripheral RB axons with longitudinal axons (arrows) and axons making apparent extended contacts with each other (arrowhead). C & D, Quantification of individual peripheral RB axon arbours demonstrate defects (among others) in the number of branches, and the percentage of crossing-over events. $*p < 0.05$, $***p < 0.005$. Numbers on graphs indicate number of axon arbours, or axon interactions. Error bars indicate SEM.

tension forces are responsible for relocating the position of the cell body and ultimately for generating clustered network topologies.[4]

Xu *et al.*[34] have gone even further, suggesting that sustained tensile stress exists in white matter of the mature mammalian brain. They applied a series of carefully directed crosscuts in the mouse brain and examined the resulting deformations. Their findings indicate that cerebral white matter is under considerable tension while the cerebral cortical gray matter is in compression. These findings could be related to previous suggested mechanisms of brain development (including cortical folding in the human brain).

Concluding remarks

All the above-reviewed studies are characterized by application of an interdisciplinary, systems-thinking (rather than reductionist) approach to the study of nervous system development. Under such an approach, the very important and even central role of biochemical–molecular mechanisms in controlling various aspects of neuronal development is not disputed. However, it is clear that a full understanding of the processes comprising not only the development of the complex architecture of the nervous system, but even that of a single neuron in our brain, will be achieved only when also taking into account the effects of physical–mechanical forces.

As described above, we have been recently presented with very compelling findings relating mechanical forces (excreted by neurons and their environment) to neuronal and network function. More work in this direction is needed; specifically, studies relating phenomena described *in vitro* to intact *in vivo* systems. Future results will greatly contribute to relocating the question of the function of mechanical tension in neuronal and network development to centre stage in the coming years.

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