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The emerging role of forces in axonal elongation

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ABSTRACT

An understanding of how axons elongate is needed to develop rational strategies to treat neurological diseases and nerve injury. Growth cone-mediated neuronal elongation is currently viewed as occurring through cytoskeletal dynamics involving the polymerization of actin and tubulin subunits at the tip of the axon. However, recent work suggests that axons and growth cones also generate forces (through cytoskeletal dynamics, kinesin, dynein, and myosin), forces induce axonal elongation, and axons lengthen by stretching. This review highlights results from various model systems (*Drosophila*, *Aplysia*, *Xenopus*, chicken, mouse, rat, and PC12 cells), supporting a role for forces, bulk microtubule movements, and intercalated mass addition in the process of axonal elongation. We think that a satisfying answer to the question, "How do axons grow?" will come by integrating the best aspects of biophysics, genetics, and cell biology.

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Abbreviations: m, meter; μm, micron; mm, millimeter; d, day; h, hour; PDMS, polydimethylsiloxane; nN, nanoNewton; P, peripheral; T, transition; C, central; pN, picoNewton; FSM, fluorescent speckle microscopy.

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1. Introduction: how do axons grow?

Despite a century of investigation since the pioneering work of Ramón y Cajal, a satisfying answer to the question "How do axons grow?" still eludes us. This question is interesting for at least two reasons: Firstly, understanding the mechanism of axonal elongation is essential for acquiring a better picture of what happens during the development of the nervous system (Lowery and Van Vactor, 2009). Secondly and perhaps more practically, if we have a better understanding of how axons grow, we can devise rational strategies to overcome intrinsic limitations to regrowth and to accelerate regeneration following injury and disease (Chen et al., 2007). Enormous gains have recently been made in our understanding of the cell biology of axonal growth: force generation by molecular motors and microtubule dynamics have emerged as crucial processes for both axonal guidance and lengthening (Conde and Caceres, 2009; Vallee et al., 2009). Yet most studies to date have used relatively simple read-outs, such as measuring changes in the rates of axonal elongation, rather than characterizing and quantifying the underlying behavior of individual axonal and growth cone components. Thus, in order to achieve a better understanding of the mechanisms of axonal elongation, quantitative biophysical and imaging methods are needed to analyze the relationship between (1) forces acting on neurons, (2) the bulk movement of cytoskeletal elements/organelles in response to forces, and (3) cytoskeletal assembly/disassembly dynamics inside living neurons. In this review, we discuss the underlying cytoskeletal mechanisms of tension-induced axonal elongation. In the first part, we highlight recent key studies providing evidence for the role of tension in driving axonal elongation. This has been an understudied problem, but we think it is very important because it offers new insights into the process of axonal elongation. We then follow with a discussion of how forces could affect microtubule polymerization/translocation dynamics during axonal elongation and growth cone advance. Lastly, we discuss potential mechanisms of how forces could translate into changes of cellular physiology and signaling as well as the question whether a universal mechanism of axonal elongation exists across different species.

2. Forces and axonal elongation

2.1. Forces cause axons to grow

It is utterly remarkable that neurons can grow to the length of 30 m in blue whales and more than 1 m in humans (Smith, 2009), perhaps even more so when considering that most of this growth happens after synapse formation and is driven by the increase in body size of the animal. This mechanism of elongation has long been recognized: Harrison (1935) called it "passive stretching" and Weiss (1941) called it "towed growth". Stretch growth of axons likely begins during embryogenesis. As the animal's body grows, the distances between neuronal cell bodies and synapses steadily increase, thereby exerting tensile forces on the axons. In a series of innovative *in vitro* studies, the growth cones of cultured chick sensory axons were attached to glass needles to examine their response to forces (Bray, 1984; Lamoureux et al., 1989). Axons could be stretched up to 100 μm over a few hours without apparent thinning or disruption of the cytoskeleton. More recent studies have confirmed that externally applied forces potently induce axonal elongation. In the context of this problem the work from Smith's group is particularly interesting. In an effort to design strategies for improved axonal regeneration following injury, they developed a specialized chamber system in which neurons are cultured on two initially contiguous platforms that are pulled apart by a stepper motor (Pfister et al., 2006, 2004). The axons of neurons plated onto these platforms can be elongated to lengths of 10 cm at a sustained rate of 8 mm/d (330 μm/h). This is approximately ten times faster than typical growth-cone mediated axonal outgrowth rates (see Table 1.1 in Gordon-Weeks, 2000) and can be continued for many days. Furthermore, these neurons tend to increase in diameter (Pfister et al., 2004) and are functionally normal in their electrophysiology (Pfister et al., 2006). In a separate study, the effect of leg lengthening on axonal stretching was examined *in vivo* (Abe et al., 2004). As an indirect way to determine if axons stretch, the distance between nodes of Ranvier was measured as an assay. In myelinated axons in the peripheral nervous system, Schwann cells are wrapped tightly around axons. Stretching of the underlying axon causes lengthening of the Schwann cells and the internodal distance. Using orthopedic leg-lengthening procedures in adult rats, it was found that applied forces could double inter-nodal distances without significant axonal thinning. An important aspect of all the studies discussed above is that forces were applied at low levels over long time periods: hours to days. Notably, acute stretching resulting in high tension, as it occurs clinically when large nerve gaps are directly joined, impairs axonal regeneration (Sunderland et al., 2004; Yi and Dahlin, 2010). Together these results indicate that forces, when carefully controlled, are powerful stimulators of axonal elongation.

2.2. Neurons generate forces

With the advent of nanotechnology and sophisticated software to track microscopic movements, there has been a surge of interest in neuronal biomechanics. Several recent reviews focused on biophysical properties of neurons. Ayali (2010) discusses the role of forces in neuronal morphology, network formation, and the effects of substrate stiffness. Franze et al. (2009) have written an excellent book chapter covering the foundations of rheology, measurement techniques, and the viscoelastic properties of neurons and the brain. Bueno and Shah (2008) discuss the effects of tensile loading on neurons and the nervous system. Lastly, Franze and Guck (2010) recently published a comprehensive review on the biophysics of neuronal growth and the susceptibility of neurons to physical cues. In brief, the methods used to study the physical properties of neurons have innovatively utilized nanowires (Hallstrom et al., 2010), force calibrated glass needles (Bernal et al., 2007), microfabricated silicon-based micromechanical force sensors (Siechen et al., 2009), optical stretchers (Lu et al., 2006), stretchable polydimethylsiloxane (PDMS) substrates (Ahmed et al., 2010), and polyacrylamide gel-based compliant substrates (Chan and Odde, 2008). Using these approaches the significant findings have been that (1) tension generation by growth cones is higher on softer (i.e. $<\sim$1 kPa) substrates (Chan and Odde, 2008), (2) glial cells provide a soft substrate that may facilitate axonal elongation (Lu et al., 2006), (3) active force generation in neurons causes them to shorten when slackened (Ahmed et al., 2010; Bernal et al., 2007), and (4) the rest tension of axons both *in vivo* and *in vitro* is in the